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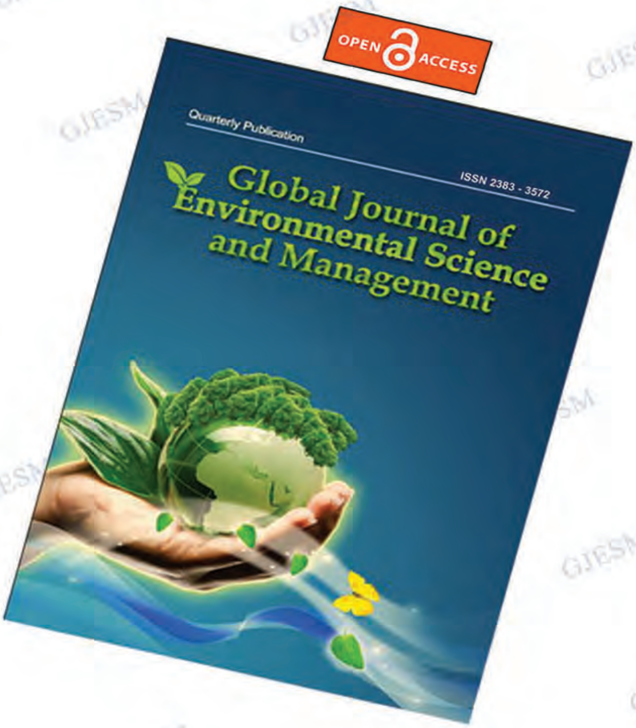

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ORIGINAL RESEARCH PAPER

Life cycle assessment of coconut plantation, copra, and charcoal production

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ABSTRACT

BACKGROUND AND OBJECTIVES: Coconuts and their derivatives, such as copra and charcoal, are leading commodities of Indonesia contributing to local consumption and exports. Life cycle assessment is a tool for evaluating the inputs, outputs, and potential impacts of a product system throughout its life cycle and is associated with product sustainability. The cradle-to-gate life cycle assessment of copra and coconut shell charcoal aims to determine the impacts of coconut, copra, and charcoal production from copra byproducts quantitatively and identify scenario improvements to reduce the impacts and enhance sustainability.
METHODS: Field observations were conducted in tall coconuts in Agrabinta, South Cianjur, and in copra and coconut shell charcoal factories in Sukabumi, West Java, Indonesia. The life cycle assessment method comprises the following four stages: goal and scope definition, inventory analysis, impact assessment, and interpretation. The scope of this study was based on land preparation, nurseries, planting, fertilization, harvesting of mature coconuts, transportation of mature coconuts, copra production, transportation of coconut shells, and charcoal production. Ten impacts were calculated using the Center of Environmental Science of Leiden University Impact Assessment baseline method with Simapro software.
FINDINGS: This study obtained ten impact categories, not only the global warming potential impact similar to most studies of perennial crop products in Indonesia. Normalization results showed that the category with enormous impacts on humans from coconut cultivation and copra processing activities had terrestrial ecotoxicity potential. The largest impact on charcoal production was on the human toxicity potential. Separated coconut factories from plantations have a high impact because of high fuel transportation. Four recommendation scenarios were formulated: 1) utilization of smoke from pyrolysis into liquid, 2) implementation of organic coconut cultivation practices, 3) integration of coconut plantations with copra and charcoal processing plants and processing smoke into liquid, and 4) combining scenarios 1, 2, and 3. In scenario 3, seven of ten impacts showed the lowest value among other scenarios. This scenario potentially decreases the impact from 68.35 to 99.62 percent. The human toxic potential of coconut shell charcoal decreased from 2.92×10^5 to 109.43 kilogram 1,4-dichlorobenzene equivalent, terrestrial ecotoxicity potential decreased from 59 to 19 kilogram 1,4-dichlorobenzene equivalent, and the global warming potential decreased from 1753.55 to 93.03 kilogram carbon dioxide equivalent.
CONCLUSION: Life cycle assessment can evaluate the impacts of copra and coconut shell charcoal from the coconut cultivation to the production stages. Opportunities for improvement can be identified from the interpretation and hotspots. Scenario analysis results showed the potential of developing integrated coconut agroindustry with coconut plantations, copra factories, and charcoal factories to produce liquid smoke in one location. This integration markedly reduces the impact due to the reduction of transportation fuel and emissions and the treatment of air pollution from pyrolysis.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
55	5	10

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INTRODUCTION

Coconuts (*Cocos nucifera* L.), one of the most important commodities of Indonesia, have a significant economic impact. Coconut is a plantation commodity with the third-largest area of production and productivity in Indonesia, after palm oil and rubber. According to the Food and Agriculture Organization (FAO), Indonesia was the largest coconut producer in 2019 and 2020, with a total production of 17.07–16.82 million tons of nuts, followed by the Philippines at 14.77–14.49 million tons and India at 14.68–14.69 million tons (FAO, 2021). The coconut area in the world was around 11.63 million ha in 2019, 79.1% of which are found in three countries, namely the Philippines (31.4%), Indonesia (29.3%), and India (18.5%) (ICC, 2022). The Directorate General of Estate Crops (DGEC) reported that Indonesia has 3.4 million ha of coconut farms, which comprise 3.27 million hectares (ha) of tall coconut plantations and the remainder being hybrid coconut plantations (DGEC, 2022). Approximately 99.4% of tall coconut plantations are smallholder plantations cultivated by farmers in gardens and their yards in monocultures or mixed gardens, involving approximately 6.1 million coconut farming families. The coconut production in Indonesia is approximately 2.86 million tons, which is equivalent to copra, or approximately 14.3 billion coconuts assuming that 1 kg of copra is obtained from five coconuts (DGEC, 2022). The largest coconut plantation areas in Indonesia are found in the following provinces: Riau, North Sulawesi, East Java, Central Sulawesi, Central Java, North Maluku, and West Java (DGEC, 2022). Cianjur is a crucial coconut production area in West Java, with 8042 ha and 4252 tons, particularly in southern Cianjur. Coconut plants can be harvested from immature and mature plants. Immature coconuts are directly consumed while mature coconuts can be used as raw materials in various products. The coconut fruit comprises 35% coconut coir, 28% endosperm, 25% coconut water, and 12% coconut shells (Mawardi *et al.*, 2016). The final production of coconut meat in Indonesia is divided into the following: copra (42.5%), coconut oil (46.9%), desiccated coconut (4.7%), and others (5.9%) (ICC, 2022). Almost every part of the coconut palm can be utilized to make high-value products; thus, the coconut is known as the "tree of life." However, the productivity of coconuts in Indonesia is still low. Therefore, increasing production by replanting unproductive palms, promoting good agricultural practices, and introducing high value in coconut plantations and products is necessary (Alouw *et al.*, 2020). Regarding coconut exports, India, Indonesia, and the Philippines export 11, 9, and 13 types of coconut products, respectively (ICC, 2022). Copra and coconut shells are among the most exported products in Indonesia. The export of coconut shell derivative products in Indonesia is still dominated by nonactivated shell charcoal because it does not require activation processes. Charcoal can be used as a raw material for other derivative products, such as activated charcoal, biogas, bio-pellets, and bio-briquettes, which can be used for various applications (Yavari *et al.*, 2021). Copra is a coconut intermediate product obtained by drying coconut meat and is typically used for cooking oil, food, beverages, and cosmetics. In 2019, 108.3 tons of copra were exported to various destination countries, such as Sri Lanka, Pakistan, and Malaysia (DGEC, 2022), increasing copra export from 2022 to 155.65 tons. A total of 0.81 tons of coconut shell can be obtained as a byproduct from 1 ton of copra (Kaseke, 2016). At the farm level, burning coconut shells into charcoal produces gas and dust pollutants, which can reduce the ambient air quality of the surrounding environment. Incomplete combustion produces various emissions, such as carbon oxide (CO), methane (CH_4), ethylene (C_2H_4), and other volatile organic compounds (VOCs) (Arena *et al.*, 2016). Thus, controlling the environmental impact of charcoal production throughout its life cycle is necessary. Environmental impacts can be calculated using the life cycle assessment (LCA) method. LCA is a compilation and evaluation of the inputs, outputs, and potential environmental impacts of a product system throughout its life cycle (ISO, 2006). Climate change, followed by extreme weather patterns, is a considerable challenge for the entire agricultural system, including coconut plantations. Climate impacts are expected to become prominent by 2050 and will affect availability and utility (Pathiraja *et al.*, 2015). Thus, a coconut development system and its derivatives must be environmentally friendly (Maliangkay and Matana, 2018). Sustainability and environment-friendly products are essential for building consumer confidence. One is the application of ecolabels (Riyanto *et al.*, 2018). Ecolabel identifies the overall environmental performance of a product

Table 1: Scope and impact categories of LCA studies in perennial crop products in Indonesia

Reference	Commodity	Scope	Impact categories									
			GWP	AP	EP	ADP	ADP-FF	HTP	TEP	FAP	POP	ODP
Suprihatin *et al.* (2015)	Palm oil	Cradle-to-gate	√									
Siregar *et al.* (2015)	Palm oil	Cradle-to-gate	√									
Siregar *et al.* (2015)	Jatropha	Cradle-to-gate	√									
Yusuf *et al.* (2019)	Sago	Cradle-to-gate	√									
Diyarma *et al.* (2019)	Arabica coffee	Cradle-to-gate	√									
Pramulya *et al.* (2022)	Gayo arabica coffee green bean	Cradle-to-gate	√									
Gunawan *et al.* (2019)	Sugarcane	Cradle-to-gate	√									
Parameswari *et al.* (2019)	Quinine	Cradle-to-gate	√	√	√							
Mila *et al.* (2020)	Green tea	Cradle-to-gate	√	√	√			√		√	√	
This study	Coconut	Cradle-to-gate	√	√	√	√	√	√	√	√	√	√

Note: abiotic depletion potential (ADP), abiotic depletion potential (fossil fuels) (ADP FF), global warming potential (GWP), ozone layer depletion potential (ODP), human toxicity potential (HTP), freshwater aquatic ecotoxicity potential (FAP), terrestrial ecotoxicity potential (TEP), photochemical oxidation potential (POP), acidification potential (AP), eutrophication potential (EP).

or service based on life cycle considerations (Setiawan *et al*., 2019). The results of the LCA calculations can be used as the basis for type III ecolabel or environmental product declaration (EPD). An LCA study of copra and coconut shell charcoal products should be conducted at the cultivation stage (cradle-to-gate). Most LCA studies on plantation products in Indonesia have been conducted on palm oil (Suprihatin *et al*., 2015; Siregar *et al*., 2015), quinine (Parameswari *et al*., 2019), jatropha (Siregar *et al*., 2015), sago (Yusuf *et al*., 2019), and arabica coffee (Diyarma and Bantacut, 2019). The impact categories from other studies generally only considered global warming potential, acidification, and eutrophication. Thus, this study considered additional impact categories, such as ecotoxicity and human toxicity potential. Table 1 shows the impact categories and scope used in the LCA study of perennial crop products.

Except for the impact categories, minimal attention has been provided to the agronomic aspects of perennial or plantation crops in LCA because of the limited data available and the lack of methodological guidelines to explain the entire life cycle, especially the agricultural/plantation aspects. Calculating the entire lifespan of perennial crops within the LCA is necessary (Diyarma and Bantacut, 2019; Bessou *et al*., 2013). LCA studies of coconut-derived products in Indonesia have been limited to gate-to-gate or production aspects, such as coconut shell liquid smoke (Yuliansyah, 2019). As a leading export product, evaluating the environmental impact of copra and charcoal products is important to improve their environmental performance such that they can increase product competitiveness and can be promoted as environmentally friendly products. This study can also be a reference to the LCA of other derived products, such as coconut cooking oil, activated carbon, bio-briquette, and bio-pellet. This study aimed to analyze inventory by identifying inputs and outputs, calculating the impacts of coconuts, copra, and coconut shell charcoal using the LCA approach, and identifying improvement scenarios to reduce these impacts and improve sustainability. This study was conducted on coconut plantations in Agrabinta, South Cianjur, and a copra-charcoal factory in Sukabumi, West Java, Indonesia, from 2020 to 2021.

MATERIALS AND METHODS

This study was based on the LCA framework following the ISO 14044 guidelines, which comprises several stages: 1) goal and scope definition, 2) life cycle inventory (LCI), 3) life cycle impact assessment (LCIA), and 4) interpretation followed by improvement recommendations.

Goal and scope definition

An LCA study was conducted to quantify the impacts of coconuts on farms, copra, and coconut charcoal. This study aimed to identify hotspots and opportunities for improvement. The scope of this study was cradle-to-gate, starting from preparation of coconut land and nurseries, planting, fertilization, and harvesting mature coconuts, which had been conducted for 42 years, to transporting coconuts from farm to factory, copra production in the factory, and charcoal production (Fig. 1). Forty-two years was considered to be the coconut age because it is necessary to calculate the entire lifespan of the perennial crop within the LCA (Bessou *et al.*, 2013).

Life cycle inventory (LCI)

The LCI stages include data collection, calculation, validation, and linkage with process and function units based on mass and energy balances. The data comprised primary (foreground) data from plantations and secondary (background) data from the literature, Ecoinvent 3.5, and agri-footprint databases. The Technical Instructions for Tall Coconut Cultivation of the Palma Plantation Research Institute (PPRI) collected data on planting, fertilization, and maintenance of coconut plants (PPRI, 2015). The output comprised primary products, co-products, and emissions. Inventory analysis also addresses allocation mechanisms to discover the processes shared with different production systems. The mass balance determines allocation. The inventory analysis of this LCA study used 1 ha of land for coconut plantations as the basic unit for the unit function of a 1 ton mature coconut on the farm. The impact study normalized the unit function based on the plantation yield of 1 ton of copra and 1 ton of coconut charcoal.

Life cycle impact assessment (LCIA)

According to ISO 14044, the mandatory elements of LCIA include the selection of impact categories, classification of LCI results, and characterization or calculation of category indicator results. The characterization is calculated using Eq. 1 (Heijungs *et al.*, 2004).

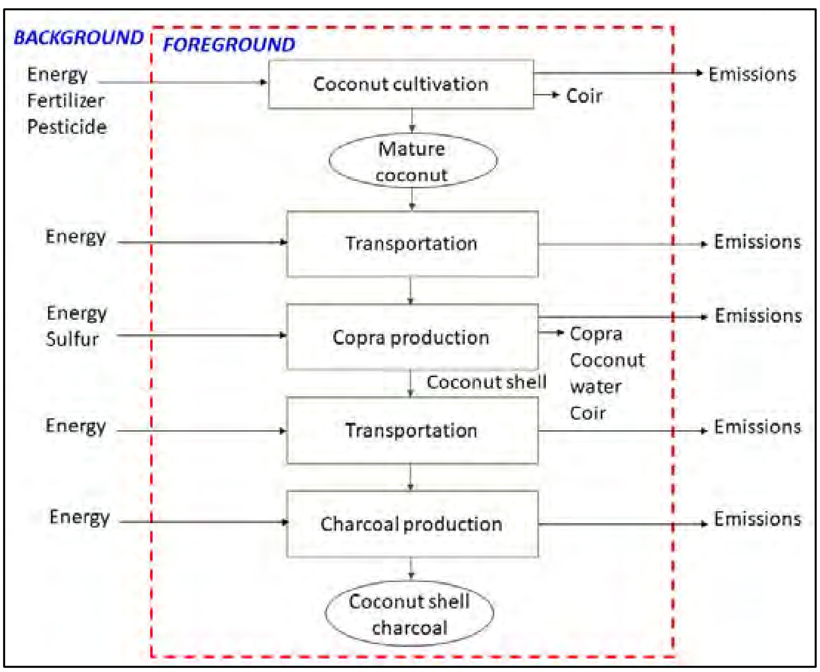


Fig. 1: System boundary (scope) of the LCA (cradle-to-gate)

$$IRc = \sum_{s} CFcs \text{ x } ms, \quad (1)$$

where:

IR_c : indicator results for impact category c;

CF_{cs} : characterization factor that connects inventory s with impact category c;

m_s : amount or mass of inventory s.

The impact assessment calculation was performed using SimaPro software version 9.3, Faculty License. The selected impact assessment method was the Center of Environmental Science of Leiden University Impact Assessment (CML 2001-IA baseline) because it is the most widely used impact calculation method in LCA studies in the agriculture and food product (agri-food) sectors (Merchan and Combelles, 2012). CML 2001 uses a midpoint approach, which includes all emission- and resource-related impacts (Guinee, 2002). The mandatory impacts (baseline) in the CML 2001-IA baseline comprise 11 midpoint categories: ADP, ADP-FF, GWP100, ODP, HTP, FAP, marine aquatic ecotoxicity potential (MAP), TEP, POP, AP, and EP. MAP was excluded from this study because the characterization model of this impact category is still debatable due to the absence of evidence regarding increased pollutants in seawater caused by atmospheric emissions (Wiloso *et al.*, 2019; Heijungs *et al.*, 2004). A normalization procedure was performed to identify the most significant impact categories for humans.

Interpretation and recommendation

The results of the LCI and LCIA stages should be interpreted considering the objectives and scope of the study. During the interpretation stage, inferences were made from the resultant hotspots, specifically the stages with the most substantial impact categories that played the most significant role. Scenarios for improvement were also identified, and the impact reduction was calculated.

RESULTS AND DISCUSSION

Life cycle inventory

The data revealed that 1 hectare (ha) of tall coconut plantations has generated an average of 1235 mature coconuts/ha/year since the sixth year of planting. The weight of each tall coconut was approximately 1.4 kg, which is equal to 1729 tons/ha/year. Table 2 shows the input and output data from the Inventory of Tall Coconut cultivation for one cycle (42 years) within the scope of the cradle-to-farm gate. These activities begin with land preparation, nurseries, planting, fertilization, and harvesting, including transportation to the warehouse and coconut husk stripping. The distance between the plantation and the warehouse was 6.2 km. The production site is in the same area as the coconut plantation; thus, the transportation of supporting materials, such as fertilizers and pesticides, is zero (0).

Land preparation comprised several stages: land clearing, establishment of plant lines, and creation of planting holes. The initial land for the Tall Coconut Plantation at the Agrabinta Plantation was an acacia wood secondary forest. Land clearing is performed by chopping trees using an ax to cut shrubs and small trees and a chainsaw to cut down giant trees. The average time to cut down acacia trees using a chainsaw on a 25% sloping topography is 0.06 h/tree (Wulan *et al.*, 2020). The number of acacia trees per 1 ha was 647; therefore, chopping an acacia tree on 1 ha of land takes 38.82 h. Logging was performed using a gasoline-fueled chainsaw with a requirement of 3 L/h and mixed with oil at a ratio of 25:1 (Dulsalam *et al.*, 2018; Faqih *et al.*, 2018). The following process involved land preparation using a diesel-fueled four-wheeled tractor with a disk plow, which takes 7.5 h/ha with a diesel fuel consumption of 6.498 L/h (Murti *et al.*, 2016). Processing 1 ha of land requires as much as 49 L of diesel fuel. The polybag used for seedlings was black polyethylene with a length of 40 cm, a height of 50 cm, and a thickness of 0.18–0.10 mm (16–17 pieces of polybag/kg). Plating 160 seeds over 1 ha took 160 polybags weighing 10 kg. Fertilization was performed until the seedlings were eight months old based on the PPRI guidelines. Table 3 shows the dosage of fertilizers during seedlings, and Table 4 shows the dosage during fertilization after planting. The types of fertilizers used are Urea (Nitrogen source), P_2O_5 or SP-36 (phosphor source), KCl (Potassium source), Kieserite (Magnesium source), and Borax (Boron source). Intensive fertilization only lasts until the fourth year, after which poultry manure is given annually at the rate of 5 kg/tree/year.

The primary outputs of tall coconut cultivation are coconut bunches and biomass from coconut leaves and fallen leaf midribs. The amount of fallen leaves is estimated to be 5 kg/ha/year, with the leaf midrib

Table 2: Inventory input of coconut cultivation (1 ha in one cycle – 42 years)

Process stage	Input/output	Amount	Unit
Input			
	Gasoline fuel	116.46	L
Land preparation	Oil	4.66	L
	Diesel fuel	49	L
	Polybag	10	kg
	Coconut seeds	160	pcs
	Urea	10.4	kg
	Phosphorous pentoxide (P_2O_5)	7.2	kg
Nursery/ seedlings	Potassium chloride (KCl)	18.4	kg
	Kieserite	4.8	kg
	Herbicide	0.6	kg
	Insecticide	0.6	kg
	Fungicide	0.6	kg
Planting	P_2O_5	4.8	kg
	Urea	272	kg
	P_2O_5	192	kg
Fertilization	KCl	464	kg
	Kieserite	80	kg
	Borax	9.6	kg
	Poultry Manure	15,200	kg
Harvesting	Fuel	2,901.6	L
Output			
Harvesting	Coconuts	62,244	ton
	- Coconut grains	41,349	ton
	- Coconut coir	20,895	ton
Biomass	Coconut leaves	95,020	ton
	Coconut midrib	52,053	ton

Table 3: Fertilizers dosage during seedlings (PPRI Guideline)

Fertilizers	Seedling age (month)							
(g/seed)	1	2	3	4	5	6	7	8
Urea	5	5	5	10	10	10	10	10
P_2O_5	0	0	15	0	0	0	0	0
KCl	10	10	10	15	15	15	20	20
Kiserit	5	0	5	0	10	0	10	0

Table 4: Fertilizers dosage during fertilization (PPRI Guideline)

Fertilizers	Amount (g/tree/year)			
	Year I	Year II	Year III	Year IV
Urea	250	250	500	700
P_2O_5	175	175	350	500
KCl	350	350	700	1500
Kieserite	50	100	150	200
Borax	-	10	20	30

weighing up to 188.56 kg/tree. The biomass falls to the ground and decomposes if left unmanaged. The harvested coconuts were transported to a copra factory in Sukabumi, West Java. Copra-processing inventories were justified on the basis of the number of coconuts per hectare in one cradle-to-gate cycle. The activity began by transporting raw materials from the coconut warehouse in the Agrabinta plantation to the copra factory at a distance of 142 km. The total input of coconut grains used was 41,349 tons.

Table 5: Inventory data of copra product (unit function: 1-ton copra)

Process	Input/Output	Amount	Unit (Per 1 ton of Copra)
Transportation of harvested coconut (142 km)	Input		
	- Mature coconut	4.168	ton/ton
	- Diesel fuel	0.045	GJ/ton
	Output		
	- Mature coconut	4.168	ton/ton
Transportation of sulfur (110 km)	Input		
	- Sulfur	0.001	ton/ton
	- Gasoline	0.036	GJ/ton
	Output		
	- Sulfur	0.001	ton/ton
Production of copra	Input		
	- Mature coconut	3.182	ton/ton
	- Sulfur	0.001	ton/ton
	- Electricity	0.023	GJ/ton
	Output		
	- Copra	1.000	ton/ton
	- Water vapor	0.953	ton/ton
	- Coconut water	0.752	ton/ton
	- Coconut coir	0.161	ton/ton
	- Coconut shell	0.989	ton/ton

Coconut grains that arrive at the factory are sorted and cleaned from the remaining fibers in the coconut shell. The coconut was split and dried in a drying chamber. Fumigation was conducted on the first and second days of drying using 0.2 kg of sulfur for each drying room with a capacity of approximately 2000 coconuts. Fumigation aims to clean and prevent fungal contamination in dried coconut meat (Najamuddin *et al*., 2020). Table 5 presents the inventory data for the copra products.

Table 5 shows that the highest material inventory hotspot was mature coconuts at 4.168 tons/ton copra. The entire copra production is traditionally processed; machines or equipment that require large amounts of energy are not used. The use of energy in the form of electricity in copra production shows a small ratio to copra products because it is only used for lighting. Coconut shell charcoal is produced by burning coconut shells sourced from the byproducts of copra processing. The coconut shell used as a raw material for charcoal affects the mass allocation of the copra. Table 6 reveals that the highest inventory hotspot is the use of coconut shells and fibers, which is 5601 tons/ton of charcoal.

Pyrolysis produces charcoal, ash, and combustible substances containing various compounds. Of the charcoal products produced, 11% are raw or immature charcoal (Darmawan *et al.*, 2015). Ash is produced from charcoal processing fuel because the furnace in which the fuel is only partially closed is still in operation. The burning substances from smoke due to pyrolysis contain various compounds, such as water vapor, gases, and VOCs. Odor emissions and the formation of photochemically reactive species are the most influential impacts associated with VOCs in the environment (Reyes *et al.,* 2020). During fuel combustion, the combustible substances contain only CO_2 and dihydrogen oxide (H_2O). H_2O and gas-containing components CO, CO_2, hydrogen (H), CH_4, and C_2H_4 were produced during pyrolysis as much as 18.97%, 35.63%, 0.23%, 4.02%, and 2.30%, respectively, and other volatile matter as much as 18.24% of the charcoal (Fagbemi *et al.,* 2001). The amounts of combustible substances in the charcoal are listed in Table 7.

Life cycle impact assessment (LCIA)

The environmental impact analysis of coconut cultivation comprised 10 impacts based on the CML 2001-IA baseline method. Table 8 shows the potential impacts of mature coconuts in this study compared with Indonesia, India, and the Philippines from the Agri footprint.

The Agri-footprint 6 database (a, b, c) uses economic

Table 6: Inventory data of coconut charcoal product (unit function: 1-ton coconut shell charcoal)

Process		Input/output	Amount	Unit (Per 1-ton charcoal)
Transportation of coconut shell from copra factory to charcoal factory (11 km)	Input			
		- Coconut shell + coconut coir	7.337	ton/ton
		- Gasoline	1.305	GJ/ton
	Output			
		- Coconut shell + coconut coir	7.337	ton/ton
Charcoal production	Input			
		- Coconut shell	3.069	ton/ton
		- Coconut shell (firing)	1.498	ton/ton
		- Coconut coir (firing)	0.105	ton/ton
		- Electricity	0.150	GJ/ton
	Output			
		- Coconut charcoal	1.000	ton/ton
		- Half-baked coconut charcoal	0.111	ton/ton
		- Ash of coconut shell	0.020	ton/ton
		- Ash of coconut coir	0.020	ton/ton
		- Burning substance from firing	1.651	ton/ton
		- Burning substance from pyrolysis	1.958	ton/ton

Table 7: Composition of burning substance (smoke) from charcoal production per 1-ton charcoal

Substances		Amount (ton)
Burning substance from firing		
-	CO_2	1.486
-	H_2O	0.166
Burning substance from pyrolysis		
-	H_2O	1.161
-	CO	0.190
-	CO_2	0.356
-	H	0.002
-	CH_4	0.040
-	C_2H_4	0.023
-	VOC	0.182

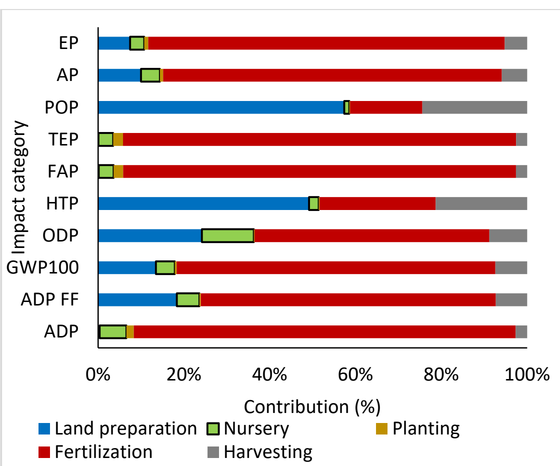
Table 8: Potential value of impact categories per 1 ton of coconut

Impact category	Units	Coconut (this study)	Coconut (Indonesia)[a]	Coconut (India)[b]	Coconut (Philippines)[c]
ADP	kg Sb eq	2.58×10^{-3}	2.43×10^{-4}	3.36×10^{-3}	2.83×10^{-4}
ADP FF	MJ	434.19	363	3.87×10^{3}	481
GWP100	kg CO_2 eq	40.02	1.06×10^{3}	413	344
ODP	kg CFC-11 eq	5.41×10^{-6}	3.35×10^{-6}	3.42×10^{-5}	4.05×10^{-6}
HTP	kg 1,4-DB eq	50.69	5.87	61.1	11.6
FAP	kg 1,4-DB eq	22.40	67.5	289	318
TEP	kg 1,4-DB eq	9.53	5.45	22.9	23.6
POP	kg C_2H_4 eq	0.05	-6.52×10^{-3}	-0.49	-5.35×10^{-3}
AP	kg SO_2 eq	0.26	0.32	8.74	0.431
EP	kg PO_4 eq	0.06	0.78	5.36	0.66

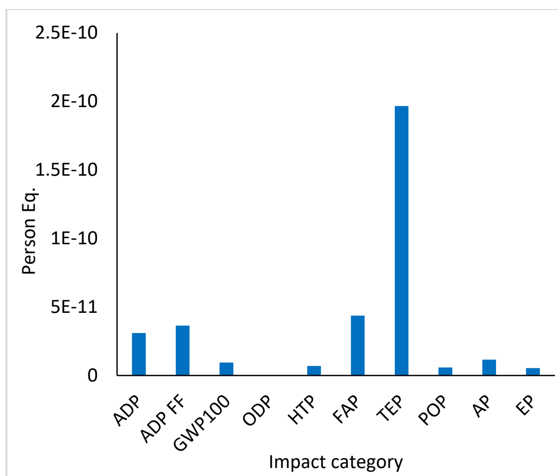
[a]Agri-footprint 6 database (coconuts at orchard {ID}, economics)
[b]Agri-footprint 6 database (coconuts at orchard {IN}, economics)
[c]Agri-footprint 6 database (coconuts at orchard {PH}, economics)

allocation, whereas the current study utilizes the mass allocation method for the LCI. This database covers various natural resources, such as water, land occupation, land transformations, and inputs, which include fertilizers, lime, capital goods, and energy use for field management and irrigation. Specific fertilizer amounts were quantified on the basis of the total NPK and the relative amounts of fertilizer consumed by type in Indonesia by the International Fertilizer Association (IFA, 2021). The total pesticide use in the Agri-footprint database is based on a pesticide model specific to crop–country combinations. Describing coconut plant varieties is necessary; thus, this study used an inventory based on PPRI guidelines for tall coconuts and live field observations. The crop yields of Agri-footprint 6 were derived from FAO statistics using a five-year average (2014–2018). This study covers land preparation and harvesting for up to 42 years. The length of the crop cycle is a key parameter that must be considered in the LCA of perennial crops (Bessou *et al.*, 2013). Fig. 2a shows the percentage impact at each stage of the coconut production process. By contrast, Fig. 2b shows the normalization results for the most influential coconut cultivation activities. LCA contribution analysis of coconut cultivation showed that fertilization was the most critical contributor to these impacts.

Fig. 2 shows that the value of any impact is unrelated to the relevance of the effect because each impact has a unique unit. Normalization was performed in accordance with the ISO 14044:2006 standard. Ranking categories with the same unit based on their influence on person-equivalent is feasible. According to the normalization, TEP has the most significant impact of 9.53 kg 1,4-DB eq/ton harvested coconut. The 1,4-DB (dichlorobenzene) eq unit is the normalized outcome of the ecotoxicity impact and is used to compute the emissions of each dangerous substance in 1,4-DB equivalent units (Singh *et al.*, 2018). TEP and FAP encompass the effects of toxicity on the environment, specifically in terrestrial and freshwater environments. Toxicity impacts involve various indicators that cause environmental harm based on the inherent toxicity and potential exposure to a compound (Tagliaferri and Lettieri, 2019). Fertilization causes the most significant ecotoxicity effects due to various chemical fertilizers (Merchan and Combelles, 2012). The effects of TEP are the most severe because they are linked to chemical fertilizers and pesticides, which directly impact the plantation land. Land exposed to chemical fertilizers for an extended period can leave harmful residues on the soil. The ADP FF impact represents the amount of energy in MJ for fossil resources utilized as energy or fuel. By contrast, ADP is expressed in stibium equivalent units (Sb eq), representing elements of abiotic energy. The stages of fertilization and land preparation primarily influenced this effect. This category of environmental consequences is impacted mainly by the rate at which nonrenewable or finite resources are extracted (Farinha *et al.*, 2019). The impact of ADP is mainly influenced by chemical fertilizers and land preparation by heavy equipment using fossil fuels. The next potential impact is acidification caused by the sulfur dioxide (SO_2) reaction with water in the atmosphere (Acero *et al.*, 2017). Acid-deposition gases include ammonia (NH_3), nitrogen oxides (NOx), sulfur oxides (SOx), and HCl.



(a)



(b)

Fig. 2: Impact of coconut cultivation a) Percentage of the contribution of the coconut cultivation process, b) Normalization of impact category

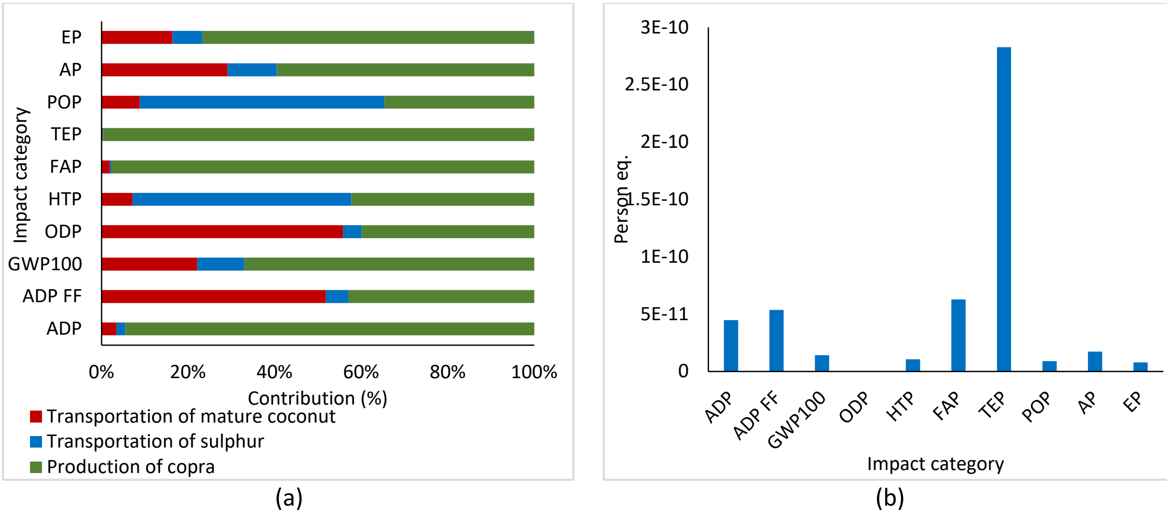


Fig. 3: Impact of copra production a) Percentage of the contribution of copra production, b) Normalization of impact category

Acidification also refers to lowering the soil pH by adding nitrogen, which can have various direct and indirect effects on plant growth (Clark *et al.*, 2013). Coconut has a relatively low GWP100 value when compared to other plantation crops, such as palm oil, jatropha, and quinine salt, with 1378 kg CO_2 eq/ton palm oil, 817.25 kg CO_2 eq/ton jatropha (Siregar *et al.*, 2015), and 1533 kg CO_2 eq/ton quinine (Parameswari *et al.*, 2019). Coconut plants do not require extensive maintenance after fruiting, particularly with chemical fertilizers and pesticides. The coconuts were analyzed at 42 years of age; thus, the age of the crop cycle also affected the degree of impact (Bessou *et al.*, 2013). Crops produce additional fruits as age increases. The influence of crops on the cultivation period was relatively minimal. The copra production system involves transporting raw coconut grains from the warehouse, transferring sulfur-supporting materials from the producer, and processing coconuts into copra. The percentage impacts of the transportation and copra production stages are shown in Fig. 3a. Meanwhile, the normalization results are presented in Fig. 3b.

The use of sulfur in the fumigation process caused a high impact on processing. By contrast, the magnitude of the impact on transportation was due to the use of gasoline to transport coconuts from the warehouse to a copra factory. The number of impact categories is listed in Table 8. The normalization results in Fig. 3b show that the categories of impacts with the most significant influence were almost the same as those on cultivation activities. TEP still demonstrated the most significant impact because most impacts were still influenced by coconut cultivation activities. The system limitation in copra production is cradle-to-gate; therefore, the impact of coconut cultivation is included in the copra product system. The ADP FF impact category remained the next most important factor due to the use of fossil fuels in the copra production system. The energy used in copra production comes from the gasoline utilized for transporting raw materials, supporting materials, and lighting. ADP FF increased to 1683.58 megajoules (MJ)/ton of copra from 434.19 MJ/ton of coconut grains. Another impact was observed for acidification. The use of sulfur in the fumigation process influences this potential impact. The potential impact of acidification has almost doubled from 0.26 kg SO_2 eq/ton coconut to 0.48 kg SO_2 eq/ton copra. The use of sulfur also affects the category of HTP impact, which increases by 30.73 kg 1.4 DB eq. Burning sulfur produces SO_2 gas, which harms human health because it can cause respiratory problems and lung damage considering long-term exposure (Cahyono, 2011). Excess sulfur can irritate the cornea and cause blindness (Najamuddin *et al.*, 2019). The subsequent impact was GWP, which has the potential global impact. The gases that affect

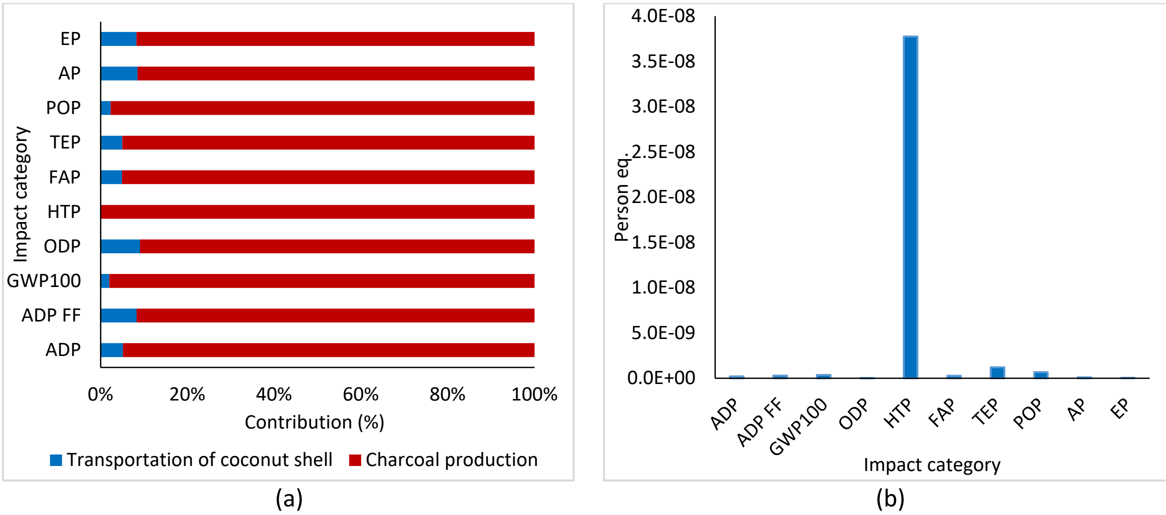


Fig. 4: Impact of coconut shell charcoal production a) Percentage of the contribution of coconut shell charcoal production, b) Normalization of impact category

Table 9: Potential value of impact categories per 1 ton of copra and 1 ton of coconut shell charcoal

Impact category	Units	Copra	Coconut shell charcoal
ADP	kg Sb eq	3.78×10^{-3}	0.02
ADP FF	MJ	1,683.58	8,651.17
GWP100	kg CO_2 eq	70.74	1,753.55
ODP	kg CFC-11 eq	1.87×10^{-5}	9.47×10^{-5}
HTP	kg 1,4-DB eq	81.42	292,487.52
FAP	kg 1,4-DB eq	32.50	140.96
TEP	kg 1,4-DB eq	13.72	58.50
POP	kg C_2H_4 eq	0.08	5.58
AP	kg SO_2 eq	0.48	2.58
EP	kg PO_4 eq	0.10	0.54

GWP include carbon dioxide (CO_2), methane (CH_3), nitrogen oxides (NO_2), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF6) (IPCC, 2006). The analysis shows that copra production produces a GWP100 of 70.74 kg CO_2 eq/ton of copra in a cradle-to-gate. If copra is only seen from the gate-to-gate scope, from raw material transportation to product processing, then GWP100 emissions are only 30.72 kg CO_2 eq/ton of copra. Copra production has a low impact because it uses traditional methods and is primarily performed by manpower. In the Republic of Fiji, copra production through solar drying with manual labor contributes zero emissions (Charan, 2020). For consumption in the UK, copra emissions are 270 kg CO_2 eq/ton copra (Audsley *et al.*, 2009). Differences in technique and process technology, use of materials and energy, and the effect of transportation distance cause variations in impact. Charcoal production comprises shell transportation from the copra factory to the charcoal factory and shell processing into charcoal. Based on the LCIA, the processing of the shell into charcoal had a substantially significant impact compared with the transportation stage for all the impact indicators. The percentage of impact from the transportation and coconut–shell charcoal production stages is shown in Fig. 4a, and the normalization results are depicted in Fig. 4b. The potential values for each impact are presented in Table 9.

On a cradle-to-gate basis, charcoal production uses copra byproduct shells as the raw material, with 23.72% of the coconut grains processed into copra. The existence of an allocation method may affect the processes of previous stages. The calculation results of potential impacts in Table 9 show that all impact categories increased, particularly HTP. The toxic impact of charcoal preparation is obtained from the accumulation of coconut cultivation, coconut transportation, copra production, and shell transportation because the scope of this study is cradle-to-gate.

Normalization was performed for the 10 impact categories to assess the importance of the different impact categories (Wagner and Lewandowski, 2017). The order of impact (from highest to lowest) was HTP, TEP, POP, GWP100, ADP FF, FAP, ADP, AP, EP, and ODP, with person-equivalent values as shown in Fig. 4b. HTP is the impact category with the largest influence on the charcoal product system, which is mostly due to pyrolysis. HTP is a calculated index that reflects the potential hazard of a chemical unit released into the environment and is based on the inherent toxicity of a compound and its potential dose (Acero *et al.*, 2017). During pyrolysis, smoke is released through kiln-drum smoke containing compounds or emissions, including CO, CO_2, H, CH_4, C_2H_4, and VOCs. CO emissions provide significant contributions under scarce oxygen, poor mixture preparation, air entrainment, and incomplete combustion in traditional pyrolysis processes (Gunasekar *et al.*, 2020). Smoke was immediately released into the air because pollution control was not conducted. This uncontrolled air pollutant is similar to biomass burning in the open field, producing primary organic aerosols and VOCs in the atmosphere (Fang *et al.*, 2021). Biomass burning is a significant air pollution source, with global, regional, and local impacts on air quality, public health, and climate (Chen *et al.*, 2017). Water vapor is produced by burning raw materials and ash from coconut shells and fiber fuels. The smoke produced from pyrolysis harms human health and the environment because it can interfere with breathing and vision (Supraptiningsih, 2020). Exposure to coconut shell charcoal smoke can cause skin, eye, and gastrointestinal irritation (Abdollahi and Hosseini, 2014). The small particle can reach the alveolar region of the respiratory system depending on the inhaled particle size distribution. The fine particles penetrate the alveolar region and might be absorbed into the bloodstream of the human body (Chen *et al.*, 2017). This impact leads to charcoal factories that are located far from copra factories and settlements to avoid exposure to pollution in the wide community. HTP is also affected by the use of fossil fuels for transportation and fossil-fueled electricity production (Acero *et al.*, 2017). In the activated carbon production from coconut shells, HTP also has the highest impact (Arena *et al.*, 2016). The impact significantly increased photochemical oxidation potential (POP), which increased to 5.58 kg C_2H_4 eq/ton coconut shell charcoal. Photochemical oxidant formation occurs in relatively stagnant air under sunlight, low humidity, nitrogen oxides, and VOCs (Wang *et al.*, 2021). The GWP100 in the charcoal product system also increased to 1.75 tons CO_2 eq/ton coconut shell charcoal. The gas emissions due to pyrolysis significantly affect the GWP potential. By contrast, the GWP study of coconut shell charcoal in other factories within the gate-to-gate scope resulted in an impact of 0.18 tons CO_2 eq/ton of coconut shell charcoal (Yuliansyah, 2019). The charcoal production system was limited to transporting coconut shells from the market to a factory located 17 km away. In pyrolysis, a kiln drum connected to a condenser produces liquid smoke. The smoke from the pyrolysis results is accommodated in the condenser using this technology such that the emissions do not pollute the environment and the resulting impact is negligible.

Interpretation and improvement recommendations

An enormous percentage of contribution to the impact of environmental pollution (hotspots) occurred at the charcoal production stage, particularly during the pyrolysis process, based on LCI and LCIA. HTP was the most significant impact category for charcoal production. The high impact of HTP is due to exhaust gas emissions in the form of smoke during pyrolysis. Copra production has negligible potential impact because the process is still traditional and does not require significant resources or energy. Compared with other plantation crops, coconuts have minimal impact because of the slightly intensive cultivation and maintenance processes and a large amount of production due to the plant age. The impact allocation is small because it uses a cradle-to-gate system limitation from the beginning of land preparation to the harvesting of a 42-year-old coconut plant due to the large number of coconuts produced. Opportunities to reduce the impacts of the coconut shell charcoal are still available based on the interpretation. Therefore, a baseline scenario, existing conditions, and four improvement scenarios were developed without considering the technical and economic aspects. Improvement scenarios were identified for all activities, including coconut cultivation, copra production, and charcoal production. Several formulation improvement scenarios are presented as: 1) utilizing pyrolysis smoke for liquid smoke production, 2) implementing organic coconut farming, 3) developing decentralized coconut plantations integrated with a copra–charcoal factory and producing liquid smoke, and 4) applying scenarios 1, 2, and 3. The 10 impacts considered in this scenario analysis are presented in Fig. 5. Normalization was performed for the four scenarios to identify the influence level of each impact category. The normalization results show that compared with the existing condition, the effect of each impact category on the person equivalent is significantly reduced (Table 10).

In Scenario 1, the smoke from pyrolysis was processed into liquid smoke. The decrease in impact was due to the pyrolysis of smoke containing H_2O, CO, CO_2, H, CH_4, C_2H_4, and VOCs connected to the condenser. The GWP of liquid smoke from coconut charcoal was studied, and the impact of pyrolysis was found to be 0.075 tons CO_2 eq/ton charcoal. This impact is smaller than that in the current study, which produced 1.65 tons of CO_2 eq/ton charcoal. The calculation results show that scenario 1 can potentially reduce the impact of GWP to 0.2 tons CO_2 eq/ton coconut shell charcoal (cradle-to-gate scope). In addition to controlling air pollution and reducing emissions, liquid smoke can also increase the added value of coconut derivatives. Coconut coir, which is another byproduct, can be used as tar adsorbent in liquid smoke (Sari *et al.*, 2021). The liquid smoke condensation process is disregarded because it is not included in the coconut shell charcoal product system. In Scenario 2, organic farming was applied to coconut cultivation. Organic coconut farming can produce organic-based products that increase with rising health awareness among people (Alouw *et al.*, 2020). Ecotoxicity and human toxicity in organic and low-input farming systems are lower than that in conventional farming systems (Alaphilippe *et al.*, 2013). The fertilization stage in coconut cultivation significantly contributed to almost all impact categories due to the LCA under existing conditions. Petroleum fuel can also be replaced with fuel from vegetable oil (bioenergy) at the land preparation stage. Gasoline and oil for cutting trees were replaced with biofuel and bio-oil (lube oil), whereas diesel used for cultivating land with tractors was replaced with biodiesel. Scenario 2 showed a decrease in most of the impacts compared to the current conditions, but the impact categories of acidification and eutrophication increased. These results indicate that manure increases the incidence of SO_2 emissions, which causes acidification, and raises the amount of N and P nutrients, which cause eutrophication. Similarly, applying organic farming to apple plantations also raises acidification due to the use of compost (Alaphilippe *et al.,* 2013). Scenario 3 applied the concepts of integrated coconut plantations, copra factories, and coconut-shell charcoal factories. Coconut plantations were maintained under conventional conditions in this scenario. The integrated coconut agroindustry is developed near the warehouse of harvested coconut, which is 6.2 km away from the coconut plantation. The copra and charcoal factories are located in one area, that is, the warehouse; thus, does not require transportation. This concept aims to reduce the use of fuel oil for the transportation of coconut grains to the copra factory and shell transportation from the copra factory to the charcoal factory. Transportation is an activity that significantly contributes to the impact, especially ADP FF. This scenario still considers the transportation of sulfur from producers in Bandung to plantations in Agrabinta at a distance of 162 km. The emission reduction from charcoal production was achieved by producing liquid smoke. If liquid smoke is produced, then the smoke due to pyrolysis containing various gas emissions will be accommodated in the condensation pipe to prevent its entrance in the air and avoid potential impacts. Liquid smoke affects the LCA only for the usage of smoke from pyrolysis, but the production process of liquid smoke is not included in the system product of charcoal LCA. Scenario 4 combines the concepts of Scenarios 1, 2, and 3. The impact analysis results in Scenario 4 also decreased the impact but required additional implementation effort. Further implementation can also utilize charcoal as biochar to replace herbicides (Yavari *et al*., 2022). Table 10 shows that the highest level of influence changed from the HTP to the TEP in all scenarios. The decrease in HTP in the individual units was due to reduced toxic chemicals from fertilizers, fossil fuels, and various gas emissions

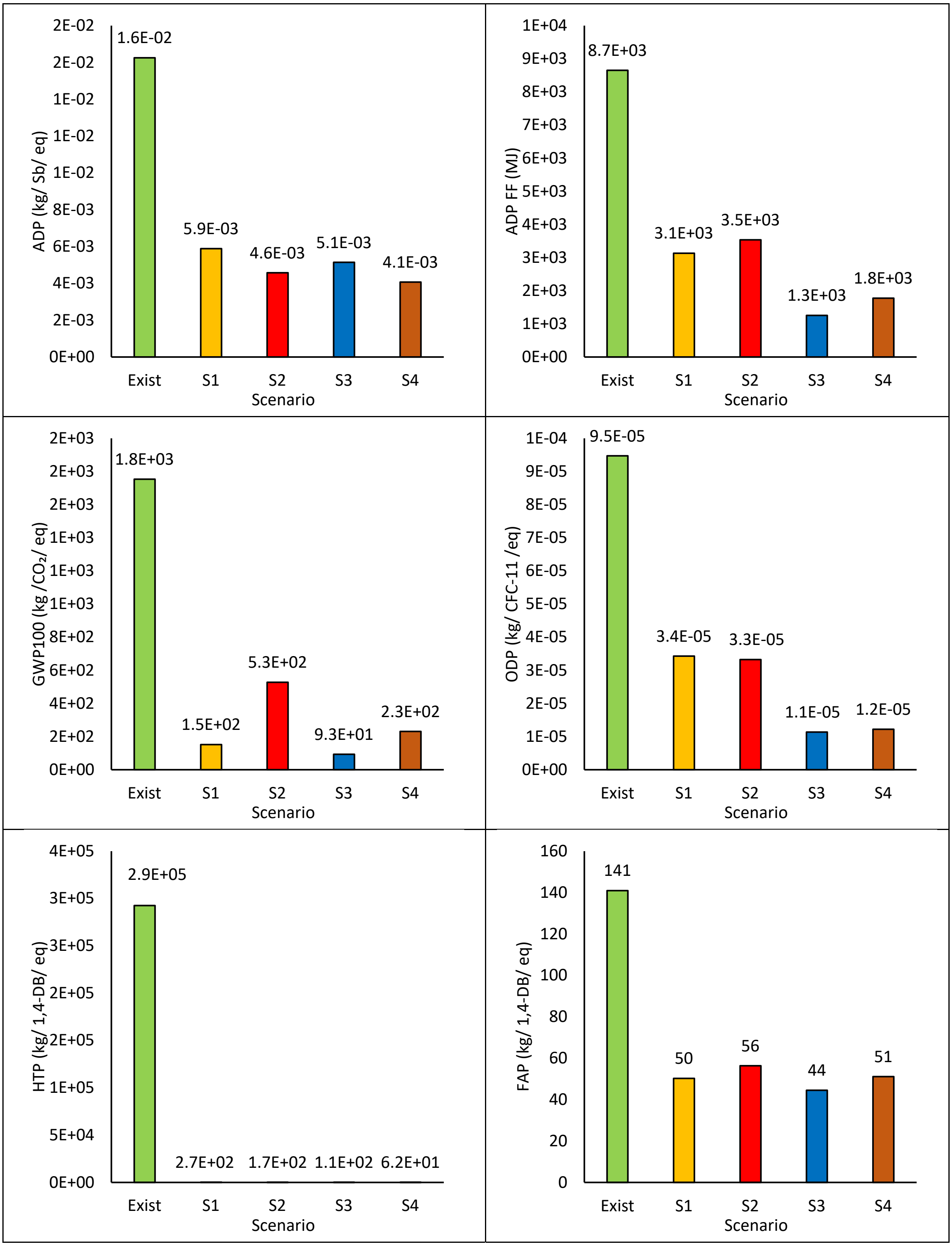
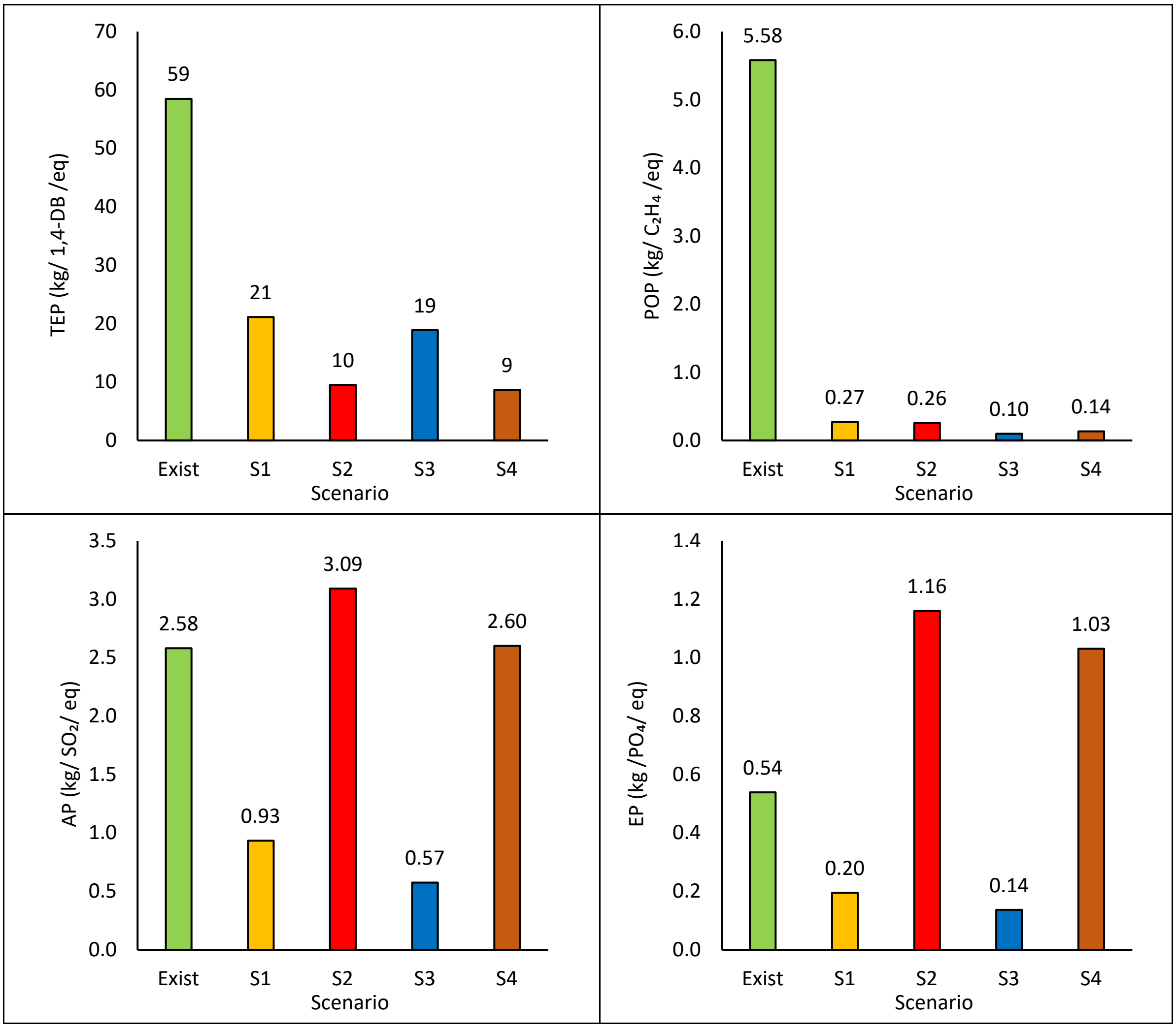


Fig. 5: Ten impact potential categories of coconut shell charcoal from each scenario compared with the existing condition



Continued Fig. 5: Ten impact potential categories of coconut shell charcoal from each scenario compared with the existing condition

Table 10: Normalization of impact category from each scenario compared with the existing condition

Impact category (*Person eq.*)	Exist	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Decrease in scenario 3 (%)
ADP	1.92E-10	6.94E-11	5.40E-11	6.07E-11	4.80E-11	68.35
ADP FF	2.75E-10	9.96E-11	1.12E-10	4.00E-11	5.65E-11	85.47
GWP100	3.49E-10	3.03E-11	1.05E-10	1.85E-11	4.59E-11	94.69
ODP	1.06E-12	3.84E-13	3.72E-13	1.27E-13	1.37E-13	88.01
HTP	**3.77E-08**	3.52E-11	2.18E-11	1.41E-11	7.99E-12	99.96
FAP	2.72E-10	9.71E-11	1.09E-10	8.59E-11	9.86E-11	68.43
TEP	1.21E-09	4.36E-10	1.96E-10	3.89E-10	1.78E-10	67.70
POP	6.59E-10	3.22E-11	3.03E-11	1.19E-11	1.60E-11	98.19
AP	9.16E-11	3.32E-11	1.10E-10	2.04E-11	9.24E-11	77.73
EP	4.09E-11	1.48E-11	8.79E-11	1.04E-11	7.81E-11	74.64

due to pyrolysis. Scenario 3 demonstrated the most significant reduction in the impact categories among all the scenarios. Scenario 3 had the lowest 7 of 10 impacts among other scenarios, namely ADP-FF, GWP100, ODP, FAP, POP, AP, and EP (Fig. 5 and Table 10). Scenario 3 integrated coconut plantations, copra factories, charcoal factories, and coconut shell liquid smoke in one location without implementing organic coconut cultivation. Conventional farming practices for coconut cultivation are currently considered beneficial because coconut cultivation does not require intensive care and is highly resistant to pests or diseases. Coconut plants can generally last for a sufficiently long period of up to 60 years. The impacts decrease when the accumulation of manufactured products increases. Organic coconut cultivation can be applied if organic certification of coconut-derived products, such as virgin coconut oil and coconut sugar, is necessary. This implementation can increase product competitiveness, especially for consumers concerned about organic products. Coconut derivatives that generally require organic certification include virgin coconut oil and coconut sugar. Under conventional conditions, integrating coconut plantations, copra factories, charcoal factories, and liquid smoke production can lower the impacts through the following: reducing transportation fuels, removing emissions to air from pyrolysis, reducing time, cost, and handling risks, lessening byproducts, and increasing value-added products. Coconut-based agroindustry can be a promising sector for integrated industrial development while implementing the zero waste concept as an important part of a sustainable industry. The environmental performance of copra and coconut shell charcoal can be declared as EPD or Type III ecolabel.

CONCLUSION

Potential impact analysis showed that the activity that produced the most significant impact (hotspot) in coconut cultivation was at the fertilization stage. Hotspot copra production occurs during the processing stage, whereas hotspot charcoal production transpires during pyrolysis. Pyrolysis for charcoal production had the largest impact on the three activities. The normalization results show that the most significant impact on humans from coconut cultivation and copra processing activities is TEP because of the use of fertilizers and transportation fuel. The largest influence of charcoal production was on the HTP because of emissions from pyrolysis. Improvement scenarios were formulated to reduce the environmental impact and improve sustainability. Scenario 1 converted the smoke due to pyrolysis into liquid smoke. Scenario 2 implemented organic coconut cultivation practices. Scenario 3 integrated coconut plantations with copra and charcoal processing plants and processed smoke into liquid smoke, and Scenario 4 combined all the scenarios. The results of the scenario implementation demonstrated the potential of developing an integrated coconut agroindustry that integrates coconut plantations, copra factories, charcoal factories, and liquid smoke in one location. The factors that affected the potential impact categories based on the scenario analysis were the type and amount of fertilizers and pesticides, transportation distance, amount of fuel, and treatment of emissions from pyrolysis. Scenario 3 can be implemented in the absence of organic farming because the cultivation of tall coconuts in Indonesia, especially in South Cianjur, West Java, does not require intensive care during its life cycle. Among all scenarios, Scenario 3 has the lowest 7 of 10 impact categories (ADP-FF, GWP100, ODP, FAP, POP, AP, and EP). The integration of coconut derivative production in one location significantly reduced the impact of fuel transportation and emissions. A further feasibility study should also consider the economic and technical aspects. The application of this scenario can help develop sustainable coconut agroindustry that produces environmentally friendly coconut derivative products and brand products using Type III ecolabel or EPD. This study can also be a reference for the next LCA of copra and shell charcoal derivatives, such as coconut cooking oil, virgin coconut oil from copra, activated carbon, bio-briquette, bio-pellet, and liquid smoke.

AUTHOR CONTRIBUTIONS

T. Puspaningrum contributed to the data collection and observation, life cycle analysis, scenario analysis, and manuscript preparation and revision. N.S. Indrasti supervised the data collection and impact analysis, extended the discussion, and improvement recommendations. C. Indrawanto processed the coconut statistical data, sharpened the background, life cycle analysis deepened the discussion, and prepared the manuscript. M. Yani, the corresponding author, supervised the life cycle analysis, revised the discussion, and proofread the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
1,4-DB	1,4 – dichlorobenzene
DGEC	Directorate General of Estate Crops (*Direktorat Jenderal Perkebunan Republik Indonesia*)
ADP	Abiotic depletion potential
ADP FF	Abiotic depletion potential (fossil fuels)
AP	Acidification potential
B	Boron
C_2H_4	Ethylene
CFC-11	Trichlorofluoromethane
CH_4	Methane
CML-IA	Center of Environmental Science of Leiden University Impact Assessment
CO	Carbon monoxide
CO_2	Carbon dioxide
EP	Eutrophication potential
EPD	Environmental product declaration
Eq.	Equation
eq.	Equivalent
FAP	Freshwater aquatic ecotoxicity potential
GJ	Gigajoule
GWP100	Global warming potential (100 years)
H_2O	Hydrogen oxide
H	Hydrogen
h	Hour
ha	Hectare
HC	Hydrocarbon
HFCs	Hydrofluorocarbons
HTP	Human toxicity potential
ICC	International coconut community
ID	Indonesia
IN	India
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organization for Standardization
KCl	Potassium chloride
kg	Kilogram
km	Kilometer
L	Liter
LCA	Life cycle assessment
LCI	Life cycle inventory

LCIA	Life cycle impact assessment
MAP	Marine aquatic ecotoxicity potential
Mg	Magnesium
MJ	Megajoule
N	Nitrogen
NO_2	Nitrogen dioxide
NOx	Nitrogen oxides
ODP	Ozone layer depletion potential
P	Phosphorus
P_2O_5	Phosphorus pentoxide
Pcs	Pieces
PFCs	Perfluorocarbons
PH	Philippines
POP	Photochemical oxidation potential
PPRI	Palma Plantation Research Institute
S1	First Scenario
S2	Second Scenario
S3	Third Scenario
S4	Fourth Scenario
Sb	Stibium
SF6	Sulfur hexafluoride
S-LCA	Social life cycle assessment
SP-36	Superphosphate (36% of Phosphorus)
SO_2	Sulfur dioxide
TEP	Terrestrial ecotoxicity potential
VOCs	Volatile organic compounds

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ORIGINAL RESEARCH PAPER

Coagulants for water based on activated aluminum alloys

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ABSTRACT

BACKGROUND AND OBJECTIVES: The reduction of fresh water deficit and water-related morbidity is the most important problem of the state's national security. The effective treatment of natural water in industrialized areas from natural and anthropogenic pollutants is the main ecological task. Coagulation is one of the effective methods used to treat water chemically to purify it. Aluminum polyoxychlorides have gained popularity because of their advantages over coagulants—aluminum and iron sulfates. No production of aluminum polyoxychloride occurs in Kazakhstan despite the need for coagulants (the minimum need is assessed at about 11 thousand tons). The work is aimed at theoretical justification and experimental proof of a principally new approach to the development of aluminum polyoxychloride production technology based on activated aluminum alloys containing metal activators, such as gallium, indium, and tin from 0.5 to 5 percent weight. In addition, the goal is solving environmental issues associated with improving the drinking water quality and related to environmental pollution with wastewater.

METHODS: The microstructures, phase components, and elemental compositions of alloys and reaction products were studied by scanning electron microscopy/energy dispersive X-ray spectroscopy. The thermal effects of alloys were investigated usin thermogravimetry methods. Oil content in wastewater was determined by spectrophotometry. Oil particle dimensions and wastewater zero potentials were determined using electrophoretic light scattering method and residual turbidity by turbidimetry. Water quality assessment was included in the purified water analysis and comparison with the sanitary and epidemiological standards established for drinking water supply and wastewater intended for water discharge.

FINDINGS: The structures and compositions of activated aluminum alloy containing metal activators - gallium, indium, and tin - from 0.5 to 5 weight percent and aluminum polyoxychlorides based on it were studied using modern instrumental methods. The efficiency of the treatment of natural and oil-contaminated wastewater with aluminum polyoxychloride was assessed. The treated water parameters were within the norms established for drinking water supply and wastewater disposal by Sanitary Rules and Norms 2.1.4.559-96. The efficiency of potable water treatment reached 90–99 percent.

CONCLUSION: An effective and technologically simple method is developed for producing aluminum polyoxychloride. It involves dissolving an activated alloy in 1–5 percent hydrochloric acid, with an aluminum content of 98.5–85 percent. Alloy processing is carried out at temperatures ranging from 60 to 65 degree celsius. The temperature rises from 20 to 25 degree celsius to the specified optimum without heat supply from the outside due to the interaction among reagents. The process is completed in 2–3 hours. The results confirm that aluminum polyoxychloride is an effective coagulant for drinking and wastewater treatment. The treated water is within the established limits in terms of hydrogen potential, chemical oxygen demand, and turbidity. The water treatment method can be easily implemented.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
39	10	10

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INTRODUCTION

Traditional coagulants based on iron and aluminum salts (Chen *et al*., 2018; Jiao *et al*., 2015), including titanium salts (Wang *et al.,* 2018; Xiao *et al*., 2008; Zhao *et al.,* 2011), are widely used in the treatment of natural water (H_2O) and wastewater in the coagulation process to remove suspended solids and reduce turbidity and dissolved substances. In the conditions of water resource scarcity, severe environmental pollution, and tightening standard for the discharge of oil-contaminated wastewater into water bodies (Lee *et al*., 2017), research on the development of methods for obtaining effective coagulants and purification process and natural and wastewater disposal is significant and relevant. Polyaluminum chloride (PAC) is one of the widespread coagulants for water and wastewater treatment. The methods of producing aluminum polyoxychloride using metallic aluminum in the form of ingots, secondary raw materials (scrap), aluminum hydroxides, aluminum chloride, and aluminum oxide are discussed in detail in the works of He *et al*. (2019); Li *et al*. (2010); Zhang *et al*. (2017). Hydrochloric acid (HCl), aluminum chloride, aluminum sulfate, and low-basic aluminum chloride are used as chlorine (Cl)-containing reagents (Sychev *et al*., 2015), which have a low corrosivity (hydrogen potential pH = 1.0–2.5), allowing the use of equipment made of ordinary or low alloy steel. The methods of obtaining PAC using metallic aluminum are limited by the high costs of aluminum, corrosive environment, and process duration and application of a complex technological scheme with the circulation of the reaction mixture through a system of two reactors (Tokareva *et al.,* 2020). Solving the issue of equipment and pipeline protection from corrosion is necessary when sufficiently concentrated HCl above 10 percent (%) is used and a reaction temperature is 95 degree celsius (°C). Special materials are used for equipment, including graphite reflux condensers, graphite grids in the reactor, and special steels. The method of obtaining PAC by the interaction of aluminum powder with an aluminum chloride solution is no less dangerous. A risk of explosion is possible due to the high activity of a specific surface area and increased reactivity. The application of aluminum oxides and hydroxides for the production of PAC is limited by the hard conditions of the process, the need to use freshly prepared aluminum hydroxide, multistage, the use of apparatus made of special materials, because the process takes place in an aggressive environment at high pressure and temperature (Masakbaeva *et al*., 2021). The cost of PAC obtained using these methods is quite high in the world market. Studing applying activated aluminum alloys (AAAs), which contain activating metals gallium (Ga), indium (In), tin (Sn) as reagents for aluminum polyoxychloride production, have not been found from the world state of art. Three, four, and multicomponent AAAs are used as sources for hydrogen storage (Sarmurzina *et al*., 2022a; Trowell *et al*., 2020); hydrogen production from water (He *et al*., 2017a, 2017b; Kumar *et al*., 2020; Pini *et al*., 2020; Qiao *et al*., 2019; Wei *et al*., 2018;); and alternative, renewable, and clean fuel sources (Xiao *et al*., 2019). Aluminum is alloyed with Ga, In, Sn, zinc (Zn), bismuth (Bi), cadmium (Cd), copper (Cu) metals for activation (Du *et al*., 2018; He *et al.,* 2020b; Wang *et al.,* 2012; Yang *et al*., 2017). The scientific novelty of this work lies in using AAAs to obtain a coagulant PAC for physical and chemical water purification from natural and anthropogenic pollutants, in improving water quality, in rejecting foreign products, in solving the problem of clean fresh water shortage, in increasing water supply sustainability to populations and industries, in improving national water security and sustainable state development, in developing efficient technological processes, in new technical solutions for the creation of AAAs with low prices, excluding the use of expensive components. The advantage of the obtained PAC with a high content of alumina (> 10%) and high basicity is that its use makes it possible to obtain high-quality drinking water with a low content of residual aluminum (0.05–0.10 milligram per liter (mg/L), which is more than two times less than the norm of the World Health Organization (0.20 mg /L). In addition, PAC is used in natural water purification at temperatures ranging from 4 °C to 8 °C, at doses 20%–25% lower than for aluminum sulfate. The pH of the treated water practically does not change. PAC has a high polymerization ability (Tang *et al*., 2015), which is accelerate flocculation and coagulated suspension settling, ensures the highest efficiency of water purification from suspended solids and metals, and reduces water corrosiveness (absence of excess sulfates), which has bactericidal properties. The mechanism of coagulation water treatment with aluminum polyoxychloride has been considered in existing works (Shamaei *et al*., 2018; Sun *et al*., 2019). According to Jiao, R *et al*. (2015), a distinctive feature of aluminum polyoxychloride is that aluminum exists in the form of aquahydroxo complexes. These complexes have high charges, molecular weights, and specific surface areas, which allow the products of their hydrolysis to capture, adsorb, and thereby remove suspended colloidal impurities from the treated water. The current study aims for the theoretical substantiation and experimental proof of a fundamentally new approach to the development of technology for the production of aluminum polyoxychloride based on AAAs containing activating metals: Ga, In, and Sn from 0.5 weight percent (wt%) to 5 wt%. It also involves the investigation of the microstructures, compositions, and thermal properties of AAAs with metal activators: Ga, In, Sn in amounts ranging from 0.5 wt% to 5 wt%; development of a method for producing aluminum polyoxychloride based on AAAs used as coagulants in drinking water purification; evaluation of the efficiency of natural and oil-contaminated wastewater purification with aluminum polyoxychloride. This study is carried out at Satbayev University, Joint-stock company (JSC) Institute of Metallurgy and Enrichment, Republic of Kazakhstan during the 2022–2023 period.

MATERIALS AND METHODS

Aluminium (purity 99.85%) in granules was purchased from Aluminium Smelter JSC, the only aluminium producer in Kazakhstan, part of the Eurasian Group. Ga (purity 99.99%), in the form of cylindrical ingots with weights ranging from 900 grams (g) to 1000 g, was purchased from Kazakhstan Aluminium Smelter JSC; its melting point was 156.59 °C and density was 7.362 (+20 °C, grams per cubic centimeter (g/cm^3). In (purity 99.999%) in the form of cylindrical ingots with weights ranging from 0.05 g to 1000 g was purchased from Aluminium Smelter JSC; its melting point was 29.80 °C and density was 5.904 (+20 °C, g/cm^3). Sn was in ingots with weights from 22 kilogram (kg) to 26 kg; Sn content was 99.565%, and its melting point was 231.91 °C. HCI (chemical purity) with azeotropic mixture boiling point (20.22% by mass) –108.6 °C, density 1.16 g/cm^3 (35%), was used without additional purification.

*Sodium hydroxide (*chemical purity) *was used without additional purification.*

AAAs - Rau-98.5, Rau-97, and Rau-85 - containing activator metals, such as Ga, In, and Sn (from 0.5 wt% to 5 wt%), were obtained by melting (Sarmurzina *et al*., 2022a; 2022b). Aluminum was melted in an inert gas atmosphere in an alundumina crucible in a muffle furnace at 850 °C. Activating additives stirred to achieve melt homogeneity were put in the molten aluminum. The heating–holding–cooling cycle lasted 1 hour, then the melt was poured into fabricated steel molds with an outer diameter of 60 millimeters (mm), inner of 40 mm, and a height of 40–80 mm. Powder with given particle sizes of 80–1250 micrometers (µm) was made from ingots by changing gaps among crusher jaws (mm): 1, 2, 5 and 10. Aluminum alloys were characterized using a scanning electron microscope with energy dispersive X-rays (SEM/EDX). The X-ray spectral analysis of alloys was performed with an X-ray fluorescence spectrometer—X-Ray Innov-X systems (United States of America). The phase compositions of alloys were analyzed using X-ray diffraction method (XRD) on a D8 ADVANCE diffractometer (Bruker, Germany), at α- Cu tube voltage 40 kilovolt (kV) and current 40 milliamp (mA). The elemental composition analysis for the samples and photographing in various radiation kinds were performed using energy dispersive spectrometer—INCA Energy by Oxford Instruments (United Kingdom) mounted on electron-probe microanalyzer Superprobe 733 by JEOL Limited trade development (Ltd) (Japan), at accelerating voltage 25 kV and probe current 25 nanoamp (nA). The thermal analysis of alloys was performed using a synchronous thermal analysis device—simultaneous thermal analyzer (STA) 449 F3 Jupiter (Germany). The furnace space was evacuated before heating (vacuum level reached ~92%) and then purged with inert gas for 5 minutes (min). Heating was performed at a rate of 10 degrees Celsius per minute (°C/min) in an atmosphere of highly purified argon. The total volume of incoming gas was kept within 110 milliliters per minute (mL/min). The results obtained with the STA 449 F3 Jupiter were processed using NETZSCH Proteus software. X-ray powder images were recorded on a D8 Advance diffractometer (Bruker), at α-Cu tube voltage 40 kV and current 40 mA. The processing of obtained data from diffractograms and calculation of interplanar distances was performed using the EVA software. Sample interpretation and phase search were conducted using the search/match program using the powder diffractometer database (PDF)-2. Aluminum polyoxychloride, with the mass fraction of Al on aluminium oxide (Al_2O_3) 33.2%–36.9% and basicity

56%–67%, corresponding to GuoBiao (GB) 15892-2009, was obtained according to the developed method used to treat aluminum-containing alloys by 1%–5% HCl solution at a ratio of 1:50. The dissolution process for aluminum-containing alloys proceeds with sufficient speed, accompanied by a uniform release of hydrogen and increasing temperature of the reaction medium. The temperature rose from room temperature to the optimum temperature (50 °C–90 °C), without heat input from outside, due to the interaction among reagents. The process was completed in 2–3 hours. The high reactivity of aluminum-containing alloys was due to the presence of activating metals (Ga, In, and Sn) contributing to the mechanical loosening of surfaces, heterogeneity of alloys, and formation of micro-galvanic elements. Activating metals form easily fusible eutectics with aluminum in the region of immiscibility in the liquid phase, resulting in the destruction of a dense layer of aluminum oxide and a sharp increase in its chemical activity. The amount of hydrogen released during the interaction of aluminum alloys with HCl was measured on a drum gas meter. All experiments were repeated at least three times and were performed at 25 °C and a humidity of 60%. The water heating temperature was measured with a thermometer with an accuracy of 0.1 °C. The hydrogen release rate was calculated using Eq. 1 in milliliters per gram per minute (mL/g/min) (Sarmurzina *et al*., 2018).

$$W_{\mathrm{H}} = \frac{V}{m\Delta t} \quad (1)$$

Where, *V* is the amount of hydrogen released; *m* is the mass of the alloy sample; Dt is time between two measurements of gas clock readings.

The theoretical amount of hydrogen was calculated based on the generation of 1.244 L of hydrogen per 1 g Al under standard conditions (273 Kelvin (K), 1 atm). Sampling; determining initial water quality parameters; and process controlling aluminum, turbidity, hydrogen potential, and chemical oxygen consumption were performed according to standard procedures. Oil content in wastewater was determined with spectrophotometer SPEKOL 1300 (Analytik Jena). Studies on oil particle size and wastewater zeta potential before and after a coagulant treatment were executed using the Malvern Zetasizer NanoZS 90 particle size analyzer. The water treatment with the obtained coagulants was performed as follows: 200 mL of test natural water was poured into a 250 mL beaker, and a given amount of coagulant solution was introduced into it under constant quick stirring (180 revolutions per minute (rpm). The sample was stirred for 1 min with a continuous pH control. Subsequently, the stirrer was switched to slow stirring −50 rpm for 10 min. The residual turbidity was measured directly using a HACH 2100Q Turbidimeter.

RESULTS AND DISCUSSION

The X-ray spectral analysis of Rau-85 aluminum alloy obtained using the X-Ray Innov-X system spectrometer showed that the main component of the activated Rau-85 alloy was aluminum with the content of 85.71%. The metal content in the alloy insignificantly differed from the initial ratio of metals taken for the alloy preparation. The Rau-85 alloy contained trace amounts of impurities of metals not used as initial components: iron (Fe), Cd, lead (Pb), and Bi in amounts from 0.01% to 0.02%, and Fe content reached 0.09. SEM images and EDXs maps of the Rau-85 alloy sample surface are shown in Fig. 1. The study allowed us to obtain a general picture of the alloy surface, fractogram of the fracture surface, distribution of dissolved elements in the alloy, and their contents in some local areas of the sample by microprobe with a diameter of 1 µm (Fig. 1). The lighter areas in the micrograph indicate the presence of liquid eutectics of metal activators on aluminum grains (He *et al*., 2020a; Sarmurzina *et al*., 2022a; 2022b). The EDXs analysis results for the samples of bright white grains and aluminum alloy matrcies are illustrated in Fig. 1 and Table 1. Based on the data presented, concluding that the bright white grains are heterogeneous in composition is possible. The

Table 1: Results of energy dispersive X-ray spectroscopy of the Rau-85 alloy (Sarmurzina *et al*., 2022a)

		Element content, weight (%)				
Spectra	Grain	Al	Ga	In	Sn	Total
Spectrum 1	Bright white grain	3.04	5.90	69.37	21.70	100.00
Spectrum 2	Bright white grain	2.26	3.59	72.42	21.70	100.00
Spectrum 3	Matrix gray in the photo	58.49	12.54	17.70	11.27	100.00

activating components—Ga, In, Sn—in the alloy participate in the formation of complex eutectics and have a different distribution in the amount and on the surface of aluminum.

The thermogravimetric analysis method was used to obtain information on the melting temperatures of AAAs. The thermograms (TGs) of activated Rau-85 alloy are shown in Figs. 2 and 3.

The differential thermal analysis (DTA) curve recorded an exothermic effect with a peak at 556 °C and an endothermic effect with an extremum at 639 °C. The dDTA curve recorded endothermic effects with extremums at 113.9 °C and 568.5 °C. The process of surface oxidation of pellets caused the exothermic effect. The endothermic effect with an extremum at 639 °C on the DTA curve could be a manifestation of monotectic reaction in the In–Al system (Lyakishev, 1996). The endothermic effect with an extremum at 113.9 °C on the DTA curve could be a manifestation of melting in the Al–Ga system compound with Ga content ~84%–86%. Alternatively, the effect could be a manifestation of melting in the Ga–In system compound with In content ~80%. The endothermic effect with the extremum at 568.5 °C on the dDTA curve could be a manifestation of the melting in the Al–Ga system compound with Ga content ~20%. Three exothermic effects were recorded on the curves obtained during cooling at a rate of 13 °C/min. The first peak on the DTA curve was 599.7 °C. It reflected solid phase precipitation from the melt and one more liquid (monotectic reaction) in the In–Al system. The second peak at 619.4 °C appeared on the DTA curve. It showed aluminum crystallization. The third peak −571.8 °C reflected the crystallization of Al–Ga alloy

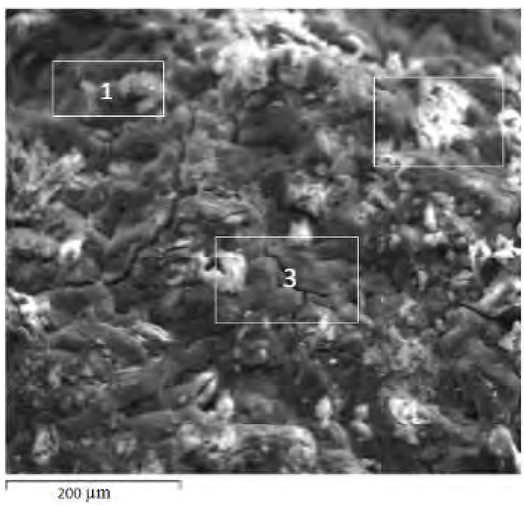


Fig. 1: SEM image of the activated Rau-85 alloy surface in secondary electrons (Sarmurzina *et al*., 2022a)

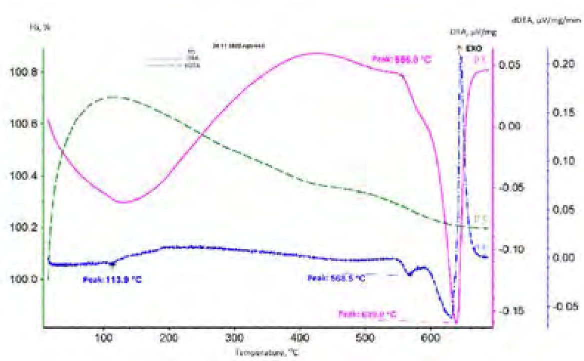


Fig. 2: Thermal analysis of activated Rau-85 alloy

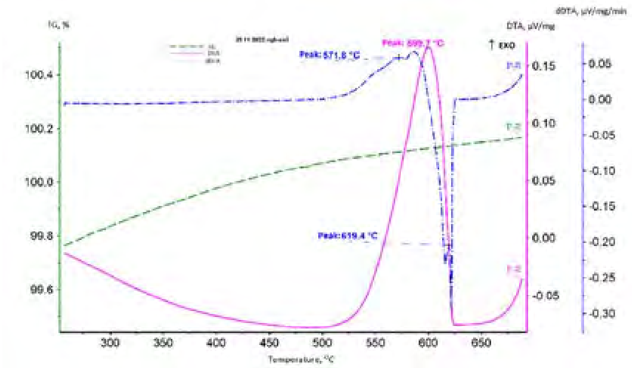


Fig. 3: Thermal analysis of activated Rau-85 alloy (cooling)

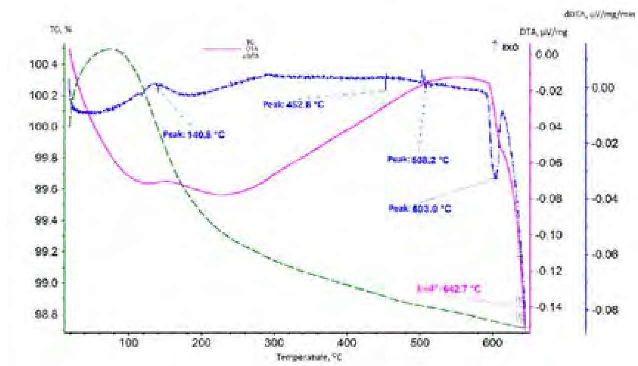


Fig. 4: Thermal analysis of activated Rau-97 alloy

with ~10%–12% Ga content.

The TGs of activated Rau-97 alloy with the content of In, Ga, and Sn 1:1:1 are shown in Figs. 4 and 5. Individual alloy pellets were selected to prevent intense surface oxidation for the thermal analysis of the activated Rau-97 alloy. The samples were heated to 643 °C. Endothermic effects with extremums at 140.8 °C, 452.8 °C, 508.2 °C, and 603 °C were recorded on the DTA curve. The first effect (140.8 °C) could be presumably attributed to the manifestation of the compound from the In–Sn system. A peritectic reaction In and liquid →ß were observed at this temperature, where ß was an intermediate phase with a face-centered cubic structure (according to other data, it may be body-centered cubic structure structure). Other effects probably belonged to compounds from the Al–Ga system. Thus, a liquid phase was formed in the Al–Ga alloy at 452.8 °C, with ~40% Ga content, and a liquid phase was formed in the Al–Ga alloy at 508.2 °C, with ~30% Ga content. The endothermic effect with extremum at 603 °C showed the formation of the liquid phase in the Al–Ga alloy, with ~10% Ga content, that is, the alloy contains Ga with different qualitative and quantitative compositions.

The curves obtained during cooling at a rate of 13 °C/min recorded two exothermic effects (Fig. 5). The first peak on the DTA curve was at 620.7 °C. It showed aluminum crystallization. The second peak at 586.6 °C appeared on the DTA curve. It showed crystallization of Al–Ga alloy with ~10% Ga.

Two endothermic effects were found on the DTA curve of Rau-98.5 alloy (Fig. 6a). The most intensive one had the maximum development at 663.6 °C. The extremum of the less intensive effect corresponded to 611 °C. The endothermic effect of 663.6 °C most likely showed aluminum melting. A bit elevated melting temperature could be caused by the presence of some impurities, specifically Al_2O_3. If the attention was paid to the dDTA curve in the

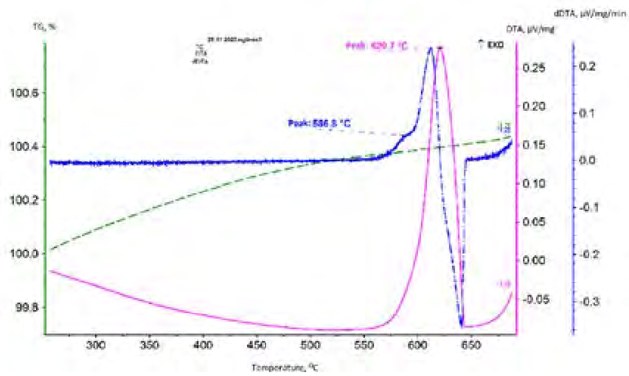
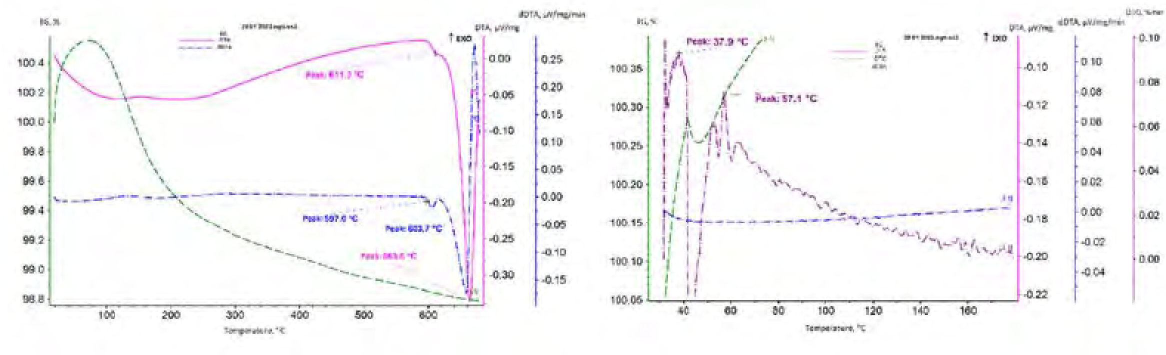


Fig. 5: Thermal analysis of activated Rau-97 alloy (cooling)



(a) (b)

Fig. 6: Thermal analysis of activated Rau-98,5 alloy (a) enlarged image of the dDTA curve within the considered temperature segment with extremum at 611 °C (b)

endothermic effect area with an extremum at 611 °C, then seeing that several effects appeared in the considered temperature interval was possible.

Fig. 6b shows an enlarged image of the dDTA curve in the temperature interval under consideration. Three endothermic effects with extremums at 597 °C, 603.7 °C, and 607 °C appeared. Presumably, this is a representation of the melting of Al–Ga system alloys with the content that is close in Ga-content value. No effects showing the presence of other phases were detected. Practically, the same effects were recorded. An exothermic effect with a peak at 596.1 °C, showing the surface oxidation in aluminum particles by residual oxygen, also appeared. An increase in the mass of the sample was recorded at the initial stage of heating. It could be seen from the thermogram (TG) curve course. The derivative thermogravimetric (DTG) curve showed maximums at 37.9 °C and 57.1 °C. We may assume that the oxidation of the In surface (37.9 °C) and the molten In surface (57.1 °C) takes place here. The presence of In was detected possibly indirectly. The X-ray diffraction analysis (XRD) of AAAs revealed that the alloys contained eutectic Ga with the composition ($Al_{0.95}$ $Ga_{0.05}$), binary intermetallic In with Sn $(In_3Sn)_{0.5}$; $(InSn_4)_{0.2}$; $In_{0.18}$ $Sn_{0.82}$, $In_{1.74}Sn_{0.26}$, $In_{0.2}Sn_{0.8}$. Their total percentage depended on the amount of activating additive in the alloy (Table 2).

The synthesis of PAC was performed by the interaction of AAAs with HCl. AAAs easily reacted with the aqueous solutions of HCl (1%–5%) at 20 °C–25 °C. The reaction was accompanied with heat and hydrogen emissions. Aluminum alloy was introduced into HCl solution in portions to prevent reaction mass overheating. Multiple injections of activated aluminum created conditions for the directional hydrolysis of aluminum chloride and formation of polyoxychloride with the desired characteristics. Basic salt was formed during the hydrolysis of aluminum chloride. Hydrolysis is assumed to proceed by Al^{3+} cation (Novakov and Radchenko, 2013; Wei *et al.*, 2015; Zhang*et al.*, 2015). During hydrolysis, the Al^{3+} ion tears off the OH^- hydroxide ions from the water molecule, releasing H^+ cations. As hydrolysis deepens and the pH of the medium rises to neutral or weakly alkaline, the interaction among products results in the formation of polymeric hydroxocomplexes or colloidal aluminum hydroxide (Dayarathne *et al.*, 2021). The reaction is complicated by acid-base equilibrium with the position that depends on many factors: the pH value and temperature of the solution, the concentration and initial form of aluminum, and the activity of the anion present (Novakov and Radchenko, 2013). The structure of PAC depends on the pH of the medium. The product is prone to hydrolysis and has a strong effect on the pH of its aqueous solution, giving it an acidic reaction. The EDX analysis of the alloy reaction product with HCl showed that the Cl content increases as the concentration of HCl solution in the product increases and is 34.34%. The Cl content is 14.45% for the product based on Rau-85 and 13.34% for the product based on Rau-97 in the alloy reaction product with HCl of lower concentration. The observed dependence is the characteristic for Rau-85 and Rau-97-based PAC (Table 3).

A study of the reactions of AAAs with aqueous solutions of HCl revealed that the addition of AAAs to HCl solutions is accompanied by a heating of the reaction mixture from 50 °C to 90 °C. The temperature depends on the HCl concentration, the alloy-to-acid ratio, and the composition of the used alloy. In this research, the reaction was heterogeneous and ran on the solid–liquid interface. The amount of heat released and the speed of the chemical reaction largely depended on the contact surface area, including the chemical composition of the aluminum alloy. The period of rapid reaction lasted about 30–40 min, then its rate gradually slowed down. Curves of heat release and volume of released hydrogen during PAC synthesis, depending on the HCl concentration and multiplicity of AAA addition to the acid solution, are shown in Figs. 7 and 8.

The analysis allowed us to conclude that the amount of gas released and the rate of its release depended on the HCl concentration and the alloy composition during the process at 25 °C. When the HCl concentration increased from 1% to 5%, the hydrogen release rate increased. The reaction temperature, the amount of H_2 released, and the rate of its release increased as the acid concentration increased, reaching the theoretically calculated value. The reaction proceeded without an induction period and ended within 20–30 min. The conversion reached 95%–100%. The results showed that the treatment of aluminum-containing alloys activated by In 0.5 wt%–5 wt%, Ga 0.5 wt%–5 wt%, Sn 0.5 wt%–5 wt%, containing 98.5%, 97%, and 85% aluminum, respectively with 1%–5% aqueous HCl solutions,

Table 2: X-ray phase analysis of AAAs

Pattern number	Compound name	Formula	S-Q,%
	Rau-85		
PDF 00-004-0787	Aluminum, syn	Al	79.0
PDF 01-074-5208	Aluminum Gallium	$(Al_{0.95}Ga_{0.05})$	11.4
PDF 01-077-2745	Indium Tin	$(In_3Sn)_{0.5}$	5.3
PDF 01-085-4243	Indium Tin	$(InSn_4)_{0.2}$	4.3
	Rau-97		
PDF 00-004-0787	Aluminum, syn	Al	87.8
PDF 01-074-5208	Aluminum Gallium	$(Al_{0.95}Ga_{0.05})$	6.4
PDF 01-077-2745	Indium Tin	$(In_3Sn)_{0.5}$	3.9
PDF 01-073-9037	Indium Tin	$In_{0.18}Sn_{0.82}$	1.8
	Rau-98.5		
PDF 00-004-0787	Aluminum, syn	Al	25.0
PDF 01-074-5208	Aluminum Gallium	$(Al_{0.95}Ga_{0.05})$	67.0
PDF 01-077-2747	Indium Tin	$In_{1.74}Sn_{0.26}$	3.4
PDF 00-048-1547	Indium Tin	$In_{0.2}Sn_{0.8}$	2.3
PDF 01-077-3457	Tin	Sn	1.5
PDF 00-037-1462	Aluminum Oxide	Al_2O_3	0.8

Table 3: Results of X-ray elemental analysis of AAAs and their reaction products with HCl

Element, W (%)	Rau-85	Product		
		Rau-85 after treatment with 5% HCl solution,	Rau-85 after treatment with 1% HCl solution	Rau-97 after treatment with 1% HCl solution
Al	53.89	20.33	21.02	23.04
O	35.76	43.11	47.44	53.19
In	4.84	0.62	0.04	0.04
Ga	2.43	0.93	1.25	0.23
Sn	3.07	0.66	0.04	0.04
Cl	-	34.34	14.45	13.34

Single addition of activated alloys to HCl solution, EDX analysis. Alloy: HCl ratio (1:50)

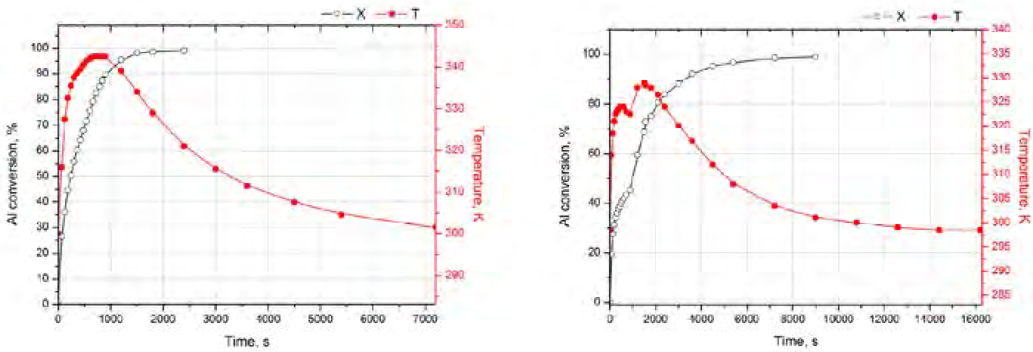


Fig. 7: Curves of process temperature and aluminum conversion vs. time, in the synthesis of coagulant based on Rau-97 and 3% HCl (1:50): a) single addition, b) double addition

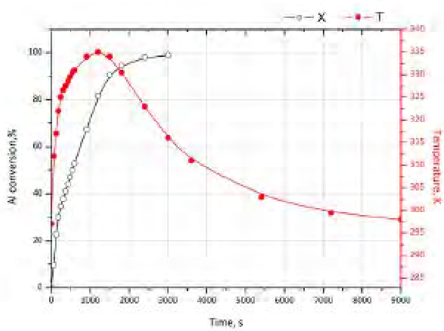


Fig. 8: Curves of process temperature and aluminum conversion vs. time for the synthesis of a coagulant based on Rau-98.5 (single addition) and 3% HCl (1:50)

resulted in sufficient heat emission to conduct the process without heating the solution and allowed to gain aluminum polyoxychloride with necessary basicity degree 41%–82.6% and with mass fraction Al_2O_3 30%–48% according to dissolution conditions. When the activating metals were dissolved in acid, they did not pass into the solution but roll into balls forming eutectic low-temperature alloys, settled at the bottom of the reactor unchanged, and could be reused for the preparation of aluminum-containing alloys. This property of a metal activator ensures the manufacturability of the process and its economic feasibility because the price of the alloy corresponds to the price of the original aluminum used. The basicity of a coagulant characterized the substitution degree of Cl atom by hydroxyl group in aluminum chloride during the basic salt formation. Aluminum polyoxychloride solutions had pH ranging from 2.3 to

4.2, and the mass fraction of aluminum in terms of Al_2O_3 from 4.5% to 15.75%. A study of diffractograms of reaction product samples of activated Rau-85 alloy with 1% HCl solution (Coagulant No. 11, Al_20_3 content [31%], basicity [66.9%]) and of activated Rau-97 alloy with 1% HCl solution (Coagulant No. 15, Al_20_3 content [33.1%], basicity [62.7%]) resulted in the conclusion that the product was a mixture of crystal and amorphous phases. The XRD results indicated that the reaction products were heterogeneous in composition (Table 4). The compositions of the phases formed and their percentages depended on the nature of the alloys used for the reactions and reaction conditions (acid concentration, temperature).

Infrared (IR)-spectral analysis of interaction products of AAAs with HCl

The IR spectra of coagulants based on aluminum alloy Rau-85 (No. 11) and Rau-97 (No. 15) were recorded on the fourier-transform IR spectrometer "Avatar 370 CsI" in the spectral range 4000-300/cm from products in the form of tablets prepared by pressing, 2 mg sample and 200 mg potassium bromate (KBr), and in Vaseline oil-based suspension in thallium bromoiodide windows. A spectrum of Vaseline oil was taken as a reference spectrum. Attachment for the experiment: Transmission Enhanced Synchronous Protocol (Fig. 9).

An intense band of valent vibrations vOH – 3451/cm and a band of strain vibrations δ HOH – 1635/cm of water molecules were found in the spectra of Coagulants Nos. 11 and 15 (Nakamoto, 2009). Poly aluminum, aluminum polynuclear hydroxo complex, aluminum hydroxide chloride hydrate $Al_{13}(OH)_{30}\ Cl_{9} \cdot 15\ H_2O$ – 3451, 1635, 983, 726, 631, 553, 366/cm. The IR spectra of the products were similar and correspond to the compound—aluminum polyoxychloride. Based on the sources (Novakov and Radchenko, 2013; Tang *et al*., 2015) and data obtained in this work, when coagulants were introduced into water, their dissociation occurred. The polyvalent cations of the coagulant Al^{3+} formed in this case entered into ionic exchange with cations of the adsorption layer of negatively charged colloidal particles of pollution, reducing their stability (Wei *et al*., 2015). When working solutions of the PAC coagulant were prepared, the aluminum ion was assumed to exist in the form of an aqua complex $[Al(H_2O)_6]^3$ of octahedral structure in dilute aqueous solutions at pH ≤ 3 (Novakov and Radchenko, 2013). During the coagulation process, PAC hydrolysis resulted in the formation of polynuclear aquahydroxocomplexes of monomeric, polymeric, or amorphous aggregates due to the presence of a surface acid shell. It increased the intensification of wastewater treatment of suspended solids and metals. The high polymerization ability accelerated flake formation and precipitation. The hydrolysis reaction of aluminum polyoxychloride occured spontaneously in an aqueous solution (Grechanikov *et al*., 2010). The products were complexes of the general formula $Al[(OH)_3Al]n^{3+}$, complex $Al_6(OH)_{15}{}^{3+}$ $Al[(OH)_5Al_2]n^{3+}$, $Al_{13}(OH)_3{}^{7+}$; $Al(OH)_2{}^{4+}$, $Al_{2+n}(OH)_{3n}Cl_6$ (Grechanikov *et al*., 2010). When a certain hydrolysis degree was reached, the polymerization stage became preferable. According to Novakov and Radchenko

Table 4: Comparative results of semi-quantitative XRD of crystalline phases of reaction products based on Rau-85 alloy (Coagulant No. 11) and Rau-97 alloy (Coagulant No. 15)

Pattern number	Compound name	Formula	S-Q (%)	
			Coagulant No. 11	Coagulant No. 15
PDF 00-061-0795	Poly aluminum, aluminum polynuclear hydroxo complex, PAL | Aluminum Hydroxide Chloride Hydrate	$Al_{13}(OH)_{30}Cl_{9}\cdot 15H_2O$	85.7	100.0
PDF 00-037-1377	δ-Al (O H)3 | Aluminum Hydroxide	$Al(OH)_3$	5.7	
PDF 01-088-1609	δ-Al Al1.67 O4 | Aluminum Oxide	$AlAl_{1.67}O_4$	4.8	
PDF 01-078-4581	δ-Al O (O H) | Aluminum Oxide Hydroxide	AlO (OH)	3.8	

Images were taken on a D8 Advance (Bruker), α-Cu tube voltage 40 kV, current 40 mA. The processing of the obtained data from diffractograms and the calculation of interplanar distances were performed using EVA software. Sample interpretation and phase search were performed by search/match using PDF-2.

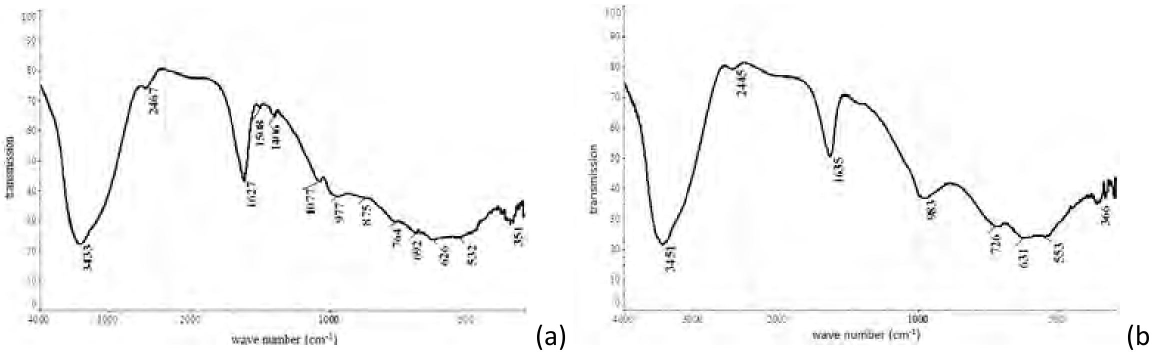


Fig. 9: IR spectra of Coagulants No. 11 (a) and No. 15 (b) as tablets with KBr

(2013) and Tang *et al*. (2015), increasing charges were strong enough to impede further cluster access. The efficiency of the aluminum oxychloride coagulant $Al_x(OH)_yCl_{3x\text{-}y}{\cdot}zH_2O$ depended on its basicity. The high efficiency of aluminum oxychloride was probably due to the establishment of equilibrium between cations in the adsorption layer of colloidal particles and hydrolysis in the solution of excess coagulants. Hydrophobic colloids of low soluble aluminum hydroxides or their basic salts were formed as results of hydrolysis, depending on the pH of the medium. These colloids had huge active surfaces and play major roles. This assumption is consistent with the existing opinions of authors (Grechanikov *et al*., 2010; Zhao *et al*., 2015). The colloidal particles of impurities were adsorbed on the surfaces of colloidal hydroxide particles coagulated under the actions of electrolytes dissolved in water to form flakes, which sorbed and captured the impurities in the water during settling. As a result of these processes, the aggregative stability of the system was disturbed resulting in the formation of macrodispersion separated through sedimentation, flotation, and filtration (Wei *et al*., 2015). As noted above, the obtained reagents were tested in the treatment of different types of natural drinking water ("Almaty Su" and "Medeu" water intakes), recycled water from water treatment units, including oil-contaminated wastewater (Sample Nos. 1 and 2) from various enterprises of Kazakhstan. Coagulants Nos. 11, 15, and 20 were tested as RAS samples. The main characteristics of coagulants are shown in Table 5.

Water quality control, from the "Almaty Su" water intake before and after treatment, was performed for the main parameters of water quality: pH International Organization for Standardization (ISO) 110523-2008, turbidity ISO 7027-1-2016, permanganate acidity; chemical oxygen demand (COD), ISO 6060-1989, salt content, oil ISO 9377-2. Tables 7 and 8 show the comparative results of water turbidity measurements after treatment with coagulants. Based on the data in Table 6, turbidity decreased from 16.8 to 0.47 Formazin Nephelometric Unit (FNU), the effectiveness of turbidity reduction reached 97.2%, and water quality improved as pH of water increased to 9–9.5. Coagulant No. 20 based on activated Rau-97 alloy was effective for the treatment of oil-contaminated wastewater (Table 7). Water turbidity was 44.4 FNU before treatment. It was 0.37 FNU after the treatment with the coagulant in the dosage of 5 milliliter per cubic decimeter (mL/dm^3)of 0.1% of Al_2O_3 solution (pH 4.35). Purification efficiency reached 99.16%.

Water from the "Almaty SU" water intake after treatment with Coagulant No. 15 at a dose of 10 mg/L had permanganate oxidation equal to −2.2 milligram of Oxygen per liter ($mg0_2$/L). The comparative results of the permanganate acidity of water depending on pH after treatment with Coagulant No. 15 enabled to conclude that the permanganate acidity of water samples increased from 1.17 $mg0_2$/L to 2.70 $mg0_2$/L with pH increase from 5.0 to 7.5. An increase in pH up to 9 with a coagulant treatment reduced permanganate oxidation to 1.87 $mg0_2$/L, which is within normal limits. The average permanganate acidity for drinking water was 2.6 $mg0_2$/L. The results of turbidity measurement, treatment efficiency assessment for natural and oil-contaminated wastewater (Sample no. 1), and circulating water of a water treatment unit after treatment with 0.1% Al_2O_3 solution of Coagulant No. 20 based on aluminum alloy Rau-97 are shown in Table 7 and Fig. 10.

Based on the data of water analysis using the industively coupled plasma method, the contents

Table 5: Main characteristics of and conditions for obtaining aluminum polyoxychloride solutions based on AAAs

Coagulant cipher	Terms of test				Main characteristics of coagulants			
	Alloy	Reaction temperature, ℃	HCl content, % by mass in solution	Amount of added HCl, mL	Mass fraction of Al converted to Al_2O_3, in ,(dry)%	Basicity, %	Electrical conductivity, mS/cm	pH
No. 11	Rau-85	25	3.0	50	31.0	66.9	30.5	3.29
No. 15	Rau-97	90	1.0	50	33.1	62.70	22.8	3.52
No. 20	Rau-97	25	3.0	50	33.0	56.20	27.8	3.28

Alloy weight: 1 g

Table 6: Comparative results of turbidity measurement and assessment of effectiveness of natural water treatment from "Almaty SU" water intake with 10% Coagulant solution No. 15 at different pH of water (coagulant dose 10 mg/L)

pH of Source water	Water pH after treatment	Water turbidity after treatment, FNU	Removal efficiency (%)
5.0	5.7	7.51	55.30
6.5	6.7	0.63	96.25
7.5	6.9	0.83	95.06
9.0	8.4	0.47	97.20
9.5	8.9	0.60	96.43

Almaty SU water (07.07.2022) (before treatment) pH: 6.5–6.8, turbidity (16.8) FNU (9.74 mg/L kaolin). According to the standards of SanPiN 2.1.4.1074-01, the turbidity of drinking water must not exceed 2.6 FNU or 1.5 mg/L kaolin (FNU 0.58)

Table 7: Results of turbidity measurement and efficiency assessment for natural water and wastewater treatment with 0.1% solution in terms of Al_2O_3 of coagulant No. 20 based on aluminum alloy Rau-97

Water sample, turbidity	Coagulant dose, mL/dm^3	Water pH		Turbidity, FNU	Removal efficiency (%)
		Before treatment	After treatment		
Natural "Almaty Su" 4.52 FNU	0.1	7.34	7.43	0.48	89.38
	0.5	7.38	7.48	0.32	92.92
	1.0	7.25	7.23	0.53	88.27
	5.0	7.25	7.13	0.62	86.28
	10	7.25	7.01	0.64	85.84
Natural Medeu 26.1 FNU.	0.02	7.12	7.15	1.98	92.41
	0.10	7.15	7.28	1.20	95.40
	1.0	7.15	7.28	0.82	96.86
	5.0	7.12	7.05	0.67	97.43
Oil-contaminated wastewater (Sample No. 1) 44.4 FNU	1.0	7.04	7.44	6.19	86.06
	1.5	7.38	7.17	0.99	97.77*
	1.5	7.38	7.24	0.69	98.45
	2.0	7.38	7.17	0.55	98.76
	3.0	7.37	7.30	0.62	98.60
	4.0	7.37	7.25	0.42	99.05
	5.0	7.04	7.54	0.37	99.17

*Coagulant PAC Aqua-Aurat-30

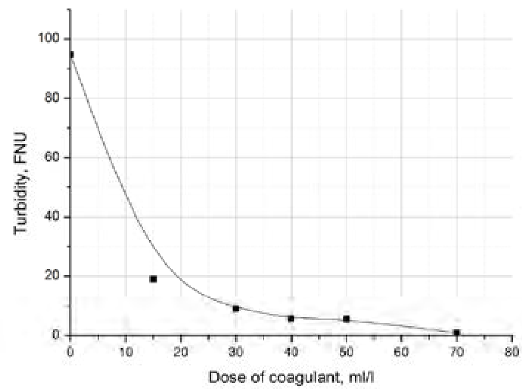


Fig. 10: Change in the turbidity of recycled water from a water treatment unit treated with 0.1% solution per Al_2O_3 of Coagulant No. 20

Table 8: Oil content in wastewater (Sample no. 1) treated with 0.1% solution in terms of Al_2O_3 of coagulant PAC No. 20.

Dose of PAC (mL/dm^3)	pH before treatment	pH after treatment	Oil content (mg/L)	Removal efficiency (%)
1.0	7.04	7.44	1.02	34.62
1.5	7.38	7.24	0.78	50.00
2.0	7.38	7.17	0.56	64.10
3.0	7.37	7.30	0.60	61.54
4.0	7.37	7.25	0.60	61.54
5.0	7.04	7.54	0.42	73.08

Oil content in water sample was 1.56 mg/L.

of Fe, calcium (Ca), and magnesium (Mg) cations significantly decreased after water sample treatment. Thus, the content of Fe cations decreased from 3.85 g/L to 0.049 g/L, Ca from 0.727 g/L to 0.713 g/L, Mg from 0.235 g/L to 0.202 g/L for oil-polluted wastewater after treatment with Coagulant No. 20 (1.5 mL/dm^3 of 0.1% solution in terms of Al_2O_3) (Sample No. 1). The oil content in wastewater (Sample no. 1) treated with 0.1% solution in terms of Al_2O_3 of Coagulant PAC No. 20 decreased from 1.56 mg/L to 0.42 mg/L (Table 8). Purification efficiency was 73.1%.

The permanganate oxidation of oily contaminated wastewater (Sample no. 1) with 0.1% solution in recalculation on Al_2O_3 of Coagulant No. 20 decreased from 50.05 mg O_2/L to 45.27 mg O_2/L. The assessment of oil-contaminated water treatment efficiency was also performed for oil-contaminated wastewater (Sample no. 2). Water characteristics before treatment with aluminum polyoxychloride are specified in Table 9.

Oil content was determined with spectrophotometer SPEKOL 1300 (Analytik Jena), extraction with chloroform. The wastewater treatment (Sample No. 2) with a coagulant in an amount of 10–20 µl/L resulted in the decrease of oil content from 8.85 mg/L to 0.86 mg/L. The efficiency of cleaning with Coagulant No. 11 was 90.28 %. The particle size of oil and zeta potential of oil-contaminated wastewater (Sample no. 2) was studied before and after the coagulant treatment using particle size analyzer Malvern Zetasizer NanoZS 90. The device enabled to measure particles from 0.3 nm to 10 microns (dynamic light scattering method) using noninvasive backscattering technology. The minimum sample amount was 20 µL. The accuracy was ± 2%. The M3-PALS method of electrophoretic light scattering was used to measure zeta potential in aqueous and anhydrous disperse systems. In this regard, the manufacturers of the device recommend using water with a particle size of 3.8 nm–100 µm for analysis. Six samples of oil-contaminated wastewater (Sample No. 2) with oil content of 8.85 mg/L) with different dosage of coagulants were prepared for measurements in the present work. Each sample was analyzed 12 times for particle size determination and 3 times for zeta potential determination with the average value derived. The working temperature of the measurement was 25 °C. The refractive index was 1.4, and the absorption coefficient was 1 for oil according to the reference data. In Grechanikov *et al*., (2010), the zeta potential is usually from −14 to −30 mV for colloids present in water having pH = 5–8. The greater its negative value, the greater the magnitude of the particle charge. Elkhova *et al*. (2006) revealed that when the zeta potential decreases, the distance between particles decreases, which increases the probabilities of their collisions. Coagulants create positive charges in water treatment systems with pH = 6–8, which decrease the zeta potential value. The authors point out that coagulation usually proceeds with a small negative zeta potential value, so that the complete neutralization of the charge is not required. However, when the coagulant is overdosed, the surface of a particle becomes positively charged, and the particles reenters the dispersed phase. The main results of the determination of oil particle sizes and zeta potentials of wastewater before and after treatment with coagulants in comparison with the Rau-85 reagent are shown in Table 10. The zeta potential of wastewater was negative –28.6 mV, the oil particle radius in the initial wastewater was 19.09 nm, the average particle radius was 90.44 nm, and the polydispersity index was 0.245. The oil particle radius in the wastewater reached 60.18 nm after wastewater treatment with the Rau-85 reagent in an amount of 10 m/L. The average radius was 100.2 nm, the polydispersity index was 0.325, and the average value of zeta potential was –28.4 mV.

After wastewater treatment with the Rau-85 reagent of 100 mg/dm^3, the particle radius increased to 647.4 nm, the average radius was 764.2 nm, the polydispersity index was 0.244, and the average value of zeta potential was −22.3 mV. The average particle radius increased up to 7901 nm, the polydispersity index was 0.101, and the average value of zeta potential reached −18.8 mV in wastewater after treatment with Coagulant No. 11. The data obtained allowed to conclude that oil particle size increased and a change occurred in the zeta potential toward zero with an increase in the dose of reagents, indicating that the system became less stable and was prone to coagulation and precipitation. Note that the most accurate particle size results can be obtained when the polydispersity index is less than 0.1, i.e., for spherical particles. The average particle size (radius) can be used for comparative purposes for slightly large polydispersity values. Relying on the average value is inappropriate, and the distribution analysis should be used to determine peak positions for broad distributions where the polydispersity is greater than 0.5.

Table 9: Physical and chemical properties of oil-contaminated wastewater (Sample no. 2)

Water sample	Density, kg/cm^3	pH	Electrical conductivity, mS/cm	Total salt content, mg/L	Oil content, mg/L
Oil-contaminated wastewater (Sample No. 2)	998	6.91	311	174.0	8.85

Table 10: Comparative results of oil particle size and zeta potential of oil-contaminated wastewater (Sample No. 2) before and after treatment with coagulants

Sample	Particle radius (nm)	Average particle radius (nm)	Polydispersity index	Zeta potential (mV)
Wastewater (Sample No. 2)	19.09	90.44	0.245	−28.6
Wastewater (Sample no. 2) treated with 10 mg/dm^3 Rau-85	60.18	100.2	0.325	−28.4
Wastewater (Sample No. 2) treated with 100 mg/dm^3 Rau-85	647.4	764.2	0.244	−22.3
Wastewater (Sample No. 2) treated with 10 µl/L of Coagulant No. 11	21.69	90.14	0.264	−29.1
Wastewater (Sample No. 2) treated with 100 µl/L of Coagulant No. 11	No peaks in the measured range	7901	0.101	−18.9
Wastewater, (Sample no. 2) treated with 100 µl/L of Coagulant No. 8	No peaks in the measured range	$2.775 \cdot 10^4$	1.0	−14.2

Particle Size Analyzer Malvern Zetasizer Nano ZS 90;
Coagulant No. 8 based on Rau-85, basicity 22.38%, mass fraction of Al_2O_3 −15.75% (solution), pH 2.24

CONCLUSIONS

A principally new approach to the development of a technology intended to obtain aluminum polyoxychloride coagulants for water treatment from natural and anthropogenic polution sources was theoretically substantiated and experimentally proven. The regularities of obtaining basic aluminum chloride (PAC) on the basis of AAAs containing metal activators—Ga, In, and Sn with contents from 0.5 wt% to 5 wt% (Rau-98.5, Rau-97, and Rau-85)—were studied. A method intended to produce aluminum polyoxychloride used as a coagulant for a potable water treatment was developed, considering existing disadvantages of known methods. The method involved treatment with 1%–5% HCl for 2–3 hours of AAAs containing metals in the following ratio (wt%): In 0.5–5, Ga 0.5–5, Sn 0.5–5, and aluminum is the rest. The developed method provided aluminum polyoxychloride with the necessary degree of basicity 41%–82.6% and with a mass fraction of aluminum in terms of Al_2O_3 of 30%–48%. The process was performed under mild conditions at an initial temperature of 20 °C–25 °C. Then, the temperature rose up to 60 °C–65 °C maintained at the expense of the exothermic dissolution reaction heat of activated aluminum-containing alloys in HCl. The microstructure, phase component, elemental composition of aluminum polyoxychloride were studied with the use of a sufficient number of analytical and instrumental methods—IR spectroscopy, XRD, SEM with EDS, XRF, and X-ray spectral analysis. The effectiveness of the purification of water samples taken from "Almaty Su" and "Medeu" water intake stations, recycled water of a cooling water treatment unit, and oil-contaminated wastewater (anthropogenic pollution sources) was assessed. Treated water parameters were within the standards established for drinking water supply and wastewater disposal intended for water discharge into the receiving facility. The reduction of the turbidity of water sampled from the "Almaty Su" water intake station reached 97.2% and that of recycled water reached −99.6%. The efficiency of the treatment of oil-contaminated wastewater from oil reached 90.27%. This research intended to determine oil particle size and the zeta potential of oil-contaminated wastewater. It concluded that oil particle size increased and the zeta potential of water toward zero changes with an increase in coagulant dosage, indicating that the system becomes less stable, prone to coagulation and precipitation. The results confirm that aluminum polyoxychloride is an effective coagulant for potable and wastewater treatment. The treated water is within the established limits for hydrogen potential, COD, and turbidity. The water treatment method can be easily implemented.

AUTHOR CONTRIBUTIONS

R.G. Sarmurzina defined the concept, selected and justified the research direction. G.I. Boiko determined the purposes and objectives of the study, supervised the research, verified and edited the manuscript. B.K. Kenzhaliyev attracted funding and perfomed the research methodologies. N.P. Lyubchenko analyzed and interpreted the data and prepared the manuscript text. P.V. Kenyaikin performed the experiments, figures, tables, and graphical abstracts. U.S. Karabalin perfomed consultation and analyzed the research results. Zh.B. Ilmaliev analysed the results of instrumental methods of the study.

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CONFLICT OF INTEREST

The author declares no conflict of interest regarding the publication of this manuscript. Ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degrees Celsius
µm	Micrometers
^{0}C/min	Degrees Celsius per minute
A.C.S.	American Chemical Society
AAA	Activated aluminum alloys
AC	Aluminum chloride
Al	Aluminium
Al_2O_3	Aluminium oxide
BCC	Body-centered cubic structure
Bi	Bismuth
Ca	Calcium
Cd	Cadmium
Cl	Chlorine
Cu	Copper
DTA	Differential thermal analysis
DTG	Derivative thermogravimetric
EDX	Energy dispersive X-rays
EDXS	Energy-dispersive X-ray spectroscopy
ERG	Eurasian Group
FCC	Face-centered cubic
Fe	Iron
FNU	Formazine Nephelometric Unit – formazine turbidity unit
FTIR	Fourier-transform infrared
g	Grams
g/cm^3	Grams per cubic centimeter
Ga	Gallium
GB	GuoBiao
HCl	Hydrochloric acid
OH	Hydroxyl
In	Indium
IR Spectrum	Infared spectrum
ISO	International organization for standardization
JSC	Joint-stock company
K	Kelvin
KBr	Potassium bromate
kg	Kilogram
kg/cm^3	Kilogram per centimeter in a cube
kV	Kilovolt
Ltd	Limited trade development
mA	Milliamp
Mg	Magnesium
mg/L	Milligram per liter
mgO_2/L	Milligram of oxygen per liter
min	Minut
mL	Millilite
mL /min	Milliliters per minute
mL/dm^3	Milliliter per cubic decimeter
mL/g min	Milliliter per gram per minute
mm	Millimeter
mV	Millivolt
nA	Nanoamper
NIBS	Non-invasive backscatter
nm	Nanometer
NTU	Nephelometric turbidity units
Pb	Lead
PDF	Powder diffractometer database
pH	Hydrogen potential
PAC	Poly aluminium chloride
rpm	Revolutions per minute
Sn	Tin
STA	Simultaneous thermal analyzer
SEM	Scanning electron microscope
TG	Thermogram
wt	Weight
XRD	X-ray diffraction analysis
XRF	X-ray phase analysis
Zn	Zinc

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ORIGINAL RESEARCH PAPER

Climate change mitigation and adaptation through livestock waste management

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ABSTRACT

BACKGROUND AND OBJECTIVES: Farmer characteristics are recognized in this study. The characteristics, perceptions, willingness to adopt climate change mitigation, and awareness of livestock farmers toward livestock waste management are the main points for determining appropriate climate mitigation rules.

METHODS: This study was conducted in Enrekang and Barru Regencies of South Sulawesi. International Business Machines- Statistical Package for the Social Sciences 27 was used for this study. In descriptive statistics, data were compiled, and the age, long husbandry experiences (year), number of family member, number of farming assistant, gender, education, farmer group participation status, side job, type of business, cattle ownership status, number of cattle (head), and weight total of cattle's manure (kilogram per day) were examined qualitatively. A chi-square test was used to compare the experimental results (perception and knowledge of livestock manure management) with practical livestock manure management.

FINDINGS: This study found that the average age of farmers in the study area is 45 and 11.2 percent received have high formal education level from a university. Most of the cattle are male at 86.7 percent. Poor manure management system at 76.30 percent manure un-managed and un-appropriate farmer groups with more than 60 percent of the farmers un-joined farmer's group. Almost 50 percent of the cattle farmers are willing to learn manure management. Nevertheless, this study found that the respondents' knowledge and practical manure management, as well as the respondents' knowledge (0.837) and perception (0.343) of practical manure management, do not have any significant connection.

CONCLUSION: This study determines the full condition of cattle farmers in Barru and Enrekang Regencies. Barriers include low level of education, age of farmers, lack of manure management, and lack of willingness to join farmers group. Nevertheless, drivers, such as willingness to adopt manure management and high levels of experience in cattle farming, were also found. Enriching the knowledge and perception of farmers is essential in managing livestock wastes to mitigateof climate change.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
91	3	6

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INTRODUCTION

Global demand for food, livestock feeds, fiber, and fuel has increased because of population growth and changing dietary patterns (Mairura *et al.,* 2022). In 2019, the demand for meat reached 70 million tonnes and is predicted to rise to 74 million tonnes by 2023 (Meat Livestock Australia (MLA), 2020). In other words, the consumption of animal proteins will soar dramatically in densely populated areas of the world (McAuliffe *et al.,* 2018). The increase is also more associated with income and urbanization than with most other food groups that actually can be recycled (Sivakumar et al., 2022). In line, livestock is truly capable of converting protein sources that are not edible to humans into high-value protein and contributes almost 37 percent (%) of the world's protein supply (Food Agriculture Organization (FAO), 2018). Nonetheless, agriculture is also predicted as the highest enhancer of methane (CH_4) pollution (International Energy Agency (IEA), 2022), with livestock, including ruminants, one of the primary sources of agricultural emissions (Frank, 2017; FAO, 2021). The ongoing climate change and livestock production make the increase in production while reducing greenhouse gases (GHGs) and climate impact emissions difficult (Cheng *et al.,* 2022). In other words, rising numbers directly related to increasing carbon dioxide (CO_2) and CH_4 emissions (Agossou and Koluman, 2022). Thus, an effort to increase livestock's productivity can lead to an enhance of emissions on earth (Beauchemin *et al.,* 2020). In this sense, livestock causes water eutrophication, as well as air pollution, such as ammonia (NH_4) and GHGs emissions, directly and indirectly (Chang *et al.,* 2019). In comparison with other livestocks, such as buffalo, swine, goat and chickens, cattle farm is a major contributor to pollution and GHGs (Scherer *et al.,* 2018). Furthermore, in the tropics, cattle and oilseed products account for almost half of the deforestation and carbon pollution that causes GHGs (Creutzig *et al.*, 2019), whereas cattle production increased 18% in 2019 (FAO, 2021), thereby reaching 978.68 million head and is projected to reach 1,9009.69 million head in 2022 (Statista, 2022). This increase occurred because to the livestock can play an important role in ensuring food security on earth (Chungchunlam *et al.*, 2020). Furthermore, in husbandry, pollution due to livestock farming in the form of water, air, and soil can disrupt the environmental balance, including the comfort of the surrounding community, which can threaten health. Water pollution caused by livestock waste is due to the fact that the liquid waste is disposed of without being processed or filtered first and then channeled into waterways around the environment, potentially causing an unpleasant odor. The smell from livestock waste is caused by NH_4 gas and other chemical compounds, including phosphate and nitrogen, which are quite dominant. Other research states that livestock waste that contain nitrogen and phosphorus causes eutrophication and the death of fishes in rivers (Biagini and Lazzaroni, 2018). Eutrophication causes the oxygen content in water to decrease, thereby making surviving difficult for biota in aquatic ecosystems. Moreover, manure management is responsible for almost 12% of emission sources in 2021 (Environmental Protection Agency (EPA), 2010). Nevertheless, the livestock sector can also make a significant contribution to reducing the global temperature increase that causes climate change (Intergovernmental Panel on Climate Change (IPCC), 2022; Reisinger *et al.*, 2021). In this sense, livestock is indicated as the main agricultural contributor of reducing almost 38% of excess CO_2 emissions (IPCC, 2022) and more than 60% of total emissions aside from CO_2 with proper management (Frank et *al.*, 2018). Livestock waste comes in various forms, namely, solid, liquid, and gas. The waste generated by livestock is in the form of solid, liquid and gaseous waste. Solid waste on cattle farms include solid animal manure, leftover feed, and livestock bones and bodies. Liquid waste includes animal urine, blood when animals are injured, and water used for washing slaughtered livestock. Overall, all livestock organic wastes, such as silage, animal feed residue, slaughterhouse bio waste, sewage sludge, molasses and others, can be processed further. A total of 34.7% of the potential amount of cow dung can be used for further processing (Priekulis *et al.*, 2021). Storage and management of slurry (a mixture of urine, feces, water, and sand bedding material) are critical factors to consider during livestock and rearing practices (Guo *et al.,* 2019) because GHGs are formed during storage. Nitrous oxide (N_2O) results from the improper storage of manure and the use of various types of fertilizers. N_2O is a compound that harms climate change and is 265–298 times higher than CO_2 (Grossi *et al.,* 2019).

Under anaerobic conditions, CH_4 emissions occur as a result of organic matter degradation. Methane, a gas emitted by animals primarily through enteric fermentation and the improper storage of manure, contributes to global warming by 25 to 28 times than CO_2 (Wang *et al.,* 2021). The nitrification of ammonium (NH_4+) and denitrification of nitrate (NO_3) processes to produce N_2O (Liebig *et al.,* 2021). Based on these facts, long-term solutions for improved manure storage management must be considered. Treatment of slurry can reduce N_2O emissions at 50% and methane emission at 36% to 63% (Ruiz, 2022). Moreover, the substrate that is converted into CH_4 during anaerobic digestion processes can be used as a renewable energy source. The higher the organic matter content in its composition is, the higher the biogas production if manure is used as a substrate or co-substrate in anaerobic digestion processes (Van den Oever *et al.,* 2021). The anaerobic digestion reduced the GHGs by relpacing the consumption of fossil fuel due to the decline in the use and production of fertilizer (Kaparaju and Rintala, 2011). Conversely, in Indonesia, livestock waste is most often only disposed of in sewers or human yards, causing pollution to the environment (Nugraha *et al.,* 2021). The total production in the country has been rising in recent years (Statistic of Indonesia, 2021). Moreover, in 2022, the Ministry of Agriculture of the Republic Indonesia has committed to make this year as the year of animal husbandry and promised to encourage more cattle production in the next year (DGLAH-RI, 2022). Hence, modifying industry management practices is essential to lessen the contribution of livestock production to climate change (Rojas-Downing *et al.,* 2017). Additionally, these efforts can also increase farm profitability and environmental sustainability by improving farm animal welfare (Dawkins *et al.,* 2017; Fernandes *et al.,* 2021). In this sense, farmers' involvement in the area's agricultural development is essential to ensure the success of agricultural development and mitigation plans (Yuniarsih *et al.,* 2021). Recognizing the characteristics of farmers is also crucial (Reddy *et al.,* 2022) because farmers are the first line of defense against climate change (Rockney, 2022). This information is essential for public decision-makers to encourage the adoption of mitigation measures (Calciolari *et al.*, 2021). The research question is, what are the correlation between the perception and awereness of livestock waste management in the Enrenkang and Barru Regencies? These data include the basic steps in formulating adequate program procedures for climate mitigation. Second, the data can be used for further research that could lead to new ideas for creating mitigation strategies. Third, the study could facilitate a proper understanding between field stakeholders in creating new solutions. This study will contribute relevant information for education. Hence, this study will elaborate on the research by interpreting the relationship between the perception or awareness of livestock waste management practices. The characteristics, perceptions, and awareness of livestock farmers toward the livestock waste management will be investifared. South Sulawesi was selected becaise it is the third highest cattle producer in Indonesia at 1.46 million heads in 2021 (Statistic of Indonesia, 2022). The current study aims to investigate climate change mitigation and adaptation in various ways. This study was carried out in Enrekang and Barru Regency in South Sulawesi in 2022.

MATERIALS AND METHODS

Description of the study area and context

The Enrekang and Barru Regencies are located in South Sulawesi, Indonesia. The coordinates of Enrekang Regency are 3° 33′ 52″ South, 119° 46′ 29″ East, and Barru Regency is 3° 14′ 36″ South, 119° 40′ 53″ East (Fig. 1). According to the 2020 Census, Enrekang Regency has a total area of 1,786.01 square kilometer (km^2) and a population of 225.172 people, whereas Barru has population of 184.452 people and an area of 1,174.72 km^2.

Data collection

This study was conducted in 2022 using the primary data from 49 selected respondents from Barru Regency and 49 selected respondents from Enrekang Regency. The selection of 98 samples was conducted by paying attention to breeders who consistently raise cattle, not seasoned breeders. Information was gathered through observation and questionnaire-assisted interviews for the survey. The study used a qualitative survey methodology, and semi-structured questionnaires were used to collect from purposively selected interviews. In terms of adoption, farmer characteristics are important factors in the success of the farming business (Small *et al.*, 2022). Observable

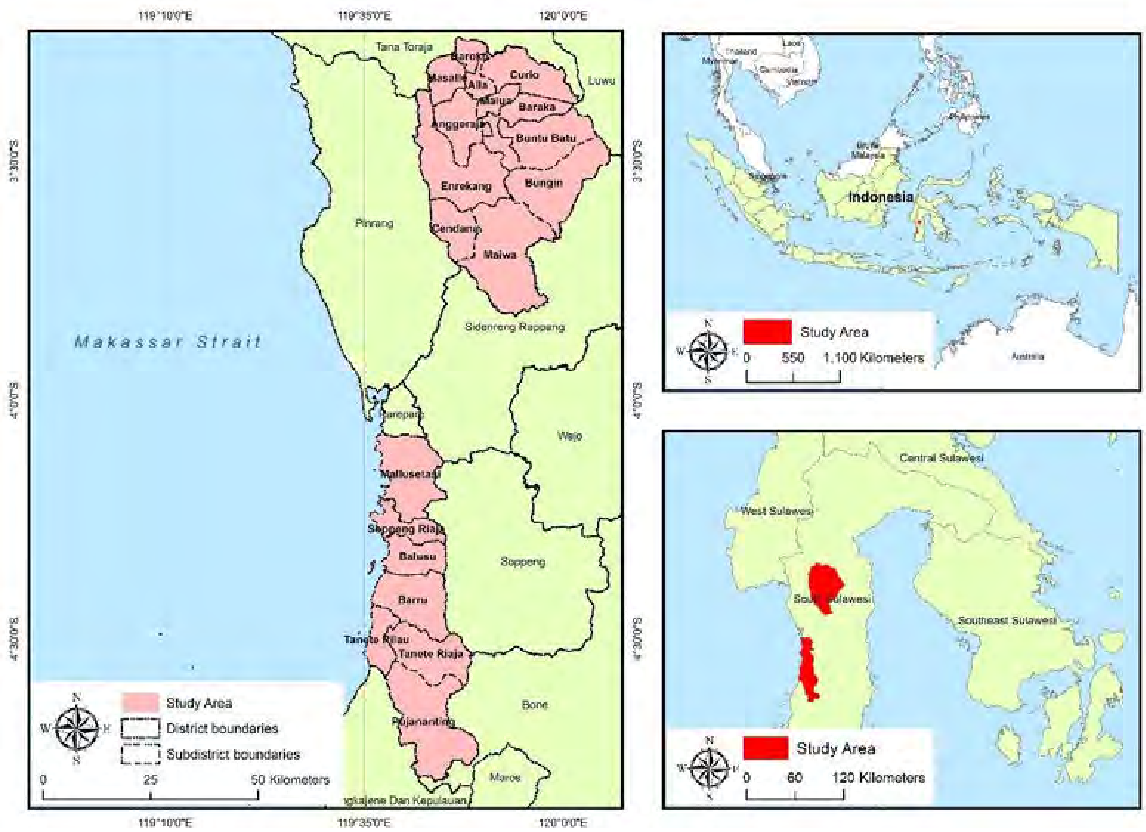

Fig. 1: Geographic location of the study area in Barru and Enrenkang, Indonesia

individual characteristics include age, education level, farming experience, side job, number of family dependents, and business scale (Etsay *et al*., 2019). A farmer's age affects his productivity when conducting business activities (Komba *et al.,* 2018). Good adaptation can be obtained through learning and education so that business activity will grow more rapidly with innovation that is driven by the ability to think creatively using that education (Ramesh *et al.*, 2019). Continuous learning activities will also improve a person's experience and help them avoid mistakes in business management (Kolapo *et al*., 2022). The number of family dependents whose needs must be met is another factor that can motivate a person to grow his or her business. The existence of income from a side business will aid in increasing capital to scale up the main business (Maake and Antwi, 2022). The data collected include gender, age, education, primer job, farmer group participation status, farming status, and cattle ownership status. Moreover, data on type, perception, and knowledge on livestock manure management are also included in this study. In addition, liquid petroleum gas (LPG) or gas energy consumption is also an important variable to emphasize a point to develop livestock manure into biogas to minimize farmer's consumption expenditure. Previous studies that describe essential variables are shown in Table 1.

Furthermore, to recognize manure management condition in these two regencies, data on perception and knowledge about this management will also be collected. In addition, the willingness to adopt new management and original manure treatment practices is included.

Data analysis

The study used International business machines (IBM) - Statistical package for the social sciences

Table 1: Variables collected

No.	Variables collected	References
1	Age	Gebre *et al.,* 2022
2	Long husbandry experiences (year)	Masumi *et al.,* 2022
3	Number of family member	Hasan and Kumar, 2022
4	Number of farming assistant	Mairura *et al.,* 2022
5	Gender	Fadeyi *et al.*, 2022
6	Education	Ramesh *et al*., 2019
7	Farmer group participation status	Goodwin *et al.,* 2022
8	Side job	Kayode *et al*., 2017
9	Type of business	Dew *et al.,* 2022
10	Cattle ownership status	Rahman *et al.,* 2022
11	Number of cattle (head)	Aken *et al.,* 2022
12	Weight total of cattle's manure (kilogram per day: kg/day)	Setoguchi *et al.,* 2022
13	Age, long husbandry experiences, total of family member, total farming assistant, LPG consumption (in a month), education, gender, job, farmer group participation status, type of cattle farming, cattle farming characteristics, manure management condition, and practical livestock waste, knowledge and perception of farmers	This study

(SPSS)- 27 to analyze the collected factors. SPSS sowftware is selected because of its simplicity, simple command language, and well-documented user manual. In addition, SPSS has all the features needed for this research, such as descriptive and bivariate analyses. The result will be categorized into three main groups. First, farmer characteristics, which will describe the age, long husbandry experiences, number of family members and farming assistants, gender, LPG consumption, education level, address, and group participation. Second, cattle farming characteristics interpret the business type, cattle owning status, number of cattle and total manure produced, livestock waste management practices, willingness to manage livestock waste, knowledge level, and perception level of manure management. Livestock waste is assessed by collecting waste every day, which is collected in a certain container, and measuring the volume of waste produced. Then, it was calculated to the body weight of each livestock. So that results in the average size of waste per head per day. For knowledge and perception levels, the results will be explained based on median score. Scores of 16 or above will be categorized as adequate, whereas scores below 16 will be categorized as inadequate. Additionally, cattle types will be specified as adult cattle (more than 2 years), young cattle (young cows, 13 weeks to 2 years), and calf (newborn to 8 months). Cow was chosen as the object of research because of the dominant breed of cattle at the research location. Thus, the cow waste produced is quite a lot and worthy of being the object of research. Third, bivariate analysis is conducted to examine knowledge and perception variables with the practice of managing manure. A chi-square test and probability-value (p-value) analysis is used to compare the observed result from the experiments (perception and knowledge of livestock manure management) with livestock manure management practices. Moreover, descriptive statistics will be used to compile and examine the data, including mean, maximum, minimum, and percentages, qualitatively.

RESULTS AND DISCUSSION

Farmer's characteristic

Climate change adaptation is critical for achieving food security and sustainable agricultural development (Das *et al.,* 2022). Climate change is sparking new levels of global concern, shifting values, preferences, and behavior (Nezhyva and Mysiuk, 2022). However, the current set of personal farmer preferences, daily practices, and belief systems have a positive impact on decision-making in farming activities (Haberl *et al.,* 2021). Specifically, factors such as farmer group intervention (Crudeli *et al.,* 2022), and basic characteristic data (e.g., main job, experiences, total family's member, land ownership and education level) are massively useful in presenting new mitigation management due to farmer's willingness to adopt programs (Wang *et al.,* 2022). Moreover, Fernández-Habas *et al.,* (2022) in their study stated that proper management

Table 2: Farmer's characteristic (descriptive analysis)

No.	Variable	Data	Minimum	Maximum	Mean	Standard deviation (SD)
1	Age	98	20	83	45.69	13.126
2	Long experience husbandry (year) *Data missing*	95 3	0	43	11.29	9.680
3	Total family member	98	1	8	4	1.560
4	Total farming assistant	98	0	3	1	0.849
5	LPG consumption (a month)	98	1	6	3	1.037

Table 3: Specific farmer's characteristic

No	Variable	F	%
1	Farmer's address (Regency)		
	Barru	49	50
	Enrekang	49	50
2	Gender		
	Male	85	86.7
	Female	13	13.3
3	Education		
	No educational attainment	4	4.1
	Elementary level	35	35.7
	Junior high school level	20	20.4
	Senior high school level	28	28.6
	University	11	11.2
4	Job		
	Farmer	85	86.7
	Employee	4	4.1
	Housewife	9	9.2
5	Farmer group participation status		
	Yes	35	35.7
	No	63	64.3

programs is needed to guarantee farmer's survival and functionality. In other relevant studies, knowing the characteristics of farmers, especially age and job can affect their perceptions (Zhang *et al.*, 2022). In the case of agriculture, describing some information, such as education level, can benefit in designing suitable training programs.

The data presented in Table 2 describe the basic information of cattle farmers in Enrenkang and Barru Regencies. The age of cattle farmers range from 20 to 83 with a mean age of 45 years Wanga *et al.*, (2022) found that most farmers under 45 years old exhibit better productivity. This range of age is important to consider with the conditions that are found. Additionally, farmers in the active age with more than 22 years of experience are more likely to recognize the importance of new farming innovations (Shah *et al.*, 2022). The average farming experience of the respondents in this study was 11.29 years, although some farmers had more than 20 years of experience in cattle farming. As mentioned in the Methodology, the research was conducted in two regencies, namely, Barru Regency and Enrekang Regency, South Sulawesi. The respondents were studied comprised

Table 4: Type of cattle farming

No	Variable	F	%
1	Type of business		
	Profitable business	72	73.5
	Non-profitable business	26	26.5
2	Cattle ownership status		
	Not an owner	1	1
	Owner	97	99

86.7% male. Furthermore, most of the respondents had elementary school education (35.7%), while only 11.2% had university education. According to Table 3, 4.1% of farmers have primary job as employees, and more than 50% farmers did not join any farmer groups in Barru Regency nor in Enrekang Regency. Some studies, such as (Tong *et al.,* 2021), suggested that farmers' associations or groups and relevant government agencies must be proactive to achieve agricultural development through education. Moreover, participation and interaction among farmers through social networks (Pratiwi and Suzuki, 2017) and farmer groups (Zossou *et al.*, 2019) play an active role in exchanging information and acquiring knowledge. Additionally, education level is an important factor in motivating farmers to adopt new farming practices (Bai *et al.,* 2022). Another study suggests that training programs and experiences can be alternatives to reduce the education gap (Abebe *et al.,* 2022).

Cattle farming characteristics

The livestock business of the respondent is dominated by the profit livestock business type in both districts, accounting for 73.5% of the total, with 99% of livestock being privately owned. The data are shown in Table 4.

Indeed, data of business type and cattle owning status are used to identify restrictions and assess scenarios of mitigation (Arata *et al.,* 2022). Furthermore, a connection is observed between climate mitigation goals and selected rules as part of a mechanistic environmental modeling tool or mitigation model that fits the business conditions (Stoian *et al.,* 2022). A related study about cattle age was conducted by (Pence *et al.,* 2022). The study showed that knowledge about animal age can be used to predict the biogas amount, CO_2 emissions, coal, electricity-thermal energy, and CH_4 values that are all modeled. This study finds that the livestock business carried out by the respondents is divided into three types, namely, adult cattle, young cattle (e.g., young cows aged 13 weeks to 2 years), and calf (e.g., newborns to 8 months). On average, farmers keep three adult cows, one young cow, and one calf. The amount of cattle's manure produced by an adult cow ranges from apprixımately 14 kg/day (average) to 125 kg/day (maximum). Meanwhile, the average manure produced by young cattle and calf is 2 kg/day. Of course, this, depends on the number of livestock kept. In addition, classifying animal age is used to create a mitigation formula that can adequately be adapted by farmers (Ghalandari *et al.*, 2021).

The husbandry business type in these regencies is small-scale farming, which, according to some studies, mean that it might restrict the professionalization of animal production (Pence *et al.,* 2022). Based on the data in Table 5, cattle farming generates enough manure to be processed productively, such as by composting and bio-energy. Biogas, which is a type of biomass, can be produced from the animal manure. Digested substrate or decay product residues can be used as a valuable fertilizer when the obtained biogas is used (Cheng *et al*. 2022). The upcycling of cattle manure has the potential to reduce greenhouse gas emissions significantly (Kim *et al.,* 2022). Based on Fig. 2, the contribution has grown slightly every year and has reached more than 2700 kilo-tonnes CO_2-equivalent (kt CO_2-eq).

Additionally, (Honorato *et al.,* 2022) described that cattle manure application in thyme farming could raise plant antioxidants. Further, (Carmo *et al.,* 2022) found that by using fertilizer from cow's manure, plants could possibly grow better based on their diameter and height. Moreover, cattle's manure application on farming system can significantly

Table 5: Cattle farming characteristics

No.	Variable	Data	Minimum	Maximum	Mean	SD
1	Total of adult cattle (head)	73	0	25	3	4,164
2	Total of adult cattle's manure (kg/day)	73	0	125	14	12,088
3	Total of young cattle (h)	73	0	7	1	1,414
4	Total of young cattle's manure (kg/day)	73	0	15	2	4,035
5	Total of calf (head)	73	0	5	1	1,279
6	Total of calf's manure (kg/day)	73	0	9	2	2,200

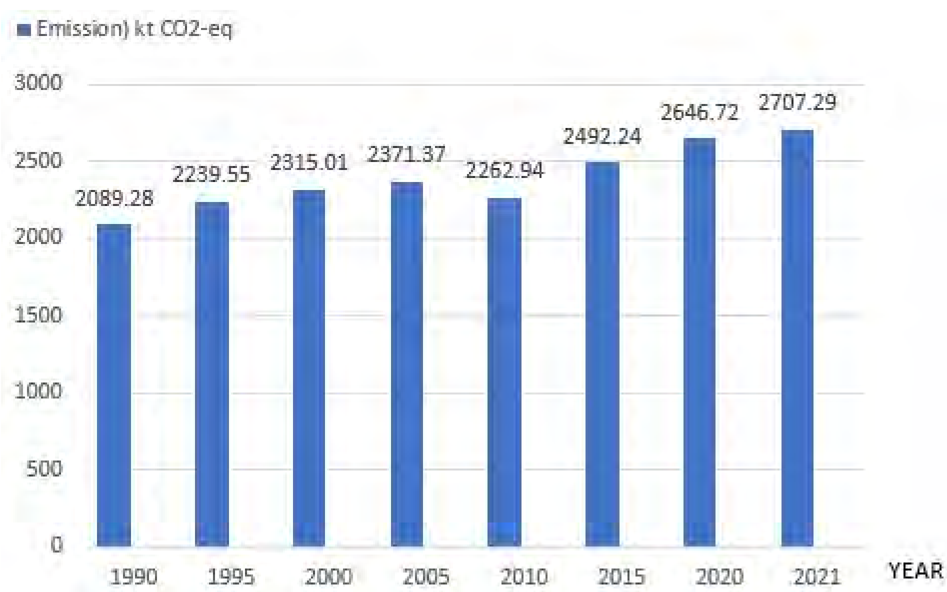

Fig. 2: Manure emission contribution to global GHGs (MLA, 2022)

reduce farming cost. Natural gas can be saved, and imports are reduced significantly if the potential of agricultural and animal waste is used effectively (Melikoglu and Menekse 2020).

Despite the potential benefits of cattle manure management in climate change mitigation, recognizing the percentage of livestock patterns that are already being used in local cattle husbandry is important. According to Table 6, only 14.3% of the respondents have already managed cattle's manure on their husbandry, whereas 85.7% have not yet applied these manure management practices. However, 50% of the respondents are willing to manage livestock manure, whereas the other 50% are not willing because of the potential costs and time involved. Notably, poor handling of waste is still being carried out by farmers in the regencies. Furthermore, only 51% of the respondents have adequate knowledge and perception of the processing and impact of livestock waste, whereas 49% have poor knowledge and perception. Nevertheless, when a large number of farmers adopt a particular practice, it may inspire others in the area to follow suit. In contrast, low participation may discourage other farmers from adopting the practices (Šūmane *et al*., 2018). In fact, based on the data in Fig. 3, farmers are predicted to accumulate livestock waste in open spaces (63.3%), disposed of into rivers (10.2%), and stockpile it (3.1%). Only a small number of farmers manage their livestock waste by processing it into compost (10.2%)

Table 6: Manure management condition

No	Variable	F-value	%
1	Livestock management practical		
	Yes	14	14.3
	No	18	85.7
2	Willingness to manage livestock waste		
	Yes	49	50
	No	49	50
4	Respondent knowledge in livestock waste management and its impact		
	Adequate	50	51
	Inadequate	48	49
5	Respondent perception of livestock management and its impact		
	Adequate	50	51
	Inadequate	48	49

or using it as a medium for earthworms (13.3%).

Although the management of cattle's manure in these areas is extremely poor, the willingness of the farmers to manage the manure may positively attract the potential of implementing mitigation programs (Lambert *et al.,* 2022). Improving socio-economic conditions, such as intensifying government extension and study programs in the husbandry area, can also increase the willingness of farmers to adopt mitigation programs (Jan, 2021).

The chi-square test (X2) results suggest no significant relationship between the respondents' knowledge and the practice of managing animal manure (p-value: 0.837). Additionally, respondents' perceptions and the practice of managing animal manure have no significant relationship (p-value: 0.343). However, a relevant study found that perception and knowledge could affect farming management, particularly when farmers understand not only the climate impact but also the economic impact of their practices (Abdollahzadeh *et al.,* 2022). Using manure in anaerobic digestion reduces the release of carbon in the atmosphere and produces biogas. The manure in anaerobic digestion also produces organic fertilizer that is valuable in the marketplace (Awasthi *et al.,* 2022). Furthermore, peer groups and field experiences can encourage the development of knowledge and perception (Hazard *et al.,* 2022). This condition happened because of the realization of new ideas that need collaboration among the farmers or community and farming policy. The policy frameworks are needed to support the adaptation of climate-friendly livestock waste management practices in agriculture. For example, China's Zero Fertilizer Increase Input Policy aimed to replace 60%–75% more friendly synthetic fertilization (Awasthi *et al.,* 2022). To improve farmers' adaptive capacity and adoption of innovations, the government and other stakeholders must increase their access to socioeconomic resources and education (Asare-Nuamah *et al.,* 2022). Similarly, providing support from institutions and improving access to proper facilitation and technology are important for bringing perception and knowledge into practical action (Bui and Do, 2022). Adequate technology will encourage bioenergy production with appropriate livestock manure management and utilization as a feedstock. Additionally, the mutual trust level among stakeholders is a factor that can generate management action in agriculture (Erkkilä-Välimäki *et al.,* 2022). Perceptions and actions show different responses in the actualization of waste management in agriculture, even though livestock and waste are not optimally managed, farmers still have a sense of responsibility to maintain their livestock. The reason is that farmers know that the waste that they produce can pollute the environment. Nitrate waste in the form of nitrogen monoxide (NO) and nitrogen dioxide (NO_2) significantly affects climate change on earth, but farmers do not understand this concept of climate change because of their livestock activities. Therefore, managing livestock waste through a

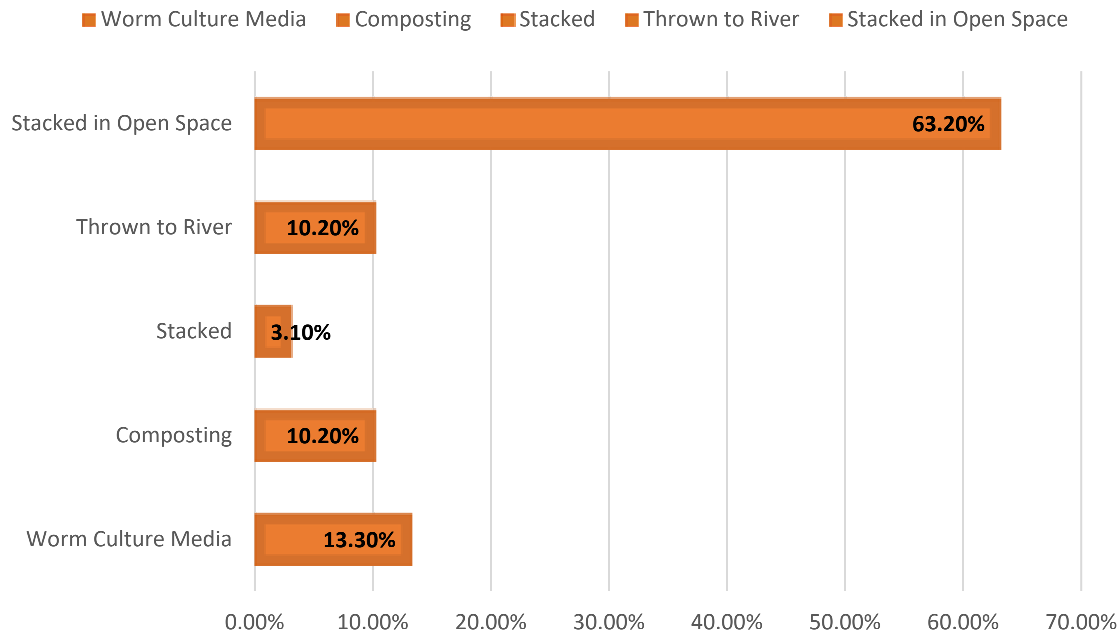

Fig. 3: Percentage of practical livestock waste

Table 7: Bivariate analysis of knowledge and perception variables with the practice of managing manure

Variable	Livestock management practical						Probability
	Yes		No		Total		
	Data	%	Data	%	Data	%	
Knowledge variable							
Adequate	8	16.0	42	84.0	50	100	0.837
Inadequate	6	12.5	42	87.5	48	100	
Perception variable							
Adequate	5	10.0	45	90.0	50	100	0.343
Inadequate	9	18.8	39	81.3	48	100	

conversion strategy into something useful is crucial to mitigate livestock waste and reduce greenhouse gas emissions. Management to achieve zero waste is focused on business owners who manage the whole process in the farm. The fact is that the conversion requires a high amount of waste, thereby giving large farmers more opportunities for development, in contrast to small farmers. However, a gap still exists in the management between small and large breeders. In industrial-scale livestock, management is more organized, thereby making business owners aware of to the importance of minimizing livestock product waste. On a small scale, farmers tend to produce minimal waste, hence, they may be reluctant to manage it because of considerations of time and cost inefficiency. However, other alternatives can be further studied through socialization and intensive assistance regarding the benefits of livestock waste management. Business owners, as leaders, have a significant influence in making decisions that greatly determine the goals of a farm (Fałkowski and Lewkowicz, 2022). Productivity should not only focus on production but also on feed yields through the concept of horizontal integration. This concept can motivate farmers because they benefit in saving costs by reducing fertilizer considering that livestock waste can be used as a substitute, leading to sustainable farming practices (Shortall, 2022). In addition, the local government's control over livestock waste management has been previously studied. Good relations between the government and the livestock private sector can contribute to socially and environmentally sound livestock management (Richards and Yabar, 2022). The public–private partnership will help the project of livestock management run productively, that is, the formulation of a firm and booming market for boosting the profit. This includes the disposal of livestock waste water, which should not be disposed directly into waterways. A strict approach to companies through government regulation is very important in regulating livestock waste contamination. With regulations in place, companies will be more disciplined in managing their wastes, because if the companies violate the regulations, their permits will be revoked. Further, if regulations are good but supervision, outreach, and assistance are weak, then it can be an opportunity for rogue companies to not manage their waste. Large-scale farmers can take advantage of this, whereas small-scale ones can lose motivation and willingness to manage their livestock wastes. Periodic inspections can help analyze the condition of large-level breeders.

CONCLUSION

This research expanded on previous studies by interpreting the relationship between perception and awareness of livestock waste management in South Sulawesi's Enrekang and Barru Regencies. Male farmer employees in these two regencies are found higher in comparison to female farmer employees, making up only 4.1% of them. According to the findings of this study, the respondents' livestock business is divided into three categories, namely, adult cattle, young cattle (young cows aged 13 weeks to 2 years), and calf (newborns to 8 months). On average, farmers keep three adult cows, one young cow, and one calf. An adult cow can produce manure at the rate of around 14 kg/day (average) to 125 kg/day (maximum). Meanwhile, young cattle and calves produce manure at average of 2 kg/day. However, manure management in these areas was not well developed. More than 63.2% of the manure was only stacked in open space. Nevertheless, other treatments, such as composting and worm culture media, were used by 23.5% cattle farmers, and 50% of the farmers are willing to manage livestock waste. The research found that more than 50% of the farmers have adequate knowledge about the impact of livestock waste. Furthermore, no significant relationship is observed between respondents' knowledge or perceptions of animal manure management practices. The results in this study contributes to the basic knowledge for stakeholders and other researchers to develop the appropriate intervention to improve livestock waste management, especially for farmers. The limitation of this study was the sole use of statistical analysis data. Furthermore, the researcher can determine the economic valuation of livestock waste management or even provide an intervention to enrich the perception and awareness toward livestock waste management. The public–private partership of the livestock waste management needs to be considered. Additional local government participation is recommended to develop good manure management in husbandry, especially centralized waste management, and to optimize biogas waste utilization.

AUTHOR CONTRIBUTIONS

E. Frimawaty performed conceptual and design, data acquisition, analysis, and interpretation of data, obtaining funding, supervision, and the corresponding author. A. Ilmika performed the data analysis and interpretation and drafted the manuscript. N. A Sakina performed the data analysis and interpretation, drafted the manuscript, and checked the article to guideline for author. J. Mustabi performed the statistical analysis and administrative, technical, or material support.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
CH_4	Methane
CO_2	Carbondioxide
FAO	Food and Agriculture Organization
GHGs	Green House Gasses
IBM	International business machines
IEA	International Energy Agency
IPCC	Intergovernmental Panel on Climate Change
kg	Kilogram
kg/Day	Kilogram per Day
KM	Kilometer
kt CO_2-eq	Kilo-ton CO_2-equivalent
LPG	Liquid Petroleum Gas
MLA	Meat and Livestock Australia
m^2	Meter square
NH_4	Ammonia
NH_4+	Ammonium
N_2O	Nitrogen oxide
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide
NO_3	Nitrate
p-value	Probability-Value
RI	Republic Indonesia
SD	Standard deviation
SPSS	Statistical package for the social sciences
X2	Chi-square

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ORIGINAL RESEARCH ARTICLE

Relationship between bacteria and nitrogen dynamics in wastewater treatment oxidation ponds

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ABSTRACT

BACKGROUND AND OBJECTIVES: The bacterial community plays a crucial role in the nitrogen cycle. Oxidation ponds act as a natural treatment system for wastewater and are designed to promote the growth and activity of certain bacterial species that remove contaminants from the water. The nitrogen cycle in these ponds involves the conversion of nitrogen compounds through biological processes by bacteria. The presence or absence of certain bacterial species can greatly influence the efficiency of the nitrogen cycle in these ponds. This research investigates the relationship between bacteria and nitrogen dynamics, the key components of wastewater treatment, in oxidation ponds. This work aims to identify the bacterial community composition in oxidation ponds, investigate the role of bacteria in the transformation and removal of nitrogen compounds from wastewater in oxidation ponds, and evaluate the impact of environmental factors on the microbial communities and nitrogen dynamics in oxidation ponds. This study was carried out in the oxidation wastewater treatment at the King's Royally Initiated Laem Phak Bia Environmental Research and Development or LERD Project, in Phetchaburi, Thailand.
METHODS: Wastewater samples were collected from the 1st–5th oxidation ponds at a depth of 30 centimeter from the water surface and analyzed for various quality parameters including temperature, dissolved oxygen, potential of hydrogen, biochemical oxygen demand, nitrates, ammonia, and total kjeldahl nitrogen. Next-generation sequencing by Illumina Miseq was used to examine the 16S ribosomal ribonucleic acid of bacteria in the collected samples. Correlation test was used for statistical analysis.
FINDINGS: The temperature, potential of hydrogen (1st to 5th ponds), and dissolved oxygen (2nd to 5th ponds) in the oxidation ponds were within the standard value. Fifteen bacterial phyla were identified in the five oxidation ponds, with phylum Proteobacteria accounting for the highest population comprising 47.56% of the total bacterial population.
CONCLUSION: Genera Novosphingobium (phylum Proteobacteria), Ammonia-11 (*phylum Verrucomicrobiota*), and *Vicinamibacteraceae* (phylum Acidobacteriota) have the strongest relationships with ammonia, nitrate, and total kjeldahl nitrogen (R^2 = 0.9710, 0.986, 0.8124). The bacterial population is a crucial factor in nitrogen nutrient and water quality. *Novosphingobium* is involved in the removal of ammoniafrom wastewater, *Verrucomicrobiota* act as denitrifiers, and *Vicinamibacteraceae* increases the total kjeldahl nitrogen levels.

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INTRODUCTION

Increasing urbanization and rapid population growth has intensified the generation of waste, including human excretion, e-waste, garbage food, fat oil and wastewater (Bajsa *et al*., 2003). Domestic wastewater is a complex mixture of suspended and dissolved materials with extremely high concentrations of nutrients and organic content, including nitrogen in the form of ammonia (NH_3), nitrates (NO_3^-), and total kjeldahl nitrogen (TKN) (Latrach *et al*., 2015; Vlyssides *et al*., 2002). These nutrients can remarkably affect water quality (Quiros, 2003; Camargo *et al*., 2006). When untreated, wastewater is discharged directly into water bodies, causing contamination and water quality issues (Wedyan *et al*., 2016; Noophan *et al*., 2018; Kumar *et al*., 2020). Excess nitrogen can harm aquatic ecosystems, causing the eutrophication of amphibian systems and the depletion of dissolved oxygen (DO) and leading to the death of fish and other marine organisms (Ahmed *et al*., 2022). In addition, nitrate leaching from soil can contaminate groundwater, which could cause methemoglobinemia in infants under 3 months upon ingestion (Ward *et al*., 2018). Wastewater treatment is essential to maintain the quality of the environment and protect public health. Oxidation ponds are a low-cost and widely used wastewater treatment system that includes five consecutive ponds and involves interactions with sunlight, wind, bacteria, and algae: the first one is for sedimentation (2.43 m in depth with 5 days of retention time), the second to fourth are for oxidation (2.23, 1.93, and 1.64 m in depth with 12, 15, and 15 days of retention time, respectively), and the fifth is for polishing (1.42 m in depth with 16 days of retention time) (Pattamapitoon *et al*., 2013; Jinjaruk *et al*., 2018; Nimtim *et al*., 2020). Biological treatment using aerobic and facultative heterotrophic bacteria is an environmentally friendly alternative method for treating domestic wastewater. This approach involves using oxygen from a thermosiphon process (Noikondee *et al*., 2019) and photosynthesis from *Cyanobacteria,* such as *Cylindrospermopsis* genus (Sukchinda *et al*., 2019), to consume biodegradable soluble organic contaminants, such as sugars, fats, and organic short-chain carbon molecules. This process oxidizes the organic matter and nutrients in the wastewater (Posadas *et al*., 2013; Bestawy *et al*., 2014; Zurano *et al*., 2021). Biological nitrogen removal is preferred over biochemical process because the former can convert nitrogen compounds to nitrogen gas (N_2). Particulate organic nitrogen is hydrolyzed to soluble organic nitrogen by aerobic and facultative heterotrophic bacteria (Sun *et al*., 2010). In particular, nitrifying bacteria play a crucial role in wastewater treatment oxidation ponds by converting ammonia to nitrite and then to nitrate. This process is known as nitrification and is essential for breaking down harmful waste products in the water. Meanwhile, denitrifying bacteria are responsible for converting nitrate to N_2, which is then released into the atmosphere. They help reduce nitrogen levels in the water. Both types of bacteria work together to provide an effective method of wastewater treatment in oxidation ponds. However, the relationship between bacteria and nitrogen dynamics in oxidation ponds is still not fully understood. This study aims to determine the relationship between bacteria and nitrogen dynamics in wastewater treatment oxidation ponds by investigating the microbial communities present in these systems and how they affect the transformation and removal of nitrogen compounds. The objectives are to identify the bacterial community composition in wastewater treatment oxidation ponds, investigate the role of bacteria in the transformation and removal of nitrogen compounds from wastewater in oxidation ponds, and evaluate the impact of environmental factors, such as temperature and DO levels, on the microbial communities and nitrogen dynamics in oxidation ponds. The results will be useful in the development and management of water quality at the LERD project in the future. This study was carried out in the oxidation wastewater treatment at the LERD project, Phetchaburi, Thailand during the dry period in 2022.

MATERIALS AND METHODS

Study area

The study area is the LERD project (Fig. 1), which aims to treat community wastewater from Phetchaburi municipal. The project has five ponds for treating community wastewater, namely, the 1st pond (sedimentation pond), 2nd to 4th ponds (oxidation pond), and 5th pond (polishing pond). The project area is located at latitude 130 02′40′′ to 130 03′20′′ N and longitude 1000 05′10′′ to 100 06′05′′ E, close to the mangrove forest and Phetchaburi coastal area.

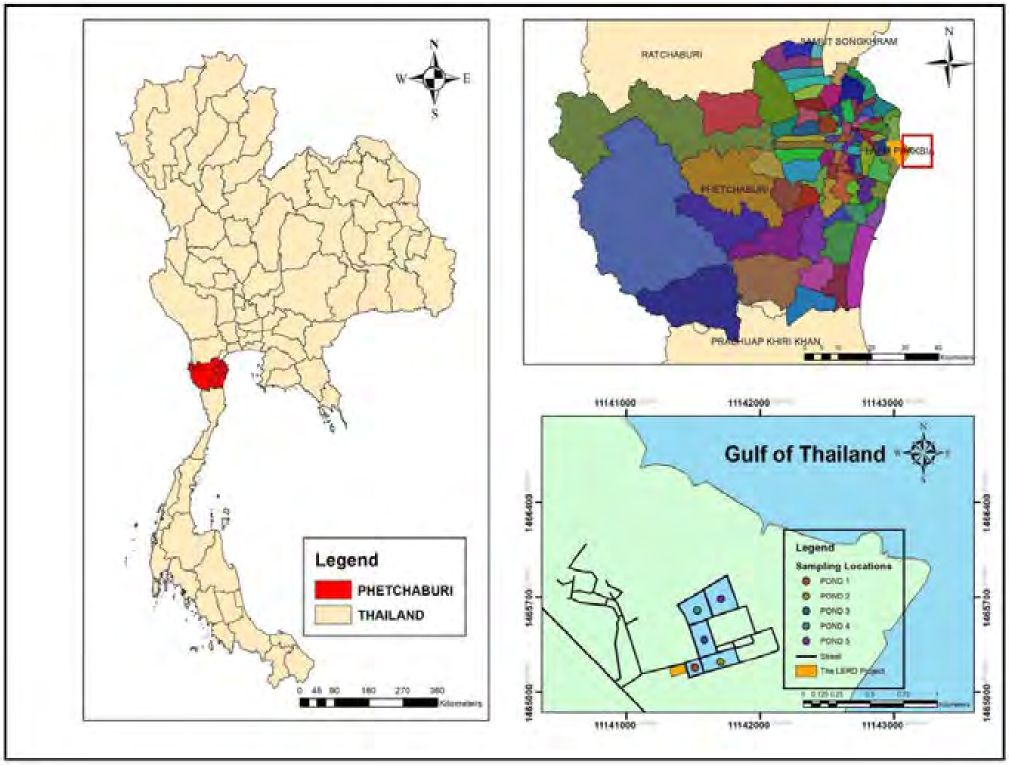

Fig. 1: Geographic location of the study area in the LERD project, Phetchaburi province, Thailand

Wastewater sampling and bacteria analysis

For wastewater collection, 1 liter polyethylene (PE) bottles were used. Samples were collected down to 30 centimeter (cm) from water surface in oxidation ponds (1^{st} to 5^{th} ponds) of the LERD project during the dry period in February 2022 because of the high nutrient concentration throughout this season. Compared with the dry period, the wet period features heavier rainfall and rain flowing into the pond that affect the performance of the wastewater treatment (Makuwa *et al*., 2022). The increased temperature in the dry period increases biological activity, respiration of organisms, and rate of organic matter decomposition (Kamarudin *et al*., 2020). The wastewater samples were kept at 4 °C during transfer to the laboratory and then centrifuged at 8,000 rpm to separate the sludge. The lower sediment was collected in a centrifuge tube for high-efficiency sequencing. For bacterial analysis using next generation sequencing (NGS) technique, deoxyribonucleic acid (DNA) was extracted and sequenced using Illumina Miseq. The generated data were assembled, annotated, and compared with other genomes.

Wastewater quality analysis

On-site measurements of the following three parameters were conducted to determine wastewater quality: temperature, DO, and potential of hydrogen (pH). Four parameters of nutrient concentrations were measured in the laboratory, namely, biochemical oxygen demand (BOD), nitrate (NO_3^-), ammonia (NH_3), and TKN. Nutrient concentrations were measured using the standard methods for the examination of water and wastewater (APHA, 2017).

Statistical analysis

A correlation test was used to describe the relationship between bacterial population and nutrients (NO_3, NH_4^+, and TKN) in an oxidation pond. Coefficients of determination are considered a measure of the degree of linear relationship between two variables. They express the extent to which two variables vary together in the same or opposite direction. They also reveal the magnitude and direction of these relationships. A correlation coefficient can have a value ranging from −1 to 1. Values close to the absolute value of 1 indicate a strong positive linear relationship between the variables, and values close to 0 indicate no linear relationship between the variables (Khambete and Christian, 2014).

RESULTS AND DISCUSSION

Bacterial population diversity

The bacterial populations in the oxidation ponds (1st to 5th ponds) consisted of normal flora bacteria. (Fig. 2) Fifteen bacterial phyla, namely, Proteobacteria, Actinobacteriota, Cyanobacteria, Planctomycetota, Verrucomicrobiota, Firmicutes, Bacteroidota, Chloroflexi, Desulfobacterota, Patescibacteria, Acidobacteriota, SAR324_clade (Marine_group_B), Fusobacteriota, Campilobacterota, and Nitrospinota, were found in the five oxidation ponds. To our knowledge, most studies on heterotrophic nitrification and aerobic denitrification have focused on phyla Proteobacteria, Actinobacteria, and Firmicutes (Huang *et al*., 2013; Srivastava *et al*., 2016; Zhou *et al*., 2017; Wang *et al.,* 2019). Proteobacteria were mainly found in the nitrification and denitrification systems and belonged to denitrifying bacteria; its large amount allowed for the effective removal of nitrate nitrogen (Bruckner *et al*., 2012). This finding is consistent with previous studies, indicating that Proteobacteria are widely spread in wastewater treatment (Wu *et al*., 2019). Verrucomicrobiota are mostly free-living bacteria and can be found in freshwater, marine water, soil, and seawater (Zwart *et al*., 1998).

Proteobacteria have the highest population, accounting for 47.56% of total bacterial population. Its population decreased from the 1st pond to the 5th pond (6.21%). Proteobacteria are one of the most dominant bacterial groups found in wastewater. Table 1 shows that under aerobic conditions, Proteobacteria use oxygen as an electron acceptor in the respiratory chain. As a result, the presence of DO is essential for their survival and growth. However, too high DO concentration can lead to the complete oxidation of organic matter, causing a decrease in the food base for Proteobacteria. This phenomenon can cause a reduction in their population. Meanwhile, the populations of phyla Actinobacteriota, Cyanobacteria, Planctomycetota, Firmicutes, Chloroflexi, Patescibacteria, Acidobacteriota, SAR324_clade (Marine_group_B), and Nitrospinota increased from the 1st pond to the 5th pond (27.74%, 22.43%, 20.12%, 6.54%, 2.53%, 0.83%, 0.91%, 0.65%, and 0.07%, respectively). Under aerobic conditions, bacteria use oxygen as an electron acceptor in the respiratory chain. As a result, the presence of DO is essential for their survival and growth. An increase in DO concentration results in an increase in the abundance of bacteria in wastewater. In addition, the BOD values in Table 1 indicated that the presence of organic matter and nutrients stimulates the growth of bacteria, which break down the organic matter and release carbon dioxide and other byproducts. The amount of BOD in the wastewater is directly related to the amount of organic matter present and hence the rate at which it is being broken down by bacteria.

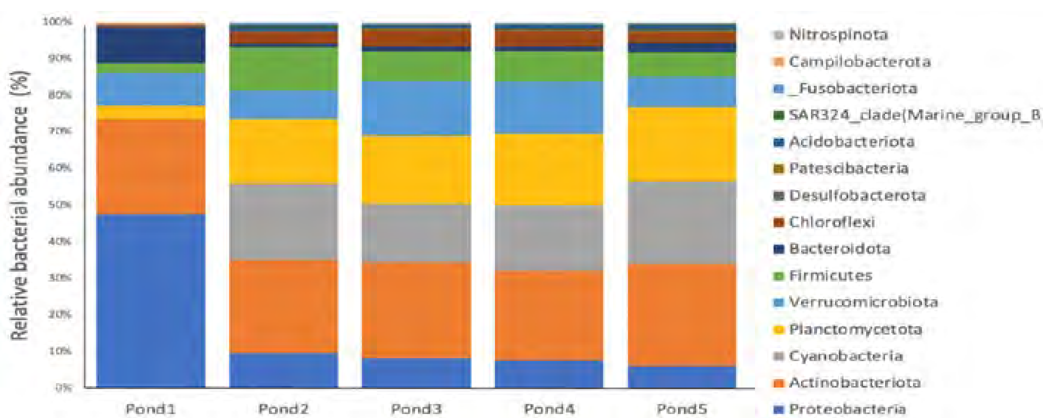

Fig. 2: Bacterial communities (phylum) at the LERD project, Phetchaburi province, Thailand

SAR324_clade is a marine bacterium species that was most commonly found in the 2nd pond (0.71%) because this pond was nearest to a mangrove forest. The population of Cyanobacteria was the highest in the 5th pond (22.43%), causing their rapid bloom. Some Cyanobacteria species can produce toxins (Sukchinda *et al*., 2019; Rastogi *et al*., 2015; Zhang *et al*., 2022; Huang *et al*., 2018; Flanzenbaum *et al*., 2022), leading to fish death during winter season (Srichomphu *et al*., 2015).

Wastewater quality

The temperature and pH of the wastewater were within the standard values (23 °C–32 °C and 5.5–9.0, respectively). The average temperatures in the 1st to 5th ponds were 27.4 °C, 27 °C, 26.7 °C, 26.8 °C, and 26.9 °C, respectively, and their pH values were 8.05, 8.92, 9.13, 9.06, and 9.21, respectively. The average DO levels in the 2nd to 5th ponds met the standard value (>3 mg/L), but that in the 1st pond failed to meet the standard (2.97 mg/L). The highest concentrations of NO_3^- and TKN were observed in the 2nd pond, and the lowest concentrations were found in the 5th pond. The highest concentrations of NH_4^+ were observed in pond 1st. The concentration of NH_4^+ decreased from the 1st pond to the 5th pond due to bacterial decomposition, resulting in a decrease in NH_3 (Table 1). DO levels increased from the 1st pond to the 5th pond due to photosynthesis activity from Cyanobacteria during daytime, resulting in high DO levels. BOD levels decreased because aerobic bacteria use a large amount of oxygen during degradation (Sukchinda *et al*., 2019; Annisa *et al.,* 2021; Boyd, 2018; Irfan, 2016). Nutrient concentrations in oxidation ponds tend to increase because Cyanobacteria can fix nitrogen gas from the atmosphere and convert it into ammonia and nitrate (Abbaszadeh *et al*., 2022; Purwono *et al*., 2017; Mahmud *et al*., 2021).

Bacterial communities and nutrient

Bacterial communities and nitrogen compounds such as NH_3, NO_3^-, and TKN were analyzed to determine their relationship with each other. As shown in Fig. 3, phyla Proteobacteria*,* Planctomycetota, Bacteroidota, Chloroflexi, Desulfobacterota, Patescibacteria, Acidobacteriota, Campilobacterota, and Nitrospinota were highly related to NH_3 in the oxidation ponds with R^2 values of 0.81, 0.84, 0.79, 0.98, 0.82, 0.81, 0.68, 0.79, and 0.75, respectively. Chhimwal *et al*. (2022) investigated the treatment efficiency of domestic wastewater using an oxidation pond and found a decrease in wastewater quality, including nitrate, phosphate, COD, and BOD, after treatment, indicating that the oxidation pond was effective in reducing nutrient concentrations. In addition, they found that the dominant bacterial phylum was Proteobacteria using 16S ribosomal ribonucleic acid (rRNA) gene sequencing. Studies of Hwang *et al*. (2005), Chen *et al*. (2014), Lawson *et al*. (2017), and Suto *et al*. (2017) on heterotrophic bacteria, such as Proteobacteria*,* Chloroflexi, Bacteroidetes, and Acidobacteria, revealed that these bacteria convert NH_3 to N_2 and play a role in adapting to environmental changes (Egli *et al*. 2001; Isaka *et al*. 2007; Jin *et al*. 2012). Nitrification mostly occurs under aerobic and anaerobic conditions. NH_4^+ oxidation can also occur, mediated by a limited number of bacteria within Planctomycetota (Strous *et al*., 1999). This phylum has a unique metabolic ability to oxidize NH_4^+ with NO_2 as electron acceptor to produce N_2 and a small amount of NO_3^- under anoxic conditions without requiring an organic carbon source (Date *et al*., 2008; Kuenen, 2008).

Fig. 4 shows that phyla Verrucomicrobiota, Firmicutes, Chloroflexi, Fusobacteriota Bacteroidota and Nitrospinota had a high correlation with a NO_3^- in the oxidation ponds (R^2 =0.88, 0.84, 0.64, 0.80, 0.73, 0.86). This finding is consistent with a previous study

Table 1: Water quality in oxidation pond at the LERD project during dry period in February 2022

Parameters	Domestic wastewater	Oxidation pond					Wastewater standard
		1st pond	2nd pond	3rd pond	4th pond	5th pond	
Temp. (°C)	28.5	27.4	27	26.7	26.8	26.9	23-32[2]
DO (mg/L)	0.264	2.97	7.45	8.92	7.7	8.31	>3[2]
pH	7.25	8.05	8.92	9.13	9.06	9.21	5.5-9.0[1]
BOD (mg/L)	64.3	21	23.1	21.3	15.8	9	< 20[1]
NO_3^- (mg/L)	0.726	0.549	2.246	0.947	0.763	0.616	-
NH_3 (mg/L)	2.728	1.842	1.553	1.165	0.591	0.282	-
TKN (mg/L)	7.0	4.8	6.2	4.8	4.2	3.9	-

[1] Ministry of Natural Resources and Environment, Thailand.
[2] Department of Fisheries, Thailand.

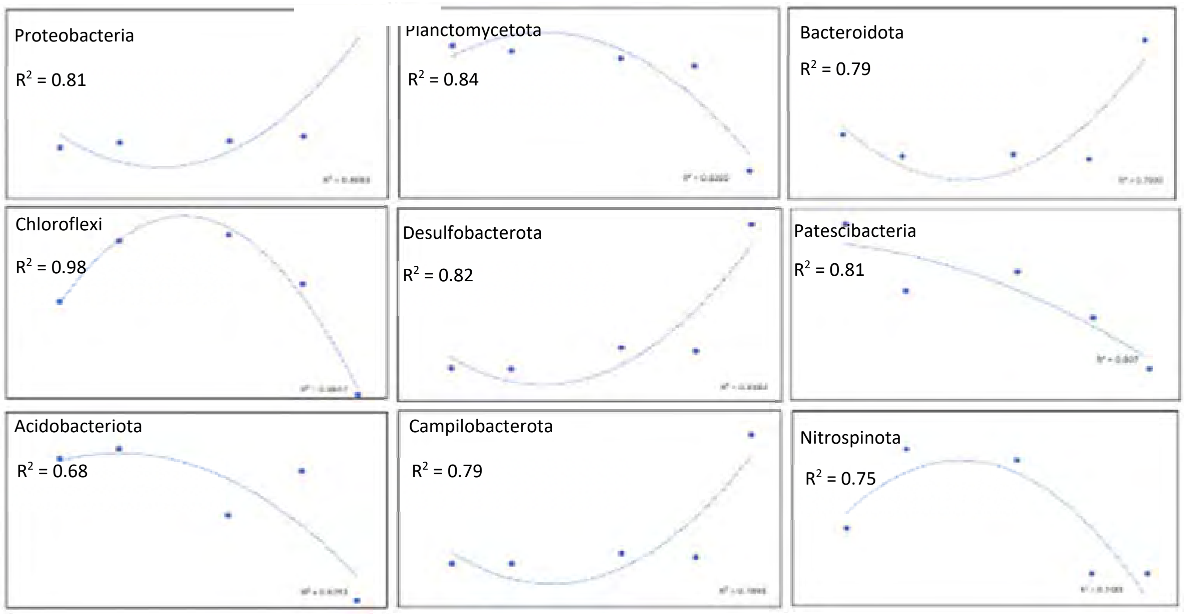

Fig. 3: Correlation between ammonia (NH_3) and bacterial communities (phylum)

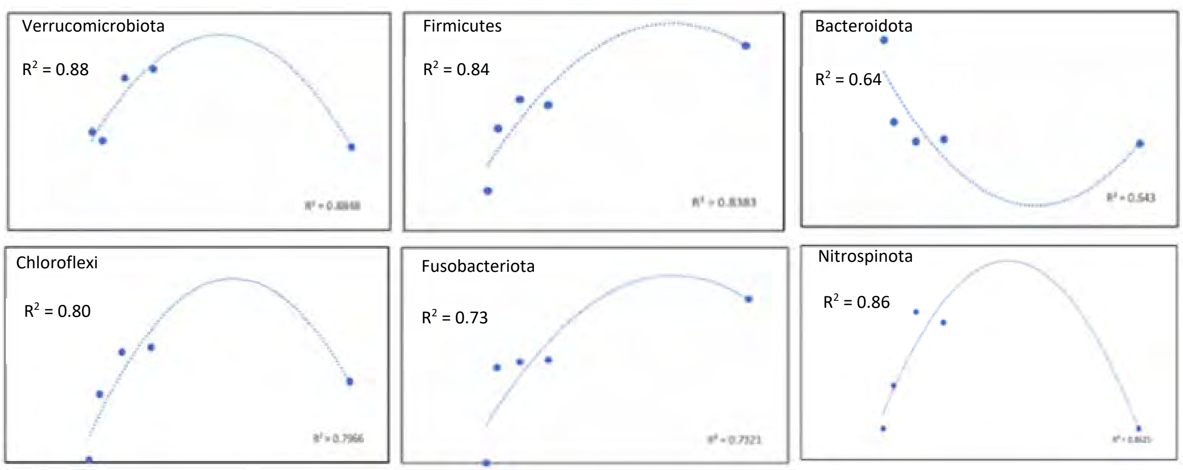

Fig. 4: Correlation between Nitrate (NO_3^-) and bacterial population (phylum)

that reported a positive correlation between NO_3^- and the abundance of Chloroflexi and Bacteroidota (Zhang *et al.*, 2018). However, Verrucomicrobiota do not appear to reduce NO_3^- or other nitrogenous compounds, but rather take up and use NH_3 (Shaomi *et al.*, 2017).

Fig. 5 shows that phyla Cyanobacteria, SAR324_ clade, and Acidobacteriota had a high correlation with TKN in the oxidation ponds (R^2= 0.60, 0.77, 0.63). Chaffin and Bridgeman (2014) studied the utilization of organic and inorganic nitrogen by Cyanobacteria and found that this phylum has preference for NH_3 but can assimilate other forms of nitrogen in the presence of ammonium. These results suggest that Cyanobacterial blooms assimilate multiple forms of nitrogen to support their growth. Genomic information indicated that *SAR324_clade* has a flexible metabolism that includes sulfur oxidation, carbon fixation, hydrocarbon

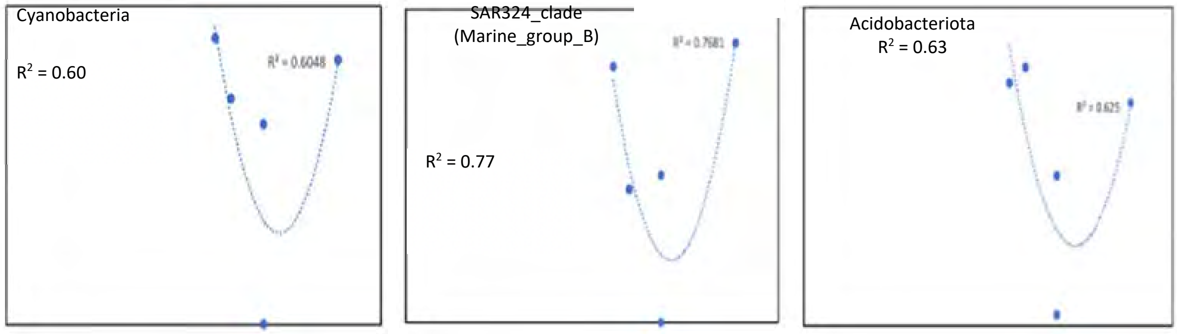

Fig. 5: Correlation between TKN and bacterial population (phylum)

Table 2: Correlation analysis between bacterial communities at the genus level and nutrient (NH_3, NO_3^-, and TKN) in oxidation ponds

Nutrient	Phylum	Genus	R^2
NH_3	Proteobacteria	*Novosphingobium*	0.8944
		Methylocystis	0.8731
		Polynucleobacter	0.8409
		Alsobacter	0.8364
		Methyloparacoccus	0.8253
		C39	0.7837
	Planctomycetota	*Roseimaritima*	0.7863
		CL500-3	0.7120
		Blastopirellula	0.6096
	Bacteroidota	*Fluviicola*	0.8909
		Flavobacterium	0.8056
		Kapabacteriales	0.7843
	Chloroflexi	*JG30-KF-CM45*	0.9551
		1-20	0.8872
	Desulfobacterota	*Desulfomonile*	0.8022
	Patescibacteria	*TM7a*	0.8707
	Acidobacteriota	*Luteitalea*	0.9120
	Campilobacterota	*Sulfurospirillum*	0.7837
	Nitrospinta	*P9X2b3D02*	0.8199
NO_3^-	*Verrucomicrobiota*	*NH3-11*	0.9886
		LD29	0.8992
		Luteolibacter	0.6767
		Akkermansia	0.6141
	Firmicutes	*Romboutsia*	0.9565
		Clostridium_sensu_stricto_1	0.9261
	Chloroflexi	*G30-KF-CM45*	0.8631
		RBG-13-54-9	0.8420
	Fusobacteriota	*Cetobacterium*	0.7321
	Bacteroidota	*Fluviicola*	0.7336
	Nitrospinta	*P9X2b3D02*	0.7789
TKN	*SAR324_clade*	*SAR324_clade*	0.7681
	Acidobacteriota	*Vicinamibacteraceae*	0.8124

utilization, and heterotrophy (Sheik *et al*., 2014).

Table 2 shows the bacterial populations at the genus level. Six genera (*Methylocystis*, *C39*, *Methyloparacoccus, Alsobacter, Novosphingobium*, and *Polynucleobacter*) in phylum Proteobacteria, three genera (*Roseimaritima, Blastopirellula*, and *CL500-3*) in phylum Planctomycetota, three genera (*Flavobacterium, Kapabacteriales*, and *Fluviicola*) in phylum Bacteroidota, two genera (*JG30-KF-CM45* and *1_20*) in phylum Chloroflexi, one genus (*Desulfomonile*) in phylum *Desulfobacterota*, one genus (*TM7a*) in phylum Patescibacteria, one genus (*Luteitalea*) in phylum Acidobacteriota, one genus (*Sulfurospirillum*) in phylum Campilobacterota, and one genus (*P9X2b3D02*) in phylum Nitrospinta had a high correlation with NH_3 in the oxidation ponds. Meanwhile, genera *LD29*, *Akkermansia, Luteolibacter*, and NH3-11 in phylum Verrucomicrobiota, two genera (*Clostridium_sensu_stricto_1* and *Romboutsia*) in phylum Firmicutes, and one genus (*G30-KF-CM45*, *RBG-13-54-9, Cetobacterium, Fluviicola,* and *P9X2b3D02*) each in phylum Chloroflexi, Fusobacteriota, Bacteroidota, and Nitrospinta had a high correlation with NO_3^- in the oxidation ponds. Finally, genera *SAR324_clade* and *Vicinamibacteraceae* in phylum SAR324_clade and Acidobacteriota, respectively, had a high correlation with TKN in the oxidation ponds (Fig. 5).

CONCLUSION

On the basis of the results of this research, oxidation ponds exhibit water quality that meets the standard value. Fifteen different types of bacterial communities were identified in the oxidation ponds. Among these communities, phylum Proteobacteria had the highest population, accounting for 45.7%. Bacterial communities showed a strong relationship with NH_3 in the oxidation ponds, and genus *Novosphingobium* in Proteobacteria phylum showed a significant correlation with NH_3 (R^2 = 0.8944). *Novosphingobium* is a genus of bacteria that is capable of metabolizing a wide range of chemicals, including ammonia. These bacteria can be found in various environments, such as soil, water, and plant surfaces, and they play important roles in nutrient cycling and biodegradation. *Novosphingobium* can use ammonia as a nitrogen source for growth and metabolism. They can convert ammonia into other nitrogen-containing compounds such as amino acids, nucleotides, and proteins. Some strains of *Novosphingobium* are involved in the removal of ammonia from wastewater and other contaminated environments. They can actively take up ammonia from the surrounding environment and transform it into less harmful forms. Overall, *Novosphingobium* is an important player in the nitrogen cycle and has versatile abilities to adapt to different environmental conditions, including in the presence of ammonia. Genus *NH3-11* in phylum Verrucomicrobiota had a high relationship with NO_3^- (R^2 = 0.9886). *Verrucomicrobiota* are a phylum of bacteria that play a role in the nitrogen cycle, including in the conversion of nitrate to other forms of nitrogen. Some species of Verrucomicrobiota are capable of reducing nitrate to nitrite or even further to nitrogen gas. This process is important in the biogeochemical cycling of nitrogen and can have implications for the availability of nutrients in ecosystems. Some studies suggested that Verrucomicrobiota play a role in the treatment of nitrate-polluted wastewater by acting as denitrifiers. Overall, the relationship between Verrucomicrobiota and nitrate is complex and not fully understood. Research suggested that these bacteria may play an important role in nitrogen cycling and environmental remediation. Genus *Vicinamibacteraceae* in phylum Acidobacteriota had a high relationship with a TKN (R^2 = 0.8124) *Vicinamibacteraceae* includes bacterial strains that are capable of degrading complex nitrogen compounds, including proteins and amino acids. As a result, the presence of these bacteria in a sample can impact the levels of TKN, a measurement of the amount of nitrogen in a sample that can be converted into ammonia. In particular, the presence of *Vicinamibacteraceae* can increase TKN levels because they break down nitrogen-containing compounds into ammonia, which contributes to the TKN content. The presence of *Vicinamibacteraceae* can be an important factor to consider when analyzing TKN levels in a particular sample. Therefore, the management of bacterial communities is a key factor for nitrogen removal in oxidation ponds.

AUTHOR CONTRIBUTIONS

S. Saneha designed the experiment, conducted field study, bacteria population and water quality analyses in the laboratory, contributed in the data analysis, interpreted the results, and prepared the manuscript. T. Pattamapitoon, the corresponding author, designed the experiment, contributed in the data analysis, interpreted the results, and examined the manuscript. S. bualert designed the experiment and contributed in the data analysis. O. Phewnil designed the experiment, contributed in the data analysis, and interpreted the results. W. Wararam designed the water quality experiment and contributed in the data analysis. N. Semvimol designed the water quality experiment and contributed in the data analysis. K. Chunkao designed the water quality experiment and interpreted the results. C. Tudsanaton contributed in bacteria population analysis in the laboratory. M. Srichomphu contributed in the water quality analysis in the laboratory. U. Nachaiboon collected wastewater and contributed in the water quality analysis in the field. O. Wongsrikaew contributed in the water quality analysis in the field. P. Wichittrakarn designed the field experiment and collected wastewater in the field. C. Chanthasoon designed the field experiment and collected wastewater in the field.

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CONFLICT OF INTEREST

The author declares no conflict of interests regarding the publication of this manuscript. Ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
1_20	Genus in the Phylum *Bacteroidota*
APHA	American Public Health Association
BOD	Biochemical oxygen demand
C39	Genus in the Phylum Proteobacteria
CL500-3	Genus in the Phylum Proteobacteria
cm	Centimeter
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
et. al.	And others
Fig.	Figure
G30-KF-CM45	Genus in the phylum *Chloroflexi*
JG30-KF-CM45	Genus in the phylum *Bacteroidota*
m	Meter
mg/L	Milligrams per liter
NH_3	Ammonia
NH3-11	Genus in the phylum *Verrucomicrobiota*
NGS	Next generation sequencing
NO_2	Nitrogen dioxide
NO_3^-	Nitrates
PE	Polyethylene
pH	Potential of hydrogen
P9X2b3D02	*Genus* in the phylum *Nitrospinta*
R^2	Coefficient of determination
RBG-13-54-9	Genus in phylum *bacteroidota*
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Rpm	Revolutions per minute
SAR324_clade	Bacteria phylum
TKN	Total Kjeldahl nitrogen
The LERD project	the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Phetchaburi, Thailand
TM7a	Genus in phylum *patescibacteria*
WPCF	Water Pollution Control Federation

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ORIGINAL RESEARCH ARTICLE

Health risk assessment and microplastic pollution in streams through accumulation and interaction by heavy metals

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ABSTRACT

BACKGROUND AND OBJECTIVES: The threat posed by microplastics to humans through fish consumption is potentially great due to microplastics' capacity to adsorb heavy metals. The Code and Gajahwong streams have suffered from plastic and heavy metal pollution as the major rivers in Yogyakarta, Indonesia. However, little is known about the cumulative danger caused by the association of the microplastic and heavy metals. A thorough analysis of the extent of the health risks that people who consume fish from these rivers may experience is urgently needed. Hence, this study aimed to study microplastic pollution accumulated by fish in Code and Gajahwong streams, analyze the interactions with heavy metals, and assess the potential health risks.
METHODS: Fish sample collection was conducted in three stations by considering the severity of plastic pollution. Microplastics were extracted from the gills, digestive tract, muscle, and water and then characterized based on the number, size, shape, color, and type of polymer. Potential health risks were evaluated based on the potential ecological risk index, polymer hazard index, pollution load index, estimated daily intake, target hazard quotient, total target hazard quotient, and target cancer risk.
FINDINGS: Microplastics have contaminated the streams and fish and were dominated by small-sized green fibers and low-density polyethylene polymer. The pollution was related to human activities around the streams. The highest accumulation in fish was found in the digestive organs. Lead and cadmium have been associated with microplastics. The calculation of the potential ecological risk index and polymer hazard index showed that the medium risk of microplastic contamination in both streams. Based on the values of estimated daily intake, target hazard quotient, total target hazard quotient, and target cancer risk, short-term consumption of fish from the streams carries a low risk, but it will increase over time and pose a serious harm in the long term.
CONCLUSION: Given that most of the microplastics found were associated with lead and cadmium, they can increase the risk to human health due to the transfer of microplastics through food chains. Mitigation efforts involving various stakeholders, community involvement, and continuous education must be continuously pursued. This study significantly contributes to the current problem of environmental pollution by means of microplastic threats associated with heavy metals and provides a thorough health risk assessment applicable to other rivers and mitigation efforts that must be exerted to achieve sustainability.

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77	9	6

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INTRODUCTION

The consumption of plastics in modern life is unavoidable. The use of plastics for public needs is considered efficient and practical given that plastics are strong and water resistant but still light and relatively cheap (Andrady and Neal, 2009). A sharp rise in plastic waste has been observed (Jambeck *et al.,* 2015). If plastics are broken down to smaller particles known as microplastics (MPs), with sizes ranging from 0.1 µm to 5 mm, the issues caused by plastic waste can be considerably aggravated (Vriend *et al.,* 2021). Plastic degradation may develop due to water currents, exposure to solar radiation (Frias and Nash, 2019), high temperatures, and mechanical abrasion by sediments (GESAMP, 2016). MP particles composed of polyethylene (PE), low-density PE (LDPE), high-density PE (HDPE), and polypropylene (PP) can adsorb heavy metals (HMs) (Rochman *et al.,* 2014). The capability of MPs to adsorb these HMs may substantially raise potential health risks. Based on previous research, fish accumulate MPs, especially in digestive organs, such as the intestines (Wootton *et al.,* 2021), and respiratory organs, including gills (Adji *et al.,* 2022). MP contamination in fish can be caused by the small size of MPs and their varying shapes and colors, which allow them to be ingested by fish (Browne *et al.,* 2008); fish accumulate MPs unintentionally because they are hard to distinguish from food (Li *et al.,* 2021). Fibers that are shaped like cloth strands have a relatively greater distribution capability than fragments, which have an irregular and rigid shape. Fibers are also often ingested by small fish when eating or hunting (Rebelein *et al*., 2021). Irregularly shaped fragments may have secondary movements that typically slow the vertical settling velocities and cause slower sinking than MPs with other shapes but comparable sizes (Yan *et al.* 2021). Within a specific size range, MPs can be absorbed into the bloodstream. They can be translocated to muscles, which tend to be eaten by other organisms in the food chain cycle (Godoy *et al.,* 2019); as a result, bioaccumulation and biomagnification potentially increase (Tosetto *et al.,* 2017). MPs associated with HMs can cause health problems for organisms, such as a decrease in their immunity to cause infertility (Al Muhdar *et al.,* 2021). For that matter, Code and Gajahwong Streams are classified as major streams in the Special Region of Yogyakarta, Indonesia. Most areas around the flow of the two streams are densely populated with various domestic and industrial activities; thus, the waste from these activities may contaminate stream waters in Code (Widodo et al., 2013) and Gajahwong Streams (Winata and Hartantyo, 2013). The dominance of plastic wastes, such as plastic bottles, plastic bags, wrappers of various daily products, and textile waste, along the Code and Gajahwong Streams is a source of MPs in these waters (Utami *et al*., 2021). According to Widagda *et al.* (2020), Code and Gajahwong Streams experienced severe pollution levels from 2013 to 2019. This finding was confirmed by the discovery of considerable amounts of organic and inorganic wastes, which exacerbated the water quality along the stream basin. On the other hand, people around the two streams catch fish from these water bodies for consumption (Priyambodo, 2010). Research evaluating MP contamination and its relationship to HMs in the streams is still limited. A previous study examined the impact of organic pollutants on soil and water quality (Widagda *et al.,* 2020; Salam *et al.,* 2019) and confirmed the presence of MPs in fish (Sulistyo *et al.,* 2020); a comprehensive assessment is urgently needed to investigate the issue of MP pollution. The research on associations of MPs and HMs in reservoirs has been conducted by Adji *et al.* (2021) and Rahmayanti *et al.* (2022), whereas no study reported streams that cross densely populated areas. This research assessed the impact of MP pollution on fish and water in Code and Gajahwong Streams. The main focuses were to evaluate the interactions between MPs and HMs, particularly lead (Pb) and cadmium (Cd), and determine any possible health risks associated with the consumption of fish contaminated with MPs and HMs. HMs were analyzed because they are prioritized in stream water quality monitoring, according to the national regulations in Indonesia. Pb and Cd are two of the ten chemical pollutants of major public health concern due to their toxicity levels and potential to trigger cancer (WHO, 2023). More specifically, characterization of MPs was carried out to determine their color, type, and size. This study characterized polymer types with Fourier transform infrared spectroscopy (FTIR). Structural analysis and spectra of the metals adsorbed onto the surface of MPs were determined by energy-dispersive scanning electron microscopy (SEM-EDS). The associations of MPs and HMs in water samples and intact fish muscle were then determined by calculating the polymer hazard index (PHI), pollution load index (PLI), potential ecological risk index (PERI), estimated daily intake (EDI), target hazard quotient (THQ), total THQ (TTHQ), and target cancer risk (TR) to assess the risk due to MP contamination in aquatic ecosystems (Adji *et al.,* 2022; Rahmayanti *et al*., 2022). This study hypothesized that MPs have polluted water and fish from Code and Gajahwong Streams. The level of MP pollution is related to the intensity of human activity around the stream's watershed, as shown the studies conducted by Babel *et al*. (2022) and Lin *et al.* (2021), which revealed that human activity is the main factor affecting MP pollution levels. MP accumulation in fish and water is dominated by small-sized MPs, and the highest accumulation of MPs is found in the digestive organs of fish (McNeish *et al*., 2018). HM association occurs due to HM adsorption onto the MP surface, which may increase HM concentrations (Naqash *et al*., 2020). Based on PHI, PLI, and PERI calculations, the risk of MP contamination in Code and Gajahwong Streams is potentially moderate. Based on the EDI, THQ, TTHQ, and TR, fish in Code and Gajahwong Streams are considered safe for consumption within a short period. This study is expected to give comprehensive information regarding MP contamination in fish and waters of the two streams and can be used as a reference to formulate policies in efforts to manage streams and conserve water resources. These study results may also be expected to be a reference in determining the quality standards for MPs and HMs in rivers. Public and community awareness regarding river flow pollution due to littering habit is expected to be enhanced through this research. Increased environmental awareness of the public is also expected to encourage more waste reduction activities in Code and Gajahwong Streams. The current study aimed to evaluate MP accumulation by fish in Code and Gajahwong Streams, analyze MP interactions with HMs (lead and cadmium), and assess the potential health risks. This study was carried out in Code and Gajahwong Streams in 2022.

MATERIALS AND METHODS

Study area

Code and Gajahwong Streams are in the Province of Special Region of Yogyakarta, Indonesia. The streams cross Sleman, Yogyakarta, and Bantul Regencies. The total population has reached more than 4 million people and is predicted to increase significantly in the next several years (CBSPSRY, 2022). In 2022, the volume of waste produced was 1,133.94 tons/day (Bappeda, 2022). Sampling was conducted from March 2022 to June 2022 at six sampling stations (Fig. 1), which were selected based on the population density around the stream basin. Each station was selected based on fishing activity. Stations of Code (C1; 7°39'31.1"S,110°23'48.2"E) and Gajahwong (G1;7°41'34.0"S,110°24'54.4"E) Streams were selected due to the relatively low-density population around them. Thus, the levels of plastic pollution at the following stations were expected to be lower than those of other stations. Stations C2 (7°44'43.6"S,110°22'37.5"E) and G2 (7°46'57.4"S,110°23'48.8"E) were located at the center of Yogyakarta City, with the area around these stations being settlements with dense populations. Based on visual observations, the levels of waste pollution at these stations were relatively higher than those at stations C1 and G1. Stations C3 (7°52'46.8"S,110°23'34.1"E) and G3 (7°52'35.3"S,110°23'45.1"E) are entry points for Code and Gajahwong Streams to Opak Stream. Fishing activities at stations C3 and G3 are relatively high than those at other stations given that the fairly wide riverside and calm water flow are very suitable fishing spots. Stations C3 and G3 are also close to residential areas.

Sample collection

Fish were collected randomly using fishing rods and nets at each station (n = 10). The samples were stored in a 1 L ziplock bag and placed in a cool box filled with ice (Asare *et al.,* 2018). In the laboratory, the samples were kept in a refrigerator at -20 °C until further analysis. The fish were dissected to obtain their gills, muscles, and digestive tract (GIT). The organs were washed with distilled water, dried, and weighed to obtain the wet weight, and they were used to extract MPs. Analysis of Pb and Cd was carried out on the muscles, and for HM analysis, the muscles were dried in an oven at 60 °C until constant weight to obtain the dry weight. Surface water was collected randomly at the same station as fish sampling (n = 3) using a 1 L water sampler and transferred to a 1000 mL glass bottle (Asare *et al.,* 2018; McNeish *et al.,* 2018; Adji *et al.,* 2022). Glass bottles filled with water samples were immediately closed to prevent contamination. Samples were stored in a cooler and brought to the laboratory for sample extraction.

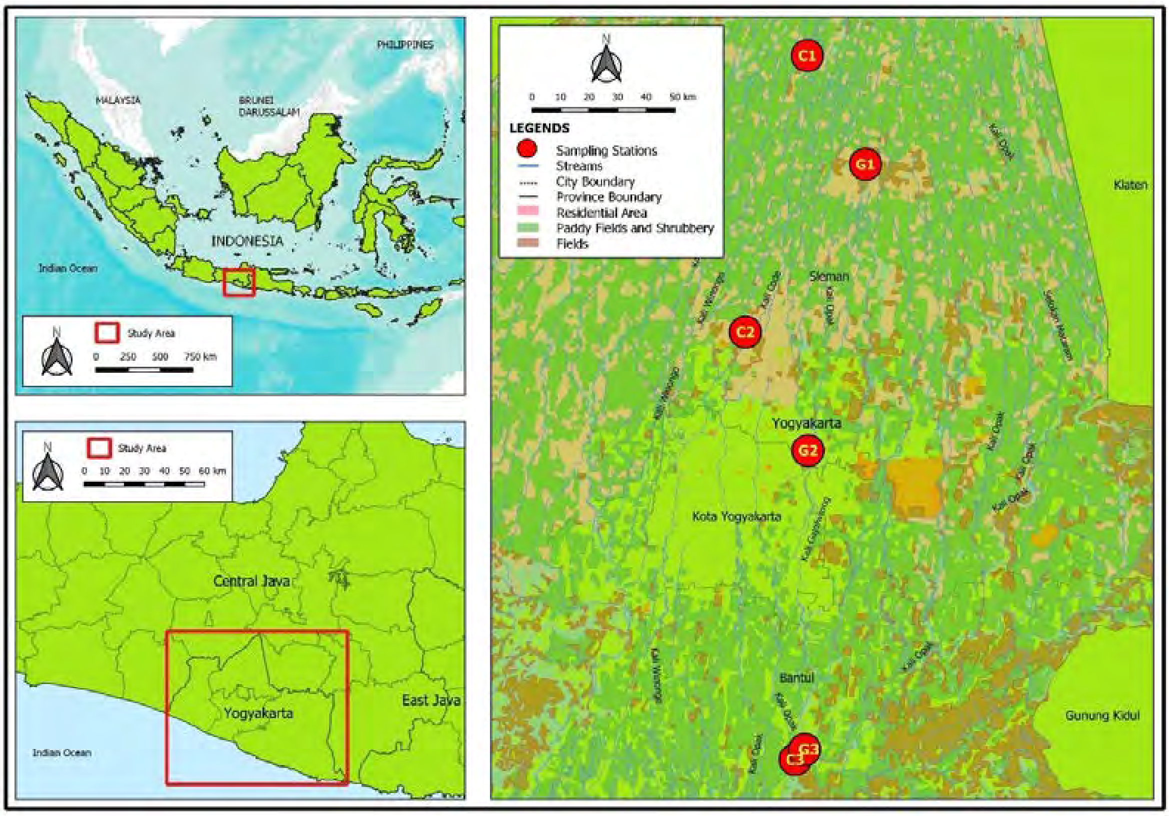


Fig. 1: Geographic location of the study area along with the sampling stations in Code and Gajahwong Streams

Extraction of MPs

The extraction of MPs in fish organs, i.e., the gills, muscles, and GIT, was performed in accordance with the work of Adji *et al.* (2022). The organs were placed in a 50 mL Erlenmeyer flask (Pyrex), and 10% potassium hydroxide (KOH) was added to the flask until the organs were submerged in the KOH solution. Next, the sample was dried in an oven for 24 h at 60 °C. After extraction, the sample was filtered with a 0.45 µm filter paper (Whatman™, UK). Each filter paper was placed in a petri dish and labeled to observe MPs in the sample. Next, water extraction was carried out by filtering with a filter paper. Again, the paper was placed in a petri dish and labeled for MP observation in the sample. During field sampling, laboratory preparation, and analysis of MPs, attention was given to quality control (QC) and quality assurance (QA). The probability of MP contamination during extraction was analyzed using the filter paper of the control (n=10). During field data collection, the sample petri dish was immediately closed and always kept in a closed condition to prevent contamination of MPs from the air. The equipment used for sample preservation was rinsed with distilled water filtered with a 0.45 µm filter paper.

Characterization of MPs

Physical characterization of MP particles was carried out by measuring the length of each particle referring to the work of Adji *et al.* (2022). MPs were classified as small if they were less than 1.5 mm, moderate if the particle size was from 1.5 mm to 3.3 mm, and large if they measured more than 3.3 mm. The color and shape MPs were also observed (Li *et al.,* 2021), i.e., fiber, fragments, films, and pellets. This observation was performed using a microscope (Leica DM 100) and Image Raster 3. The determination of MP polymer was carried out by FTIR analysis (Nicolet iS10). The particles used in FTIR analysis were selected randomly, with adjustment of the number, size, and diversity of particles among samples. SEM-EDS (Jeol-JSM-

Table 1: QC measurements for FAAS

Parameters	Units	Pb	Cd
Blank filters	μg/L	0.006±0.0001	0.006±0.0001
Blank filtrate	μg/L	0.02±0.01	0.01±0.00
Reference values	μg/L	2.00±0.01	1.50±0.03
Measured value	μg/L	1.95±0.1	1.57±0.05
Recovery	%	97.55	105.23

6510LA) analysis was also carried out to determine the surface characteristics of selected MP particles from water and fish muscle samples. This analysis was also conducted to identify the association between MPs and HMs by showing the adsorption of HMs to MPs (Kim *et al.* 2022).

Determination of HMs

Analysis of HMs Pb and Cd in fish muscle was conducted referring to the method of Asare *et al.* (2018). The samples (dry weight: 0.2 g) were mashed with a pestle and mortar and placed in a 50 mL Erlenmeyer flask (Pyrex). Then, 5 mL concentrated H_2SO_4 (Merck) and 10 mL concentrated HNO_3 (Merck) were added, and the mixture was heated on a hotplate at 130 °C for 20 min. Afterward, the sample was filtered with a 0.45 μm filter paper in a 50 mL volumetric flask (Pyrex) and added with bidistilled water up to the mark. HM contents (Pb and Cd) were determined using a flame atomic absorption spectrometer (FAAS). The detection limits for Pb and Cd were 0.5 and 0.1 mg/L, respectively. The HM concentration was expressed as μg/g. HMs adsorbed onto the surface of MPs were analyzed using a weak acid extraction. The MP particles were placed in a 50 mL Erlenmeyer flask, added with 10 mL 10% HNO_3, and left for 2 h at 30 °C. The sample was filtered with a 0.45 μm filter paper in a 50 mL volumetric flask (Pyrex) and added with distilled water up to the mark. To ensure the correctness of measurement procedure, a calibration curve was created using standard solution concentrations (Titrisol®, Germany) and compared it with standard calibration measurements (certificate number: SN.115–028/ILS/IV/2021). Blank solutions were defined to ensure that each analysis meets QA and QC. Table 1 describes the blank solution measurements.

Assessment of health risks

PHI was calculated by referring to the research of Ranjani *et al.* (2021). It was computed using Eq. 1 to determine ecological hazard through the toxicity of MP polymer types (Ranjani *et al.* 2021).

$$\mathrm{PHI} = \Sigma \mathrm{Pn} \times \mathrm{Sn} \tag{1}$$

According to the formula proposed by Meng *et al.* (2023), ratio of MPs abundance to MPs minimum abundance at each sampling point (CFi) is the ratio of MP abundance (Ci) to MP minimum abundance (Coi) at each sampling point.

PLI shows the level of MP pollution in Code and Gajahwong Streams. The mathematical model was used to calculate the PLI value using Eqs. 2 and 3 (Meng *et al.*, 2023).

$$\mathrm{CFi} = \frac{\mathrm{Ci}}{\mathrm{Coi}} \tag{2}$$

$$\mathrm{PLI} = \sqrt[n]{\mathrm{CFi}} \tag{3}$$

The MP CFi describes the quotient between the MP concentration at each station (Ci) and the minimum MP concentration (Coi).

PERI is a parameter for assessing the ecological hazard category caused by MP contamination. In this study, PERI value was calculated through the model proposed by Ranjani *et al.* (2021) in Eqs. 4, 5, and 6.

$$C_f^i = C^i / C_n^i \tag{4}$$

$$T_f^i = \sum_{n=1}^{n} \frac{P_n}{C^i} \times S_n \tag{5}$$

$$E_r^i = T_r^i \times C_f^i \tag{6}$$

Next, calculations were conducted for the EDI of MP and HM samples. The EDI of MP extraction samples was calculated based on a mathematical formula introduced by Barboza *et al.* (2020) (Eq. 7), whereas that of HMs was calculated in accordance with the work of Salam *et al.* (2020) using Eq. 8 for the average body weight (61.4 kg) and consumption rate (130 g/day/individual) (Adji *et al.,* 2022).

EDI MP = MP particles (particles⁄g) × consumption rate (g⁄d⁄individual) (7)

$$\text{EDI HM} = \frac{\text{HM concentration}\left(\frac{\mu g}{g}\right) \times \text{Consumption rate}\left(\frac{g}{d}\right)}{\text{Weight (kg)}} \quad (8)$$

THQ was calculated using Eq. 9 (Salam *et al.* (2020).

$$\text{THQ} = \frac{\text{EF} \times \text{ ED} \times \text{ FIR} \times \text{ C}}{\text{RfD} \times \text{ WAB} \times \text{ TA}} \times 10^{-3} \quad (9)$$

The EF represents the frequency of exposure (156 days/year, assuming three fish meals per week). ED is the exposure period (70 years, with a lifetime of 70 years), and FIR is the level of food consumption (130 g/day/ for Indonesian adults (Firmansyah *et al.,* 2019). C represents the content of HMs in fish muscle (µg/g wet weight), WAB denotes the average weight (NCD-RisC, 2020), and TA indicates the average period of exposure to non-carcinogens (365 days/year×ED). The RfD states the reference dose of each HM, and the RfD values for Cd and Pb are 0.5 and 4.0 /kg/day, respectively (DeForest *et al.,* 2007). Fish is declared dangerous for consumption when the THQ value exceeds 1 (Khan *et al.,* 2008). TTHQ was calculated based on the equation formula proposed by Salam *et al.* (2020) to determine non-carcinogenic health risks due to consumption of fish with more than one type of HM contamination. Eq. 10 was used for the calculation (Salam *et al.* 2020).

$$\text{TTHQ} = \text{THQ}_{\text{Cd}} + \text{THQ}_{\text{Pb}} \quad (10)$$

TR was calculated using the formula described by Salam *et al.* (2020). Carcinogenic cancer risk is determined to assess the possibility of cancer caused by certain carcinogens in a certain period. The TR was calculated using Eqs. 11 and 12 (Salam *et al.* 2020).

$$\text{TR} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{C} \times \text{CSF}}{\text{WAB} \times \text{TA}} \text{X } 10^{-3} \quad (11)$$

$$\Sigma\, \text{TR} = \text{TR}_{\text{Pb}} + \text{TR}_{\text{Cd}} \quad (12)$$

Data analyses

Principal component analysis (PCA) was performed using XLStat Premium by Lumivero to evaluate the relationship between MP concentrations, fish species, and sampling stations.

RESULTS AND DISCUSSION

MPs in water

Analysis of MPs in the water samples showed that MPs have contaminated the waters of Gajahwong and Code Streams at all sampling stations (Fig. 2).

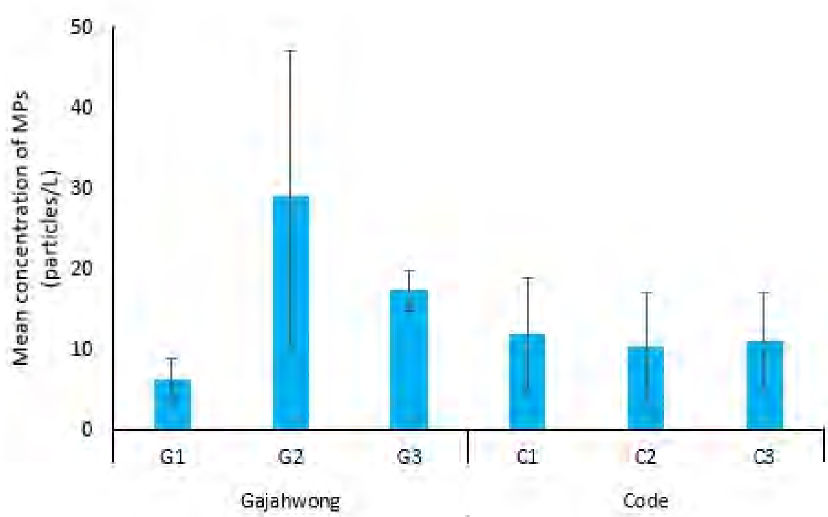


Fig. 2: Concentration of MPs in the waters of Gajahwong and Code Streams

The highest MP contamination was observed at stations G2 (Gajahwong Stream) and G3, with MP concentrations reaching 29 ± 18.19 and 17.3 ± 2.51 particles/L, respectively. The highest concentration observed at station G2 may be due to its location, that is, around residential areas and fishing activities. This notion was supported by the map of sampling stations (Fig. 1), which demonstrated that station G2 is near downtown Yogyakarta, which is a densely populated area. The station receives plastic waste from resident activities. After station G2, a floodgate can inhibit the distribution of MPs. Station G3, which is the entry point from Gajahwong Stream to Opak Stream and is relatively wider compared with other stations, may receive more materials from upstream. The station is also a fishing spot for the local community. The mean concentrations of MPs in Code Stream water significantly differed between the three stations (C1, C2, and C3), with values of 12 ± 7, 10.33 ± 6.65, and 11 ± 6.08 particles/L, respectively. Stream water with the lowest average concentration of MP contamination was found at G1 with a MP concentration of 6.33 ± 2.51 particles/L (Palupi, 2022).

The concentrations of MPs in the waters of Gajahwong and Code Streams were relatively lower compared with other streams, such as Surabaya River (Lestari *et al.,* 2021), Citarum River (Ali *et al.,* 2021), and three other streams in Southeast Asia (Babel *et al*., 2022). However, the values are still relatively higher than those of the inlet and outlet networks of Rawa Jombor Reservoir (Rahmayanti *et al*., 2022), several streams in Chicago, United States (McCormick *et al.,* 2016), and Ottawa Stream in Canada (Vermaire *et al.,* 2017). These studies were conducted in urban areas. Still, several factors can cause heterogeneity in the concentration of MPs that contaminate streams; such factors include population density (Mani *et al.,* 2015), human activities around stream basins (Kataoka *et al.,* 2019), the presence of dams (Watkins *et al.,* 2019), and wastewater treatment systems (McCormick *et al.,* 2016). The study's results regarding the concentration of MPs in Code and Gajahwong Streams (Fig. 2) showed a fluctuating trend, one of the reasons being the difference in population density around the stations. According to the Central Bureau of Statistics for the Special Region of Yogyakarta (2022), in two years (2020–2022), the population has increased to more than 100,000 residents. The increased population density of Yogyakarta has added to the intensity of human activities around the two streams, such as household activities, fishing (Sulistyo *et al.,* 2020), and public facilities in tourist areas (Zaman *et al.,* 2021). In this study, fibers were the dominant form of MPs contaminating Gajahwong and Code Streams (Fig. 3). The MPs found at stations G2 and three stations of Code Stream were 100% fiber, whereas at stations G1 and G3, the percentage of fragments accounted

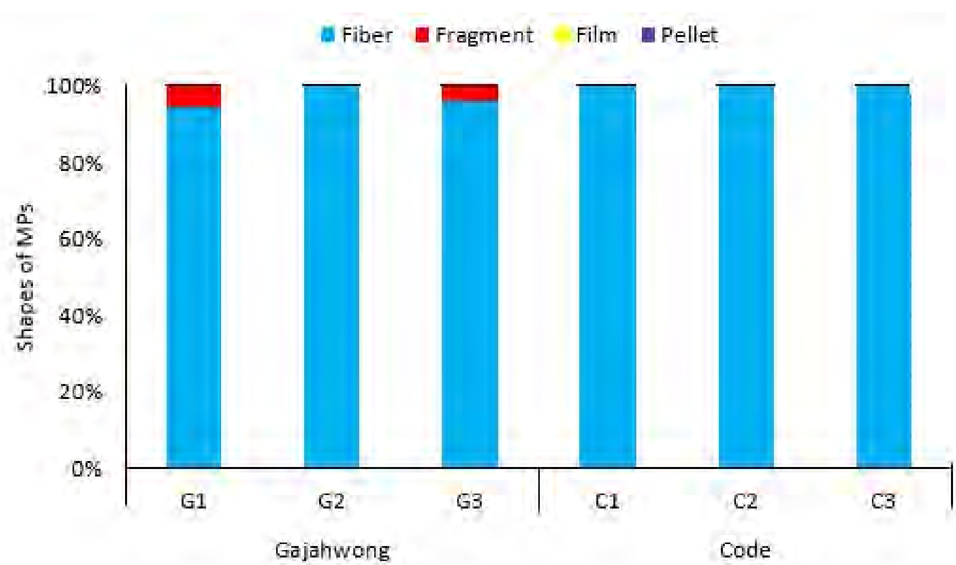


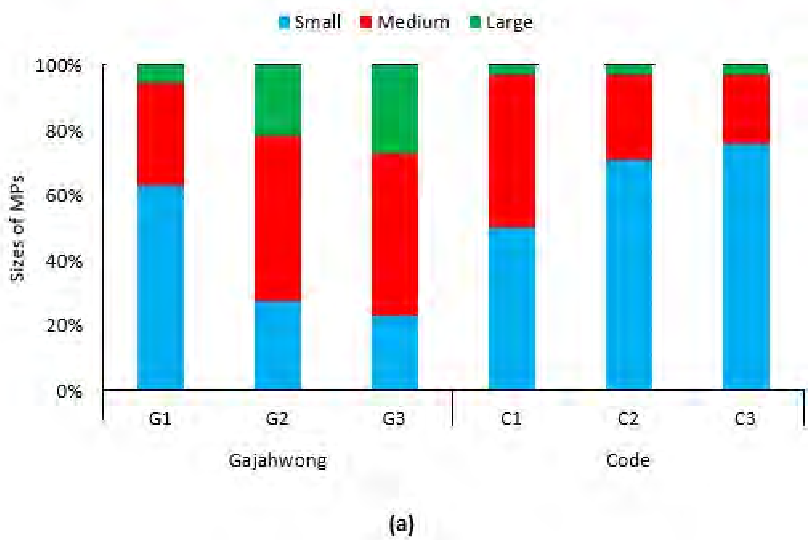
Fig. 3: Shape of MPs found in the waters of the three stations of Gajahwong and Code Streams

for approximately 5%. Similar results were reported by Su *et al*. (2019), who observed that MP fiber dominated the stream waters. Fiber contamination can be caused by the high intensity of human activities, which can produce wastes in the form of fiber; these activities include fishing with fishing rods or fishing nets (Basri *et al.,* 2021), washing clothes (Yang *et al.,* 2021), and waste production from household clothing or the textile industry (Alam *et al.,* 2019). Different results have been reported for Code and Gajahwong Streams (Utami *et al*., 2021) and three other streams in Southeast Asia (Babel *et al*., 2022), with findings indicating that fragments can also dominate the streams. According to Clere *et al.* (2022), fibers are relatively more flexible than fragments; thus, the former are more easily carried away by water currents and more difficult to get stuck or deposited. Rahmayanti *et al*. (2022) also reported different results; film-shaped MPs dominated the waters compared with fibers and fragments. This finding proves that the type of MP is greatly influenced by the type of waste that pollutes waters.

The constituent polymers also influence the distribution of MPs given that each polymer has a different density; thus, the type of polymer affects the buoyancy of MP particles (Wu *et al.,* 2018). Polymers, such as PE and PP, have a relatively low density compared with other polymers, i.e., 0.91–0.96 gram per cubic centimeters (g/cm^3) for PE and 0.90 g/cm^3 for PP (Andrady, 2017); therefore, both polymers float easily and are carried away by water. The results of FTIR analysis showed that the MP particles in the water samples from the two streams were dominated by LDPE, a copolymer of PE. About 5.26% and 3.84% of the particles at stations G1 and G3 were fragments with LDPE polymer, respectively. Most of the plastic waste found in both study areas were bottles, plastic bags, and other product packaging. According to GESAMP (2015), PE and PP are primary raw materials for product packaging; given their relatively short shelf life, they will be disposed of as waste relatively quickly. Bordos *et al.* (2019), Liu *et al.* (2021), and Garcés-Ordóñez *et al.* (2022) also showed the dominance of PE and PP in MP contamination in various aquatic ecosystems. MPs are formed as a result of plastic degradation physically, chemically, or biologically (Andrady, 2017; GESAMP, 2015); therefore, various factors, such as the duration of MPs carried by stream currents, the distance traveled by MPs, and intensity of ultraviolet radiation affect, the size of MP particles (GESAMP, 2016). In this study, the percentage size of MPs varied among stations (Fig. 4). Stations C2 and C3 were dominated by small MPs (<0.5 mm), with percentages reaching 70.97% and 75.76%, respectively (Palupi, 2022), whereas in Gajahwong Stream, small MPs dominated station G1 with the percentage reaching 63.16%. Different results were shown at stations G2 and G3; medium-sized MPs dominated the two stations at 50.57% and 50%, respectively. The results of this study regarding the percentage size of MPs (Fig. 4a) showed a different trend between Gajahwong and Code Streams. This trend can determine which station plastic waste enters the stream. The dominance of small MPs that pollute the waters was also reported by He *et al*. (2021). The high percentage of small-sized MPs indicated that streams carried plastic waste from long distances or for a long time given the long period required to degrade plastics into small sizes. As shown in Fig. 4a, the activity of garbage disposal by residents may be relatively high at stations G2 and G3, with the percentages of prominent MPs at these stations reaching 21.84% and 26.92%, respectively, which were considerably higher than those of other stations, in which large MPs accounted for less than 6%. In this study, MP particles that contaminated stream water had various colors, i.e., green, black, red, and blue (Fig. 4b). Green color dominated the MPs found in all stations (70%–90%).

MPs in fish

MP contamination was found not only in the water but also in six species of fish found in the two streams (Fig. 5). Small particles dominated MP contamination in the organs of each species (<1.5 mm); with this size, MPs can easily contaminate fish being eaten by other fish and enter the filtration process in gills (Jabeen *et al.,* 2017). The average concentrations of MPs in the GIT were relatively higher compared with those in gills and muscles. The dominance of green MPs (Fig. 4b) in stream waters can lead to an increase in MP contamination in the digestive organs of fish given the similarity of green MPs, especially fiber MPs, to microalgae or phytoplankton. The green color causes difficulty for herbivorous fish to distinguish between microalgae and MPs. The color of MP particles also affects the fish's ability to select their food. Predatory fish species that rely on their visual abilities to find



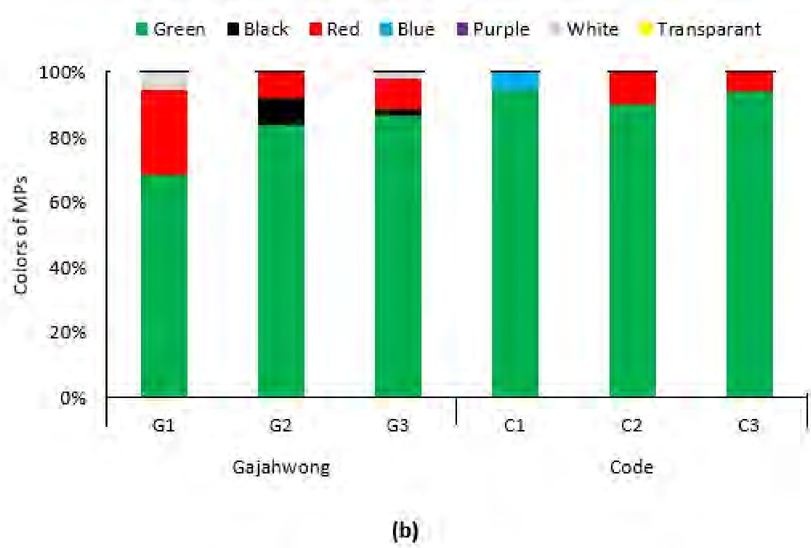
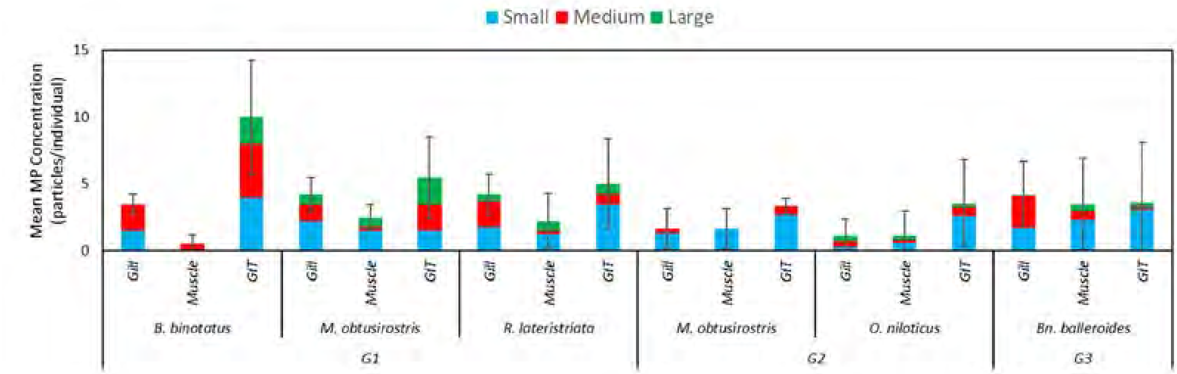


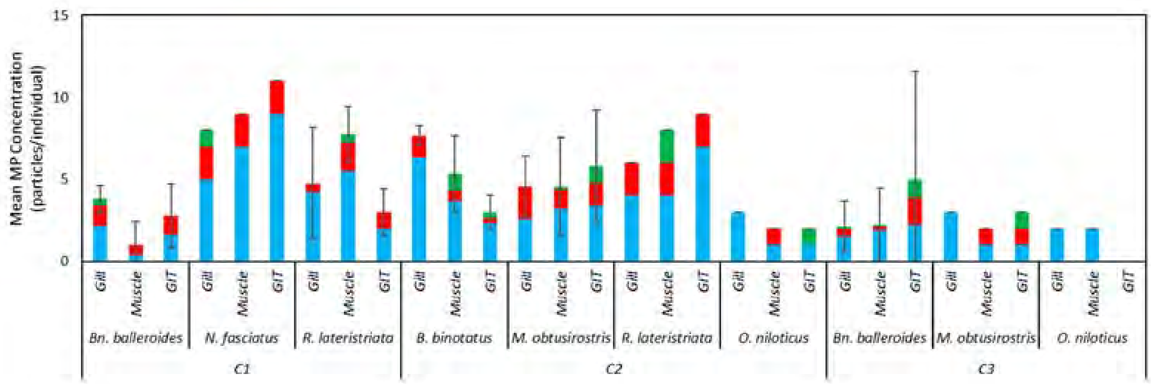
Fig. 4: (a) Size and (b) color of MPs found in waters at the three stations of Gajahwong and Code Streams

prey can be confused by MP particles that have a color resembling that of their prey (de Sa *et al.*, 2015). MP contamination in muscles is the result of absorption of MPs through the blood; thus, the MP particles can be spread in the muscles (Aryani *et al.*, 2021); MPs also contaminate gills, which are the respiratory organs of fish, and enter through these sites incidentally when fish swim; MP particles can contaminate fish muscles if they are absorbed into the blood vessels of fish, either through the digestive or respiratory tract (Su *et al.*, 2019; Jaafar *et al.*, 2021; Makhdoumi *et al.*, 2021; Adji *et al.*, 2022).

Barbodes binotatus, which lives in Gajahwong Stream, had the highest level of MP accumulation compared with other species. In Code Stream, the highest accumulation of MPs was found in *Nemacheilus fasciatus, Rasbora lateristriata,* and *Barbodes binotatus* (Palupi, 2022). *Barbodes binotatus* (Situmorang *et al.*, 2013), *Rasbora lateristriata* (Djumanto and Setyawan, 2009), and *Nemacheilus fasciatus* (Elinah *et al.*, 2016) are omnivores and feed on the same type of food. In this study, other species,



(a)



(b)

Fig. 5: Concentrations of MPs found in fish at the three stations of (a) Gajahwong and (b) Code Streams based on particle size.

namely, *Barbonymus balleroides*, *Mystacoleucus obtusirostris*, and *Oreochromis niloticus*, were also contaminated by MP particles but at relatively lower values than the three previous species. *M. obtusirostris* is an omnivore but shows carnivorous tendencies given its preference for animals, such as worms, zoobenthos, or insects, compared with algae or aquatic plants (Djumanto *et al.,* 2014). *M. obtusirostris* is less attracted to green MP particles. This species can still be contaminated with MPs if it preys on other contaminated fish. *B. balleroides* and *O. niloticus* are omnivores but tend to become herbivores (Temesgen *et al.,* 2022); thus, the chance of contamination between fish species is relatively lower than that between other species. Wu *et al.* (2021) confirmed that herbivorous fish species more easily consume MPs that resemble phytoplankton or plant litter; thus, the concentrations of MPs in herbivorous fish species can increase. According to Mizraji *et al.* (2017), omnivorous fish species contain more MP than herbivorous or carnivorous fish species. This finding is caused by a wider food source for omnivorous species, such as zooplankton and benthos organisms contaminated with MPs, which can potentially cause bioaccumulation in fish (Adji *et al.,* 2022). In this study, fiber was the most common type of MP found. The most dominant MP colors were green and black (Fig. 6a and 6b, respectively). Transparent MP fragment types were also found in this study (Fig. 6c).

In this study, green, black, and red fibers were the most abundant MP particles detected in fish bodies (Fig. 7). The dominance of green MP particles in the fish body can indicate the tendency of fish to eat microalgae. The black and red MPs can confirm that several fish species were omnivores and mistook MPs as prey.

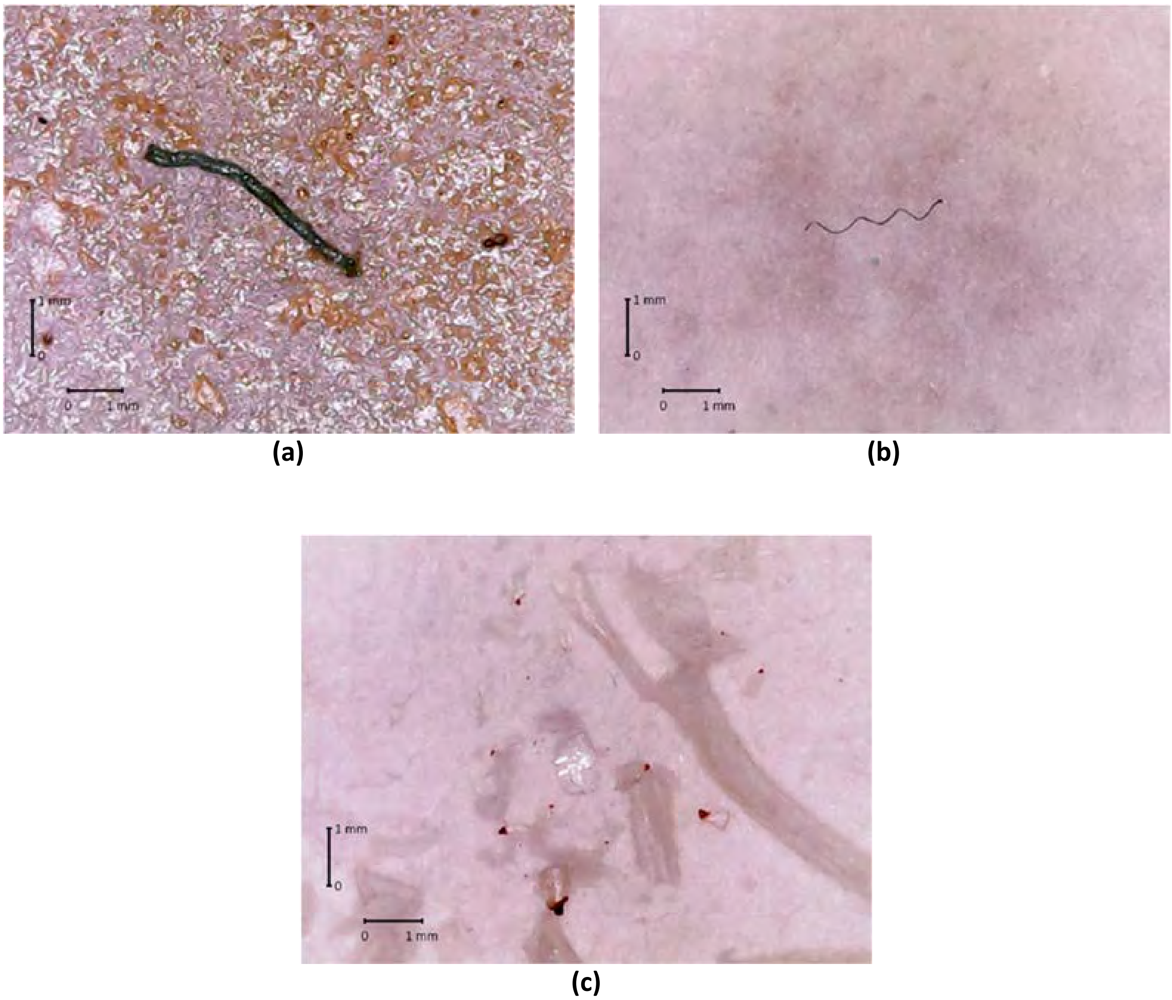


Fig. 6: MP particles in the form of (a) green fibers, (b) black fibers, and (c) transparent fragments observed during the study

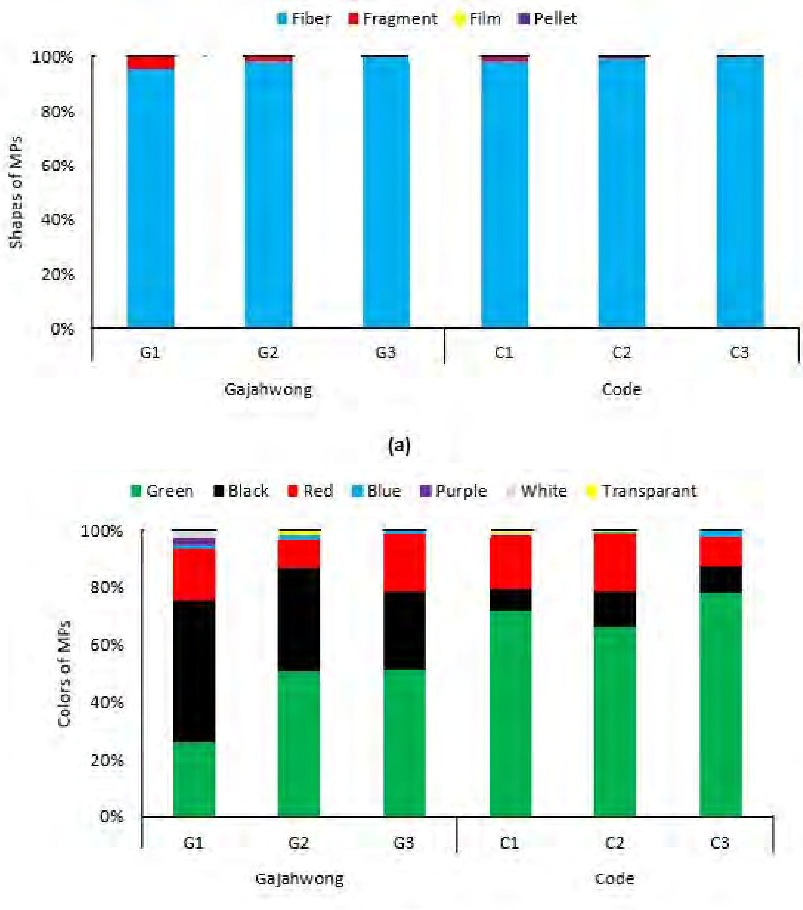
Principal component analysis

PCA showed that components F1, F2, and F3 produced eigenvalues of more than 1 (Fig. 8). In addition, F4 and F5 had eigenvalues of 0.58 and 0.35, respectively (Fig. 8a). The biplot construction in the following study used components F1 and F2 given that the eigenvalues of the two components indicate a significance between data.

The results of biplot construction in the following study showed a variation of 81.49% in the research data (Fig. 8b). In the biplot, stations G1, G2, and C2 were in positive positions from the F1 axis, which indicates the high abundance of MPs in these stations. By contrast, stations G3, C1, and C3 were in the opposite position, which implied their low MP abundance. This finding showed that the concentration of MPs found in water samples was relatively lower than those that contaminated most of the fish samples. *B. binotatus, M. obtusirostris,* and *O. niloticus* were associated to stations G1 and C2, which indicated that these species are common at both stations.

Determination of HMs in water and fish muscles and their association with MPs

Pb and Cd can be detected in water samples, MPs in water, fish muscle, and MPs contaminating fish muscle (Table 2). IGR (2021) stated that the threshold



(a)

(b)

Fig. 7: (a) Shape and (b) color of MP particles contaminating fish

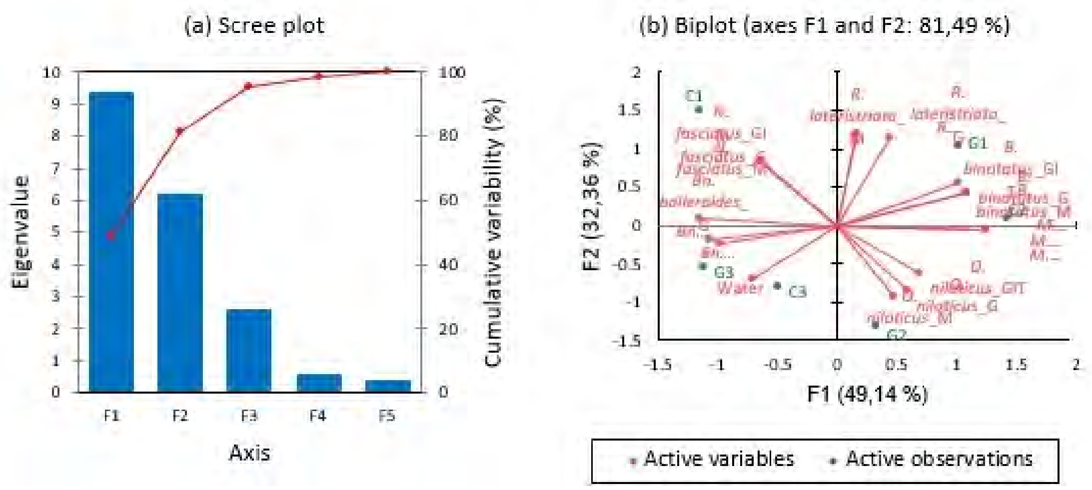


Fig. 8: (a) Scree plot and (b) biplot result of PCA of MP contamination in water and fish organs from Gajahwong and Code Streams

values for Pb and Cd in waters are 0.03 and 0.01 mg/L, respectively. In accordance with the regulation, the concentrations of HMs in the water of the two streams exceeded the threshold values. The broadest range of Pb concentrations in Gajahwong Stream waters was found at station G1 (0.25–0.40 mg/L), whereas the highest concentration of Cd was observed at station G2. In Code Stream, station C3 was the stream waters with the highest contaminations of Pb and Cd (Palupi, 2022).

The difference in the contaminations of Pb and Cd from the two streams can be caused by differences in sources of Pb and Cd. Based on the locations of streams that cross densely populated areas (Fig. 1) and field observations during sample collection, the activities and habits of the surrounding community have the potential to increase the concentrations of Pb and Cd as stream pollutants. Several activities, such as smoking, throwing electrical waste into rivers, and disposal of steel waste, are sources of Cd contamination, whereas others, such as the use of leaded fuel and leaded pipes and disposal of paint waste, also increase Pb levels in streams. HM contamination in streams or other aquatic environments that exceeds threshold values or quality standards will have direct and indirect impact on aquatic organisms (Velusamy *et al.*, 2014). HMs were also detected in MP samples from water. In Gajahwong Stream, the waters at G2 and G3 stations showed MP adsorption for Pb and Cd, respectively, whereas in Code Stream, station C3 showed adsorption for both metals (Palupi, 2022). The metals also accumulated in fish muscles, from which MPs were extracted (Table 2). *O. niloticus* in Gajahwong Stream was contaminated with Pb and Cd. Meanwhile, in Code Stream, Pb contamination in fish muscle was detected in *O. niloticus*, whereas *B. balleroides* exhibited the highest Cd contamination (Palupi, 2022). The concentration of HMs associated with the MP surface was higher than that in water samples. The results also confirmed that the range of concentrations of the two metals adsorbed by MPs in Gajahwong and Code Stream waters was higher than that of HMs in the water samples. Based on research conducted by Naqash *et al.* (2020), concentrations of HMs increased when they were adsorbed with MP particles. The effects caused by HM contamination associated with MPs are more complex. SEM-EDS observations were carried out to confirm the adsorption of HMs onto the surface of MP particles (Fig. 9). Based on the analysis, Pb and Cd were adsorbed onto the surface of MPs. The blue color

Table 2: Concentrations of Pb and Cd in water, MPs in water, fish muscle, and MPs in the muscle of fish species in Gajahwong and Code Streams

Sample		Gajahwong (G)		Code (C)	
		Pb	Cd	Pb	Cd
HMs in water (mg/L)					
Station 1		0.25–0.40	0.02–0.04	0.01–0.03	0.03–0.05
Station 2		0.27–0.31	0.03–0.05	0.03–0.05	0.03–0.04
Station 3		0.27–0.34	0.00–0.01	0.01–0.08	0.01–0.05
HMs associated with MPs in water (μg/g)					
Station 1		0.57–0.76	0.09–0.11	0.16–0.31	0.03–0.07
Station 2		0.59–1.65	0.09–0.12	0.15–0.18	0.02–0.06
Station 3		0.46–0.82	0.08–0.16	0.17–0.28	0.02–0.66
HMs in fish muscle (μg/g)					
1.	*Rasbora lateristriata*	5.10–6.61	1.18–1.55	6.13–6.50	1.09–1.60
2.	*Mystacoleucus obtusirostris*	5.08–7.32	1.18–1.87	5.79–6.91	1.35–1.48
3.	*Barbodes binotatus*	6.52–6.83	1.22–2.2	6.00	1.09
4.	*Oreochromis niloticus*	5.23–8.04	1.11–2.37	6.27–10.75	1.29–1.37
5.	*Barbonymus balleroides*	5.70–6.94	0.99–2.36	5.06–6.69	1.19–1.83
HMs associated with MPs in fish muscle (μg/g)					
1.	*Rasbora lateristriata*	0.02	0.05	0.09–0.68	0.07–0.09
2.	*Mystacoleucus obtusirostris*	0.06–0.25	0.03–0.05	0.11–0.67	0.08–0.13
3.	*Barbodes binotatus*	0.07	0.06	0.18–0.55	0.10–0.15
4.	*Oreochromis niloticus*	0.09	0.03	0.66	0.07
5.	*Barbonymus balleroides*	0.42–0.58	0.12	0.45–0.72	0.08–0.13
6.	*Nemacheilus fasciatus*	N/A	N/A	0.06	0.07

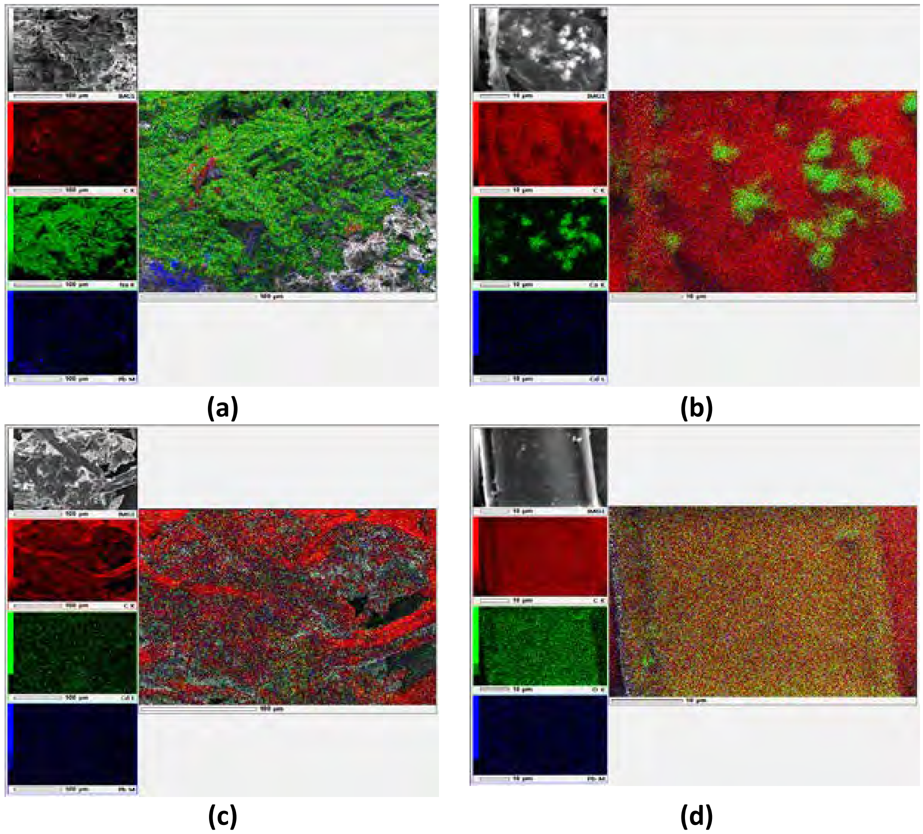


Fig. 9: SEM-EDS of MP samples in (a) Code Stream's water, (b) muscles of fish from Code Stream, (c) Gajahwong Stream's water; (d) muscles of fish from Gajahwong Stream

denotes the adsorption of Pb on MP samples in Code Stream water (Fig. 9a) (Palupi, 2022). Meanwhile, Cd accumulation onto the surface of MP in fish muscles was also marked in blue (Fig. 9b). The adsorption of Pb in the MP samples from Gajahwong Stream water was indicated by a blue color. By comparison, Cd was marked in green (Fig. 9c). Meanwhile, the accumulation of Pb onto the surface of MP in muscles of fish from Gajahwong Stream was evidenced by a blue color (Fig. 9d).

Assessment of health risks

The dominance of PE as a polymer composing MP particles has led to the two streams having level II and I hazard based on the PHI and PLI, respectively, whereas based on the PERI, Gajahwong and Code Streams had a medium level of risk (Table 3). The level of hazard and risk at the six stations was based on the presence of LDPE. Thus, the existence of other polymers may significantly affect the results of these calculations.

The highest PHI was observed at station G2 and all stations in Code Stream, where all stations had level II hazard. The highest PLI was detected at station G2. Meanwhile, the highest PERI was recorded at station G2, and all stations in Code Stream had the same value, i.e., 173.77 (Palupi, 2022). The six research stations had a medium-category risk level. However, this level did not rule out the possibility of high MP contamination in the area. The fish species sampled at the station were classified as small fish. This finding increases the potential threat because small fish have been contaminated at medium levels. Through food chains, fish consumption may also lead to biomagnification. Biomagnification incidents are possible and may have dangerous and complex consequences. As a carrier of HMs, biomagnification of MP contamination may lead to malnutrition

Table 3: PHI, PLI, and PERI and their hazard and risk levels at six stations

Stations	PHI	Hazard level	PLI	Hazard level	PERI	Risk categories
G1	8.61	II	3.56	I	165.62	Medium
G2	9.09	II	7.62	I	173.77	Medium
G3	8.74	II	5.89	I	167.09	Medium
C1	9.09	II	4.90	I	173.77	Medium
C2	9.09	II	4.55	I	173.77	Medium
C3	9.09	II	4.69	I	173.77	Medium

Table 4: MP and HM EDI in Code and Gajahwong Streams

Stream	Species	MPs (particles/day/individual)	HM on fish muscle (µg×kg/day)		HM on muscle's MPs (µg×kg/day)	
			Pb	Cd	Pb	Cd
Gajahwong	*Barbodes binotatus*	190.98	14.13	3.62	0.15	0.13
	Barbonymus balleroides	46.77	13.57	3.00	1.06	0.25
	Mystacoleucus obtusirostris	116.98	13.53	2.88	0.30	0.08
	Oreochromis niloticus	73.55	13.54	3.05	0.19	0.06
	Rasbora lateristriata	409.59	12.06	2.86	0.04	0.11
Code	*Barbodes binotatus*	125.55	12.70	2.31	0.77	0.26
	Barbonymus balleroides	100.51	12.63	2.93	1.24	0.20
	Mystacoleucus obtusirostris	90.82	13.57	3.03	0.89	0.23
	Nemacheilus fasciatus	2800.00	0.00	0.00	0.13	0.15
	Oreochromis niloticus	31.57	18.02	2.82	1.40	0.15
	Rasbora lateristriata	774.89	13.39	2.79	0.83	0.17

(Roman *et al.*, 2020), increased oxidative stress, behavioral abnormalities (Naqash *et al.*, 2020), neurotoxicity, decreased enzymatic activity (Barboza *et al.*, 2020), and cancer (Cox *et al.*, 2019).

The daily human intake (EDI) in Code and Gajahwong Streams varied depending on the species (Table 4). The highest EDI in MP samples of fish in Gajahwong Stream was observed in *R. lateristraiata*. Meanwhile, *N. fasciatus* showed the highest EDI in Code Stream (Palupi, 2022). *B. binotatus* had the highest HM contamination of Pb and Cd in the muscle of fish sampled in Gajahwong Stream, whereas *B. balleroides* exhibited the highest HM contamination associated with MPs (Palupi, 2022). In Code Stream, *O. niloticus* had the highest Pb content in fish muscle, whereas Cd presented the highest muscle content in *M. obtusirostris.* For the association of HMs with MPs, the highest concentration of Pb was observed in *O. niloticus*, and that for Cd was noted in *B. binotatus* (Palupi, 2022). These results were higher than those of seafood consumed by individuals in America (106–126 particles/day/individual) (Cox et *al.,* 2019). Complex digestion processes and continuous and dynamic habitats can cause variations in contamination values in fish. According to the Indonesian National Standard (SNI) 7387:2009 for the maximum limit of HM contamination in food, the limit for Cd contamination in the fish category and its processed products is 0.1 mg/kg. By contrast, in the same category, the limit value for Pb is 0.3 mg/kg. Suppose that the sample's EDI exceeds the acceptable daily intake (ADI). In such case, harmful effects can occur in humans due to consumption of contaminated products. Based on the results obtained, no samples had an EDI exceeding the ADI. Thus, consumption of these fish from Code and Gajahwong Streams is not potentially hazardous to health. However, the consumption of contaminated

Table 5: THQ, TTHQ, and TR HMs in fish muscle due to consumption of fish from Code and Gajahwong Streams

Stream	Species	HM on fish muscle					
		THQ (10^{-3})		TTHQ (10^{-3})	TR		∑TR
		Pb	Cd		Pb	Cd	
Gajahwong	*Barbodes binotatus*	1.51	3.09	4.60	5.13×10^{-5}	9.74×10^{-3}	9.79×10^{-3}
	Barbonymus balleroides	1.45	2.56	4.01	4.93×10^{-5}	8.07×10^{-3}	8.12×10^{-3}
	Mystacoleucus obtusirostris	1.45	2.46	3.91	4.91×10^{-5}	7.77×10^{-3}	7.80×10^{-3}
	Oreochromis niloticus	1.47	2.61	4.05	4.92×10^{-5}	8.21×10^{-3}	8.25×10^{-3}
	Rasbora lateristriata	1.29	2.45	3.74	4.38×10^{-5}	7.71×10^{-3}	7.75×10^{-3}
Code	*Barbodes binotatus*	1.38	1.98	3.33	4.61×10^{-5}	6.21×10^{-3}	6.26×10^{-3}
	Barbonymus balleroides	1.35	2.51	3.85	4.59×10^{-5}	7.88×10^{-3}	7.92×10^{-3}
	Mystacoleucus obtusirostris	1.45	2.59	4.04	4.92×10^{-5}	8.15×10^{-3}	8.20×10^{-3}
	Nemacheilus fasciatus	0.00	0.00	0.00	0.00×10^{-5}	0.00×10^{-3}	0.00×10^{-3}
	Oreochromis niloticus	1.93	2.41	4.33	6.55×10^{-5}	7.58×10^{-3}	7.64×10^{-3}
	Rasbora lateristriata	1.43	2.39	3.82	4.86×10^{-5}	7.51×10^{-3}	7.56×10^{-3}

Table 6: THQ, TTHQ, and TR association of HM and MPs in fish muscle due to consumption of fish from Code and Gajahwong Streams

Stream	Species	HM associated MPs in muscle					
		THQ (10^{-3})		TTHQ (10^{-3})	TR		∑TR
		Pb	Cd		Pb	Cd	
Gajahwong	*Barbodes binotatus*	0.02	0.11	0.12	5.38×10^{-7}	3.42×10^{-4}	3.43×10^{-4}
	Barbonymus balleroides	0.13	0.22	0.33	3.85×10^{-6}	6.84×10^{-4}	6.88×10^{-4}
	Mystacoleucus obtusirostris	0.03	0.07	0.09	1.09×10^{-6}	2.11×10^{-4}	2.12×10^{-4}
	Oreochromis niloticus	0.02	0.05	0.08	6.92×10^{-7}	1.71×10^{-4}	1.72×10^{-4}
	Rasbora lateristriata	0.01	0.09	0.09	1.54×10^{-7}	2.85×10^{-4}	2.85×10^{-4}
Code	*Barbodes binotatus*	0.09	0.23	0.31	2.81×10^{-6}	7.13×10^{-4}	7.15×10^{-4}
	Barbonymus balleroides	0.13	0.17	0.30	4.49×10^{-6}	5.36×10^{-4}	5.40×10^{-4}
	Mystacoleucus obtusirostris	0.09	0.12	0.29	3.22×10^{-6}	6.27×10^{-4}	6.30×10^{-4}
	Nemacheilus fasciatus	0.01	0.13	0.14	4.62×10^{-7}	3.99×10^{-4}	4.00×10^{-4}
	Oreochromis niloticus	0.15	0.13	0.28	5.08×10^{-6}	3.99×10^{-4}	4.04×10^{-4}
	Rasbora lateristriata	0.08	0.15	0.23	3.00×10^{-6}	4.56×10^{-4}	4.59×10^{-4}

products will cause detrimental effects if consumed excessively and continuously in the long term.

The highest TTHQ of Pb and Cd was observed in *B. binotatus* muscle samples from Gajahwong stream. By contrast, in Code stream, *O. niloticus* and *M. obtusirotris* had the highest THQ for Pb and for Cd, respectively (Table 5) (Palupi, 2022). The highest TR due to contamination of the two metals in Gajahwong Stream was observed in *B. binotatus* and in *M. obtusirotris* in Code Stream. For HM associations and MPs in fish muscle, the highest TTHQ was detected in *B. balleroides* for both metals in Gajahwong Stream. Meanwhile, *B. binotatus* attained the highest TTHQ for both metals in Code Stream (Table 6) (Palupi, 2022). For the TR values, *B. binotatus* had the highest potential value due to contamination of the two metals in Gajahwong Stream, whereas *M. obtusirotris* attained such result in Code Stream (Palupi, 2022). Determination of THQ for Pb and Cd in muscle samples (Table 5) and muscle MP and HM associations (Table 6) revealed that all values were lower than 1. These results indicated that the potential for harm caused by fish consumption decreased. For both HMs, the TTHQ were less than 1 for all fish species, which implies that the species from Code and Gajahwong Streams are safe for consumption. If the TR value is less than 10^{-4}, it is considered safe against cancer risk. Meanwhile, if the TR value is in the interval of 10^{-3}–10^{-4}, it is considered to have not met the standard. If the value is greater than 10^{-3}, the contamination can pose a significant potential risk of cancer (Salam *et al.*, 2020). In this study, the concentration of HMs in fish muscle samples in both streams showed a less safe value. Meanwhile, the accumulation of HMs on the surface of MPs in all fish species found in both streams was considered safe. This finding needs attention because fish in both streams may also be exposed to toxicants besides HMs. The comprehensive evaluation showed that the long-term impacts caused by environmental pollution are complex and disastrous. Uncontrolled

MP pollution in streams affects water and fish quality, which increases the risk to human health. These major streams flow into the Indian Ocean (Fig. 1). If this pollution occurs continuously without any concrete mitigation measures, it will expand MP contamination and cause a systematic impact on marine ecosystems. Mitigation action involving several stakeholders must be carried out immediately by improvising waste collection, reducing disposal activities, and tightening the regulations of waste production as an effective strategy.

CONCLUSION

MPs have been confirmed to have moderately polluted the Code and Gajahwong Stream waters. These locations have relatively the same level of MP pollution, given the characteristics of the watershed and their location in a densely populated area. MPs in the waters indicate that plastic degradation processes have occurred along the two streams. The primary source of MP pollution is waste disposal by people who less awareness of environmental sustainability. As a result, fish that live in the streams are also contaminated with MPs. MPs polluting the waters and those accumulated by fish mainly include small-sized, green, and fiber-shaped MPs consisting of LDPE polymer. These characteristics cause difficulty among fish to distinguish MPs as food or waste. Thus, avoiding the accidental consumption of MPs is difficult. The MPs found were associated with HMs (Pb and Cd). Pb and Cd are priority HMs to be assessed in water quality assessment and may trigger cancer. The adsorption of HMs with MPs causes the concentration of HMs to increase significantly due to the increased surface area of MPs, as shown by the results of plastic degradation. In this case, *Rasbora lateristriata* and *Oreochromis niloticus* were found to accumulate significant amounts of MPs. Both types of fish are consumed by the local community, which thereby increases health risks. Although the accumulation of HMs tested is not yet at an alarming level, in the long term, health risks can be faced by people consume these fish from the streams. The research on MP pollution and its association with HMs in relation to health risk assessment is still limited. Recent studies on the capability of MPs as adsorbents pique interest because they are not only associated with HMs but also organic pollutants, such as hydrocarbons or pesticides. This phenomenon offers great opportunities for future MP research. Exposure to MPs as an adsorbent that can absorb various kinds of pollutants is presumed to have a negative impact on fish body systems histologically, biochemically, and physiologically, although further research still needs to be conducted to study the long-term effects of this pollution. Researchers are recommended to address knowledge gaps in the understanding of cumulative exposure toxicity of MPs and various pollutants to fish and humans. This research significantly contributes to the recent issue of environmental pollution and environmental risk assessment in freshwater ecosystem by means of the danger of MP associated with HMs and provides a thorough health risk assessment and mitigation efforts that must be taken to achieve sustainability. Sustainable plastic waste management is needed as a comprehensive interdisciplinary framework to address the complex problem of plastic waste management. Increasing the scope of plastic waste management and services and their efficiency are prerequisites for the improvement of environmental quality. The involvement of various related parties is needed to formulate good policies regarding the management of stream ecosystems. Mitigation efforts including various stakeholders, community involvement, and continuous education must be continuously pursued. This study is expected to be used as a scientific basis to assist the authorities in making policies regarding the management and control of pollution in river ecosystems and as a strong reference for conducting similar research to assess the potential risk of MP pollution.

AUTHOR CONTRIBUTION

A.M. Sabilillah performed sample collection, extraction, and characterization of MPs. F.R. Palupi conducted the determination of HMs and assessment of health risks. B.K. Adji interpreted research results and prepared the map of sampling stations. A.P. Nugroho supervised the project and contributed to managing and developing research ideas, verifying research methods, and reviewing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. The ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely witnessed by the authors.

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ABBREVIATIONS

μg/g	Microgram per gram
Mm	Micrometer
%	Percent
°C	Degree Celsius
ADI	Accepted Daily Intake
C	HM content in fish muscle
C1	Code Station 1
C2	Code Station 2
C3	Code Station 3
Cd	Cadmium
Ci	Concentration of MPs at each station
Cfi	Ratio of MPs abundance to MPs minimum abundance at each sampling point
Coi	Background MPs concentration
CSF	Oral carcinogenic slope factor
ED	Period of exposure
EDI	Estimated daily intake
EF	Exposure frequency
Eq	Equation
F	The features in dataset (X)
Fig	Figure
FIR	Food Ingestion Rate
FT-IR	Fourier transform infrared spectroscopy.
G	Gram
G1	Gajahwong Station 1
G2	Gajahwong Station 2
G3	Gajahwong Station 3
g/cm^3	Gram per cubic centimeters
GIT	Gastrointestinal tract
H_2SO_4	Sulfuric acid
HDPE	High-density polyethylene
HM	Heavy metals
HNO_3	Nitric acid
i.e.	Id Est (that is)
Kg	Kilogram
Kg/day	Kilogram per day
KOH	Potassium hydroxide
L	Liter
LDPE	Low-density polyethylene
mg/kg	Milligram per kilogram
mg/L	Milligram per liter
mL	Milliliter
Mm	Millimeter
MPs	Microplastics
N	Entire quantity
Particles/l	Particles per liter
Pb	Lead

PCA	Principle Component Analysis
PE	Polyethylene
PERI	Potential ecological risk index
PHI	Polymer hazard index
PLI	Pollution load index
Pn	Percent of specific types of polymers in each sampling station
PP	Polypropylene
QA	Quality assurance
QC	Quality control
RfD	Reference dose of individual HMs
SEM-EDX	Scanning electron microscopy-energy-dispersive x-ray spectroscopy
Sn	Hazard score of MP polymers
SNI	Indonesian National Standard
TA	Mean period of exposure to non-carcinogens
THQ	Target hazard quotient
TLV	Threshold limit value
TR	Target cancer risk
TTHQ	Total target hazard quotient
WAB	Mean weight

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ORIGINAL RESEARCH PAPER

Exploring the dynamics of seasonal surface features using coastal and regional ocean community model

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ABSTRACT

BACKGROUND AND OBJECTIVES: Studying the monthly variations in the surface features of the Bay of Bengal is a complex task that involves numerous large-scale ocean-atmosphere dynamics. This study identified the bay's changing circulation patterns over recent decades as a crucial study area requiring in-depth research. Understanding the changes in circulation patterns provides valuable insights into the Bay dynamics. It helps identify the potential impacts of climate change, ocean currents, and other factors on the bay's ecosystem. This study aims to understand the seasonal variability of the Bay of Bengal's surface circulation features using a high-resolution numerical Coastal and Regional Ocean Community simulations model.

METHODS: To conduct the study in the Bay of Bengal, the Coastal and Regional Ocean Community model, a numerical ocean model, was utilized. The high-resolution numerical model for ocean circulation is three-dimensional and uses hydrostatic primitive equations in generalized curvilinear coordinates. Simulations were conducted over 8 years using a grid comprising 256 x 249 horizontal surface points to model a range of ocean-atmospheric parameters. This grid provided an approximate resolution of 10 kilometers.

FINDINGS: The findings are based on the model's enhanced performance compared to previous study results. It was observed that the sea surface temperature remains above 28 degrees Celsius throughout the bay except in winter. During the monsoon season, surface salinity was observed to be reduced in the Bay of Bengal's northern region and western and eastern boundaries. Surface eddies along the western bay extend to deep waters before the onset of monsoon. The net heat flux in the bay has been determined as positive before monsoon, negative post-monsoon, and mixed during the monsoon season.

CONCLUSION: This analysis focuses on the ocean surface layer with more prominent dynamics. Various surface parameters were calculated, and discussions on surface temperature, salinity, D20, D26, and net heat flux across seasons have been presented.

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49	8	0

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INTRODUCTION

The Bay of Bengal (BoB) is a large inlet of the Indian Ocean located in the northeastern part of the Indian subcontinent. To the west, India and Sri Lanka form the borders, while Bangladesh is situated to the north. On the eastern side, Myanmar marks the boundary, and the Andaman and Nicobar Islands are closer to the Myanmar boundary. The bay has a total area of approximately 2,172,000 square kilometers (km^2) and a maximum depth of about 5,000 m (meters) (Chakraborty and Gangopadhyay, 2016). Besides significantly impacting regional climate patterns, the BoB is a significant source of fisheries. Its warm waters are home to numerous fish species and other marine life. Additionally, its waters serve as convenient transport with shipping routes between India, Bangladesh, Sri Lanka, and other Southeast Asian countries (De and Carvalho-Junior, 2022; Sharada *et al*., 2015). The potential role of the BoB in climate change has also been extensively investigated (Li *et al*., 2018; Salathe Jr *et al*., 2008). Studies have found that the BOB's large surface area and warm waters can significantly affect regional climate patterns. Moreover, scientific investigations have demonstrated that the BoB, proximal to the equator, can be a potential origin for severe weather phenomena like cyclones. Various facets of the BoB have been explored in studies, including its ecology, fisheries, coastal resources and management, climate change impacts, and oceanography. (Yadav, 2022; Sardessai *et al*., 2007). Research has also been conducted on prevalent topics such as marine pollution, sedimentation processes, sea-level rise, and coastal erosion. The BoB is characteristically tropical and humid, with temperatures ranging from 23 to 32 degrees Celsius (°C). Monsoons bring heavy rains to the region from June to October, while a dry season extends from December to April (Akhter *et al*., 2021; Cherian *et al*., 2020; Potemra *et al*., 1991; Suprit and Neelakandan, 2016). The bay is an important part of the surrounding countries' climate system because it helps drive the monsoon rains, which are essential for agriculture in the region. Monsoons bring heavy rains to India, Bangladesh, and Myanmar during the summer months providing respite from the heat and necessary water for agriculture (June through September). (Jongaramrungruang *et al*., 2017; Krishnamurti *et al*., 2017; Schott and McCreary, 2001; Wainer and Webster, 1996; Webster *et al*., 1998). The BoB plays a significant role in this process by helping to create a low-pressure area over the Indian subcontinent. This low-pressure area draws in moisture from the ocean, resulting in heavy rains over land. Studies have shown that sea surface temperature (SST) changes in the BoB can significantly impact monsoon rainfall patterns. (Bartzokas *et al*., 2018; Dyn, 2010; Gordon *et al*., 2016; Lee *et al*., 2000; Setiawan *et al*., 2022; Vinayachandran *et al*., 2014;). The BoB region is particularly prone to cyclone genesis, with April–December being the typical peak period for cyclone formation. Cyclone genesis in the BoB is closely linked to the region's oceanic and atmospheric conditions (Ali *et al*., 2007; Fan *et al*., 2020; Girishkumar and Ravichandran, 2012; Kikuchi *et al*., 2009; Kodunthirapully Narayanaswami and Ramasamy, 2022; Lin *et al*., 2009). Being one of the warmest waterbodies, with SST often exceeding 30 °C during the peak cyclone season, o cean heating is an important factor contributing to the genesis of hurricanes in the BoB. (Amsalia *et al*., 2022; Echols, 2020; Horii *et al*., 2016; Kawai and Wada, 2007; Segele *et al*., 2009; Sen *et al*., 2021; Thadathil *et al*., 2002; Vecchi and Harrison, 2002; Yoo *et al*., 2010; Yu and Hole, 2015). This warm water provides a significant energy source for the formation and intensification of cyclones, providing the necessary heat and moisture for convective processes. (Jyothi *et al*., 2019) In addition to ocean heating, other factors such as wind shear, atmospheric instability, and pre-existing disturbances also contribute to the origin of cyclones in the BoB. These factors can interact in complex ways, creating conditions required for cyclones to form and intensify. (Maneesha *et al*., 2012; Roy Chowdhury *et al*., 2021; Ts *et al*., 2020). The climatological significance of the cyclones in the bay cannot be overstated. Cyclones in this region can significantly impact the surrounding coastal communities through floods, storm surges, and high winds. (Jourdain *et al*., 2013). In addition, the heavy rainfall associated with cyclones can have significant adverse implications on the region's agriculture and water resources (Ali *et al*., 2007). Given the potential for significant impacts from cyclones in the BoB, monitoring and forecasting these events as accurately as possible is imperative. Accurate forecasting requires a comprehensive understanding of the oceanic and atmospheric conditions contributing to cyclone formation (Anandh *et al*., 2018; 2020) and the ability to model and predict how these conditions may evolve progressively. Seasonal dynamics in ocean communities can affect various surface features, such as temperature, salinity, nutrient concentrations, and oxygen levels, which may affect populations of phytoplankton and marine organisms. These variations in ocean surface features, including temperature, nutrient availability, and currents, can significantly impact marine ecosystems and their productivity. Such changes can inadvertently disturb the growth and distribution of phytoplankton, the base of the marine food web, and in turn, upset the entire ecosystem. The ocean community models' influence on these surface features was investigated by reviewing the literature and analyzing models. Key environmental factors driving the changes in ocean communities and surface features, such as temperature, nutrient availability, and ocean currents, were also identified. Seasonal surface features in coastal and regional ocean environments, such as sea surface temperature, currents, and wind patterns, can significantly impact ocean-atmosphere interactions and climate patterns. Also known as air-sea interactions, these involve the exchange of energy, heat, and moisture between the ocean and the atmosphere. The study aims to understand the sensitivity of the upper BoB by analyzing surface variables, temperature, salinity, and other parameters using model simulations to explore seasonal variability. This study was carried out in India's BoB region using the numerical model simulation in 2022.

MATERIALS AND METHODS

Numerical model, data, and methodology

The Coastal and Regional Ocean COmmunity (CROCO) is a state-of-the-art numerical ocean model that simulates the dynamics of coastal and regional ocean systems. The CROCO model is based on the Regional Ocean Modeling System (ROMS), a free-surface, terrain-following, hydrostatic, primitive equation ocean model. It was developed as an extension of the ROMS model to include a range of physical and biogeochemical processes important for simulating coastal and regional ocean systems. The model has been applied to various research problems, including coastal erosion, oil spill modeling, and marine ecology. The CROCO is a hybrid model that combines the primitive equations of ocean dynamics with a free surface and a hydrostatic assumption and includes various parameters of physical processes such as turbulence, mixing, and surface fluxes. These parameterizations are based on empirical relationships and theoretical model approaches. The CROCO modeling system estimates the ocean and atmospheric state of the BoB. The model is based on a stretched terrain-following hydrostatic approach designed to solve the momentum and primitive equations at high resolution. The Arakawa C-grid, staggered horizontally, is employed to solve primitive equations. It also includes finite-discretized coastal boundaries acquired through a sea/land mask. An accurate representation of coastal dynamics, such as coastal upwelling and downwelling, and mixing processes, requires the inclusion of coastal boundaries. The CROCO model is a powerful tool for simulating ocean dynamics in coastal and regional areas. Also, the model's high resolution and accurate representation of coastal boundaries make it particularly useful for studying the BoB. A regional model of the BoB region was established, covering a range of latitudes 4˚N to 24˚N and longitudes 72˚E to 100˚E. The model comprised a grid of 256 x 249 horizontal surface points, providing an estimated resolution of 10 kilometers (km). While the northern, eastern, and a significant portion of the western boundaries were closed, the southern boundary was left open. The Levitus monthly climatology was employed to facilitate the temperature and salinity values on the open boundary. In contrast, the model utilized 32 S-coordinate layers in the vertical and incorporated Earth Topography 2–minutes (ETOPO2) data to establish the bottom topography. The study area is illustrated in Fig. 1. his study entailed an 8-year climatological simulation using the CROCO model. A 3-year spin-up period was also performed to examine the monthly variability of the ocean's upper surface in the bay. Additionally, simulations were conducted for monthly analyses of the salinity, temperature, depth of the 20˚C and 26˚C Isothermal Layer (D20 and D26), and net heat flux. This study's methodology ensured that the model produced reliable results, which were used to gain insights into the dynamics of the ocean-atmosphere system in the BoB. Figs. 2 and 3 show the volume-averaged salt content and temperature outputs from the CROCO model. Both results demonstrate that the model stabilized from the second year onward.

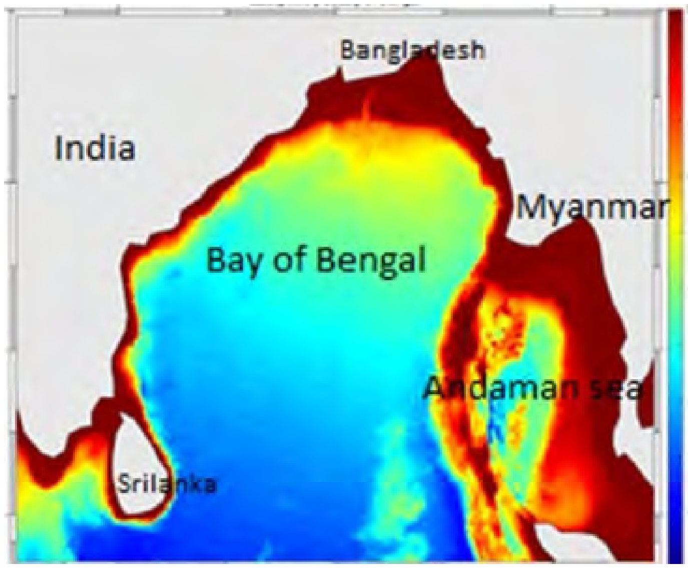

Fig. 1: Geographical location of the study area in the Bay of Bengal in India

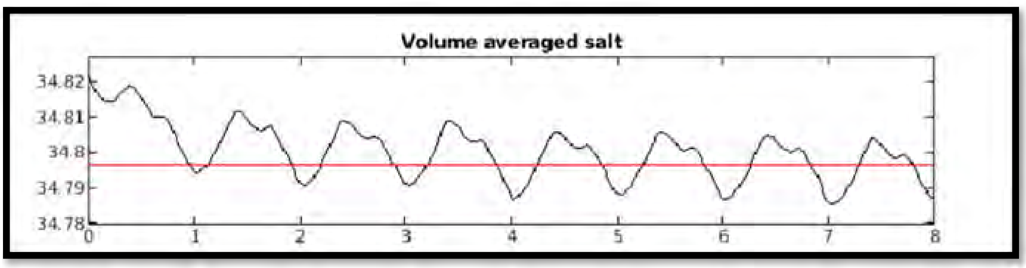

Fig. 2: Volume averaged salt for 8 years

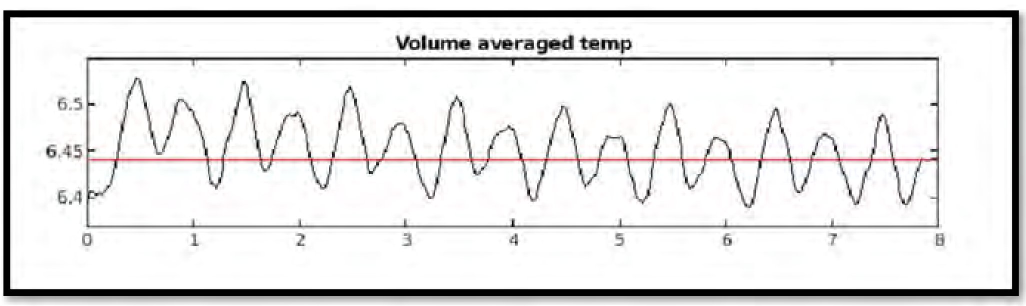

Fig. 3: Volume averaged temperature for 8 years

RESULTS AND DISCUSSION

Sea surface temperature (SST)

The surface temperature of the BoB varies significantly throughout the year, with the western region experiencing high temperatures during certain months. Fig.4 demonstrates the monthly climatological variations of SST. During the winter, the SST ranges from 26 °C to 29 °C throughout the bay, with the south and east experiencing higher temperatures. During the pre-monsoon months of March to May, the temperature in all three bay regions is the highest, ranging from 29 °C to 32 °C.

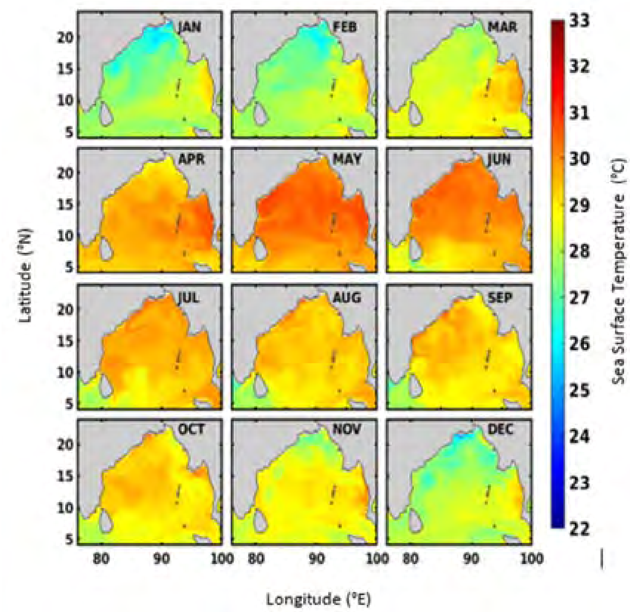

Fig. 4: Monthly climatological variations of sea surface temperature (°C)

In March, the temperature ranges from 27 °C to 31°C, with the central bay presenting a 1°C bias. In April, the temperature ranges from 29 °C to 30 °C, and this increase in temperature causes cyclones to intensify when they cross near the western coast. In May, the western coast experiences the highest temperatures of 30 °C to 32 °C, while the northern, eastern, and southern coastal areas range from 29 °C to 30 °C. In the central bay region, the temperature ranges from 30 °C to 31 °C. During the monsoon season (June to September), the temperature in the western, northern, and eastern bays ranges from 30 °C to 32 °C in June, influenced by the summer season. Due to rainfall from July to September, the surface and subsurface temperatures become cooler, ranging from 29°C to 30°C in the northern, eastern, and western bay regions. In contrast, the south bay region experiences temperatures ranging from 29 °C to 27 °C at the bottom tip. According to Girishkumar and Ravichandran (2012), the pre-and post-monsoon seasons over the bay are characterized by high sea surface temperatures and winds, resulting in frequent air-sea interactions that facilitate the formation and development of cyclones during these periods.

Sea surface salinity (SSS)

The monthly climatological variations in salinity are represented in Fig. 5. During the winter season (December to February), the salinity in the northern and eastern regions of the BoB has a low range of 30-31 Practical Salinity Unit (psu), while in the western bay, it ranges from 33 to 34 psu, and in the southern bay, it reaches its highest values of 34-35 psu. These values represent the post-monsoon conditions; there is no rainfall contribution, but the after-effects of the monsoon rains can still be perceived in the northern bay's low salinities. In the pre-monsoon season (March to May), the northern and eastern bay regions' salinity ranges around 31–32 psu. The salinity distribution ranges from 33 to 34 psu in the southern and central regions, with a bias of 1 psu in the central bay and the western region. During the pre-monsoon season, the sea surface salinity increases due to the relative minimum freshwater flow. The influx of freshwater still affects salinity, but cool and dry air over the bay reduces evaporation (Dandapat *et al*., 2021) and mixes fresh water with salt water. Even though freshwater from rivers is not included, the models show a reduction in surface salinity during monsoon months owing to rainfall and moisture flux. In June, the salinity in the northern and eastern bay regions ranges from 30 to 32 psu, from 33 to 34 psu in the central bay, from 33 to 35 psu in the southern bay, and from 33 to 35 psu in the western

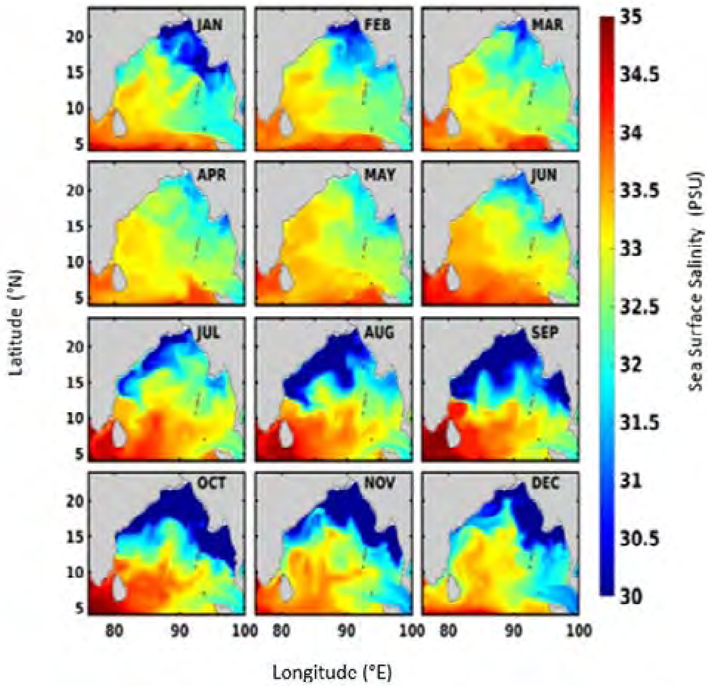

Fig. 5: Monthly Climatological Variations of Sea Surface Salinity (PSU)

bay. In July, the salinity in the northern, eastern, and western bay regions ranges from 30 to 32 psu, while in the southern bay, it ranges from 33 to 35 psu. The central bay's salinity ranges at 33 psu. During the monsoon season, freshwater starts impacting the western bay. In August, the salinity in the northern and western boundaries ranges from 30 to 32 psu, while the southern bay exhibits the highest salinities at 33–34 psu. In September, the northern, eastern, and southern bays range from 30 to 32 psu due to river water mixing in the ocean from rainfall and moisture flux. In the central bay, there is a mixing of 31 to 33 psu. Similar to September, the monsoon's influence in October causes slight changes in the central bay, ranging from 32 to 33 psu with a bias of 1 psu. In November (winter season), the lowest salinity of 30-31 psu is observed in the northern and eastern bay, while in the central bay, it ranges at 32 psu due to eddies. The surface salinity during monsoons these low in the north and the western and eastern boundaries, about 15°N. This information has been obtained from a previous study (Chakraborty and Gangopadhyay, 2016).

Isothermal layer depth (D20)

The isothermal layer depth is a substitute for the thermocline. The isothermal layer depths D20 and D26 are critical indicators of the extensions of surface eddies in the deep waters of the BoB. These eddies can store significant amounts of heat energy that can affect the oceanic environment and potentially influence cyclones passing over them. Deep warm eddies in the west side of the BoB can persist for up to 9 months, impacting the overall oceanic conditions. The monthly climatological variations in the isothermal layer at D20 are shown in Fig. 6. During the winter months (December, January, and February), D20 depths range from 60 m to 180 m across the BoB region. There is also a sudden temperature reversal along India's east coast from December to January. In the central bay, D20 depths can reach up to 180 m, with surface eddies extending to the deep waters. As winter progresses into spring, surface eddies are observed to extend deeper into the western bay areas, reaching depths of 180 m to 200 m. In the northern and southern bay regions, eddies are observed at depths of 100 m to 140 m,

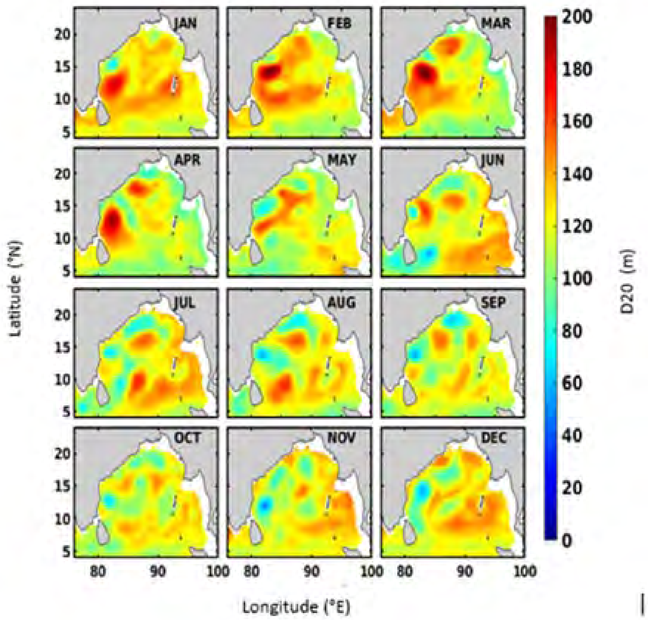

Fig. 6: Monthly Climatological Variations of the Isothermal Layer Depth D20 (m)

while in the central bay, they can reach up to 200 m. D20 depths remain relatively stable in March and April, ranging from 80 m to 120 m in the northern, eastern, and southern bay regions and from 140 m to 200 m in the western bay region. Surface eddies along the western bay extend to even deeper waters until April, storing significant amounts of heat energy. The D20 layer starts shifting to shallower depths with the onset of monsoon except near the deepest regions of the bay. A report has presented a distinctive feature of the BoB: forming a basinwide subsurface cyclonic gyre from June to November and an anticyclonic gyre from December to May (Sil and Chakraborty, 2012).

Isothermal layer depth (D26)

In the winter (December to February), the D26 depth of the isothermal layer ranges from 40 m to 80 m below the surface, with the western bay at 40 m to 80 m and the central bay at 80 m to 100 m deep. The surface eddies extend into deeper waters near the bay's western boundary, storing more heat energy. November–December marks the region's cyclone season. Fig. 7 shows the monthly climatological variations of D26. In February, the D26 depth ranges from 100 m to 120 m in the central region, while the western bay experiences warm eddies up to April, even at 120 m depth or more. In March, the D26 depth ranges from 40 m to 80 m in the northern, eastern, and southern bay regions and from 80 m to 120 m in the western bay region. With the surface temperatures increasing in April, the D26 gets shallower except in the western boundary, where it stays at more than 100 m depth. It may be noted that April is also the beginning of cyclone season in this region. In May, the depth ranges from 60 m to 40 m throughout, except in the west-central region where the D26 is slightly deeper, at 80 m to 100 m depth. From June to September, the D26 of the BoB almost divides into two distinct regions, the western boundary (D26 < 60 m) and the central and eastern regions (D26 > 80 m or more). The D26 continues to get shallower through October and November except in the south and east. It has been reported that (Swain and Navaneeth Krishnan, 2013) during the inter-monsoon period spanning October and November, a significant portion of the North Indian Ocean experiences low wind speeds and relatively low net heat loss, and moderate peak radiation during the day. These combined factors contribute to the shallow depth of the D26 thermocline, typically less than 60 m in most parts of the North Indian Ocean during this period.

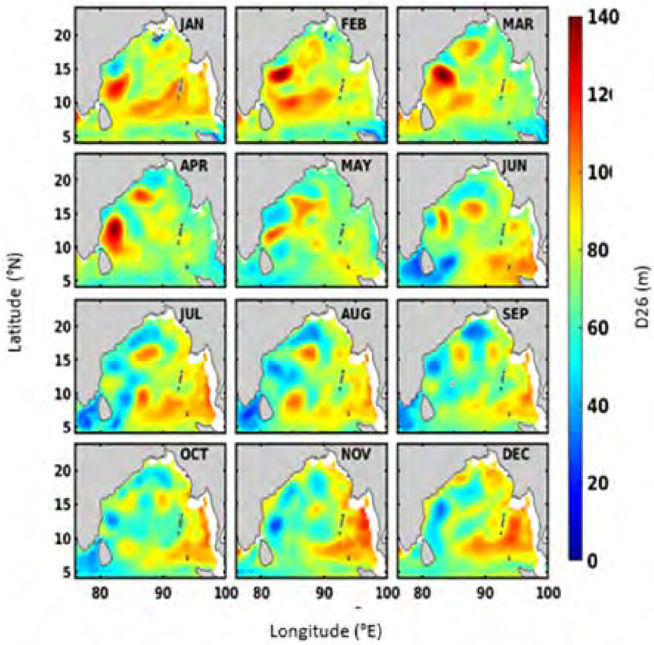

Fig. 7: Monthly climatological variations depth of the isothermal layer D26 (m).

Net heat flux

The net heat flux refers to the exchange of heat between the atmosphere and the ocean, affecting the atmospheric and oceanic processes and their interaction within the ocean-atmosphere system. Previous studies have indicated that theworld's oceans' mixed layer depth (MLD) is affected by external local forces, such as net heat flux (Evaporation minus precipitation plus wind stress). Heat transferred into the ocean is considered positive for downward flux, while heat released from the ocean is considered negative for upward flux. Fig. 8 displays the monthly climatological variations of net heat flux. Throughout the pre-monsoon season (March-May), the net heat flux is positive and ranges from 50 to 100 W/m^2 in all four bay regions (North, East, South, and West), with the maximum exchange occurring due to shortwave radiation. In May, slight changes are observed in the heat flux of the western and northern bay regions, where the range remains 50 to 100 W/m^2, whereas the heat flux from the eastern bay ranges from 0 to 50 W/m^2. The southern bay experiences a heat flux of approximately 50 W/m^2, attributable to the negative latent heat flux occurring at the interface of the atmosphere and ocean. This phenomenon also contributes to the negative Qnet. In the monsoon season (June to September), the net heat flux ranges from 0 to 100 W/m^2 in the western boundary, with negative values noted in June and July due to upward longwave radiation flux. Only the south bay region ranges negatively in August and September, with upward flux contributing more to the net heat flux. In the post-monsoon season (October and November), the heat transmitted into the ocean results in a positive net heat flux due to downward flux in the western, northern, and eastern bays. A mix of negative and positive values is observed in the central and southern bays due to latent heat flux. In the winter, the net heat flux ranges from 0 to -100 W/m^2 in December and January, with heat stored in the subsurface due to upward flux. Heat is diffused from the ocean and liberated in February as a positive heat range in all four bay areas. The North BoB experiences a maximum deepening of minus 50 m during winter, notably characterized by the negative net heat flux, with negative latent heat flux at the atmosphere-ocean interface contributing significantly to the negative Qnet. The North BoB experiences a maximum deepening of ~ 50 meters in the winter monsoon season. This deepening can be primarily attributed to the dominance of negative Qnet in the North BoB (Sadhukhan *et al*., 2021).

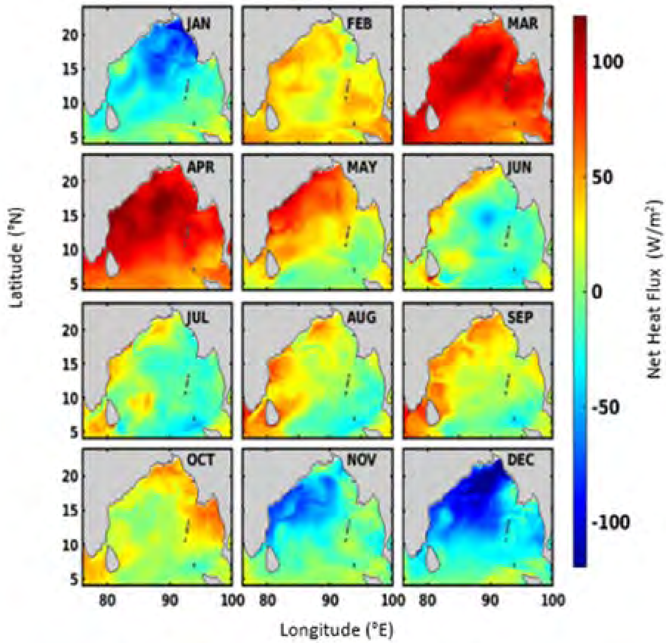

Fig. 8: Monthly climatological variations net heat flux (W/m^2)

CONCLUSION

The numerical model CROCO was utilized to establish a regional model for the BoB covering an area ranging from 4˚N to 24˚N in latitude and 72˚E to 100˚E in longitude. The model comprised a grid of 256 x 249 horizontal surface points, providing an estimated resolution of 10 km. ETOPO2 was used for the model bathymetry and Levitus climatology to assess the temperature and salinity of the open boundaries. The simulation demonstrated higher sea surface temperatures in the BoB, with a value of 28 °C or higher for most of the year, except during winter. The onset of the monsoon season was observed to cause a reduction in the surface salinity in the north, west, and eastern boundaries. Apart from the bay's deepest regions, the D20 layer shifted towards shallower depths with the onset of monsoon. This shift can be attributed to the significant amount of freshwater input during the monsoon season, which reduces the density of the surface waters, causing them to rise and mix with the underlying waters. Additionally, the D26 layer became shallower from October through November, except in the south and east, which may be caused by the cooling of the surface waters during this period, increasing their density and causing them to sink, leading to the shoaling of the D26 layer. During winter, the northern BoB experienced negative net heat flux, which was reversed before the onset of the monsoon. This reversal is due to the atmospheric circulation changes associated with the monsoon season, which leads to heat transfer from the atmosphere to the ocean, resulting in a positive net heat flux. The findings of this study provide important insights into the physical processes governing the surface characteristics of the BoB. Using the CROCO model, accurately simulating the BoB's surface features, including salinity, temperature, D20, D26, and net heat flux, contributed to a better understanding of the region's dynamics. These conclusions contribute to a good understanding of the surface characteristics in the BoB and can aid in predicting its future behavior, particularly regarding climate change and sea level rise, and can inform the development of effective adaptation and mitigation strategies. Global warming causes increased global temperatures and heat, 90 % of which is transferred to the ocean, and the rest of the 10 % is transferred to air and land. Given the earlier study demonstrating a global increase in heat in the air, land, and sea, a climatological study is vital to raise societal awareness.

AUTHOR CONTRIBUTIONS

D. Jaishree performed the literature review, preprocessing for simulations, processing model output, and data analysis, prepared the manuscript text and edition. P.T. Ravichandran contributed to the supervision of the work and preparation of the manuscript. V. Deeptha Thattai contributed to the conceptualization, pre-processing, data analysis, and manuscript review for publication.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and falsification, double publication and submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
BoB	Bay of Bengal
CROCO	Coastal and Regional Ocean COmmunity model
D20	Depth of the Isothermal Layer-D20
D26	Depth of the Isothermal Layer-D26
ETOPO2	Earth TOPOgraphy 2–minutes
km	Kilometer
Km^2	Square Kilometer
m	Meter
psu	Practical Salinity Unit
SSS	*Sea surface salinity*
SST	Sea Surface Temperature
W/m^2	Watt per square meter

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ORIGINAL RESEARCH ARTICLE

Microplastic contamination and growth pattern of oyster; Crassostrea gigas in a coastline

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ABSTRACT

BACKGROUND AND OBJECTIVES: Oysters (Crassostrea gigas) are one food source commonly consumed by the community and an important commodity with high economic value. Environmental issues, such as microplastics, have become a worldwide concern for its implications for aquatic organisms, especially oysters. This study aims to identify the microplastics and growth patterns of oysters in Aceh Province, Indonesia's north and east coasts. This study aims to determine which oysters are suitable for consumption and food health based on research locations along the east-north coast of Aceh Province.
METHODS: The locations in this study include nine regencies/cities, which are directly facing the Malacca Straits. Microplastic isolation from oysters using 10 percent potassium hydroxide and incubation process were done to dissolve the organic materials. The growth pattern was analyzed to determine the growth rate of oysters at each study location.
FINDINGS: The results of this study showed that oysters in all sampling locations were contaminated with microplastics, with a high prevalence of 48 percent found in Langsa, followed by Aceh Timur and Pidie each (40 percent), Banda Aceh (38 percent), Aceh Utara (32 percent), Aceh Besar and Bireun (30 percent), Lhokseumawe (12 percent), and Aceh Tamiang (8 percent). The analysis of the growth patterns revealed that the growth of oysters at each location was not optimal (b <3 or negative allometric).
CONCLUSION: In 500 oyster samples collected, 139 were contaminated with microplastics. The most dominant type of microplastic contaminating oysters is fiber up to 170 particles, followed by films 28 particles, and fragments 19 particles. Negative allometric growth pattern might correlate with microplastics that contaminate the waters and enter the oyster's digestive organs. The results of this study reveal that oysters consumed by people have been contaminated with microplastics, so stakeholders must carry out socialization for early prevention to be realized.

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52

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INTRODUCTION

Plastic overuse in daily activities has become a serious environmental problem worldwide (Singh and Sharma, 2016; Nielsen *et al*., 2020). It is estimated that every year, around 155–266 million tons of plastic are produced, with 19–23 million tons ending up in the sea (Onoja *et al*., 2022), leading the plastic waste the most dominant type of waste, which is around 80 percent (%) of all waste in the sea (Alabi *et al*., 2019). Plastic waste accumulating in the sea threatens the survival of marine biota, as it can be swallowed or entangled around the biota (Kurtela and Antolovic, 2019). Marine biota that ingests plastic waste will disrupt their digestive system and cause death (Cole *et al*., 2011). The plastic waste entangled in the marine biota will interfere with the growth and development process, movement, and reproductive and eating activities (Hammer *et al*., 2012; Vermeiren *et al*., 2016). Fragmented plastic waste will form microplastics with a size <5 millimeter (mm) (Patti *et al*., 2020). Microplastics can be divided into two groups: the primary microplastics originating from polyethylene fibers found in beauty products, cleansers, toothpaste, etc., and secondary microplastic originating from decomposing larger plastics which then accumulate in the sea (Moore, 2008). Microplastics can enter the digestive system of a marine organism due to an error in the food identification process or the existence of a food chain associated with microplastics (Mercogliano *et al*., 2020). Microplastics can also enter the gill organs, causing blockages in the biota's blood flow, disrupting the oxygen distribution, antioxidant capacity, oxidation, neurotoxicity, and immune response (Kim *et al*., 2021). Microplastics in aquatic organisms can also affect the immune system, causing tissue damage, nervous system disorders, and a negative impact on reproduction (Wang *et al*., 2021). Generally, high concentrations of microplastics in fish, mollusca, or marine biota can also impact humans through the food chain, causing health problems in the community (Browne *et al*., 2013). Danopoulos *et al*. (2020) showed that the highest microplastic content found in shellfish has an average amount of 0–10.5 microplastics per gram (MPs/g), followed by 0.1–8.6 MPs/g in crustaceans and 0–2.9 MPs/g in fish. This content value influences the rate of microplastics spread to humans because shellfish are generally consumed whole, unlike fish (except for small ones), which are first separated from their stomachs and intestines, where microplastics are stored in the fish's body. *Crassostrea gigas* is one kind of shellfish susceptible to microplastic contamination. Oysters have a way of eating as a filter feeder (Kasmini *et al*., 2019a). Adult oysters can filter up to 50 gallons of seawater daily (Koenig, 2018). This fact showed that oysters are more susceptible to microplastic contamination than other marine biotas, especially in waters that microplastics have polluted, including Indonesia. With the status of the second largest producer of plastic waste in the world (Eko and Dwiyitno, 2020), Indonesia's sea waters have been recorded as contaminated with microplastics. Microplastic contamination in Indonesia's seawater was shown in the research of Meirenno *et al*. (2022) in Tambak Lorok, Semarang City, which found that microplastics contaminated oysters. Research on microplastic contamination in oysters is still relatively rare in Indonesia, especially in Aceh waters. This research is crucial considering that Aceh produces and consumes a high amount of oysters and can potentially become an export commodity from Aceh (Razali, 2019). Monitoring this issue to maintain the quality of oysters is crucial. Aceh waters have been recorded as contaminated with microplastics. The research results of the Nusantara River Expedition Team, who conducted a water quality and microplastic contamination test on the Krueng Aceh River from upstream (Aceh Besar) to downstream (Banda Aceh), concluded that the river had been contaminated with microplastics, with the concentrations getting high as the water flows to the downstream (Habibi, 2022). The same conclusion was also obtained based on research at Krueng, Langsa, wherein the river had been contaminated with microplastics and was considered unfit for consumption (Waspada, 2022). Microplastics in the river will flow into the sea, contaminating Aceh's sea waters. Hence, it has the potential to contaminate oysters, especially in the north-east coast of Aceh. This study aims to identify microplastic content in oysters in the north-east coast of Aceh. The current study aims to identify microplastics and growth patterns of *Crassostrea gigas*. This study was carried out in Aceh Province, Indonesia's north and east coasts in 2022.

MATERIALS AND METHODS

Time and location

This study was conducted along the north and east coasts of Aceh Province, which led to the water of the Malacca Strait (Fig. 1). Sample collection was done from June to August 2022. The microplastic identification process was conducted at the Marine Biology Laboratory, Faculty of Marine and Fisheries, Universitas Syiah, Banda Aceh, Indonesia. The description of the numbers in the figure can be seen in Table 1.

Sample collections

Purposive sampling determined the sampling locations in each district along the north and east of Aceh Province. Fifty oyster samples were collected in each visited district. Sampling was carried out using shovels, machetes, and knives to separate oysters from its attachment to hard media such as stone, wood, and concrete. The collected samples were then placed into plastic samples and coded according to the sampling location. The packaged samples were stored in a styrofoam box and then transported to the Laboratory for further analysis.

Microplastic extractions

Before the extraction process, the total length of the sample was measured using a digital caliper (Error = 0.01 mm), and the weight was measured using a digital scale (Error = 0.01 g). Accordingly, the oysters were dissected to separate the shell and flesh, soaked in a 10% potassium hydroxide (KOH) solution (1:10 ratio), and then incubated for 12 hours at 60 degrees Celsius (°C) (Karami *et al*., 2017). The meat that decomposes into liquid is filtered using Whatman filter paper (No. 540) assisted by a vacuum

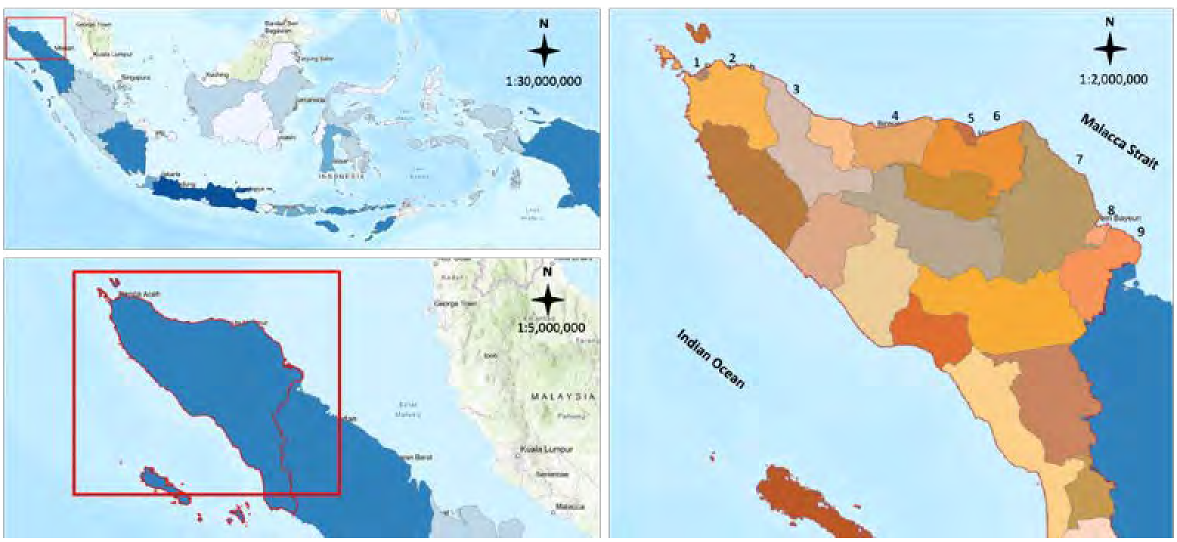

Fig. 1: Geographic location of the study area in the north and east coasts of Aceh Province in Indonesia.

Table 1: Location and coordinates of the study.

No.	Location	Coordinate
1.	Banda Aceh	5^{0}33’1.782”N 95^{0}17’12.128”E
2.	Aceh Besar	5^{0}37’18.258”N 95^{0}23’53.407”E
3.	Pidie	5^{0}24’18.663”N 95^{0}56’6. 853”E
4.	Bireuen	5^{0}14’1.493”N 96^{0}41’42. 54”E
5.	Lhoksemawe	5^{0}9’54.772”N 97^{0}8’32.899”E
6.	Aceh Utara	5^{0}11’46.199”N 97^{0}22’5.571 “E
7.	Aceh Timur	4^{0}40’32.543”N 97^{0}55’0.465”E
8.	Langsa	4^{0}51’89.082”N 98^{0}01’55.416”E
9.	Aceh Tamiang	4^{0}45’52.175”N 98^{0}13’89.004”E

machine. The compound results from that filter were then incubated at 50^{0}C for 5 hours to evaporate the liquid on the filter paper. The dried filter paper was stored in a petri dish for microplastic identification through a microscope examination.

Observational parameters

a. *Intensity and prevalence of microplastic*

Microplastic particle identification and measurement were carried out using binocular (Zeiss Primo Star) and stereo (Meiji Techno EM-32) microscopes. Calculation of microplastic particles was carried out based on the type (fragments, fibers, and films), according to Song *et al*. (2015). The prevalence and intensity were analyzed using the formula Karami *et al*. (2018) and Roch *et al*. (2019), where Prevalence = Total sample contaminated by microplastic/Total sample x 100; Intensity = Total amount of microplastic/Total sample contaminated by microplastic.

b. *Length-weight relationship (LWRs)*

Length-weight relationship analysis was done using Linear Allometric Model (LAM) using Eq. 1 (Muchlisin *et al*., 2010).

$$W = a.L^b \tag{1}$$

Where,

W = oyster weight (g), L = oyster length (mm), a and b = constant.

RESULTS AND DISCUSSION

500 oyster samples (50 samples from each location), with a total length between 14–98 mm and a range of weight between 6.3–95.42 g, were collected during the study at 9 research locations. Based on the size of the oysters collected during the study, it was estimated that they were 1–2 years old, according to the previous study of Lili *et al.* (2019b). The results from the analysis showed that oysters collected in Langsa accumulated microplastics reaching 48%, followed by Aceh Timur and Pidie each 40%, Banda Aceh 38%, Aceh Utara 32%, Aceh Besar and Bireun 30% each, Lhokseumawe 12%, and Aceh Tamiang 8% (Table 2).

The 139 oysters contaminated with microplastics yielded a total of 28 films, 170 fibers, and 19 fragments, where detailed data on microplastic types by location, as Table 3 shows. The dominance of fiber-type microplastics is possibly due to the high concentration of these contaminants in the waters at the research location, originating from laundry house waste. Previous research revealed that fiber-type microplastics generally come from polyester textiles for people's clothing during washing (S Cesa *et al*., 2017; Stanton *et al*., 2019; Chai *et al*., 2020). Film-type microplastics originate from agricultural

Table 2: Prevalence of microplastic contamination in oysters based on sampling location.

Location	Sample (n)	Total Length (mm)	Weight (g)	Accumulation (individu)	Prevalence (%)
Banda Aceh	50	31–81 64.82 ± 0.83	10.41–46.84 21.73 ± 7.11	19	38
Aceh Besar	50	35–61 46.44 ± 0.67	6.62–55.5 15.17 ± 8.44	15	30
Pidie	50	34–98 54.08 ± 1.39	6.3–68.77 22.84 ± 14.77	20	40
Bireun	50	33–62 47.08 ± 0.74	9.34–51.85 23.82 ± 8.92	15	30
Lhokseumawe	50	26–59 40.38 ± 0.74	8.55–37.81 20.92 ± 7.39	6	12
Aceh Utara	50	34–72 51.78 ± 1.04	10.19–62.24 27.49 ± 13.35	16	32
Aceh Timur	50	34–93 66.8 ± 1.43	8.99–95.42 43.31 ± 20.51	20	40
Langsa	50	14–79 50.1 ± 1.07	9.4–63.52 21.04 ± 9.52	24	48
Aceh Tamiang	50	43–82 61.68 ± 0.96	15.03–57.35 33.39 ± 10.03	4	8

Table 3: The amount of microplastic type that accumulated in oysters.

Location	Microplastic (particles/location)		
	Film	Fiber	Fragment
Banda Aceh	16	30	1
Aceh Besar	1	16	1
Pidie	0	38	0
Bireun	0	14	1
Lhokseumawe	0	10	0
Aceh Utara	0	20	0
Aceh Timur	2	24	1
Langsa	9	14	15
Aceh Tamiang	0	4	0
Total	28	170	19

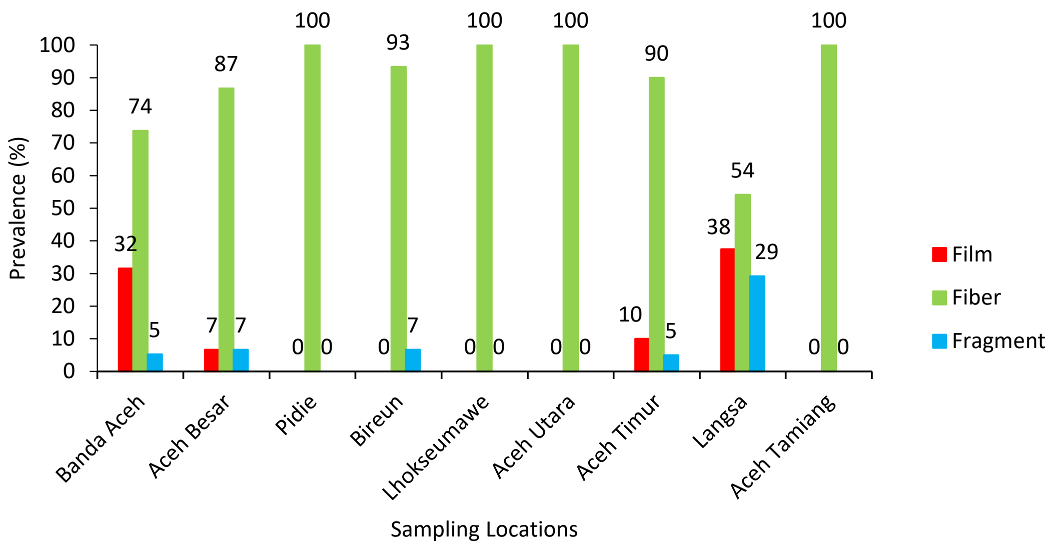

Fig. 2: The prevalence value of microplastic in oysters based on the type of microplastic.

activities (agricultural mulch film) and are released into the waters during rainfall (Qi *et al*., 2020; Ren *et al*., 2021). Fragment-type microplastics are derived from mechanical properties, crystallinity, and crack propagation from various activities on roads, buildings, and waters (Julienne *et al*., 2019; Gaylarde *et al*., 2021). Based on the research location, the intensity of microplastics in oysters showed that the highest film type was found in Banda Aceh (0.84 microplastics/oyster), the highest fiber type was found in Pidie (1.90 microplastics/oyster), and the highest type of fragment was found in Langsa (0.63 microplastics/oyster) (Table 4). Data showed that 139 of 500 total oyster samples collected had been contaminated by microplastic. From those 139 oysters, fiber-type microplastics dominated oysters with prevalence values between 54%–100%, followed by film-type between 0%–38% and fragments between 0%–29% (Fig. 2).

These results also indicate that fiber-type microplastics have contaminated oysters in all study locations. The results of the LWRs analysis showed that the oyster growth pattern was negative allometric (b < 3) (Table 5 and Fig. 3). It can be concluded that the growth of oysters is not optimal in all research locations. The highest b-value was shown in Pidie (b = 2.1) and the lowest was obtained in Lhokseumawe (b = 0.1). Previous research reported that microplastics could impair and inhibit the growth of *C. gigas* oysters, depending on the size of the microplastic particles (Bringer *et al*., 2020a). Other studies also reported the significant effect of microplastic contamination

Table 4: Microplastic intensity in oysters based on the type of microplastic and sampling location.

Location	Sample (n)	Accumulation (individu)	∑ Microplastic			Intensity (Microplastic/oyster)		
			Film	Fiber	Fragment	Film	Fiber	Fragment
Banda Aceh	50	19	16	30	1	0.84	1.58	0.05
Aceh Besar	50	15	1	16	1	0.07	1.07	0.07
Pidie	50	20	0	38	0	0	1.90	0
Bireun	50	15	0	14	1	0	0.93	0.07
Lhokseumawe	50	6	0	10	0	0	1.67	0
Aceh Utara	50	16	0	20	0	0	1.25	0
Aceh Timur	50	20	2	24	1	0.10	1.20	0.05
Langsa	50	24	9	14	15	0.38	0.58	0.63
Aceh Tamiang	50	4	0	4	0	0	1.00	0

Table 5: Analysis of LWRs, including minimum value, maximum value, mean, and standard deviation.

Location	Sample (individual)	TL (cm)	W (g)	b	R^2
Banda Aceh	50	5.1–8.1 (6.5 ± 0.7)	10.4–46.8 (21.7 ± 7.1)	0.8	0.1
Aceh Besar	50	3.5–6.1 (4.7 ± 0.7)	6.6–33.5 (14.4 ± 6.1)	1.6	0.3
Pidie	50	3.4–9.8 (5.4 ± 1.4)	6.3–68.8 (22.8 ± 14.8)	2.1	0.8
Bireun	50	3.3–6.2 (4.7 ± 0.7)	9.3–51.8 (23.8 ± 8.9)	1.5	0.5
Lhokseumawe	50	2.6–5.9 (4.0 ± 0.7)	10.8–37.8 (21.0 ± 7.3)	0.1	0
Aceh Utara	50	3.4–7.2 (5.2 ± 1.0)	10.2–62.2 (27.5 ± 13.3)	1.4	0.4
Aceh Timur	50	3.4–9.3 (6.7 ± 1.4)	8.9–95.4 (43.3 ± 20.5)	1.9	0.6
Langsa	50	3.2–7.9 (5.1 ± 0.9)	9.4–63.5 (21.0 ± 9.5)	1.4	0.4
Aceh Tamiang	50	4.3–8.2 (6.2 ± 09)	15.0–57.3 (33.4 ± 10.0)	1.1	0.3

TL = total length, W = body weight, b = coefficient of growth pattern, R = coefficient of determination

on valve activity and daily growth of *C. gigas* (Bringer *et al.*, 2021).

Fig. 4 reveals that the observed growth of oysters was similar to the predicted results. Microplastic pollution in Aceh waters is apprehensive and requires more attention, as most of the oysters had an accumulation of microplastics internally. Oyster meat separated from the shell is generally directly consumed by humans. All parts of the oyster meat are not discarded, allowing the microplastics to be transferred from the food route. According to Van-Cauwenberghe and Janssen (2014), it is estimated that Europeans can be contaminated with 11,000 microplastics within a year due to consuming contaminated oysters. Microplastics that enter humans through food, in the long term, have the potential to pose health risks, especially cancer (Sabilillah *et al.*, 2023). According to Teng *et al.* (2019), the main factor for microplastic accumulation in oysters is the oyster's natural way of feeding, namely the filter feeder. The analysis showed that microplastics had contaminated 139 of 500 oyster samples collected. Another report by Maulana *et al.* (2023) revealed that fish distributed in Aceh (the current study location) were also contaminated with microplastics in the digestive tract of fish. The prevalence value based on location shows that the highest value was found in Langsa (48%), and the lowest was found in Aceh Tamiang (8%). The phenomenon of microplastic accumulation in oysters also occurs in other regions of the world,

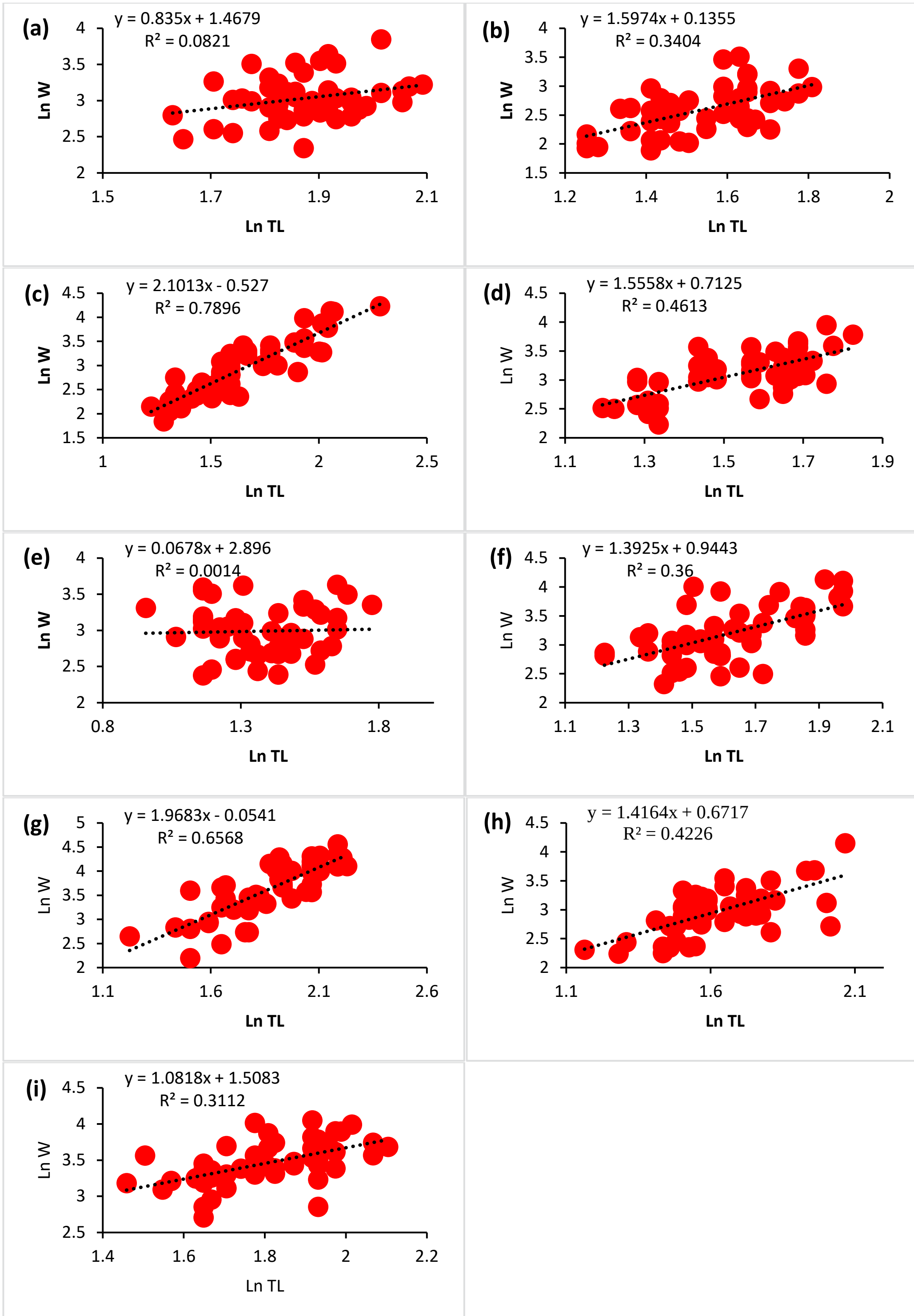

Fig. 3: LWRs of oyster based on sampling location, (a) Banda Aceh, (b) Aceh Besar, (c) Pidie, (d) Bireuen, (e) Lhokseumawe, (f) Aceh Utara (g) Aceh Timur, (h) Langsa, and (i) Aceh Tamiang

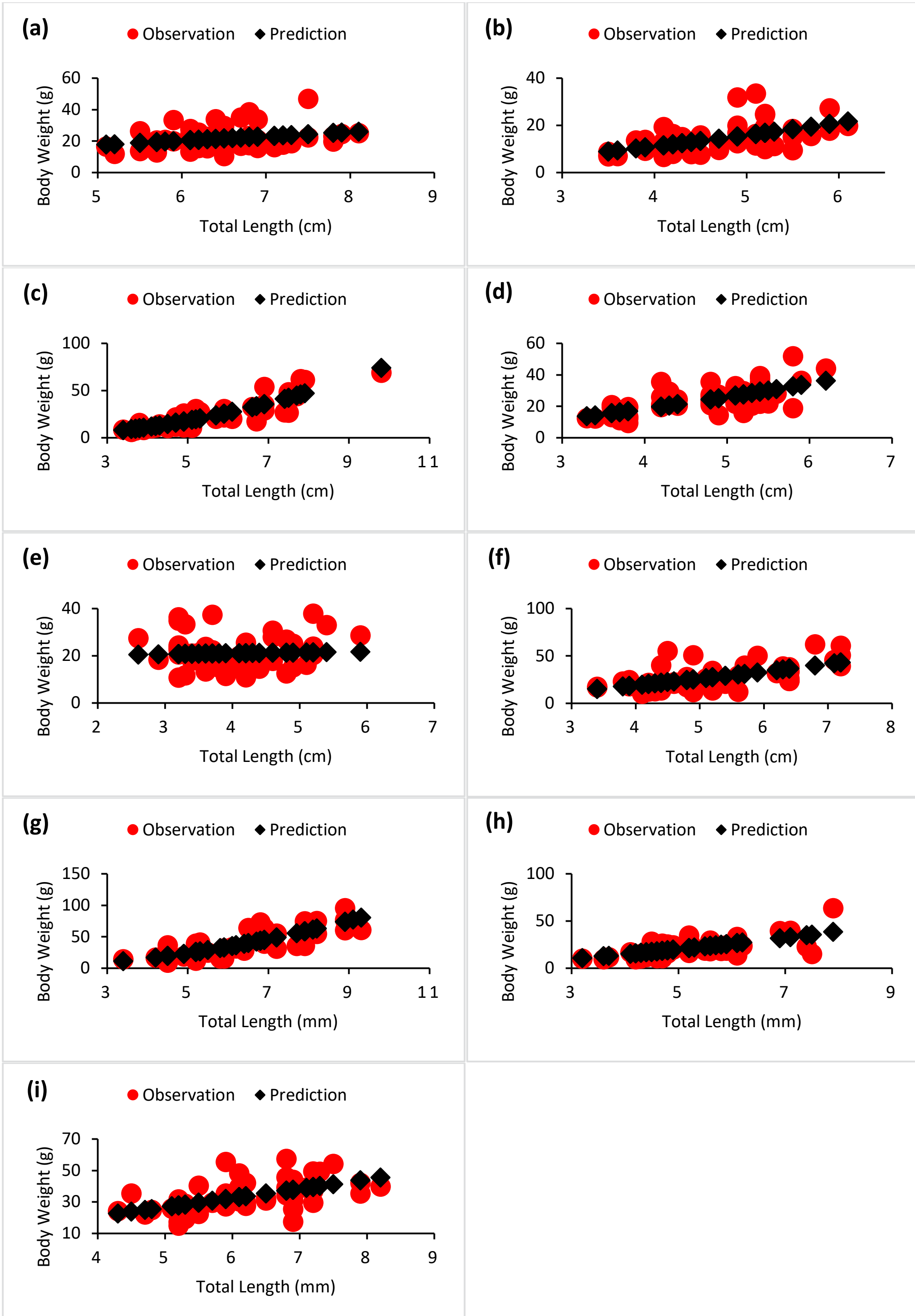

Fig. 4: Growth pattern based on sampling location, (a) Banda Aceh, (b) Aceh Besar, (c) Pidie, (d) Bireuen, (e) Lhokseumawe, (f) Aceh Utara (g) Aceh Timur, (h) Langsa, and (i) Aceh Tamiang

such as China (Li *et al*., 2018; Teng *et al*., 2019; Wang *et al*., 2021b), Australia (Jahan *et al*., 2019), and Taiwan (Liao *et al*., 2021). The microplastic polymers that contaminated the oysters in China are cellophane (CP), polyethylene (PE), and polyethylene terephthalate (PET). According to Wootton *et al*. (2022), oysters in Australia were exposed to polyethylene microplastic from aquaculture activity nets. Liao *et al*. (2021) revealed PET accumulation in Taiwan's oysters. Other studies revealed the prevalence of microplastics found in *Crassostrea gigas* reaches up to 63% in the Salish Sea, USA (Martinelli *et al*., 2020), 91% in Bahía Blanca Southwestern Atlantic (Severini *et al*., 2019), and 100% in southern Brazil (Saldaña-Serrano *et al*., 2022). Corami *et al*. (2020) also conducted a study in Venice Lagoon, Italy, which aimed to identify microplastics (<100µm in size) in the gills and hepatopancreas of *Crassostrea gigas*. This study's results showed that contamination prevalence values reached 41% and 12% for each organ. The highest intensity values for film, fiber, and fragment microplastics were obtained as 0.84, 1.90, and 0.63 microplastics/oyster, respectively. Saldaña-Serrano *et al*. (2022) revealed 0.33–0.75 microplastic/oyster in the form of fiber type were found in *Crassostrea gigas* from the six cultivation sites in southern Brazil, which was lower than the findings of this study. Dang *et al*. (2022) revealed that microplastics had contaminated *Crassostrea gigas* with an intensity value of 18.54 microplastics/oyster in Danang Bay, Vietnam. Oyster growth patterns in all study sites showed negative allometric values ($b < 3$). This fact represents that oysters got insufficient food, as they required. The microplastics ingested by the oysters may cause disturbances in their digestive process because microplastics are non-nutritional materials. These results are confirmed by previous studies, which reported the negative impact of microplastics, including hepatic stress, intestinal alteration, and digestive organ damage in Bivalvia and fish (Hoyo-Alvarez *et al*., 2022; Hollerova *et al*., 2023). Oyster larvae contaminated with microplastics can suffer from growth disorders such as malformations and abnormalities (Bringer *et al*., 2020b). Microplastic contamination also causes increased mortality in adult oysters, besides causing decreased locomotor activity and developmental abnormalities in oyster larvae (Bringer *et al*., 2022).

CONCLUSIONS

The study results revealed that microplastics had contaminated oysters throughout the north-eastern region of Aceh Province, with the highest prevalence reaching 48% found in Langsa and the lowest found 8% in Aceh Tamiang. Based on the intensity value, fiber-type microplastics dominate with a value of 1.33 particles/oyster. Fiber-type microplastics that contaminate oysters were found in all research locations, where the highest was found in Pidie with 38 particles and the lowest in Aceh Tamiang with 4 particles. Fragment-type microplastics contaminate oysters only in five research locations (Aceh Besar, Aceh Timur, Banda Aceh, Bireun, and Langsa), where the highest was found in Langsa with 15 particles and nil in 4 other research locations (Aceh Tamiang, Aceh Utara, Lhokseumawe, and Pidie). Film-type microplastics contaminated oysters only in four research locations (Aceh Besar, Aceh Timur, Banda Aceh, and Langsa), where the highest was found in Banda Aceh with 16 particles and nil in 5 other research locations (Aceh Tamiang Aceh Utara, Bireun, Lhokseumawe, and Pidie). The presence of microplastics in oysters can be potentially transferred to humans via food since humans eat oysters as a whole except for the shell, which is separated from the oyster's body. Conversely to fish, organs that are generally contaminated with microplastics, such as the digestive tract, gills, and several other organs, are removed when the fish is cleaned to minimize the transfer of microplastics to humans through the mouth. Thus, the transfer of microplastics to the human body is greater if people eat oysters than fish. Although the direct impact of microplastics on the contaminated human body is inconclusive, several studies have revealed that microplastics that accumulate in the human body have a negative impact in the long term. In the growth patterns analysis, the development of oysters at each study location was not optimal ($b < 3$ or negative allometric). This phenomenon might correlate with microplastics contaminating the waters and entering the oyster's digestive organs. Based on the results of the current study, oyster farming technology in the pond area must be developed, where the water used in the ponds is filtered to minimize microplastic contamination.

AUTHORS CONTRIBUTIONS

L. Kasmini developed the study concepts, survey, data collection and analysis, and approved the manuscript's final draft. A.S. Batubara performed survey, data collection and processing, and prepared the manuscript's first draft.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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ABBREVIATIONS

μm	Micrometer
%	Percent
°C	Degrees Celsius
a	Constanta
b	Constanta
CP	Cellophane
g	Gram
KOH	Potassium hydroxide
L	Length
LAM	Linear allometric model
LWRs	Length-weight relationship
mm	Milimeter
MPs	Microplastics per gram
No.	Nomor
PE	Polyethylene
PET	Polyethylene terephthalate
W	Weight

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ORIGINAL RESEARCH ARTICLE

Mapping and identifying heavy metals in water use as chemicals of potential concerns in upper watershed

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ABSTRACT

BACKGROUND AND OBJECTIVES: Excessive presence of heavy metals in water sources can reduce water quality and harm human health. However, research on heavy metals from water sources for sanitation and hygiene purposes and drinking water in the Upper Citarum Watershed remains limited. This study focuses on the distribution of heavy metals and chemicals that have potential health risks.
METHODS: Ten heavy metals, namely, lead, cadmium, chromium, copper, cobalt, iron, mercury, manganese, arsenic, and zinc, were analyzed. Groundwater samples were collected from 160 locations, and drinking water samples (for respondents who do not drink groundwater) were collected from 98 locations. Heavy metal concentrations were detected using inductively coupled plasma optical emission spectrometry.
FINDINGS: The levels of arsenic, cadmium, cobalt, iron, mercury, manganese, and lead exceeded the quality standards for drinking water, while those of arsenic and cobalt did not exceed the quality standards for water hygiene and sanitation. Arsenic and cobalt quality standards were more stringent for drinking water compared with those for water hygiene and sanitation. Lead–cadmium and iron–manganese in groundwater showed a positive Spearman correlation ($p<0.05$) and may originate from the same source. Copper and zinc did not exceed the quality standard in 100% of drinking water samples. Iron and zinc in groundwater differed significantly due to variations in topography and soil type ($p<0.05$). This study reveals that 6 out of 10 heavy metals are chemicals of potential concern and are sorted based on potential risks to health, that is, arsenic > mercury > lead > cobalt > manganese > cadmium. Ingestion is the main pathway for potential risk, and children are more likely to be at risk than adults.
CONCLUSION: Stakeholders and decision makers must immediately implement sustainable actions to protect public health. Evaluation of water sources, technology, maintenance processes, and water quality should be conducted before and after technology use from Refill Drinking Water Depots to ensure that raw and processing water meets the quality standards.

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INTRODUCTION

The Citarum River is one of the rivers that support the lives of most people in West Java (Salami and Pradita, 2020). However, it has been a polluted river due to the impact of human activities. In the Action Plan for Control of Pollution and Damage to the Citarum River Basin for 2019–2025 published by the Regional Government of West Java Province, the status of the Citarum River is polluted, where domestic and industrial activities within the area cause contamination. Based on data from the Coordinating Ministry for Maritime Affairs in Kusuma *et al*. (2018), in at least 3,236 textile industries, 90% do not have waste water treatment plants (WWTPs) and 280 tons of chemical wastes per day are discharged into the Citarum River. The Upper Citarum watershed is an area that has a high industrial distribution. The high number of industries in Bandung Regency will lead to a high potential for heavy metal pollution levels around the area. Various types of heavy metals in the environment originate from the textile industry (Van Ginkel, 2015; Bielak and Marcinkowska, 2022; Sumantri and Rahmandi, 2020; Bhardj *et al*., 2014; Thanki and Bhattacharya, 2022; Baek *et al*., 2020). Coal fuel is used for steam generators (boilers) in the textile industry in Bandung Regency, and burning coal increases exhaust gas emissions in the form of particles containing heavy metals (Febrion and Falah, 2018). Heavy metal concentrations in fly ash after coal combustion are high in small particles (<1 m) (Czech *et al*., 2020). Heavy metal pollution is a serious concern in Bandung Regency, especially in groundwater. The groundwater system is related to air, soil, and river water, so the potential for heavy metal pollution in groundwater is very large. Heavy metal contamination in soil occurs because wastewater effluent containing high concentrations of heavy metals is released into the drainage system and has a negative impact on community soil and groundwater. Groundwater can be polluted due to the infiltration of water containing contaminants into an aquifer (Van Ginkel, 2015; Hartmann *et al*., 2021; Araiza-Aguilar *et al.,* 2020). Heavy metal pollution in rivers can occur by releasing wastewater effluent directly into receiving river water bodies and/or through drainage systems. The content of heavy metals in the Upper Citarum watershed will also affect the quality of groundwater. The interaction between river water and groundwater can occur with groundwater filling river water (gaining stream) through streambed and/or river water filling groundwater (losing stream) through streambed and saturation zone (Safeeq and Fares, 2016), so rivers can be the main source of groundwater recharge (Gao *et al*., 2022). Air pollution can occur due to exhaust gas emissions from industries in the form of particulates in air containing heavy metals and can experience deposition in soil/groundwater (Jarsjo *et al*., 2020; Adnan *et al*., 2022; Masum and Pal, 2020; Karbassi *et al.,* 2015). Air pollution in the industrial environment can affect people living within the vicinity through the absorption of heavy metals into the human body (Manisalidis *et al*., 2020; Zhao *et al*., 2022). About 55.86% of the population in Bandung Regency still use groundwater (shallow well water and pumping wells) as a source of clean water; the use of groundwater containing heavy metals in daily life has negative impacts. Groundwater is used by the community for sanitation and hygiene, and some people still use it for drinking and other consumption purposes. Communities also use other sources of water for drinking, and the quality of water is unknown. Heavy metal contaminants in groundwater can be a source of danger to the surrounding community. Heavy metals in environmental media are very dangerous because they are toxic, persistent in the environment, and bioaccumulative, so they pose a potential threat to public health (Ali *et al*., 2019; Fahimah *et al*., 2020; Mitra *et al*., 2022). Heavy metal exposure pathways from groundwater to the community can occur through ingestion (oral, usually from exposure to the food chain) and dermal absorption (usually from the use of water) (Olawoyin, 2018; Briffa *et al*., 2020). Heavy metals are cytotoxic, hepatotoxic, nephrotoxic, neurotoxic, and carcinogenic (Anyanwu *et al.,* 2018). Heavy metals contribute to many cases of noncommunicable disease (NCDs), including neurobehavioral disorders (lead, mercury), cardiovascular diseases (lead, cadmium), kidney diseases (lead, cadmium), and some cancers (arsenic, chromium) (Nordberg *et al.,* 2015). Heavy metals can also affect the formation of clefts in the lips and palate (Pi *et al*., 2018; Takeuchi *et al*., 2022). The incidence of cleft lip and palate is caused by 22% genetic factors and 78% environmental factors (Loho, 2013). Evidence indicates the association between maternal and paternal exposure to environmental pollutants and the risk of cleft palate in offspring (Hao *et al*., 2015). According to Martin and Griswold (2009), chronic lead exposure can cause autism, kidney damage, brain damage, birth defects, and even death. Several researchers have also found a relationship between stunting and an increase in heavy metals in the body (Gardner *et al*., 2013; Gleason *et al*., 2017). The Upper Citarum watershed has 10 heavy metals, namely, lead (Pb), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), cadmium (Cd), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As) (Sukarjo *et al*., 2021; Sumantri and Rahmani, 2020; Utami, 2009; Fadhilah *et al.,* 2018; BRES, 2018; Komarawidjaja, 2017; Septiono and Roosmini, 2015; Budiman *et al*., 2012; Salam *et al.,* 2019). Despite the presence of various heavy metals in the Upper Ciltarum Watershed, only few reports are available regarding the determination of which heavy metals have the potential to pose a health risk (Septiono and Roosmini, 2015; Thufailah, 2020; Tamang *et al.,* 2020). Bandung Regency Health Profile Data show that the incidence of several types of NCDs increased from 2015 to 2020. Therefore, scholars should study the concentrations of various types of heavy metals in groundwater and determine the chemicals of potential concern (COPC). Analysis of heavy metals is conducted in groundwater prior to its use for sanitation and hygiene; analysis of other water sources is also important to assess the potential cumulative risk that society accepts and other water sources still indicate local pollution. This study is a preliminary work prior to comprehensive examination through epidemiological studies of heavy metals. Results can be used by other researchers who are interested in environmental epidemiology issues that have not been carried out in this study area. In this context, this study aims to determine the concentration and distribution of Pb, Co, Cr, Fe, Mn, Cd, Cu, Zn, Hg, and As in groundwater and other water sources (for people who do not use groundwater for drinking); compare concentration data with the quality of water used for sanitary hygiene and consumption; and determine heavy metals that have high risks to public health (or referred to as COPCs) in adults and children. COPCs were determined based on two exposure pathways, i.e., oral and dermal absorption. The study was conducted in seven sub-districts located in the Upper Citarum River Watershed, Bandung Regency in Indonesia, in 2022.

MATERIALS AND METHODS

Site description and sampling

The research was conducted in the Upper Citarum Watershed, Bandung Regency, precisely in seven sub-districts, namely, Pangalengan, Rancaekek, Ciparay, Pacet, Baleendah, Majalaya, and Soreang. The number of sampling points in seven sub-districts is 160 points spread over 24 points in Ciparay, 27 points in Majalaya, 25 points in Rancaekek, 24 points in Baleendah, 22 points in Soreang, 20 points in Pacet, and 18 points in Pangalengan. The number of samples is not evenly distributed in several sub-districts due to lack of people who use groundwater for daily life in areas with high topography, such as Pacet and Pangalengan Sub-districts, and people are more likely to use mountain springs. From 160 sampling points, 160 groundwater samples were collected at all points and 98 other water sources for consumption were also taken from respondents who use water sources other than groundwater for consumption. The study area covers seven sub-districts located at 6°57’ 30” S–7° 17’ 30’’ S; 107° 30’ 30” E–170° 50’ 0” E (Fig. 1). Overall, the study area is 47,119.98 Ha. Pangalengan has an area of 19,540.93 Ha (41.47% of the total area of the study area), Pacet is 9,193,965 Ha (19.51%), Ciparay is 4,617.57 Ha (9.80%), Rancaekek is 4,524.83 Ha (9.60%), Baleendah is 4,155.54 Ha (8.82%), Soreang is 2,550,679 Ha (5.41%), and Majalaya is 2,536.46 Ha (5.38%).

From 22 March 2022 to 10 April 2022, 160 groundwater samples and 98 consumption water samples (taken from respondents who use water sources other than groundwater for consumption) were collected in the study area. The latitude and longitude at each sampling site were recorded. The study was conducted during the dry season by considering the higher concentrations of heavy metals due to the dilution of rainwater. High rainfall can cause the dilution of heavy metals in groundwater (Long *et al*., 2021; Aithani *et al*., 2020), leading to higher heavy metal concentrations in the dry season than in the rainy season. Written surveys and sampling of groundwater and drinking water were carried out simultaneously in seven sub-districts. The survey forms were distributed at the sampling locations, precisely at 160 sampling points to ask questions related to water source use and its designation. Each respondent signed an informed consent form after receiving a thorough explanation of the survey. The percentages of respondents who use dug wells as water for sanitation and hygiene (SH) in Ciparay, Majalaya, Rancaekek, Soreang, Pacet,

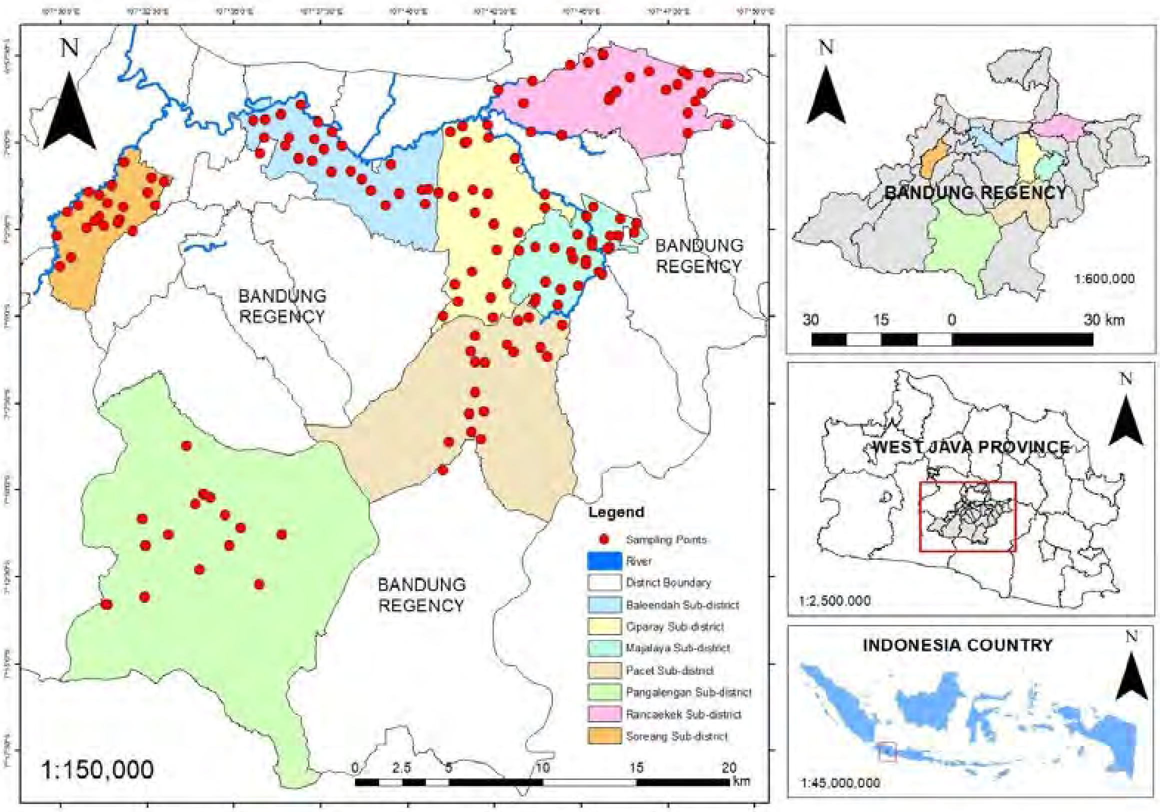

Fig. 1: Geographic location of the research area and distribution of sampling points in the Upper Citarum Watershed, Bandung Regency in Indonesia

and Pangalengan are 70.83%, 77.78%, 64%, 59.09, 60%, and 100% of the respondents, respectively. In Baleendah, the majority of respondents (56%) used bore wells for SH. People rarely use pump wells to meet their water needs for SH and they were only found in Ciparay (8.33%) and Baleendah (16%). The type of groundwater used by the respondent will affect the quality of water; for example, bore well water has better water quality than water from dug wells (Alina and Oshunrinade, 2016; Lestari *et al.*, 2021). Respondents who use groundwater for SH do not necessarily use it for consumption. In addition to groundwater, other sources of water for consumption include refilled water, spring water, and rainwater. Most respondents in Ciparay, Majalaya, Rancaekek, Baleendah, and Soreang use refilled water for consumption, with sequential percentages of 50%, 66.67%, 80%, 88%, and 50%, respectively. Some respondents use groundwater for consumption, that is, 50% in Ciparay Sub-district, 22.22% in Majalaya, 12% in Rancaekek, 12% in Baleendah, and 40.91% in Soreang. In Pacet and Pangalengan, most respondents still use groundwater for consumption (80% and 76.47%, respectively). In addition, some respondents use spring water for consumption, that is, as much as 3.70% in Majalaya. Meanwhile, about 4% of the respondents in District of Rancaekek use rainwater for consumption.

Water sampling and preservation

Groundwater sampling is conducted by taking a momentary sample (grab sample) following the Indonesian National Standard Index (SNI) 6989.58: 2008 concerning Groundwater Sampling Methods. Groundwater sampling is also carried out by SNI (2008) concerning Groundwater Sampling Methods, where a bailer is used to collect groundwater. For deep well water (from drilled wells), sample is collected by opening the well water faucet and flowing it for 1–2 minutes and is placed in a container.

Groundwater samples are collected in 250 mL high-density polyethylene (HDPE) bottles, which are rinsed with the sampled water in advance. Refilled water (gallon water) is sampled by rinsing first the HDPE bottle with sample water and then filling it with the sample 1 to 2 inches from the top (USEPA, 2016). The sampling location is recorded using global positioning system (GPS). Water samples are preserved by adding 6–7 drops of nitric acid (HNO_3) to pH < 2 and placed in a cool box at 4 °C (Standard Method for the Examination for Water and Wastewater 22nd Edition, 2012). Water samples are stored not exceeding the maximum storage time of 6 months for heavy metal analysis (SNI, 2018).

Heavy metal concentration analysis in the laboratory

Sample is prepared at the Laboratory of Industrial Hygiene and Toxicology, Institute Technology Bandung, Indonesia. Sample (digest with HNO_3) is prepared by transferring 100 mL of homogenized water with preservative into a beaker, adding 3 mL of sensitive HNO_3, and covering it with a beaker glass. The beaker is heated, evaporated to less than 5 mL, and then cooled. The walls of the beaker and beaker glass are rinsed with distilled water, added with 5 mL of concentrated HNO_3, and reheated to boiling. Heating is carried out until NO_2 gas is formed (generally indicated by a color change to brownish yellow followed by the reflux process). After cooling, the sample is added with 10 mL of 1:1 HCL and 15 mL of distilled water, reheated for 15 minutes, and cooled again. Beaker glasses are washed again with distilled water and filtered. The filtrate is transferred to a 100 mL volumetric flask (SNI, 2018). The concentration of heavy metals is measured by ICP-OES at the Central Laboratory, Directorate of Research and Community Service, Padjadjaran University, Bandung City, West Java, Indonesia. Prior to measurement, a calibration blank solution and a calibration curve are prepared.

COPC analysis

COPCs are substances that are potentially hazardous to human health. The COPCs identified will be used for further evaluation in the risk assessment process. COPCs are selected from the chemical of interest (COI) by using the following general approach (Woodward-Clyde, 1998; MDEP, 2021).

§ If the concentration of heavy metals exceeds the applicable screening level (SL) or risk-based concentration (RBC), then these heavy metals are considered as COPCs.

§ If the concentration of heavy metals is less than the applicable SL or RBC, then these heavy metals are not considered as COPCs.

RBC or SL is a risk-based concentration (benchmark) from a standard equation that combines the assumptions of exposure information with toxicity data. This RBC equation is adopted from the EPA Superfund Program Risk Assessment Guide from USEPA (2022b). RBC is a useful protector for humans (including sensitive groups). RBCs can help identify areas, types of contaminants, and conditions that require further attention. In this study, the calculated RBC is the RBC on tap water–residents. These receptors are exposed to chemicals in water that is delivered to the residence from sources such as groundwater or surface water. Swallowing drinking water is the proper pathway for all chemicals. Inhalation exposure pathway is considered for volatile compounds but is not considered in the present study. Activities such as bathing and washing contribute to dermal absorption. RBC will be calculated for cancer effects and non-cancer effects and on the pathway of exposure through ingestion (oral) and skin contact (dermal). The equation used is a special equation for inorganic compounds (not organic) because the 10 heavy metals analyzed (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn) are inorganic compounds. The equations used to calculate RBC are as follows:

A. Non-cancer effects

a. Oral (Ingestion) pathway, using Eq. 1 (USEPA, 2022b).

$$\left(RBC_{oral}\right)_{NC} = \frac{THQ\ x\ AT\ x\ BW}{\left(\frac{1}{RfD_o}\right) x\ EF\ x\ ED\ x\ IRW} \tag{1}$$

b. Dermal absorption pathway, using Eq. 2 (USEPA, 2022b).

$$\left(RBC_{dermal}\right)_{NC} = \frac{DA_{event}}{Kp\ x\ ET} \tag{2}$$

Where it is used as Eq. 3 (USEPA, 2022b).

$$DA_{event} = \frac{THQ\ x\ AT\ x\ BW}{\left(\frac{1}{RfD_o\ x\ GIABS}\right) x\ EF\ x\ ED\ x\ EV\ x\ SA} \tag{3}$$

c. Non-cancer effects RBC, using Eq. 4 (USEPA, 2022b).

$$RBC_{NC} = \frac{1}{\frac{1}{(RBC_{oral})_{Nc}} + \frac{1}{(RBC_{dermal})_{Nc}}} \quad (4)$$

B. Cancer effects

a. Oral (Ingestion) pathway, using Eq. 5 (USEPA, 2022b).

$$(RBC_{oral})_C = \frac{TR\, x\, AT\, x\, LT}{CSF_o\, x\, IFW} \quad (5)$$

b. Dermal absorption pathway, using Eq. 6 (USEPA, 2022b).

$$(RBC_{dermal})_C = \frac{DA_{event}}{Kp\, x\, ET} \quad (6)$$

Where, it is used as Eq. 7 (USEPA, 2022b).

$$DA_{event} = \frac{TR\, x\, AT\, x\, LT}{\left(\frac{CSF_o}{GIABS}\right) x\, DFW} \quad (7)$$

c. Cancer effects RBC, it is used as Eq. 8 (USEPA, 2022b).

$$RBC_C = \frac{1}{\frac{1}{(RBC_{oral})_c} + \frac{1}{(RBC_{dermal})_c}} \quad (8)$$

C. RBC Total, it is used as Eq. 9 (USEPA, 2022b).

$$RBC = RBC_{NC} + RBC_C \quad (9)$$

The information or assumptions used for the analysis and the default values used in the above equation are sourced from the USEPA (User's Guide – Risk Assessment - Regional Screening Levels) and USEPA (IRIS – Integrated Risk Information System – IRIS Assessments). Table 1 shows information related to the default values used to calculate RBC non-cancer and cancer effects. Table 2 shows the RfD, Kp, GIABS, and CSF values of each heavy metal.

Based on the results of a review from the USEPA (IRIS – Integrated Risk Information System – IRIS Assessments), the types of heavy metals that are carcinogenic are As, Cd, Cr, and Pb, while Cu, Hg, Mn, and Zn are not carcinogenic. Information on the types of heavy metals such as Co and Fe is not available in the USEPA (IRIS Assessments).

Table 1: Default values used to calculate RBC USEPA (2022c)

Symbol	Information	Unit	Adult	Child
Non-cancer effects				
THQ	Target hazard quotient	Unitless	1	1
BW	Body weight	kg	80	15
AT	Averaging time (365 days/y*ED)	days	9490	2190
IR	Ingestion rate	L/day	2.5	0.78
ET	Exposure time	hours/event	0.71	0.54
EF	Exposure frequency	days/y	350	350
ED	Exposure duration - resident	years	26	6
EV	Resident events	event/day	1	1
SA	Resident surface area water - adult	cm^2	19652	6365
Cancer effects				
TR	Target Risk	Unitless	1×10^{-6}	*
ET	Resident water exposure time	hours/event	0.71	*
DFW	Resident water dermal contact factor	cm^2-event/kg	2610650	*
LT	Lifetime	years	70	*
IFW	Resident drinking water ingestion rate	L/kg	327.95	*
AT	Averaging time	days/y	365	*

Table 2: RfD, Kp, GIABS and CSF values of each heavy metal

Heavy metals	RfD	RfD Source	Volatile	Chemical type	Kp (cm/h)	GIABS	CSF oral	CSF source
As	0.0003	IRIS	No	Inorganics	0.001	1	1.5	EPA IRIS
Cd	0.0005	IRIS	No	Inorganics	0.001	0.05	15	CAL EPA[2]
Co	0.0003	PPRTV	No	Inorganics	0.0004	1	*	*
Cr	0.003	IRIS	No	Inorganics	0.001	0.013	0.5	CAL EPA[2]
Cu	0.04	HEAST	No	Inorganics	0.001	1	*	*
Fe	0.7	PPRTV	No	Inorganics	0.001	1	*	*
Hg	0.00016	Cal EPA[1]	Yes	Inorganics	0.001	1	*	*
Mn	0.14	IRIS	No	Inorganics	0.001	0.04	*	*
Pb	0.0014	Nag and Cummins, 2022	No	Inorganics	0.0001	1	0.0085	Cal/OEHHA
Zn	0.3	IRIS	No	Inorganics	0.0006	1	*	*

RfD = Reference dose (mg/kg-day); Kp = Dermal Permeability Constant (cm/h); GIABS = Fraction of Contaminant Absorbed in Gastrointestinal Tract (unitless); CSF = Cancer Slope Factor (mg/kg-day); CAL EPA[1] = The California Environmental Protection Agency Office of Environmental Health Hazard Assessment; CAL EPA[2] = California Environmental Protection Agency, U.S in Zeng *et al*., 2015; Cal/OEHHA = California Office of Environmental Health Hazard Assessment in Parker *et al*., 2022; PPRTV = Provisional peer reviewed toxicity values; IRIS = Integrated risk information system; HEAST = The EPA superfund program's health effects assessment summary table

Spatial distributions

Heavy metal concentrations are visualized into a spatial distribution map. Interpolation is a process where the value of an attribute in a non-sampled area is predicted using data that can be accessed from another sampling area. Inverse distance weighted (IDW) is the interpolation method used to visualize heavy metal concentrations and evaluate water quality. Interpolation considers the geographic location of the sample points. IDW is an optimal interpolation model used to assess the spatial distribution pattern of heavy metal concentrations in the study area (Saha *et al*., 2022). This IDW interpolation technique assumes that variables that are close to each other are more similar than those that are far apart. This technique analyzes the values obtained around the predicted point to estimate the value for each unobserved point. Measured values closer to the predicted location have a greater impact on projected values than those further away (Saha *et al*., 2022). Eq. 10 is used in this interpretation (Bhunia *et al*., 2018).

$$Z = \frac{\sum_{i=1}^{n}\left(\frac{Z_i}{d_i^p}\right)}{\sum_{i=1}^{n}\frac{1}{d_i^p}} \quad (10)$$

where z is the estimated value at the interpolation point, z_i is the measured value at point i, n is the total number of measured values used in the interpolation, d_i is the distance between the interpolated value and the measured value z_i, and p represents the weighting power that determines how the weight is reduced by increasing the distance.

Statistical analysis

Data are statistically analyzed using the statistical package for the social sciences (SPSS) version 26.0 with correlation analysis to investigate the possibility of a positive or negative relationship between heavy metal concentrations in groundwater. Significance is set at $p<0.05$. Kruskal–Wallis test is used to analyze significant differences in heavy metal concentrations in areas with different topographies, namely, high topography (Pacet and Pangalengan) and low topography (Majalaya, Rancaekek, Ciparay, Baleendah, and Soreang). The test is used for data that are not normally distributed (non-parametric test) and continued with a follow-up test to determine the location of the difference in heavy metal concentrations. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Heavy metal concentration and spatial distribution map

For sanitation and hygiene (SH)

Table S3 shows the statistical characteristics of the

Table 3: Matrix that presents the order of the average concentration of heavy metals in sanitation and hygiene water from highest to lowest

Sub-districts	As*	Cd	Co*	Cr*	Cu*	Fe	Hg	Mn	Pb	Zn*
Ciparay	2	7	5	1	2	2	4	4	6	5
Majalaya	7	2	6	6	6	1	5	6	2	6
Rancaekek	1	1	7	2	5	3	6	5	1	4
Baleendah	4	4	4	5	1	4	1	7	5	1
Soreang	5	3	2	7	7	5	3	2	3	3
Pacet	3	5	3	4	3	6	7	1	4	7
Pangelangan	6	6	1	3	4	7	2	3	7	2

*The concentration complies the water quality standard "Regulation of the Health Minister of the Republic of Indonesia No. 32 of 2017" at 100% samples.
**The concentration complies water quality standard Environmental Working Group - EWG (2021) at 100% samples.

1 2 3 4 5 6 7 = Order from highest to lowest concentration

Average concentration of heavy metals (HMs) < Quality standard and all samples < Quality Standard.
Average concentration of HMs < Quality standard and some samples > Quality Standard
Average concentration of HMs > Quality standard and some samples > Quality Standard

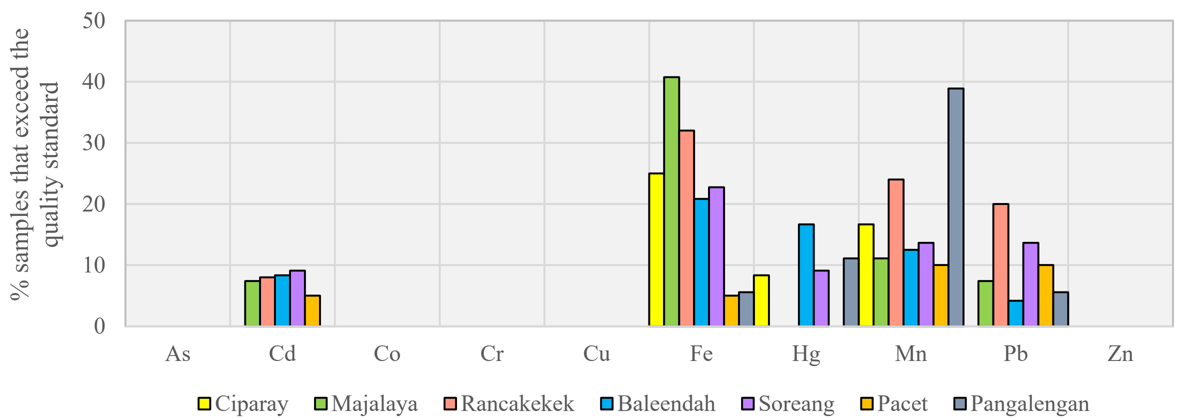

Fig. 2: Percentage of the number of samples whose concentration of heavy metals exceeds the sanitation and hygiene water quality standard in seven sub-districts

analyzed heavy metal concentrations in groundwater used for SH activities. In terms of the minimum and maximum concentration values of the 10 heavy metals, all of them were detected in groundwater samples used for SH; hence, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn are COI in this study. In detail, the order of the average concentration of heavy metals from the highest to the lowest is presented in Table 3 and Fig. 3.

Table 3 shows that in 100% of the SH water samples analyzed, the contents of As, Co, Cr, Cu, and Zn still meets the water quality standards for sanitation hygiene based on the Regulation of the Minister of Health of the Republic of Indonesia No. 32 of 2017, although the average concentration has been sorted from the highest to the lowest. In contrast to Cd, Fe, Hg, Mn, and Pb, some of the samples do not meet the water quality standards for SH. The highest average concentration (±SD, in mg/L; Table S3) of Cd was found in Rancaekek (0.0015±0.0025), Fe in Majalaya (1.7465±3.1291), Hg in Baleendah (0.0135 ±0.0571), Mn in Pacet (1.2254±3.3744), and Pb in Rancaekek (0.0291±0.0506). Fig. 2 shows the percentage of samples whose heavy metal concentrations exceed the SH water quality standard. The heavy metals of concern in groundwater for SH purposes are Cd, Fe, Hg, Mn, Pb and Zn. The level of Cd exceeds the quality standard in five sub-districts, namely, Soreang (9% of groundwater samples exceeds the quality standard) > Rancaekek (8%) = Baleendah (8%) > Majalaya

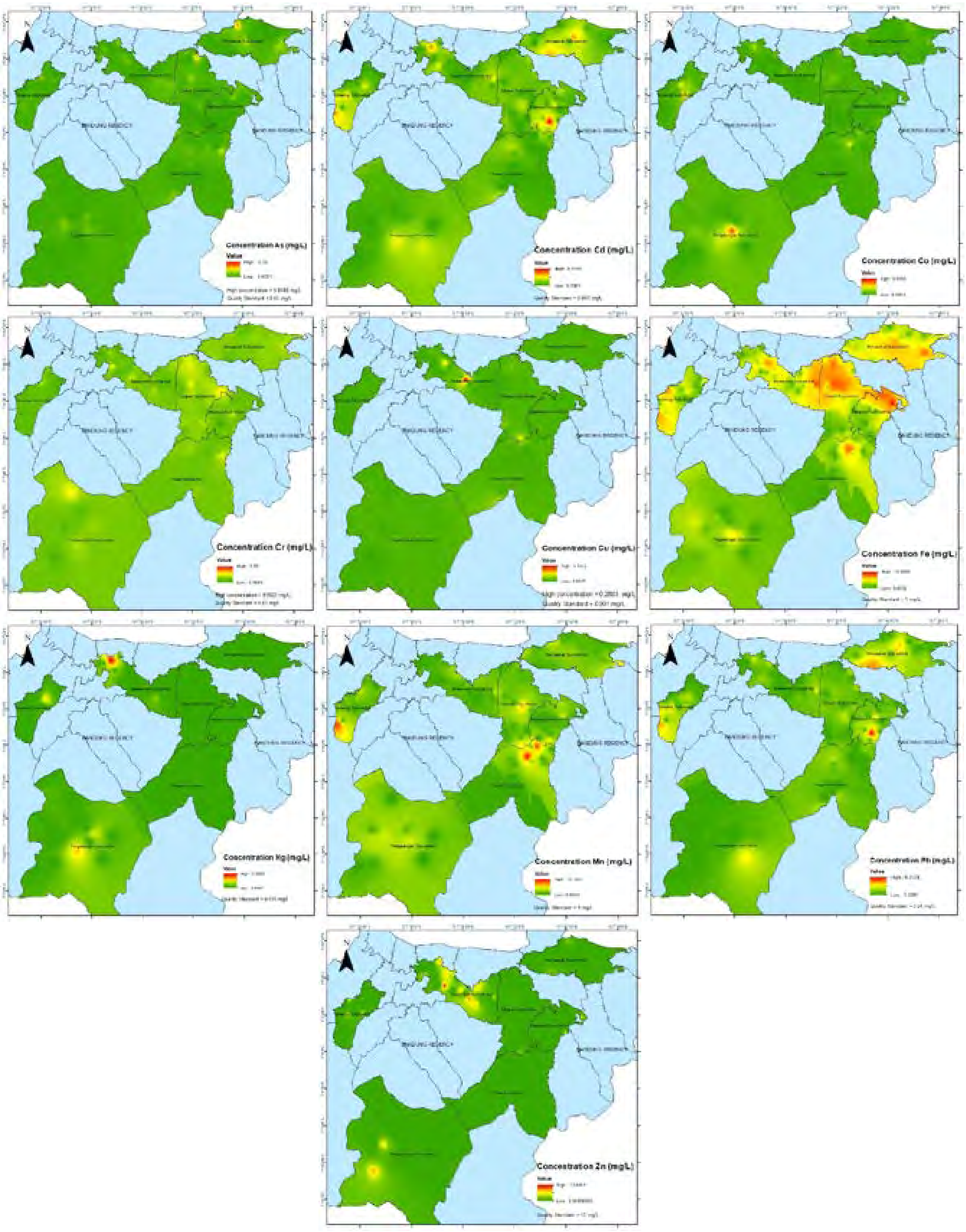

Fig. 3: Spatial distribution map of 10 heavy metals in sanitation and hygiene water

(7%) Pacet (5%); Fe in seven sub-districts, namely, Majalaya (41%) > Rancaekek (32%) > Ciparay (25%) > Soreang (23%) > Baleendah (21%) > Pangalengan (6%) and Pacet (5%); Hg in four sub-districts, namely, Baleendah (17%) > Pangalengan (11%) > Soreang (9%) > Ciparay (8%); Mn in seven sub-districts, namely, Pangalengan (39%) > Rancaekek (24%) > Ciparay (17%) > Soreang (14%) > Baleendah (13%) > Majalaya (11%) > Pacet (10%); and Pb in six sub-districts, namely, Rancaekek (20%) > Soreang (14%) > Pacet (10%) > Majalaya (7%) > Pangalengan (6%) > Baleendah (4%). In particular, although the average concentration of

Mn is the highest in Pacet, the highest percentage of groundwater samples exceeding the quality standard was found in Pangalengan. This finding could be due to the distribution of high Mn concentrations (marked in red in Fig. 3) in Pacet.

Table 3 and Fig. 2 show that Rancaekek, Majalaya, and Soreang sub-districts need attention because of their simular patter their higher average concentrations of Pb and Cd than the other sub-districts. They also have the same pattern (Fig. 3):

1. Rancaekek has the highest average concentrations of Pb and Cd in groundwater; about 20% of groundwater samples exceeded the Pb quality standard and 8% exceeded the Cd quality standard.

2. Majalaya has the second highest average concentrations of Pb and Cd in groundwater; 7% of groundwater samples exceeded the Pb quality standard and 7% exceeded the Cd quality standard.

3. Soreang has the third highest average concentrations of Pb and Cd in groundwater; 14% of groundwater samples exceeded the Pb quality standard and 9% exceeded the Cd quality standard.

Pb and Cd were found to have a significant positive correlation (Table 7; $p<0.05$; $r=0.575$). Pb and Cd in the three sub-districts may have originated from anthropogenic sources. Based on Table S2, these sub-districts have high distributions of the textile industry. The release of large amounts of wastewater containing heavy metals, such as Pb and Cd, is unavoidable from the textile industry because their raw materials are mixed with fibers during the dyeing process (Velusamy *et al*., 2021). Another possible source of Pb and Cd in groundwater is river water. The distribution pattern of high Pb and Cd concentrations is located in the area adjacent to the river (Fig. 3 and Fig. S1). Heavy metal pollution in rivers may enter the groundwater system through infiltration and percolation. The West Java government has implemented the Citarum Harum Program as an effort to improve water quality in the Citarum River and its surroundings. This program has been regulated through Presidential Regulation Number 15 of 2018 concerning the Acceleration of Pollution Control and Damage to the Citarum River Basin. The program includes handling industrial waste, livestock waste, domestic waste, law enforcement, and others. The program succeeds in improving the water quality of the Citarum River, from moderately polluted in 2019 to slightly polluted in 2020 (JOD, 2020). However, heavy metals are non-degradable and persistent in the environment (Ali *et al*., 2019; Jeyakumar *et al*., 2023) and are difficult to clean completely in the environment; this phenomenon may be one of the reasons for the discovery of heavy metals in the present study. In addition, the presence of Hg in Baleendah, Pangalengan, Soreang, and Ciparay needs attention; in particular, Baleendah has the highest average concentration of Hg (Fig. 3), with 17% of groundwater samples exceeding the quality standard (Fig. 2); Hg in Pangalengan has the second highest average concentration, with 11% of groundwater samples exceeding the quality standard; Hg in Soreang has the third highest average concentration, with 9% of groundwater samples exceeding the quality standard; and Hg in Ciparay has the fourth highest average concentration, with 8% of groundwater samples exceeding the quality standard. The high concentrations of Hg in these sub-districts can be sourced from natural and anthropogenic sources. Naturally, Hg originates from volcanoes, geothermal springs, geological deposits, and oceans (USGS, 2019; Karbassi and Heidari, 2015). Given that Hg in the sub-district exceeds the water quality standard, it possibly originates from human activities (anthropogenic). Hg is thought to have come from illegal small-scale gold mining or from burning coal. Globally, artisanal and small-scale gold mining (ASGM) is the largest source of anthropogenic mercury emissions (37.7%), followed by stationary burning of coal (21%) (USEPA, 2022a). Based on Fig. 2 and Table 3, Fe in groundwater also shows a certain pattern from the seven sub-districts studied; it has a high average concentration from order 1 to sequence 4 in Majalaya, Ciparay, Rancaekek, and Baleendah (areas with low topography) and a low mean concentration from order 5 to 7 in Soreang, Pacet, and Pangalengan (high topography) (the concentration distribution pattern can be seen in Fig. 3). Based on the results of the statistical analysis in Table 8, Fe significantly differed among different topographical areas ($p<0.05$). The number of groundwater samples that exceed the quality standard is higher in Majalaya (41%), Ciparay (25%), Rancaekek (32%), Baleendah (21%). and Soreang (23%) than in Pacet (5%) and Pangalengan (6%). In contrast to Fe, Mn has high average concentrations from order 1 (highest) to 3 (Table 3) in Pacet, Soreang, and Pangalengan (high topography) and low average concentrations (orders 4 to 7) in Ciparay, Rancaekek, Majalaya, and Baleendah (low topography); however, the difference in the concentrations is not significant (*p>0.05*; Table 8). When viewed from the percentage of samples that exceeded the groundwater quality standard for SH purposes (Fig. 2), the result has no pattern that can inform factors that influence or the source of Mn in groundwater. Fe and Mn in groundwater have a significant positive correlation (see Table 7, *p<0.05*; *r=0.498),* indicating that they may have originated from the same source (*natural source*) and influenced by topography, soil properties/types, and/or rock formations. The high average concentration of Fe in the present study (especially in Majalaya, Ciparay, Rancaekek, and Baleendah) is thought to be more influenced by topographic factors and soil properties/types. These sub-districts are areas with low topography (Fig. S2) and low slope (Fig. S3) and are dominated by alluvial soil types (soil derived from sediment processes or textured sediments such as clay) (Fig. S4). Meanwhile, Soreang, Pacet, and Pangalengan are located in a high topographic area; the latter two are dominated by andosol soil types (soil originating from volcanic activity), and the former is dominated by latosol soils (soil formed from weathering with high intensity and overgrown by trees). Fe in groundwater may increase with decreasing altitude, indicating that the concentration of Fe is high in areas with low topography. In areas with high topography, the residence time of Fe is relatively lower because dissolved Fe will migrate to areas with lower topography. By contrast, the concentrations of Fe and Mn are higher in areas with low topography because they have a longer residence time in groundwater (Zhai *et al*., 2021). The main mineral in clay is layered aluminosilicate containing Fe. Clay soil contains fine particles and small pores between particles so it is easy to become a reducing environment that is conducive to the reductive dissolution of solid Fe (Zhai *et al*., 2021). The average concentration of Mn is higher in Pacet, Soreang, and Pangalengan than in Majalaya, Ciparay, Rancakekek, and Baleendah. This finding is thought to be influenced by rock formations found in the area. The Mn content is high in limestone or limestone formations (Force and Cox, 1916). Pacet, Soreang, and Pangalengan sub-districts are areas dominated by Plio-Plistocene volcanic rock formations (Fig. S5). Volcanic rock products deposited during the Plio-Plistocene period can be in the form of breccia rocks (MEMR, 2014), which are composed of limestone. Geological factors contribute to the composition of groundwater through the influence of water–rock interactions in aquifer, where Mn in rocks can be released into the aquifer at pH 4–7, namely, the decoupling of Mn (IV) in an insoluble form in rock to Mn (II) dissolved form in aquifers (Kousa *et al*., 2021).

For drinking water or consumption water

Table S4 shows the statistical analysis of the concentrations of heavy metals in drinking water. In detail, the order of the average concentration of heavy metals from the highest to the lowest is presented in Table 4 and Fig. 5.

Table 4 shows that 100% of the consumption water samples analyzed for Cr, Cu, and Zn content still meet the drinking water quality standards in accordance with the Regulation of the Minister of Health of the Republic of Indonesia No. 492 of 2010, although the average concentrations are sorted from the highest

Table 4: Matrix that presents the order of the average concentration of heavy metals in drinking water from highest to lowest

Sub-districts	As	Cd	Co	Cr	Cu*	Fe	Hg	Mn	Pb	Zn*
Ciparay	1	3	4	1	3	1	5	3	4	5
Majalaya	7	5	6	5	5	7	7	6	5	6
Rancaekek	5	1	2	2	4	3	1	4	1	4
Baleendah	4	7	7	7	6	6	3	7	7	1
Soreang	3	6	1	6	7	5	2	5	3	2
Pacet	2	2	3	4	2	2	6	1	2	7
Pangelangan	6	4	5	3	1	4	4	2	6	3

*The concentration complies the water quality standard Regulation of the Health Minister of Indonesia No. 492/2010 at 100% samples

The quality standard for heavy metal concentrations referes to Regulation of the Health Minister of Indonesia No. 492/2010, except for Co which refers to the Agency for Toxic Substances and Disease Registry – ATSDR (2004).

1 2 3 4 5 6 7 = Order form highest to lowest concentration

Average concentration of heavy metals (HMs) < Quality standard and all samples < Quality standard

Average concentration of HMs < Quality standard and some samples > Quality standard

Average concentration of HMs > Quality standard and some samples > Quality standard

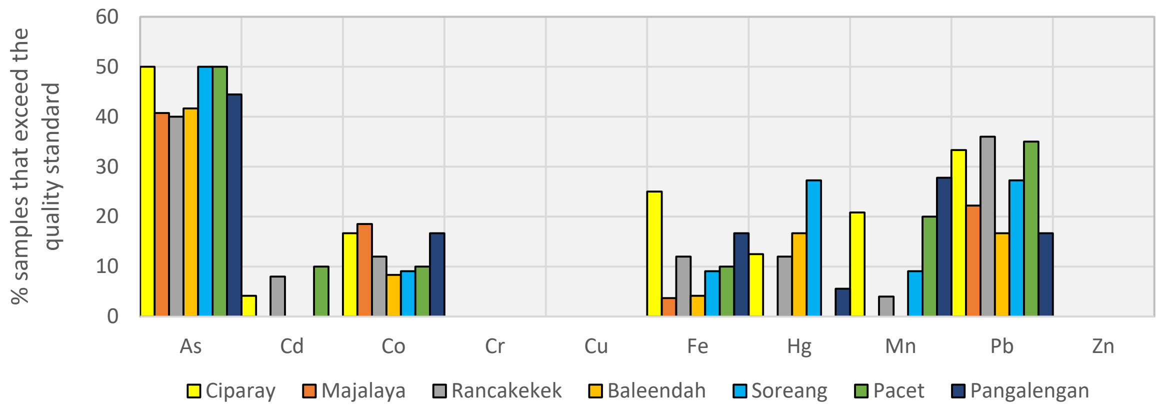

Fig. 4: Percentage of the number of samples whose heavy metal concentrations exceed the consumption water quality standard in seven sub-districts

Table 5: Percentage of total consumption water sample that exceed the quality standard for groundwater and from refill water/spring/rainwater

Heavy Metals	Type of water consumption	Ciparay	Majalaya*	Rancakekek**	Baleendah	Soreang	Pacet	Pangalengan
As	Well water	41.67	18.18	10.00	30.00	27.27	100.00	50.00
	Refill water, etc	58.33	81.82	90.00	70.00	72.73	0.00	50.00
Cd	Well water	0.00	0.00	0.00	0.00	0.00	100.00	0.00
	Refill water, etc	100.00	0.00	100.00	0.00	0.00	0.00	0.00
Co	Well water	75.00	0.00	0.00	50.00	100.00	100.00	100.00
	Refill water, etc	25.00	100.00	100.00	50.00	0.00	0.00	0.00
Fe	Well water	83.33	100.00	33.33	100.00	50.00	100.00	100.00
	Refill water, etc	16.67	0.00	66.67	0.00	50.00	0.00	0.00
Hg	Well water	66.67	0.00	0.00	25.00	16.67	0.00	100.00
	Refill water, etc	33.33	0.00	100.00	75.00	83.33	0.00	0.00
Mn	Well water	60.00	0.00	100.00	0.00	100.00	100.00	100.00
	Refill water, etc	40.00	0.00	0.00	0.00	0.00	0.00	0.00
Pb	Well water	50.00	0.00	11.11	25.00	33.33	100.00	33.33
	Refill water, etc	50.00	100.00	88.89	75.00	66.67	0.00	66.67

* In Majalaya, 1 sample of drinking water from springs did not detect various types of heavy metals

** In Rancaekek, 1 sample of drinking water from rainwater detected As and Pb with concentrations exceeding the quality standard.

to the lowest. The levels of As, Cd, Co, Fe, Hg, Mn, and Pb do not meet the drinking water quality standards in few samples, similar to previous reports (Abeer *et al*., 2020; Hossain *et al*., 2023; Rahman *et al*., 2023). As and Co do not exceed the quality standard for SH purposes (Table 3, Fig. 2) but exceed that of the quality standard for drinking water; this finding is due to the fact that the quality standard for drinking water is stricter than for SH purposes. The highest average concentrations (±SD, in mg/L; Table S4) of As were found in Ciparay (0.0129±0.0108), Cd in Rancaekek (0.0017±0.0047), Co in Soreang (0.0018±0.0041), Fe in Ciparay (0.5528±1.5324), Hg in Rancaekek (0.0232±0.1016), Mn in Pacet (1.2254±3.3744), and Pb in Rancaekek (0.0171±0.0316) (details regarding the spread of high concentrations in Fig. 5). The percentage of samples whose heavy metal concentrations exceed the quality standards for consumption water is presented in Fig. 4.

Among the seven heavy metals that were found to be above the quality standards, As and Pb were detected in the largest number of samples and exceeded the quality standards for drinking water, with average values of 45% and 27%, respectively (Fig. 4). Other types of heavy metals are sorted as follows: Co (13%) > Mn (11.7%) > Fe (11.52%) > Hg (10.57%) > Cd (3.17%). Based on Table 5, As in drinking water is more dominant, with concentrations exceeding the quality standards in water other than groundwater (refill water or rainwater) (90% in Rancaekek >

81.82% in Majalaya > 72.723 in Soreang > 70% in Baleendah > 58.33% in Ciparay > 50% in Pangalengan), compared with groundwater (100% in Pacet > 50% in Pangalengan > 41.67% in Ciparay > 30% in Baleendah > 27.27% in Soreang > 18.18 % in Majalaya > 10% in Rancaekek). Pb with concentrations that exceed the quality standard is more dominant in drinking water from water sources other than groundwater (Table 5). In refill water, the heavy metals detected may be related to the raw water source used at the Refill Drinking Water Depot (DAMIU); the technology used cannot remove heavy metals, and its maintenance process is not optimal. According to Hasanawi and Salami (2022), ultrafiltration (UF) is the processing technology generally used by DAMIU in Bandung Regency as reported by 83% of the respondents, and ozonation is used by 17% of the respondents. The UF process can effectively remove microorganisms and other pathogenic bacteria (Pei and Duo, 2022) and colloidal particles (Nguyen, 2014) but cannot remove heavy metals in certain speciation forms, such as dissolved charged ions (Bernat *et al*., 2007). Hence, UF can remove heavy metals in particulate form (adsorbed on particulates) but cannot remove those in dissolved forms because UF does not have a charged membrane on the surface and its pore size is too large (Nguyen, 2014). In drinking water sourced from groundwater, the sources of heavy metals have been described previously, except for As and Co. The source of arsenic in groundwater is thought to be of natural origin. As shown in Table 7, As and Fe are positively and significantly correlated in the Spearman test, but the correlation is very weak ($p<0.05; r=0.162$). As can bind to Fe(III)-oxyhydroxides or Fe oxides in soils. The reductive dissolution of Fe(III)-oxyhydroxides or iron oxides is the main geochemical mechanism of the release of soil As into groundwater (Maity *et al*., 2011). Arsenopyrite (FeAsS) is the most abundant mineral that contains As and is generally found in the environment and in various rock-forming minerals, such as sulfides, oxides, phosphates, carbonates, and silicates (Smedley and Kinniburgh, 2002). Given the weak correlation between As and Fe, other sources of As in groundwater may exist, such as from textile industry (Singha *et al*., 2021), use of pesticides in agriculture (Hooda, 2010; DHSS, 2013; Kayode *et al*., 2020), use of fertilizers in agriculture (Jayasumana *et al*., 2015), and coal-fired power plants (DHSS, 2013). Furthermore, the source of Co in groundwater is difficult to discuss because the results of the Spearman correlation were not significant compared with those of the other heavy metals ($p>0.05$ in Table 7). The results of the Kruskal Wallis analysis did not show any significant differences based on topography ($p>0.05$ in Table 8), but try to explain based on the dominant land use. Table 3 shows the average concentration of Co in seven sub-districts, where the highest average concentration was found in Pangalengan, Soreang, and Pacet (dominant in areas with a high percentage of agriculture; Table S1) and the lowest concentration was detected in Rancakekek, Ciparay, Majalaya, and Baleendah (predominant in areas with a low percentage of agriculture; Table S1). Thus, Co is thought to originate from agricultural activities, such as from the use of fertilizers. Co is also an essential micronutrient for plant growth (Hu *et al*., 2021). One of the fertilizers sold freely in Indonesia contains 0.27 ppm Co. However, the type of fertilizer used in the study area remains unknown. Rainwater is another source of drinking water for one of the respondents in Rancaekek; the sample has As concentration that exceeded the quality standard. The increased concentration of arsenic (As) is due to mineral dust particles entering the system, when rainwater interacts with the roof catchment (Quaghebeur *et al*., 2019) and may be the reason for the high Pb in rainwater.

Chemical of potential concerns

For sanitation and hygiene (SH)

Groundwater is used by respondents for sanitation and hygiene. Heavy metals in water have the potential to enter the respondent's body through the dermal pathway because water is only used for SH and not used for drinking, so the RBC calculated refers to only the RBC of the dermal pathway and does not consider the RBC of the oral pathway. Based on information from IRIS USEPA, metals that have carcinogenic risks are As, Cd, Cr, and Pb, while Co, Cu, Fe, Hg, Mn, and Zn are not carcinogenic; hence, RBC is calculated only on non-cancer effects and does not consider cancer effects. Table S5 shows the matrix for determining COPCs based on the comparison of the concentration values of heavy metals from SH water with RBC or SL in adults. Table S5 shows that the maximum concentration of As is smaller than that of RBC As (0.0448 mg/L < 1.80298 mg/L), and the same is true for other metals such as Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and

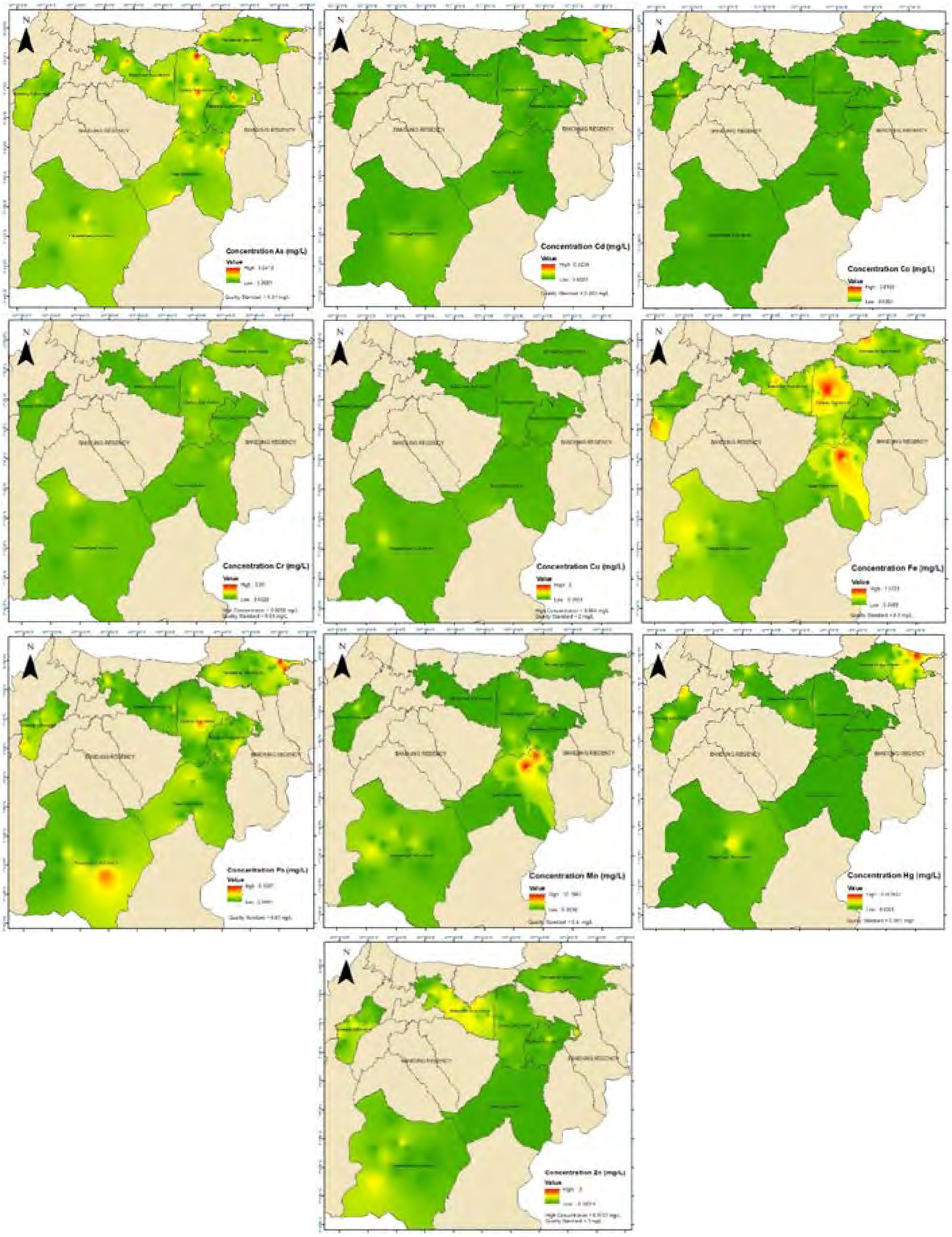

Fig. 5: Spatial distribution map of 10 heavy metals in drinking water

Zn, with values of 0.0157 mg/L < 59.7928 mg/L; 0.0171 mg/L < 4.48447 mg/L; 0.0323 mg/L < 1379.84 mg/L; 0.1414 mg/L < 239.172 mg/L; 14.9385 mg/L < 4185.54 mg/L; 0.2803 mg/L < 0.95669 mg/L; 12,2943 mg/L < 20927.52 mg/L; 0.2588 mg/L < 99.92687 mg/L; and 1.0918 mg/L < 2989.65 mg/L, respectively. All heavy metal concentrations were concluded to be lower than the RBC values, so they were not retained as COPCs.

Skin contact (dermal), in contrast to oral consumption, does not pose a potential risk (Luo *et al*., 2022). As such, determination of COPCs will be continued by calculating RBC from oral intake. In addition, the potential risk of children is higher than that of adults (Gao *et al*., 2019; Xiao *et al*., 2019), so determination of COPCs will be continued by calculating RBC in children. Table S6 shows the matrix for determining COPCs based on the comparison of the concentrations of heavy metals from SH water with RBC or SL in children. Similar to the conclusion of COPCs in adults, Table S6 shows that the concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn in SH water are smaller than the RBC in children. Thus, these metals are not retained as COPCs. The results of determining COPCs cannot be seen only by the comparative analysis of the use of SH water for adults and children. Without considering the risk of oral intake, the heavy metals inferred as COPCs have not been seen because oral intake is the main pathway for potential health risks. Table S6 shows that the order of RBC from highest to lowest: Mn (15929.126 mg/L) > Fe (3185.8253 mg/L) > Zn (2275.5895 mg/L) > Cr (1050.2721 mg/L) > Cu (182.0472 mg/L) > Pb (63.7165 mg/L) > Cd (45.5118 mg/L) > Co (3.4134 mg/L) > As (1.3654 mg/L) > Hg (0.7282 mg/L). The smaller the RBC value is, the more likely it is to pose a risk to human health, so it needs to be maintained as COPCs; however, it will depend on the concentration of heavy metals detected in the field.

For drinking water or consumption water

The RBC of drinking water was calculated for direct contact (oral pathway) with drinking water to determine COPCs posing a risk to human health. These RBCs were calculated in adults (Table 5) and children (Table 6). In drinking water, the RBC on skin contact (dermal) was not considered and only referred to RBC from the oral pathway and RBC cancer effects only.

Table 5 confirms that heavy metals such as As, Cd, Co, Hg Mn, and Pb can be maintained as COPCs because their maximum concentrations are greater than RBC, with values of 0.0416 mg/L > 0.0101 mg/L; 0.0234 mg/L > 0.0167 mg/L; 0.0171 mg/L > 0.0100 mg/L; 0.5090 mg/L > 0.0053 mg/L; 12.2943 mg/L > 4.6720 mg/L; and 0.1229 mg/L > 0.0559 mg/L, respectively. From the 160 samples analyzed, the concentration of As greater than the COPC value is 45.57%, Cd is 0.63%, Co is 1.27%, Hg is 7.59%, Mn is 1.27%, and Pb is 5.06%. Therefore, heavy metals such as As, Cd, Co, Hg, Mn, and Pb have the potential to pose a risk to human health; moreover, some sample points need attention because 45.57% have the potential to pose health risks from As (less than 10%) and from low percentage (Cd, Co, Hg, Mn and Pb). Arsenic concentrations in drinking water to levels that pose a health risk were found in Pakistan (Abeer *et al*., 2020), India (Ravindra and Mor, 2019),

Table 5*:* Matrix for determining COPCs from the comparison of heavy metal concentrations for consumption water with RBC or SL for adults; n = 160

Const.	Max. Conc.	Cancer Effects			Non Cancer Effects			RBC = RBC_C + RBC_{NC} (Screening Level)	Retain as COPC		
		RBC oral	RBC dermal	Calc. Goals	RBC oral	RBC dermal	Calc. Goals		Based on Max. Conc.	Based on 160 samples	
		$(RBC_o)_C$ mg/L	$(RBC_d)_C$ mg/L	RBC_C (mg/L)	$(RBC_o)_{NC}$ mg/L	$(RBC_d)_{NC}$ (mg/L)	RBC NC				
		TCR = 1 x 10-6			THQ = 1					Yes (%)	No (%)
As	0.0416	0.0001	**	0.0001	0.0100	**	0.0100	0.0101	Yes	45.57	54.43
Cd	0.0234	0.0000	**	0.0000	0.0167	**	0.0167	0.0167	Yes	0.63	99.37
Co	0.0171	*	**	-	0.0100	**	0.0100	0.0100	Yes	1.27	98.73
Cr	0.0268	0.0002	**	0.0002	0.1001	**	0.1001	0.1003	No	0.00	100.00
Cu	0.0840	*	**	-	1.3349	**	1.3349	1.3349	No	0.00	100.00
Fe	7.6741	*	**	-	23.3600	**	23.3600	23.3600	No	0.00	100.00
Hg	0.5090	*	**	-	0.0053	**	0.0053	0.0053	Yes	7.59	92.41
Mn	12.2943	*	**	-	4.6720	**	4.6720	4.6720	Yes	1.27	98.73
Pb	0.1229	0.0092	**	0.0092	0.0467	**	0.0467	0.0559	Yes	5.06	94.94
Zn	0.9703	*	**	-	10.0114	**	10.0114	10.0114	No	0.00	100.00

*RBC $_{oral}$ is not calculated because water is used only for SH
**RBC for cancer effects is not calculated because heavy metals is non-carcinogenic

Table 6: Matrix for determining COPCs from the comparison of heavy metal concentrations for consumption water with RBC or SL for children; n = 160

Const.	Max. Conc.	Cancer Effects: RBC oral $(RBC_o)_c$ mg/L	Cancer Effects: RBC dermal $(RBC_d)_c$ mg/L	Cancer Effects: Calc. Goals RBC_c (mg/L)	Non Cancer Effects: RBC oral $(RBC_o)_{NC}$ mg/L	Non Cancer Effects: RBC dermal $(RBC_d)_{NC}$ (mg/L)	Non Cancer Effects: Calc. Goals RBC NC	RBC = RBC_c + RBC_{NC} (Screening Level)	Retain as COPC: Based on Max. Conc.	Retain as COPC: Based on 160 samples Yes (%)	Retain as COPC: Based on 160 samples No (%)
		TCR = 1 x 10-6			THQ = 1						
As	0.0416	*	*	-	0.0060	**	0.0060	0.0060	Yes	63.29	36.71
Cd	0.0234	*	*	-	0.0100	**	0.0100	0.0100	Yes	0.63	99.37
Co	0.0171	*	*	-	0.0060	**	0.0060	0.0060	Yes	2.53	97.47
Cr	0.0268	*	*	-	0.0602	**	0.0602	0.0602	No	0.00	100.00
Cu	0.0840	*	*	-	0.8022	**	0.8022	0.8022	No	0.00	100.00
Fe	7.6741	*	*	-	14.0385	**	14.0385	14.0385	No	0.00	100.00
Hg	0.5090	*	*	-	0.0032	**	0.0032	0.0032	Yes	8.86	91.14
Mn	12.2943	*	*	-	2.8077	**	2.8077	2.8077	Yes	1.27	98.73
Pb	0.1229	*	*	-	0.0281	**	0.0281	0.0281	Yes	9.49	90.51
Zn	0.9703	*	*	-	6.0165	**	6.0165	6.0165	No	0.00	100.00

*) For Children, $RBC_{\text{cancer effects}}$ are not calculated
**) RBC_{oral} is not calculated because water is used only for SH

China (Jiang *et al.*, 2021), and Iran (Maleki and Jari, 2021). The results in children (Table 6) are the same as that in adults (Table 5), that is, the types of heavy metals that are retained as COPCs are As, Cd, Co, Hg, Mn, and Pb, but the percentage of sample points that have potential health risks children are bigger than adults, with values of 63.29% for As, 0.63% for Cd, 2.53% for Co, 8.86% for Hg, 1.27% for Mn, and 9.49% for Pb. Children have underdeveloped immune systems and unique activity patterns that make them more susceptible to heavy metal exposure (Wang *et al.*, 2019). Compared with adult body size, children absorb 40% to 90% more heavy metals ingested (ATSDR, 1990). Low-income children may be at particular risk due to poor diet lacking nutrients that can otherwise help inhibit heavy metal absorption (Bradman *et al.*, 2001). Exposure to the risk of heavy metals through consumption water intake can threaten the welfare of the community; the groups most vulnerable to this risk are children so they require special treatment (Ismael *et al.*, 2022). This study reveals that six of 10 types of heavy metals are chemicals of potential concerns (COPCs) and are sorted based on potential risks to health: As > Hg > Pb > Co > Mn > Cd. Ingestion is the main pathway for potential risk, and children are more likely to be at risk than adults. Heavy metals detected in drinking water can accumulate in the human body and can cause side effects on human health. The bioaccumulation of heavy metals Hg, Pb, Cd, and As has a variety of toxic effects on tissues and organs of the body. They can damage tissues and even organs, reduce organ function, and cause neurological disorders, cancer, and even death (Malik and Sandhu, 2023; Pandey and Kumari, 2023). Hg, Pb, Cd, and As interfere with cellular activities including growth, proliferation (cell cycle repetition), differentiation, damage repair processes, and apoptosis. The mechanism of action for heavy metals is similar, namely, by inducing toxicities including reactive oxygen species (ROS) generation, weakening of antioxidant defenses, enzyme inactivation, and oxidative stress. Heavy metals can induce toxicity in biological systems by binding to sulfhydryl groups and forming ROS. Several toxic metals including Cd and As can cause genomic instability. Defects in deoxyribonucleic acid (DNA) repair after the induction of oxidative stress and DNA damage by Cd and As have been considered to be the cause of their carcinogenicity (Balali-Mood *et al.*, 2021). Carcinogenicity of As, Cd, and Pb may occur due to ROS generation in cells by selectively activating transcription factors, indicating that cell death may be associated with exposure to carcinogenic metals. The mechanism of carcinogenesis induced by As, Cd, and Pb is as follows.

- As can bind to DNA-binding proteins and interfere with the DNA repair process, thereby increasing the risk of carcinogenesis (Engwa *et al*., 2019).

- Generation of ROS by Pb is the key in changing the structure and sequence of chromosomes (Silbergeld *et al*., 2000; Ohiagu *et al*., 2022)
- Cd has been implicated in promoting apoptosis, oxidative stress, DNA methylation, and DNA damage (Engwa *et al*., 2019).

Long-term arsenic exposure can cause skin lesions (pigmentation, keratosis, and skin carcinoma cancer) (Banerjee *et al*., 2023). The effects of Hg poisoning on the human body are not limited to redness of the hands and feet; kidney failure; cardiovascular, liver, brain, and hormonal problems; and intestinal ulceration (Jyothi and Farook, 2020). Hg poisoning causes psychiatric disorders due to central nervous system (CNS) dysfunction and affects listening and speaking disorders. Intention tremor (often shaking) is a disorder that occurs in speech and mouth disorders (Kark *et al*., 1971). Attention-deficit hyperactivity (ADHD) and ASD (autism spectrum disorder) are some of the disorders associated with mental retardation; people who are exposed to Hg also manifest abnormal behavior (Jyothi and Farook, 2020). Co can be absorbed through the gastrointestinal tract and accumulate in the liver, kidneys, pancreas, heart, skeleton, and skeletal muscles. Chronic increases in serum Co may result in adverse long-term biological effects such as immune modulation and oxidative DNA damage. Higher Co concentrations are associated with neurologic, cardiac, hematological, and endocrine toxicity. Higher Co levels in tissues compete with calcium uptake and affect other signaling processes involving hypoxic responses, oxidative stress, and energy metabolism (Uddin and Rumman, 2020).

Statistical results

Based on the results of normality testing with Shapiro Wilk, the concentration of 10 types of heavy metals in groundwater has an abnormal distribution (*$p<0.05$*), so analysis was carried out using Spearman's correlation (non-parametric). The Spearman correlation coefficient between heavy metals in groundwater is presented in Table 7.

In Table 7, a significant positive correlation exists between heavy metals. As is significantly positively correlated with Fe (*$p<0.05$*), but the correlation is very weak (*$r=0.162$*). Significant and weak positive correlations also occur in Co and Mn, Cr and Fe, Cr and Hg, Cr and Mn, Cr and Pb, and Cu and Pb. Cd has a significant and quite strong positive correlation with Pb (*$p<0.05$*; *$r=0.575$*). A strong and significant positive correlation also occurrs between Cr and Cu (*$p<0.05$*; *$r=0.567$*) and Fe and Mn (*$p<0.05$*; *$r=0.498$*). The strong correlations among heavy metals indicate that they may have originated from the same source (Zhao *et al*., 2021).

Kruskal–Wallis analysis was used to test differences in heavy metal concentrations in groundwater in the seven sub-districts (Table 8). Cr, Cu, Fe, and Zn are significantly different among several sub-districts *($p<0.05$).* Fe and Zn are significantly different in areas with high and low topography. High concentrations of Fe and Zn are found in areas at low topography and vice versa. Low concentrations of Fe and Zn are found in areas of high topography. In addition to topographic differences, the two divisions of the region have

Table 7: Spearman correlation coefficient between heavy metals in groundwater

	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Pb	Zn
As	1.000	-0.034	-0.097	0.080	0.035	0.162*	-0.140	0.082	0.105	0.079
Cd		1.000	0.071	0.063	0.106	0.060	0.071	0.094	0.575*	0.070
Co			1.000	0.006	0.040	0.092	0.024	0.266*	-0.022	0.022
Cr				1.000	0.567*	0.327*	0.208*	0.177*	0.238*	0.123
Cu					1.000	0.072	0.113	0.086	0.210*	0.060
Fe						1.000	-0.025	0.498*	0.125	0.101
Hg							1.000	0.043	0.083	0.009
Mn								1.000	0.139	0.024
Pb									1.000	0.090
Zn										1.000

*. Correlation is significant at the 0.05 level (2-tailed).

Table 8: Results of the Kruskal – Wallis analysis to determine significant differences in heavy metal concentrations in groundwater in regions with different topography

Heavy Metals	Kruskal-Wallis H	df	Asymp. Sig.
As	0.009	1	0.923
Cd	1.254	1	0.263
Co	1.465	1	0.226
Cr	0.016	1	0.898
Cu	2.980	1	0.084
Fe*	18.133	1	0.000
Hg	0.057	1	0.811
Mn	2.237	1	0.135
Pb	3.773	1	0.052
Zn*	3.939	1	0.047

*indicates significant value (≤0.05)

different soil types (Fig. S4). In low topography, the dominant type of soil is clay. According to Zhai *et al*. (2021), Fe is found to be high in clay soils and has a lower residence time in high topography because it can migrate to areas with low topography; meanwhile, Zn is suspected of migrating even though the concentrations in all samples do not exceed the quality standard (Table 3). The concentrations of Cr and Cu still meet the quality standards for drinking water and for sanitation and hygiene (Table 3). The concentrations of Cr and Cu significantly differ among several sub-districts ($p<0.05$) and have a strong positive correlation, indicating that they originate from natural sources, such as the main material in soil and/or geological activity. Hence, the significant difference between Cr and Cu is thought to be influenced by the characteristics of each sub-district.

CONCLUSION

This study analyzed water quality through comparison of quality standards in regulations, determined chemicals of potential concerns, and statistically analyzed 10 heavy metals in groundwater used for sanitation and hygiene as well as water sources other than groundwater for consumption. The areas with the highest average concentrations of heavy metals in groundwater for hygiene and sanitation purposes were as follows: in Rancaekek (0.0015±0.0025 mg/L) for Cd, Majalaya (1.7465±3.1291 mg/L) for Fe, Baleendah (0.0135±0.0571 mg/L) for Hg, Pacet (1.2254±3.3744 mg/L) for Mn, and Rancaekek (0.0291±0.0506 mg/L) for Pb. The concentrations of Fe in groundwater for SH at 23%, Mn at 18%, Pb at 9%, Hg at 6%, Cd at 6% of the study area were higher than the maximum allowable limits. Moreover, 46%, 27%, 13%, 11%, 11%, 11%, and 3% of the consumption water samples contained As, Pb, Co, Fe, Hg, Mn, and Cd, and the values were higher than the maximum limit. In drinking water, samples that exceeded the maximum limits of As, Cd, Hg and Pb were dominant sourced from refill water, while Fe, Mn and Co were sourced from groundwater. Thus, As, Cd, Co, Fe, Hg, Mn, and Pb exceeded the quality standards for drinking water, while only As and Co did not exceed the quality standards for water hygiene and sanitation. Arsenic and cobalt quality standards are more stringent for drinking water compared with water sanitation and hygiene. Lead–cadmium and iron–manganese in groundwater showed a positive Spearman correlation ($p<0.05$) and may have originated from the same source. About 100% of the drinking water samples had copper and zinc concentrations that did not exceed the quality standard. Iron and zinc in groundwater differed significantly based on differences in topography and soil types ($p<0.05$). This study reveals that six out of 10 heavy metals are chemicals of potential concern and are sorted based on potential risks to health, that is, arsenic > mercury > lead > cobalt > manganese > cadmium. Ingestion is the main pathway for potential risk, and children are more likely to be at risk than adults. Therefore, evaluating the quality of community drinking water in Bandung Regency should be conducted in collaboration with stakeholders and decision makers, who must immediately take sustainable actions to protect public health; possible actions include determining areas that have a high level of vulnerability of groundwater to heavy metal pollution and are unfit for use as a source of drinking water, considering alternative sources of drinking water, and/or the application of engineering to reduce health risks. Evaluation of water sources, technology, and maintenance processes and checking of water quality before and after technology application from Refill Drinking Water Depots should be conducted on a regular basis to ensure that raw and processing water meets the quality set out in regulations. These processes will accelerate the cessation of use in the event of water quality deterioration and trigger the necessary actions to prevent further deterioration and remedy. People need to be careful in choosing drinking water sources that are free from heavy metal pollution. Communities should be provided with information on how to determine the quality of water suitable for drinking.

AUTHORS CONTRIBUTION

N. Fahimah, the first author and corresponding author, has contributed with conceptualization, data curation, formal analysis, methodology, software, visualization, roles/writing - original draft, writing – review, and editing. I.R.S. Salami, has contributed with supervision, writing – review, editing, and funding acquisition. K. Oginawati has contributed with supervision, writing – review, and editing. S.J.Yapfrine and Y.N. Thaher have contributed with data curation. A. Supriatin has contributed with project administration and data curation.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors. The author's statement there are no human or animal respondents in this study.

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APPENDIX. SUPPLYMENTARY DATA

The following is the Supplementary data to this article can be found from the below link:
https://docs.google.com/document/d/1320cCjD-9myvdB0h36i4EgKGHCW4aR50M/edit?usp=share_link&ouid=110524797063764488087&rtpof=true&sd=true

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ABBREVIATIONS

%	Percent
ADHD	Attention deficit hyperactivity
$(RBC_d)_c$	RBC from dermal absorption route for cancer effects
$(RBC_d)_{NC}$	RBC from dermal absorption route for non cancer effects
$(RBC_o)_C$	RBC from oral route for cancer effects
$(RBC_o)_{NC}$	RBC from oral route for non cancer effects
As	Arsenic
ASD	Autism spectrum disorder
ASGM	Artisanal and small-scale gold mining

AT	Averaging Time
AT	Averaging time
BW	Body weight
C	Cancer effect
CAL EPA	California Environmental Protection Agency
Cal/ OEHHA	California Office of Environmental Health Hazard Assessment
Cd	Cadmium
cm/h	Centimeters per hour
cm^2	Square centimeter
CNS	Central nervous system
Co	Cobalt
COI	Chemical of interest
COPCs	Chemical of potential concerns
Cr	Chromium
CSF	Cancer slope factor
Cu	Copper
DAMIU	Refill drinking water depot
Days/y	Days per year
DFW	Resident water dermal contact factor
df	Degree of freedom
d_i	The distance between the interpolated value and the measured value zi
DNA	Deoxyribonucleic acid
ED	Exposure Duration - resident
EF	Exposure frequency
ET	Exposure time
ET	Resident water exposure time
EV	Resident events
Fe	Iron
GIABS	Fraction of contaminant absorbed in gastrointestinal tract
GPS	Global positioning system
HEAST	The EPA superfund program's health effects assessment summary table
Hg	Mercury
HMs	Heavy metals
ICP OES	Inductively coupled plasma optical emission spectrometric
IDW	Inverse distance weighted
IFW	Resident drinking water ingestion rate
IR	Ingestion rate
IRIS	Integrated risk information system
Kp	Dermal permeability constant
L/day	Liters per day
L/kg	Liters per kilogram
LT	Lifetime
mg/L	Milligram per liter
mg/L	Milligrams per liter
Mn	Manganese
NC	Non cancer effect
NCDs	Noncommunicable diseases
NH4+	Ammonium
p	The weighting power that determines how the weight is reduced by increasing distance.
Pb	Lead
PPRTV	Provisional peer reviewed toxicity values
RBC	Risk based concentration
RfD	Reference of dose
ROS	Reactive oxygen species
SA	Resident surface area water - adult
SD	The standard deviation
SH	Sanitation and hygiene
SL	Screening level
SNI	Indonesian national standard
THQ	Target hazard quotient
TR	Target risk
WWTPs	Waste water treatment plants
z	The estimated value at the interpolation point
z_i	The measured value at point
Zn	Zinc

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ORIGIBNAL RESEARC PAPER

Bioenergy potential of *Chlorella vulgaris* under the influence of different light conditions in a bubble column photobioreactor

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ABSTRACT

BACKGROUND AND OBJECTIVES: Recent investigations indicated that continuous use of fertilizers and pesticides in agricultural fields not only deteriorated soil health but also caused a deleterious effect on surface and groundwater bodies. Treating such wastewater using microalgae has shown higher nutrient removal and biomass efficiency. Moreover, microalgae are proven to be miniature factories that augment the huge potential of biofuel. The aim of this study is to evaluate the different light intensities required for *Chlorella vulgaris* algae to remove nutrients from synthetic agricultural wastewater in a fabricated bubble column photobioreactor. Additionally, the research findings focus on assessing the degradation of organic pollutants and biomass generation under different light conditions.

METHODS: In this study, synthetic agrochemical wastewater was treated in a bubble column photobioreactor with blue, red, sunlight, and white light conditions. The treatment was conducted in a batch process with a hydraulic retention time of 21 days, using light intensity of 1800–2800 luminescence and a temperature maintained at 25–28° degrees Celsius.

FINDINGS: Under different lighting conditions, the blue light condition exhibited a higher biomass concentration of 3.99 gram per liter, with an estimated heat energy value of 1.278 kilojoule per liter. Moreover, in the blue light condition, scanning electron microscopy analysis showed no significant changes in the shape of *Chlorella vulgaris* and energy-dispersive X-ray analysis elemental composition exhibited the lowest oxygen-to-carbon ratio (1.03). Fourier transform infrared spectroscopy was used to illustrate the functional group of microalgae under different lighting conditions. The lipid, protein, carbohydrate, and amino acid contents were 3329–3332, 2116–2139, 1636–1645, and 545–662 per centimeter, respectively. The higher biomass potential from the wastewater treatment shows significant benefit in terms of feedstock and biofuel production.

CONCLUSION: The present investigation identified the nutrient reduction and biomass productivity to be more in blue light condition for *Chlorella vulgaris* algae. The investigation also assessed the potential of lipid, carbohydrate, and protein content in *Chlorella vulgaris*, which indirectly evaluates the biofuel potential of the species.

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INTRODUCTION

In recent years, energy has become a most valuable product, and many research studies are focused on generating sustainable energy for replacing fossil fuel. Conversely, recent investigations indicated that continuous use of fertilizers and pesticides in agricultural fields not only deteriorated soil health but also caused a deleterious effect on surface and groundwater bodies. Specifically, the nutrient-laden runoff from agricultural fields poses a great threat due to the excessive content of phosphate and nitrate, which are carried to natural water bodies (Díaz *et al*., 2012; Khalid *et al*., 2019). This leads to eutrophication in water bodies, causing an ecological imbalance that leads to water pollution. Integrated systems are found to be effective in treating agricultural wastewater and generating biomass, which produces value-added products such as biogas, biofertilizers, and biofuel. Bioenergy and bioeconomy from biomass have great scope to satisfy the need of energy demand in the future (Driver *et al*., 2014; Shahid *et al*., 2019). Compared with constructed wetland treatment, wastewater treatment using microalgae had shown the highest biomass efficiency with a pollution removal efficiency of 80–90 percent (%). Energy recovery from algal biomass has also become one of the sustainable ways for harvesting renewable energy processes (Cai *et al*., 2013; Hoang *et al*., 2022). Because of declining petroleum reserves, increasing fuel prices, and depleting natural resources, renewable energy has become an essential global factor. In recent years, microalgae's biofuel potential had attracted considerable commercial interest due to its carbon-neutral ecosystem and indigenous production (Kunjapur *et al*., 2010; Moshood *et al*., 2021). Microalgae that are capable of growing very rapidly can accumulate bioproducts, and they do not require either large quantities of freshwater or fertile land to grow. Hence the algae can be conveniently grown in municipal or industrial wastewater and can assist in bioremediation (Borowitzka, 1999; Cai *et al.,* 2013). Microalgae also helps in tackling global warming by reducing atmospheric carbon dioxide (CO_2) and serves as an alternative animal feedstock due to its high harvesting index (Chen *et al*., 2011). It is also emphasized that algal biomass can be used as a supplement for proteins to animals as it contains a high protein concentration of 40%–70% (Amaral *et al*., 2020; Maryjoseph and Ketheesan, 2020). Microalgae had a great potential of lipid (fat) accumulation 1%–70% in their cell density and can convert waste organics into bioenergy (H. Kamyab *et al*., 2017). Photoautotrophic microalgal growth depends on light intensity, CO_2, temperature, and nutrient availability in the photosynthesis process (Martínez Sancho *et al.,* 1999). Photobioreactors (PBRs) are enclosed systems that help in the growth of photoautotrophic organisms to treat wastewater without any external containment with the aid of an artificial light source to facilitate photosynthesis (Pulz and Scheibenbogen, 2007). PBRs are of two types: a) an open PBR normally a raceway pond and b) closed PBR, which includes a bubble column, tubular flat plate, and spiral (Acién *et al*., 2017; de Vree *et al*., 2015). Considering the several limitations of open PBR, closed PBR is often preferred for bioremediation. Among different closed PBRs, bubble column reactors are generally preferred because of their simple design and construction. Besides, these reactors consume less floor space, are less prone to contamination, and are efficient in CO_2 utilization (Chinnasamy *et al*., 2010; Gupta, Lee, and Choi, 2015). Because light is an energy source that serves as an environmental factor for developing photosynthetic organisms in bubble column PBRs (BC-PBRs), it can be provided either naturally or artificially using lamps (Pulz and Scheibenbogen, 2007). The use of sunlight as a light source for microalgae is advantageous as it is free, cost effective, and abundant, but its temperature should be considered for microalgal growth systems (Xin *et al*., 2011). However, the duration of day and night periods, changing weather and climatic conditions, nonuniform light intensity, and other seasonal changes may influence the efficiency of the reactor (Singh and Singh, 2015). These drawbacks can be avoided by installing artificial lighting systems with continuous or intermittent illumination in PBRs to enhance biomass productivity (Jung *et al*., 2019). The biofuel ability of certain microalgae can be enhanced by modifying the artificial light supply and factors such as culture conditions, nitrogen depletion, and temperature. Several studies investigated the effects of light, temperature, and CO_2 on the growth of microalgae, such as *Scenedesmus*, *Spirulina platensis*, *Dunaliella salina*, *Nannochloropsis oceanica*, and mixed cultures of the *Chlorella* species (Masojídek and Torzillo, 2014; Mohsenpour *et al*., 2021; Xin *et al*., 2010, 2011). Generally, light-emitting diodes

(LEDs) are more frequently used than traditional light sources for microalgae cultivation. LEDs consume less power and are energy efficient. They are characterized by their long lifespan, less heat or no ultra voilet (UV) emissions, and instant lighting condition. They are also highly reliable in case of system-level failures. Because the absorption bands are seen in blue and red spectral areas of the chlorophyll molecule, LEDs are considered a good source compared with fluorescent light due to their broad visible spectrum. Indeed, microalgae require optimal irradiation conditions with narrow bands of light to maximize their photosynthetic rates, which can be achieved using LEDs (Borella *et al*., 2022). Because microalgae are a photoautotrophic organism, the effects of light, such as white, blue, and red light and sunlight conditions: white light conditions (WC), blue light conditions (BC), red light conditions (RC), and sunlight conditions (SC), respectively on microalgae are investigated to understand its influence in biomass production. The experiments were conducted in BC-PBRs to understand the optimum light intensities and favorable conditions required for the algae for nutrient removal, degradation of organic pollutant, and biomass generation. This study aims to treat high wastewater nutrients using *Chlorella vulgaris* in a BC-PBR and evaluate the effect of different light intensities required for *C. vulgaris* algae to remove nutrients from synthetic agricultural wastewater, assess nutrient pollutant degradation, and determine biomass generation. This study has been conducted in the Environmental Engineering Laboratory, Department of Civil Engineering, SRM Institute of Science and Technology, Kattankulathur, India, in 2022.

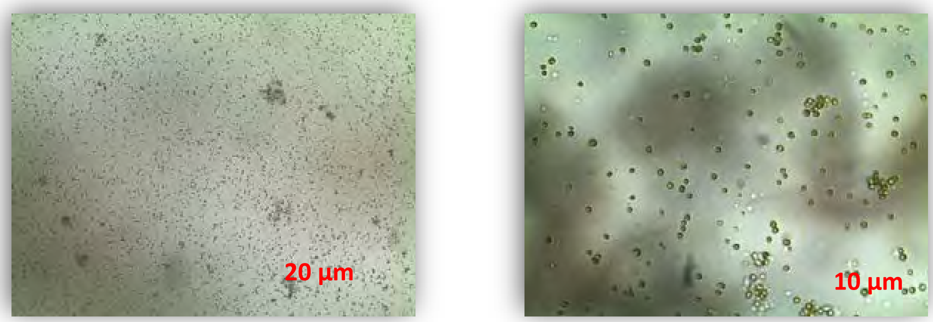
MATERIALS AND METHODS

Microalgae strains and culture conditions

The microalgal strain *C. vulgaris* was considered for this study. The algal strain was obtained from the Annakili Algal Research Institute, Chennai, India. Fig. 1 shows the algal strain culture under 20- and 10-micrometer (µm) magnifications obtained using the Hover labs Trinocular Research Coaxial Microscope. The algae were precultivated using culture medium with the following operating conditions: white LEDs with a light intensity of 1300–1800 luminescence (lux) were used under a light-to-dark period of 16:8. The optimum room temperature varied from 23°C ± 2°C (degrees Celsius) (Tripathy and Kumar, 2022). Bold's Basal medium (BBM) was used to cultivate the strains of microalgae that were then centrifuged (Remi R-8C, India) at 1,957 × *g* for 5 min before being rinsed in deionized water and centrifuged again for 5 min. The suspended microalgae were collected and inoculated in BBM. Later, the cultured microalgae strains were introduced into the BC-PBRs with a 10-milliliter per liter (mL/L) dilution (Sevugamoorthy and Rangarajan, 2023).

Synthetic agrochemical wastewater

Synthetic agrochemical wastewater (SACWW) was prepared by slightly modifying the compositions of ammonium dihydrogen phosphate ($[NH_4]\ H_2PO_4$) and phosphorus pentoxide (P_2O_5). Because SACWW acts as a source of nutrients for microalgal cells,



(a) **(b)**

Fig. 1: Light microscopic images of *C. vulgaris* under (a) 20-µm and (b) 10-µm magnifications

high quantities of ammonium and phosphorus were obtained as per the American Public Health Association (APHA) method in BC-PBRs (Martínez Sancho *et al.,* 1999). Glucose (carbon source) and chemicals such as ammonium chloride (NH_4Cl), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), copper sulfate pentahydrate ($CuSO_4.5H_2O$), and cobalt(II) nitrate hexahydrate ($CO[NO_3]_2.6H_2O$) were also added in BC-PBRs with pH kept near 7. The initial characterization of the wastewater for pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium, and phosphorus were 6.8, 1900, 6500, 90, and 23 mg/L, respectively (Anusha Gowri *et al*., 2022).

Experimental setup and reactor conditions

For the experimental setup, four BC-PBRs with 190-millimeter (mm) inner diameter, 5-mm thickness, and 500-mm depth were made with a transparent acrylic sheet. The reactor had a total volume of 20L. During the treatment process, a liquid and gas volume of 12 and 8 liter (L), respectively, was maintained at a temperature of 23°C ± 2°C in laboratory conditions to ensure uniform luminescence throughout the research period. From previous studies, it is learned that the support system of cylindrical or tubular BC-PBRs occupied more land space (Díaz, Inostroza, and Acién Fernández 2019; Camacho *et al*., 2011), and to overcome this, BC-PBRs were designed to stand on the ground without any external support (Fig. 2). The reactors were exposed to cycles of 16 h of light followed by 8 h of darkness, using external different-colored LED lights emitting BC, RC, and WC; lights providing a medium illuminance of approximately 2 kiloluminescence (klux); and a natural SC. The cultures in BBM were grown in 2-L Erlenmeyer under ideal SC for a photoperiod of 16:8-h light-to-dark phases. The initial cell concentration of microalgae for all lighting conditions in BC-PBR was adopted in the ratio of 1:10 (100 mL of cultured microalgae to 1 L of synthesized wastewater). An air diffuser motor supplying 3-L/min rate of air was used in PBR to prevent the accumulation of algae at the bottom and to ensure complete light supply to the microalgae inside the reactor. (Fig. 2) shows the experimental setup of *C. vulgaris* under different light conditions.

Lighting conditions and its configurations

Light is one of the most important factors in PBR. The light intensity, photoperiod, and light wavelength also constitute vital elements in PBRs (Czeczuga, 1986). LED bulbs were considered for this study. One of the major advantages of LED was its low energy consumption and in providing high illumination. These bulbs are readily available in the market and are 40%–60% cheaper than compact fluorescent light bulbs (Janssen *et al*., 2000; Ra *et al*., 2016; Borella *et al*., 2022). LED lights for three BC-PBRs arranged with 20 diodes spaced at 2-cm intervals and one BC-PBR setup at sunlight were used as the light source for photoperiodic effect for growth of the microalgae for this study (Fig. 2) and their labeling is discussed in (Table 1). LED strips emitting white 450-, blue 465-, and red 660-nanometer (nm) light were evaluated for the effects on algal growth rate with a 12-h light and 12-h dark photoperiod (de Mooij *et al*., 2016; Pulz and Scheibenbogen, 2007; Silva *et al*., 2022).

Analytical techniques

APHA 2012 was used for monitoring the physiochemical parameters, namely, pH, organic pollutants, and nutrients like ammonium and phosphorus concentrations, before and after the treatment. A pH probe (SYSTRONICS, India) was used to monitor the pH variations in the sample. COD and BOD were evaluated using the open reflux method and Winkler's method, respectively. UV spectroscopy operating at 620 and 420 nm was used to monitor phosphorus and ammonium, respectively (Shimadzu UV-VIS 1900i, Japan) (APHA 2012 "WPCF 2012).

Growth estimation

The growth pattern of the inoculated culture was determined using the optical density (OD) at 680 nm (Shimadzu UV-VIS 1900i, Japan) daily for 24 days until the culture reaches its stationary phase.

Biomass analysis

A Whatman filter (GE Healthcare Lifesciences, Grade 4) with a diameter of 125 mm was used to filter the microalgal biomass that had grown in wastewater. The empty oven dry filter paper weight (W_1) and drying the algal biomass at 105°C for 24 h, the dry weight of the filter containing algal biomass (W_2) were measured. The microalgal concentration (mg/L) was obtained using Eq. 1 (Pruvost *et al*., 2009).

$$\text{Biomass analysis (mg/L)} = (W_2 - W_1)/V \qquad (1)$$

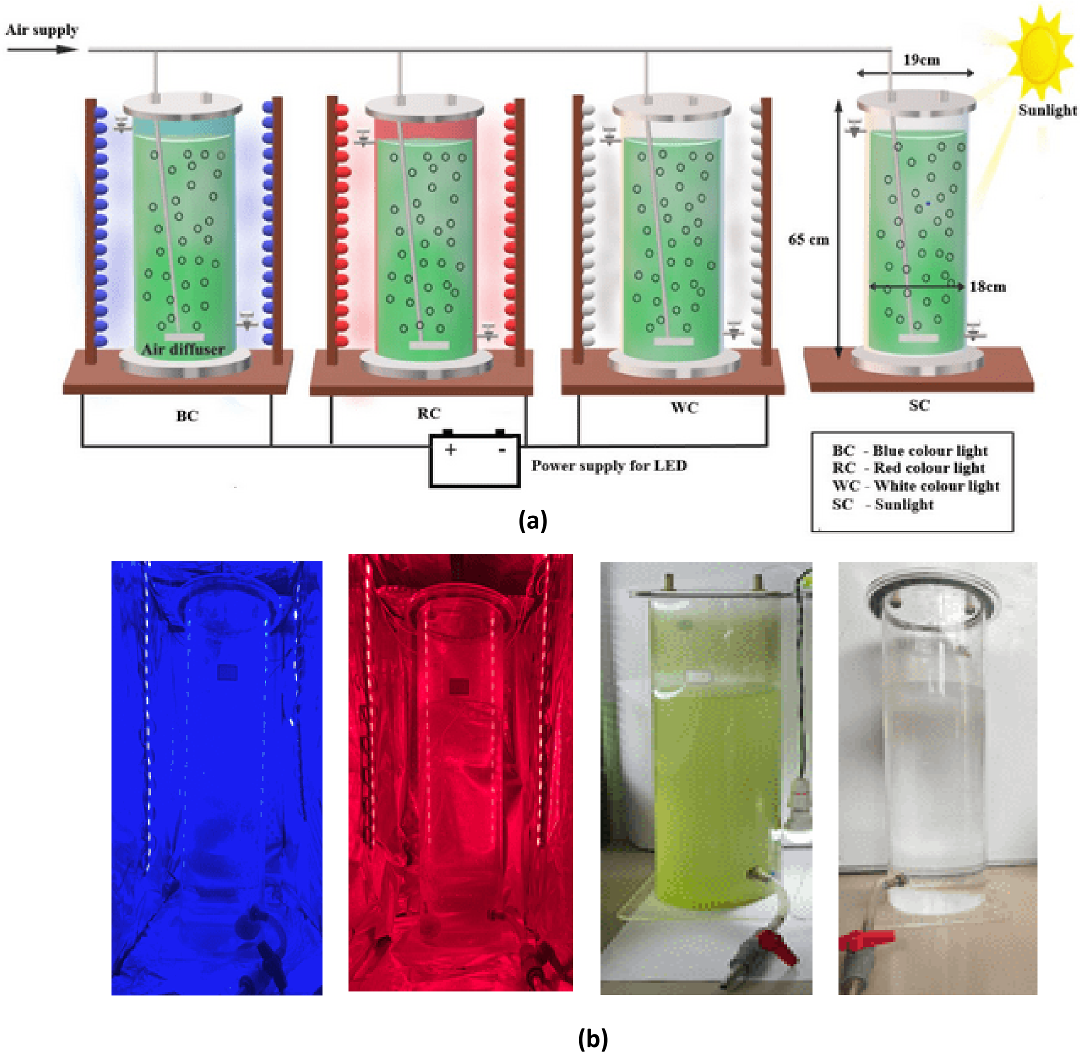


Fig. 2: (a) Pilot scale bubble column photobioreactor (BC-PBR) model for microalgal growth with different lighting conditions and (b) photographic view of BC-PBRs

Where, W_1 is the empty oven dry weight of filter paper, W_2 is the dry algal weight of filter paper (after oven drying at 105°C), and V is the volume of wastewater after the treatment.

Nutrient and organic pollutant removal

The nutrient removal efficiency of ammonium and phosphorus was calculated in alternate days of sampling, and organic pollutant removal was identified by influent and effluent sampling based on Eq. 2 (Pruvost *et al*., 2009).

Nutrient and organic pollutant = (Initial concentration − Final concentration)/(Initial concentration) (2)

where the initial concentration is the influent wastewater before treatment in BC-PBRs and the final concentration is the effluent wastewater from the reactor after the treatment in BC-PBRs.

Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analyses

SEM and EDX analysis images were considered to

Table 1: Lighting parameters and their intensities

Symbol	Lighting conditions	Temperature (°C)	Wavelength (nm)	Light intensity (klx)	pH
BC	Blue light	24 ± 2	400–500	1.8–2.8	6.9–7.2
RC	Red light	24 ± 2	600–700	1.8–2.8	6.9–7.2
WC	White light	24 ± 2	300–400	1.8–2.8	6.9–7.2
SC	Sunlight	28 ± 4	520–700	1.8–4.8	6.9–7.2

illustrate the algal cell morphology under treated and untreated conditions. Algal cells were treated with 2.5% glutaraldehyde and dehydrated using ethanol (30%–100% concentration). The cells were then dried in a hot air oven, mounted on protective film in the molds, and sputtered with chromium. SEM micrographs of algal cells and their elemental composition were captured using Thermo Fisher Apreo S, USA.

Differential scanning calorimetry (DSC) analysis for thermal properties of microalgae

For the DSC analysis, microalgae from the late exponential phase were harvested from the BC-PBRs and were centrifuged using REMI R8C at a rate of 5000 revolutions per minute for 15 min. Later, the biomass from the instrument was collected and washed twice with deionized water and then dried in a hot air oven at 80°C for a day. The dried biomass was then pulverized using porcelain mortar and stored in a desiccator. The stored biomass was then assessed for its combustion property in a Differential Scanning Calorimeter (NETZSCH, Germany) under 10°C/min and 30°C/min with nitrogen gas supply. The correlation between weight loss and its respective temperature was recorded continuously, and the DSC plot was established to determine the calorimetric value of the algal biomass to understand its bioenergy potential.

Fourier transform infrared (FTIR) spectroscopy *measurements*

The FTIR spectra of all algal consortia were investigated to identify the shifts in different functional groups. FTIR spectra were obtained at ambient temperature, using a (Bruker Alpha, Germany), and the FTIR spectrometer wavelength ranges between 500 and 4000 /cm.

RESULTS AND DISCUSSION

Comparison of OD values in different light conditions

The colorimetric method was used for analyzing OD with the range of 680 nm. SACWW was introduced in BC-PBR and was operated in batch mode until it reached the stationary phase. The growth ability of microalgae was monitored under different lighting conditions (Fig. 3) because it enhances the growth cell structure along with nutrients. A comparison of OD values between different light conditions exhibits the growth variation in SACWW. Under BC and RC, OD at 680 nmshows a similar result by achieving a maximum value of 0.34 on the 18th day of treatment, which indicates the higher BC and RC wavelengths of 500 and 600 nm, respectively, with a uniform average intensity of 2 klx. Followed by that, SC showed 0.33 on the 19th day of treatment, although the intensity varied from time to time due to the availability of daylight conditions. WC showed a lesser value of 0.24 on the 17th day of the treatment process. The OD results clearly showed that the algal cells placed in different lighting conditions achieved their exponential growth between the 10th and 17th days.

Comparison of algal biomass in different light conditions

Biomass productivity is one of the major benefits of PBRs. A comparison of algal biomass in different lighting conditions is shown in Fig. 4. The biomass productivity of 4.15 g/L at the end of 24 days obtained in SC was better than that in other lighting conditions. BC produced a biomass of 3.99 g/L, followed by both RC and WC with biomass of 2.55 g/L. Although the experimentation was limited to 24 days, the microalgal growth rate was also found to be in its declining phase after this period. The results confirmed the biomass potential of *C. vulgaris* under all lighting conditions.

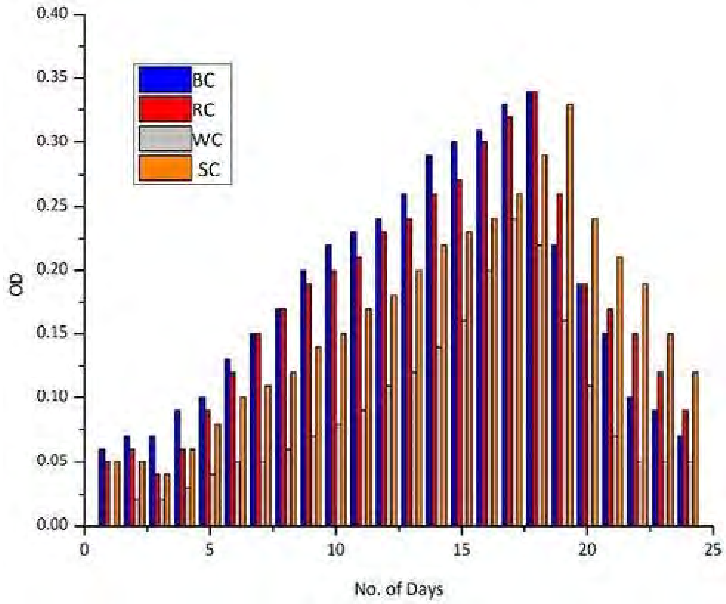


Fig. 3: The OD values of *C. vulgaris* under different light conditions

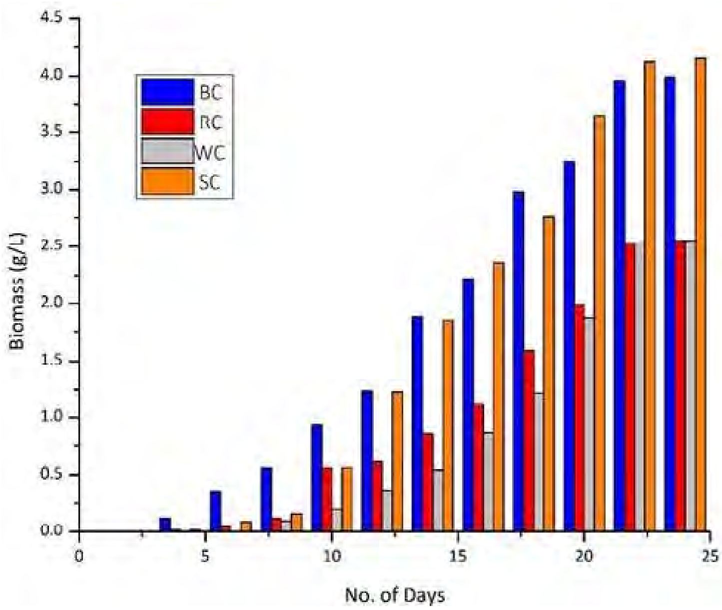


Fig. 4: Biomass concentration of *C. vulgaris* in different lighting conditions

Comparison of nutrient removal under different light conditions

Fig. 5 depicts the significant reduction in the ammoniacal nitrogen concentration in all four lighting conditions. In the first 12 days of exposure, the nutrients present in the wastewater were removed quite similarly in all lighting conditions. The NH_4–N concentration on the 12th day in the BC was reduced from 90 to 14 mg/L, signifying an 85% nutrient reduction in the initial growth phase of microalgae.

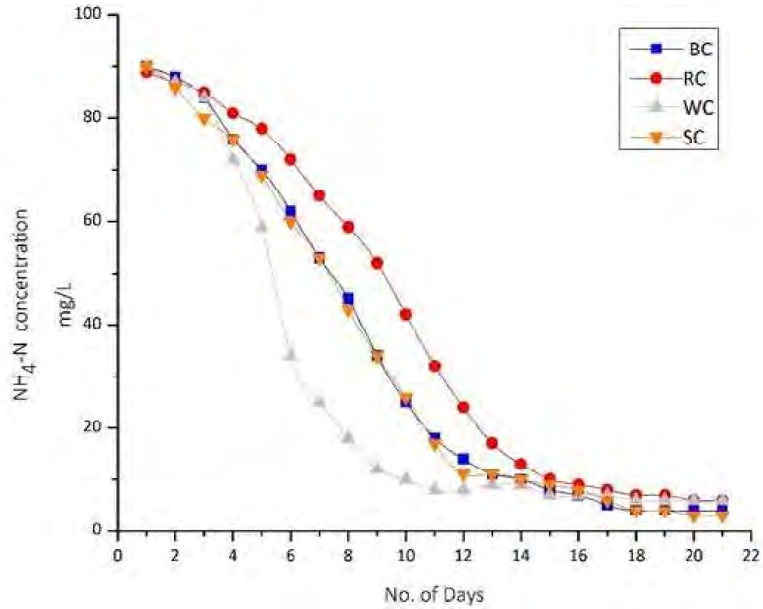


Fig. 5: NH_4–N removal of *C. vulgaris* in different lighting conditions

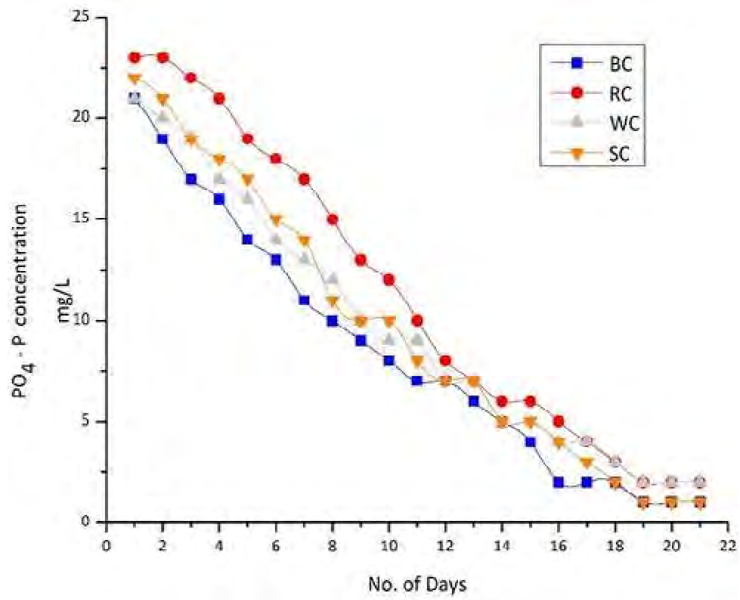


Fig. 6: PO_4–P removal of *C*. vulgaris in different lighting conditions

The overall NH_4–N removal rate was found to be 95.5% in the BC. In SC, the nutrient reduction was 87% at the end of the 12th day with an overall removal rate of 96.6%. RC and WC showed 73% and 91% nutrient reduction on the 12^{th} day, respectively. In all lighting conditions, *C*. *vulgaris* showed significant NH_4–N removal and is found to be significantly high at ~93% from treating SACWW in BC-PBR with a retention time of 24 days.

Fig. 6 shows the variation of PO_4–P in different lighting conditions for the retention period of 24 days. The nutrients present in wastewater

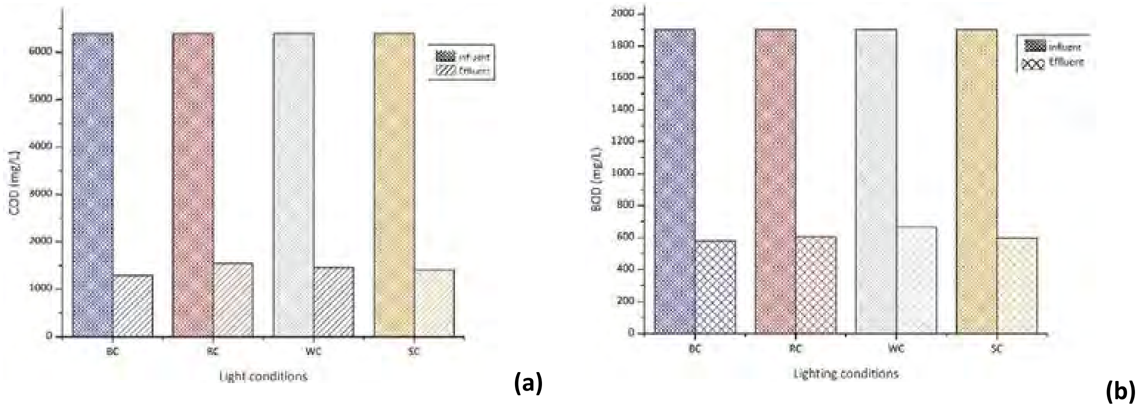


Fig. 7: (a) COD removal rate of *C. vulgaris* in different light conditions and (b) BOD removal rate of *C. vulgaris* in different light conditions of BC-PBRs

Table 2: Comparison of SACWW using *C. vulgaris* in BC-PBRs under different light conditions

Light condition	Initial concentration (before treatment (mg/L)				Final concentration (after treatment (mg/L)				Reduction (%)			
	NH_4–N	PO_4–P	COD	BOD	NH_4–N	PO_4–P	COD	BOD	NH_4–N	PO_4–P	COD	BOD
BC	90	21	6400	1880	4	1	1210	578	95.5	95.2	81	69
RC	89	23	6394	1990	5	2	1340	603	93.2	91.3	79	65
WC	90	21	6389	1910	5	2	1415	665	93.3	90.4	77	68
SC	90	22	6397	1900	3	1	1329	597	96.6	95.4	79	68

were significantly removed in the first 14 days of exposure under different lighting conditions, and the overall removal rate was approximately 90% for the retention period. At the end of 14 days, the PO_4–P concentration was reduced from 21 to 5 mg/L (i.e., 76%) with an overall removal rate of 90% under BC. The nutrient reduction at the end of 14 days and the overall removal rate were found to be 77% and 90%, 73% and 91%, and 76% and 90% in SC, RC, and WC, respectively.

Comparison of COD and BOD removal efficiency under different lighting conditions in BC-PBRs

The COD and BOD represents the organic matter found in the wastewater. The air supplied inside the reactor not only enhances the mixing regime but also initiates the oxidation of organic compounds inside the reactor (Ting *et al*., 2017). Besides, CO_2 fixation and O_2 transformation during biodegradation indirectly enhance the degradation of organic matter in the reactor. Before and after the treatment, the COD removal rate in BC-PBRs under different light conditions was analyzed (Fig. 7a). BC exhibited 81% COD removal, followed by SC with 79%. The COD removal rate was 78% for both RC and WC. The BOD in BC-PBRs analyzed before and after the treatment under different light conditions is depicted in Fig. 7b. The maximum BOD removal rate of 70% was exhibited by BC. The BOD removal rate in SC, RC, and WC were 69%, 68%, and 65%, respectively.

Comparison of pollutant removal before and after treatment under different light conditions using BC-PBRs

Table 2 shows the pollutant removal before and after the treatment of SACWW using *C. vulgaris* under different light conditions. As per the Environmental Protection Rules (1986) standard of India, the concentration of ammoniacal nitrogen should be 50 mg/L, dissolved phosphate should be <5 mg/L, and COD and BOD should be <250 and 30 mg/L, respectively. Although COD and BOD are slightly higher than the stipulated standard, it is required to be treated before being discharged in freshwater bodies. Although microalgae are an efficient medium for treating nutrient pollutants, they have limitations in COD and BOD removal. In general, primary and secondary treatment plants remove 60%–80% of COD and BOD, and when the effluent reaches the tertiary PBR units using microalgae, the remaining organic pollutants will be treated.

Comparison of SEM and EDX analyses under different light conditions

SEM analysis was performed to investigate the variations in surface morphologies of *C. vulgaris* strains before and after the treatment of SACWW under different lighting conditions in BC-PBRs. To investigate the changes in the algal strain after the treatment, SEM images were obtained in different magnifications. Fig. 8 shows SEM and EDX analyses of *C. vulgaris* under BC, RC, WC, and SC after wastewater treatment with 20- and 5-µm magnifications. It is observed that under BC and SC, the algal strains exhibited distinct shapes, rigid cell walls, and

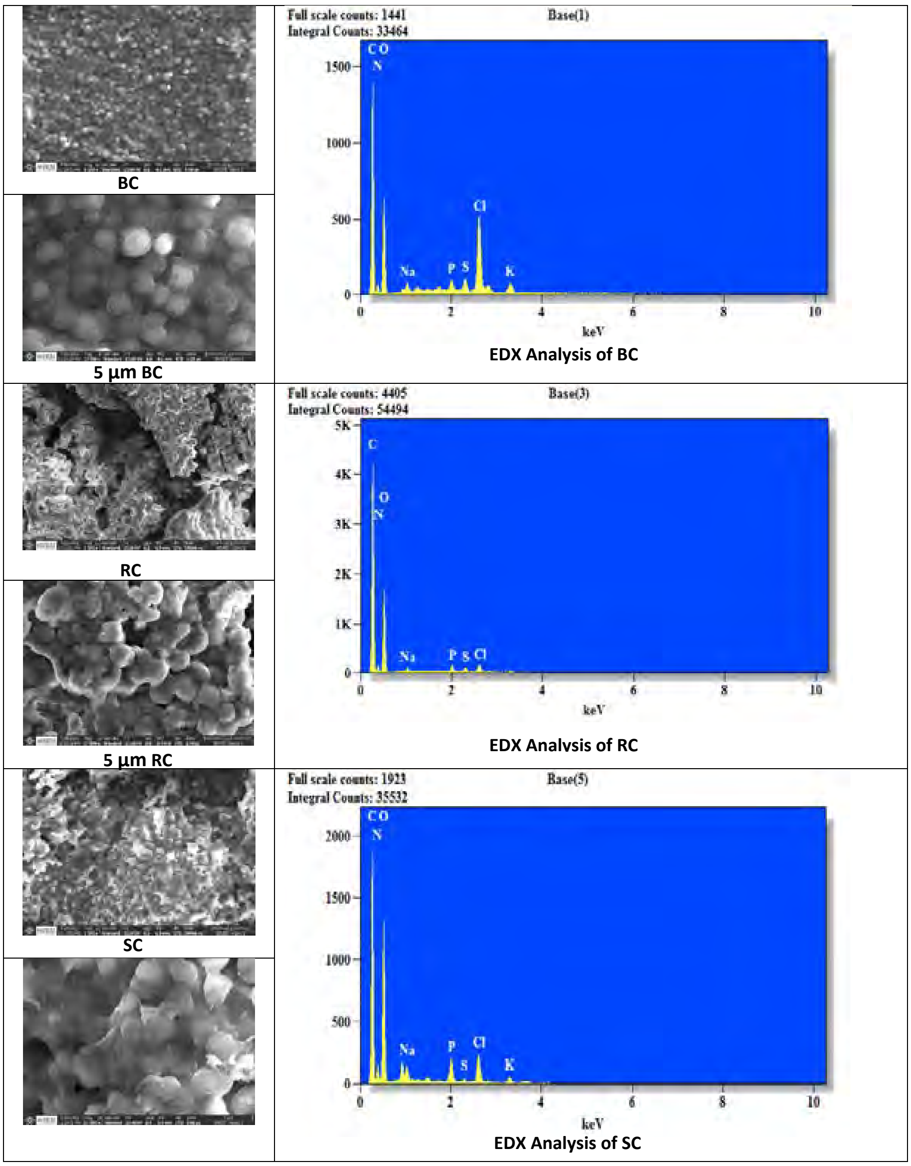
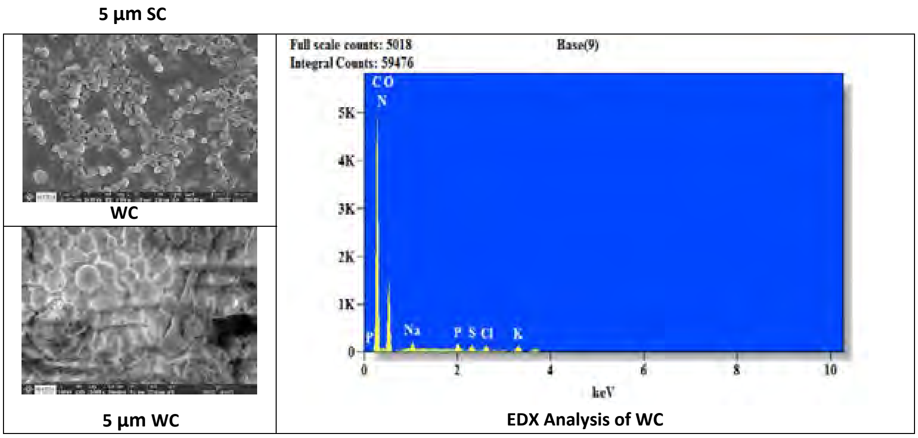


Fig. 8: SEM images of BC, RC, WC, and SC under 20- and 5-µm magnification and EDX analyses of BC, RC, SC, and WC



Continued Fig. 8: SEM images of BC, RC, WC, and SC under 20- and 5-µm magnification and EDX analyses of BC, RC, SC, and WC

smoothness, which were comparatively equal to those of algal cells in a culture condition. Conversely, algal strains under RC and WC displayed a nondistinct shape with slightly damaged cell walls after the treatment, signifying their stressed condition.

Elemental analysis of C. vulgaris *under different lighting conditions using SEM-EDX*

The organic elements for *C. vulgaris* under different light conditions are shown in Fig. 8, which mainly contains carbon (C), oxygen (O), and nitrogen (N). Other insignificant elements such as sodium (Na), chlorine (Cl), phosphorus (P), potassium (K) were also identified. Table 3 shows the percentage weight of elements harvested under different light conditions: the elements C, O, and N ranges between 37.33 and 28.17, 48.23 and 35.51, and 15.85 and 14.71, respectively. In BC, the percentage atomic weight of C, O, and atomic oxygen-to-carbon (O/C) ratio is 34.44%, 43.54%, and 1.03%, respectively, which is slightly higher than the O/C value exhibited by terrestrial crops such as sugarcane bagasse (0.88), corn (0.8), and pine waste (0.88) (Hossain *et al*., 2019). The lowest O/C ratio (1.03%) is achieved in BC, which is equal to the biomass of rice husk (Bousdira *et al*., 2014). Phukan *et al*. (2011) documented that O/C ratios were directly associated with the energy content of solid fuel.

DSC analysis of biomass under different light conditions

DSC analysis under different light conditions is shown in Table 3. In a heating cycle, the microalgae exhibits peak stretches, which indicate the decomposition process, with its respective enthalpies. The harvested biomass from BC-PBRs is evaluated for its energy potential, and the exothermic events with enthalphy (ΔH) are shown in Table 4. The peak ΔH was observed as 308.2 J/g at 115.6°C, followed by a second exothermic event of 50.96 J/g at transition temperature of 197.1°C under RC. But the biomass generation was high under BC, which is shown in Fig. 4. Overall, BC showed a high estimated enthalpy of 1.278 kilojoule per liter (kJ/L). Although algal biomass has bioenergy potential, the results infer that light conditions will have a significant effect on their nature.

FTIR spectroscopy of C. vulgaris *under different lighting conditions*

FTIR spectroscopy assessed the properties of *C. vulgaris* before and after treatment under different light conditions with various characteristic functional groups in the range of 4000–450/cm, as shown in Fig. 9. FTIR spectra indicated the presence of lipid, protein, carbohydrate, and amino acid stretching vibration peaks in cells at 2800–3300, 1700–2800, 1500–1700, and 700–500/cm, respectively. The determination of lipid, carbohydrate, and protein content using FTIR has been well documented by many researchers (Sharma *et al*., 2018, 2019). In a previous study, Sharma *et al*. (2018, 2019) documented the characteristic peaks of O–H, C–H, C≡C, C=O, and N–H amide after wastewater

Table 3: Elemental analysis of biomass from BC-PBRs under different light conditions using SEM-EDX

Elements	Light conditions	Weight (%)	Atomic (%)
Carbon (C)	BC	34.44	43.54
	RC	34.88	41.53
	SC	28.17	35
	WC	37.33	44.60
Oxygen (O)	BC	35.51	33.70
	RC	45.34	40.52
	SC	48.23	44.98
	WC	41.22	36.98
Nitrogen (N)	BC	14.71	15.95
	RC	15.85	16.19
	SC	15.30	16.30
	WC	15.26	15.63
Chlorine (Cl)	BC	10.22	4.38
	RC	1.79	0.72
	SC	3.74	1.58
	WC	1.16	0.47
Sodium (Na)	BC	0.67	0.44
	RC	0.42	0.26
	SC	0.51	0.33
	WC	1.14	0.71
Phosphorus (P)	BC	1.22	0.60
	RC	0.95	0.44
	SC	2.27	1.09
	WC	1.15	0.53
Sulfur (S)	BC	1.55	0.73
	RC	0.78	0.35
	SC	0.40	0.19
	WC	0.86	0.39
Potassium (K)	BC	1.69	0.66
	RC	-	-
	SC	1.38	0.53
	WC	1.88	0.69
O/C (%)	BC	1.03	0.77
	RC	1.30	0.97
	WC	1.71	1.28
	SC	1.10	0.82

Table 4: DSC analysis of biomass from BC-PBRs after treatment under different light conditions

Light conditions	ΔH (J/g)			Temperature (°C)		Biomass generated (g/L)	Estimated ΔH (kJ/L)
	1st peak	2nd peak	Total	1st peak	2nd peak		
BC	283.4	37.02	320.42	112.2	194.8	3.99	1.278
RC	308.2	50.96	358.98	115.6	197.1	2.55	0.915
SC	252.7	46.44	299.47	105.4	198.9	4.15	1.242
WC	278.4	-	278.4	105.4		2.55	0.71

treatment to be 3300, 2900, 2100, 1600, and 600/cm, respectively. In this study, after treating SACWW with different light conditions, the exhibited peak of the O–H, C≡C, C=O, and N–H functional groups was in the range of 3329–3332, 2116–2139, 1636–1645, and 545–662/cm, respectively (Miglio *et al.*, 2013).

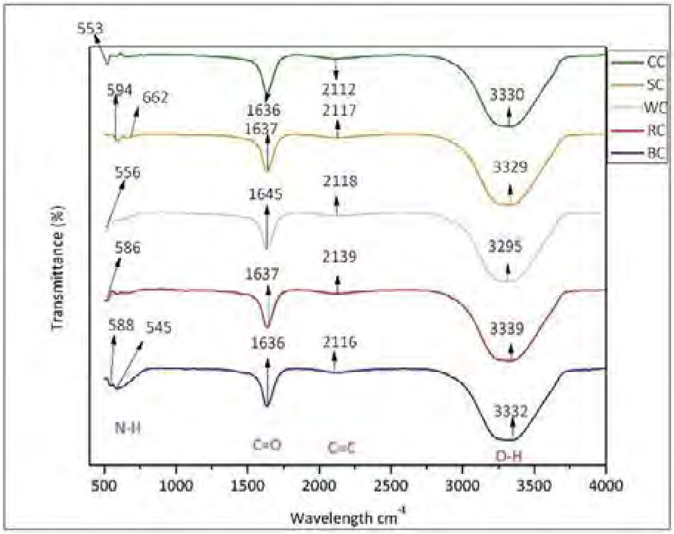


Fig. 9: FTIR spectroscopy of *C.* vulgaris under different light conditions after SACWW treatment

CONCLUSIONS

In this study, *C. vulgaris*, a blue–green microalgal species that have a tendency to treat wastewater, were cultivated under different light conditions, namely, BC, RC, SC, and WC, in BC-PBRs to treat SACWW. Among the four different lighting conditions, *C. vulgaris* under BC was found to be the most efficient for treating synthesized agricultural wastewater. The algae exhibited the highest growth rate under BC (400–500 nm), followed by SC (520–700 nm), with more effective treatment processes compared with those of RC (600–700 nm) and WC (300–400 nm). OD values clearly showed the exponential growth of algae falls between the 10th and 17th day irrespective of the lighting conditions. SC (91%) showed the highest COD removal rate, followed by BC, RC, and WC with a removal rate of 81% and 78%, respectively. In BOD removal, BC and SC showed similar degradation (~70%), followed by RC (68%) and WC (65%). The maximum ammonium nitrate (NH_4–N) and phosphorus (PO_4–P) removal rates were found in SC (96.6% and 91%, respectively), followed by BC (95.5% and 90%, respectively), RC (73% and76%, respectively), and WC (91% and 90%, respectively). SEM and EDX analyses in SC and BC indicated a more rigid cell structure and higher O/C ratio after SACWW treatment. FTIR spectroscopy of algal consortia helps to understand the biochemical functional group of microalgae before and after treatment, revealing their potential for biofuel production. Vibration peaks of lipids, proteins, carbohydrates, and amino acids in cells were 2800–3300, 1700–2800, 1500–1700, and 700–500/cm, respectively. The application of LEDs in algal culturing has become quite common. This study investigated the biomass growth under different-colored LEDs and demonstrated the bioenergy potential of algal biomass as a resource for biofuel production due to the high lipid, carbohydrate, and protein content in *C. vulgaris.* The biomass produced from the algae can be converted to biofuel, such as bioethenol and biobutanol, which has a huge potential to convert light energy into sustainable bioenergy through wastewater treatment. Although nutrient removal is the primary focus of the research work, the generation of algal biomass as a supplementary by-product enhances the biofuel capability of the algae.

AUTHOR CONTRIBUTIONS

S. Dhanasekar, the first author, has contributed in conceptulization, experimentation, data analysis, manuscript preparation and interpolation of results. R. Sathyanathan corresponding and second authour has contributed supervising and revising the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
°	Degree
-	Single bond
=	Double bond
≡	Triple bond
°C	Degree Celsius
°C/min	Degree Celcius per minute
ΔH	Enthalpy
~	Approximately equal
Abs	Absorbance
APHA	American public health association
BBM	Bold's Basal Medium
BC	Blue light conditions
BC-PBRs	Bubble column photobioreactors
BOD	Biochemical oxygen demand
C	Carbon
C. vulgaris	Chlorella vulgaris
Cl	Chlorine
/cm	Per Centimeter
cm	centimeter
CO_2	Carbondioxide
COD	Chemical oxygen demand
d	Day(s)
DSC	Differential Scanning Calorimetery
e.g	Exempli gratia (for example)
EDX	Energy dispersive X-ray analysis
eq	Equation
FTIR	Fourier transfrom infra red spectroscopy
g	Gram (s)
g/L	Gram(s) per liter
H	Hydrogen
i.e.	Id est (that is)
K	Pottasium
J/g	Joules per gram
kJ/L	Kilojoule per liter
Klux	KiloLuminescence
km	Kilometer
L	Liter
L/D	Light/Dark
LED	Light emmiting diodes
lx	Luminescence
m	Meter
m^2	Meter square
mg/g	Miligram per gram
mg/L	Milligram per liter

mL/L	milliliter per liter
mm	Millimeter
mm/g	Millimeter per gram
min	Minute
N	Nitrogen
Na	Sodium
$NH_4H_2PO_4$	Ammonium dihydrogen phosphate
NH_4-N	Ammonium nitrate
nm	Nanometer
No.	Number
O	Oxygen
O/C	Atomic oxygen-to-carbon ratio
OD	Optical density
P	Phosphorous
pH	Potential of hydrogen
P_2O_5.	Phosphorus pentoxide
PBRs	Photobioreactors
PO_4-P	Phosphate
RC	Red light conditions
rpm	Revolution per minute
S	Sulphur
SACWW	Synthetic agrochemical wastewater
SC	Sunlight conditions
SEM	Scanning electron microscopy
UV	Ultra voilet
UV–Vis	Ultravoilet-Visible spectroscopy
V	Volume
Viz	Videlicet
W	Weight
WC	White light conditions
µm	Micrometer

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CASE STUDY

Healthcare waste characteristics and management in regional hospital and private clinic

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ABSTRACT

BACKGROUND AND OBJECTIVES: Good health and a safe environment are essential for sustainable development, including the appropriate management of healthcare wastes. The study intends to assess the generation rate and management methods of healthcare waste in the regional hospital center and a private clinic in Tangier, Morocco, with a focus on potential risks to health workers from infectious diseases.
METHODS: The study collected data on healthcare waste generation over a period of two months by measuring and analyzing general and hazardous waste using an electronic scale. The data was presented as averages in kilograms per bed per day and as percentages. A questionnaire was provided to 100 healthcare workers. It included questions on their sociodemographic characteristics and their knowledge and attitudes regarding healthcare waste management.
FINDINGS: The case study revealed that the healthcare waste production in the two institutions varied, with the private clinic producing 0.76 kilograms per day per bed and the regional hospital center producing 1.84 kilograms per day per bed. The survey also discovered that the hazardous fraction of waste generated in the regional hospital center was 40 percent, which was much higher than the World Health Organization's estimation. The daily amount of hazardous waste generated increased from 260.49 kilograms to 436.81 kilograms post-COVID-19. The survey found gaps in knowledge, attitudes, and daily challenges in waste management practices among the health workers in both facilities.
CONCLUSION: The survey findings suggest that the healthcare waste management methods in Tangier are unsafe and may endanger the health workers and patients. The study found that the lack of monitoring and control contributed significantly to noncompliance with good practices. These findings can be used by the regional divisions of the Ministry of Health to develop specific protocols for managing sanitary emergencies and perform routine observation and training at all levels in the two facilities studied.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
51	7	4

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INTRODUCTION

Healthcare activities are essential for global public health. The rising number of hospitals and medical facilities has increased the healthcare waste (HCW) generation (Gunawardana, 2018). The quantity of HCWs has increased in many nations due to the spread of the coronavirus disease (COVID-19) (WHO, 2022; Tamang *et al.,* 2020). As a result, HCW generation in China (Wuhan) increased significantly from 40 tons to 240 tons per day (Ilyas *et al.,* 2020). Prior to the pandemic, in India, waste production averaged 1.93 kilograms per bed per day (kg/bed/day). A profound increase in waste production (7.76 kg/bed/day) was recorded during the outbreak (Gowda *et al.,* 2021). Turkey recorded an average waste production of 1.34 kg/bed/day in 2021, whereas it was 0.86 kg/bed/day in the pre-COVID-19 period (Hanedar *et al.,* 2022). The volumes of HCW generation have increased during the past two years in Morocco (Khazraji *et al.,* 2022). The national average estimated HCW increased from 1.5 to 3 kg/bed/day (Bouzid *et al.,* 2016). Decree No. 2-09-139 took effect on May 21, 2009, and acted for the laws governing the classification, collection, transportation, and disposal of HCW (El Morhit *et al.,* 2021). HCW includes all the wastes generated by the hospitals, medical centers, clinics, and laboratories (Gunawardana, 2018). It is categorized into general or nonhazardous waste, such as household waste (Mugabi *et al.,* 2018; Masum and Pal, 2020) which is generated by dietary, pharmacy, and administrative departments. The second category is hazardous waste, which includes infectious wastes, anatomical wastes, sharps, pharmaceutical, chemical, radioactive wastes, and many other physically harmful substances (Andeobu *et al.,* 2022). The World Health Organization (WHO) estimates, 75%-85% general waste and 15%-25% hazardous waste are produced (Alwabr *et al.,* 2016). Approximately 80% of the general waste is mixed with a hazardous component (Uloma *et al.,* 2022), which amplifies the rate of waste generation and increases threats to the safety of health workers and patients (Al-Khatib *et al.,* 2020 ; Araiza-Aguitar *et al.,* 2020). Workers in contact with hazardous medical waste (HMW) are at the highest risk of acquiring human immunodeficiency virus (HIV), hepatitis B and C, and bacterial infections (Begum *et al.,* 2021). According to the WHO reports, in developing countries, 21 million new cases of hepatitis B, 2 million new cases of hepatitis C, and 260.000 new cases of HIV were caused by unsafe injections with sharps (contaminated syringes and needles), which represented 32% of all new hepatitis B infections, 40% of all new hepatitis C infections, and 5% of all new HIV infections (Pépin *et al.*, 2014). Various other health problems, including genital deformities, hormone-related cancers, neurological disorders, asthma, and dermatitis, have been reported, which put healthcare workers, patients, and the public at risk (Javid and Manoj, 2019). Healthcare workers includes the primary handlers of HCW, and their role in appropriate waste management is crucial. Good practice depends on their level of expertise and attitude toward HCW management (Deress *et al.,* 2019). Singh *et al.* (2022) showed that a significant amount of HCW generated in healthcare facilities in countries with transitional economies is inadequately handled. Only 45% of health workers were aware about appropriate HCW management. A recent study has shown that the private sector has difficulty implementing national and international protocols, with particularly negative responses from healthcare workers compared with the other sectors (Alzghoul *et al.,* 2022). Previous investigations conducted in various healthcare facilities in Morocco have revealed that the health workers lack adequate knowledge about efficient procedures of waste handling and separation. Bajjou *et al.* (2019) highlighted the importance of establishing a biosafety policy and conducting training programs. Oussibrahim and Nadir (2020) demonstrated a significant deficiency in internal waste management systems, as well as gaps in regulatory knowledge among the staff. In their case survey, Mbarki *et al.* (2013) showed that the management practices in the hospitals selected did not adhere to the guidelines of the Moroccan Decree. An assessment of the current medical waste management systems is crucial for national policymakers to define specific protocols. The data regarding the volume of HCW production in the city are inadequate and require updating. The present study aimed to assess the quantity of medical waste and the effectiveness of the waste management system. The waste generation rate was investigated in two health institutions: the public regional hospital center (RHC) and a private clinic. Then, the knowledge, status, and practices of health workers in the HCW management were investigated. This study is expected to provide a different perspective in developing a strategy for HCW management. The study was conducted in the RHC and a private clinic in Tangier, north of Morocco in 2022.

MATERIALS AND METHODS

Study location

The RHC is regarded as the city's most important public health institution, with a 250-bed capacity and several departments. It offers services to other provinces. The private clinic (44-bed capacity) provides pregnancy services and care for infants. Each location has been visited for two months to conduct interviews, observations, and discussions. In the studied institutions, HMW collection, transport, and treatment are provided by the accredited private company ATHISA.

Waste production rate

Obtaining precise information on the medical waste generation rate is critical to improve techniques for appropriate waste management. Bakiu and Durmishaj (2018) affirm that the common norm for weighing the amount of HCW is kg/bed/day. Waste collection and separation were performed by cleaning agents twice a day for four weeks to obtain the typical waste quantity. Wastes were separately disposed in a particular container, where the type of healthcare unit is identified. The containers were removed and measured on an electronic scale. In this study, no anatomical, radioactive, or chemical wastes were included. A descriptive analysis was conducted using computer software (Excel) for data summary and configuration into necessary graphs. The quantitative data are expressed as averages, kilogram per week (kg/week), kg per day (kg/day), kg/bed/day, and percentages. The HCW daily production (P) per bed was determined using Eq. 1 (Bouzid *et al.,* 2016).

$$P = \frac{\frac{Q}{OR \times LC}}{D} \tag{1}$$

Where, the quantity of HCW (Q) is measured in kg; the occupancy rate (OR) is measured as %; the litter capacity (LC) represents the total number of beds in the facility; and (D) represents the number of days.

Socio-professional survey

An anonymous questionnaire was provided to 100 healthcare workers (depending on availability and willingness to participate), including doctors, nurses, and sanitation workers, via a hard copy and an electronic version of Google forms to evaluate their practices, behaviors, and knowledge regarding HCW management. The questionnaire was designed to evaluate the characteristics including in the following sections:

Section A: Included items on sociodemographic information: profession, age, sex, and years of experience.

Section B: Consisted of questions on HCW management based on many factors, such as understanding of legislation references, definition of the word "waste," risk assessment for healthcare professionals, and risk of disease transmission. The questions covered issues including waste management training, worker vaccination status, attitudes, and practices related to HCW labeling and color-coding, and challenges faced in efficient waste management.

RESULTS AND DISCUSSION

Production of healthcare waste

The quantity of waste generated were recorded using sheets made specifically for this purpose and expressed in kg/week, kg/bed/day scale, and % for total general and hazardous wastes (infectious, pathological waste, and sharps). Table 1 shows the quantity of HMW generated in various healthcare facilities of the RHC of Tangier.

In the first level, a high amount of HMW was found to be generated by patients in the critical care unit (3.40 kg/bed/day), followed by those in resuscitation room (3.12 kg/bed/day), hemodialysis (1.60 kg/bed/day), emergency service (1.12 kg/bed/day), and pediatric surgery (0.37 kg/bed/day). Intensive care and resuscitation room (reserved for critically ill patients) services compose the hospital interface; they can handle the most extreme situations, which requiere personal protective equipments (PPE), supplies, medical devices, and therapies that produce a sizeable amount of HMW. The emergency and hemodialysis services use a uniform infrastructure, enabling simultaneous management of several patients, thereby increasing the daily output of HMW, which mostly comprises dressing care products, plaster waste, and PPE. The HMW production rate (kg/bed/day) in different care units varied depending on

Table 1: Hazardous medical waste production in different units of the regional hospital

Healthcare unit	HMW (kg/week)	Active bed	HMW (kg/bed/day)
Emergency	102.24	13	1.12
Intensive care	190.15	8	3.40
Resuscitation	109.24	5	3.12
Woman surgery	91.74	24	0.55
Man surgery	82.80	29	0.41
Pediatric surgery	78.12	30	0.37
Hemodialysis	90.05	8	1.60
Maternity	168.89	50	0.48
Pediatric/Neonatology	105.90	33	0.45
Traumatology	91.19	26	0.50
Neurosurgery	85.12	24	0.51

Table 2: Total generation of general and hazardous waste per week and the ratio average (kg/bed/day)

Hospital	Week	General waste (kg/week)	HMW (kg/week)	Total waste (kg/day)	OR (%)	Ratio average (kg/bed/day)
Regional	Week 1	1960.00	1193.12	436.81	94.5	1.84
	Week 2	1890.23	1416.83			
	Week 3	2000.60	1290.20			
	Week 4	2050.98	1302.46			
Private	Week 1	153.00	10.74	18.60	55	0.76
	Week 2	110.65	17.15			
	Week 3	120.56	16.62			
	Week 4	106.13	23.22			

activities, specificity of services, and LC. The large LC at the level of the services reduces the importance of the quantity of HCW. The quantity of HMW generated as a result of the maternity service was the largest of all units, at approximately 170 kg/week. The quantity suggests merely 0.48 kg/bed/day with an overall 50-bed capacity. The amount collected remains within the national average regarding the care and treatment activity performed in these services.

Table 2 shows that the average quantity of HCW generated at the RHC was 1.84 kg/bed/day with a high litter OR at 94.5%. The RHC of Tetouan City (Morocco) recorded an average waste generation rate of 3.82 kg/bed/day; the hospital has a higher LC (330 beds) with litter OR at 66% (Raoui *et al.,* 2018). During the COVID-19 pandemic, the average waste generation rate at the King Abdullah University Hospital in Jordan is 3.95 kg/bed/day in terms of 100 active beds (Abu-Qdais *et al*., 2020). The total amount of HCW and HMW (kg/day) generated have doubled as compared with those before the pandemic. The amount of HCW has increased from 260.49 kg/day to 436.81 kg/day, whereas the hazardous fraction has increased from 78.14 kg/day to 173.42 kg/day in the RHC (Ouzekhti Yettefti *et al.,* 2019). The outcomes of the present study are analogous to the previous findings by Khazraji *et al.* (2022) made at the Provincial Hospital Center Moulay Abdellah in Sale, Morocco; the daily production increased from 266 kg to 433 kg in the post-pandemic period. The widespread use of PPE as a preventive measure against the second wave of the COVID-19 pandemic and the provisional inclusion of surgical intervention units in the RHC have increased the workload for the new staff of the University Center Hospital (UCH), including resident and intern physicians, faculty members, and nurses. Morocco's public health facilities are currently working at capacity. The total waste collected was 18.53 kg/day in the private clinic, and the average rate was 0.76 kg/bed/day with 55% of litter OR. Oduro-Kwarteng *et al.* (2021) found that the average waste produced in a private hospital specializing in gynecology in Ghana was 1.34 kg/bed/day. The average rate less than that was found by Rani and Rampal (2019) in the gynecology and obstetrics hospital department with an average of 0.31 kg/bed/day.

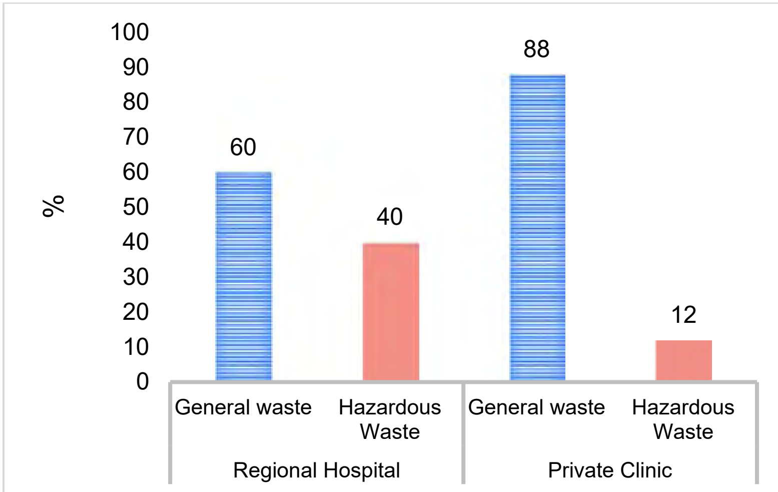


Fig. 1: Distribution of general and hazardous medical waste

Fig. 1 demonstrates that the general waste quantity collected daily for a month is more than the HMW amount collected for the same time in the two institutions. At the RHC, 60.30% of the waste was general, and 39.70% was hazardous. Al-Khatib *et al.* (2019) found that the HMW was in the range of 42%–55%. The RHC generates greater amounts of HMW than the private clinic. It might be primarily explained by several variables, including LC (Zamparas *et al.*, 2019), bed OR, instrumentation level, and healthcare category (Al-Momani *et al.,* 2019). In the private clinic, 88.11% of the waste was general, and only 11.82% was hazardous; these results were consistent with the WHO estimation (WHO, 2014).

Socio-professional survey

The purpose of conducting a socio-professional survey was to better understand the participants' backgrounds and experiences. This information can be used for decision-making and identifying areas of improvement.

Demographic characteristics of participants

The study included 100 participants from various departments of the two hospitals, and were categorized depending on their occupation, age, sex, and seniority of position (Table 3).

In the present study, most participants were nurses (49.60%), and the nursing staff frequently come into contact with medical waste. A total of 37.50% of the participants aged less than 30 years, 35.40% aged 30-39 years, and 25.40% aged 40-49 years, whereas only 3.40% of them were older than 50 years. Approximately 65.10% of the participants were females, and 34.90% were males. More than half (53.30%) of the participants from the RHC had between five and 14 years of work experience, 28.30% had more than a year of work experience, and 6.70% of them had less than a year in their position. Whereas 53.80% of the private clinic participants had seniority between one and four years, 20.60% between five and 14 years, and 20.50% less than a year.

Knowledge of HCW management

Table 4 assesses the knowledge of the participants regarding HCW management – legislative references, risk assessment, definition of "waste," and risk of disease transmission – to determine their needs and offer them training and courses to fulfill those needs.

Knowledge of legislative references

Among the 100 participants, 70 from both institutions lacked knowledge regarding national regulations, whereas only 29 participants were familiar with the regulations, including law 28_00 addressing the HCW administration.

Table 3: Demographic characteristics of participants

Characteristics		Regional hospital (sum= 60) Staff (%)	Private clinic (sum=40) Staff (%)
Profession	Doctors	18.30	17.50
	Nurses	46.70	52.50
	Nursing major	11.70	7.50
	Caregiver	13.30	10
	Sanitation staff	6.70	12.50
Age	<30	15	60
	30-39	58.30	12.50
	40-49	23.30	27.50
	>50	3.40	-
Sex	Male	44.80	25
	Female	55.20	75
Seniority of position	Less than year	6.70	20.50
	Between one and four years	28.30	53.80
	Between five and nine years	25	10.30
	Between 10-14 years	28.30	10.30
	More than 15 years	11.70	5.10

Table 4: Knowledge of healthcare workers regarding HCW management

Variables	Frequency % (N)		Total
	Public	Private	
Knowledge of legislative references			
Yes	45% (24)	12.8% (5)	29
No	60% (36)	87.2% (34)	70
Significance of the word "Waste"			
Dirt	61.7% (37)	69.2% (27)	64
Unusable residue	66.7% (40)	46.1% (18)	58
Worthless/Valueless objects	55% (33)	30.8% (12)	45
Disposable items	76.7% (46)	79.5% (31)	77
Risk assessment for the health workers			
Biological	95% (57)	50% (20)	77
Radioactive	61.7% (37)	22.5% (9)	46
Chemical	86.7% (52)	37.5% (15)	67
Psychological stress	50% (30)	52.5% (21)	51
Infectious disease risk	96.7% (58)	92.5% (37)	95
Risk of disease transmission			
Hepatitis B	95% (57)	66.7% (26)	83
Hepatitis C	85% (51)	51.3% (20)	71
HIV	50% (30)	89.7% (35)	65
Others	1.7% (1)	23.2% (9)	10

Definition of "Waste"

According to the findings, 77 participants from both institutions viewed medical waste as something that can be eliminated, 64 considered it as dirt, 58 viewed it as residue and useless, and 45 perceived it as worthless.

Risk assessment of health workers

In general, 94.6% of the participants recognized the risk of infectious diseases, 72.5% were aware of biological risks, 51.25% acknowledged the psycho-emotional problems associated with medical waste contamination, and only 42.1% were cognizant of the radioactive risks.

Risk of disease transmission

In both hospitals, the participants' knowledge of different types of disease transmission revealed that the awareness level of HIV and hepatitis C and B is above 50%. The transmission of other infections (COVID-19, tuberculosis, and tetanus) is at 23.2% for the private clinic and 1.7% for the RHC staff.

Status, attitudes, and practices of the HCW management

HCW management is a critical component for ensuring patient safety and preventing the spread of infectious diseases in healthcare settings. The status, attitudes, and practices of health workers in HCW management affect patient outcomes and the overall quality of the care provided. Understanding these factors can help healthcare organizations identify areas of improvement and implement effective strategies for HCW management.

Status of healthcare worker vaccination

The status of vaccination of health workers is the key indicator of the overall health and safety of the workplace. Most private clinic participants (77.50%) were unvaccinated against hepatitis B, C virus, and tetanus. Whereas 71.20% of RHC participants stated they had received all recommended doses of hepatitis B and C and tetanus vaccines. By contrast, 28.80% of the participants were unvaccinated (Fig. 2). Approximately 60% of total participants reported cases of blood exposure accidents. This estimate was higher than that reported by a study conducted in Ethiopia, where the exposure prevalence was 46.5% (Semere *et al.,* 2021). Previous investigations showed that the main blood-borne infection to which the health workers were exposed was hepatitis B (Deuffic-Burban *et al.,* 2011; Yuanyi *et al.,* 2022). Complete vaccination of the workers of the studied institutions is necessary to prevent infections (Maltezou *et al.,* 2019).

Status of the training of the healthcare workers

As depicted in Fig. 3, 74.40% of the private clinic staff had never benefited from any training on medical waste management; it could be attributed to most of the clinic staff having a maximum of four years of work experience. On the contrary, 46.70% of the RHC healthcare workers had already received training on medical waste practices.

Attitude and practices on HCW labeling and color-coding

The attitude toward the color coding of various waste categories was determined to demonstrate that the medical staff can adequately separate HCW in the hospital (Letho *et al.,* 2021).

The medical staff from the private clinic displayed a positive attitude toward color-coding (Fig. 4). The percentages of correct responses about the use of yellow bins and various colored plastic bags were 52.40%, 82.20%, and 60.40% (Fig. 4). A survey found that 69.1% of healthcare workers accurately categorized waste into color-coded containers (Jalal *et al.,* 2021). By contrast, 35.10% of the RHC staff responded that sharp waste was disposed into yellow bins, 31% knew that red

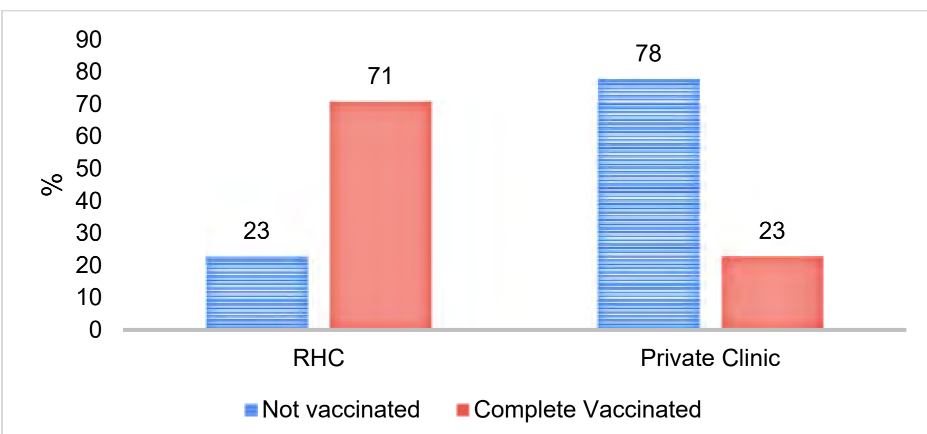


Fig. 2: Status of health workers' vaccination in the regional hospital and the private clinic

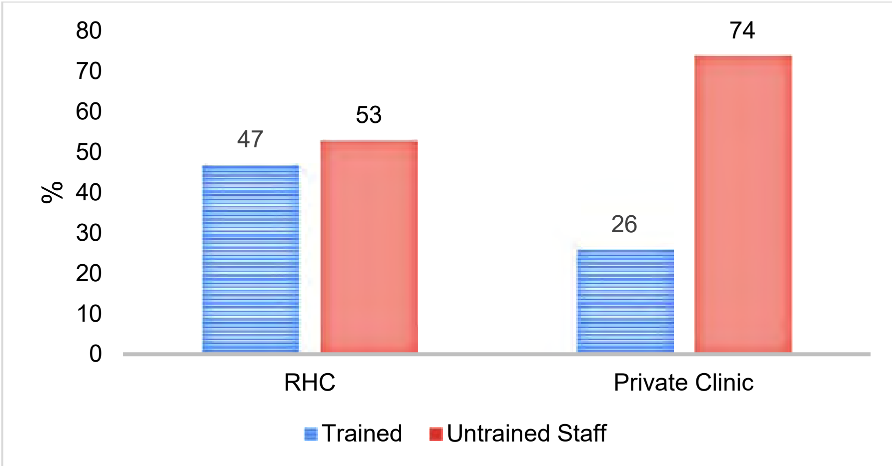


Fig. 3: Status of health workers' training in the regional hospital and the private clinic

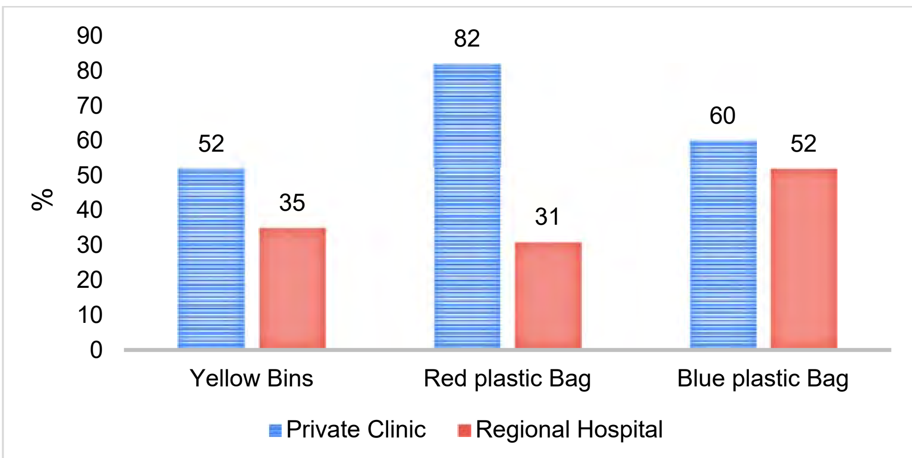
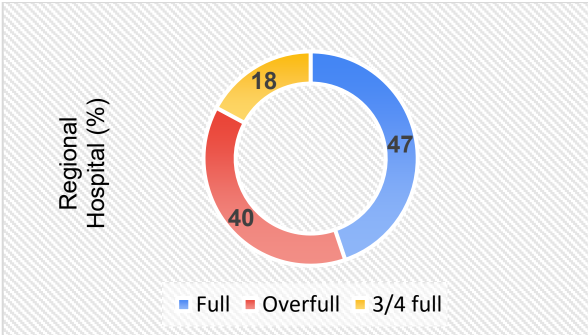


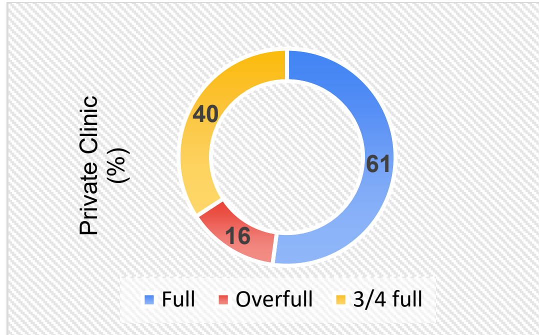
Fig. 4: Adequate responses on color-coding (Frequency in %)

plastic was for infectious waste disposal, and 51.70% of the participants could distinguish general waste from blue plastic bags. The remaining participants were unfamiliar with color-coding.

Fig. 5 demonstrates the disposal of sharps by health professionals. In the RHC (Fig. 5a), 46.7% confirmed that the collectors of sharp objects are filled, and 40% confirmed the overfilling of the collectors; only 18.30% said that three-quarters (3/4) of the collectors are filled. A total of 60.50% of the private clinic staff (Fig. 5b) acknowledged that the collectors are full and 15.80% are overflowing; 39.50% said that the collectors are filled, respecting the 3/4 standards. Sharp collectors must be filled up to the point that they are 3/4 full to reduce the likelihood of needles and syringes poking out of the bin and endangering the user (Torkashvand *et al.,* 2022). These findings are believed to pose a serious infectious risk for the health staff and patients (Andeobu *et al.,* 2022). During our visits to the hospitals, waste disposal and separation procedures were evidently subpar (Fig. 6). Appropriate segregation of waste using color-coded plastic bags and the suitable disposal of sharps in designated bins constitute an essential component of an effective waste management strategy (Barbosa and Mol, 2018; Kalpana *et al.,* 2016).



(a)



(b)

Fig. 5: Disposing of sharps in yellow bins: (a) in the regional hospital and (b) in the private clinic.

Fig. 6: Improper disposing and separation of medical waste in the hospitals sampled

Identification of healthcare waste management challenges in the two institutions studied.

The final part of Section B required participants to select from a list of obstacles: the difficulties they encountered in their daily work activity (Fig. 7). The findings were compared between RHC and the private clinic and found that they were experiencing similar issues to inadequate training/information (84.25% on average), absence of protocols/procedures (67.20% on average), and ineffective monitoring (61.25% on average). For the RHC, the critical hurdle was the lack of equipment/materials (84.70%). The increase in

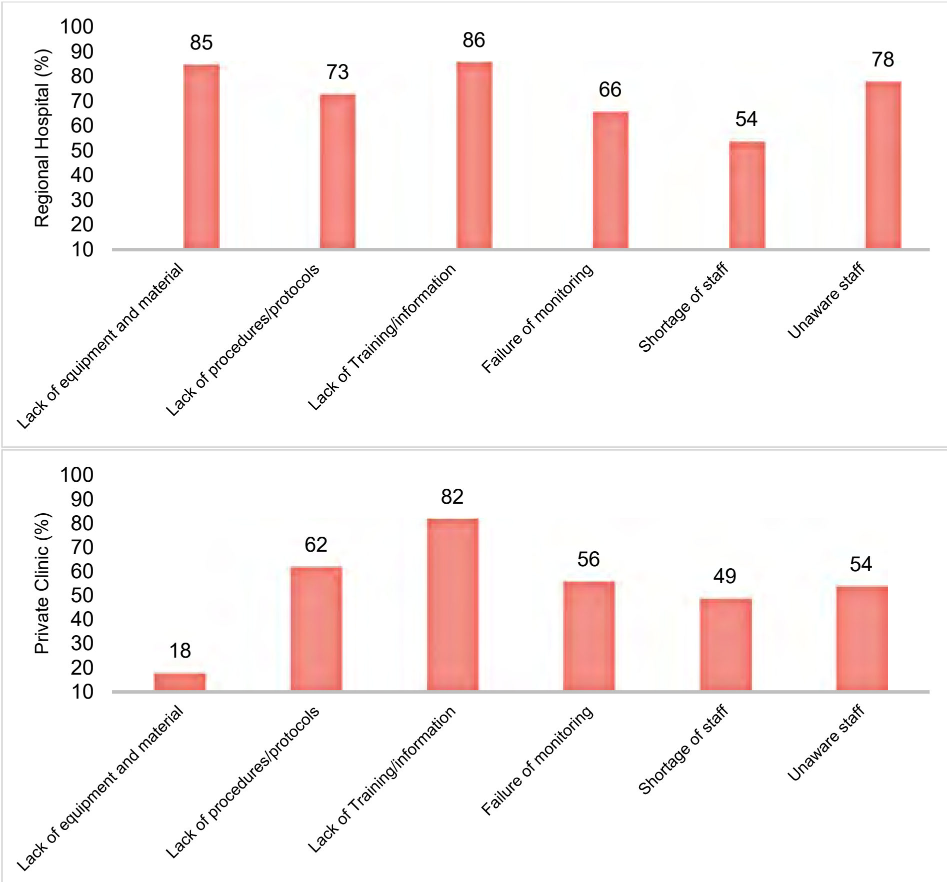


Fig. 7: Comparison of HCW challenges facing the RHC and the private clinic (%)

the number of UCH healthcare workers and medical activities in the hospital during the pandemic has led to a lack of tools and supplies required for medical waste management. A case study from various healthcare facilities in Sudan has documented the HCW management workers' challenges including lack of waste segregation at the source, lack of policies, failure of planning, inadequate training, lack of awareness of the hazardous nature of waste, weak infrastructure, and lack of suitable treatment technologies (Hassan *et al.*, 2018).

The management of HCW represents a serious concern for health professionals to protect themselves against acute infections and the environment from any potential contaminations (Kumarasamy and Jeevaratnam, 2017). The responsibility to achieve this condition is not limited to the health professionals but also the decision-makers in authority (Nangbe, 2018). The process for sustainable management of HCW requires planning at all levels: enforcement of policies, appropriate handling, and segregation at the source (Olaniyi *et al.*, 2021), continuous training programs of healthcare personnel, quantification, and strengthening of monitoring (Yousefi and Rostami, 2017). The health facilities studied should enhance their waste segregation practices by providing necessary materials, equipment, and appropriately labeled containers for storage. PPE, such as gloves, masks, clinical coats, and shoes, should be available to reduce the risk of infection and injury. Vaccination campaigns for all health professions should be organized. Surveys and quality assurance monitoring can improve healthcare worker management (Solomon *et al.*, 2019). Staff training in appropriate hazardous waste handling and disposal, including emergency response procedures, is crucial.

Accurate records of HMW management practices should be maintained, including waste generated and any incidents that occur. Clear guidelines for managing hazardous waste should be established in each facility. International organizations, such as the WHO, can support these efforts by providing funding and expertise to create good practice guidelines for managing medical waste during health crises (Chisholm *et al.,* 2021).

CONCLUSION

HCW management practices in Tangier (Morocco) are unsafe and may negatively impact the health of staff and patients. As a result of the COVID-19 pandemic, an exponential increase in waste output has been observed, and various management procedures have become dysfunctional. Healthcare workers discussed the challenges they encounter daily, the majority of which are brought on by the lack of training, absence of protocols, and lack of monitoring. The institutions assessed herein had no waste management officer. The finding evidently shows the non-application of the national Decree n°2-09-139. HCW management is a critical aspect of maintaining a safe and healthy community and environment. In the lack of financial resources, steps including adequate waste source separation; waste reduction, such as single-use medical devices; and promoting the use of environmentally friendly products can help lessen the environmental impact of the HCW, as well as the cost of their treatment. Continuous training of the responsible staff is also important. In conclusion, secure management of medical waste necessitates the formulation of proactive strategies. To date, this subject has received minimal consideration and requires increased awareness. By cooperating with the regional divisions of the Ministry of Health, and international organizations such as the WHO or the environmental protection agency, a thorough investigation can help in the enforcement and implementation of a national management plan for healthcare workers during healthcare crisis. The perspectives of this work recommend the following: 1) adequate and ongoing training for the healthcare staff, 2) appropriate procedures and protocols on good practices, 3) appointment of a person responsible for safety and hygiene in the institution, 4) organization of mandatory vaccination campaigns for all health workers, 5) provision of the necessary materials and equipment, and 6) an efficient protocol for measuring and quantifying waste. Effective HCW management benefits the community and wider society by reducing the risk of environmental pollution, preventing the spread of disease, and conserving resources. It also contributes to a cleaner, safer, and healthier environment, which can have positive impacts on the health and well-being of the local communities.

AUTHOR CONTRIBUTIONS

S. Ajbar El Gueriri, the first author, has conducted the survey research, contributed to analyzing data, interpreted the results, and prepared the manuscript. F. El Mansouri, the corresponding author, has participated in the preparation, creation, and presentation of the published work by those from the original research group, specifically critical review, commentary, or revision – including pre- or post-publication stages. S. Lachaal, performed the management activities to annotate (produce metadata), scrub data, and maintain research data. F. Achemlal, designed the field experiment and conducted field data acquisition. J. Brigui, contributed to the preparation, creation, and presentation of the published work, specifically writing the initial draft. A. Fakih Lanjri, has contributed to supervising the first author in the data analysis, and interpreting the results.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

¾	Three quarters
%	Percent
COVID-19	Coronavirus disease
D	Day
Eq.	Equation
Fig.	Figure
HCW	Healthcare waste
HIV	Human immunodeficiency virus
HMW	Hazardous medical waste
kg	Kilogram
kg/bed/day	Kilogram per bed per day
kg/day	Kilogram per day
kg/week	Kilogram per week
LC	Litter capacity
N	Number of staff
NO.	Number
OR	Occupancy rate
P	Daily production
PPE	Personal protective equipment
Q	Quantity of waste
RHC	Regional hospital center
UCH	University center hospital
WHO	World health organization

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CASE STUDY

The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove

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ABSTRACT

BACKGROUND AND OBJECTIVES: Mangroves are known to contain tannins, flavonoids, and quinones, which have the potential to be antibacterial, effective even against multidrug-resistant bacteria. Mangroves also have antifungal and antiviral properties. Although, mangroves are known for their use as medicinal ingredients, information regarding symbiont bacteria's antibacterial and antifungal potential is still scarce. Therefore, this study aimed to examine symbiont bacteria in the fruit and leaves of *Xylocarpus* granatum as additional raw materials for anti-acne cosmetic creams and moisturisers.

METHODS: Symbiont bacteria were isolated using the pour plate method through Zobell 2216E and incubated for 2 x 24 hours at 27.5 Celcius degree. Afterwards, 13 isolates were successfully isolated and characterised based on their morphology. Further, everal tests were conducted, including the antibacterial test, antifungal test, molecular identification, and gas chromatography-mass spectrometry. The pathogenic bacteria used in the antibacterial test were *Staphylococcus aureus*, *Vibrio harveyi*, and *Vibrio alginolyticus*

FINDINGS: The antibacterial test results showed that eight isolates were capable of producing an inhibition zone against S. aureus, seven isolates were positive for antibacterial activity against *Vibrio harveyi*, and 10 isolates were positive for antibacterial activity against *Vibrio alginolyticus*. The pathogenic fungi used in the antifungal test were *Malassezia furfur* and *Candida albicans*. The antifungal test results demonstrated that six isolates could produce inhibition zones against *Malassezia furfur* and *Candida albicans*. Furthermore, molecular identification was carried out on six potential isolates based on the antibacterial and antifungal tests, which were X2.52, X1.65, X1.64, X1.53, X1.54, and X1.63. The molecular identification results revealed the occurrence of four species in the *Xylocarpus granatum* mangroves, namely, *Sinomicrobium oceani*, *Proteus mirabilis*, *Pseudomonas khazarica*, and *Alcaligenes aquatilis*.

CONCLUSION: The study found that the mangrove symbiont bacteria had antibacterial and antifungal potential. The compound with the highest concentration in six isolates was 9-octadecenoic acid, methyl ester. This type of content has antibacterial potential and is also predicted to have antifungal potential.

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INTRODUCTION

Indonesia is listed as a mega-biodiverse country with abundant natural wealth in terms of macro as well as microorganisms, which have not been widely studied. Marine and coastal environments have the potential to be the source of natural antioxidants, antibacterial agents, and enzyme inhibitors for the pharmaceutical and cosmetic industries (Simlaiet *et al.,* 2014; Eswaraiah *et al.,* 2020). Mangrove plants that inhabit these environments contain tannins, flavonoids, and quinones (Pringgenies *et al.,* 2018), micro and macro element content (Ariyanto *et al.,* 2019) and amino acid contents (Ningsih *et al.,* 2020). As flavonoid compounds function as antibacterial, antifungal, and antiviral agents, they can be pharmacologically accepted to treat human diseases. In comparison, tannin compounds are biomaterial components with antiviral and antibacterial properties (Kaczmarek, 2020), while quinone derivatives have antifungal and antibacterial properties (Amani, 2014). However, there is a fear that the continuous usage of mangrove plants in large quantities can cause the overexploitation of these species, thereby disrupting the balance of the ecosystem. In order to avoid this issue, many researchers have used microbes associated with marine and coastal natural resources, especially marine facultative moulds, as a source of active ingredients because of their well-known ability to produce potential bioactive compounds (Darmadi *et al.,* 2021). Marine mangrove symbiont bacteria are eukaryotic microorganisms from the microbial kingdom isolated from coastal and marine environments. According to a study, the fruit extract of *X. granatum* has medicinal properties that can be used to heal wounds (Das *et al.,* 2019; Dey *et al.,* 2021, Pringgenies *et al.,* 2021). Moreover, bacterial symbionts in mangroves have the potential as multidrug resistance (MDR) antibacterial agents, along with the potential to decompose organic matter. Furthermore, symbiotic bacteria found in mangroves have demonstrated antibacterial activity against *Pseudomonas aeruginosa*. The bacterial gene contains L-2-amino-4-methoxy-trans-3-butenoic acid l-2-Amino-4-methoxy-trans-3-butenoic acid (AMB), which is a powerful antibiotic and toxin (Pringgenies *et al.,* 2021). The supernatant obtained from the extraction of *P. aeruginosa* in symbiosis with *Cerithidea sp.* in the mangrove area was found to have pyocyanin (PYO) and phenazine-1-carboxylic (PCA) pigments. The emerald colour of the sample culture indicated the presence of the phenazine pigment in the bacterial sample (Pringgenies and Setyati, 2021). Phenazine is a natural pigment that plays an important role in health as an anti-cancer, anti-malarial, anti-tumour, and potentially antibiotic agent. The fruit of *X. granatum*, also known locally as *buah pengantin* (the bride's fruit), is often used as a mask to make the bride's face smooth and look beautiful, which is everyone's desire on their wedding day. All these biological activities can be utilised in the cosmetic production industry. As time goes by and the public's awareness about the importance of natural ingredients for health is increasing, the demand for natural-based products is also expected to increase. This is due to consumer concerns regarding synthetic cosmetics made from artificial ingredients that are widely circulated in the market and can cause skin irritation and, at worst, skin cancer (Panico *et al.,* 2019). Antibacterial and antifungal bacteria are natural ingredients (Kokoska *et al.,* 2019; Qadri *et al.,* 2022) that are rarely utilised for cosmetic purposes, especially for body and skin care products; therefore, it was challenging opportunity to conduct preliminary research using antibacterial and antifungal bacteria as raw material to produce anti-acne cream cosmetic products, with moisturising cosmetics using *X. granatum* mangrove fruit symbiont bacteria, which have potential as antibacterial and antifungal agents. Based on this potential, it is possible that the fruit of this species can be applied as a source of medicine and cosmetics. The research consisted of nanosystems against a wide variety of fungal species (Jangjou *et al.,* 2022), antibacterial and antibiofilm activities against clinical pathogens (Balaraman *et al.,* 2022), antimicrobial activity of flavonoids (Cushnie and Lamb, 2005), and hazardous ingredients in cosmetics (Zulaikha *et al.,* 2015). The present study aimed to investigate symbiont bacteria in the fruit and leaves of *X. granatum* as an additional raw material for cosmetic anti-acne creams and moisturisers. The samples of *X. granatum* mangrove fruit and leaves were collected from the water of the Baturusa River, Merawang District, Bangka Regency, Bangka Belitung Islands, Indonesia in 2022.

MATERIALS AND METHODS

Sample collection

Samples of *X. granatum* mangrove were taken from the river flow area (RFA) of Baturusa, Merawang, Bangka, Bangka Belitung Islands, Indonesia. In this process, leaves and fruits were picked using a knife, put in a dark-coloured plastic ziplock, and stored in a coolbox filled with ice at 4 ˚C on the way to the laboratory. Subsequently, the samples were washed thoroughly with distilled water to remove the attached epiphytes and dried until no water was left.

Bacterial procedure

Bacterial isolation was performed using the pour plate method. For this, the incubation process was carried out at 27.5 ˚C for 2 x 24 hours (Pringgenies *et al.,* 2021), while the separation and purification of bacterial isolates that managed to grow were performed using the streak method on a petri dish with incubation at 27.5 ˚C for 48 h. The antibacterial activity was measured using the disc diffusion method (Balouiri *et al.,* 2016). For this, 100 microliter (µL) of pathogenic bacteria were spread on agar media, and several paper discs (8 mm, Advantec, Toyo Roshi, Ltd, Japan) containing 10 microliter of bacterial isolates were placed on each surface of the paper discs. The Petri dishes were incubated at room temperature for 48 h. Antibacterial activity was defined as the formation of an inhibition zone greater than 9 milimeter around the paper disc. The positive control test was conducted using the antibiotic amoxicillin with a concentration of 20 µg/disk. This aimed to determine the existence of an inhibition zone formed by antibiotics that are available on the market so that it can be used for the comparison of antibacterial abilities. The negative control test was carried out using a sample of the symbiont bacteria tested against the test bacteria. The pathogens used in testing antibacterial activity were *Staphylococcus aureus*, *Vibrio harveyi*, and *Vibrio alginolyticus*, while the pathogenic fungi used were *Malassezia furfur* and *Candida albicans*. When isolating pathogenic bacterial and fungal species, the protective measure is to mix antibiotics with as much as 2% of the culture medium used. Therefore, nystatin-type antibiotics were mixed for the bacterial test media, while penicillin-type antibiotics were mixed for the anti-fungal test media.

DNA extraction, PCR, and sequencing

First, a bacterial colony isolate was taken and dissolved in 1 mililitre (mL) of sterile water in a micro-centrifuge tube with a volume of 1.5 mL before being centrifuged for 1 minute at 10,000–12,000 rotation per meter. Then, the supernatant obtained was discarded, and 200 µL of instance matrix pellet (bacterial precipitate) was added before being vortexed and incubated at 56 °C using a heat block for 30 minute. It was vortexed again at high speed for 10 second. The tube was placed at 100 °C on the heat block for 8 min, then vortexed at high speed for 10 s and centrifuged at 12,000 rpm for 3 min. Later, 20 µL of the resulting supernatant (genome DNA solution) was used per 50 µL of the PCR reaction. PCR analysis and electrophoresis were conducted afterwards (Pringgenies *et al.,* 2021; Yuan *et al.,* 2021). The amplification results were sent to PT Genetika Science Indonesia to identify the nucleotide base sequence based on the Sanger dideoxy method.

Data analysis

Molecular data processing

The sequencing results of each sample were aligned and analysed using the molecular evolutionary genetics analysis (MEGA) 11 software. Then, the FASTA (nucleotide base sequences matched against data from GenBank) file from the analysis results was matched with the data in the National Center for Biotechnology Information (NCBI)'s GenBank database to determine the species. Several comparison species from the BLAST results were downloaded, and their phylogenetic trees were compiled using MEGA 11 in order to identify the kinship between the sample species, control species, and species from the other samples.

Gas chromatography—mass spectrometry (GC—MS) analysis

The bacteria were cultured in liquid using the marine broth, after which the bacteria were extracted with methanol using the soxhletation method. The products of soxhletation were treated with a rotary evaporator in order to obtain the extraction products. The gas chromatography—mass spectrometer (GC—MS) analysis was conducted using a GC—MS QP2010S (column: Rtx-5MS 30 m, diameter: 0.25 mm), programmed at 80–300 °C with a temperature rise of 10 °C/min and helium carrier gas while the pressure was 22 kilopascal. The number of compounds present in the extract was indicated by the number of peaks on the chromatogram, while the names/types of compounds present were interpreted based on the data spectra of each of these peaks using the approximation method library on the GC—MS database (Fox 1999; Hsouna and Alayed, 2012).

RESULTS AND DISCUSSION

Isolate morphology

13 isolates were successfully obtained from the leaf

and fruit extract of *X. granatum* from Bangka Regency. Morphologically, each isolated sample was mostly circular and white with an entire margin, convex elevation, and moderate, small, large, and punctiform. Leaf bacterial isolates were coded as X1, while flower bacterial isolates were coded as X2.

Antibacterial and antifungal test

Antibacterial and antifungal tests were carried out qualitatively. Pure isolates were tested on three pathogenic bacteria, namely, *V. harveyi*, *V. alginolyticus*, and *S. aureus*. The qualitative test results demonstrated that eight isolates were capable of producing an inhibition zone against *S. aureus*, seven isolates were tested positive for antibacterial activity against *V. harveyi*, and ten isolates were tested positive for antibacterial activity against *V. alginolyticus*. The antifungal test was conducted on two pathogenic fungi, namely, *M. furfur* and *C. albicans*. The qualitative test results demonstrated that six isolates could produce zones of inhibition against *M. furfur* and *C. albicans*. The results of the qualitative antibacterial and antifungal tests are presented in Table 1.

The antibacterial qualitative test is a preliminary test that aims to ensure the ability of bacterial secondary metabolites against pathogenic bacteria. According to Table 1, there was more antibacterial activity against the pathogen *V. alginolyticus* than against the other pathogens. This could be because *V. alginolyticus* is a gram-negative bacterium with thinner peptidoglycan than that in *S. aureus*, which is a gram-positive bacterium. Quantitative tests were conducted on the obtained pure isolates, and the pure isolates with positive results in the qualitative test were proceeded by the quantitative test to observe the diameter of the inhibition zone. The antibacterial activity was declared positive if a clear zone was formed. The quantitative test results of the mangrove bacterial isolate *X. granatum* against pathogenic bacteria *S. aureus*, *V. harveyi*, and *V. alginolyticus* are presented in Table 2.

According to the test results, 8 out of 13 isolates showed antibacterial activity against *S. aureus*, and isolates X1.62 and X1.131 did not show any inhibition zones at 24 h of observation. The highest isolates at 24 h were X1.53 (3.31 ± 0.12 mm), X1.65 (2.99 ± 0.01 mm), and X1.54 (2.52 ± 1.30 mm). The isolates with the largest inhibition zone area at 48 h of observation were X1.53 (3.39 ± 0.42 mm), X1.54 (3.1 ± 1.13 mm), and X1.62 (2.8 ± 5.66 mm), while the isolates with the largest inhibition zones at 72 h of observation were X1.53 (3.09 ± 0.55 mm), X1.631 (1.82 ± 0.45 mm), and X1.131 (1.52 ± 0.45 mm). Table 2 presents results related to the mangrove symbiont bacterial isolates *Xylocorpus sp*, which is known for its antibacterial activity against pathogenic bacteria *S. aureus*, as it is known to exist in other mangrove species (Pringgenies *et al.,* 2021). *S. aureus* pathogenic bacteria are gram-positive bacteria that are round, golden in colour, and anaerobic. Although these bacteria can secrete endotoxin, they are unable to form spores. Moreover, *S. aureus* die when kept at 60 ˚C for 60 min (Tam and Torres, 2019). The antibacterial test result regarding the pathogenic bacteria *V. harveyi* showed that 8 out of 13 isolates had positive results. *V. harveyi* is a gram-negative bacterium that has a positive oxidase, can ferment glucose, and produces lysine decarboxylase. These bacteria can also produce catalase, degrade gelatin, are motile, and have flagella (Yuan *et al.,*

Table 1: Antibacterial and antifungal qualitative test

Isolate	Pathogenic bacteria			Pathogenic fungi	
	S. aureus	*V. harveyii*	*V. alginolyticus*	*M. furfur*	*C. albicans*
X1.212	-	+	+	-	-
X1.63	+	+	+	-	+
X1.54	+	+	+	-	+
X1.62	+	+	+	-	-
X1.631	+	+	+	-	-
X1.632	-	+	-	-	-
X1.131	+	-	-	-	-
X1.64	+	-	-	+	+
X1.53	+	-	+	+	-
X1.65	+	-	+	+	-
X2.54	-	-	+	+	+
X2.52	-	-	+	+	+
X2.55	-	-	+	+	-

Table 2: Antibacterial quantitative test on *staphylococcus aureus, vibrio harveyi, vibrio alginolyticus* pathogen

Isolate	Pathogenic bacteria (mm)								
	Staphylococcus aureus			*Vibrio harveyi*			*Vibrio alginolyticus*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
X1.212	0±0	0±0	0±0	2.15±3.04	2.87±0.61	2.4± 0.14	1.43±2.02	1.43±0.66	1.6± 0.42
X1.63	2.15±0.09	2.25±0.07	1.5± 0.42	0±0	0,48±0	1.55±0.77	1.51±1.25	0.14±0.19	4.64±1.66
X1.54	2.52±1.30	3.1±1.13	1.34±0.50	1.21±1.71	0.85±1.2	1.1±0.84	2.4±0	1±1.41	2.25±0.35
X1.62	0±0	2.8±5.66	1.26±1.78	1.71±2.42	0.9± 1.27	1.2± 0.71	0.3± 0.42	1.24±1.07	1.53±0.32
X1.631	0±0	1.67±0.81	1.82±0.45	2.37±0.49	1.29±1.15	1.8± 1.15	0.7± 2.08	0.71±0.99	2.08±0.37
X1.632	0±0	0±0	0±0	3.05±2.59	1± 1.15	2± 1.15	0±0	0±0	0±0
X1.131	2.45±3.46	2.25±1.62	1.52±0.51	0±0	0±0	0±0	0±0	0±0	0±0
X1.64	1.75±2.47	2.10±1.41	1.50±1.13	0±0	0±0	0±0	0±0	0±0	0±0
X1.53	3.31±0.12	3.39±0.42	3.09±0.55	0±0	0±0	0±0	1.33±0.24	148±0.68	1.72±0.11
X2.54	0±0	0±0	0±0	0±0	0±0	0±0	0.63±0.89	0.66±0.91	0.24±0.34
X1.65	2.99±0.01	1,11±1.56	0.75±1.06	11±15.5	3.25±4.59	6.35±2.33	0.5±0.7	0.66±0.9	1± 1.35
X2.52	0±0	0±0	0±0	0±0	0±0	0±0	1.23±0.89	0.91±1.29	2.17±0.13
X2.55	0±0	0±0	0±0	0±0	0±0	0±0	0.7± 0.99	0.86±0.48	1.22±0.17

2021). Isolate X1.63 did not form any inhibition zone at 24 h of observation. The highest isolates at 24 h were X1.65 (11 ± 15.5mm), X1.632 (3.05 ± 2.59 mm), and X1.631 (2.37 ± 0.49 mm). Isolates with the largest inhibition zone area at 48 h of observation were X1.65 (3.25 ± 4.59 mm), X1.212 (2.87 ± 0.61 mm), and X1.31 (1.29 ± 1.15mm), while the isolates with the largest inhibition zone at 72 h of observation were obtained by X1.65 (6.35 ± 2.33 mm), X1.212 (2.4 ± 0.14 mm), and X1.632 (2 ± 1.15 mm). Furthermore, according to the antibacterial test conducted on the pathogenic bacteria *V. alginolyticus*, 10 isolates showed positive results. *V. alginolyticus* is a gram-negative bacterium that is halophilic and has a crooked (i.e., comma) shape with a length of about 1.4–5.0 micrometer and a width of 0.3–1.3 µm, while, *V. alginolyticus* is motile and has a flagellum with a protective sheath. The highest isolates at 24 h were X1.54 (2.4 ± 0 mm), X1.632 (1.51 ± 1.25 mm), and X1.53 (1.33 ± 0.24 mm). The isolates with the largest inhibition zone area at 48 hours (h) of observation were X1.53 (1.48 ± 0.68 mm), X1.212 (1.43 ± 0.66 mm), and X1.62 (1.24 ± 1.07mm), while the isolates with the largest inhibition zone at 72 hours of observation were obtained by X1.63 (4.64 ± 1.66 mm), X1.54 (2.25 ± 0.35 mm), and X2.52 (2.17 ± 0.13mm). According to the test results, there were isolates that showed bacteriostatic and bactericidal antibacterial activity. One such example of bacteriostatic antibacterial activity is X1.63 against the pathogen *S. aureus*. As the growth of the inhibition zone on isolate X1.63 tended to increase, it can be said that X1.63 showed bacteriostatic ability. Additionally, the isolates X1.54, X1.62, X1.131, X1.64, X1.53, and X1.65 showed bacteriocidal activity against *the S. aureus* pathogen in the antibacterial test, while X1.63, X1.54, and X2.54 showed bacteriocidal activity against *V. alginolyticus pathogen*. Only isolate X1.63 showed bacteriostatic activity against the *V. harveyi* pathogen in the antibacterial test. Thus, based on the results, mangrove symbiont bacteria *Xylocorpus sp* has potential as an antibacterial agent, especially against the test bacteria *S. aureus*, *V. alginolyticus*, and *V. harveyi*. Moreover, mangrove plants contain alkaloids, flavonoids, saponins, and tannins (Pringgenies *et al.,* 2021). As this compound has potential as an antibacterial agent, the bacteria found were also suspected to have potential as antibacterial agents.

Antifungal test

The antifungal quantitative tests were conducted on the obtained pure isolates. The antifungal activity was tested on pathogenic fungi *M. furfur* and *C. albicans*. The results of the quantitative tests conducted on *M. furfur* and *C. albicans* can be observed in Table 3.

According to the results of the antifungal test, six isolates exhibited antifungal activity against the two types of fungi tested, namely, *M. furfur* and *C. albicans*, while the remaining seven isolates out of thirteen only showed activity on one of the tested fungi. *M. furfur* is a pathogenic fungal species and is a constituent of the human microflora (Rojas *et al.*, 2014). Moreover, *M. furfur* is a lipophilic dimorphic fungus belonging to the flora group. Isolates X1.54, X1.53, and X2.52 did not show any inhibition zones at 72 h of observation. This was predicted due to the inability of pure isolates to fight pathogenic bacteria. The highest isolates at 24 h were X1.53 (3.65 ± 5.16 mm), X1.64 (2.05 ± 2.33 mm), and X1.63 (1.65 ± 0.78 mm). The isolates with the largest inhibition zone area at 48 h of observation were X1.64 (2.8 ± 3.95 mm), X1.53 (2.55 ± 3.60 mm), and X1.63 (2.32 ± 0.45mm), while the isolates with the largest inhibition zone at 72 hours of observation were obtained by X1.64 (4 ± 0 mm) and X2.54 (2.73 ± 1.03 mm). According to the antifungal test results, 6 out of 13 isolates exhibited antifungal activity against *C. albicans*. *C. albicans* is a fungal pathogen that belongs to the Ascomycota group and has opportunistic characteristics. Isolate X1.53 did not show any inhibition zone at 24 h of observation. The highest isolates at 24 h were X2.55 (4.8 ± 3.68 mm), X2.52 (3.81 ± 0.26 mm), and X2.54 (2.85 ± 4.03 mm). The isolates with the largest inhibition zone area at 48 h of observation were X2.52 (3.75 ± 0.35 mm), X1.53 (3.5 ± 0.70 mm), and X2.55 (3.25 ± 1.06 mm), while the isolates with the largest inhibition zone at 72 h of observation were obtained by X2.52 (3.6 ± 0.56 mm), X1.64 (3 ± 0.56 mm), and X2.55 (2.6 ± 0.85 mm). The bacterial isolates tested were assumed to produce clear zone areas that inhibited the growth of pathogens in the test medium due to secondary metabolite processes. Secondary metabolites that have bioactive compounds are produced by bacteria as a self-defence response. These bioactive compounds inhibit the growth of fungal cells by damaging the cell walls, causing the fungal cells to either experience inhibited growth or even die. Based on their activity, antifungal drugs are grouped into two types: fungicidal and fungistatic. While fungicide antifungals kill fungal growth, fungistatic antifungals inhibit fungal growth without killing the fungal population (Cowen *et al.,* 2015; Revie *et al.,* 2018; Hossain *et al.,* 2022). All the isolates that were tested against *M. furfur* and *C. albicans* produced an inhibition zone of <5 mm, indicating that the three isolates possessed weak antibacterial property.

Molecular identification

The analysis of the qualitative and quantitative bacterial tests conducted on pathogenic bacteria and pathogenic fungi selected the three best isolates had antibacterial properties and the best isolates had antifungal properties. However, each of these isolates has the potential to be an antibacterial and antifungal agent. In other words, the six selected bacteria simultaneously have antibacterial and antifungal potential. The conditions of these selected bacteria are specific because of their potential against bacterial and fungal pathogens. The discovery of bacteria that have antibacterial and antifungal potential can lead to their use as ingredients for moisturising cosmetic formulas, which can be expected to prevent acne growth, although this hypothesis needs to be tested by further studies.

Through the methods of molecular identification, we identified six potential isolates in mangrove *X. granatum,* including *Sinomicrobium oceani* (99%), *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceani* (85%), *Sinomicrobium oceani* (99%), and *Alcaligenes aquatilis* (98%), as presented in Table 4. Based on the data from

Table 3: Antifungal Quantitative Test on *Malassezia furfur* and *Candida albicans* Pathogens

Isolate Code	Pathogenic bacterial (mm)					
	Malassezia furfur			*Candida albicans*		
	24 h	48 h	72 h	24 h	48 h	72 h
X1.212	0±0	0±0	0±0	0±0	0±0	0±0
X1.63	0±0	0±0	0±0	1.65±0.78	2.32±0.45	0.3±0.42
X1.54	0±0	0±0	0±0	1.7±0.57	2.15±0.92	0±0
X1.62	0±0	0±0	0±0	0±0	0±0	0±0
X1.631	0±0	0±0	0±0	0±0	0±0	0±0
X1.632	0±0	0±0	0±0	0±0	0±0	0±0
X1.131	0±0	0±0	0±0	0±0	0±0	0±0
X1.64	1.32±1.86	3.2±0.28	3±0.56	2.05±2.33	2.8±3.95	4±0
X1.53	0±0	3.5±0.70	1.85±0.21	3.65±5.16	2.55±3.60	0±0
X2.54	2.85±4.03	2.8±3.96	1.9±2.69	1.38±1.95	1.5±2.12	2.73±1.03
X1.65	1.91±2.70	1.6±2.26	1.75±2.47	0±0	0±0	0±0
X2.52	3.81±0.26	3.75±0.35	3.6±0.56	0.59±0.014	0.5±0.70	0±0
X2.55	4.8±3.68	3.25±1.06	2.6±0.85	0±0	0±0	0±0

Table 4: Identification Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

Code	Relative similarity	Scientific name	Query cover	E-value	Percent identify	Acc number
X2.52	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	99%	0	89.84%	NR_109592.1
X1.65	*Proteus mirabilis* strain JCM 1669 16S ribosomal RNA, partial sequence	*Proteus mirabilis*	100%	0	98.52%	NR_113344.1
X1.64	*Pseudomonas khazarica* strain TBZ2 16S ribosomal RNA, partial sequence	*Pseudomonas khazarica*	100%	0	99.53%	NR_169334.1
X1.53	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	85%	1.00E-153	84.15%	NR_109592.1
X1.54	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	99%	3.00E-165	83.60%	NR_109592.1
X1.63	*Alcaligenes aquatilis* strain LMG 22996 16S ribosomal RNA, partial sequence	*Alcaligenes aquatilis*	98%	3.00E-155	83.47%	NR_104977.1

NCBI, *Proteus mirabilis* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Enterobacterales (order), Morganellaceae (family), and *Proteus* (genus). *Sinomicrobium pectinilyticum* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteriia (class), Flavobacteriales (order), Flavobacteriaceae, (family) and *Sinomicrobium* (genus). *Alcaligenes aquatilis* is classified as follows: Bacteria (kingdom), Pseudomonadota (phylum), Betaproteobacteria (class), Burkholderiales (order), Alcaligenaceae (family), and *Alcaligenes (genus)*. *Sinomicrobium oceani* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteria (class), Flavobacteriaceae (order), and *Sinomicrobium* (genus). *Pseudomonas khazarica* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Pseudomonas (order), Pseudomonas (family), and *Pseudomonas* (genus). Similarly, based on the data from NCBI, *Proteus mirabilis* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Enterobacterales (order), Morganellaceae (family), and *Proteus* (genus). *Sinomicrobium pectinilyticum* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteriia (class), Flavobacteriales (order), Flavobacteriaceae (family), and *Sinomicrobium* (genus). *Alcaligenes aquatilis* is classified as follows: Bacteria (kingdom), Pseudomonadota (phylum), Betaproteobacteria (class), Burkholderiales (order), Alcaligenaceae (family), and *Alcaligenes* (genus). The *Sinomicrobium oceani* species is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteria (class), Flavobacteriaceae (order), and *Sinomicrobium* (genus). *Pseudomonas khazarica* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Pseudomonas (order), Pseudomonas (family), and *Pseudomonas* (genus). Based on the compilation results of a phylogenetic tree using MEGA 11, *Sinomicrobium oceani* (isolate X1.52; bootstrap value: 90) had the closest kinship with *S. pectinilyticum* (isolate X1.54; bootstrap value: 90) and *P. mirabilis* (isolate X1.53; bootstrap value: 83), while *Proteus mirabilis* (isolate X1.65; bootstrap value: 99) was related to *Sinomicrobium oceani* (isolate X1.64; bootstrap value: 99). These five isolates had a kinship that was still in a large clade. Additionally, *Alcaligenes aquatilis* (isolate X1.63; bootstrap value: 55) had a very different kinship with other isolates. A high bootstrap value (i.e. over 70%) indicates that the kinship shown by the phylogenetic tree in figure x has a relatively good level of trust (Park *et al.*, 2010). There are six types of mangrove symbiont bacteria *X. granatum* that have potential as antibacterial and antifungal agents. These are *Sinomicrobium oceani* (99%), *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceani* (85%), *Sinomicrobium oceani* (99%), and *Alcaligenes aquatilis* (98%), all of which have been registered at the NCBI with the submission number SUB12685225.

GC—MS analysis was conducted using bacterial extracts with methanol solvent. The GC—MS results from a total of six identified isolates showed 20 types of compounds for each tested bacterium. The compounds with the highest concentration as revealed by analysis of the content in the isolates are as follows: *Sinomicrobium oceani* (isolate X1.53) containing Hexadecanoic acid, methyl ester (CAS) 27,52%; *Sinomicrobium pectinilyticum* (isolate X1.54) containing 9-octadecenoic acid (Z)-, methyl ester (CAS) 35,61%; *Alcaligenes aquatilis* (Isolate X1.63) containing 9-octadecenoic acid, methyl ester, (E)- (CAS) 44,02%; *Pseudomonas khazarica* (isolate X1.64) containing 9-octadecenoic acid, methyl ester, (E)- (35,64% area); Proteus mirabilis (isolate X1.65) with the highest content of 9-octadecenoic acid, methyl ester, (E)- 33,28%; and *Sinomicrobium oceani* (isolate X2.52) has the highest content of 9-octadecenoic acid, methyl ester, (E)- 46,58%. The biggest and highest compound based on the six isolates was 9-octadecenoic acid, methyl ester, (E)-. 9-Octadecenoic acid, methyl ester, (E)- is a compound derived from unsaturated fatty acids that has potential as an antifungal and antibacterial agent (Tahir *et al.,* 2018). The biosynthesis process of 9-octadecenoic acid, methyl ester, (E)- generally involves the conversion of stearic acid into unsaturated fatty derivatives due to the dehydrogenation process of the 9-desaturase stearoyl-CoA enzyme (Zahara *et al.,* 2022). *Sinomicrobium oceani* is a bacterium commonly found in marine sediments (Xu *et al*., 2013). It is known that this type of *S. oceani* bacteria has a wide distribution, ranging from the waters of China to Indonesia. Moreover, these bacteria have the potential to degrade alginate as well as seaweed; therefore, they can be used for the degradation of seaweed biomass using non-toxic and environmentally safe biological methods (Jegatheesan *et al.,* 2017). However, in the present study, the bacterium *S. oceani* has potential as an antifungal agent, while the bacterium *Proteus mirabilis* is a gram-negative, facultative anaerobic, rod-shaped bacterium. These bacteria are known to have urease activity, which causes all infections in human proteus. The present review focused on the mechanism that forms *Proteus mirabilis* biofilms, along with a state-of-the-art update regarding the prevention of biofilm formation and reduction of mature biofilms. These treatment approaches include natural and synthetic compounds targeting virulence factors and quorum sensing, along with other strategies that include carrier-mediated diffusion of antimicrobials into biofilm (Wasfi *et al.,* 2020). Moreover, the bacterium *Pseudomonas khazarica* is also found in the Khazar (Caspian) sea, which is probably how it got its name. These bacteria have the potential to degrade polycyclic aromatic hydrocarbons (Tarhriz *et al.,* 2020). *Alcaligenes aquatilis* is a motile Gram-negative, catalase-positive, and cytochrome oxidase-positive bacterium, with peritrichous flagella and of the genus *Alcaligenes*. These bacteria are found in sediments in the waters of the United Kingdom and even Japan, meaning that this type of bacteria has a wide distribution in the waters (Durán *et al.,* 2019). Furthermore, *Alcaligenes aquatilis* are also bacteria that are commonly found in sediments. These bacteria show lignin peroxidase activity, which is responsible for the bronze green decolourisation that was detected. The dominant ingredients found in bacterial isolates that have antibacterial and antifungal potential are hexadecanoic acid, methyl ester (CAS), and 9-octadecenoic acid (Z). These types of content have potential as both antibacterial and antifungal agents. Skin infections are caused by pathogenic bacteria (Chiller *et al.,* 2001), which cause a red rash filled with fluid; if the red rash breaks out, it can leave sores on the skin. Moreover, if not treated properly, skin infections can cause further complications, resulting in skin and fat tissue infections. For instance, pathogenic fungal infection is one of the most challenging diseases to treat in humans today. The increase in the cases of pathogenic fungal infections has been caused by the need for existing antifungal compounds which can result in unwanted side effects and are no longer effective against new pathogenic fungal strains. Thus, the present study is a comprehensive exploration of the natural resources of Indonesian microorganisms for microbes producing bioactive compounds as raw materials for anti-acne cosmetics, skin lightening, and skin moisturisers. The study found bacteria with potential anti-bacterial and antifungal properties, namely, *Sinomicrobium oceani, Proteus mirabilis*, *Pseudomonas Khazaria*, and *Alcaligenes aquatilis*. Bacteria contain 9-octadecenoic acid compounds, namely, the C18 straight-chain saturated fatty acid component. Additionally, the compound 9-octadecenoic acid, methyl ester, contains compounds with potential anti-bacterial and anti-cancer properties (Krishnamoorthy and Subramaniam, 2014). The high levels of nutrients in mangrove areas are caused by mangrove waste, which turns into mangrove litter and is decomposed by bacteria so that it can be used as a nutrient. Mangrove extract has

Tabel 5: GC-MS Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

S. oceani (99%) (X2.52)		*P. mirabilis* (100%) (X1.65)	
46.58	9-Octadecenoic acid, methyl ester, (E)-	33.28	9-Octadecenoic acid, methyl ester, (E)-
30.78	Hexadecanoic acid, methyl ester (CAS)	31.49	Hexadecanoic acid, methyl ester (CAS)
7.99	Octadecanoic acid, methyl ester	14.91	9-Octadecenoic acid (Z)-, methyl ester (CAS)
3.21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	9.34	Octadecanoic acid, methyl ester
1.76	Eicosanoic acid, methyl ester (CAS)	1.95	Eicosanoic acid, methyl ester (CAS)
1.54	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	1.14	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)
1.31	9-Octadecenal, (Z)- (CAS)	0.89	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
1.26	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	0.88	Tridecanedial
0.93	Tetradecanoic acid, methyl ester (CAS)	0.83	Tetradecanoic acid, methyl ester (CAS)
0.88	Octadecanoic acid, 10-oxo-, methyl ester	0.72	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.78	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (CAS)	0.68	9-Octadecenoic acid (Z)-, methyl ester (CAS)
0.76	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.66	DI-(9-OCTADECENOYL)-GLYCEROL
0.49	Dodecanoic acid, methyl ester	0.65	7,7-dimethyl-3-vinyl-bicyclo[4.1.0]hept-3-ene
0.35	3,9-Dodecadiyne	0.60	Dodecanoic acid, methyl ester
0.31	METHYL 3-acetylhydroxypalmitate	0.51	1,2-Benzenedicarboxylic acid, 3-nitro- (CAS)
0.30	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha.]	0.43	9,12-Octadecadienoic acid, methyl ester
0.24	Docosanoic acid, methyl ester (CAS)	0.31	Cyclopentadecanone, 2-hydroxy-
0.23	methyl dihydromalvalate	0.24	RANS-2-TRIDECENAL
0.18	Tetracosanoic acid, methyl ester (CAS)	0.24	DECANOIC ACID, METHYL ESTER
0.12	01297107001 TETRANEURIN - A - DIOL	0.24	Octadecanoic acid, 3-oxo-, methyl ester (CAS)
Pseudomonas khazarica (100%) (X1.64)		*Sinomicrobium oceani* (85%) (X1.53)	
35.64	9 -Octadecenoic acid, methyl ester, (E)-	27.52	9-Octadecenoic acid, methyl ester, (E)-
27.68	Hexadecanoic acid, methyl ester (CAS)	25.06	Hexadecanoic acid, methyl ester (CAS)
13.34	9-Octadecenoic acid, methyl ester, (E)-	10.12	9-Octadecenoic acid, methyl ester, (E)-
8.62	Octadecanoic acid, methyl ester	7.26	Octadecanoic acid, methyl ester
2.54	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	4.68	Oleic Acid
2.17	Eicosanoic acid, methyl ester (CAS)	3.70	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)
1.32	13-Docosenoic acid, methyl ester, (Z)-	3.09	9-Octadecenal, (Z)- (CAS)
1.20	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	2.79	2-NORPINENE-2-ETHANOL, 6,6-DIMETHYL-, ACETATE
1.16	Docosanoic acid (CAS)	2.53	9,12-Octadecadienoic acid, methyl ester
1.01	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	2.32	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.93	9-Octadecenal, (Z)- (CAS)	2.00	Ethyl linoleate
0.88	Heptadecanoic acid, 8-oxo-, methyl ester (CAS)	1.66	Dodecanoic acid, methyl ester
0.72	Tetradecanoic acid, methyl ester (CAS)	1.56	Eicosanoic acid, methyl ester (CAS)
0.59	(Z)14-Tricosenyl formate	1.41	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (CAS)
0.46	Nopyl acetate	1.40	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
0.43	Dodecanoic acid, methyl ester	1.19	Tetradecanoic acid, methyl ester (CAS)
0.41	Tricosanoic acid, methyl ester (CAS)	0.67	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.40	Cyclohexadecanone	0.61	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.27	9-Octadecenoic acid (Z)-, methyl ester (CAS)	0.34	7-Octylidenebicyclo[4.1.0]heptane
0.24	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha.]	0.09	LIMONENE DIOXIDE 1

Continued Tabel 5: GC-MS Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

Sinomicrobium oceani (99%) (X1.54)		*Alcaligenes aquatilis* (98%) (X1.63)	
35.61	9-Octadecenoic acid (Z)-, methyl ester-(CAS	44.02	9-Octadecenoic acid, methyl ester, (E)- (CAS
26.72	Hexadecanoic acid, methyl ester-(CAS)	29.98	Hexadecanoic acid, methyl ester (CAS)
11.50	9-Octadecenoic acid, methyl ester, (E)-	6.98	Octadecanoic acid, methyl ester
8.14	Octadecanoic acid, methyl ester (CAS)	4.01	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
2.94	9,12-Octadecadienoic acid, methyl ester, (E,E)- CAS	2.94	9-Octadecenal, (Z)- (CAS)
1.83	12-Octadecadienoic acid (Z,Z)-, methyl ester-(CAS)	2.35	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
1.95	Eicosanoic acid, methyl ester	1.41	Tetradecanoic acid, methyl ester (CAS)
1.54	13-Octadecenal,(Z)-	1.26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
1.53	Cyclopropaneoctanoic acid, 2-hexyl-, methyl-ester	1.10	Eicosanoic acid, methyl ester (CAS)
1.53	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	0.90	Dodecanoic acid, methyl ester
1.02	Tetradecanoic acid, methyl ester- (CAS)	0.81	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.88	Dodecanoic acid, methyl ester	0.75	Ethyl linoleate
0.84	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.67	Benzoic acid, methyl ester
0.75	Heptadecanoic acid, 8-oxo-, methyl ester CAS	0.50	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.69	9-Octadecenal, (Z)-	0.50	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester (CAS)
0.63	Docosanoic acid(CAS)	0.49	9-Hexadecenoic acid, methyl ester, (Z)- (CAS)
0.59	Tricosanoic acid, methyl ester	0.41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS
0.46	2-Pentadecanone, 6,10,14-trimethyl-(CAS)	0.36	Cyclohexasiloxane, dodecamethyl
0.43	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	0.33	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha
0.41	2-NORPINENE-2-ETHANOL, 6,6-DIMETHYL-, ACETATE	0.23	14-.BETA.-H-PREGNA

been shown to have potential as an antibacterial agent that is resistant to various drugs, and mangrove symbiont bacteria are thought to have potential as anti-bacterial agents because the active compounds in mangrove symbionts are similar to their host. The mangrove symbiont's antimicrobial properties can fight against MDR bacteria, namely, S*taphylococcus aureus, Escherichia coli*, and *Vibrio haryeyi* (Pringgenies *et al.,* 2021). Additionally, dead mangrove leaves decompose on the ground and become a source of nutrition, contributing to their immediate vicinity (Ariyanto *et al.,* 2018). The decomposition process by microbes is found in the mangrove ecosystem. Symbiont microbes of marine life can act as antimicrobial agents even against MDR strains. The MDR microbes are pathogenic strains that have mutated and become immune to various antibiotics. Several studies have been conducted on mangrove symbiont bacteria, and the results have demonstrated that the symbiont bacteria could show antibacterial activity against pathogenic bacteria. Even actinomycetes from mangrove sediments have been discovered. The present study concluded that the products were estimated to be in the NRPS, thiopeptide, RiPP-like, siderophore, beta lactone, terpene, Type III PKS, CDPS, and lasso peptide groups. Moreover, DNA identification of the isolates found three species of actinomycetes with antibacterial potential: *Virgibacillus salaries*, *Bacillus licheniformis*, and *Priestia flexa* (Setyati *et al.,* 2021), while molecular identification found that the bacteria were similar to *Brachybacterium paraconglomeratum* (99.92%), *Streptomyces pluripotent* (100%), and *Micromonospora chersina* (99.08%). Therefore, the study concluded that the three bacterial isolates with bacterial activity have similar genes with known antibiotic-producing genes and can potentially provide new antibiotic candidates (Anggelina *et al.,* 2021). Furthermore, the findings of this study prove that mangrove symbiont bacteria are more potent than synthetic antibiotics because of their ability to fight MDR bacteria. Bacteria contain 9-octadecenoic acid (oleic acid), which is in the group of fatty acids, i.e., unsaturated fatty acids— the most widely distributed and abundant in nature. Fatty acids and their derivatives have potential as antifungal agents. Notably, several types of fatty acids and their derivatives—oxylipins—have been found to have an inhibitory effect towards fungal growth and the production of mycotoxins. The use of fatty acids as antifungals could fulfil consumers' requests for more natural and environmentally friendly compounds while being less likely to promote fungal resistance. In addition, fatty acids as food additives due to their nature (Guimarães and Venâncio, 2022). Although, the findings related to the studies conducted on mangrove symbiont bacteria have not been used in treating bacterial or fungal infections, consortium symbiont bacteria have been used for composter production and deodorising, while reuse composter liquid bio-activator has been produced for wastewater treatment, which consists of four probiotic bacteria that function as pathogenic antibacterial agents (Pringgenies *et al.,* 2021). Additionally, natural products continue to be an excellent choice, especially those extracted from actinobacteria as they are a major source of metabolites with multiple biological activities, especially marine actinobacteria, which are less studied than their terrestrial counterparts. As regards this connection, studies have been conducted on marine organisms, which have the potential to fight skin diseases and inflammation (Chen *et al.,* 2020). Moreover, studies have also been conducted on marine bacteria, which have the potential to become skin pathogenic bacteria, such as anti-infection against bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* (De La Hoz-Romo MC *et al.,* 2022). Thus, the results of research conducted on the mangrove symbiont bacteria *X. granatum*, which has antibacterial and antifungal potential, are promising for use as cosmetic ingredients for natural products because they are usually safe and do not cause pollution. To continue the research in this area, we will use references from existing references despite the difficulties in cultivating symbiont bacteria and then use the metagenomic technology of marine species to make products that are greener and more environmentally friendly through biotransformation into things that are need by cosmetic manufacturers. In this case, natural products from marine microorganisms are reviewed and evaluated for various cosmetic applications (Ding *et al.,* 2022). Thus, the existing research became a trigger for further research into the use of mangrove symbiont bacteria *X. granatum* as a cosmetic ingredient. Therefore, this study aimed to obtain materials that can be used in further research into natural cosmetic formulas made from marine nature. Thus, this study is a reference material for further research into the bacterial material found as a reference for obtaining cosmetic formulations, moisturising tests, and the characterisation of cosmetic preparations. The study found that the mangrove symbiont bacteria of the *X. granatum* type have potential as antibacterial and antifungal agents. These findings will be used as a reference for further research on cosmetics.

CONCLUSIONS

The results of bacterial isolation from the leaf and fruit symbiont bacteria of the mangrove *X. granatum* collected from the Baturusa River, Merawang District, Bangka Belitung Islands, Indonesia, demonstrated 13 bacterial isolates originated from the leaf and fruit extract. It was found that circular bacterial isolates dominated. The morphological features of the isolated samples were as follows: white in colour, whole margin, convex elevation, and medium, small, large, and punctiform sizes. It was observed that six isolates showed potential as antibacterial agents against pathogenic bacteria *S. aureus, V. Harvey,* and *V. alginolyticus,* while also showing potential as an antifungal agent against pathogenic fungi *M. furfur* and *C. albicans*. The six isolates of symbiont bacteria collected from mangrove leaf and fruit extracts of *X. granatum* that have antibacterial and antifungal potential are *Sinomicrobium oceani* (99%). *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceanis* (85%), *Sinomicrobium oceanis* (99%), and *Alcaligenes aquatilis* (98%). The Gas chromatography-mass spectrometry (GC-MS) analysis of six identified isolates showed 20 types of compounds for each of the bacteria tested. The analysis of the content of bacteria that have antibacterial and antifungal potential in the fruit and leaf extract of the mangrove *X. granatum* revealed several compounds, namely, hexadecanoic acid, methyl ester (CAS) (27.52%) in *S. oceani*; 9-octadecenoic acid (Z)-, 35.61% methyl ester (CAS) in *S. pectinilyticum*; 9- octadecenoic acid compound, methyl ester, (E)- (CAS) 44.02% in *A. aquatilis*; Octadecenoic acid, methyl ester, (E) – (35.64 % area) in *P. khazarica*; 9-octadecenoic acid compound, methyl ester, (E)- 33.28% in *P. mirabilis*, and the highest compound content in *S. oceani*, i.e. 9-octadecenoic acid compound, methyl ester, (E)- (46.58%). These bacteria have the highest content of 9-octadecenoic acid compound, methyl ester, which is a straight-chain saturated fatty acid component of C18. 9-octadecenoic acid compound, methyl ester, contains compounds that have antibacterial and anticancer properties.

AUTHOR CONTRIBUTIONS

D. Pringgenies contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation literature review, validation, review and editing. W. Ari Setyati contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation. F. Feliatra collected the resources. D. Ariyanto contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation literature review, validation, review and editing. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
AMB	l-2-Amino-4-methoxy-trans- 3-butenoic acid
BLAST	The Basic Local Alignment Search Tool
⁰C	Celsius degree
CAS	Hexadecanoic acid, methyl ester
DNA	deoxyribonucleic acid
GC-MS	Gas Cromatography and Mass Spectroscopy
h	Hours
kPa	Kilopascal
MDR	Multi-Drug Resistance
MEGA	Molecular evolutionary genetics analysis
μg	Micrograms
μg/disk	Microgram per disk
μL	Microliter
mL	Mililiter
mm	Milimeter
min	Minute
NCBI	National Center for Biotechnology Information
PCA	Phenazin I-carboxylic
PCR	polymerase chain reaction
pyo	Pyocyanin
rpm	rotation per minute
RNA	Ribonucleic acid
s	Second

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Podcasts

CASE STUDY

Prediction models of iron level in beef muscle tissue toward ecological well-being

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ABSTRACT

BACKGROUND AND OBJECTIVES: Elemental status is associated with the biochemical processes occurring in the body. Beef, consumed worldwide, is an excellent source of iron in terms of quantity and bioavailability, providing up to 18 percent of the daily requirement. The level of iron in muscle tissue affects beef quality. Current methods used to assess iron content in cattle muscles are laborious and complex. Accordingly, the current study aimed to develop a fast and simple method to assess the elemental status of animals in vivo and in a minimally invasive way based on an effective model for iron-level prediction by using blood-analysis results toward ecological well-being. This method can overcome the shortcomings of currently used approaches.
METHODS: Samples of diaphragmatic muscle weighing 100 grams, as well as blood samples, were obtained from Hereford cattle bred under typical conditions of an industrial complex in the south of Western Siberia, Russia. Elemental analysis was performed by atomic absorption method with electrothermal atomization. Regression analysis was conducted to estimate the relationships between iron level in the muscle tissue of Hereford cattle and independent values (blood parameters). An optimum model for predicting the iron level was established. The coefficients of regression models were calculated using the least squares method, and the values of the dependent variable corresponded with the Gaussian ones. A high correlation existed between independent variables.
FINDINGS: An optimum model for predicting the iron level in the muscle tissue of Hereford cattle was established. It contained three predictors, namely, number of erythrocytes, color index, and globulin, as a result of selection based on internal and external-quality criteria. The model meets the necessary assumptions: the residuals are normally distributed, no autocorrelations exist, and the observations are influential. Furthermore, no signs of multicollinearity exist between the main effects of the model (variance-inflation factor = 1.2–1.7).
CONCLUSION: The model can be used for the intravital analysis of iron level in the muscle tissue of cattle. In contrast to currently used methods, the approach proposed can be used for intravital analysis of the level of iron in muscle tissue, which is the most important advantage of the developed approach. The results can be used in ecology to assess ecological well-being and determine the allowable load of iron in animals. For veterinary medicine, the resulting model enables the evaluation of the iron level in the muscle tissue of Hereford cattle during their lifetime. Studying the effect of different factors on meat quality may allow to decrease or avoid useless measures used in farming, such as the excessive use of feed additives. In turn, these measures can decrease resource exploitation and increase farming productivity. Therefore, the results can guide the further development of sustainable farming.

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INTRODUCTION

In the body of adult animals, the average concentration of iron (Fe) is 0.005 percent (%) –0.006% in natural humidity. Most iron is in the form of organic compounds that can be divided into two groups: porphyrin (heme iron) (70%–75%) and nonheme iron (25%–30%). Hemic iron is represented by hemoglobin, myoglobin, and heme-containing enzymes (cytochromes, cytochrome oxidase, catalase, and peroxidase). Nonheme iron comprises transferrin, ferritin, hemosiderin, and some iron proteinates, including ferroflavoprotein (Diniz *et al.*, 2019). This element involves many biochemical processes: oxygen transport, blood production, energy metabolism, immune functions, and many others (Diniz *et al.*, 2016; Kupczyński *et al.*, 2017). According to a meta-analysis (Institute of Medicine, 2001), the median dietary intake of iron is approximately 16–18 milligrams per day (mg/day) for men and 12 mg/day for women, whereas the tolerable upper intake level for adults is 45 mg/day. Excessive intake of iron has a harmful effect and causes symptoms of poisoning, despite its crucial role in living organisms (Institute of Medicine, 2001; Khaleghnia *et al.*, 2021; Garmyn *et al.*, 2011). Excessive iron has various adverse effects, including gastrointestinal and cardiovascular diseases caused by oxidative stress (Institute of Medicine, 2001; Geissler and Singh, 2011). Moreover, iron content in food usually does not exceed 5 milligrams per 100 grams (mg/100 g) (Table 1), so the problem owing to excessive iron consumption with food is not relevant in most cases. Different types of meat are the main source of iron for human population. Thus, the content of iron (and other elements) in different types of meat, as well as other types of food, should be controlled to solve several tasks. These tasks include regulating human diet, estimating the effect of different factors on iron content in food, and avoiding excess of iron and other metals in food (Table 1).

Iron content in beef

Beef, consumed worldwide, is an excellent source of iron in terms of quantity and bioavailability because iron content in beef exceeds that in other types of meat, providing up to 18% of the daily requirement (Valenzuela *et* al., 2009; Duan *et al.*, 2012; Mateescu, 2014). Recent studies have shown that muscle iron content can affect beef-quality parameters. Iron content in meat including beef is associated with flavor and juiciness (Mateescu *et al.*, 2013a), meat structure (Kim *et al.*, 2010), palatability (Garmyn *et al.*, 2011), red color intensity, and lipid oxidation (Purohit *et al.*, 2015). Monounsaturated fatty acid levels are positively associated with semitendinosus iron content in crossbreeds of beef cattle, whereas cholesterol and polyunsaturated fatty acid levels are negatively correlated (Ahlberg *et al.*, 2014). Molecular mechanisms of the effect of iron and other mineral contents on different gene expression,

Table 1: Summary of the studies on iron content in different type of food

Subject of the study	Summary	Reference
Raw and cooked red	The effect of different factors on the analysis of iron accuracy is studied.	Lombardi-Boccia *et al.*,
Beef meat and viscera	Bovine cuts of meat have a low variation in total Fe, and heme Fe comprises more than 60% of the total Fe.	Valenzuela *et al.,* 2009
Soil, pasture sward, and blood plasma of extensive reared bulls	No relationships exist among iron soil, forage, and blood concentration in beef cattle.	Pavlík *et al.*, 2013
Rice, wheat, or corn-containing products.	A novel method for iron extraction and determination is proposed.	Niedzielski *et al.*, 2014
Fish, shrimp, and prawn.	A modified heme-iron-extraction method has been proposed. It reveals the underestimation of previous analyses of iron content in seafood.	Wheal, *et al.*, 2016
Calf blood	The effects of feeding protein–iron complex on productive performance and indicators of calf metabolism are studied. Low doses of iron in diet positively affect calf metabolism parameters.	Kupczyński *et al.*, 2017
Paste from poultry and cattle bone	The potential role of bone paste as a source of minerals in the meat industry is demonstrated.	Kakimov *et al.*, 2021
Beef	Metal content (including iron) in beef samples from different locations is studied.	Sabow *et al.*, 2021
Reindeer meat	A meta-analysis of reindeer meat samples reveals significant differences in iron content among meat from different regions	Andronov *et al.*, 2022

which in turn affect meat quality, are considered in previous works (Diniz *et al.*, 2019; Mateescu *et al.*, 2013b). Iron may affect the expression of various genes through different mechanisms. Conversely, some genes may affect iron content in muscle tissues, which may be used to regulate iron content in meat in the future (Mateescu *et al.*, 2013b). Thus, iron content is one of the key characteristics affecting meat quality. The technique for assessing the interior by the elemental status of animals by blood parameters has yet to become widespread in agricultural production, even though the elemental status is associated with the biochemical processes occurring in the body (Kupczyński *et al*., 2017; Diniz *et al.*, 2019). This technique is due to the high financial costs and laboriousness of studying the chemical composition of animal organs and tissues (Miroshnikov *et al*., 2019; Miroshnikov *et al*., 2020). Currently used approaches to determine metal (including iron) content in different types of meat are based on sample preparation involving stages of meat sample freezing, drying, and metal extraction with different reagents. Further analysis of the obtained extracts through atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry methods or other methods is also needed. This scheme is used to analyze element content in different types of food (Table 1), as well as animal hair (Miroshnikov *et al*., 2019; Miroshnikov *et al*., 2020). Currently, this approach may be considered as the main one for meat analysis (King *et al*., 2023). Depending on the sample properties and the aim of the analysis, all stages of the analysis may be optimized, for example, more effective reagents for metal extraction may be used (King *et al*., 2023). However, it does not significantly decrease the time and labor consumption to determine the elemental status of meat. Thus, the main shortcomings of currently used techniques for analyzing iron (and other elements) in meat is complexity, presence of several stages, and high costs. Notably, another disadvantage of currently used methods to determine iron content in meat is the impossibility of intravital assessment of iron content, which in turn does not allow correct iron content in cattle meat before slaughter. Thus, the development of fast and simple approaches, which may enable the determination of iron content in meat including that in beef, is an urgent task and investigation in this area. Animal blood is an easily accessible, simple, and easy-to-select biological material. Furthermore, hematological and biochemical analyses do not require expensive laboratory equipment, are fast in sample preparation and execution, and do not require high financial costs. Therefore, this biomaterial is well suited for the role of an in vivo indicator of the content of heavy metals in the organs and tissues of animals. Furthermore, no data exist on robust relationships between blood-analysis results and iron content in meat, which does not allow using these data for iron-content estimation. The current study aimed to establish an optimum and effective model for predicting iron level in the muscle tissue of Hereford cattle, which enabled the assessment of the elemental status of animals in vivo and in a minimally invasive way toward food health consumption and ecological well-being. Notwithstanding the importance of other elements' contents in meat that determine food quality, It was focused on iron because it is the main element affecting the nutritional value of food, and beef is one the main sources of iron for the human population. This study was performed in in the Novosibirsk region of Russia in 2022–2023.

MATERIALS AND METHODS

The study was approved by the expert commission of the Federal State State-Funded Educational Institution of Higher Education "Novosibirsk State Agricultural University." Hereford cattle (n=30) bred in the south of Western Siberia (Russia) were studied. The animals were kept under standard conditions of an industrial complex in compliance with veterinary and zootechnical requirements following the law and under normal conditions for each species and breed. Feeding was performed with a typical complete feed, considering the age, live weight, and direction of animal productivity.

Sample selection

The animals were slaughtered through the current requirements, technological instructions, and legal documents in force on the territory of the Russian Federation. Before slaughter, studies on their mucous membranes, skin, and derivatives were conducted. At the time of slaughter, the animals were clinically healthy. The animals were placed on a 12–18-hour preslaughter starvation diet. Organs and tissues were sampled immediately after the massacre; they were frozen and stored at -18 degrees Celsius (°C) to -24 °C. Samples of skeletal muscles weighing 100 g were collected from the diaphragmatic muscle. Blood samples were collected from the jugular vein of the animals and stabilized with 5% sodium citrate. Blood samples were delivered to the laboratory within 6–12 hours, where hematological and biochemical analyses was performed.

Sample preparation for analysis

A portion of muscle tissue was crushed for atomic absorption analysis. From a homogeneous mass, a sample weighing 2–5 grams was selected and placed in a 50 milliliter (mL) quartz cup and poured with 25–50 mL of ethanol, covered with filter paper, and left at an ambient temperature of 10–25 °C for 24 hours. Then, the samples were dried at low heat. After cooling, a solution of nitric acid (HNO_3) (1:1) was added in small portions, and the oxidation reaction was monitored, preventing the rapid evolution of foam. After the samples reacted with the HNO_3 nitric acid solution, their heating slowed to charring. Quartz cups with charred pieces were placed in a muffle furnace heated to 250 °C. They were tested, gradually raising the temperature to 510 °C, and kept for at least 4 hours until complete ignition. The samples were then treated with 5 mL of concentrated nitric acid HNO_3 and 1 mL of perchloric acid $HClO_4$, the lids were covered, and the solution was heated strongly with fire until the answer became clear. After removing the caps, the samples were evaporated to a dry residue, which was treated with 5 mL of hydrochloric acid solution (1:1) and evaporated to wet salts. Then, the resulting sample was transferred into a container with 10 mL volume. The resulting working solution was analyzed on an MGA-1000 atomic absorption spectrometer (Lumeks LLC (Limited Liability Company)), Russia). The hematological parameters determined were the level of erythrocytes, leukocytes, and hemoglobin. An automatic hematology analyzer PCE-90VET (Hight Technology Inc, USA) was used. Biochemical parameters were determined using photometric methods on a Photometer-5010 semiautomatic biochemical analyzer (Robert Riele GmbH and Co KG, Germany) using reagents manufactured by Vector-Best CJSC (Closed Joint-Stock Company) and Olvex Diagnosticum LLC. Hemoglobin level was assessed at a wavelength of 540 nm by the Hemichromes method using a Hemoglobin-Novo reagent kit manufactured by Vector-Best CJSC. Hemoglobin concentration was proportional to the hemichrome color intensity.

Statistical analysis

The original data were processed using the statistical programming language R. Model-fitting conditions were tested according to the exploratory data analysis protocol (Zuur *et al*., 2010). Potential outliers were analyzed using the Grubbs test (Adikaram *et al*., 2015). They determined whether the data distributions are Gaussian using the Shapiro–Wilk test (Razali *et al*., 2011; da Silva Diniz *et al*., 2020). The correlation coefficient between variables was calculated using the Spearman test (Xiao, 2019). Multicollinearity was assessed by calculating the variance-inflation coefficient for each parameter (Zuur *et al*., 2010) and by using a graphical method with the scatterplot matrix of regression-model variables. The model coefficients were calculated by the least squares method. Multiple comparisons of influential observations were made by Bonferroni correction (Aickin and Gensler, 1996). The conditions for the independence of the model's residuals were tested using the Durbin–Watson test (Chen, 2016).

RESULTS AND DISCUSSION

Model fitting

According to the methodology, regression analysis began with the creation of a complete model containing all predictors by using Eq. 1 (Zuur *et al*., 2010).

$$y \sim x1+x2+x3+x4+x5+x6+x7+x8+x9+x10+x11+x12 \qquad (1)$$

Where, y is the response variable, and x1–x12 are independent variables.

For convenience, the independent variables are renamed. According to Table 2, the dependent variable (y) is the muscles' level of iron in milligram per kilogram (mg/kg).

An essential step in exploratory analysis is the selection of regression models and the choice of predictors to assess multicollinearity. The models are unstable in the presence of multicollinearity in estimating coefficients. Consequently, analyzing individual factors' contribution to the response variable's variance is challenging. A paradox may arise

Table 2: Designation and interpretation of independent variables used to select regression models

Indicator	Unit of measure	Variable value in the model
Fe level in blood	millimoles per liter (mmol/L)	x1
Leukocytes	$\times 10^9$ pieces (pcs.)	x2
Erythrocytes	$\times 10^{12}$ pcs.	x3
Hemoglobin	gram per liter (g/L)	x4
Erythrocyte sedimentation rate	millimeters per hour (mm/h)	x5
Color indicator of blood		x6
Protein	g/L	x7
Albumin	g/L	x8
Globulin	g/L	x9
Urea	mmol/L	x10
Uric acid	micromole per liter (μmol/L)	x11
Cholesterol	mmol/L	x12

when the coefficients of the regression model are statistically insignificant. At the same time, the model as a whole is significant (the null hypothesis tested by F-statistics about the equality of all coefficients to zero is rejected). Accordingly, the values of the Spearman correlation coefficient were calculated and a correlation matrix and scatterplots were built to assess the linear relationship between variables (Figs. 1 and 2). The calculated correlation coefficients are in the lower triangle on a red background, and the significance levels for these coefficients are in the upper triangle. Analysis showed a relatively large number of relationships among variables, many of which are physiologically determined. For example, the color index of blood reflects the degree of saturation of red blood cells with hemoglobin. It represents the ratio of red blood cells and hemoglobin in blood. Likewise, the concentration of iron in blood is related to the number of red blood cells, and the total protein level is associated with the concentration of globulins, one of its main fractions. Naturally, these indicators have a high correlation. In other cases, the connection could be more precise. For example, a positive relationship between the level of iron in the blood and the concentration of uric acid may be due to the possibility of creating complexes from them, thereby increasing the antioxidant activity of the latter (Davies *et al*., 1986).

Another complex way to assess the degree of multicollinearity of a complex of predictors is to calculate the variance-inflation factor (VIF). A higher VIF for each predictor corresponds with a closer linear relationship with the rest of the independent variables. The VIF values of predictor dispersion were calculated for all candidate models (Table 3).

The example of the general model clearly shows the high multicollinearity of the model parameters, especially the protein and its constituent fractions of globulins and albumin. The correlation is less pronounced in the value of the color index of blood, calculated from the number of erythrocytes and hemoglobin. Including a complete set of predictors in the model leads to duplication of the influence of independent variables on the response value and creates excessive information noise. The rest of the indicators have a relatively low VIF weight, so the estimate of the remaining coefficients should be statistically significant.

Regression-model selection

Table 4 gives estimates of the coefficients of the complete model containing all independent variables. Consequently, a predictable situation is observed when, owing to the high multicollinearity, the estimates of all coefficients of the complete linear regression model turn out to be statistically insignificant according to the t-test. The F-test also indicates the statistical significance of the entire

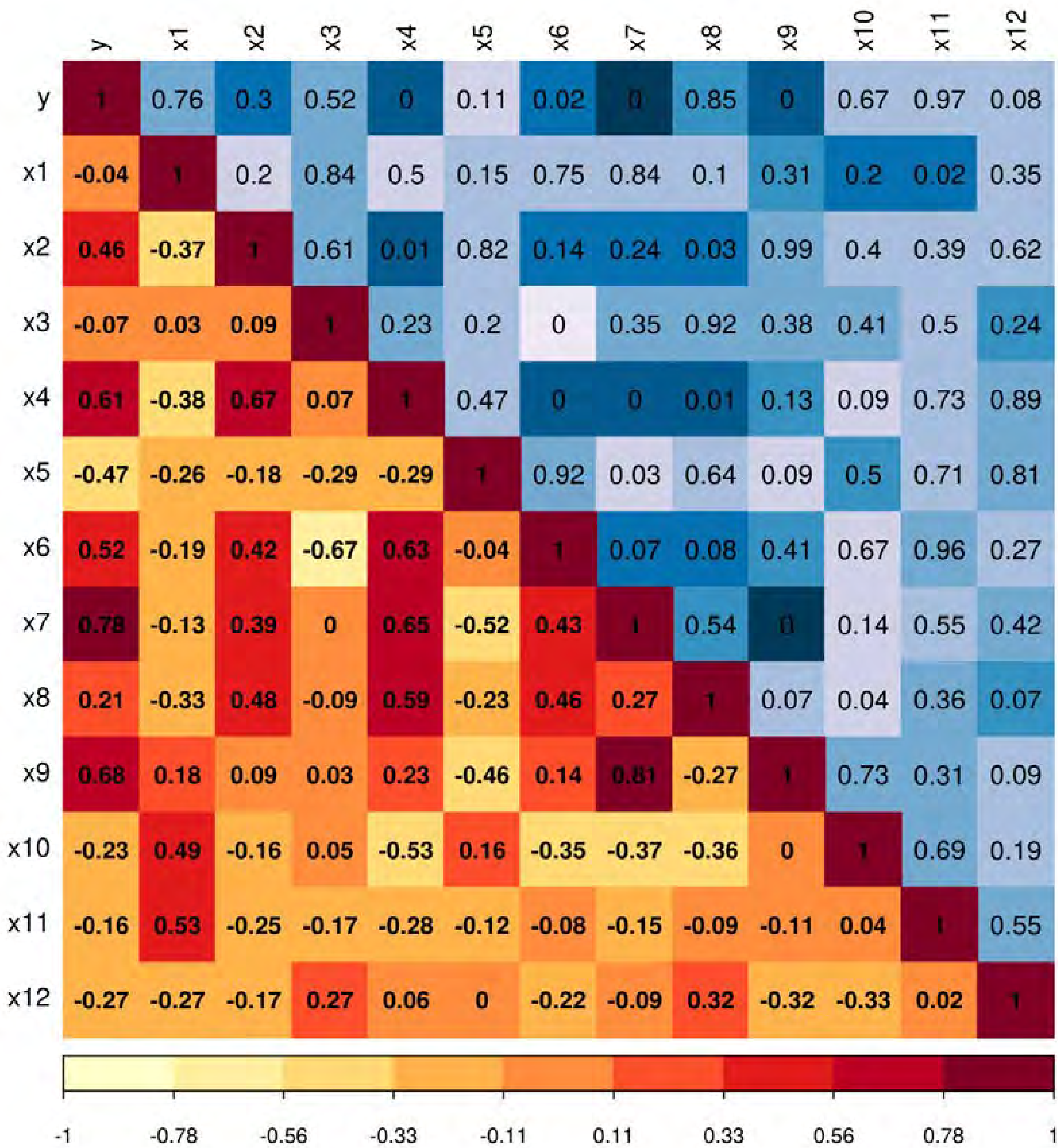

Fig. 1: Correlation matrix of regression-model variables

model. One of the most effective ways to reduce multicollinearity is to select an informative set of predictors.

The optimum structure model with tuned parameters should provide the "best" value of a particular quality criterion. However, with many variables, achieving the optimum value for all quality criteria is almost impossible, so several suboptimal candidate models are created for the subsequent selection of the working model. Stepwise regression

Fig. 2: Matrix of scatterplots of regression-model variables

Table 3: Dispersion of inflation-factor values for regression models' coefficients to assess iron level in muscle tissue

Predictor	Complete model	y~x3+x4+x6+x7+x12	y~x3+x4+x6+x8+x9+x12	y~x3+x6+x9
x1	2.1	–	–	–
x2	4.1	–	–	–
x3	27.2	13.8	15.5	1.7
x4	34.2	14.5	18.1	–
x5	2.3	–	–	–
x6	37.9	21	22.4	1.7
x7	631.5	1.8	–	–
x8	156.6	–	2.8	–
x9	697.5	–	2.1	1.2
x10	3.4	–	–	–
x11	1.8	–	–	–
x12	1.9	1.2	1.5	–

Table 4: Parameters for estimating the coefficients of the complete model to predict the iron level in muscle tissue from blood parameters

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	P_t*
Int.	-25.655	25.605	-1.002	0.338
x1	-2.483	45.661	-0.054	0.958
x2	-0.181	0.345	-0.525	0.61
x3	3.166	2.865	1.105	0.293
x4	-0.094	0.179	-0.523	0.611
x5	0.523	2.608	0.2	0.845
x6	17.562	15.91	1.104	0.293
x7	-0.573	1.308	-0.438	0.67
x8	0.888	1.265	0.702	0.497
x9	0.823	1.288	0.639	0.536
x10	0.54	0.903	0.598	0.562
x11	-0.001	0.015	-0.09	0.93
x12	-1.856	1.66	-1.118	0.288

RSE^2 – 4.95; F-statistic – 3.98, P = 0.015.

Here and below: [1]Int. Is the free term of the equation; RSE^2 is the estimate of the standard deviation of residuals (residual standard error); * P_t – significance level of t-statistics; SE (standard Error).

Table 5: Parameters for estimating the coefficients of the candidate model selected by the combined algorithm

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	P_t
Int.	-21.170	14.601	-1.450	0.165
x3	3.555	1.830	1.943	0.069
x4	-0.140	0.110	-1.267	0.222
x6	21.055	10.373	2.030	0.058
x8	0.217	0.144	1.509	0.150
x9	0.231	0.059	3.899	0.001
x12	-1.735	1.233	-1.407	0.180

RSE – 4.20; F-statistic – 10.78, P < 0.001.

analysis is performed using a combined algorithm "stepwise forward and backward selection". In the first step, the best one, according to the Akaike information criterion, is selected from all predictors. Then, the following variable with the optimum solution with the first coefficient of the model is set. The algorithm stops when the extremum of the criterion value is reached. Afterwards, the exclusion stage replaces the location of the inclusion of variables. In this case, all combinations of variables are sorted out. Then, a less informative predictor is excluded from the model by the value of the specified quality criterion, and so on, until the criterion extremum is reached. The model obtained by this method is presented in Table 5. Compared with the entire model, the estimate of the standard deviation of the residuals and the F-statistic is significantly lower, showing the superiority of this model over the general one.

A more illustrative method for selecting the optimum regression model is to sequentially build all possible regression models with an assessment of the quality of each of them. The method's main disadvantage is the need to use significant computing power. In this case, when using 12 independent variables, 4096 regression models were built. Consequently, the best models were ranked according to the main internal-

Table 6: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) according to the value of the Akaike information criterion

Model equation	df	p	SSE	MSE	R^2	R^2_{adj}	AIC	BIC
y ~ 1+x3+x4+x6+x7+x12	18	5	310.281	17.238	0.785	0.725	143.535	159.946
y ~ 1+x3+x6+x9	20	3	372.917	18.646	0.741	0.702	143.948	158.003
y ~ 1+x3+x6+x9+x12	19	4	348.338	18.334	0.758	0.707	144.312	159.545
y ~ 1+x3+x6+x7+x12	19	4	350.013	18.422	0.757	0.706	144.427	159.66
y ~ 1+x2+x3+x6+x7+x10+x12	17	6	296.436	17.437	0.794	0.722	144.44	162.028

Here and below: df is degrees of freedom, p is the number of model coefficients, SSE is the sum of squared errors, and MSE is the mean-squared error.

Table 7: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) according to the value of the Bayesian information criterion

Model equation	df	p	SSE	MSE	R^2	R^2_{adj}	AIC	BIC
y ~ 1+x3+x6+x9	20	3	372.92	18.65	0.74	0.7	143.95	158
y ~ 1+x7+x12	21	2	436.75	20.8	0.7	0.67	145.74	158.62
y ~ 1+x4+x9	21	2	439.64	20.94	0.7	0.67	145.9	158.78
y ~ 1+x7	22	1	509.75	23.17	0.65	0.63	147.45	159.15
y ~ 1+x4+x7+x12	20	3	392.96	19.65	0.73	0.69	145.21	159.26

Table 8: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) by the value of the adjusted coefficient of determination

Model equation	df	p*	SSE	MSE	R^2	R^2_{adj}	AIC	BIC
y ~ 1+x3+x4+x6+x7+x12	18	5	310.28	17.24	0.79	0.73	143.54	159.95
y ~ 1+x2+x3+x6+x7+x10+x12	17	6	296.44	17.44	0.79	0.72	144.44	162.03
y ~ 1+x3+x4+x6+x7+x8+x9+x12	16	7	281.01	17.56	0.81	0.72	145.16	163.92
y ~ 1+x3+x4+x6+x8+x9+x12	17	6	299.91	17.64	0.79	0.72	144.72	162.31
y ~ 1+x3+x4+x6+x7+x10+x12	17	6	303.93	17.88	0.79	0.72	145.04	162.63

quality criteria, namely, Akaike information criterion (AIC) (Table 6), (Bayesian information criterion (BIC) (Table 7), and R^2 adjusted (adj) (Table 8).

The adjusted coefficient of determination is the greediest; that is, a model with many parameters will often be preferred, even though each criterion considers the number of predictors in the model. For example, the best model for R^2 adj includes five predictors. A more balanced approach is implemented using the information criteria AIC and BIC, imposing a "penalty" for adding new parameters. Their main difference is that the BIC is more sensitive to adding new parameters and prefers the most compact models. This criterion is found when analyzing Tables 3 and 4. According to the AIC, the best model contains five coefficients, and the following has 3. Moreover, according to the BIC, the best model includes three independent variables. Although the value of the standard deviation of the residuals is slightly higher than that of the previous model, the value of the F-statistic is higher (Table 9).

Thus, for further analysis, three models can be selected (the best according to internal-quality criteria), and the whole model with all coefficients can be left for comparison. The most compact models with minimal multicollinearity are worth giving preference. Therefore, if it is considered the selected candidate models based on the VIF (Table 2), multicollinearity would be absent only in the model with three predictors (the best according to the BIC). When using the Mallow test (Fig. 3b), the best model has the same variables as the Bayesian information test (Fig. 3a).

The above estimates of the quality of fit of the

Table 9: Parameters for estimating the coefficients of the candidate model to predict the iron level in muscle tissue (mg/kg) from blood parameters

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	P_t^*
Int.	-9.583	6.925	-1.384	0.182
x3	1.551	0.631	2.456	0.023
x6	11.503	2.966	3.878	0.001
x9	0.212	0.046	4.570	<0.001

RSE^2 – 4.318; F-statistic – 19.1, P < 0.001.

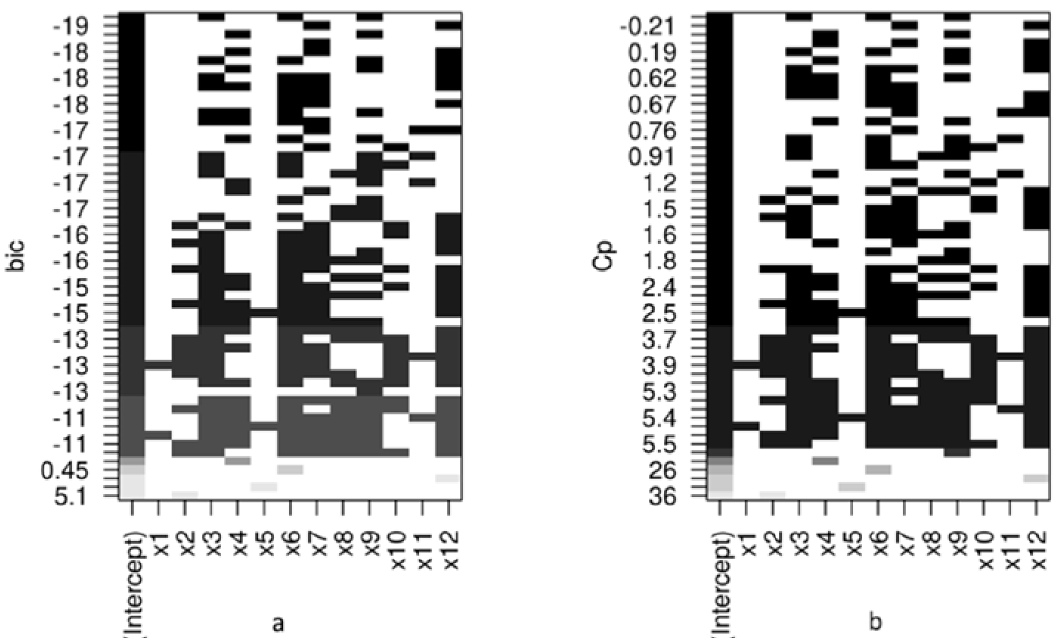

Fig. 3: Ranking models for predicting iron level in muscle tissue by using the Bayesian information criterion (a) and the Mallow criterion (b)

regression model refer to "internal" criteria because their calculations are based on the same data used to calculate the model. Therefore, estimates often provide biased measures of the actual function of the process, which are based on sampled empirical values of small samples. Unbiased forecasts can be obtained only by applying external-quality criteria. They are also effective against model overcomplication and allow model selection with an optimum number of parameters. The most informative external criterion is cross-validation (CV). The prediction error of the response variable is estimated during the course of multiple random splitting of the initial sample into training and testing. Some of them are based on leave-one-out CV: n (sets) of regression models are fitted on (n–1) sample values, and the excluded observation is used each time to calculate the prediction error. Visualization of the best candidate models, divided into three blocks (k=3) by the CV method, shows that the regression lines of the model for BIC and Mallows's *Cp* (Cp) (Fig. 4a) provide a more accurate forecast relative to the others (Fig. 4b and c), and the complete model (Fig. 4d) is the worst fit to predict the level of iron in muscle tissue.

The CV error for the model has decreased by more than 110 times relative to the overall model selected based on the BIC. This error is also 26% better than the closest model selected based on the adjusted

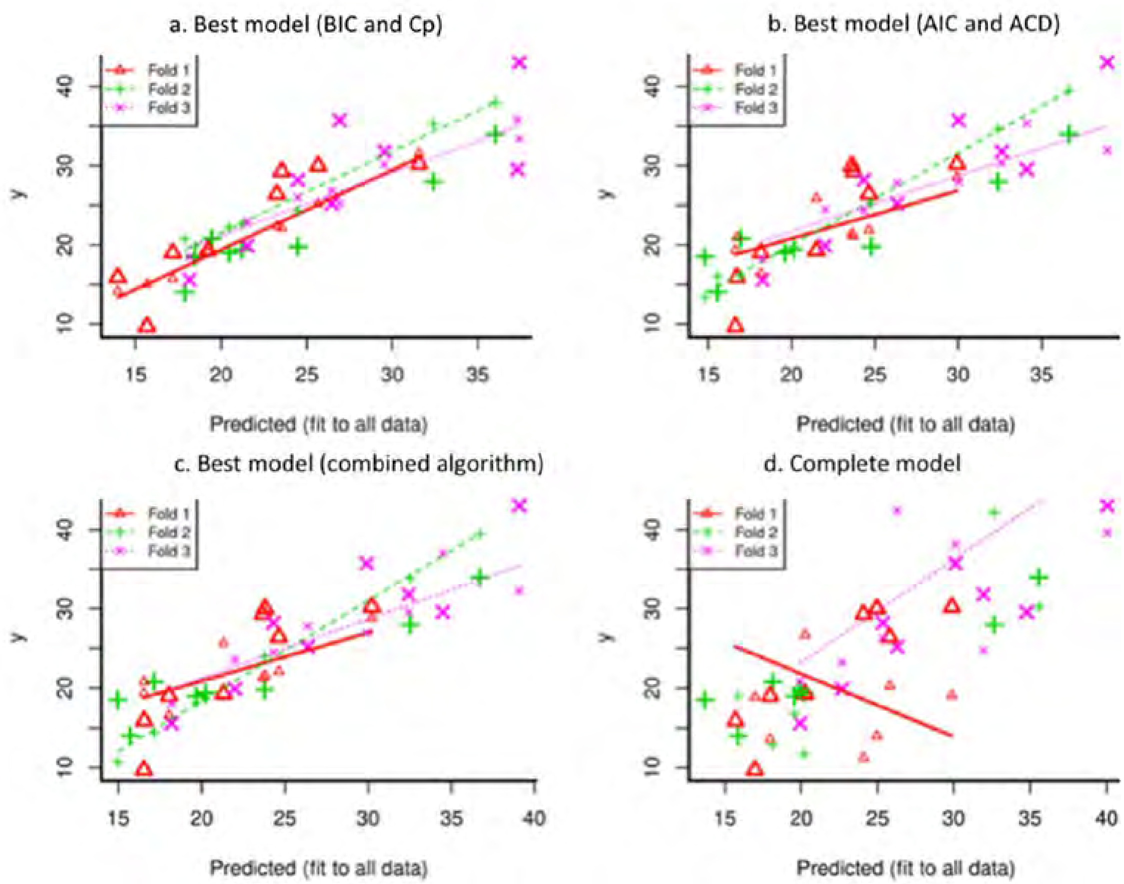

Fig. 4: Visualization of candidate models for assessing the level of iron in muscles by the cross-validation method: BIC and Cp (a), AIC and ACD (b), combined algorithm (c), and complete model (d)

Table 10: Estimated error in the cross-validation of regression models for predicting the level of iron in muscle tissue (mg/kg)

Model Formula	SS	df	MS
y ~ 1+x3+x6+x9	561	24	23
y ~ 1+x3+x4+x6+x7+x12	756	24	31
y ~ 1+x3+x4+x6+x7+x8+x9+x12	2360	24	98
y ~ 1+x2+x3+x4+x5+x6+x7+x8+x9+x10+x11+x12	62126	24	2589

Note: SS is the sum of squares, MS is the mean square.

determination coefficient and the Akaike information criterion (Table 10).

Thus, the optimum model includes three predictors (x3, x6, x9).

Verifying assumptions about model residuals

Verifying the assumptions regarding the model's residuals is necessary to determine the adequacy of applying the least squares method. Using graphical methods, one can evaluate the distribution normality of residuals and the dependence on predicted values. (Figs. 5 and 6). Thus, the probability density curve of the residual distribution practically repeats the Gaussian distribution's confidence region (Fig. 5). The quantile plot of the standardized residuals

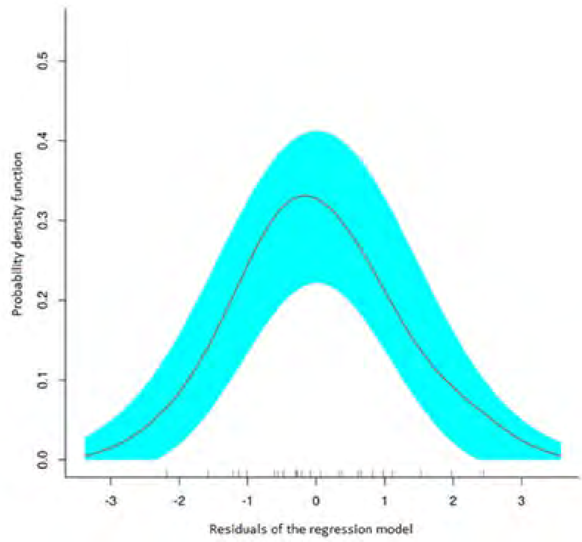

Fig. 5: Distribution of residuals of the regression model to assess the level of iron (mg/kg) in muscle tissue

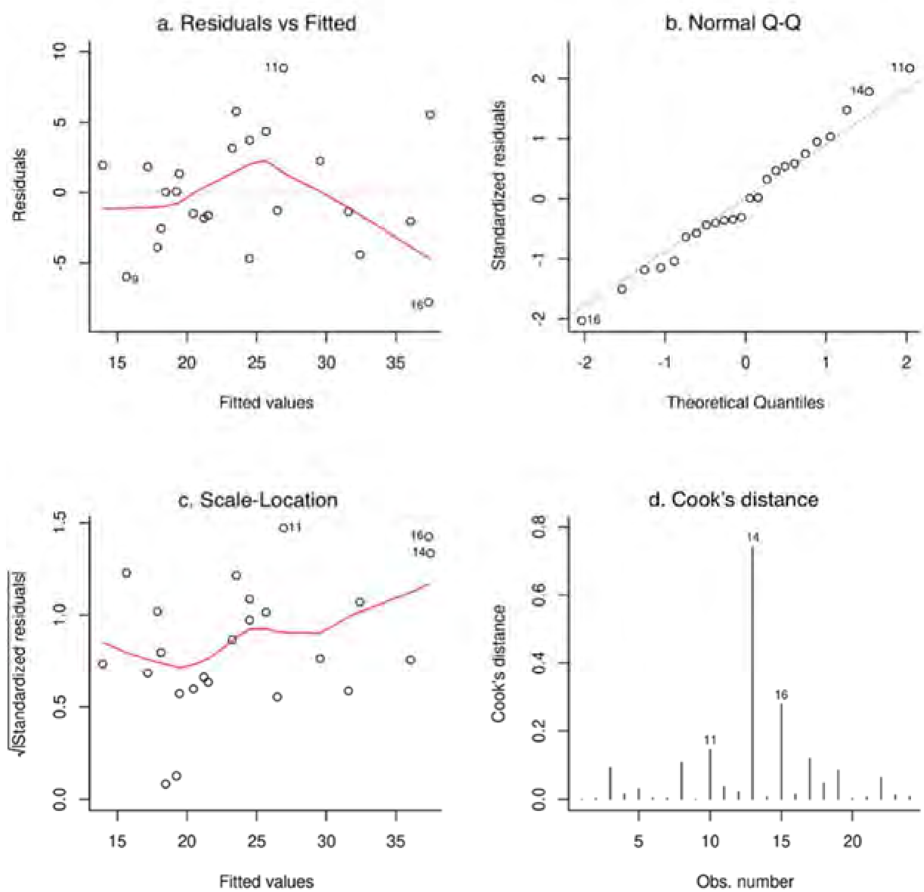

Fig. 6: Residuals versus response (a), quantile plot (b), square root of standardized residuals versus response (c), and Cook's distances (d)

and theoretically expected quantiles show that the values are distributed relatively normally (Fig. 6, top left field). This plot is also confirmed by the formal Shapiro–Wilk test (W = 1; P = 1).

The model's dispersion of residuals versus predicted values is shown in Fig. 6a, indicating the homogeneity of the variance. A smoothing line is plotted in red, which facilitates the analysis. It is close to a horizontal line, so the condition for uniform dispersion of residuals is satisfied. Fig. 6c also indicates the variance homogeneity of the residuals, the y-axis shows the square root of the standardized residuals, which are standardized by dividing each residual by its standard deviation. The standardization procedure improves the heterogeneity detection of their variance. The smoothing line, in this case, is also close to horizontal. Fig. 6d is built to identify "influential" observations. The visualized Cook's distance values show that three comments (highlighted by ordinal values) require careful consideration. First, student-t residuals are compared with the theoretically expected values of the t-distribution to ensure that they are not outliers. The significance level is calculated considering the Bonferroni correction for the observation with maximum deviation. In the selected model, the deal with the maximum deviation is 2.42. Its adjusted level of significance (P) is 0.62. This value is similar to the theoretically expected one, so none of the potentially influential observations is an outlier. The values of the criterion d=1.92 are obtained, and they correspond with an autocorrelation coefficient of 0.025 (p=0.67) owing to the Durbin–Watson test. Such a high significance level of the statistical error of the first kind indicates the absence of autocorrelation. Therefore, the condition for the independence of the model's residuals is satisfied. As a result of the selection and evaluation of the quality of models, the best predictive model, taking into account internal and external-quality criteria, contains three predictors: the number of erythrocytes, color index, and globulin. To predict the level of iron in the muscle tissue of cattle, it is proposed to use Eq. 2 which is made by the author based on data shown in Table 9.

$$y = -9{,}583 + 1{,}551 \times RC + 11{,}503 \times CCR + 0{,}212 \times G \quad (2)$$

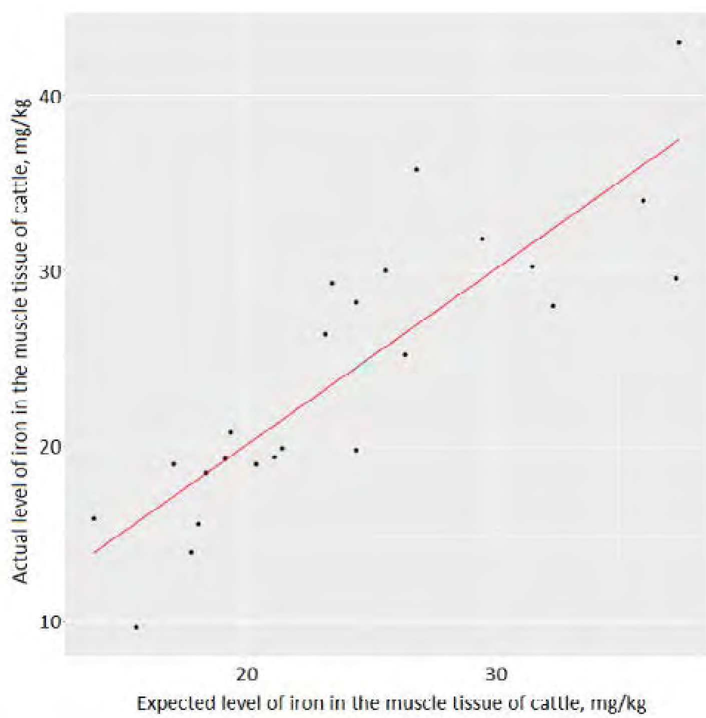

Fig. 7: Model of the expected and actual level of iron in the muscle tissue of cattle

Table 11: Summary of the studies on meat quality using novel techniques

Subject of the study	Method used and summary	Reference
Chicken breast and beef chops	Optomagnetic methods can be used to evaluate the spoilage of fresh beef and chicken meat when stored in a refrigerator.	Mileusnić *et al.,* 2017
Beef	The predictability of a detailed mineral profile of beef using different portable near-infrared spectrometers is studied. The methods can predict Fe and P contents.	Patel *et al.,* 2020
Different types of food	The work summarizes the results of studies on different nondestructive spectroscopic and imaging techniques for food quality. The authors conclude that these techniques may be used for food analysis, but the commercialization of these techniques is prevented by the high-cost equipment and generation of large data sets.	Edwards *et al.,* 2021
Different types of meat	The work summarizes results of studies on different techniques for meat quality (electronic nose, computer vision, spectroscopy, hyperspectral imaging (HSI), and multispectral imaging technologies). The authors conclude that future studies are required to enhance the accuracy, scalability, robustness, and simplicity of these technologies.	Khaled *et al.,* 2021
Pork and beef	The review concluded that HSI and visible/near-Infrared spectroscopy are the leading techniques for monitoring pork and beef quality and safety.	Sanchez *et al.,* 2022
Different types of meat	The work shows that different nondestructive spectroscopic and imaging techniques are promising for analyzing the quality of different types of meat.	Wu *et al.,* 2022

Where, y is the concentration of iron in muscle tissue (mg/kg), RC is the red count, CCR is the cell–color ratio, and G is globulin.

Visualization of the resulting model is displayed as a scatterplot of predicted and observed values (Fig. 7). The results obtained show a sufficient level of approximation and the absence of outliers.

Advantages of the proposed approach

A fast, simple, and reliable method of predicting iron content in cattle meat was proposed. The method is based on blood analyses according to commonly used methods and further use of the results obtained to calculate iron content in cattle meat. Notably, many studies have focused on the development of methods for meat-quality analysis because currently used methods are characterized by high consumption of time and labor (King *et al.*, 2023). Additionally, in literature, no data are available on the use of blood analysis to predict meat quality. Most studies have focused on the use of different spectroscopic, biochemical, and optical methods that allow fast and nondestructive meat analysis to estimate main quality parameters without tedious sample preparation (Table 11).

Despite the various methods proposed, they cannot solve the main disadvantage of currently used methods, i.e., they do not allow the prediction of elemental status of meat before slaughter. Moreover, they have certain advantages on currently used methods as they do not require long-term preparation. The method developed in this work avoids long-term preparation and application of complex and expansive equipment and can be used to predict iron content in meat before slaughter. In turn, it enables the correction of the final iron content in meat by optimizing feed livestock and obtaining meat with required quality. Notably, iron-content control in meat (particularly beef) is one of the elements of so-called sustainable beef production (Purchas and Busboom, 2005; Broom, 2021; Hubbart *et al.,* 2023) because iron content is one of the key meat-quality indicators. Thus, controlling the intravital iron content may be one the most effective tools to correct beef quality by understanding the effect of different factors on the final product quality, which may enable the development of approaches to obtain the meat of required quality through targeted methods of influence on certain meat-quality indicators. This in turn will allow only required methods to control food production and decrease the possible environmental impact of beef production, thereby avoiding the use of extensive methods to improve the quality and amount of meat produced, which can contribute to ecological well-being.

Using the method proposed, the conditions of cattle keeping may be regulated to improve the goal parameter (iron content) through a minimally invasive approach (blood sampling and analysis), which is currently used routinely in veterinary practice. Thus, applying the proposed method will not include any special ethical implications.

Limitations of this study and further research directions

This study presents initial results on the development of the proposed method. Indeed, the model accuracy and the effect of different factors should be further studied in detail by using a large sample. In the present work, a comparatively small sample was used. Based on the results, it cannot unambiguously evaluate these factors affecting the model performance and the limitation of its application. Nevertheless, the results obtained clearly suggest that the proposed method is promising for future research and may be improved for further commercialization. Further development of the method proposed also requires study of the mechanisms affecting relationships between blood parameters and iron content in muscle tissues of cattle, which in turn may improve the proposed model and avoid its shortcomings owing to factors that decrease model accuracy. The results showed only empirical patterns observed in this work but cannot provide an explanation for them. The iron amount in the diet of calves affects key blood parameters (Kupczyński *et al*., 2017). Some mutual effects probably determine blood parameters and iron content in livestock feed and muscle tissues, but their mechanisms should be studied. In addition to direct contributions to the increase in beef quality by regulating one of the key parameters of meat quality, further studies in the area of the study may be used as element of sustainable beef production. Given that the prediction of iron content cannot be used to directly solve most ecological problems of livestock farming (for example, gas emission), studying the effect of different factors on meat quality may allow to decrease or avoid useless measures used in farming, such as the excessive use of feed additives. Consequently, resource exploitation can decrease and farming productivity can increase.

CONCLUSION

A fast and simple method of assessing the elemental status of animals in vivo and in a minimally invasive way is developed. The method is based on an effective model for predicting the iron level in the muscle tissue of Hereford cattle by using blood-analysis results. The coefficients of regression models using the least squares method and the values of the dependent variable corresponding with the Gaussian are calculated. A high correlation between independent variables is revealed. An optimum model for predicting the iron level in the muscle tissue of Hereford cattle was identified, containing three predictors: the number of erythrocytes, color index, and globulin as a result of selection based on internal and external-quality criteria. The model meets the necessary assumptions: the residuals are normally distributed, no autocorrelations exist, and the observations are influential. Furthermore, no signs of multicollinearity exist between the main effects of the model (VIF = 1.2–1.7). The resulting model can be used for the intravital analysis of iron level in the muscle tissue of cattle, which is an important advantage of the developed approach to currently used methods. In the future, verifying this model on a test sample, increasing the accuracy of the forecast, and continuing to train the model are necessary. The results can be used to assess ecological well-being and determine the allowable load of iron on animals and its transfer to human. For veterinary medicine, the resulting model enables the evaluation of the iron level in the muscle tissue of Hereford cattle during their lifetime. Moreover, similar studies must be conducted on large populations and mixed linear models considering random effects must be established. It is believed that the method proposed may be used for cattle and for other types of meat animals. Analysis of literature reveals that the article possesses scientific novelty as it has not been found work based on similar approaches. The method is proposed may also be the simplest and fastest technique among currently available methods of predicting the content of one the key elements in meat. Therefore, the method has practical significance for commercialization.

AUTHOR CONTRIBUTIONS

K. Narozhnykh, as the study single author performed the study conceptualization and design, data collection, analysis and interpretation of results, and manuscript writing up.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

µmol/L	Micromole per liter
%	Percent
°C	Degree Celsius
AAS	Atomic absorption spectroscopy
ACD	Adjusted coefficient of determination
adj	Adjusted
AIC	Akaike information criterion
BIC	Bayesian information criterion
CCR	Cell-color ratio
CJSC	Closed Joint-Stock Company
Cp	Mallows's *Cp*
CV	Cross-validation
df	Degrees of freedom
Eq.	Equation
Fe	Iron
G	Globulin
g/L	Gram per liter
$HClO_4$	Perchloric acid
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
LLC	Limited Liability Company
mg/100 g	Milligram per 100 grams
mg/day	Milligram per day
mg/kg	Milligram per kilogram
mL	Milliliter
mm/h	Millimeters per hour
mmol/L	Millimoles per liter
MS	Mean square
MSE	Mean squared error
nm	Nanometer
pcs	Pieces
RC	Red count
RSE	Residual standard error
SE	Standard error
SS	Sum of squares
SSE	Sum of squared errors
VIF	Variance inflation factor

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Podcasts

CASE STUDY

Sustainability index analysis of the black soldier fly (Hermetia illucens) cultivation from food waste substrate

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ABSTRACT

BACKGROUND AND OBJECTIVES: Most Food Waste (FW) is dominated by domestic activities consisting of large numbers of organic pollutants, such as nitrogen and phosphate potentially hazardous to the environment. Domestic waste can be used as a feed source in Black Soldier Fly (BSF) cultivation with utility in pollutant removal, animal feed production, and compost fertilizer. Therefore, this study aimed to determine sustainability of larvae from BSF cultivation by calculating and analyzing index.

METHODS: Data collection was conducted using the scientific judgment of experts and business actors in BSF through Focus Group Discussion and the filling out of questionnaires consisting of 31 attributes connected with environment or ecology, economics, social, and technology dimensions. Furthermore, the data were calculated using the multi-dimensional scale approach with rapid appraisal software. Sustainability status and leverage attributes were analyzed by Monte Carlo analysis, and alternating least-squares algorithm.

FINDINGS: Sustainability index for larvae of BSF production was 89.69%. The result suggested that the technique in several stages of operation including waste collection, cultivation, harvesting, and commercialization contributed to sustainability development when the elements of strength of each dimension are considered. From the analysis of the four dimensions, economic dimension had 100% or maximum leverage value. The environmental and social dimensions had the same leverage values of 92.02%, while the technological dimension had 74.74%. The results indicated that management experiences and techniques, potential for odor generated, family member involvement, productivity level, and managers level, warrant further attention to improve sustainability of BSF production.

CONCLUSION: Production, productivity, land conversion, and population were identified as significant or dominating factors impacting the supply framework of BSF production by the intended investigation inside the display ponders. Therefore, study should be encouraged to effectively integrate BSF biomass as a value-added component in an ideal environmental, social, economic, and technical system. The results are significant in providing insights into the possibility of feasible BSF biomass production in Indonesia, which can inform government policies and programs.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
71	5	6

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INTRODUCTION

In 2022, Indonesia was estimated to generate 13 million tons of Food Waste (FW) annually, equivalent to 115-184 kg/capita/year. This resulted in losses of about USD 14.2 billion to USD 36.7 billion, equivalent to 4-5% of the annual Gross Domestic Product (GDP) (Bappenas, 2022). In addition to the enormous costs of processing, FW results in several environmental problems, including the use of landfills, odors, and the production of leachate and gas (Rehman *et al*., 2023). According to Food and Agriculture Organization (FAO) study, about 33% of all food produced and consumed is lost or wasted, equivalent to 1,300 million tons of waste per year. This means that the resources needed to produce food are also wasted, with the resulting emissions (Aziz *et al*., 2022). However, burning FW decreases its value economically, and can have negative health and environmental effects, such as dioxin release (Roy *et al*., 2023). Increased pesticide usage, biomass burning, and FW contribute significantly to an estimated 23% of total annual greenhouse gas emissions (Golasa *et al*., 2021). To address this, it is vital to find cost-effective and ecologically beneficial alternatives (Roy *et al*., 2023). The treatment of FW has become a critical concern on a global scale due to the significant expenses associated with environmental management (Aziz *et al*., 2022). Furthermore, composting has become an important approach to FW management because it can reduce the amount and weight while providing non-hazardous, stable, and nutrient-rich soil stabilization materials (Roy *et al*., 2023). The possibility of using insects as a food source (Hoang Ly *et al*., 2023) and a resource for environmental remediation has also resulted in the development of insect breeding (Balaraman *et al*., 2023) and bioconversion entrepreneurs (Zang *et al*., 2022). One of these insects, BSF (*Hermetia illucens* L.), is essential in developing a circular economy in agri-food production systems (Cohn *et al*., 2022). BSF can eat a variety of organic compounds and ingest more than twice the feed of its weight (Surendra *et al*., 2020). The larvae may alter different organic substances (Manojkumar et al., 2023), recover nutrients, and reduce the biomass of organic waste by 50–60% (Franco *et al*., 2021). Meanwhile, BSF Larvae (BSFL) create a highly useful protein biomass by converting an organic molecule, reaching 40–50% (Rini, 2021). BSF frass is also a beneficial by-product of composting in addition to protein larval biomass (Luperdi *et al*., 2023). Depending on the composting substrate used, BSF frass of the experiment is collected after 9 to 23 days and may weigh more than 33% of the original weight of substrate (Basri *et al*., 2022). Furthermore, the use of organic waste for the process is a sustainability and cost-effective means of resource recovery, which can produce value-added products and create business opportunities in the industrial sector (Rehman *et al*., 2023). BSF can also compost organic waste, and convert it into biomass. The development is controlled by substrate nutrition, Potential of hydrogen (pH), temperature, moisture content, Relative Humidity (RH), and light intensity, hence resulting in difficult cultivation. Protein and fat are crucial nutrients for BSF development (Aziz *et al*., 2022), which may devour more than twice its body weight and feed on a variety of organic compounds (Surendra *et al*., 2020). Additionally, the larvae can change various organic components into nutrients and reduce the biomass of organic waste by 50–60% (Franco *et al*., 2021). According to Ermolaev *et al*., (2019), methane emissions from FW composted by fry larvae were 99.5% lower than those from windrow composting. Mertenat *et al*., (2019) also reported that methane levels from the use of BSFL to treat kitchen trash were comparable to those measured in the air. However, (Bava *et al*., 2019) did not detect the gas emissions following co-product treatment with BSFL. Guo *et al*. (2021) also stated equivalent findings when reporting the emission levels below the detection limit of 100 ppm. Table 1 presents data on methane emissions resulting from the use of different substrate. The concept of circular economy is rooted in waste management through the use of BSFL due to its ability to promote circularity, close the loop, and transform waste into valuable resources. (Lopes *et al*., 2022).

BSF works to lower gas emissions (Gao *et al*., 2019) and FW (Giannetto *et al*., 2020) with expected larval feed conversion values of 1.5 to 12.5. Therefore, in the prepupa phase, the larvae are expected to reach a length of 27 mm and a width of 6 mm (Lievens *et al*., 2021), with body weights ranging from 113 to 225mg (Gligorescu *et al*., 2019). The widespread use in waste management occurs because it can lead to the conversion of low-quality carbon sources and produce high-quality larval biomass. After undergoing metamorphosis, BSF adults do not possess a mouth

Table 1: Comparative data on methane (CH_4) emission reduction per kilogram of the treated substrate from bioconversion of different substrate by BSFL

Substrate	Moisture (%)	pH	Amount of substrate (kg)	CH_4 emission substrate (mgCH$_4$/kg)	Reference
FW	75	4.4	0.3-1.1	2.4	Ermolaev *et al*. (2019)
Kitchen waste	-	-	15	0.4	Mertenat *et al*. (2019)
FW (90%) and rice straw (10%)	65	3.0-11.0	1.2	0.2-2.6	Pang *et al.* (2020)

Table 2: Proximate composition of BSF biomass

Material	Composition (%)	Reference
Crude protein	30–52	Surendra *et al.*, 2020
Fat	15–50	Surendra *et al*, 2020; Franco *et al.*, 2021; Mai *et al.*, 2019
Carbohydrates	8–24	Soetemans *et al.*, 2020
Amino acid Essential	40.54-40.89	Huang *et al.*, 2019
Non-Essential	59.11-59.46	Huang *et al.*, 2019
Glutamic acid	12.2	Wang *et al.*, 2020
Aspartic acid	10.3	Wang *et al.*, 2020
Leucine	7.7	Wang *et al.*, 2020
Lysine	7.4	Wang *et al.*, 2020
Arginine	6.2	Wang *et al.*, 2020
Phenylalanine	6.2	Wang *et al.*, 2020
Proline	6.2	Wang *et al.*, 2020
Glycine	5.4	Wang *et al.*, 2020
Histidine	4.8	Wang *et al.*, 2020
Isoleucine	4.8	Wang *et al.*, 2020
Threonine	4.5	Wang *et al.*, 2020
Serine	4.1	Wang *et al.*, 2020
Methionine	0.6	Wang *et al.*, 2020
Cysteine	0.5	Wang *et al.*, 2020

and are incapable of feeding, rendering the lifespan to less than a week. Due to the absence of a mouth, fly cannot act as a vector for disease transmission (Lievens *et al*., 2021). Male BSF typically mates at the age of three days and dies shortly after. On the other hand, female BSF lay eggs three days after mating and typically produce between 500-1,000 eggs before dying. The eggs hatch after three days and grow into prepupa in 18 days. Subsequently, it develops into a pupa, and metamorphoses into a fly for 15 days, then mates and dies after three days as an adult. The life cycle of BSF is short at 45 days and does not have a mouth, or perch on garbage. Therefore, they are clean fly and are not vectors of disease. In contrast, ordinary fly eat and perch on trash. These fly possess a wide range of mobility and can spread disease through feces attached to their mouth and feet. They are small in size and become larvae in only 4-7 days, with a longer pupa phase of 10-20 days. In addition, essential and non-essential amino acids (Kawasaki *et al*., 2019), as well as significant amounts of polyunsaturated fatty acids are found in BSFL (Gao *et al*., 2019). BSFL proximate has the composition as shown in Table 2.

Solid waste engineers have reevaluated and designed waste management plans using alternative processes due to environmental concerns related to FW composting. Some of the processes used to treat FW in the past 20 years have been approved by the solid waste sector (Aziz *et al*., 2022). Meanwhile, the potential for FW utilization is quite large because of the characterization of useful materials, as shown in Table 3. Several studies have reported that the composition of FW varies in terms of its organic components such as protein, and carbohydrates, namely starch, cellulose, hemicellulose, lignin, fat, and organic acids (Suhartini *et al*., 2022). Although the government typically bears the cost of waste processing in high-income nations, the situation is different in low-income nations where limited resources hinder the development of trash management infrastructure (Rindhe *et al*., 2019).

Table 3. Characteristics of FW and analytical measures (Suhartini *et al.,* 2022)

Parameters	Value	Analytical Measurement
TS (percent wet weight; % ww)	22.1	American public health association (APHA) method
VS (% ww)	20.4	APHA method
MC (% ww)	78.9	APHA method
VS (percent total solid; %TS)	92.2	VS divided by TS multiplied with 100
pH	5.92	Digital pH meter
Crude fat/ lipids (percent total solid, %TS)	31.8	NA*
Crude protein (%TS)	15.5	TKN multiplied with a factor of 6.25
Carbohydrate (%TS)	41.6	NA*
C (%TS)	50.84	Elemental analyzer
H (%TS)	7.2	Elemental analyzer
N (%TS)	1.8	Elemental analyzer
S (%TS)	0.24	Elemental analyzer
O (%TS)	32.03	By the difference of 100 minus C, H, N, and S concentration
Cellulose (%TS)	4.7	Automatic cellulose analyzer
Hemicellulose (%TS)	10.05	Automatic cellulose analyzer
Lignin (%TS)	2.12	Automatic cellulose analyzer
C/N ratio	28.2	Calculation

*Not applicable (NA)

Waste transportation and collection account for more than 70% of the costs (Roy *et al.,* 2023). In developing nations, biodegradable organic waste consists of 50 and 80% of municipal garbage production (Rehman *et al*., 2023). Furthermore, Mahmood *et al*., (2021) claimed that more marketing opportunities, a limited incentive system, and a lack of legislative backing from the government are barriers to mainstreaming biowaste processing. Evaluation of sustainability of FW composting process is possible by calculating sustainability index using Multi-Dimension Scaling (MDS). Meanwhile, the instruments that enable the evaluation are increasingly needed since the importance of sustainability is progressively acknowledged. A statistical analysis tool known as MDS describes patterns of similarity or resemblance that are close to one another. MDS can convert scientific assessments of their opinions or preferences into distances represented on a multi-dimensional scale. It formally refers to a collection of statistical methods that simplify preference information by providing a numerical representation of the underlying relationships between groups (Wan *et al.,* 2021). In this situation, MDS can translate and develop the opinions or preferences of respondents regarding BSF cultivation themes into sustainability index. This tool has broad applicability in the management of natural resources, marketing, political science, sociology, and ecology. Study on MDS method has been widely used for various objects in agribusiness (Ningsih *et al.,* 2021), natural resources (Narendra *et al.,* 2019), and tourism (Saputro *et al.,*2023). However, the aims of the study were almost similar in providing recommendations and considerations for decision-makers in the framework of sustainability development. The current study aims to assess the feasibility of a sustainability BSF production supply system. Previous analysis related to sustainability of agricultural products using MDS method are presented in Table 4.

The use of insects as a source of protein has been widely discussed around the world. The quality and quantity of fly larvae development media greatly affect the nutrient content of the body and the survival at each instar and subsequent metamorphosis stage (Suardi *et al*., 2022). Efforts to obtain alternative sources of protein are meaningless when the raw materials cannot be mass-produced on an industrial scale. Meanwhile, BSFL development media based on organic waste is an important factor in the production process because it does not compete with human needs. The type of FW media produces the highest water, fiber, and carbohydrate content in BSFL compared to vegetable and fruit waste (Andari *et al.,* 2021). Since Indonesia is estimated to generate 13 million tons of FW annually, equivalent to 115-184 kg/capita/year (Bappenas, 2022), then the production of maggot larvae in a sustainability

Table 4: Previous study utilizing MDS analysis

No.	Title/Topic of Study	Dimensions	References
1	Using MDS preference plot as visual analytics of data	Environmental, social, economic	(Zhang and Ding, 2023)
2	Policy-related biodiesel sustainability in Indonesia	Ecological, social, economic	(Dharmawan *et al.,* 2020)
3	Assessing sustainability of Plants and Facilities	Ecological, social, economic	(Narendra *et al*., 2019)
4	The study of social network analysis	Ecological, social, economic, institutional	(Chen and Chen, 2021)
5	Sustainability agricultural development	Ecological, social, economic, institutional	(Suardi *et al*., 2022)
6	Sustainability microalgal biomass production	Ecological, social, economic, technological	(Santoso *et al*., 2023)

manner is very critical. However, sustainability can be affected by environmental (Arifudin *et al.*, 2023), economic (Hanim *et al.,* 2021), social (Herlinda and Sari, 2021), and technological factors (Ginanti and Kusuma, 2020). In this study, the four factors were studied to determine sustainability index in providing recommendations to increase maggot production. Many studies on the potential for sustainability of BSF cultivation have been reported but none has analyzed index using MDS. Therefore, this study hypothesizes that the use of FW for BSF maggot-growing media in addition to reducing pollution also improves the quality. The practical approach of MDS provides information that can assist waste management decision-makers in BSF cultivation businesses. This study also assesses the feasibility of BSF production supply system and seeks to 1) identify the dimensions that influence sustainability index, 2) determine sustainability index for environmental, social, economic, and technological dimensions and 3) pinpoint the key variables influencing BSF production systems. Furthermore, it was performed from November 2022 to March 2023 to determine BSF biomass production by calculating and analyzing sustainability index. This study was carried out in the study group on sustainability and Life Cycle Assessment (LCA) analysis of integrated agricultural production systems, National Study and Innovation Agency, Indonesia from 2022 to 2023.

MATERIALS AND METHODS

Study procedure

The process of data collection was executed through the Focus Group Discussion (FGD) at two distinct locations, namely Biomagg Sinergi International Ltd. situated in the Depok district of West Java, and Giri BSF Malang Ltd. situated in East Java. In addition, questionnaires were administered to seven experts associated with BSF workers, maggot and solid waste experts, and business actors. The FGD was conducted to identify the existing conditions of business actors and support for BSF farming resources at the study site as material for preparing dimensions and sustainability attributes. Furthermore, there were four dimensions, including environment/ecology, economics, social, and technology, and the number of attributes used was 31. These dimensions and attributes are then outlined in a questionnaire with answer choices using a Likert scale. The questionnaire used four dimensions with several attributes, and the expert respondents answered the questions in the questionnaire with a score of 0, 1, and 2 for bad, moderate, and good. Questionnaires on biomass production were made available in BSF and solid waste study communities as well as the scientific opinion of professionals. In addition, MDS approach was used to process and evaluate the data. Rapid appraisal for fisheries software was used to conduct an MDS analysis (Lloyd *et al*., 2022). The current study examined 31 attributes connected to social, economic, ecological, and technological dimensions. Attributes identified on the environmental dimension are material efficiency, use of chemicals in electricity, fuel, and water, the potential for air and water pollution, utilization of waste (feed scraps, dead maggot remains, BSF bodies), exploitation level of natural resources, potential damage to biodiversity, and spread of disease. Attributes of social dimensions included management education, involvement in cultivation of BSF, the level of economic interests, the potential for mass protests, the possible loss of more labor, the possibility of work accidents, and the possibility of creating jobs for locals. Furthermore, attributes in the economical dimension were cultivation productivity level, potential increase in business scale and success rate, contribution to improving the welfare of managers/workers, efficiency of obtaining raw materials, and level of market absorption of maggot and compost. Attributes in the technology dimension were the success rate of adopting BSF production system, the level of specialization/expertise/skill required for managers, the availability of production facilities and infrastructure, the potential for technology improvement, and the level of technical sensitivity to the quality and quantity of maggot and compost production.

Data analysis

The value of sustainability status and leverage attributes of BSF cultivation from FW substrate through Rapfish analysis has several steps according to (Lloyd *et al*., 2022). These include a) selecting attributes for the assessment of sustainability status and leveraged attributes of BSF farming technology referring to good benchmarks, b) assessing attributes on an ordinal scale referring to each dimension sustainability criteria, c) compiling an index of sustainability status and leverage attributes of BSF cultivation from FW substrate. Furthermore, analysis results show a) the status or index of each dimension and b) the leverage/sensitive attributes affecting sustainability status of BSF farming technology. The position of sustainability status level can be described in vertical and horizontal ordinates, and represented by a flat line. The lousy and excellent extreme has index value of 0% and 100%, respectively. Meanwhile, the scale of value in sustainability status index of intensive duck-rearing technology ranges between 0-100%. It is only sustainability with a more excellent value of 50%. Sustainability status ordination is an overview of each dimension, referring to the attribute of the dimension. Index value point on the axis (x) reflects sustainability status of intensive duck farming technology, and the ordinate (y) shows the variation in scores. This supplements the ordination analysis by testing the normalization of the S-stress value (S) and squared correlation (R^2) of the model. Furthermore, the model is rated good when the S<0.25% value and R^2 are close to 1. The value of S and the R^2 also determine the need to add attributes and simultaneously reflect the accuracy of the dimensions studied with the actual state. Leverage analysis is used to determine attributes sensitive to sustainability, and analysis selects the attribute with the highest influence on each dimension as a lever factor influencing the value. The most sensitive indicators are indicated by the highest Root Mean Square (RMS) values (Borg *et al*., 2018). Meanwhile, Monte Carlo analysis examines a) the Influence of attribute scoring errors, b) the effect of scoring variations due to differences of opinion or judgments by experts, c) the stability of MDS analysis process, d) data entry errors or the presence of missing data, and e) the high-stress value of MDS analysis results. The system under study is excellent or corresponds to actual conditions when the difference between MDS and Monte Carlo calculation results is less than one. MDS ordination is represented by a circle with variable values, references, and anchors. The x-axis for Good and Bad has a maximum and minimum value of 100 and 0, while the y-axis for Up and Down is half the maximum and minimum attribute scores of 50 and -50. In MDS method, points are plotted for the distance between objects to become proportional to their similarity. In addition, the ordination technique or distance determination is based on the Euclidian distance in n-space using Eq. 1 (Borg *et al*., 2018).

$$d = \sqrt{\left(|x_1 - x_2|^2 + |y_1 - y_2|^2 + |z_1 - x_2|^2 + \dots\right)} \tag{1}$$

where, configurations of objects or points in MDS are approximated by regressing Euclidian distance (d_{ij}) from point i to j with point of origin (o_{ij}) using Eq. 2 (Borg *et al*., 2018).

$$d_{ij} = \alpha + \beta\delta\beta_{ij} + \varepsilon \tag{2}$$

the ALSCAL algorithm is used for regression in the equation, and the method optimizes squared distance (d_{ij}) against data (o_{ij}), which in three dimensions ($_{I,\, j,\, k}$) are recognized as S-Stress using Eq. 3 (Borg *et al*., 2018).

$$s = \sqrt{\frac{1}{m}\sum\nolimits_{k=1}^{m}\left[\frac{\sum_i\sum_j\left(d_{ijk}^2 - o_{ijk}^2\right)^2}{\sum_i\sum_j o_{ijk}^4}\right]} \tag{3}$$

where, the Euclidian distance is given a value using Eq. 4 (Borg *et al*., 2018).

$$d_{ijk} = \sum\nolimits_{a=1}^{r} w_{ka} \left(x_{ia} - x_{ja} \right)^2 \qquad (4)$$

RESULTS AND DISCUSSION

MDS method was used to calculate the level of sustainability, where the dimensions and attributes are determined by identifying their influence on sustainability of BSF cultivation. Meanwhile, dimensions and attributes influencing sustainability are determined specifically and temporally (Lloyd *et al.*, 2022). The attributes which are indicators of each dimension are the results of experts in the field of BSF cultivation. This study includes environmental, social, economic, and technological dimensions, as well as 31 attributes in total. The data in MDS calculation is the assessment of the attributes asked in the questionnaire, and the list of dimensions and attributes is shown in Table 5.

The features in each dimension were organized into a sheet questionnaire and given to pertinent specialists to obtain their expert opinion on the scientific viability of the biorefinery method employed in the manufacture of BSFL. Additionally, MDS approach of Rapfish software was used to examine the outcomes of the expert evaluations. Table 6 displays the derived sustainability indices for each dimension.

The conditions for sustainability BSF cultivation are influenced by environmental carrying capacity, production input availability, production processes, product processing, marketing of BSFL, and the function of relevant organizations. By considering

Table 5: Dimensions and Attributes of BSF cultivation sustainability

Environmental	Social	Economical	Technological
1. Material utilization efficiency (biodegradable) for the production of BSF and compost 2. Efficiency in the use of chemicals during cultivation process and post-harvest 3. Efficiency in the use of electrical energy and fuel during cultivation and post-harvest processes 4. The efficiency of water use during cultivation process and post-harvest 5. The potential for air pollution (odor) generated 6. The potential for water pollution 7. Utilization of waste, consisting of feed scraps, dead maggot remains, and BSF bodies, produced from maggot cultivation 8. Exploitation level of natural resources (land, plants) for maggot cultivation 9. Potential damage to biodiversity due to the maggot business 10. The potential for the spread of disease due to the existence of a maggot business	11. Management education level 12. Involvement of family members in maggot cultivation 13. Level of business motivation 14. The possibility of widespread unrest brought on by BSF cultivation system 15. Level of knowledge of managers/workers on environmental conservation and restoration 16. Potential for work accidents 17. Potential job creation for residents	18. Maggot cultivation productivity level 19. Production management level 20. Potential increase in business scale/business success rate 21. Contribution to improving the welfare of managers/workers 22. The efficiency of production of raw materials 23. The efficiency level of obtaining raw materials 24. The level of market absorption of maggot production product 25. The market absorption rate of compost production	26. The success rate of adopting the maggot production system for the surrounding community 27. The level of specialization/expertise/skill required for managers 28. Availability of production facilities and infrastructure 29. Potential for maggot cultivation technology improvement 30. The level of technical sensitivity to the quality and quantity of maggot and compost production

Table 6: Sustainability index results for all dimensions with data quality indices

Dimension	Index (%)	Stress	R^2(SQR)
Environmental	92.02	0.131	0.947
Social	92.02	0.137	0.948
Economical	100.00	0.137	0.947
Technological	74.74	0.148	0.948
Average	89.69	0.138	0.947

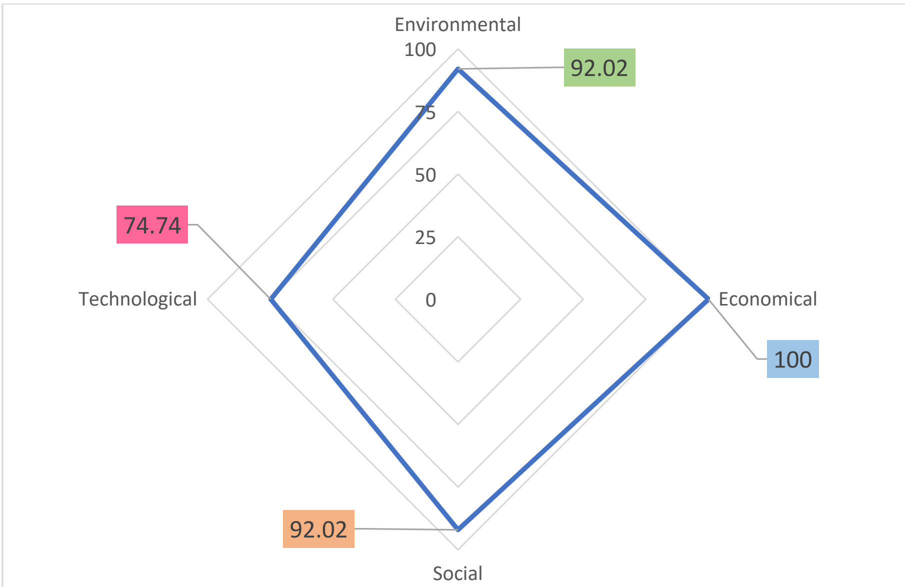

Fig. 1: Sustainability status of BSFL and compost production

these factors and adopting sustainability practices, BSF cultivation can be a more environmentally-friendly and economically-viable alternative to traditional animal feed production (Rehman *et al.*, 2023). Fig. 1 displays the findings of MDS study of environmentally friendly BSF farming. A stress value of 0.14 (stress 50%) was observed with comparable findings from the Monte Carlo test between four dimensions as measures of validity and accuracy. Furthermore, BSF production system is in a sustainability condition with a value of 89.69. The technology dimension has the lowest sustainability index but is still in a fairly sustainability condition. The other three dimensions, namely environmental, social, and economic, are included in sustainability category, as shown in Table 6. Leveraging analysis showed that eight attributes have a sensitive influence on BSF production supply system.

Environmental dimension

According to the findings of MDS analysis, the ecological dimension has an index value of 92.02, satisfying the criteria for sustainability. This is a very good index status and is worth maintaining. It has been reported that the utilization of waste can be used for BSF cultivation, with a positive impact on the environment (Iqbal *et al.*, 2020).

Leverage analysis shows that 1 attribute has a sensitive influence on sustainability of BSF. It is the potential for air pollution (odor) generated with an RMS value of 7.80, as shown in Fig. 2. For other attributes, the value does not have a significant effect, but attention needs to be paid to maintaining environmental dimension in BSF sustainability. The results reported by Hana and Kriswibowo, (2022) stated that the existence of a waste management program with BSF through corporate social

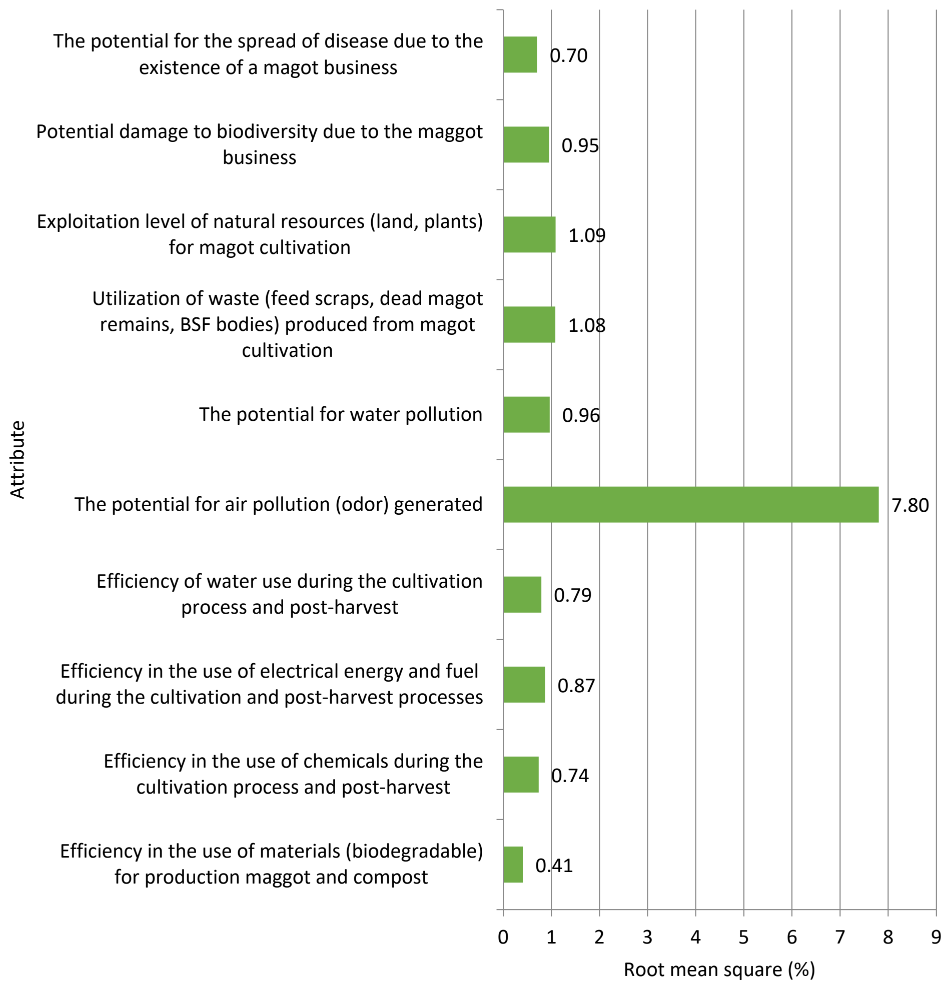

Fig. 2: Leverage of environmental attributes

responsibility (CSR) had a good influence on the quality of the village environment to keep it clean and reduce pollution due to waste. BSFL degrades and consumes different types of organic waste and reduces the initial weight by about 50% in a shorter time than conventional composting methods (Amrul *et al.,* 2022). In addition, the larvae do not cause a pungent odor and no smell complaints are observed (Raksasat *et al.*, 2020). Recently, the use of BSFL to process organic waste has been developing worldwide. The technology has great potential to overcome problems especially related to sanitation infrastructure for waste disposal in tropical countries with low and middle incomes supported by favorable climatic conditions. In addition, BSFL can be used as an alternative feed containing amino acid protein and can be a solution to the high cost of feed (da Silva and Hesselberg, 2020).

Social dimension

Social sustainability can be attained by considering policies that minimize adverse effects on society and support family enterprises. The participation of family members is very important in the characteristics of the social dimension. Many employees working for family businesses are prepared to contribute their resources to lower the financial strain on the enterprise. Furthermore, the structure also enables

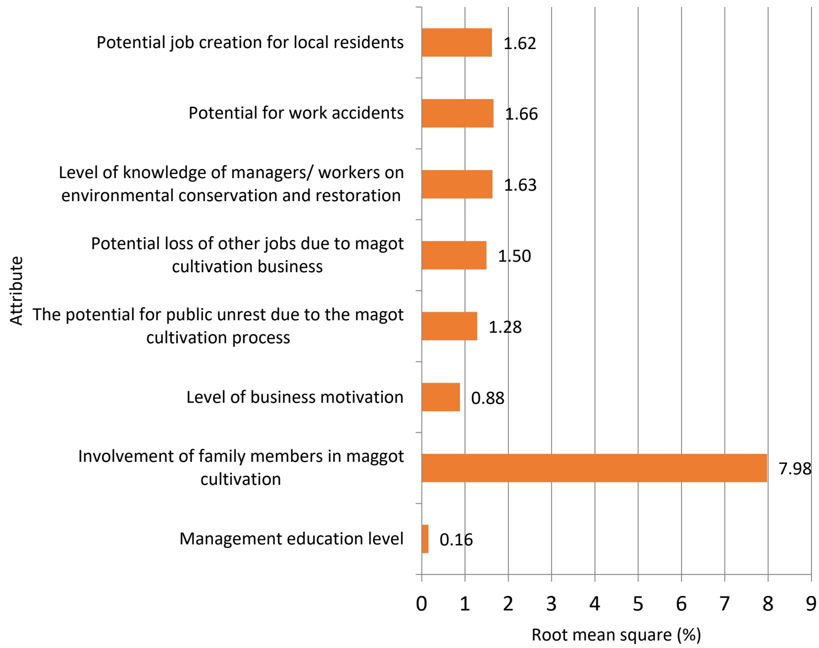

Fig. 3: Leverage of social attributes

management to concentrate more on running the business.

Sustainability index on the social dimension based on MDS analysis shows a value of 92.2, which is included in sustainability criteria. In the leverage analysis, the social dimension consists of 8 attributes. Furthermore, two attributes have the most significant RMS value gap, namely the involvement of family members in BSF cultivation (7.98) and management education level at 7.98 and 0.16, as shown in Fig. 3. The environmental and social dimensions have a direct relationship with the development of BSF cultivation and the same leverage values of 92.02%. The highest attributes for environmental and social dimensions were potential for air pollution generated and involvement of family members in maggot cultivation at 7.8% and 7.98%, respectively. In addition innovation and creativity are required to solve environmental problems in increasing community of family member involvement. Individual involvement in waste management is closely related to the perceptions and expectations, background, social values prevailing in society, and changes in behavior. Factors driving individual involvement are obtained from concern for a problem that directly encourages changes in behavior to be involved in specific actions or programs. Meanwhile, changes in attitude can be made through education programs and other instruments related to the economy. (Fadhullah *et al*., 2022). A study by Noufal *et al*., (2020) showed several factors that influence households in carrying out waste management programs (for example, composting). Some of the challenges identified are the unavailability of land, inadequate time to engage in such practices, absence of practical objectives, unsanitary and unhygienic conditions, and insufficient knowledge on how to effectively implement the program. Furthermore, the involvement of local authorities is needed to support and encourage households through environmental education, training, and awareness campaigns, which provide knowledge on the importance of implementing a program. Fadhullah *et al*., (2022) studied household involvement in waste sorting activities. This study states that education level is an important factor influencing the perception of household waste management. Education has a negative relationship with waste sorting activities, where people with low education are more willing to sort their waste than people with higher education. Possible reasons can be

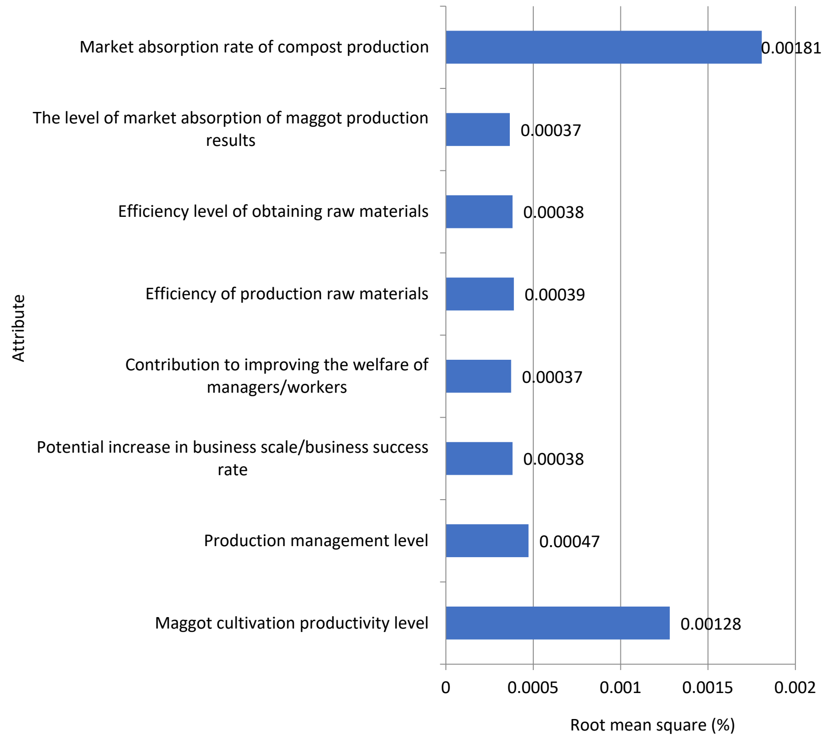

Fig. 4: Leverage of economical attributes

attributed to different lifestyles and time constraints deliberately allocated for waste sorting activities. Garbage is one of the environmental problems faced by almost many countries, hence an effective and sustainability waste management technology is needed. Salam *et al.,* (2022) stated that waste treatment techniques in developing countries do not meet standards. BSF has a good and proven ability to degrade waste and its biotransformation, which provides a potential and economical alternative to recycling biological waste. The results showed that BSFL can improve the quality of the final product and encourage the degradation of organic waste. It can also reduce municipal waste by efficiently degrading organic waste in the environment. From an environmental aspect, BSFL can degrade waste, reducing pollution, and the production process can create job opportunities with an increased value from a social aspect. Furthermore, it can also be used as feed for slag and fish with economic value (Abro *et al.,* 2020). BSFL digests nutrients from waste and produces protein and fat used in animal feed processes and organic fertilizer (Amrul *et al.,* 2022).

Economical dimension

MDS analysis for the economic dimension produces a sustainability index value of 100.00. In this case, the expert assessment of 8 attributes shows that the prospects for developing BSF business are very good. Leverage analysis for the economic dimension shows that there are attributes influencing sustainability, namely market absorption for compost products and the productivity level of BSF cultivation, as shown in Fig. 4. Environmentally compliant BSFL levels can also reduce organic waste and greenhouse gas emissions from landfills to produce sustainability animal feed. Furthermore, it reduces the need for forage production and provides employment opportunities for people in rural areas. The use of BSFL in animal feed production reduces the cost of animal products to become more accessible to low-income consumers. The values correspond to ecosystems and reduce the dependence on fishmeal and soybean meal associated with environmental

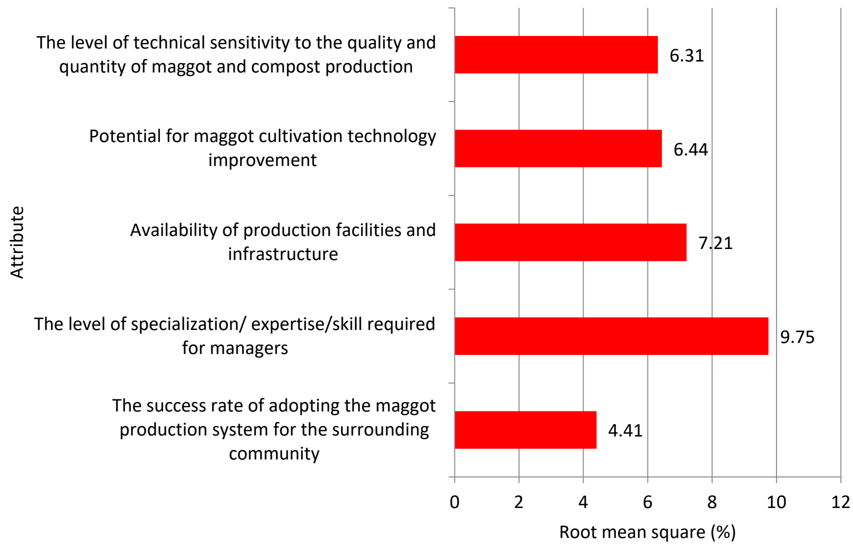

Fig. 5: Leverage of technology attributes

impacts such as overfishing and deforestation. BSFL farming also improves soil health, producing nutrient-rich frass as fertilizer. Finally, these values correspond to technological advances in BSFL farming techniques such as automated harvesting and processing systems to increase efficiency and productivity. Genetic engineering can be used to improve the nutritional content of BSFLs or improve the ability to digest certain types of waste, further improving sustainability of their use.

According to the business and financial analysis conducted, BSF cultivation is quite feasible with a good profit level in a growing, healthy, and advanced condition (Supena *et al.*, 2021). Meanwhile, the availability of capital, labor, and technology affects the efficiency of BSFL produced (Prihartini *et al.*, 2022). On a smaller scale, productivity standards are used to evaluate the conversion of inputs into desired outputs using equipment, facilities, businesses, organizations, systems, or individuals. Sustainability in the production of BSFL is determined by productivity, particularly in terms of obtaining FW as a raw material that satisfies the requirements for a high-quality product. Furthermore, it is also impacted by the degree of management of BSFL generation. Planning, organizing, directing, and efficiently controlling production are parts of production management. To accomplish organizational objectives, the management operates to coordinate activities. A method to overcome the management of household waste is through BSF Bioconversion Technology, which needs a very short time compared to traditional forms (Cohn *et al.*, 2022). Moreover, the advantage of using BSFL bioconversion technology is waste media as feed material for cultivation. BSF is a source of animal and fish feed because the protein content is not relatively different from soybean meal (Luperdi *et al.*, 2023). Therefore, it is very prospective as a feed ingredient, especially for poultry. This is a great opportunity to replace soybean meal with BSF flour as a source of animal feed which is relatively cheaper. The potential of BSF flour is not only as poultry feed but also as fish feed. It can also be used as ornamental animal feed for birds, reptiles, cats, and fish. To further increase profits, BSF cultivation business needs to be integrated with poultry, fish, and agricultural farming businesses. This is because solid and liquid fertilizers produced are very good for plant and reduces inorganic fertilization costs (Rehman *et al.*, 2023). Therefore, BSF bioconversion technology is an integrated circular solution that can provide sustainability food and feed. It is necessary to increase understanding of good and correct BSF cultivation management by avoiding growing media from heterogeneous organic waste contaminated with heavy metals and toxic chemicals.

Technological dimension

The technological dimension had a comparatively high sustainability index score of 74.74%, according to the current investigation. Therefore, the technological effects of producing BSFL are extremely sustainability, as shown in Table 6. The greatest influence on sustainability was from management expertise and abilities in BSFL generation. Another significant factor with a favorable impact on job prospects for the local people involved in the production is the accessibility of BSFL-producing facilities.

Sustainability of BSFL productivity is influenced by the availability of capital, human resources, and technology. For BSFL production on a small scale, productivity standards are used to evaluate the effectiveness of the workforce, businesses, or systems to convert inputs into desired outputs. To produce sustainability BSFL, it is necessary to pay attention to the level of productivity, and the importance of selecting and obtaining leftover feed as raw materials to produce high-quality products and management levels. Planning, organizing, directing, and efficiently managing production are all parts of the management. BSF technology is an environmentally friendly and low-cost method due to its fast transition rate, high sustainability, and low cost-effectiveness compared to existing and other operational technologies (Salam *et al*., 2021). Furthermore, sustainability of BSF cultivation can be improved by performing some aspects of key areas which can be addressed, such as feedstock selection, energy efficiency, water conservation, waste reduction, and potential disease management. The development of BSFL production for different applications such as animal feed, fertilizer, and waste disposal involves several technical issues. Some of the key technical aspects to consider when developing a BSF are *a). Habitat design*: BSFL needs specific environments to thrive, and habitat design must provide optimal conditions such as temperature, humidity, and light. Meanwhile, BSF and housefly (HF) larval setups were fed poultry manure aged 0-8 days at 16 hours of light and 8 hours of darkness, with temperature of 26°C, and 70% relative humidity (Miranda *et al*., 2019). *b). Feeding system*: BSF biomass development requires a consistent food source, and feeding systems must be designed to provide optimal nutrition while maintaining cleanliness and preventing contamination (Meneguz *et al*., 2018). Manojkumar *et al*., (2023) indicated that cauliflower (*Brassica oleracea*) as well as the species of seaweed *Sargassum myriocystum* (Balaraman *et al*., 2022) contain substances used as bactericidal or larvicidal. Therefore, these plants can endanger the life of BSFL and should not be used as a food source. *c). Harvest*: Harvesting BSF biomass can be a difficult task and the techniques used must be efficient and cost-effective. Furthermore, the harvesting system must reliably separate larvae from substrate and other contaminants. The method should be designed according to the intended use of the biomass and the processing method. For example, when BSF biomass is to be used as animal feed, processing methods should ensure the larvae are pathogen-free and safe for consumption (Gold *et al*., 2021). *d). Automation*: This technology increases efficiency, reduces labor costs, and ensures consistent quality of BSFL (Vuoang, 2019). *e). Waste management*: Waste is generated during the production of BSFL, and the management systems should be designed to prevent pollution and ensure sustainability (Amrul *et al*., 2022). *f).* Q*uality management*: Quality control measures should be taken to ensure that BSFL biomass meets the standards required for the intended use. This may include regular testing for nutrients, moisture, and contamination. Developing BSF biomass requires careful consideration of several technological points to ensure optimal production and sustainability (Siddiqui *et al*., 2022). In addition, assessing the quantity of BSF in a sustainability study can be conducted by several methods by counting the larvae, measuring the growth rate (Meneguz *et al*., 2018) and biomass (Smetana *et al*., 2019), and estimating the population density (Surendra *et al*., 2020).

Environmental dimension

The lowest attribute is efficiency in the use of materials for the production of BSF biomass and compost by 0.41 as shown in Fig. 2. This dimension can be improved with an organic waste processing program through regular and comprehensive training and coaching conducted by the empowerment organization to improve the capabilities of its human resources and make the community more active and empowered in organic waste processing activities. This is conducted through cultivation which produces BSF for animal feed and compost from manure.

Social dimension

The lowest attribute is management education level at 0.16, as shown in Fig. 3. After the community is given education and guidance on waste management with BSF biomass cultivation, the economic business group can be assisted in its implementation. Therefore, BSF biomass production scale becomes increased with more organic waste.

Economical dimension

The weaknesses in Fig. 4 include the attribute market level absorption of BSF production (0.00037), the efficiency level of obtaining row materials (0.00038), the efficiency of row production materials (0.00037), contribution to improving the welfare of managers or workers (0.00038), and a potential increase in business scale and production management level (0.00047). Furthermore, improvements in the economic dimension can be made through cooperatives to play an active role in improving and maintaining price stability.

Technological dimension

The weakest technological dimension is the attribute of the success rate of adopting BSF production system for the surrounding community at 4.41, as shown in Fig. 5. These weaknesses can be overcome by maximizing the potential starting from adequate facilities and infrastructure, developing BSF which can be used for fish and livestock feed. In this case, Village Owned Enterprises must assume a role in management and marketing. Additionally, it is imperative to establish cooperation with BSF cultivators and the farming community to enhance sales and foster a sense of community encouragement. In addition, all dimensions need to be fully supported by the Regency Government, especially the Environment and Sanitation Service and several related agencies. Therefore, the implementation is more effective to reduce the amount of waste, especially organic waste. It is imperative to diversify the processing technology of BSF in a manner that can extend its shelf life, thereby ensuring greater market absorption. One approach to achieve this goal is the production of BSF biomass flour.

CONCLUSIONS

MDS method was used to calculate the level of sustainability of BSF cultivation, where the dimensions and attributes are determined by identifying the influence of each dimension. Furthermore, environmental, social, economic, and technological dimensions were determined. The approach of Rapfish software was used to evaluate the scientific viability of the biorefinery method employed in the manufacture of BSFL. Sustainability index of biomass production was estimated to be 89.69%, hence the process has the potential for sustained development when the leverage factors described in each dimension are considered. Analysis showed that the economic dimension had the highest leverage value of 100%. The environmental and social dimensions had a value of 92.02% with the highest attribute being the potential for air pollution generated and involvement of family members in maggot cultivation by 7.8% and 7.98%. The lowest is the technological dimension with a value of 74.74%. Competencies and experiences in management, family member involvement, and productivity level need to be improved to increase sustainability of BSF biomass production. Finally, Quality control measures should be considered to ensure that BSFL biomass meets the standards required for its intended use. Developing BSF biomass requires careful consideration of several technological points to ensure optimal production and sustainability. In the future, BSF cultivation has the potential to be developed because of the increasing need for animal feed. Moreover, national protein requirements represent a crucial element in animal feed, serving as a vital source of nutrition for body growth and brain development. BSF cultivation produces organic fertilizers rich in nutrients required by agricultural countries using the potential of "organic waste" resources.

AUTHOR CONTRIBUTIONS

A.D. Santoso performed the literature review, experimental work, drafting of the manuscript, obtaining funding, interpretation of MDS data and made critical revision of the manuscript for important intellectual content; T. Handayani performed the literature review, designed the study, drafting of the manuscript, experimental work and made critical revision of the manuscript for important intellectual content; R. A. Nugroho performed drafting of the manuscript, and supervised all experiments and manuscript preparation; A.I.

Yanuar performed analysis and interpretation of MDS data, experimental work, statistical analysis and supervised manuscript preparation; Nadirah performed the literature review, experimental work, manuscript preparation and critical revisions; E. Widjaja performed the literature review, manuscript preparation, analysis and interpretation of MDS data, critical revisions and supervised manuscript preparation; E.S. Rohaeni performed the literature review, manuscript preparation, analysis data, interpretation of MDS data, critical revisions and supervised manuscript preparation; M.A.M. Oktaufik performed analysis and interpretation of MDS data, experimental work, and supervised statistical analysis; U. Ayuningtyas supported the administrative, technical, or material support; Y.P. Erlambang supported the administrative, technical, or material support; R. Herdioso performed analysis and interpretation of MDS data, and statistical analysis; M.N. Rofiq performed the literature review, analysis and interpretation of MDS data, statistical analysis, experimental work, and supervised manuscript preparation; R. Hutapea performed the literature review, experimental work, manuscript preparation and critical revisions; A.L. Sihombing performed the literature review; B. Rustianto performed the literature review; I.M.A.D. Susila performed the literature review, and acquisition of data BSF production; D. Irawan supported experimental and administrative work, provided technical and material assistance; D. Iskandar performed the literature review and acquisition of data BSF production; S. Indrijarso performed the literature review and acquisition of data BSF production. G.D. Widiarta supported experimental, administrative work and provided technical assistance.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%	Percent
°C	Degree Celsius
ASCAL	Alternating least-squares algorithm
BSF	Black soldier fly
APHA	American public health association
BSF frass	Material organic fertilizer from solid excreta of BSF
BSFL	Black soldier fly larvae
C	Carbon
CH_4	Methane
C/N ratio	Carbon-nitrogen ration
CSR	Corporate social responsibility
d	Euclidian distance
d_{ij}	Euclidian distance from point i to point j

d_{ijk}	Squared distance
FW	Food waste
FAO	Food and agriculture organization
GDP	Gross domestic product
H	Hydrogen
HF	House fly
kg	Kilogram
LCA	Life cycle assessment
Ltd	Limited
Maggot	BSF larvae
MC	Moisture content
MDS	Multidimensional scaling
mg	Miligram
$mgCH_4/kg$	Miligram methane per kilogram substrate
N	Nitrogen
NA	Not applicable
O	Oxygen
pH	Potential of hydrogen
ppm	Part per million
Rapfish	Rapid appraisal for fisheries, an analytical method to assess the sustainability of fisheries based on a multidisciplinary approach
RH	Relative humidity
RMS	Root mean square, a frequently used measure of the differences between values
S	Sulphur
SR^2	Squared correlation
SQR	Structured query reporter, a programming language designed for generating reports from database management systems
TKN	Total Kjeldahl Nitrogen
TS	Total solid
USD	United states dollar
VS	Volatile solid
ww	Wet weight
x-axis	Horizontal number line
y-axis	Vertical number line

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CASE STUDY

The impact of fruit and vegetable waste on economic loss estimation

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ABSTRACT

BACKGROUND AND OBJECTIVES: Receiving nutrients from fruits and vegetables are essential for public health. However, a large amount of waste is produced during producing, supplying, and consuming these fruits and vegetables. Water, fertilizers and pesticides used for the production of agricultural products can affect the soil and their cultivation environment and finally lead to environmental pollution. Therefore, this study aimed to investigate the amount of fruit and vegetable waste caused by corruption and evaluate its economic loss and health damage.

METHODS: The data were collected using the observation technique aided by observation tools and weighing tests to physically analyze and determine the quantity and quality of waste from Tehran's fruit and vegetable distribution centers. In each center, the waste obtained from fruits and vegetables was stored in special waste tanks at the end of the day, and the waste was transported to outside the center by special waste disposal vehicles. The economic loss of unusable fruits and vegetables was calculated according to the weight of their waste in the fields of agricultural product supply. The data were analyzed using statistical software SPSS, ANOVA statistical test, and Excel software.

FINDINGS: The results revealed that the amounts of fruit and vegetable wastes were 12 percent and 24 percent, respectively, accounting for a total loss of 54,891,539 USD. The highest quantity of fruit waste (15 percent) was observed in summer, and the economic loss due to fruit waste was higher in this season. Also, the amounts of vegetable waste in summer and spring were equal to 28 percent and 24 percent, respectively, indicating the higher economic loss due to vegetable in these seasons.

CONCLUSION: The results showed that the economic loss due to vegetable waste was greater than the economic loss due to fruit waste. The amount of vegetable waste was 24 percent, representing a high economic loss. The generation and disposal of these wastes caused a great economic loss and health problems due to their unpleasant odor, release of leachate into the environment, and landfill pollution. Therefore, it was recommended to follow appropriate production principles and supply operations. Moreover, a comprehensive waste disposal management, as a practical measure, should be used to prevent and control these problems. The use of modern technology in harvesting, transporting and supplying fruits and vegetables could reduce their lesion, and subsequently reduce the economic loss.

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46	4	7

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INTRODUCTION

Food safety is one of the most important issues in human life (Ahmadi *et al.*, 2020). One-third of the food is wasted worldwide, while 1 billion people are malnourished, and this food disposal can feed 1.9 billion people (Shafiee-Jood and Cai, 2016; Joardder and Masud, 2019; Elik *et al.*, 2019). The consumption of agricultural products is growing each year (Sosalagere *et al.*, 2022), and the continuous increase in population, despite technological advancements, has led to an imbalance in supply, demand, and consequently increased food waste globally (Ashokkumar *et al.*, 2022; Ganesh *et al.*, 2022). The Food and Agriculture Organization (FAO) of the United Nations declared that food waste included all things that could change accessibility, edibility, health, and safe or quality of a product and prevent the consumer from consuming it (Girotto *et al.*, 2015). There is a limitation for natural resources of agricultural products, and the required range for these resources to produce agricultural products is related to the increase of population (Gholifar *et al.*, 2010; Tien *et al.*, 2019). On the other hand, fruits and vegetables are major sources of nutrients and minerals. More than 42 percent (%) of the food waste is made up of fruits and vegetables, as the most consumed food, in the world (Pushparaj *et al.*, 2022; Ganesh *et al.*, 2022.; Coman *et al.*, 2020). They have the highest amount of waste among food products, which can be due to high sensitivity to spoilage. FAO estimated that 40-50% of fruits and vegetables were wasted throughout the food supply chain worldwide (Gustavsson *et al.*, 2011). In other words, more than one-third of the fruits and vegetables produced worldwide are destroyed before reaching the consumer (Yavari *et al.*, 2022; Plazzotta *et al.*, 2020). This amount is equivalent to 28 million tons of waste (Abadi *et al.*, 2021). Fruits and vegetable waste include withered, shriveled, rotten, and decayed food products that have been provided with suitable conditions for spoilage in the stages of handling, transportation, packaging, storage, sale, and consumption (Gholifar *et al.*, 2010; Kitinoja and Kader, 2015; Shafiee-Jood and Cai, 2016; Ganesh *et al.*, 2022). The average fruit and vegetable wastes in developed and developing countries have been reported to be in the range of 2%-20% and 24%-40%, respectively (Golshan Tafti *et al.*, 2017). The amount of waste from the agricultural sector in Iran is 35%, equal to 28 million tonnes (t) of waste (Abadi *et al.*, 2021). It has been reported that about 25%-30% of Iran's garden products are lost only because of the lack of maintenance and storage equipment (Taghizadeh-Alisaraei *et al.*, 2017). The survey conducted on the components of fresh urban solid waste in one of the waste disposal centers of Tehran (Kahrizak) revealed that more than 60% of waste is made up of food waste, fruits, vegetables, and organic materials (Shariatmadari *et al.*, 2015). In Iran, about 90% of the raw materials are supplied from the agricultural sector, and increase of agricultural waste has negative effects on social and environmental issues such as production of a large amount of methane gas (MG) (Hawkins, 2013), large amounts of loss of water, soil, fertilizer, seeds, and labor (Ganesh *et al.*, 2022). Fruits and vegetables, as one of the most consumed foods in the world, account for more than 42% of food waste (Ganish et al., 2022; Kuman *et al.*, 2020). They have the highest amount of waste among food products, which can be due to their high sensitivity to corruption. Large quantities of organic waste are produced daily in fruit and vegetable fields in big cities such as Tehran. To the best of the authors' knowledge, no comprehensive study has been performed regarding fruit and vegetable wastes in the Tehran Municipality so far. In mega cities of Iran, vegetables are sold in the centers named "vegetable squares"· Unsuitable transportation service, inadequate process, and bad storage space are responsible for 35% of fruit and vegetable waste produced, which is a significant amount (Omrani *et al.*, 2008; Nawab Akbar, 2014). According to national reports, more than 51,000 tons of fruit and vegetable waste is being produced in fruit and vegetable fields and subsidiaries of Tehran, the value of which is estimated to be over 14,000 USD per year. In Iran, fruits and vegetables are collected from the farm in bulk and are transferred to sales centers inappropriately. Considering the sensitivity of fruits and vegetables to corruption in the farm, inappropriate transportation conditions, unsuitable maintenance conditions, and improper supply conditions in sales centers, wastes of fruit and vegetable are higher compared to other foods. Specifying the amount of fruit and vegetable waste and identifying its damage in Tehran can be extended to other cities of Iran. This study aimed to investigate the amount of fruit and vegetable waste caused by corruption and wastage in Tehran and evaluate its economic loss and health damage. The study has been carried out in Tehran, Iran in 2021.

MATERIALS AND METHODS

This descriptive-analytical study was performed in a cross-sectional design in Tehran in 2021. Investigations were first conducted in a library and then in the field using the observation technique with the help of observation tools, tests, and checklists. Finally, the obtained data were analyzed using the statistical software and tests. Product input data to all fruit and vegetable distribution centers of Tehran Municipality (250 centers) during 2022 were collected to physically determine the quantity and quality of waste. Likewise, they were analyzed and evaluated using software and statistical tests while registering in the computer system. All the stages and required information are recorded by hundreds of people in the field of health and exploitation of the Tehran Municipality Fruit and Vegetable Organization.

The organization of fruit and vegetable fields

Tehran, as the capital of Iran, is one of the largest and most populous cities with 22 municipal districts (Fig. 1). The Fruit and Vegetable Fields of Tehran Municipality (FVFTM) were established according to the resolution of Tehran Municipality in 1979. Food supply centers, including 22 fields and 228 fixed and neighborhood markets (a total of 250 centers) under the Tehran Municipality's direct management, provide citizens with services such as distributing and supplying all the necessary food and agricultural products. The organization of FVFTM is the largest supply network of agricultural and food products in Iran, providing the daily food needs of more than 2 million people. In these markets, other food groups, including eggs, poultry meat, red meat, and meat products, milk and dairy products, fish and aquatic products, commercial products, basic goods, nuts and dried fruits, bakery, and confectionery products, and summer foods are also sold. In other words, all products of food and agricultural fruits and vegetables needed are offered in these centers.

Data collection

Data related to the amount of arrival, sale, and waste of fresh agricultural products of various types of

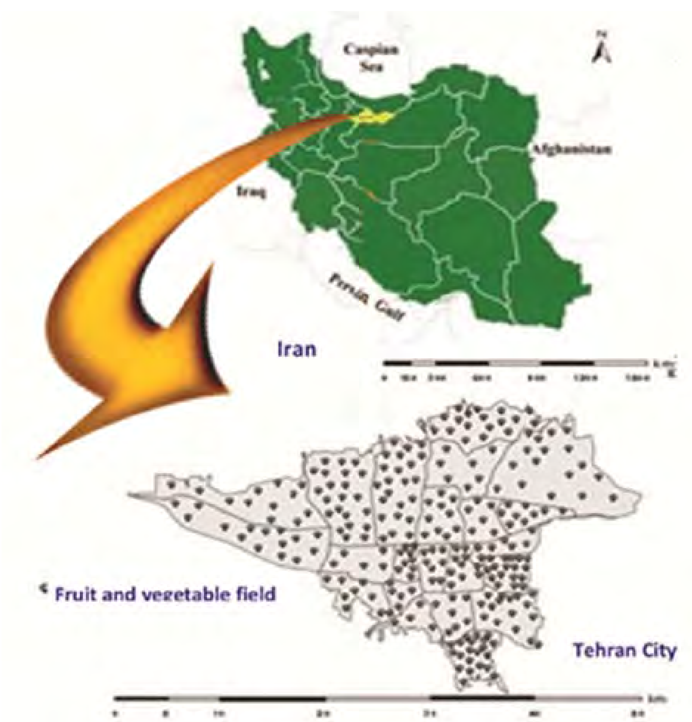

Fig. 1: Geographic location of the study area and spatial distribution of fruit and vegetable fields in Tehran, Iran

vegetables (such as tomato, carrot, cabbage, potato, onion, garlic, radish, eggplant, cucumber, pepper, pumpkin, lettuce, turnip, green bean, bean, beet, celery, other types of bean), green vegetables for table, stew and soup (including parsley, chive, spring onion, garden cress, cilantro, spinach, dill, tarragon, savory, horseradish, mint, and basil), and fruits (including apple, orange, grape, peach, tangerine, banana, pear, pomegranate, persimmon, fig, watermelon, melon, cantaloupe, apricot, cherry, strawberry, sour cherry, kiwi, dew melon, sweet lemon, rose apple, nectarine and other types of fruit) in 250 existing centers (fields and markets) located in Tehran were collected daily during the year for four seasons of spring, summer, autumn, and winter. Usually, fresh fruit and vegetable products are separately transported by trucks and weighed via digital scales, and their details are recorded in all the FVFTMs. The consignment is unloaded from the trucks and transferred to storage. The information and input data of products (amount and type of products) are recorded and stored by each center's head of administrative affairs in the administrative system and the comprehensive management system of fruit and vegetable fields (tonnage system). While the agricultural products are ready for sale, the price approved by the organization and the weight of the sold product are recorded and stored in the scale's memory. In addition to the product weight, the purchase slip provided to the customer includes the price, date of purchase, and the name or number of the stall or market. At the end of the working day, all the sales information stored on the digital scales is transferred and recorded by the person in charge of administrative affairs of the field or market in the comprehensive management system of the fruit and vegetable fields (tonnage system). Furthermore, to maintain this information in the system of each center, all the information and tonnage of sales in the fields are printed daily and preserved physically. The information and data registered in the comprehensive management system or the tonnage system of the fields of Tehran Municipality are checked, summarized, and stored by the tonnage officials as the organization's deputy of the fields and tonnage. The price list of the products offered in fruit and vegetable markets available in the comprehensive management system of the agricultural products markets is updated daily by the officials of the commercial department of the organization of FVFTM. Since the price of each product is recorded by the officials of the fields and markets, the product is always offered based on the approved price.

Recording the amount of the produced waste

At the end of each day, the amount of fruit and vegetable waste is separately weighed by a digital scale and recorded, and the waste tonnage is automatically sent to the Department of Health, Safety, and Environment (DHSE) and health department of FVFTM. In each center, the waste from fruits and vegetables is held in special waste tanks, and at the end of the day it is transported to outside the center by special waste disposal vehicles.

Estimation of economic loss

All the data related to the entry and sale of fruit and vegetable products of each center registered in the comprehensive management system of the fields were analyzed separately. The acceptance price of the fruit and vegetable products was obtained from the commercial vice-chancellor of the FVFTM. In 2021, the new price lists were approved 63 times (20 times in spring, 16 times in summer, 13 times in autumn, and 14 times in winter). The average selling price of the product was considered in the price list for each season. Furthermore, the number of Kilogram (KG) of waste of each product was multiplied by its price to get the amount of loss caused by the produced waste. The economic loss of unusable fruits and vegetables was calculated according to the weight of the fruit and vegetable waste in the fields of agricultural product supply. It is worth mentioning that the total amount of costs was considered based on the exchange of the Iranian currency (Rial) to the American dollar (USD).

Statistical analysis

The results were analyzed using statistical software SPSS, analysis of variance (ANOVA), and Excel software. The abundance of production waste was calculated as the amount of fruits and vegetables that could not be consumed (expressed as a percentage of the total amount of the fruits and vegetables entered into the market). The economic importance of the problem was analyzed based on the information obtained from the market and calculated annually in each season.

RESULTS AND DISCUSSION

The FVFTM has a completely traditional structure, and the method of sending products by manufacturers to these centers causes the production of large amounts of waste before they reach the consumer, as they are thrown away as fruit and vegetable waste.

Findings related to fruits

In spring, out of 68,605 t of fruit sold in the FVFTM, 7,258 t of waste (12%) was produced, and its economic value was 5,338,558 USD. According to Fig. 2, the amount of waste for apple, orange, grape, peach, and tangerine was 15%; and for apricot, cherry, sour cherry, kiwi, and nectarine was 12%. Likewise, among the fruits of this season, lemon and banana had the highest and the lowest amounts of waste of 18% and 5%, respectively.

In summer, out of the 82,343 t of fruit sold in the FVFTM, 12,244 t of waste (15%) was produced, and its economic value was 819,948 USD. According to Table 1, the amount of waste for pear, cantaloupe, and watermelon was 10%. In summer, the highest amount of waste was related to grape, peach, rose apple, and sweet lemon (20%) , and the lowest amount of waste was related to banana (8%) and melon (9%).

In the autumn, out of the 3296128 t of fruit sold in the FVFTM, 6,422 t of waste (10%) was produced, and its economic value was 3,605,404 USD. As can be seen in Table 2, peach and grape had the highest amount of waste (15%). Moreover, melon and banana have the lowest amount of waste (5%) in this season.

In winter, out of the 59,848 t of fruit sold in the FVFTM, 5,828 t of waste (10%) was produced,

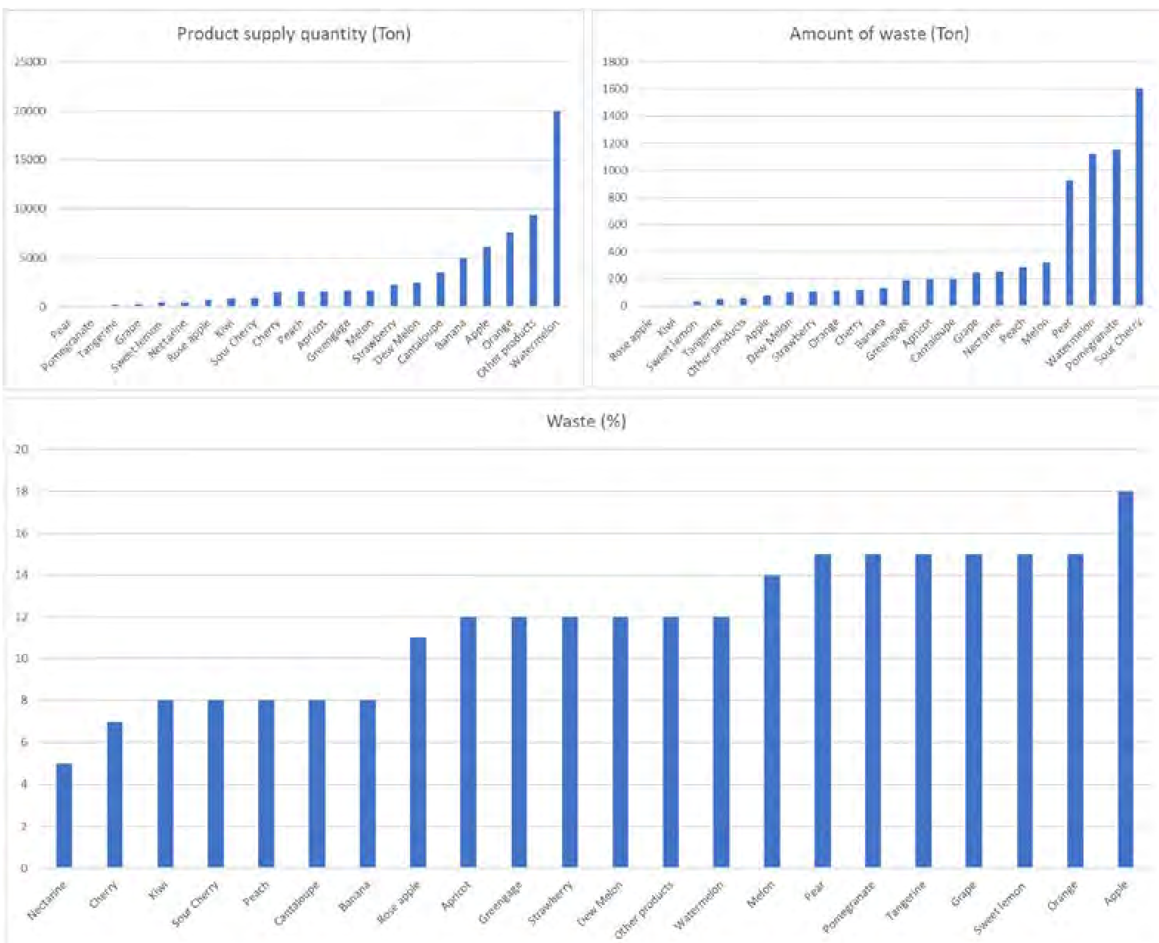

Fig. 2: Fruit waste in spring in the fruit and vegetable fields organization of Tehran Municipality

Table 1: Fruit waste in summer in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Apple	3998	600	15	258318
Orange	40	6	15	1846
Grape	6422	1284	20	864600
Peach	7095	1419	20	1047917
Tangerine	2	0.328	16	137
Banana	5624	450	8	401476
Pear	1402	140	10	126204
Pomegranate	335	40	12	20056
Fig	1345	202	15	162919
Watermelon	17431	2743	10	527520
Melon	6832	615	9	201014
Cantaloupe	4921	492	10	160852
Apricot	1036	155	15	217054
Cherry	1252	188	15	250601
Strawberry	106	16	15	17396
Sour cherry	1113	167	15	141262
Kiwi	0.588	0.086	15	41
Greengage	29	4	15	5767
Sweet lemon	874	175	20	58974
Rose apple	3506	701	20	372157
Nectarine	5773	866	15	666117
Other products	13207	1981	15	2590710
Total	82343	12244	15	8119948

Table 2: Fruit waste in autumn in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	loss value (USD)
Apple	9470	947	10	385948
Orange	6421	642	10	216176
Grape	3665	550	15	379875
Peach	1626	244	15	167664
Tangerine	9909	991	10	400322
Banana	5766	289	5	262588
Pear	671	67	10	59448
Pomegranate	6871	687	10	365252
Persimmon	1968	236	12	149822
Fig	251	30	12	21358
Watermelon	2313	162	7	27540
Melon	706	36	5	10009
Cantaloupe	319	22	7	7046
Strawberry	0.217	0.017	8	13
Kiwi	790	63	8	28002
Sweet Lemon	4194	419	10	227423
Nectarine	202	24	12	8601
Other products	8445	1013	12	915849
Total	63589	6422	10	3605404

and its economic value was 7,072,040 USD. Based on the data presented in Table 3, grape and melon had the highest and the lowest amounts of waste of 15% and 4%, respectively.

Despite the long storage life and average shelf life of citrus fruits (Terry *et al.*, 2011), the waste of these fruits was low in autumn, due to their harvest in this season, and high in spring, due to the long storage time or improper storage conditions. Banana has low waste due to having a relatively constant demand pattern

Table 3: Fruit waste in winter in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Apple	12731	1273	10	621029
Orange	18537	1854	10	980732
Grape	745	112	15	61613
Tangerine	5290	529	10	301718
Banana	5727	286	5	281529
Pear	264	26	10	24793
Pomegranate	2981	298	10	159956
Persimmon	183	22	12	18120
Watermelon	354	18	5	3315196
Melon	3	0.121	4	31
Strawberry	305	30	10	49294
Kiwi	2535	203	8	109569
Sweet lemon	13	1	8	292
Dew melon	2289	229	10	168335
Other products	7891	947	12	1261354
Total	59848	5828	10	7072040

throughout the year (Terry *et al*., 2011). The high perishability of rose apple is influenced by its thin and dehydrated skin (Techakanon and Sirimuangmoon, 2020). Since grape, as a perishable fruit, mainly suffers from decline of quality after harvesting, its waste was significant in all seasons. The waste of grape can be reduced by the prevention of the factors that reduce its quality, mainly driven by the gray mold fungus *Botrytis cinerea* (Shen and Yang, 2017). It is not far from the idea that peach, as a highly perishable fruit with a short shelf life, has a higher loss under environmental conditions, and after harvesting, its nutritional and organoleptic quality decreases rapidly (Mahajan *et al*., 2015). High shelf life (Pech *et al*., 2008) and hard skin (rind) are the factors that reduce the waste volume of melon as a non-climacteric fruit.

Findings related to vegetables

In spring, out of 89,577 t of vegetables sold in the FVFTM, 21,171 t of waste (24%) was produced, and its economic value was 6,705,072 USD. Table 4 shows that the waste is 30% for potato and 25% for onion and garlic. In this season, the highest and the lowest amounts of waste were related to lettuce (50%) and radish (5%), respectively.

In summer, out of 97,340 t of vegetables sold in the FVFTM, 26,994 t of waste (28%) was produced, and its economic value was 9,178,741 USD. According to Table 5, the amounts of waste for celery, potato, and onion were 40%, 35% and 30%, respectively. In this season, the highest and the lowest amounts of waste were related to lettuce (55%) and radish (6%), respectively.

In autumn, out of 104668 t of vegetables sold in the FVFTM, 27,783 t of waste (22%) was produced, and its economic value was 7,101,533 USD. According to Table 6, the amount of waste was 30% for celery and 25% for potato and tomato products. In this season, the highest and the lowest amounts of waste were related to lettuce (50%) and radish (5%), respectively.

In winter, out of 99,956 t of vegetables sold in the FVFTM, 23,834 t of waste (23%) was produced, and its economic value was 9,178,741 USD. According to Table 7, the amount of waste was 30% for celery and 25% for potato and tomato. Moreover, in this season, the highest and the lowest amounts of waste were related to lettuce (50%) and radish (5%), respectively.

Although potato and onion are semi-perishable (Terry *et al*., 2011, Tyovenda *et al*., 2022), their storage (Saha *et al*., 2014) can spoil them, especially under air heat (Maroušek et al., 2020, Asif et al., 2022). In addition, spring onion, like garlic, is highly perishable (Kłebukowska *et al*., 2015). Since there are no suitable conditions for maintaining the quality of agricultural products in the FVFTMs, the observed amounts of waste are expected, not only for tomato but also for other perishable products. In addition to the fact that most of the lettuce waste is produced at the end of the distribution chain (Terry *et al*., 2011), its waste volume is high due to its specific volume, high humidity, and lack of packaging (Lopez *et*

Table 4: Vegetable waste in spring in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Green vegetables (table, stew, and soup)	6076	1215	20	374045
Tomato	12117	2666	22	1160411
Carrot	5561	1112	20	337417
Cabbage	2152	387	18	105622
Potato	15107	4532	30	1175779
Onion	11739	2935	25	554878
Garlic	747	187	25	135431
Black radish	3	0.157	5	33
Eggplant	3687	479	13	101172
Cucumber	9507	1426	15	426397
Pepper	1590	207	13	95410
Courgette	1763	247	14	69445
Lettuce	6886	3443	50	1090036
Turnip	6	0.680	11	95
Green beans	978	98	10	55460
Broad bean	3323	499	15	131524
Beetroot	14	2	14	308
Celery	721	216	30	56142
Other products	7600	1520	20	835458
Total	89577	21171	24	6705072

Table 5: Vegetable waste in summer in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Green vegetables (table, stew, and soup)	5352	1338	25	427174
Tomato	15158	3941	26	1728087
Carrot	4101	1026	25	485079
Cabbage	1967	433	22	139789
Potato	18494	6473	35	1518686
Onion	14100	4230	30	764683
Garlic	361	90	25	69488
Black radish	12	0.007	6	113
Eggplant	4355	697	16	150077
Cucumber	9754	1658	17	529372
Pepper	1774	266	15	106449
Courgette	2194	351	16	99915
Lettuce	5129	2821	55	Turnip
Turnip	12	2	17	406
Green beans	1387	208	15	103231
Broad bean	60	9	15	2319
Beetroot	10	1	10	365
Celery	1133	453	40	95900
Other products	11987	2997	25	1959430
Total	97340	26994	28	9178741

al., 2000). Black radish had little waste in all seasons. Radish skin is a protective barrier for this product (Yücetepe *et al.*, 2021). The large volume of celery waste can be reduced by packaging and providing suitable temperature conditions (0 to 0.6 °C) and the required amount of relative humidity (RH)

Table 6: Vegetable waste in autumn in the fruit and vegetable fields organization of Tehran municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Green vegetables (table, stew, soup)	6077	1094	18	348876
Tomato	12472	3118	25	1259686
Carrot	5839	1051	18	361294
Cabbage	5035	856	17	269966
Potato	16001	4000	25	1389321
Onion	14063	3094	22	581100
Garlic	193	19	10	16098
Black radish	283	14	5	2605
Eggplant	4134	496	12	94935
Cucumber	9849	1379	14	390915
Pepper	1540	185	12	75302
Courgette	3534	424	12	130755
Lettuce	8804	3902	50	1064778
Turnip	2591	337	13	70421
Green beans	1031	103	10	46772
Beetroot	2206	309	14	65663
Celery	1990	597	30	126942
Other products	9026	1805	20	806096
Total	104668	22783	22	7101533

Table 7: Vegetable waste in winter in the fruit and vegetable fields organization of Tehran Municipality

Product	Product supply quantity (Ton)	Amount of waste (Ton)	Waste (%)	Loss value (USD)
Green vegetables (table, stew, and soup)	6395	1151	18	475133
Tomato	13047	3262	25	1701195
Carrot	5923	1066	18	228034
Cabbage	4014	682	17	215107
Potato	17183	4296	25	1740828
Onion	12921	2843	22	618632
Garlic	190	19	10	15257
Black radish	423	21	5	3491
Eggplant	3218	386	12	85495
Cucumber	9437	1321	14	495759
Pepper	1523	183	12	67872
Courgette	2156	259	12	101918
Lettuce	9122	4561	50	941294
Turnip	1573	204	13	70617
Green beans	424	51	12	26909
Broad bean	67	10	15	2977
Beetroot	1724	241	14	62504
Celery	1547	464	30	80114
Other products	9069	1814	20	837096
Total	99956	22834	23	7770243

(92%-95%) (Rizzo and Muratore, 2009). Undoubtedly, the amount of waste would be reduced by controlling the diseases that reduce the quality of vegetables after their harvesting (Soleimani *et al.*, 2021). According to Fig. 3, the amount of fruit waste in the FVFTM during 2021 was 12%, which led to an economic loss of 24,135,950 USD. The highest amount of fruit waste (15%) was observed in summer, in which the economic loss was higher compared to other seasons. Considering that fruit ripening is affected by high temperatures, the softness of ripe fruit reduces the transportability and shelf life of the product (Mulholland *et al.*, 2003) and leads to an increase in waste in this season. It is suggested

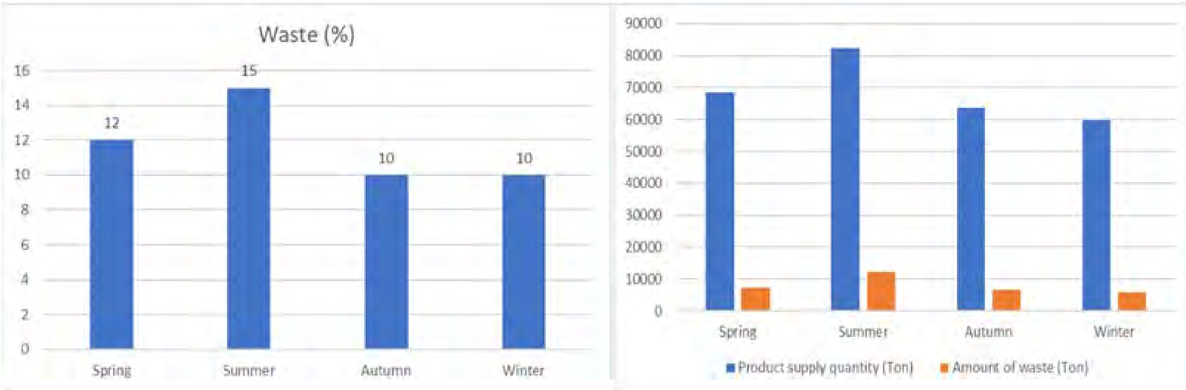

Fig. 3: Fruit waste in fruit and vegetable fields organization of Tehran Municipality

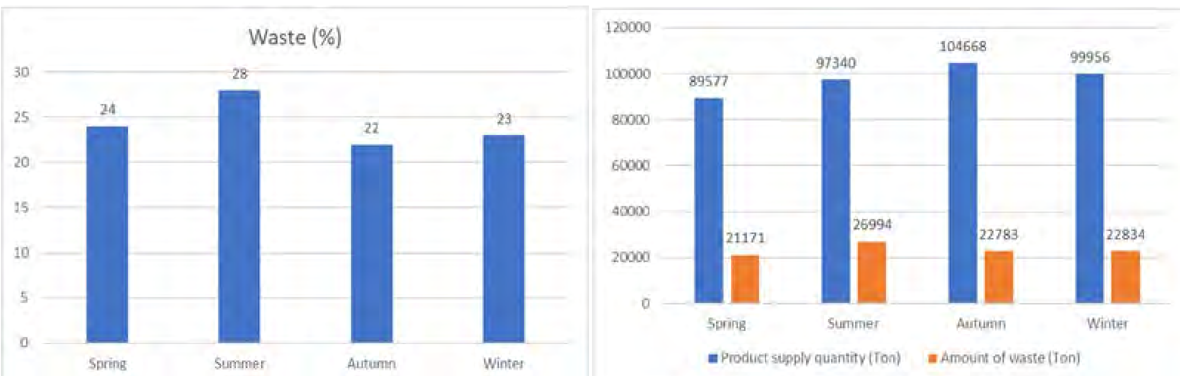

Fig. 4: Vegetable waste in fruit and vegetable fields organization of Tehran Municipality

to pack the summer fruits which do not have proper packaging using suitable technologies such as vacuum cooling and ethylene control.

According to the values reported in Fig. 4, the amount of vegetable waste in the FVFTM in 2021 was 24%, causing a high economic loss of 30,755,589 USD. Vegetable wastes in summer and spring were 28% and 24%, respectively, resulting in a higher economic loss compared to other seasons.

The economic loss due to fruit and vegetable waste was estimated as 54,891,539 USD in a year. Indeed, the costs associated with seeds, pesticides, staffing, transportation, and the amount of water wasted to produce these products are added to this economic loss (Liu *et al*., 2013, Joardder and Masud, 2019). The results of this study showed that the economic loss caused by vegetable waste was greater than that of fruit waste, while vegetables have a major contribution to the health of the people in the world (Kalia and Singh, 2018). Since the decrease of moisture content in agricultural products has a significant role in their quality loss (Al-Tayyar *et al*., 2020), it is possible to control the moisture content of these products by providing favorable conditions in terms of temperature and humidity and by suitable packaging (Rizzo and Muratore, 2009). Even though the main priority is to prevent the food waste production (Abadi *et al*., 2021), inventing the useful methods to separate and transform food waste into a valuable substances (Gustavsson *et al*., 2011) is important to reduce the economic loss and environmental issues (Liu *et al*., 2013). Food waste and bulking materials may contain trace amounts of contaminants and pathogens. These contaminants have long half-lives and are easily move within soil and plants. They can accumulate within the food supply chain and cause moderate to high toxicity levels (O'Connor *et al*., 2022). According to the findings of the present study, a large amount of waste is produced daily in Tehran. This waste is mixed with urban waste and transported from different areas of the city to landfill centers by garbage trucks with high fees. The accumulation of such wet organic materials has resulted in adverse environmental effects including leachate leakage, attracting insects, stench (Neff *et al*., 2015), and producing harmful greenhouse gases (Joardder and Masud, 2019). They also cause a considerable economic loss which needs to be prevented. In this sense, a proper waste disposal management system seems to be vital (Neff *et al*., 2015). Since fruit and vegetable wastes contain organic and protein substances, they can be used to prepare organic fertilizers; biofuels for electricity generation, cooking, and heating (Kuwahara *et al*., 1999); biopolymers (Girotto *et al*., 2015); pectin (Khoshnevisan *et al*., 2020); and to produce hydrogen and volatile fatty acids (Scotto di Perta *et al*., 2022). The results indicated that the economic losses caused by fruit and vegetable wastes in the fruit and vegetable fields organization of Tehran Municipality were 24,135,950 USD and 30,755,589 USD, respectively, totally amounting to 54,891,539 USD in 2021.

CONCLUSION

Fruit and vegetable wastes can be a fundamental problem due to the continuous increase of population in cities. The FVFTM plays a vital role in producing fruit and vegetable waste, and reducing this waste is important due to food safety and environmental issues. The high amount of waste from the supply and sale of fruits and vegetables in the FVFTM differs from season to season, and causes a huge economic loss each year. The present study revealed the increment of fruit and vegetable waste in sale fields and economic loss in hot seasons of the year. The amounts of waste from some fruits and vegetables in the FVFTM reached over 20% and 50% in summer, respectively. Since fruit ripening is affected by high temperatures, the softness of ripe fruits reduces their transportability and shelf life and leads to increase of fruit waste in this season. The amount of vegetable waste in the FVFTM was 24%, representing a high economic loss. Vegetable wastes in summer and spring were 28% and 24%, respectively, and therefore their economic loss was higher in these seasons. The total economic loss caused by fruit and vegetable wastes was estimated as 55 million USD in a year. Indeed, the costs associated with seeds, pesticides, staffing, transportation, and the water wasted to produce fruits and vegetables would add to their economic loss. The economic loss caused by vegetable waste was greater than that of fruit waste. The increase of fruit and vegetable waste imposes serious threats such as environmental pollution, health risks, need for more transportation equipment, and lack of burial grounds. Therefore, there is an urgent need to reduce the amount of fruit and vegetable waste and minimize their economic loss by adopting standard management practices and executing different approaches in food waste management. Proper methods for collecting and disposing the food waste can be effective in reducing economic and environmental losses. It is also suggested to apply appropriate policies for good agricultural and post-harvest practices, such as the use of ethylene synthesis inhibitors or absorbents to reduce the ripening speed in fruits or the use of packaging for reducing fruit and vegetable wastes in the storage, distribution and supply stages. In addition, to prevent the environmental problems, it is necessary to implement more accurate management principles for waste disposal.

AUTHOR CONTRIBUTIONS

B. Parsafar performed the data gathering, data analysis and preparing the manuscript. M. Ahmadi supervised the design of the study, its reviewing and editing. Gh.J. Jahed Khahiki, the corresponding author, contributed by supervising the design of the study and the original draft, rendered data analysis, interpreted the results, performed writing, reviewing and editing of the manuscript. N. Shariatifar participated in the design of the study, reviewing and editing. A. Rahimi Foroushani contributed to the methodology, and software and data analysis.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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ABBREVIATIONS

%	Percent
ANOVA	Analysis of variance
°C	Degree Celsius
*DHS*E	Department of health, safety, and environment
FAO	Food and Agriculture Organization
Fig.	Figure
FVFTM	Fruit and Vegetable Fields of Tehran Municipality
KG	Kilogram
MG	*Methane gas*
RH	Relative humidity
SPSS	Statistical package for the social sciences
t	Ton
USD	United States Dollar

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Podcasts

CASE STUDY

Harbor water pollution by heavy metal concentrations in sediments

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ABSTRACT

BACKGROUND AND OBJECTIVES: *The Belawan Harbor is the third largest port, which is located in an estuary, causing the port water area to be vulnerable to pollution, especially heavy metals. Conflicts between the community and the port authorities often occur due to pollution. Heavy metals are dangerous contaminants for waters, and total organic carbon in waters is needed but will cause eutrophication if the concentration is excessive in the environment. The level of heavy metal pollution in the waters of the Belawan Harbor and the factors that cause the pollution should be analyzed, because the level of heavy metal pollution has not been measured in the sediments of harbor waters. This study can be used as a reference for the actions of related agencies in dealing with heavy metal pollution in waters.
METHODS: *Sampling of sediments was performed at 10 locations, starting before the harbor activity began and moving toward the open sea. Sampling was conducted using Van Veen grab. Heavy metal concentrations were analyzed in the laboratory using the atomic absorption spectrometer method to assess the essential heavy metal copper and non-essential heavy metal lead, cadmium, and mercury. Heavy metal pollution in sediments was assessed by analyzing sediment pollution index. The multivariate statistical analysis on the relationship among factors was conducted using Pearson correlation matrix method, principal component analysis, and cluster analysis.
FINDINGS: *The environmental quality standards used indicate average concentration of heavy metals; lead (28,869 milligram per kilogram) and copper (8,003 milligram per kilogram) are below the quality standard. The mercury concentrations are undetectable (<0.00011 milligram per kilogram) at each station. By comparison, the concentration of cadmium (1,455 milligram per kilogram) exceeded the Interim Sediment Quality Guidelines from the Canadian Council of Ministers of the Environment. Results of the index analysis show that the average value of the pollution factor of copper is −0.177 (low contamination), that of lead is −1.433 (moderate contamination), and that of cadmium is −4.850 (high contamination); the geoaccumulation index value of copper is −5.328. (not polluted), that of lead is −0.190 (unpolluted), and that of cadmium −1.657 (moderately polluted). As mercury concentration in sediments is relatively low, it is not considered when calculating pollution levels. Overall, on the basis of a pollution index of 1.033 (1 < pollution load index ≤ 2), this condition indicates that the waters of the Belawan Harbor are categorized as not polluted to lightly polluted. The highest total organic carbon is at the estuaries of the Belawan and Deli Rivers. The sediment fraction is 72.2 percent sandy, 16.4 percent sludge, and 11.4 percent clay substrate.
CONCLUSION: Pollution in the waters of the Belawan Harbor is in the category of not polluted to slightly polluted. Although the pollution is still in the light category, this must be of particular concern to the relevant agencies, especially the local government, to make the right policies to overcome this pollution immediately. Pollution problems increase with the anthropogenic activities around coastal areas, as well as activities in the Belawan and Deli River watersheds, because the pollutant will flow from the upstream to the estuary area.

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INTRODUCTION

The Belawan Harbor is Indonesia's third busiest harbor and is the gateway to the economy of Medan City, North Sumatra. The working area (DLKr) is 12072.33 hectare (ha), which has more than one base and more than one terminal. On the basis of the existing data in the Indonesian central statistic agency; Badan Pusat Statistik BPS, in 2019, The Belawan Harbor loads and unloads 11,979,268 tons per year, and it has 128,396 passengers overall in annual arrivals and departures. Harbors have various supporting facilities, such as passenger terminals, international container terminals, ship repair sites, storage tanks for hazardous and toxic materials, and offices. Various harbor activities have a negative impact on the environment, especially harbor waters. Pollutants from various sources, such as manufacturing processes, passenger waste, oil spills, terminal construction, and ballast water, can affect the aquatic environment. The maintenance and repair of ships near harbors also contribute to water pollution (Pourabadehei and Mulligan, 2016a; Salam et al., 2019). The Belawan Harbor is unique, because it is located between two major river estuaries, namely, Belawan and Deli Rivers. The pollution in the waters of the Belawan Harbor is caused by anthropogenic activities, either directly or indirectly, directly or indirectly, in the watersheds of Belawan and Deli Rivers. These activities include industrial, agricultural, and urban activities. Human interference, tourism, and fisheries are potential long-term sources of organic and inorganic pollutants that can degrade harbor sediments and increase the levels of polycyclic aromatic hydrocarbons and metals (Edge *et al*., 2021; Ojemaye and Petrik, 2019). Due to its location at the confluence of estuaries, the waters of the Belawan Harbor are vulnerable to pollution because of the settling of various pollutant types (Hanggara *et al*., 2021). The Belawan Harbor, which is located in the river estuary area, is often faced with social conflicts from the community because it is considered a contributor to pollution around the waters. For example, communities complain about the difficulty in catching fish around Belawan waters, which makes it challenging for fishermen to support their livelihoods. Heavy metal contamination in waters will make endemic fish migrate to uncontaminated waters, but species such as clams, shrimp, and crabs will survive in that environment. If humans consume contaminated aquatic species, then they will accumulate in their bodies and eventually have an adverse effect on human health (Pourabadehei and Mulligan, 2016a; Zhu *et al*., 2022). According to Chen *et al*. (2016), the steel, chemical, and plastic industries—all of which are located in watersheds that also produce industrial wastewater and domestic waste—are the main sources of heavy metal pollution (Araiza-Aguilar *et al*., 2020; Karbassi *et al.*, 2015). Medan industrial Park and other urban activities can be found along Belawan and Deli Rivers, which may result in the entry of heavy metals in to Belawan Harbor's waters, and heavy metal particles will be deposited in the sediment due to tidal activity (Mignard *et al*., 2017). The dangers associated with heavy metal contamination, which will have an impact on continuing social conflicts, will be in conflict with the goals of sustainable development, which considers environmental, economic, and social aspects of society. Hence, whether Belawan Harbor's waters are contaminated by heavy metals must be determined. The analysis in question calculates heavy metal concentrations in sediments because sediment deposits, as opposed to water columns, are more reliable indicators of heavy metal contamination in waters (Rochyatun and Rozak, 2008; Banu *et al*., 2013). Given that concentration analysis and studies on heavy metal pollution have never been conducted in the sediments of Belawan Harbor waters, the concentration of heavy metals in sediments, the level of heavy metal pollution, and the influence of factors affecting heavy metal contaminants in sediments must be investigated. Non-essential heavy metals have essentially less benefit from being present in water. Thus, only one form of necessary heavy metal, namely, copper (Cu), and three types of non-essential heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg), were chosen to be measured. This study aims to 1) analyze the concentration of heavy metals in the sediments of Belawan Harbor waters; 2) assess the level of heavy metal pollution in sediments; and 3) examine the factors that affect heavy metal contamination in harbor water sediments. The study was conducted in the Belawan Medan Harbor in November 2018 and January 2019.

MATERIALS AND METHODS

The geographical location of Belawan Harbor block extends from 03°47′N to 98°42′E. Sediment samples were collected from 10 locations in Belawan Harbor, Medan, North Sumatra (Fig. 1). Ten locations

Fig. 1: Geographic location of the study area in Belawan Medan Harbor along with the sediment sampling locations for heavy metals

Table 1: Location of water sediment parameter collection

Site	Description	Coordinates	
		Latitude	Longitude
1	Belawan River Estuary	3°46’27, 39”NL	98°40’26, 15”EL
2	Passenger terminal-LANTAMAL	3°47’02, 63”NL	98°40’47, 55”EL
3	Unloading dock	3°47’29, 11”NL	98°42’41, 42”EL
4	Reclamation project	3°48’12, 20”NL	98°43’42, 50”EL
5	Deli River Sea	3°47’07, 10”NL	98°43’47, 70”EL
6	Pacific Ocean Tour	3°47’28, 60”NL	98°43’10, 30”EL
7	Belawan fishing harbor (PPS)	3°46’39, 40”NL	98°42’44, 30”EL
8	Deli River Estuary I	3°46’02, 28”NL	98°42’14, 77”EL
9	Deli River Estuary II	3°46’12, 70”NL	98°42’55, 20”EL
10	Belawan River Sea	3°48’53, 90”NL	98°43’27, 90”EL

in Belawan Medan Harbor’s DLKr and DLKp waters served as sampling points. Details description of the site sites seen in Table 1.

Targeted sampling was used to pinpoint the area between the Belawan and Deli rivers. The areas encompass the sea around Belawan Harbor and span the time before and after harbor activities. Global positioning system coordinates were used to pinpoint the locations of samples.

Sampling and handling sediment

At low tide, sediment samples were collected using the Van Veen grab from a boat. The tool was lowered to the bottom of the water, and sediment samples were then taken from the upper layers (depths between 0 centimeter: cm and 250 cm). Sediment samples were packed in polyethylene containers with a volume of 250 mL, which had been sterilized beforehand with 95% alcohol and distilled water and then put into an ice box. The sample was then taken to the laboratory for analysis. After being put in the laboratory in the freezer with a temperature of −20 °C, the heavy metal content was measured using a spectrophotometer using the ASTM C1301-95 Reav 2001 testing method. The sediment sample was used for chemical analysis, which consisted of digesting aliquots of 5 g by adding 2 mL of 4:1 of nitric acid: perchloric acid (HNO_3: $HClO_4$) solution to the samples for 3 h at 140 °C (Yap and Pang, 2011). After the sample had cooled, a 250 mL Erlenmeyer tube was prepared, in which the sample was added and then diluted with 20 mL of double distilled water and shaken gently. The sediment sample was filtered with Whatman paper, and the filtered results were put into a 100 mL volumetric flask and up to 50 mL of distilled water was added. Then, the sediment sample was ready to be measured using atomic absorption spectrometry (AAS). The AAS method is generally used for the analysis of heavy metal content because it is characterized by good precision and accuracy, as well as a percentage recovery of more than 90% of the amount of analyte (Feist *et al*., 2007). Strict quality control was maintained during the experiment. The blank samples and standard substances were progressed using the same method. Four duplicates were used for each sample. The repeat sample analysis error and the analytical precision for replicate samples were below 2% the relative standard deviation (SD). Method of blank operation showed no detectable metals (Men *et al*., 2018).

Pollution index calculation

Heavy metal pollution evaluation was conducted using Pb, Cu, Cd, and Hg geoaccumulation index (Igeo), pollution load index (PLI), and contamination factor (CF). The Igeo value is calculated using Eq. 1 (Shams El-Din *et al*., 2014; Rabee *et al*., 2011; Veerasingam *et al*., 2012).

$$\text{Igeo} = \log_2\left[\frac{C_n}{1.5B_n}\right], \tag{1}$$

where Cn is the measured concentration of metal n in the sediment, and Bn is the geochemical background values of the metal *n*. Factor 1.5 compensates for possible fluctuations in the background values for a given substance in the environment, as well as highly anthropogenic influences. The Igeo consists of seven classes: Igeo ≤ 0 is Class 0 (unpolluted); 0 < Igeo < 1 is Class 1 (unpolluted to moderately polluted); 2 < Igeo < 3 is Class 3 (moderately to heavily polluted); 2 < Igeo < 4 is Class 4 (heavily polluted); 4 < Igeo < 5 is Class 5 (heavily to extremely polluted); and 5 > Igeo is Class 6 (extremely polluted; Ke *et al.,* 2017; Dai *et al*., 2018). For each observation site, the level of CF is calculated using Eq. 2 (Lars, 1980).

$$\text{CF} = \text{C heavy metal/C background}. \tag{2}$$

Where, C heavy metal is the concentration of metals in the sediment samples, and C background is the ambient environmental metal concentration (background metal concentration; Earth's crust; Effendi *et al*., 2017; Ogbeibu *et al*., 2014). Comparison between normal concentrations of metals (C background) in nature according by Turekian and Wedepohl (1961), namely Cu = 45 mg/kg, Pb = 20 mg/kg, Cd = 0.3 mg/kg, and Hg = 0.4 mg/kg. The CF values were interpreted as follows: low contamination (CF < 1), moderate contamination (3 < CF < 6), and extremely high contamination (CF > 6). PLI is calculated using Eq. 3 (Sivakumar *et al*., 2016).

$$\text{PLI} = (\text{CF1} \times \text{CF2} \times \text{CF3} \times \ldots\ldots \text{CFn})^{1/n}. \tag{3}$$

TomLinson *et al.* (1980) were the first to use PLI. The CF multiplied by the number of heavy metals n yields CF * n. The PLI method was used in the study of heavy metal contamination. The PLI values were classified into four groups: no pollution load (PLI < 1), low pollution load (1 < PLI < 3), moderate pollution load (3 < PLI < 6), and high pollution load (6 < PLI).

Statistical analysis

Pearson's correlation matrix is a statistical technique for illustrating associations between sets of data. The degree of significance is used in decision making when the significance value is correlated ($p < 0.05$) or when it is not correlated ($p > 0.05$). If R^2 equals 1, then the relationship is completely positive; if it equals −1, then the relationship is completely negative. Low (0.00–0.20), moderate (0.40–0.60), high (0.60–0.80), and extremely high (1.0–1.0) are the typical ranges assigned to correlation coefficients (0.80–1.00; Bush and Guilford, 1956). Principal component analysis (PCA) is widely used in conducting research on the environment (Abollino *et al*., 2003; Lucho-Constantino *et al*., 2005; Pourabadehei and Mulligan, 2016b; Rognerud and Fjeld, 2001; Sundaray *et al*., 2011). Descriptive statistics, such as PCA, are useful for elucidating as much information as possible inside a data matrix. Specifically, the accompanying data matrix has rows that represent study sites and columns, which represent quantitative data for environmental factors (physical chemistry). By comparing the contamination intensity (Igeo) of sediment samples, cluster analysis (CA) was used to classify contaminated and clean sites. Statistics were calculated using Excel 2016 and XLSTAT 2022.

RESULTS AND DISCUSSION

Table 2 displays the percentage results of the sediment fraction. The proportion of sand (72.20% on average) is the most dominant in aquatic sediments. Total organic carbon (TOC) is important for analysis; it is considered a modifier of sediment toxicity because it can change contaminant distribution and bioavailability (Watson-Leung and Picard, 2016). The TOC concentration in the sediment around Belawan Harbor waters varies between 0.26% and 2.74%. The categories of TOC assessment in sediments are low = TOC ≤ 1%, moderate = 1 < TOC <3%, and high = TOC > 3% (USEPA, 2002). The average TOC value measured in the Belawan Harbor waters is in the low category at 1.74%. The highest TOC value is at Station 2; this station is at the DLKr, that is, at the passenger terminal. The high TOC concentration in this area is caused by the changes in the flow of river water toward the sea resulting from bends in the river flow. The existence of river water bends in the estuary due to harbor activities can affect the river water discharge, and the sediment transported from the upstream river will significantly be retained in that area (Miao *et al*., 2010; Zheng *et al*., 2019). Generally, marine organic matter has TOC/total nitrogen (TN) values of 4–10, and the TOC/TN values of terrestrial plants are 20 and above (Meyers, 1994; Lamb *et al*., 2006). The TOC/TN values of marine phytoplankton are close to 6.6 (Redfield *et al*., 1963). The TOC/TN ratios of fresh vascular plants and their degraded detritus are 23–1,560 and 14–47, respectively (Tyson, 1995; Usui *et al*., 2006). The TOC/TN ratios in soils generally range from 8 to 15 (Kendall *et al*., 2001). The highest average TOC concentration at each sites does not have considerably different values except at Sites 4 and 5, which are reclamation areas and seas off. The sediment substrate affects the TOC concentration, as observed in sites that have sandy substrates with a lower TOC concentration. This phenomenon will be analyzed further in a multivariate statistical analysis. Extracted organic matter may come from natural or manmade activities. Natural sources of organic compounds can be terrestrial and aquatic organisms

Table 2: Physicochemical concentration of sediment in Belawan Harbor waters

Site	Heavy metal concentration (mg/kg)				Sand	Sludge	Clay	TOC
	Pb	Cu	Cd	Hg				
1	20.99	5.64	0.90	<0.00011	67	15	18	2.10
2	24.69	5.80	1.37	<0.00011	69	17	14	2.74
3	19.82	3.42	1.32	<0.00011	67	19	14	1.42
4	15.50	6.86	1.22	<0.00011	99	1	0	0.26
5	23.97	1.68	1.30	<0.00011	79	9	12	0.76
6	31.90	4.53	1.53	<0.00011	61	29	10	2.09
7	28.00	16.24	1.35	<0.00011	69	19	12	1.89
8	68.43	21.49	2.10	<0.00011	61	23	16	2.65
9	29.46	9.82	1.92	<0.00011	77	15	8	1.96
10	25.93	4.55	1.54	<0.00011	73	17	10	1.54
Minimum (Min.)	15.5	1.68	0.9	<0.00011	61	1	0	0.26
Maximum (Max.)	68.43	21.49	2.1	<0.00011	99	29	18	2.74
Average	28.869	8.003	1.455	<0.00011	72.200	16.400	11.400	1.741
SD	14.712	6.234	0.3446	-	11.1235	7.5454	4.9933	0.7778
Interim Sediment Quality Guidelines (ISQG)								
CCME (2002)	ISQG	30.2	18.7	0.13	0.7			
	PEL	112	108	0.7	4.2			
ANZECC/ARMCANC (2000)	Low	50	65	0.15	1.5			
	High	220	270	1	10			

(Gao *et al*., 2012).

Belawan Harbor water sediments contain Pb concentrations of 15.5–68.43 mg/kg, Cu concentrations of 1.68–21.49 mg/kg, and Cd concentrations of 0.9–2.1 mg/kg; conversely, Hg concentrations are not detected because the value is relatively small (below 0.00011 mg/kg), but the concentration is not 0 in the sediment. The data obtained show that in this case, Hg is considered not to pollute and not harmful to the aquatic life in Belawan Harbor. The heavy metal concentrations of Pb, Cu, Cd, and Hg were compared with the sediment quality standards from the Canadian Council of Ministers of the Environment (CCME, 2002) and the quality standard guidelines from Australia and New Zealand (ANZECC/ARMCANZ, 2000). The obtained metal concentrations were compared using the two quality standard guidelines from other countries because Indonesia does not have its own quality standard guidelines yet. A small set of quality benchmarks for the heavy metal concentration represents the heavy metal pollutant load. The heavy metal concentrations of Pb (28,869 mg/kg) and Cu (8,003 mg/kg) were below the quality limits, according to the CCME and ANZECC/ARMCANZ (2000) sediment quality standards. Moreover, the average Cd concentration (1,455 mg/kg) exceeds the CCME (2002) ISQG and is becoming close to the low thresholds of ANZECC/ARMCANZ.

The concentration levels in other metal-polluted harbors from different regions of the world are shown in Table 3 to understand the extent of metal pollutions in the harbor area of this study. The contaminant of Cd in the Belawan Harbor sediments for all observation stations is above the quality standard threshold of the CCME (2002) compared with other heavy metals. The Cd concentration in this study is not considerably different from that in the Gironde Estuary, Yangtze River Estuary. Two harbors on the Egyptian Mediterranean Coast with a relatively high Cd content compared with other harbors worldwide exhibit different characteristics in their Cd concentration. This finding supports the claim that sediment contamination in Belawan Harbor waters is influenced by the rate of pollutants produced from anthropogenic activities that enter through the Belawan and Deli Rivers. Overall, the Pb, Cu, Cd, and Hg concentrations in the sediments of Belawan Harbor are relatively lower compared with other harbors in the world. This phenomenon is influenced by the level of harbor activities and dense urban activities around harbors, such as Rize Harbor, 12 South Korea harbors, Eastern Harbor, and Kaohsiung Harbor. The Belawan Harbor is still the third busiest harbor in Indonesia. Thus, heavy metal pollution can be caused by development in many sectors (e.g., industrial, agriculture, urbanization, and navigation), accompanied by the high rate of population growth that acts as environmental stressors on aquatic systems, particularly for coastal countries (Ebeid *et al*., 2022). Overall, the extent of metal pollution in the sediments of the Belawan Harbor is comparable to those found in other metal-contaminated harbors worldwide. Table 4 shows the Pb, Cu, and Cd values for pollution factor, geological accumulation index, and pollution index.

The average CF value of Pb is 1.4437, which is more than 1 ($1 < CF < 3$), so the Pb contamination level is moderate. The Igeo of Pb typically falls in the range of −0.1907 to 0; the sediment has an unpolluted status because this value is less than 0 (Igeo < 0, Class 1). The CF value of Cu has an average value of 0.1778, which is less than 1 ($CF < 1$), so the sediment contamination of Cu in Belawan Harbor is considered

Table 3: Concentration ranges of heavy metals reported in Belawan Harbor compared with other regions of the world

Regions	Pb	Cu	Cd	Hg	References
Gironde Estuary, France	5.0–84	0.5–40	0.01–2.1	0.001–0.37	Larrose *et al*. (2010)
Rize Harbor, Turkey	15.9–33.0	33.9–279.1	0.1–1.4	0.01–0.07	Gedik and Boran (2012)
12 South Korea harbors	20–599	5–2,360	0.03–15	0.003–3	Choi *et al*. (2012)
Eastern Harbor, Egypt	1.3–112	3.80–129	0.3–1.8	-	Ghani *et al*. (2013)
Kaohsiung Harbor, Taiwan	14–219	12–2,840	0.05–7.3	0.03–10	Chen *et al*. (2016)
Yangtze River Estuary, China	1.12–2.20	1.12–71.80	0.02–2.20	0.001–0.556	Li *et al*. (2019)
Pearl River Estuary, China	36.1–51.2	26–47.7	0.29–0.82	-	Ye *et al*. (2020)
2 harbors in the Egyptian Mediterranean Coast	50.5–134.4	828–1,537	26.1–59.5	-	Ebeid *et al*. (2022)
Belawan Harbor, Indonesia	15.5–68.43	1.68–21.49	0.9–2.1	<0.00011	This study

to be relatively low. The Igeo of Cu has a mean value of −3.4327, which is lower than 0, so the sediment does not contain Cu contaminant (Igeo < 0, Class 1). The CF value of Cd is 4.850, which is greater than 4 (3 < CF < 6), indicating a highly contaminated sediment level. The mean Igeo value is 1,657, which is greater than 1 and less than 2, so Belawan Harbor's sediment is considered to be highly contaminated (1 < Igeo < 2, Class 3). The obtained values for Igeo can be negative or positive. The results demonstrate that the sediment in these waters is cleaner (less polluted) when the Igeo is negative, whereas a higher value indicates that the sediment is more contaminated (polluted). The PLI analysis result, with values ranging from 0.579 to 2.2253, show an average value of 1.033, which is between 1 and 3, so the Belawan Harbor has a low pollution load. The human activities in DLKr and DLKp in Belawan Harbor have been increasing every year. As a local industrial cluster develops in the Deli River watershed, a harbor reclamation project (site 4) is changing the shoreline in a manner that traps airflow and sedimentary material at the river mouth. The Deli River watershed, from which the wastewater flows into the harbor area, has three of the four indicated pollutants. These contaminants may have been introduced by illicitly generated industrial output effluent (Sites 7–9). The Environment Agency of North Sumatra estimated in 2019 that there would be 54 industrial and 27 household waste canals along the Deli River. Businesses from various sectors (e.g., painting and electroplating, loose iron, food processing, and food and beverage sectors) have established themselves along the Deli River. One way in which manufacturing contributes to air pollution is through the use of Cd in the raw material trade or as a carrier material in the industry (Masum and Pal, 2020). Of all Cd, 75% of it is used to make batteries (especially Ni–Cd batteries). Pigment mixtures containing this metal are used in the production of ceramics, electroplating, low-melting-point alloys, nuclear-reaction cleavage, paint, metal coatings, semiconductors, PVC stabilizers, and cigarettes; but they are not useful in the production of phosphate fertilizers or anti-malarial drugs, such as those used to treat syphilis and malaria (Bonberg *et al*., 2017; Hill *et al*., 2010; Rodrigues *et al*., 2020). After entering the river, this toxic metal is carried by the tides to the estuary's sediments. Restoring a harbor can affect more than just the shoreline; it can also modify the oxygen content of the soils and sediments below the water. Hence, in biologically polluted estuaries (Sites 7–9), heavy metals are absorbed by the sediment substrate; especially, the silt sediment fraction has better absorption (Chen and Jiao, 2008). Heavy metal contamination in sediments is highly influenced by anthropogenic activities, which include allowing the construction of houses and businesses near watersheds and orienting most buildings almost fully away from the river. The procurement of domestic wastewater treatment plants (WWTPs) has also not

Table 4: Analysis results of CF, Igeo, and PLI of Belawan Harbor sediments

Site	Pb		Cu		Cd		PLI
	CF	Igeo	CF	Igeo	CF	Igeo	
1	1.050	−0.515	0.125	−3.581	3.000	1.000	0.733
2	1.235	−0.281	0.129	−3.541	4.567	1.606	0.899
3	0.991	−0.598	0.076	−4.303	4.400	1.553	0.692
4	0.775	−0.954	0.152	−3.299	4.067	1.439	0.783
5	1.199	−0.324	0.037	−5.328	4.333	1.531	0.579
6	1.595	−0.089	0.101	−3.897	5.100	1.766	0.936
7	1.400	−0.100	0.361	−2.055	4.500	1.585	1.315
8	3.422	1.190	0.478	−1.651	7.000	2.222	2.253
9	1.473	−0.026	0.218	−2.781	6.400	2.093	1.272
10	1.297	−0210	0.101	−3.891	5.133	1.775	0.876
Min.	0.775	−0.954	0.037	−5.328	3	1	0.579
Max.	3.422	1.190	0.478	−1.651	7	2.222	2.253
Rerata	1.443	−0.190	0.177	−3.432	4.85	1.657	1.033
SD	0.735	0.561	0.138	1.071	1.148	0.341	0.489

Note: The level of Hg contamination was not further analyzed, because the Hg concentration is extremely low (undetected at <0.00011 mg/kg) at all observation sites and considered not to contaminate the sediment.

Table 5: Personnel correlation matrix parameter of sediment quality in Belawan Harbor waters

Parameter		Pb	Cu	Cd	Sand	Sludge	Clay	TOC
Pb	Pearson Correlation	1						
	Sig. (2-tailed)							
Cu	Pearson Correlation	0.779**	1					
	Sig. (2-tailed)	0.008						
Cd	Pearson Correlation	0.783**	0.594	1				
	Sig. (2-tailed)	0.007	0.070					
Sand	Pearson Correlation	−0.519	−0.261	−0.259	1			
	Sig. (2-tailed)	0.125	0.466	0.475				
Sludge	Pearson Correlation	0.522	0.275	0.405	−0.928**	1		
	Sig. (2-tailed)	0.122	0.441	0.246	0.000			
Clay	Pearson Correlation	3.67	0.166	−0.036	−0.826**	0.556	1	
	Sig. (2-tailed)	2.97	0.647	0.922	0.003	0.095		
TOC	Pearson Correlation	0.564	0.451	0.390	−0.815**	0.751	0.679*	1
	Sig. (2-tailed)	0.089	0.191	0.265	0.004	0.012	0.031	

been implemented, especially in controlling household waste pollution. This situation has resulted in the community to consider disposing household waste (*e.g.,* plastic, food waste, cable scraps, and corrosion of water pipes) directly into river water bodies the most effective technique to handle their waste problem. Industrial waste also contributes to contamination in Belawan Harbor waters. According to BPS data in 2018, Deli Serdang Regency had 444 industries, Karo Regency had 7, and Medan City had 270. The Deli and Belawan Rivers flow in these regencies. The data from the Belawan Ular Padang resource management pattern by the Minister of Public Works in 2012 indicate that many industries have a WWTP capacity smaller than the waste they produced, such that their waste did not meet the established quality book. Anthropogenic impacts accelerate the rate of toxicity in the food web in water sediments and reduce the quality of the water environment through the transportation of pollutants through land by humans (Leung *et al*., 2021; Azizi *et al.,* 2016).

Pearson correlation matrix analysis

The correlations between heavy metals are depicted by the Pearson matrix (Table 5). Heavy metals, sediment fraction, and TOC, as well as the significance level of the correlation matrix, are described. Having a value of 1 for the degree of significance indicates an extremely high level of relevance.

The Pearson correlation value for several metals is presented in Table 5. Pb and Cd have the strongest heavy metal correlation value (R^2 = 0.783, p = 0.007), as well as Pb and Cu (R^2 = 0.779, p = 0.008). A significant degree of association is observed. The lowest value of correlation is found between Cu and Cd (R^2 = 0.594, p = 0.070). For all metals, a p value of 0.05 is considered significant. Thus, a relationship exists between these factors. No statistically significant difference exists between the sediment and heavy metal fractions and TOC (p > 0.05). Sludge has the highest R^2 values for Pb, Cd, and Cu among the sediment fractions studied (0.522, 0.122, 0.405, 0.26; 0.275). After Pb and Cu, clay exhibits a weak negative association with Cd (R^2 = −0.036, p = 0.922) and a moderately positive correlation with Pb (R^2 = 0.367, p = 0.297). Thus, the Cd output decreases with the increase in the fraction of clay sediment. Pb (R^2 = −0.519, p = 0.125), Cu (R^2 = -0261, p = 0.466), and Cd (R^2 = −0.259, p = 0.471) have a negative connection with sand fraction. Given that sand cannot bind with heavy metals, as the sand percentage in the water becomes more concentrated, the heavy metal content will also drop. Heavy metals in TOC have a significance level of p > 0.05. The significance threshold for heavy metals in sediment fractions is equivalent to this finding. Considerable differences between the two are not observed. Pb (R^2 = 0.564, p = 0.089), Cu (R^2 = 0.451, p = 0.191), and Cd (R^2 = 0.390, p = 0.265) have the highest Pearson correlation values of TOC with the highest heavy metals. Heavy metal concentrations tend to be greater in fine-grained, high-organic-matter sediments (Dan *et al*., 2022). There exists a high degree of relationship between TOC and sludge (R^2 = 0.751, p = 0.12), and there exists a similarly high correlation value between TOC and clay (R^2 = 0.679, p=0.31). The degree of connection between TOC and sand fraction is extremely strong (R^2 = −0.815, p=0.004). Thus, the TOC concentration in sediment

Table 6: Principal component análisis

Parameters	F1	F2
Pb	0.672	0.227
Cu	0.385	0.365
Cd	0.359	0.462
Sand	0.786	0.188
Sludge	0.726	0.068
Clay	0.473	0.326
TOC	0.779	0.038
Eigenvalue	4.180	1.674
Variance (%)	59.708	23.915
Cumulative (%)	59.708	83.623

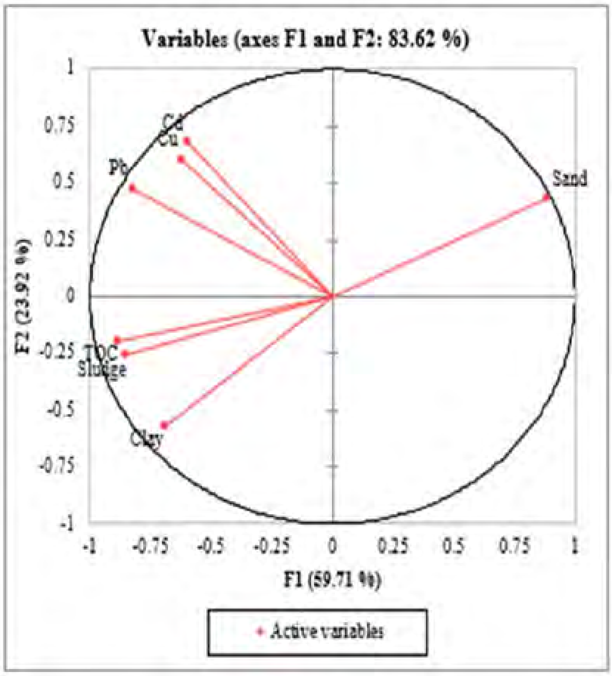

Fig. 2: Analysis of the main components of sediment quality parameters in the waters

decreases with the increase of sand content. The sludge fraction often contains a high concentration of organic and inorganic materials (Quaty *et al.*, 2022).

Principal component analysis

PCA should be performed on the normalized data to compare the compositional patterns between sediment samples and identify the factors that influence each sample. Results of PCA analysis of this study can be seen in Table 6 and Fig. 2.The PCA has revealed two principal components with eigenvalues greater than 1, with a contribution of 83.63%. The first component (F1) contains 59.71% of Pb, Cu, sand, mud, clay, and TOC. The second component (F2) contains 23.92% of Cd. F1, which accounts for 59.708% of the total dispersion, has strong positive charges (>0.70) on Pb, Cu, sand, sludge, clay, and TOC. F2, which accounts for 23.915% of the total dispersion, has a strong positive charge on Cd. Cd is the most polluting heavy metal in Belawan Harbor water based on CF, Igeo, and PLI calculations. The correlation analysis has revealed that Cd has the lowest Pearson correlation with heavy metals and sediment fractions, TOC, and other heavy metals.

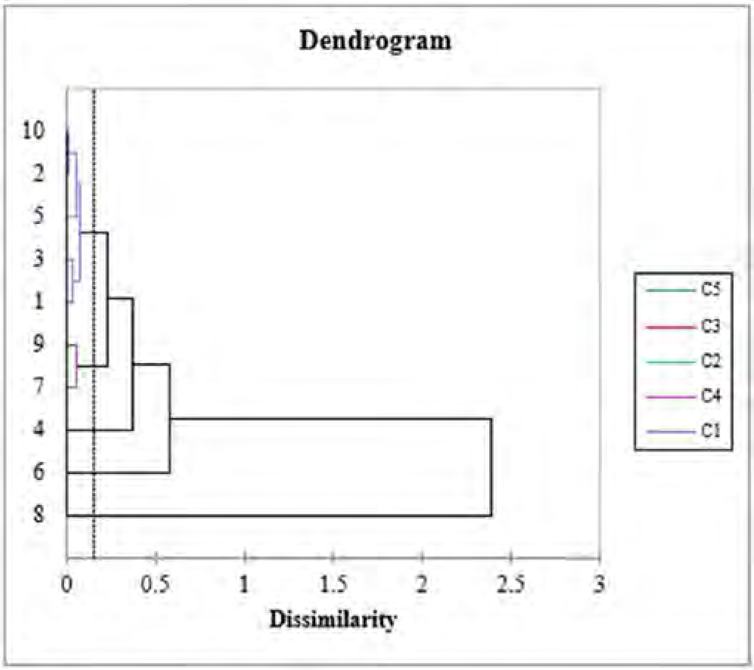

Fig. 3: Cluster analysis of observation sites

Thus, the environmental conditions for Cd and its compounds are found in many layers. Cd is known to be common in landfills, stormwater streams, and sewage (Wardani et al., 2018).

Cluster analysis

Similarity between data points is used as a criterion in the CA. The correlation between the sites is investigated using CA and the centroid linkage technique (Fig. 3). The spatial CA is shown in a dendrogram, where the 10 sediment sample locations have been partitioned into five groups.

Cluster 5 (Site 8) is located in a highly polluted area, which receives the most waste disposal. This finding is in accordance with the PLI results, which indicates that Site 8 is the most polluted area of the other sites. Cluster 4 (Sites 7 and 9) is where the two sites are at the same estuary. Cluster 3 (Site 6) is where the difference in clusters is a Pacific Ocean tourism area, and the tourism management here, in relation to waste management, is also still subpar. Cluster 2 (Site 4) is a harbor reclamation area, so the sand substrate in this area is extremely high compared with other sites. Finally, in Cluster 1 (Sites 1, 2, 3, 5, and 10), Sites 1, 2, 3, and 10 are in the same stream (*i.e.,* Belawan River), whereas Site 5 is in the sea area. Therefore, river flow influences sediment concentration in these waters.

CONCLUSIONS

The content of heavy metals in the sediments of Belawan Harbor varies; that is, essential heavy metal Cu does not contaminate the sediments. Conversely, non-essential heavy metals Cd and Pb contaminate them, but different from the concentration of Hg, which has a value greater than 0 but is undetected in the sediment. The content of heavy metals and TOC will be absorbed in the muddy sediment substrate rather than the sand substrate. Given that the smaller pores in the mud substrate are smaller, they can better trap heavy metals and TOC into the sediment. As a result, heavy metal pollution is often detected in estuary and coastal areas rather than the open sea, which has sand substrate. Harbor pollution levels are in the following order: Site 8 > Site 7 > Site 9 > Site 6 > Site 2 > Site 10 > Site 4 > Site 1 > Site 3 > Site 5. The highest pollution is at the mouth of the Deli River; this area is on the DLKp not the DLKr, which shows that harbor activities do not contribute significantly to heavy metal pollution in the waters. The high TOC in the DLKr area is due to changes in the river trajectory area. On the basis of the results of a study on heavy metal pollution in sediments, water pollution at Belawan Harbor is included in the category of lightly polluted levels. This study can be used as a reference for the government to make regulations or even wiser actions, especially related to the management of the aquatic environment in estuary areas. If not taken seriously, the pollution level will increase with the anthropogenic activities along the Belawan River and coastal watersheds every year. The long-term impact that can occur is that many endemic biota will disappear due to not being able to survive in an environment exposed to heavy metals. Thus, people who depend on their lives as fishermen will find it increasingly difficult to find fish, and social conflicts between the community and local companies will occur. Spatial planning in watersheds, especially in residential areas, might raise concerns by preventing the construction of settlements that face away from the river. Indonesia's Ministry of Environment Regulation No. 03 of 2010 concerns wastewater quality standards for industrial areas. If these regulations are obeyed by every company, then heavy metal pollution in waters can be reduced. The reason is several research results claim that the largest heavy metal contaminants come from industrial waste and residential areas. The need for periodic monitoring of all companies and the provision of communal WWTP in each settlement area is a considerably important concern for creating sustainable management of the aquatic environment. This research suggests Indonesia to have its own sediment quality standard guidelines to have a more accurate results of the analysis of pollution levels. Quality standards are made by the state with various aspects related to the country. The lower the value of the set quality standard, the more serious the pollution that can occur in the country. For future research, this study can be continued to measure the concentration of other heavy metals. In addition to Cd and Pb, the concentration of other heavy metals that mostly pollute waters can be investigated.

AUTHOR CONTRIBUTIONS

The corresponding author, L. Sulistyowati, helped with data analysis and writing. Y. Yolanda reviewed the relevant literature, gathered relevant data, and examined the extent to which water was polluted. N. Andareswari analyzed statistically the principal component analysis and cluster analysis.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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ABBREVIATIONS

%	Percent
^0C	Degree celsius
'E	East
'N	North
AAS	Atomic absorption spectrometry
ANZECC	Australia New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Reource Management Council of Australian and New Zealand

BPS	Badan Pusat Statistik: Central statistic agency in Indonesia
CCME	Canadian for Toxic Substance and Disease Registry
CA	Cluster analysis
Cd	Cadmium
CF	Contamination factor
cm	Centimeter
Cu	Copper
DLKr	Work Authority area
DLKp	Interest Authority area
EL	East longitude
Eq.	Equality
e.g.	Exempli gratia (for example)
F	Component of principal component analysis
GPS	Global positioning system
ha	Hectare
$HClO_4$	Perchloric acid
Hg	Mercury
HNO_3	Nitric acid
i.e.	Id est (that is)
Igeo	Geoaccumulation index analysis
ISQG	Interim Sediment Quality Guidelines
Max.	Maximum
mg/kg	Milligram per kilogram dry wight
Min.	Minimum
mL	Mililiter
NL	North latitude
p	Significance value
PAH	Polycystic aromatic hydrocarbons
ppb	part per billion
Pb	Lead
PCA	Principal component analysis
PEL	Probable effect levels
PLI	Pollution load index
r	Pearson's correlation coefficient
Site	Station sampling
SD	Standard deviation
TOC	Total organic carbon
TN	Total nitrogen
USEPA	United States Environmental Protection Agency
WWTP	Wastewater treatment plants

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CASE STUDY

The influences of environmental awareness on green performance

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ABSTRACT

BACKGROUND AND OBJECTIVES: Despite the various environmental challenges the hotel sector faces, there is a significant need to resolve them by applying sustainable techniques like green human resource management. As a result, there is a pressing need to investigate how green human resource management may improve environmental performance. This study investigates the causal linkage among environmental awareness, green human resource management, green behaviours, green performance, and servant leadership.
METHODS: The data were collected via Questionnaires obtained from employees working in hotels in Amman, Jordan (52.5 per cent response rate). To evaluate the model, Smart Partial Least Square was employed to conduct validity and reliability testing and develop structural equation modeling.
FINDINGS: According to the results, Environmental awareness mediated the effects of green human resource management and green behaviours on green performance. Green HRM was found to have a direct and substantial impact on Green Performance ($\beta = 0.109$, $p < 0.005$). A positive and statistically significant relationship between Green Behaviors and Green Performance was also found ($\beta = 0.338$, $p < 0.000$). Additionally, Green HRM has a favorable and significant effect on Environmental Awareness ($\beta = 0.176$, $p < 0.0001$). Furthermore, Environmental Awareness is positively and significantly influenced by Green Behaviors ($\beta = 0.743$, $p < 0.000$). Green performance positively relates to environmental consciousness ($\beta = 0.186$, $p < 0.000$).
CONCLUSION: Based on social cognition, social exchange, and social learning theory, this study contains theoretical insights, practical implications, and positive recommendations for hospitality managers and scholars. Current research is critical because it emphasizes environmental stewardship in industries that directly connect to and influence the environment, such as tourism and hospitality. The study does not examine environmental performance and behavior in general but instead evaluates pro-environmental behaviors in depth by considering green behaviour.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
69	2	4

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INTRODUCTION

Many countries have experienced substantial environmental challenges as a result of industrial expansion. Climate change, depleted natural resources, and pollution is only some environmental problems that may be traced back to tourist activity. The entire environment is threatened by these industrial activities and their negative impacts. For example, fresh drinking water is a basic requirement of growing human civilization and a major challenge in many developing countries. As living standards improve and the world population expands (Moradi *et al.,* 2023).This demand increased public awareness of environmental or green issues, such as energy conservation and renewable energy sources (Darvishmotevali and Altinay, 2022). Future perspectives based on the impact of COVID-19 on the solar energy industry can focus on solar energy efficiency measures; these challenges should be addressed less as a technology optimization challenge and more as an organization skills (behaviors) test and political commitment (Kamyab *et al.,* 2022). Due to increased commercial competition, companies compete to supply consumers with appealing value, distinctiveness, and novelty. Globalization and environmental challenges have always been major themes in society. One of the company's values is environmentally-based business management, which is focused on improving the company's performance. There is a growing need to understand better and shape employee behaviour to reduce their work's negative environmental implications (Islam *et al.,* 2020). According to experts and environmental policymakers, the causes of environmental degradation, such as resource shortages, increased pollution, and biodiversity loss, are deeply ingrained in human behaviour. Environmentally conscious business practices can be found at all levels of the organisation, from the employee to the strategy. It will promote the development of green workplace behaviour and environmental awareness, resulting in improved green performance. Selection, training, empowerment, and performance are all covered by green human resource management (HRM) (Ahmad *et al.,* 2021). Green HRM is the process of hiring, educating, rewarding, and creating a green workforce that incorporates environmental awareness throughout the entire HRM process. Green HRM and its role in promoting green employee behaviour in the workplace has become a research issue (Islam *et al*., 2021). Employees' pro-environmental behaviours are becoming increasingly important in all organisations, including tourism. Studies on Green HRM have concentrated more on the corporate sector than the tourism sector. Jordan, which is more prone to pollution and environmental degradation, has paid less attention to environmental management. Researchers in the fields of tourism and hospitality have studied a variety of topics related to "green" human resource management, including "green" policies and practices, "green" innovation, "green" attitudes, and "green" outcomes (Ali *et al.,* 2021). Technology and innovation can help businesses lessen their negative effects on the environment, increase their efficiency with scarce resources, and foster a culture of sustainability. Businesses can increase their eco-awareness and green performance by implementing sustainable technology and practices and partnering with other organisations (Xie *et al.,* 2019). Several researchers specialising in this field have contributed to these studies (Ababneh, 2021; Alkhodary, 2022; Farooq *et al.,* 2022). "Green HRM" refers to an increasingly important aspect of HR management: environmental consciousness. Ultimately, green HRM aims to foster a more environmentally conscious work environment. To achieve success, businesses need green management practices, which include informing workers about environmental objectives and finding ways to turn those objectives into marketable asset. Management of this kind emphasizes environmental responsibility and collaboration among employees. (Mohanty *et al.,* 2021). Servant leadership attributes include self-sacrifice and a greater likelihood of instilling a feeling of community engagement among personnel (Alafeshat and Tanova, 2019). None of the previous studies on the effects of servant leadership on behaviour and attitude examined the effect on environmentally friendly output (Afeshat, 2019). When it comes to promoting a sustainable and environmentally friendly workplace, Green HRM, Green Behaviors, and Green Performance are all interconnected. Furthermore, servant leadership can help foster a sustainability culture within an organisation. Green HRM refers to an organization's policies and practices that promote sustainability and reduce environmental impact. Green Behaviors are employee actions that contribute to sustainability goals, such as turning off lights and electronics when not in use and reducing paper consumption. Green Performance is the overall environmental impact of an organisation, including its carbon footprint, waste generation, and use of natural resources. On the other hand, servant leadership emphasises the importance of putting employees' needs first and giving them the authority to make decisions that benefit the organisation as a whole. Green HRM practises when combined, can help promote green behaviours among employees, which can lead to improved green performance. Furthermore, servant leadership can aid in developing a sustainable culture within the organisation, where employees feel empowered and encouraged to contribute to environmental goals. Organisations can achieve their sustainability goals and promote a greener environment by combining green HRM, green behaviours, and servant leadership. This study analyses how green HRM, environmental awareness, green behaviours, and servant leadership interact to close this gap. Human resource management, green practices, green behaviour, and environmental management challenges have all been identified as essential aspects in the greening of organisations. Theoretically, this research will contribute to eco-friendly performance knowledge and illuminate these topics. Furthermore, social interaction, cognition, and learning theories are used to generate hypotheses for investigating the direct and indirect channels through which green HRM, environmental consciousness, and environmentally responsible habits influence green performance. Additionally, this study will provide light on an understudied topic and, as a result, underappreciated: the effects of green HRM in hotels in developing countries. The current study intends to learn if and how green HRM and green behaviors might boost green performance. This study aims to investigate how green human resource management and green behaviours improve green performance and investigates the causal linkage among environmental awareness, green human resource management, green behaviours, green performance, and servant leadership. This study was carried out in a five-star hotel in Amman, Jordan, in 2022.

Theoretical framework and hypothesis

Individual behaviour is validated using social cognitive theory (SCT), a widely accepted model (Hagger *et al.,* 2020). Environmental effects, personal characteristics, and behaviour are all bidirectionally influencing determinants in the social cognitive model (Brown and Lent, 2019). One of the most popular conceptual models for analysing workplace behaviour is a social exchange concerned with intrinsic rewards (Naz *et al.,* 2021). It varies from economic trade, however, in that the former implies vague duties, the benefits do not have a precise value in the perspective of a particular quantitative medium of exchange, and the character of the exchange cannot be negotiated for (Nisar *et al.,* 2021). Moreover, corporate social responsibility (CSR) can aid firms in spreading green practices and boosting green performance. To help ensure a more sustainable future, businesses should make CSR one of their guiding principles by emphasizing environmental responsibility, transparency and accountability, stakeholder engagement, reputation enhancement, innovation, and collaboration. As a result, only social exchange theory tends to elicit feelings of personal responsibility, gratitude, and trust. According to social exchange theory, social behaviour is the product of an exchange process (Aboramadan, 2020). It could be the most effective technique to comprehend effort–reward relationships and workplace fairness. Because failures in fairness, i.e. injustice, lead to theft, sabotage, and even violence, moral considerations in workplace fairness are critical. Following the ability motivation opportunity (AMO) theory, high employee performance requires an organisation to improve employees' abilities, motivate employees' engagement, and provide appropriate possibilities for employee involvement (Yang *et al.,* 2018). Green HRM, on the other hand, isn't always enough to give businesses a green competitive edge. Other organisational environments may need interaction to increase employees' competencies, motivation, and green creativity chances (Zhu *et al.,* 2021). HRM is an approach to human resources to help a company obtain an advantage in the marketplace. In human resources, "green HRM" refers to policies and practices that help a business while also seeking to counteract the negative consequences of anti-environmental workplace behaviour (Islam *et al.,* 2020). An important part of Green HRM is teaching workers to take ecologically friendly measures to increase their environmental knowledge, efficiency, involvement, and performance (Ababneh, 2021). Green HRM is largely seen as one of the most successful ways to help organisations achieve ecologically friendly operations (Ali *et al.,* 2021). Green HRM is a relatively unexplored area (Afeshat, 2019). Ecologically conscious employees can benefit from green HRM practices such as employee training and empowerment and an environmentally conscious corporate culture (Ahmad *et al.,* 2021). When it comes to establishing workplace sustainability programmes, green behaviours, which are those that are designed to lessen a person's negative influence on the natural and built world, can be a useful method (Ababneh, 2021). Examples include recycling, decreasing rubbish, conserving water, and lowering energy consumption, which lessens human activity's impact. (Francoeur and Paillé, 2022). There are two types of environmental activities in this category: those focused on completing a specific task and those proactive (Francoeur *et al.,* 2021). "Task-related Pro-Environmental Performance" refers to behaviours that are explicitly enforced by the firm and expressed in terms of employee responsibilities (Rubel *et al.,* 2021). Task-related Pro-Environmental Performance refers to employees who do their work ecologically sustainably. Also, it refers to an employee's readiness to go above and beyond their job duties regarding green behaviour, known as "Proactive Pro-Environmental Performance (P-EP)". Due to personal involvement in dealing with unexpected situations rather than a workplace or job definitions, this type of behaviour occurs (Soomro *et al.,* 2021). Efforts to improve the environment are critical in this study because they necessitate applying environmental recommendations, implementing essential adjustments, and detecting and resolving environmental issues (Azhar and Yang, 2021). Psychological variables influence people's willingness to engage in environmentally friendly activities, attitudes, and behaviours (Ahmed *et al.,* 2021). This person participates in various activities and has strong environmental ideas and values (Kousar *et al.,* 2022). Environmental awareness and associated challenges have resulted in a greater understanding of the significance of environmental conservation for human well-being. Reduce, reuse, recycle, and rethink are the four R's of environmental awareness (Rotaris *et al.,* 2021). Environmental awareness refers to the understanding that our planet is delicate and that it is imperative to protect it. Understanding environmental issues is essential to promoting environmentally-friendly behaviour and performance, which can be achieved through education. HRM is an essential component of this investigation because it creates awareness and greening the company and society while also addressing the underlying issues of sustainability and environmental awareness (Darvishmotevali and Altinay, 2022). In conclusion, Servant leadership emphasises serving individuals rather than leaders serving people (Gui *et al.,* 2021). For those who believe in "Servant Leadership," an unselfish disposition, a willingness to serve others, and an openness to new experiences are the characteristics of a servant leader (Ozturk *et al.,* 2021). Servant leaders strive to instil social responsibility in their subordinates through their deeds (Khan *et al.,* 2021). It is one of the study's primary constructs because of its uniqueness and ability to better explain a wide range of outcomes than other leadership styles (Li *et al.*, 2021).

Green HRM, green behaviors, environmental awareness, and green performance

The ability-motivation-opportunity hypothesis, the most prevalent paradigm in understanding the impact of HRM practises on organisational performance in empirical studies, can help better understand the greening of HRM and the resulting environmental impacts (Darban *et al.,* 2022; Haldorai *et al.,* 2022). In organisations, environmental awareness and green performance are inextricably linked. Environmental awareness is defined as the understanding the impact of human activities on environment and the need to mitigate that impact. In contrast, green performance refers to an organization's ability to implement environmentally sustainable practices and achieve positive environmental outcomes. Ability, motivation, and opportunity are the three main components that make up high-performance job practice, according to the AMO theory (Muisyo and Qin, 2021). There are numerous ways for a company to raise environmental awareness and improve its green performance. Perform an environmental audit, Create an environmental policy. Provide environmental education, Implement environmentally friendly practices. Environmental performance should be measured and reported on. Engage stakeholders and invest in green technologies (Gadenne *el al.,* 2009). Employee participation can be encouraged through involvement, information exchange, and autonomy-enhancing behaviours, all elements of opportunity (Naz *et al.,* 2021). As stated by Makhloufi *et al.* (2021) in their AMO paradigm, HRM practises that enhance employees' talents, passion for working, and involvement in available possibilities contribute to organisational success. As a result, high-performance work systems (HPWS) and organisational performance are linked via employee organisational citizenship behaviours (Gill *et al.,* 2021). Researchers have used AMO theory to examine Green HRM in various businesses (Afeshat, 2019; Ababneh, 2021; Ali *et al.,* 2021; Darvishmotevali and Altinay, 2022; Farooq *et al.,* 2022). People's ability to choose, implement, and control their own actions to accomplish desired outcomes is affected by external variables. From a socio-cognitive perspective, people are flexible and responsive to their environment (Naz *et al.,* 2021). For employees to be more engaged in environmental issues, it is vital for them to better appreciate their environment's value and their significant role in maintaining it. SCT suggests that Green HRM indirectly impacts employee environmental behaviour by raising environmental awareness. Employees are encouraged to develop their environmental knowledge and abilities to assist the organisation in achieving its objectives more successfully through Green HRM. Consequently, the study hypothesis is:

H1: Green HRM positively and significantly influence Green Performance

H2: Green Behaviors positively and significantly influence Green Performance

H3: Green HRM positively and significantly influence Environmental Awareness

H4: Green Behaviors positively and significantly influence Environmental Awareness

H5: Environmental Awareness positively and significantly influence Green Performance

Environmental awareness as mediator

Employees' awareness of the environment and motivation to enhance green performance are boosted (Ahmed *et al.,* 2021; Song *et al.,* 2019; Kousar *et al.,* 2022). Environmental awareness will be encouraged in the company's numerous operations, according to Galli *et al.* (2020), by hiring and training people who are ecologically concerned. As a result of these initiatives, employees' behaviour is more ecologically friendly. Environmentally responsible tasks that increase the organisation's environmental performance are rewarded to employees (Yang *et al.,* 2021). Employees actively work to lessen human activity's negative influence on the environment or to enhance its quality. A study has found that employees who are well-informed on environmental issues and problems tend to take measures to protect the environment; the goal of green human resources management is to raise employees' environmental consciousness and commitment through education and outreach (Rotaris *et al.,* 2021). Green HRM's main objective is to teach workers about the many facets of environmental management, such as why it's important, what they can do to help, and how things get done (Islam *et al.,* 2020). Human resource managers should provide employees with green systems and environmental protection training programmes (Farooq *et al.* (2022). For workers to gain knowledge of environmental legislation and appreciate the importance of doing their part to safeguard the environment, inspiring them to act in P-EB design. According to Rustam *et al.* (2020), environmental knowledge is so critical that lacking it could lead to Task-related Pro-Environmental Performance avoidance. Environment-friendly behaviour becomes second nature and part of the daily routine when employees have access to information about environmental challenges at work and are informed. Though it is unclear exactly how green HRM might help raise Proactive P-EP, it appears that there has been insufficient study linking PPE among environmentally aware employees (Rubel *et al.,* 2021). One sector where these concerns might be examined is the hospitality industry. As a result, green HRM is thought to increase workers' awareness of environmental issues, boosting their task-related and proactive Pro-Environmental Performance at work. As a result, the following hypotheses were advanced in this investigation:

H6: Environmental awareness mediated the influence of green HRM on green performance

H7: Environmental awareness mediated the influence of green behaviors on green Performance

Servant leadership, as a moderator

Servant leadership is a powerful motivator because it may supply employees with critical tools and information, such as professional development resources and educational possibilities (Darvishmotevali and Altinay, 2022). Social learning, which emphasises the role of leaders in an organization, improves performance by encouraging workers to work together more productively and retain more of the information they are exposed to. The importance of leaders in every organization is one of the main reasons for the development of the idea of social learning. In other words, servant-leaders aid their teams in acquiring and retaining the skills they need to perform their jobs successfully and efficiently (Divya and Suganthi, 2018). As a servant leader, it is your responsibility to help those under you develop professionally by giving them access to resources and making them aware of opportunities that could benefit them and the organization as a whole (Gnankob *et al.,* 2022). Suppose the company's values depend on a certain type of performance, like eco-performance. In that case, the company's service leader will surely take steps to educate personnel on green performance concepts and environmental practices. Inspiring and convincing others to work together toward a single goal is a hallmark of effective leadership. Instead of being based on rank or seniority, leaders are chosen by their ability to influence others. As a result of its correlation to many measures of employee performance, this issue is of paramount importance to the business's success (Divya and Suganthi, 2018; Kiker *et al.,* 2019; Darvishmotevali and Altinay, 2022). Servant leadership is one of the most effective leadership styles, which has been extensively researched. Servant leadership has been shown to positively affect followers' attitudes and behaviours, such as psychological empowerment (Azila-Gbettor, 2022), organisational commitment (Irfan *et al.,* 2021), work engagement (Ozturk *et al.,* 2021), job satisfaction (Rivaldo *et al.* 2021) and organisational citizenship behaviour (Gnankob *et al.,* 2022). Serving leaders help their followers realise their full potential, enable them to engage in service-oriented behaviours in the workplace, and allow their followers to watch and mimic the leader's behaviours through role-modeling processes, according to social learning theory (Bentein *et al.,* 2022). By employing a reward and punishment system to reinforce desired behaviours, servant leaders can motivate people and get the organisation closer to its goals (Kauppila *et al.,* 2022). Various methods, such as surveys, interviews, and behavior observation, can be used to assess servant leadership. However, each servant leader's behavior can vary depending on their characteristics and the context in which they operate. One approach to linking servant leadership abilities to environmental sustainability is to identify specific behaviors and actions associated with servant leadership and aligned with environmental sustainability. Some servant leadership characteristics that may be relevant in a green context include, for example, empathy, listening, foresight, and stewardship (Ying *et al*., 2020). The effectiveness of servant leadership in promoting ecological sustainability can also be gauged by direct observation of its workers. A researcher might follow a leader to see how they handle input from employees and other stakeholders, how they make decisions, and whether or not they value environmental sustainability (Reed *et al*., 2011). However, despite the relevance of green behaviour in organisations and some scholars' works on environmentally specific servant leadership (Li *et al.,* 2021; Neubert *et al.,* 2021). Therefore, this study proposed the following hypotheses:

H8: Servant leadership moderated the influence of green HRM on green performance

H9: Servant leadership moderated the influence of green behaviors on green performance

MATERIALS AND METHODS

This study was conducted in five-star hotels in Amman, Jordan. Because Amman is one of Jordan's most visited cities, hotels in the city were picked for the sample—Jordan's financial, tourist, and cultural hub in Amman. The city's five-star hotels attract international tourists. Looking at the numbers, Jordan has come a long way towards implementing environmentally friendly business practices. With an eye towards sustainable development, Jordan has enacted several environmental laws and regulations, including greening the economy, environmental monitoring and related activities, public engagement, and several training and education initiatives. Jordan is trying to include environmental considerations in its energy, industrial, agricultural, and health programs. Additionally, its attempts to adapt to and mitigate climate change and its participation in international processes are important. Amman (Jordan's capital) is the country's most important scientific, cultural, and financial centre, with a population of 2.21 million at the start of 2022. The top ten five-star hotels were chosen. With approval from human resource departments, questionnaires were distributed to available employees. Respondents were required to complete and return the questionnaire to the person in charge. Staff were given 600 surveys in person, and 350 questionnaires were returned, 315 of which were legitimate (52.5 per cent response rate). Respondents were asked to answer all questions on a five-point Likert scale. Consistent Partial Least Squares Structural Equation (CPLSSE) bootstrapping was used to test the hypotheses' causal linkages. Partial Least Square Structural Equation (PLS-SEM) is a multivariate statistical method for modelling connections between latent (unseen) constructs and the observable variables that act as proxy measures of those constructs. Latent variable measurement model, structural model and direction of these relationships, model fit, bootstrap resampling, and cross-validation are just some of the statistical components that make up PLS-SEM. Publications employing PLS-SEM have seen a dramatic uptick in recent years. Organizational management (Sosik *et al.,* 2009), marketing management (Bernarto *et al.,* 2020), and human resource management (Fawehinmi *et al.,* 2020) are just a few examples of the many social science fields where SEM is now routinely utilized. Fig. 1 illustrates the research model, demonstrating a structural analysis of the study constructs (green HRM, environmental awareness, servant leadership, green behaviors , and green performance) (Hair *el al.,* 2017).

RESULTS AND DISCUSSION

Measurement model

The construct validity and reliability were assessed using the Partial Least Square (PLS) structural equation model. The Cronbach's alpha coefficient and composite reliability were employed to certify and verify item dependability and a standardised item loading greater than 0.7. According to the findings, both alpha and composite reliability exceeded 0.7. (Memon *et al.,* 2021). Cronbach's alpha (α) and composite reliability (CR) were determined to be high, ranging from 0.81 to 0.96 were above 0.70, which meets the minimum requirement of 0.70 (Table 1). The construct validity was checked using convergent and discriminant methods. Convergent validity was assumed for items with standardised loadings of at least 0.7 and average variance extracted (AVE) values for the research variable of at least 0.5. The factor loading, AVE, and CR were used to assess the convergent validity. The CR and AVE values of the constructs were 0.938 and 0.580 for green HRM, 0.957 and 0.585 for green Behaviors (Gb), 0.952 and 0.689 for green performance (GP), 0.916 and 0.687 for Environmental awareness (EA), and 0.860 and 0.553 for Servant leadership (SL). As

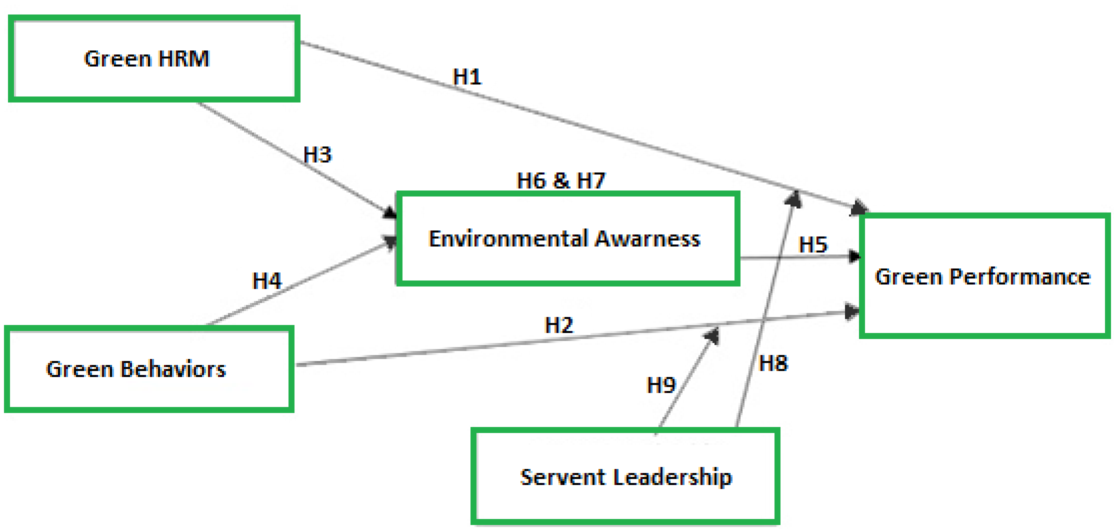

Fig. 1: Proposed moderating model of servant leadership

shown in Table 2, all factor loadings were significant, with AVE values above 0.5 and all the CR values above 0.7, as shown in Table 1. Hence, the two conditions for convergent validity were satisfied. Cross-loadings show where items of one reflective construct should load higher than items of other reflective constructs, as shown in Table 2. Fornell and Lacker's discriminant validity could be evaluated using the square root (√AVE) of average AVE >= association between constructs shows that the square root of the correlation between two measures of a construct is far larger than the correlation between the construct

Table 1: Measurement model

	Items	Factor loading	Cronbach Alpha	CR	AVE
Green HRM					
	GHRM1	0.744	0.927	0.938	0.580
	GHRM2	0.811			
	GHRM3	0.824			
	GHRM4	0.746			
	GHRM5	0.762			
	GHRM6	0.767			
	GHRM7	0.849			
	GHRM8	0.773			
	GHRM9	0.740			
	GHRM10	0.786			
	GHRM11	0.794			
	GHRM12	0.773			
Green Behaviors					
	GB1	0.773	0.952	0.957	0.585
	GB2	0.83			
	GB3	0.809			
	GB4	0.794			
	GB5	0.821			
	GB6	0.772			
	GB7	0.731			
	GB8	0.774			
	GB9	0.788			
	GB10	0.805			
	GB11	0.763			
	GB12	0.794			
Green performance					
	GP1	0.831	0.943	0.952	0.689
	GP2	0.872			
	GP3	0.873			
	GP4	0.843			
	GP5	0.717			
	GP6	0.790			
	GP7	0.854			
	GP8	0.836			
	GP9	0.845			
Environmental awareness					
	EA1	0.810	0.886	0.916	0.687
	EA2	0.869			
	EA3	0.808			
	EA4	0.834			
	EA5	0.822			
Servant leadership					
	SL1	0.796	0.807	0.860	0.553
	SL2	0.718			
	SL3	0.697			
	SL4	0.794			
	SL5	0.805			

and any other construct value below, as shown in bold in Table 2, indicating that all constructs share more variance with their own measurements than they do with any other constructs. Every one of the three requirements for construct validity has been satisfied. And as shown In Table 3, a complete list of all cross-loading values employed by reflective measurement model frameworks' metrics. Table 4 shows that each indicator (scale item) has a higher loading on its underlying latent construct in reflective measurement models than any other construct. Heterotrait-Monotrait correlation ratios (HTMTs) of less than 0.85 were used to assess if factors were distinct, as Seidu *et al.* (2022) recommended. Each HTMT value was less than 0.85, and Table 1's cross-loading results demonstrated that individual measurement items loaded more heavily on their latent constructs than on any other latent constructs. Anjum *et al.* (2020) claim that the PLS assessment model's convergent and discriminant validity would suffer if three personality traits were included.

Testing of hypothesis

The results of this study are shown in Table 4, where green HRM was found to have a direct and substantial effect on Green Performance (β = 0.109, p < 0.005). This provided further evidence in favor of hypothesis 1. A positive and statistically significant relationship between green behaviors and green performance was also found (β = 0.338, p < 0.000). Hence, H2 was confirmed. Additionally, green HRM has a favorable and significant effect on Environmental Awareness (β = 0.176, p < 0.0001). This led to a confirmation of H3. Furthermore, Environmental Awareness is positively and significantly influenced by Green Behaviors (β = 0.743, p < 0.000). Hence, H4 was accepted. Green performance positively relates to environmental consciousness (β = 0.186, p < 0.000). As a result, we can confirm H5. Lastly, Cohen *et al.* (2003) method was used to elucidate the moderating influence in this investigation. Table 4 shows that the data indicate that servant leadership is a negative moderator of the relationship between Green HRM and Green

Table 2: Discriminants validity

Variable	Environmental awareness	Green behaviors	Green HRM	Green performance	Servant leadership
Environmental awareness	**0.829**				
Green behaviors	0.672	**0.765**			
Green HRM	0.521	0.533	**0.762**		
Green performance	0.604	0.612	0.615	**0.830**	
Servant leadership	0.634	0.515	0.625	0.692	**0.744**

Table 3: Hetromonotrait

Variable	Environmental awareness	Green behaviors	Green HRM	Green performance
Environmental awareness	-			
Green behaviors	0.95	-		
Green HRM	0.797	0.783	-	
Green performance	0.875	0.863	0.765	-
Servant leadership	0.827	0.78	0.684	0.856

Table 4: Path co-efficient

Variable	Co-efficient	T-statistics	P-values
Green HRM -> Green Performance	0.109	2.99	0.003
Green Behaviors -> Green Performance	0.338	7.197	0.000
Green HRM -> Environmental Awareness	0.176	6.387	0.000
Green Behaviors -> Environmental Awareness	0.743	29.221	0.000
Environmental Awareness -> Green Performance	0.186	4.166	0.000
GHRM*SL -> Green Performance	-0.117	3.024	0.003
GB*SL -> Green Performance	0.118	2.82	0.005

Performance. Meanwhile, the connection between green actions and results is controlled in a favorable way by servant-oriented management. Hence, both H6 and H7 are correct.

Even though servant leadership acts as a negative moderator of the relationship between green HRM and green performance. Servant leadership can help an organisation foster a culture of sustainability in which employees feel empowered and encouraged to contribute to environmental goals. As a result, servant leadership rarely has a negative impact on the relationship between green HRM and green performance. The relationship between green actions and results, which can be positively controlled by servant-oriented management, can be used to evaluate the green environment. Organizations can better understand their environmental impact and identify areas for improvement by tracking green actions and measuring their impact on green performance. Servant-oriented management can aid in these efforts by empowering employees to take ownership of sustainability initiatives and providing the resources and support required to meet sustainability targets. Servant-oriented leadership can help an organisation promote sustainability and improve its green performance. Furthermore, for mediating effect, this study used the variance accounted for (VAF) as recommended by (Mahmud *et al.* 2022). The formula for computing VAF using Eqs. 1 and 2 (Hair *et al.,* 2014). The mediating effect of environmental awareness on the relationship between green HRM and green performance.

$$\mathrm{VAF} = \frac{\text{The size of the indirect effect}}{\text{The Total Effect}\left(\text{Indirect effect} + \text{Direct effect}\right)} \tag{1}$$

$$\mathrm{VAF} = \frac{0.176}{0.176 + 0.109} = \frac{0.176}{0.285} = 0.618 = 62\% \tag{2}$$

The mediating effect of environmental awareness on the relationship between green behaviors and green performance, using Eq. 3 (Hair *et al.,* 2014).

$$\mathrm{VAF} = \frac{0.743}{0.743 + 0.338} = \frac{0.743}{1.081} = 0.687 = 69\% \tag{3}$$

Consequently, 62 and 69 % of the relationship between Green HRM, Green Behaviors, and Green Performance can be explained by the mediating effect of Environmental Awareness. Because the VAF was less than 80%, the mediating effect can be characterized as partial mediation. Fig. 2 displayed the path coefficient analysis of the study graphically.

In terms of green HRM, green behaviors, and green performance correlations in the Amman hotel sector, this study examined the mediating and the moderating role of environmental awareness and servant leadership. This means that the results back up each of the working hypotheses. All of the research's hypotheses about the link between green HRM and green performance in the workplace were confirmed. Knowledge of the advantages and disadvantages of green practices increases the probability that workers will demonstrate awareness of green on the job. Therefore, green HRM practices influence Green Performance-friendly behaviors and strengthen businesses' environmental productivity initiatives. This study's findings corroborated those of Darban *et al.* (2022), who proposed that environmental productivity policies and activities promote environmentally aware, resource-efficient, and socially responsible business practices and encourage individuals to adopt ecological worldviews on the job (Ababneh, 2021; Ali *et al.,* 2021; Farooq *et al.,* 2022). The results of this study align with those of other research that has shown that an organisation's green HRM policies can affect how its personnel carry out their daily duties (Azhar and Yang, 2021; Rubel *et al.,* 2021; Francoeur *et al.,* 2021). Worker participation in the company's environmental initiatives is confirmed (Afeshat, 2019). Prior research has linked green HRM to Green Performance strongly and transparently. By offering a shared environmental vision, purpose, and target and teaching workers about environmental issues, "green HRM" fosters a more inclusive and productive workforce. According to the social learning theory, servant leaders enable their followers to develop to the fullest extent possible. Managers in these positions are in a place to encourage employee compliance with business policies. Although green HRM and servant leadership have a positive and statistically significant interaction effect on green performance and employee environmental consciousness, the results show that this effect is smaller than the sum of the individual effects of the two elements. Consequently, while servant leaders and green HRM strongly supported the study sample, the interaction effect was only partially supported. This shows that while servant

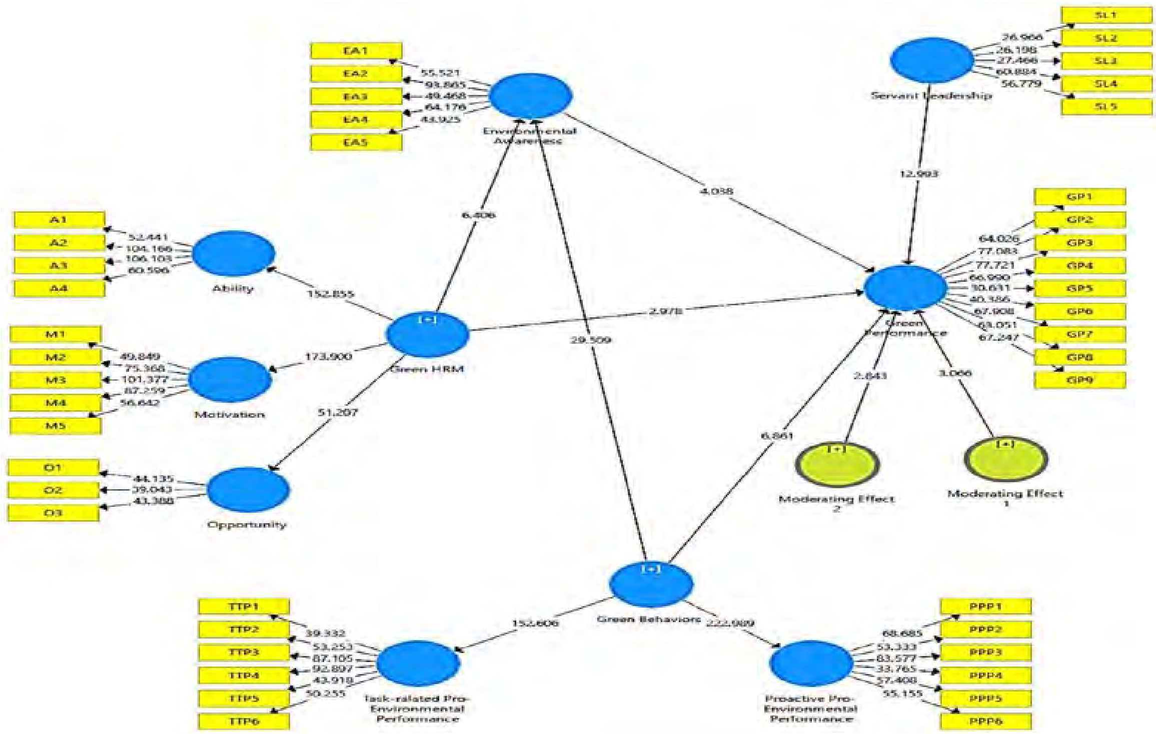

Fig. 2: Path coefficient

leaders may help employees in numerous ways to achieve their tasks, proactive green behaviour or environmental awareness does not. Servant leaders can provide an example for their followers by "doing and showing," or they can offer moral support and encouragement to persuade people to adopt environmentally friendly activities. This study adds to the growing body of literature on the importance of environmentally conscious servant leadership in boosting green performance in the hospitality sector (Tuan, 2020). Additionally, government regulations and policies can significantly influence environmental awareness and green performance in organisations. Governments can help create a more sustainable future by setting environmental performance standards, providing incentives for sustainability, encouraging transparency and reporting, promoting innovation and research, and raising public awareness (Ramanathan *el al.,* 2017). Instead of looking at environmental performance and overall behaviour, this study assesses green behaviors in detail. Alzubaidi *et al.* (2021) and Kim and Stepchenkova (2020) are just a few of the latest experimental researchers to find similar results about the environmental behaviors of employees. Secondly, the SCT research model uses environmental consciousness to explain the link between green HRM and P-EP (Bandura, 2001). In addition, the authors of this study aimed to fill a knowledge gap by investigating a potential mediator of the relationship between green HRM and environmental consciousness: servant leadership (Tian *et al.,* 2020).

CONCLUSION

The findings of this study will have a considerable impact on HRM literature in general and hospitality HRM literature in particular. This study is the first to focus on hotel performance rather than staff performance, as has recently happened in the hospitality management research stream. Current research is critical because it emphasizes environmental stewardship in industries that directly connect to and influence the environment, such as tourism and hospitality. The significance of environmental consciousness strengthens SCT's tenets as a mediating factor in the relationship between green HRM and green performance. Third, we used SLT to describe how servant leadership acts as a moderator in the model. This study found that employees' tendencies to focus on green behaviors above and beyond only doing their job can be influenced by servant leadership. Employees can contribute significantly to increasing environmental awareness and green performance in organisations. Employees can help create a culture of sustainability within the organisation and contribute to a more sustainable future by implementing sustainable practices, participating in training and awareness programmes, providing feedback and suggestions, advocating for sustainability, participating in green initiatives, and taking ownership of sustainability. In sum, the findings of this study provide empirical evidence for thriving green performance in the hospitality sector by analyzing the effect green-oriented businesses have on encouraging individuals to adopt environmentally friendly practices. Also, it demonstrates the roles played by organizations and individuals in constructing P-EP. First investigations on green performance centred on standard HRM procedures. Most effective strategies can assist organisations in measuring and evaluating their environmental impact, tracking their progress, and identifying areas for improvement. Organizations can improve their green performance and contribute to a more sustainable future by implementing effective measurement and evaluation strategies. Consumer behaviour can be essential in increasing environmental awareness and business green performance. Consumers can encourage companies to prioritise sustainability and contribute to a more sustainable future by demanding sustainable products, pressuring for transparency, influencing supply chains, and impacting reputation. Second, additional factors, such as a green mentality and empowerment, can be incorporated into the current findings to explain mediation better. To further strengthen the connection between green HRM and P-EP, additional study of moderating factors, such as intrinsic rewards and supervisors' personality traits, is advised. Instead of doing a single large-scale quantitative study, it is recommended that researchers use qualitative methods or a mixed-methods approach that incorporates a time lag in the data collection process. Managing the root causes of the green environment will necessitate a commitment to sustainability and a willingness to invest in new technologies and practices. Collaboration with stakeholders is also required, as is a focus on measuring and reporting environmental performance.

Lack of Environmental Management Systems, for example (EMS), Organizations may not have a comprehensive approach to managing their environmental impact if they do not have a formal EMS in place. Unnecessary waste and pollution can result from poor resource management, such as managing energy, water, and materials. This will allow them to see where to make changes, establish goals, and measure their success as they work towards a more sustainable future. Environmental consciousness is one of the most important links connecting Green HRM, Green Behaviors, and Green Performance. To achieve true sustainability, businesses must adopt a holistic strategy incorporating Green HRM, Green Behaviors, and Green Performance. This can be done by raising environmental awareness, aligning HR policies and procedures with sustainability goals, and encouraging employees to engage in green activities. Lastly, this research is the first to examine green human resource management in Jordan. The study focuses on green HRM strategies and outcomes for the hospitality industry in Jordan. Important and novel discoveries were made Further research on these variables in the same context is needed to allow for the widest possible generalization of the findings.

AUTHOR CONTRIBUTIONS

M.H Mansour, the corresponding author, has conducted the conception and design, acquisition, analysis and interpretation of data, drafting and critical revision of the manuscript and statistical analysis.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/

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ABBREVIATIONS

%	Percent
AMO	The ability-motivation-opportunity
AVE	Average variance extracted
B	*Beta*
Co-efficient	Correlation coefficient
CPLSSE	Consistent partial least squares structural equation
CR	Composite reliability
CSR	Corporate social responsibility
EA	Environmental awareness
Fig.	Figure
*GB*SL*	Green behavior*Servant leadership
GHRM	*Green* human resource management
*GHRM*SL*	Green human resource management*Servant leadership
GP	Green performance
H1	First hypothesis
H2	Second hypothesis
H3	Third hypothesis
H4	Fourth hypothesis
H5	Fifth hypothesis
H6	Sixth hypothesis
H7	Seventh hypothesis
H8	The eighth hypothesis
H9	The ninth hypothesis
HPWS	high performance work systems
HR	Human resource
HRM	Human resource management
HTMT	Heterotrait monotrait ratio of correlations
P	P-value
P-EB	Pro-environmental behavior
PLS	Partial least square
PLS-SEM	Structural Equation modelling partial least square
PPE	Proactive pro-environmental performance
R's	Word starts with "R"
SCT	Social cognitive theory
SL	Servant leadership
SLT	Servant leadership theory
T-statistics	Test- statistics
VAF	Variance accounted for

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CASE STUDY

Effects of citizen participation on urban water management based on socioeconomic factors

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ABSTRACT

BACKGROUND AND OBJECTIVES: In this era of globalization, clean water management is considered a complex problem requiring the strategic management of various aspects. Citizen participation in managing polluted waters is a critical, determining factor in preventing water crises. This study predicts the socioeconomic factors influencing citizens' behaviors in polluted water management.
METHODS: A survey on Jakarta's clean water was conducted with 503 respondents in 2022. Three interest variables were studied: first action taken during contamination, water nuisance level, and willingness to pay for clean water. Control variables were also explored, including daily income, education level, age, marital status, and gender. Data were analyzed using logistic regression.
FINDINGS: In general, socioeconomic factors influence citizens' behaviors in dealing with polluted water. The specific findings regarding the probabilities for the first action on the basis of asking for immediate action from local authorities, namely, by asking other citizens, waiting for information from other citizens, and looking for sources of water pollution were –2.21, –3.50, and 0.61, respectively. The results also revealed the probabilities of nuisance level (0.07), willingness to pay for clean water (0.0495), daily income (–0.02), educational level (–0.429), and age (0.01). The probabilities for married citizens (–2.845) and men (–0.268) were lower than those for unmarried citizens and women, respectively.
CONCLUSION: The findings of this study can be used to predict the management of water pollution among Jakarta citizens, as well as serve as a reference for related stakeholders. Socioeconomic factors can affect citizens in various aspects of life, including participation in water management. However, not all socioeconomic factors are directly proportional to citizen participation. In fact, other socioeconomic indicators are inversely proportional to what are expected based on theoretical assumption. Finally, educational level and income do not always translate to behavioral changes linked to water pollution management.

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INTRODUCTION

Lack of access to hygienic water and the ensuing water crises are significant issues in many nations (Muñoz *et al.,* 2020; Czajkowski *et al.,* 2021). Water is essential for many activities, and a water crisis arises if demand exceeds clean water supplies. Environmental sustainability may be negatively impacted by this phenomenon. Despite the fact that water is renewable and can be used again, the global water crisis is expected to worsen in the years (Biswas and Tortajada, 2019). According to the Brazilian and international press (BIP), water crisis is caused by climatic, geomorphological, hydrometeorological, anthropogenic, and water management factors (Milano *et al.,* 2018; Mena and Quiros, 2018; Abiriga *et al*., 2020). Several examples, such as industrial waste, landfills, agriculture, and global warming, have been identified as popular factors exacerbating this issue. Additionally, anthropogenic factors are considered the most influential due to the uncertainties they possess (Akhtar *et al.,* 2021). Over time, their impacts have increasingly become a cause for concern as many resources and infrastructure once taken for granted are now becoming scarce or damaged. As a result, ensuring clean water sources has become a complex and challenging task (Shepherd, 2019). Nonetheless, a study by Hoekstra *et al.* (2019) reported that the water crises can be lessened with the aid of water footprints, which gauge how much water is used in the production of goods and services. However, ensuring clean water supply requires contribution from various sectors. While the government has a critical role in managing polluted waters, citizen participation is also essential. In urban water management, citizen involvement plays a crucial role by allowing stakeholders to contribute their knowledge and perspectives to the decision-making process. According to Von Korff *et al.* (2012), citizen participation in water management can ensure fair and socially equitable water planning and management, as well as form participatory modeling and decision-making processes. In another study in Australia (Koop *et al.,* 2019), citizen participation is effective in promoting water conservation at the individual level. This study also found that improving household water conservation at the individual level provides a promising alternative to reduce costs and stimulate pro-environmental behaviors. Citizen participation plays a major role in averting “day zero,” which refers to the day when water scarcity is expected to occur (Shepherd, 2019; Yu *et al.,* 2021). This has also been found to influence the trends and characteristics of water pollution incidents (Huang *et al.,* 2022). Citizen participation can take many forms, including public hearings, workshops, focus groups, online platforms, and social media. For example, Evans and Hollander (2010) examined public participation using social media and found that the majority of citizen initiatives focused on planning issues. Those forms of citizen participation include monitoring (Alvarado *et al.,* 2020) and compliance (Fu and Geng, 2019). On the one hand, water quality monitoring can be achieved through observing physical, chemical, and biological parameters of water. On the other hand, “compliance” refers to the implementation of rules that have been set by default. Monitoring and compliance serve as measures to control water pollution and are instrumental in preventing diseases caused by polluted water. Citizen participation in controlling water pollution cannot be separated from citizen science. Citizen science is a rigorous process of scientific discovery, indistinguishable from conventional science apart from the participation of volunteers” (McKinley *et al.,* 2017). Cultural factors, such as religious factors, impact citizens’ participation via their habits and behaviors. Among these, religious obligation in terms of water conservation activities is the most likely factor affecting the rise of water conservation (Boazar *et al.,* 2019). Unfortunately, citizens’ knowledge of water management has been rarely studied (Dean *et al.,* 2016). Thus, in addressing water pollution, there is a need to conduct further research on all aspects of water management (Cosgrove and Loucks, 2015), particularly in the area of citizen participation. Many factors, including internal ones related to citizens’ awareness and external factors associated with socioeconomic, political, and religious factors (Akpabio *et al*., 2021), affect citizens’ participation in managing polluted waters. Therefore, these also need further investigations. In this sense, socioeconomic factors influencing low-income and marginalized communities, for example, may be less likely to have access to information and resources required to participate in decision-making processes related to water management (Ferrar *et al.,* 2013). Previous studies conducted across various cultures have examined the link between willingness to pay (WTP) for clean water and citizen participation in urban water management, showing a consistent positive relationship between them. A study conducted in Switzerland found that individuals who were WTP more for clean water were also more likely to participate in activities aimed at protecting water resources (Veronesi *et al.,* 2014). In China, individuals who were WTP higher water tariffs were more likely to support water conservation policies and initiatives and take individual actions to reduce water consumption (Wang and Jia, 2012). In another study conducted in the Mediterranean region, citizens who were WTP more for clean water also tended to engage in water conservation behaviors, choosing accommodations that provide water-saving rooms (Casado-Díaz *et al*., 2020). These findings suggest that citizens' WTP for clean water can be a key driver of citizen participation in urban water management. In particular, when citizens understand the value of clean water and the importance of protecting water resources, they are more likely to engage in efforts to manage and reduce water pollution, including activities like water quality monitoring, conservation efforts, and public education campaigns. Other socioeconomic factors, such as education, race, and gender, can also influence citizen participation in water management. For example, a study by Miguel (2018) in a rural community in Mexico found that women were less likely to participate in water management due to cultural norms and gender roles that limit their mobility and ability to participate in public decision-making processes. Jablonski *et al*. (2021) found that color-based mental constructs and racial stereotypes persist, with some being stronger in certain places than others. This finding indicates that those who comprise the major racial group in an area still maintains their power compared with other groups. The persistence of racial differences will affect various vitalizations in political, economic, and social activities, including participation in water management. Meanwhile, the role of community organizations and social networks is to promote public innovations in water management (Leavell *et al*., 2019). Furthermore, education is another crucial factor in promoting public awareness of water management issues and encouraging active participation in relevant efforts, such as conservation campaigns and community decision-making processes (Shiklomanov and Rodda, 2003). Education can also provide citizens with the knowledge and skills needed to participate effectively in decision-making processes related to water management by providing workshops and trainings for promoting awareness and understanding (Chess and Purcell, 1999; Mukhtarov *et al.,* 2018). Although socioeconomic factors are quite influential in other fields, such as malnutrition (Emamian *et al.,* 2014), gradient in cancer (Singer *et al.,* 2017), and energy consumption of citizens (Zhou and Shi, 2019), there remains a lack of research on how these factors can influence citizen participation in managing polluted waters. In a non-probabilistic study conducted by de Lazaro Torres *et al.* (2020), citizens' views on autonomous communities were identified, and good and bad practices stemming from water management were detected, including efficiency gains and special problems. Feng *et al.* (2021) examined the effect of socioeconomic development on water quality, while other researchers studied the effects of polluted waters on citizens' socioeconomic development. It can be concluded that citizens' good and bad practices in water management affect water quality, which in turn, influence socioeconomic development. If citizens' practices in managing polluted waters are influenced by socioeconomic factors, then it follows that such factors can both cause and be affected by citizen water pollution management. Given the prediction regarding the importance of controlling water pollution by citizens to mitigate the water crisis, along with the influence of socioeconomic factors in various aspects, it is necessary to investigate how socioeconomic factors influence citizen participation in managing water pollution. The ever-rising rates of population, globalization, and industrialization have increasing socioeconomic impacts as well. Consequently, there should be a renewal of existing water governance, policies, and management strategies from various socioeconomic perspectives to avoid the impending water crisis. The megacity of Jakarta is at high risk of experiencing a water crisis in 2040. Limited sources of raw water have resulted in poor coverage of clean water of up to 64% (Muhtarom, 2022). Jakarta's impending water crisis has a potential effect on the city's suitability as a living space (Colven, 2022). Meanwhile, several developed countries have used tools to detect water pollution. For example, in the United Kingdom (UK), planar microwave sensors were used as a first measure (Frau *et al.,* 2021). Several other studies have reported that WTP increases with income, education, respondents' perceptions of water quality and risk, health problems, and family structure (Jianjun *et al*., 2016; Chatterjee *et al.,* 2017; Tanellari *et al.,* 2015). This indicates that the behaviors of citizens in managing water pollution cannot be separated from the socioeconomic factors that influence them. This study contributes to the literature in several ways. First, this work predicts the influence of socioeconomic factors on citizens' water management behaviors, thus filling gaps from previous research that focused on the correlation between socioeconomic factors and water quality as well as the impact of water pollution on socioeconomic growth. Second, this study uses a probabilistic survey, whereas previous studies used non-probabilistic online surveys. Third, the study is conducted in Jakarta, the capital city of Indonesia, which has a high risk of clean water shortage. Based on the above discussion, this paper examines how socioeconomic factors influence citizens' behaviors in dealing with water pollution. This study poses several research questions: 1) what is the first action taken by citizens when water is polluted? 2) What is the value level of water pollution that disturbs citizens? 3) Is there a positive relationship between WTP for clean water and citizen participation in urban water management? Other socioeconomic factors (daily income, education level, age, marital status, and gender) were also examined. This study contributes to the literature by providing various water management perspectives through an examination of how communities respond to water pollution and the availability of clean water to reduce the impact of a water crisis. This is significant for several reasons. First, the importance of citizen involvement in managing water problems (Dean *et al.,* 2016) makes an actual contribution to achieving sustainable development goals (Fritz *et al.,* 2019). Second, continuing research on how the actions of citizens at different spatial levels help create water footprints (Hoekstra *et al.,* 2019). Third, it is important to provide another perspective in mitigating the impending water crisis in Jakarta (Colven, 2022). The current study focuses on the factors affecting citizens' participation in water pollution management given the perceived importance of citizen engagement in water management based on socioeconomic factors. The aims of the current study is focuses on what affects citizens' participation in handling polluted water. Given the importance of citizen engagement in water management based on socio-economic factors. This study has been carried out in Jakarta, Indonesia in 2022.

MATERIALS AND METHODS

Study area

The study location is in Jakarta, the capital of Indonesia. As shown in Fig. 1, Jakarta hosts an extensive array of economic activities that contribute to its rapid urbanization. According to Badan Pusat Statistik (BPSb, 2021), the most recent total population of Jakarta is around 10.6 million people, representing an increase of 0.1 million compared with the previous year (around 10.5 million). Various industries are located in Jakarta, attracting citizens from other regions to live in the city. The dense population, combined with various large-scale socioeconomic activities, has produced significant amounts of pollutants. The city's citizens have been the primary contributors to water pollution for the last few decades, although the amount of water pollutants they generate is not directly proportional to their participation in managing polluted waters. Therefore, this research provides suggestions and recommendations that can be applied to other areas facing similar problems in creating effective strategies to address water pollution.

Survey

The data used in this research were gathered from the Jakarta Clean Water Survey and included the population in the north, central, south, east, and west areas of the city. The sample consisted of 503 respondents. The logistic regression method to estimate was used (Hosmer *et al.,* 2013) how variables of interest and other socioeconomic factors affect citizens' probability of managing polluted waters. The dependent variable used in this study was the probability of managing polluted water. There were three other variables of interest in this study: 1) first action taken during water contamination, 2) citizens' nuisance score related to polluted water, and 3) citizens' WTP for clean water. Additionally, the study used control variables, including income per day (IPD), educational level, age, marital status, and gender. To estimate citizens' probability of managing polluted waters, the model specified in this study is expressed using Eq. 1 (Afroz *et al.,* 2016):

$$Y=\beta_1X1+\beta_2X2+\beta_3X3+\beta_4X4+\beta_5X5+\beta_6X6+\beta_7X7 \quad (1)$$

Where,

Y	Probability of managing polluted waters
β_1X1	Income, respondents' IPD, stated in \$US (US Dollar)
β_2X2	Education, respondents' education level, stated in year(s) of schooling
β_3X3	Respondents' age, stated in year(s)
β_4X4	Respondents' marital status, binary dummy variable
β_5X5	First action taken during contamination, in categorical dummy
β_6X6	Nuisance level, indicates respondents' level of tolerance to polluted waters, wherein a higher score indicates low tolerability
β_7X7	WTP for clean water, stated in \$US

RESULTS AND DISCUSSION

Issued linked to water pollution

The high rates of urbanization and population growth in Jakarta (Remondi *et al.,* 2016; Alzamil, 2017; Rustiadi *et al.,* 2021) have led to the production of large amounts of anthropogenic waste, and waste products that have not been managed properly are likely to pollute the environment, including the waters. The chemical, physical and biological components produced from waste cause damage to water bodies. Apart from anthropogenic waste, other factors also contribute to water pollution, including the heavy metal arsenic (As), which contaminates groundwater (Adeloju *et al.,* 2021; Mena and Quiros, 2018).

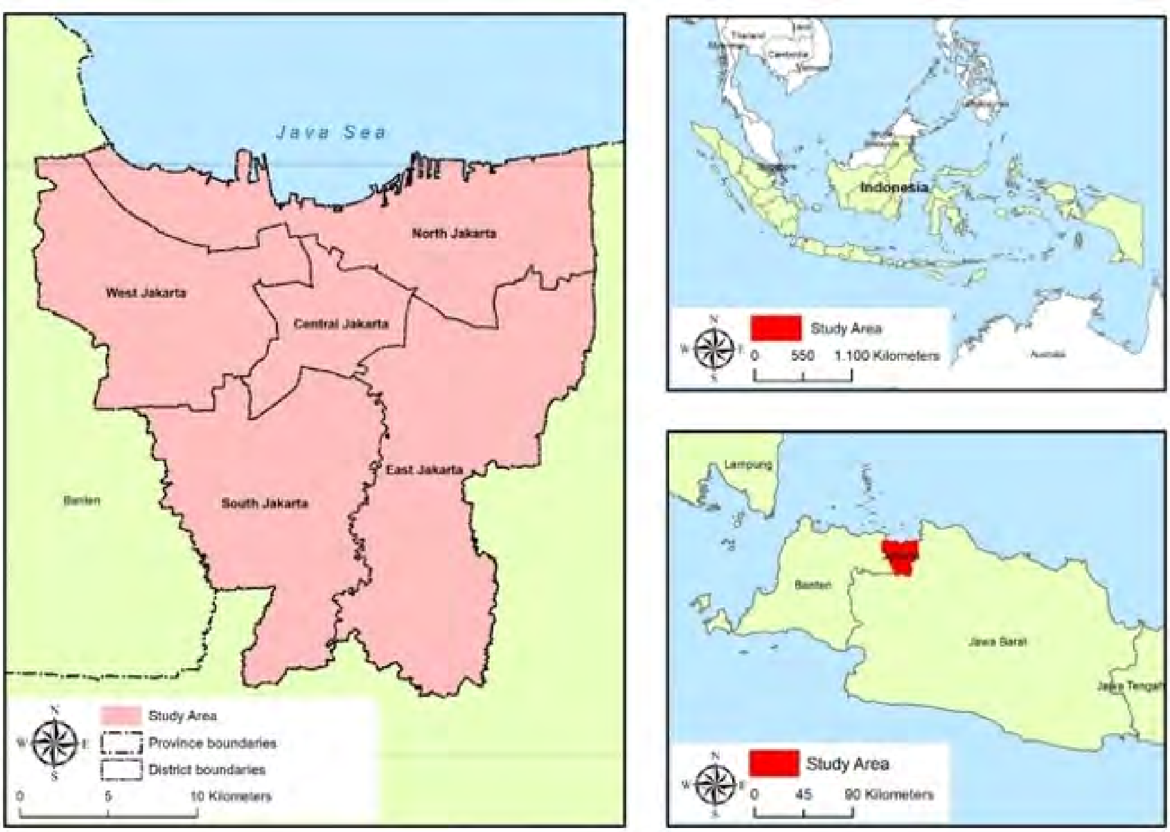

Fig. 1. Geographic location of the study area, Jakarta, Indonesia

Although most Jakarta citizens (about 60% of the population) rely on groundwater (Furlong and Kooy, 2017), their activities such as bathing in upstream rivers lead to fresh water pollution (Garg *et al.,* 2018). Citizens in the upstream who depend on the river for most of their activities also use polluted water. Based on data BPSb (2020), 219 urban villages in Jakarta have rivers, while 48 urban villages do not have them. This highlights the fact that rivers are the primary water source for Jakarta citizens. Due to the high levels of water pollution in many of these rivers, they have resorted to using groundwater as an alternative. However, the quality of groundwater for daily needs is not always guaranteed, and this poses an additional challenge that must be addressed, including the risk of waterborne diseases.

Possibility of citizens managing water pollution

Data were analyzed using logistic regression, and odds ratios were used to interpret the results. Predictor variables, including marital status and gender (represented as dummy variables), were also tested. This study identified four key estimates that can explain the impact of socioeconomic factors on water pollution management. Several continuous and dummy variables were collected from the survey to provide a more comprehensive understanding of water pollution management. The regression estimates are presented below.

Table 1 presents the regression results of the predictor variables on the probability of managing water pollution. The predictor variables that are discussed here are the variables of interest (the first action when contamination occurs, the disturbance score for polluted waters, and the WTP for clean water). The control variables were also discussed, namely, IPD, educational level, age, marital status, and gender. The first interest variable is the categorical

Table 1: Regression results (dummy variables were interpreted through odds ratio)

Variables	Probability to handle polluted water			
	-1	-2	-3	-4
	Estimate-1	Estimate-2	Estimate-3	Estimate-4
Income	-6.84e-06***	-6.05e-06***	-6.20e-06***	-2.05e-05***
	-1.56E-06	-1.54E-06	-1.58E-06	-2.54E-06
Education	-0.367***	-0.341**	-0.341**	-0.429***
	-0.137	-0.149	-0.149	-0.165
Age	0.0119	0.0119	0.0113	0.0106
	-0.0139	-0.015	-0.0151	-0.0179
Marital Status	-1.229	-1.552*	-1.542*	-2.845***
1=married; 0=else	-0.748	-0.802	-0.803	-0.884
Gender	-0.689	-0.409	-0.396	-0.268
1=male; 0=female	-0.764	-0.766	-0.765	-0.879
First action taken during water contamination Base: 1. Asking for immediate steps to local authority				
2. Asking other citizens		-2.528***	-2.556***	-2.212**
		-0.908	-0.911	-1.082
3. Looking for source of polluted water		0.404	0.406	0.61
		-0.608	-0.609	-0.67
4. Waiting information from other citizens		-2.566**	-2.563**	-3.502***
		-1.064	-1.064	-1.347
Nuisance level			-0.0463	0.0708
			-0.109	-0.125
WTP for a clean water				4.95e-05***
				-6.33E-06
Constant	8.461***	7.925***	8.382***	7.405***
	-1.961	-2.247	-2.503	-2.711
Observations	504	503	503	503

*p<0.1, **p<0.05, ***p<0.01

dummy of first action taken by the respondents. In the model, there are four types of first action taken: (1) asking local authorities for immediate steps to be taken, (2) asking other citizens, (3) looking for the source of water pollution, and (4) waiting for information from other citizens. The model used “asking for immediate steps” as the base option. According to the regression table, individuals who choose to ask other citizens (Option 2) have a lower probability of dealing with water pollution compared with those who ask local authorities for immediate action. The probability is 2.21 times lower than the baseline, and this result is statistically significant at the 95% confidence level (estimate 4). At estimates 2–3, people with these characteristics have a much lower probability than the baseline and are statistically significant at the 99% confidence level. In addition, people waiting for information from other citizens have a much lower probability of managing water pollution, where the probability is –3.50 times lower than the baseline, which is statistically significant (estimate 4) with a 99% confidence level. Meanwhile, communities that exhibit both characteristics of asking other citizens and waiting for information from other citizens theoretically have a lower probability of effectively dealing with water pollution compared with communities that request immediate action from the local government. On the one hand, these findings indicate that passive behaviors (waiting and asking) will result in significantly lower participation in managing water pollution (i.e., the possibility of managing water pollution always decreases). On the other hand, individuals who actively seek sources of water pollution tend to have a higher probability of effectively dealing with polluted waters, with a probability that is 0.61 times higher than the baseline (estimate 4). These findings suggest that proactive behaviors may increase the probability of effectively dealing with water pollution, although the effect is not statistically significant. This finding is relevant to the study of Hur *et al.,* (2019) about proactive behaviors. Such behaviors have a positive relationship with perceptions related to water pollution management. In turn, better perceptions on water management may increase the probability of dealing with water pollution through proactive behaviors. Notably, being proactive does not always guarantee an increase in the probability of managing water pollution, as there are other factors at play. To further strengthen the analysis, this research also conducted graphical analysis to investigate how the probability of water pollution management differs among the kinds of first action taken. The analysis indicates that individuals who actively seek the source of water pollution have the highest probability of effectively dealing with polluted waters compared with the baseline. Conversely, individuals who choose passive actions (options 2 and 4) have a much lower probability. This graphical analysis is consistent with the regression results as previously stated.

Based on the information presented in Fig. 2, the basic option of asking local authorities for help has a probability of more than 60% in effectively dealing with water pollution. At the same time, asking questions and waiting for information from other citizens has a probability of more than 20%, but it is lower than the basic option. These findings suggest that seeking immediate action from local authorities may be the most effective approach in managing water pollution. Meanwhile, looking for sources of water pollution has more than 80% likelihood, which is higher than the basic option in terms of dealing with water pollution. Looking for sources of water pollution has a higher probability of effective management than passive behavior (waiting and asking). This finding is consistent with both the regression results and the graphical analysis presented in the study. Aprile and Fiorillo (2017) stated that environmental knowledge and active participation through donations of time and money are the main drivers of water conservation behaviors. Participation is mediated by increasing environmental knowledge and connectedness to nature (Otto and Pensini, 2017). This shows that increasing environmental knowledge by finding sources of water pollution encourages better water conservation behaviors. Furthermore, active participation from citizens is needed in managing water pollution. The next variable of interest is respondents’ nuisance score related to polluted waters, which indicates how much pollution a certain respondent can tolerate. In particular, a higher nuisance score indicates lower tolerance. According to the regression table, a higher nuisance score is associated with a higher probability of managing water pollution, although this relationship is not statistically significant. An increase of nuisance level by 1 will increase the probability by 0.07 or 7% (estimate 4). As the result

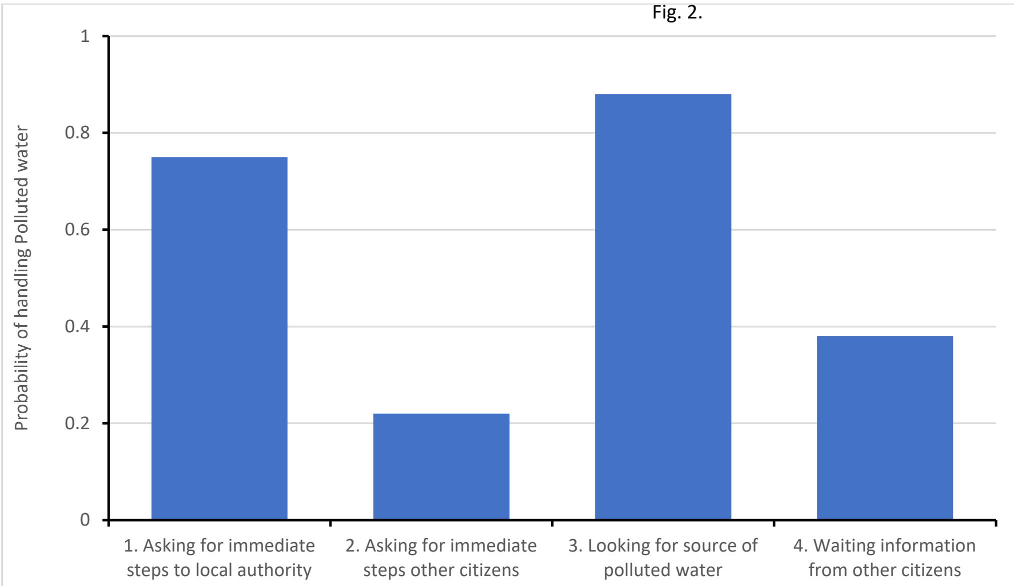

Fig. 2. Probability of managing water pollution based on first action taken

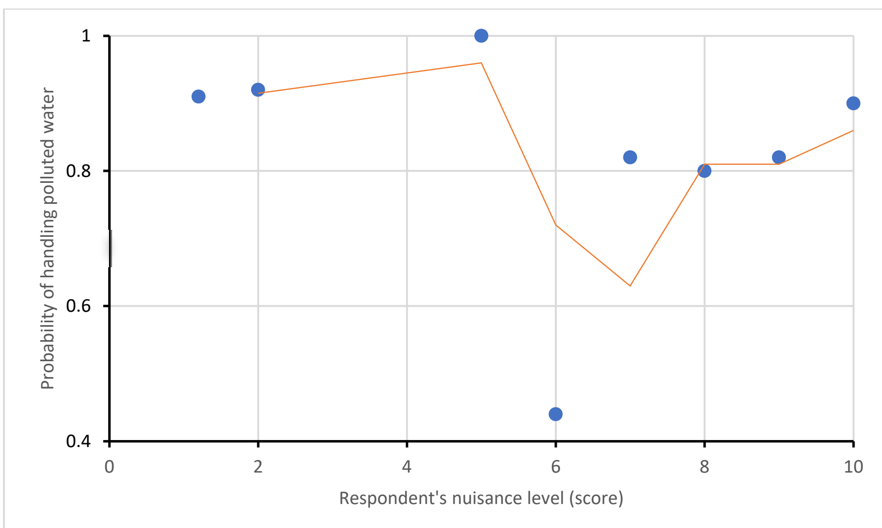

Fig. 3. Probability of managing water pollution based on respondents' nuisance level

is not statistically significant, we conducted graphical analysis to confirm how nuisance level affects citizens' probability of managing water pollution.

As shown in Fig. 3, the probability of managing water pollution decreases until the nuisance level reaches level 7, at which point the probability increases. This finding indicates that citizens' actions to handle water pollution will increase after a certain level of nuisance. This confirms the theoretical notion that people will take counteractions when they become annoyed by a certain level of nuisance. The level of respondents' nuisance related to water is positively related to the probability of managing water pollution, in which the higher the respondent's level of nuisance, the higher the probability of managing pollution and the lower their tolerance. This can be likened to Newton's third law, which is the law of action and reaction. The reaction is obtained when the action is given. The action here is the level of disturbance related to water, while the reaction is an act of managing water pollution. This is in accordance with the study conducted by Schielen *et al*., (2022) regarding the reactions of participants. They found that the participants' reaction times correlated most with the reaction times of agents that expressed functional behavior (true action). This indicates that their responses depend on what is considered correct. The same thing can be said with the level disturbance in terms of the emergence of reactions from participants when the perceived level of water disturbance is appropriate to manage. The next interest variable is citizens' WTP for clean water. According to the regression table, WTP for clean water has a positive relationship to the probability of citizens managing water pollution, although it is not statistically significant. In estimate 4, an increase of $US 0.66 on citizens' WTP will increase the probability of managing water pollution by 0.0495. As the regression result is not significant, we conducted graphical analysis to confirm the relationship of citizens' WTP to the probability of them handling polluted waters.

As shown in Fig. 4, the probability of managing water pollution increases at a higher rate until WTP reaches $US 13.16. At this point, it further increases but at a slower rate. This finding indicates that higher WTP indicates high personal valuation for clean water, resulting in higher effort to handle water pollution, as citizens with high WTP for clean water value water more than those with lower WTP. Those with high WTP have a high probability of managing water pollution, while lower WTP indicates that controlling water pollution is not a main priority (Choe *et al.,* 2019). It is assumed that people's WTP shows their concern for clean water. One study has shown that WTP is most strongly influenced by behavior control (Wang *et al.,* 2020). Furthermore, it has been reported that WTP is due to individual insights and anticipation of future improvements in water quality (Makwinja *et al.,* 2019). In other words, behavioral control from individual insights into clean water and anticipation of water quality lead to a high WTP for clean water and a greater probability of managing water pollution. Trust is the foundation for excellent cooperation with one another. Shi (2001) stated that trust is an important ingredient for social and economic progress in public institutions. Greater public trust has been shown to increase policy

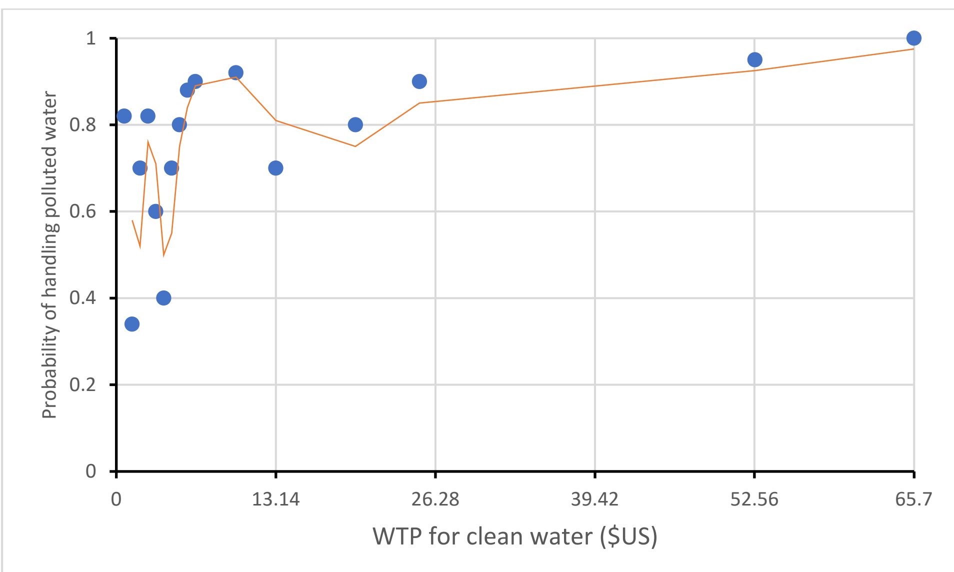

Fig. 4. Probability of managing water pollution based on WTP ($US)

compliance, such as active participation in urban water management programs. After analyzing the variables of interest, the current research analyzes the control variable and how it affects the probability of citizens managing water pollution. The first control variable is the daily income of citizens, and the results show that it has a negative impact on the likelihood of citizens dealing with polluted water. According to estimate 4, an increase in income of $US 0.66 reduces the probability of treating water pollution by –0.0205, which is statistically significant at the 99% confidence level (estimate-4). Estimates 1–3 indicate an even greater probability of decline at –0.06, which is significant with a 99% confidence level. This finding means that high-income citizens tend to have a lower probability of dealing with polluted waters because they have the ability to pay others to treat it or to buy clean water. To deepen the analysis, a graphical analysis of citizens' IPD and their probability of managing water pollution were also contacted. The graph below shows a U-shaped curve, indicating that the probability increases again after the IPD reaches a certain value. This additional finding reveals that Jakarta citizens with higher incomes will always have a lower probability of managing polluted waters. In contrast, their probability of handling polluted waters will increase again past a certain level of income. This indicates that citizens' behaviors in managing water pollution might change as their IPDs increase.

Based on Fig. 5, the probability of managing water pollution among citizens with an income of less than $US 13.17 tends to increase. This means that those with low income have a higher probability of dealing with water pollution, because they cannot afford to buy clean water, while the need for clean water is essential. At the same time, there are certain respondents who have a higher probability of managing water pollution even though their daily incomes are greater than $US 26.34. This suggests that their perception of controlling water pollution is high, even though they can afford clean water. The high probability at low-income levels indicates that people with middle to lower economic status are more likely to manage water pollution. However, this does not rule out the possibility that some citizens with high income levels can also handle polluted waters. Gomez *et al*. (2019) stated that income plays a role in forming perceptions and behaviors related to water management. Those with high incomes have greater access to clean water than those with low incomes. This means that the level of daily income does not have a linear relationship with the possibility of citizens dealing with water pollution. Another study reported that health problems due to water pollution arise from low-income respondents (Wang and Yang, 2016). This reinforces the idea

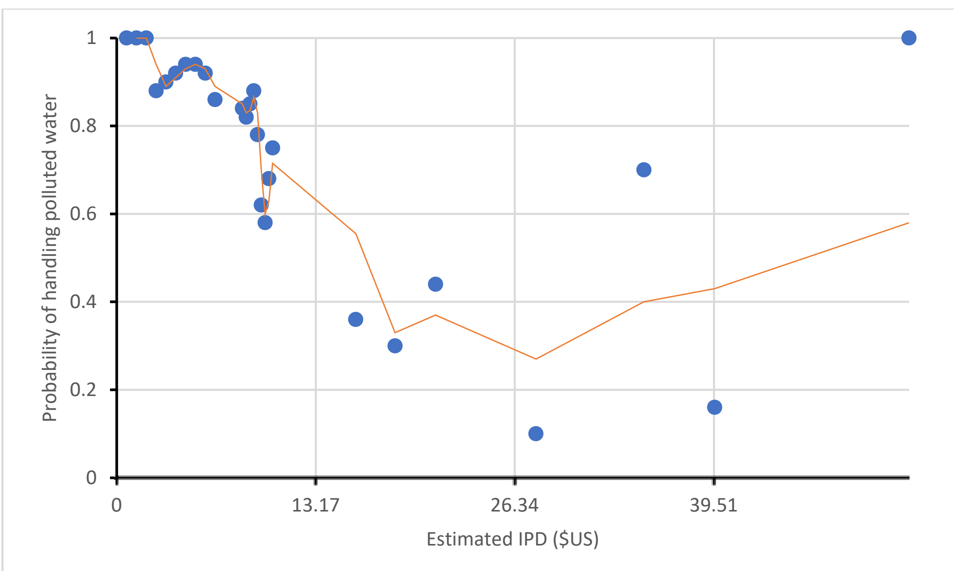

Fig. 5. Probability of managing water pollution by Estimate IPD ($US)

that low-income citizens tend to experience health problems due to water pollution, so they have greater awareness of and ability to overcome these problems. The next control variable to be analyzed is the level of education. Based on the regression table above, education level has a negative and statistically significant effect on the possibility of managing water pollution. An increase in education level of 1 year will reduce the probabilities by –0.429 (estimate 4) and –0.367 (estimate 1), which are statistically significant at the 99% confidence level. Estimates 2 and 3 reduce the probability –0.341 which is statistically significant with a 95% confidence level. These four estimates indicate that the higher the level of education, the lower the probability of dealing with water pollution. This is certainly a strange phenomenon. In theory, the higher the educational level of citizens, the greater the opportunity to deal with water pollution. To investigate this phenomenon further, it was conducted a graphical analysis to estimate how an increase in educational level would affect the possibility of dealing with polluted waters.

As shown in Fig. 6, the results are the same as those in the regression table, where more educated Jakarta citizens tend to have a lower probability of dealing with polluted waters. Those who have an educational level at the elementary school level have the greatest estimated probability of managing water pollution. In contrast, those who have a higher educational level have a low probability of doing the same. Theoretically, this is odd because ideally, highly educated citizens should have a higher environmental awareness than less educated citizens. Meyer (2015) has proven that educational level influences pro-environmental behaviors based on causal estimation not only based on descriptive relationships. Causal estimation can give us a better idea of what would happen to the extent of individuals' pro-environmental behaviors. Muttarak and Lutz (2014) found 11 original empirical studies set in diverse geographic, socioeconomic, cultural, and hazard contexts, which provide consistent and robust evidence of the positive impact of formal education on reducing vulnerability to environmental problems. Educational level affects perceptions regarding protecting the environment, which in turn, impacts individuals' willingness to participate in urban water management programs. It is necessary to consider whether water education is should be added in the education curriculum. For example, Compulsory 9-year water education programs in China are extremely rare (representing 0.2%–1.4% of the curriculum; Xiong *et al.,* 2016). Moreover, the education curriculum focuses more on theoretical than practical aspects

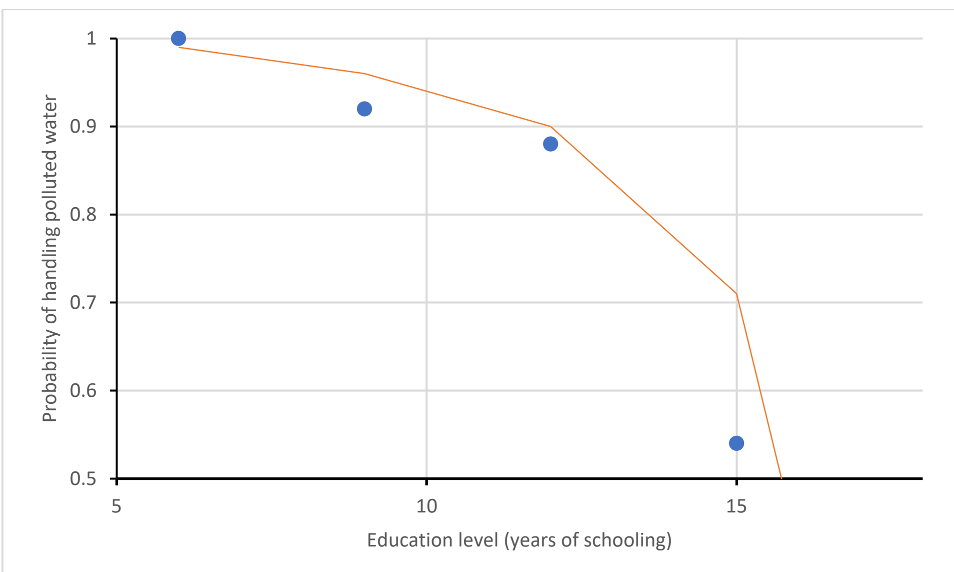

Fig. 6. Probability of managing water pollution based on educational level

(Al-Maliki *et al.,* 2021). The results of a recent study show that environmental education is just starting to be included in educational programs, taking green buildings as a reference, and that there has been an increase in efforts to reduce the environmental footprint through education in 21 countries (Díaz *et al.,* 2023). It can be concluded that the importance of including water education in the curriculum is not limited to theoretical but should also include practical education. This finding suggests that, in the case of Jakarta, citizens with higher education might be more apathetic to managing polluted waters. However, it does not mean that they are not bothered by water pollution or do not care about water conservation at all. More educated citizens might be involved in more strategic activities to handle water pollution or they could pay or ask someone else to handle pollution when contamination occurs. The next control variable is age. Based on the regression results in the previous discussion, age has a positive effect on the possibility of managing water pollution, although it is not statistically significant (it can reduce the probability and does not always increase). Based on estimates 1–4, an increase in age of 1 year will increase the likelihood of water pollution management by 0.01 or 1%. To further analyze this, it is conducted a graphical analysis.

Fig. 7 shows that the effect of age on the probability of managing water pollution has a very small level of decline when the age is between 0–50 years. Once citizens pass the age of 50, their chances of dealing with polluted waters decrease at a higher rate. This finding confirms that most of the water management activities are conducted by people of productive age. After passing such an age, the possibility of handling polluted waters will shift to younger and more productive citizens. At the same time, findings from Malaysia reveal that age has a significant effect on individuals' perceptions of water pollution (Afroz *et al.,* 2016). This suggests that the perception of water pollution increases with age, accompanied by a higher probability of managing water pollution. However, for the elderly who may have difficulty carrying out physical activities, their probability of water pollution management may be smaller, even though their perceptions of water pollution are high. The next control variable is marital status, which has a negative effect on the probability of managing polluted waters based on the regression results. In particular, married citizens tend to have a lower probability of managing water pollution by up to –2.85 times than unmarried citizens, which is statistically significant at the 99% confidence level (estimate 4). Estimates 2 and 3 both have a probability of –1.5

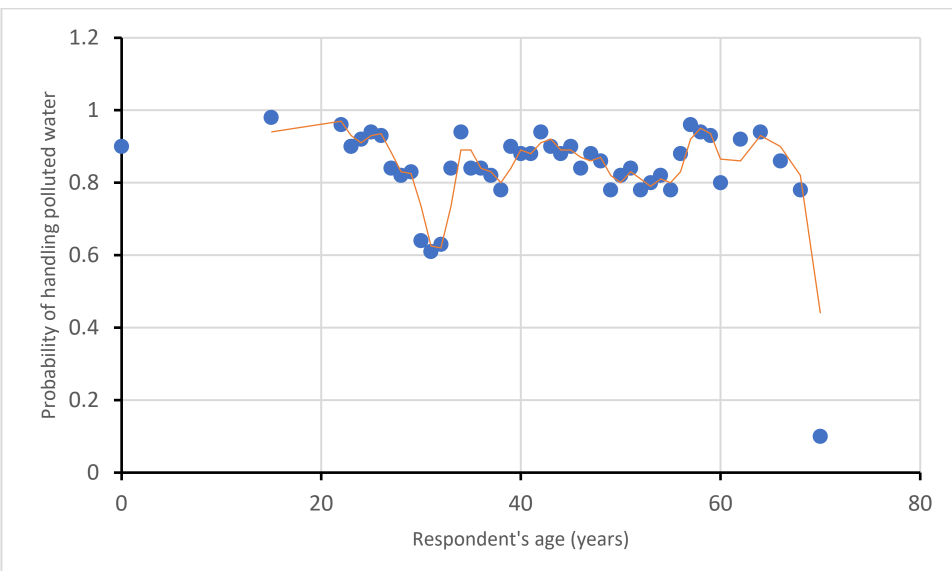

Fig. 7. Probability of managing water pollution based on respondents' age

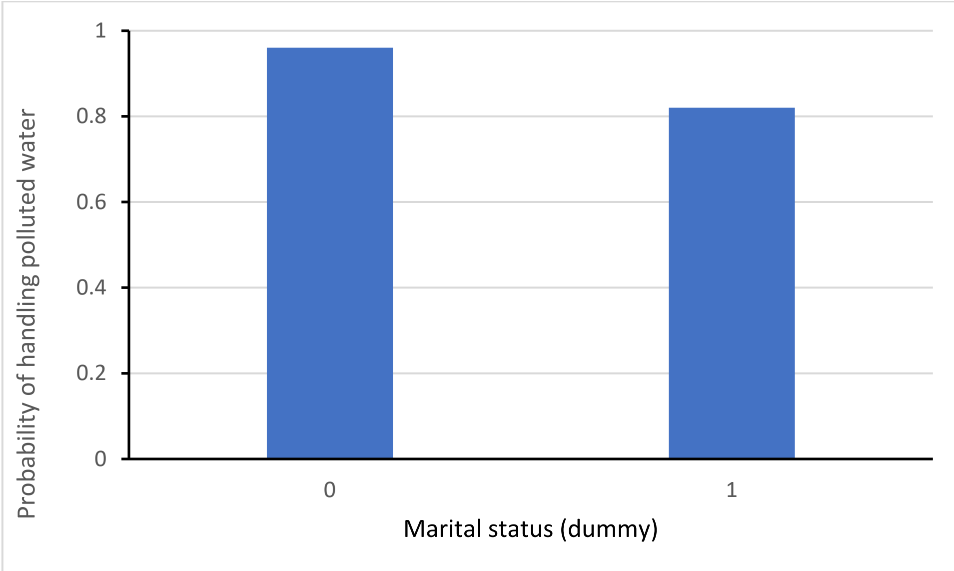

Fig. 8. Probability of managing water pollution based on marital status

times lower with a confidence level of 90%, while estimate 1 has a probability of –1.229 times lower but is not statistically significant. This suggests that married citizens have a lower probability of dealing with water pollution than single people, although it is possible that the opposite may occur in other areas. A graphical analysis was also conducted to examine how the probability of water pollution management differs between married and unmarried citizens.

As shown in Fig. 8, married citizens in Jakarta (dummy = 1) have a lower probability of handling water pollution than unmarried ones. This finding indicates that married citizens tend to pass on the water conservation activities to unmarried citizens. This is supported by the statement that the intentions to participate and consequent behaviors of citizens are highly limited by the objective conditions of the citizens themselves (Huang *et al.,* 2021). In this study, the objective condition is being married, which means that when a citizen is married, their focus shifts to the interests of their family. The last control variable is gender. Based on the regression results shown in Fig. 9, gender has a negative but statistically insignificant effect on the probability of managing water pollution. Male citizens tend to have –0.27 times lower probability of dealing with polluted waters than female citizens which is not statistically significant (estimate 4). Estimate 1 has a lower probability at –0.689. Furthermore, estimates 2 and 3 have –0.409 and 0.396 times lower probabilities, respectively. Although not statistically significant, this phenomenon is quite strange.

Based on Fig. 9, the graphical analysis results seem to be convergent, in which male citizens (dummy = 1) have a lower probability of handling water pollution than women. Theoretically, men should have a higher probability of doing so because water conservation activities require more physical abilities. Men tend to be more sensitive to symptoms that try to change their behaviors (Vicente *et al.,* 2018). Another study finding that strengthens the results of the study states that gender does not have an effect (De Lázaro Torres *et al.,* 2020). Gender refers to the different roles, rights, responsibilities, behaviors, and identities of men and women and the relationships between them. These relationships and responsibilities can and do change over time (UNDESA, 2014). Differences and inequalities between women and men affect how individuals respond to changes in the management of water resources. In recent years, it has been increasingly accepted that women must play an important role in water management, and certainly,

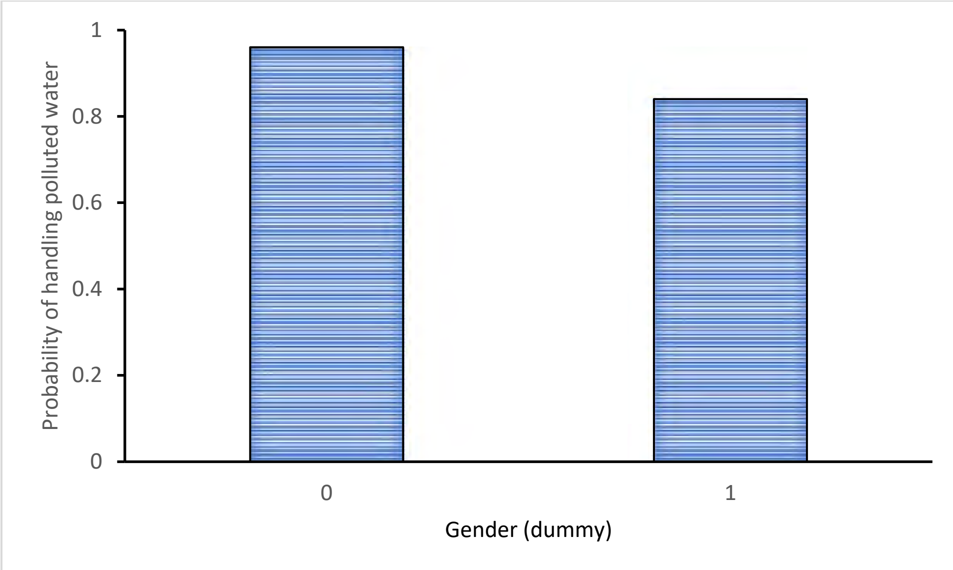

Fig. 9. Probability of managing water pollution based on gender

the response given by women is different when they are not accepted. The gender gap in environmental concern and political knowledge is explored by Lafuente *et al.* (2021) who found that women's preferences are related not only to their greater environmental proactiveness in the domestic sphere, but also to variables related to political knowledge. In the current study, gender does not have a significant relationship with the dependent variable; thus, it may not be concluded which gender tends to have a greater probability of managing polluted waters.

CONCLUSION

This study was conducted to add to the perspective that socioeconomic factors can influence behaviors in managing water pollution, filling gaps from previous research that focused on the correlation between socioeconomic factors and water quality and the impact of water pollution on socioeconomic growth. The study was conducted through a clean water survey held in Jakarta in 2022 using the probabilistic survey method. Interest and control variables were tested against this possibility. The interest variables tested were initial action, level of water disturbance, and WTP for clean water. The results showed that people with higher income and educational levels had a lower probability of dealing with polluted waters, while those with lower income and educational levels had a higher probability of doing the same. This suggests that education and income do not always translate to behavioral changes in managing water pollution. The study also found that age played a role, with older citizens having a higher perception of water pollution but lower probability of managing it due to physical limitations. Finally, married citizens were found to have a lower probability of managing polluted waters than unmarried citizens. Overall, this study adds to the understanding that socioeconomic factors and personal characteristics play a significant role in shaping behaviors related to water pollution. It further highlights the importance of considering these factors in designing interventions to promote water conservation and pollution reduction. In particular, a higher WTP reflects a high personal valuation of clean water, resulting in a higher effort to handle polluted waters, mainly because citizens with a high WTP for clean water value water more than those with a lower WTP. Jakarta citizens with higher incomes are less likely to handle polluted water, but their probability of doing so will increase again after a certain income level. Moreover, citizens with higher educational levels may have a lower probability of dealing with water pollution compared with those with lower education levels. However, this does not necessarily mean that the former do not care about water conservation or are completely apathetic towards polluted water. It is possible that more educated citizens are involved in more strategic activities related to water conservation or may delegate the task of water pollution management to others. Thus, the relationship between educational level and the probability of dealing with water pollution still needs to be explored**.** In the case of Jakarta, water management activities are generally carried out by people of productive age. After passing their productive age, their ability to treat polluted waters will shift to younger and more productive citizens. In addition, the results revealed that married Jakarta citizens are less likely to be affected by water pollution than unmarried people. In terms of gender, it cannot be concluded which gender is more likely to handle polluted waters. Overall, this study contributes to the literature by examining the possibility of socioeconomic factors influencing actions taken to deal with water pollution. This can help stakeholders in designing and implementing strategies to mitigate short-term clean water crisis in the capital region and support relevant decision-making processes.

AUTHOR CONTRIBUTIONS

D. Elfina Purba perfomed conceptualization,; methodology, literature review and project administration. F. Madianti performed the validation and formal analysis. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABREVIATIONS

%	Percentage
$US	US Dollar
As	Arsenic
BIP	Brazilian and International Press
BPS	Badan Pusat Statistik (Statistics Indonesia)
C.S.	Citizens science
E or e	10 to the power of
e.g.	For example
Eq.	Equation
Est.	Estimation
Fig.	Figure
i.e.	That is or that are
IPD	Income per day
p	Level of significance
UK	United Kingdom
WTP	Willingness to Pay

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CASE STUDY

Health risk assessment through probabilistic sensitivity analysis of carbon monoxide and fine particulate transportation exposure

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ABSTRACT

BACKGROUND AND OBJECTIVES: The rising number of vehicles used for transportation, which is attributed to the steady increase in population, is known to be a major contributor of air pollution, which, in turn, can have adverse effects on the environment and human health. Therefore, in this study, we aimed to evaluate the concentration of carbon monoxide and fine particulate matter in the air and their potential health risks and further examine the use of probabilistic methods to simulate the sensitivity of people living in communities and school children to these pollutants.
METHODS: This study collected carbon monoxide and fine particulate matter samples from 32 stations near community houses and 14 sites near schools located along roads. Hazard quotient and target hazard quotient calculations were used to estimate the non-carcinogenic health risks associated with exposure to these substances for both community adults and school children. Finally, Monte Carlo simulations were applied to analyze the sensitivity and uncertainty risks.
FINDINGS: As per the results, the highest level of carbon monoxide was recorded in station 22, with 6729 microgram per cubic meter, while the lowest was in station 24, with 1037 microgram per cubic meter. Station 10 had the highest concentration of fine particulate matter at 116 microgram per cubic meter, as opposed to station 2 with the lowest level at 10 microgram per cubic meter. In children, the hazard quotient value for carbon monoxide was found to be highest at 3.013, with the lowest at 0.614. Similarly, the highest level of target hazard quotient for carbon monoxide in children was 7.370, whereas the lowest was 1.522. For fine particulate matter, the highest risk level was 0.180. Additionally, the highest, and lowest levels of target hazard quotient for fine particulate matter were 0.311 and 0.037, respectively. Deterministic and probabilistic approaches were used to assess the risks these pollutants impose on adults and school children based on their daily inhalation rate. The results revealed that the 5th and 95th percentiles of cancer risk for carbon monoxide in adults were 2.85 and 6.11, respectively, indicating medium risks. However, for fine particulate matter, the 5th, and 95th percentiles were 0.09 and 0.19, respectively, signifying lower risks. For school children, the percentiles for carbon monoxide and fine particulate matter were 1.20 and 2.50, respectively, demonstrating higher risks.
CONCLUSION: As per the results, it was determined that the hazard quotient risk for carbon monoxide in adults exceeded the standard, >1, thus posing a risk. Only three stations had hazard quotient values lower than 1, which is deemed of safe level. Most of the fine particulate matter risk assessment results had hazard quotient values lower than 1, indicating a safe level. However, all other 30 stations had exceeded the World Health Organization standard (>1), thus demonstrating risks. The likelihood of the inhabitants being at risk increased as the frequency of discrete exposure occurrences increased; this is evidenced by target hazard quotient calculation results for both carbon monoxide and fine particulate matter at the 32 monitored station areas. These results warrant that future research should focus on reducing carbon monoxide and fine particulate matter in the environment by fostering awareness among local and national stakeholders as well as the academe; this may allow South Tangerang to become a center of excellence for green schools in the area.

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INTRODUCTION

The dependence of people on private vehicles instead of public transportation presents a significant public health challenge (Wang *et al.,* 2019; Gao *et al.,* 2020). The use of motorized vehicles on roads has been strongly linked to greenhouse gas emissions and climate change (Filigrana *et al.,* 2022; Bath *et al.,* 2022). In urban areas where the majority of the population resides (Sarigiannis *et al.,* 2016), private cars and two-wheeled vehicles are the most commonly used means of transportation (Nagpur *et al.*, 2016; Lin *et al.,* 2019). The transportation sector has been identified as a major contributor of air pollution (Roth *et al.,* 2019; Song *et al.,* 2021; Zeydan *et al.,* 2023). According to the US Department of Transportation (2022), emissions from transportation consist of 61 percent (%) carbon monoxide (CO), 53% nitrogen dioxide (NO_2), 17% volatile organic compound, and 13%–15% fine particulate matter ($PM_{2.5}$) and particulate matter (PM_{10}) (USDT, 2022). The primary source of traffic-related air pollution in ambient air is the combustion of fuel in vehicles, with CO, and $PM2_{.5}$ being the primary pollutants. Community exposure to pollutants depends on their physical activity and mode of transportation used, as well as the location of schools close to main roads for school children (Khreis, 2020). Vehicle emissions can disperse based on meteorological conditions such as wind speed and humidity (Li *et al.,* 2022; Sbai, *et al.,* 2022). Low wind speed causes the concentration of air pollution to remain in the area (Lai *et al.,* 2020), Air pollution is known to have significant negative impacts on human health (Requia *et al.,* 2018; Mak *et al.,* 2021). Street vendors and people living along the main road are among the most affected population groups. Children, elderly, and individuals with heart or lung conditions are more susceptible and have higher risk to the harmful effects of particle pollution. According to estimates, air pollution is responsible for 7 million premature deaths yearly (Mason *et al.,* 2020; Hein *et al.,* 2022). According to the World Health Organization (WHO), air quality guideline levels for $PM_{2.5}$ and CO in 2021 are set at 15 microgram per cubic meter (µg/m^3) and 4 µg/m^3, respectively (WHO, 2021). Meanwhile, the quality standard for CO for 1 hour is 10,000 µg/m^3, 8 hours is 4,000 µg/m^3, and $PM_{2.5}$ is 55 µg/m^3 (Government Regulations, 2021). Examining its inhalation process, CO pollutant enters the body and then binds to hemoglobin, forming a carboxyhemoglobin (COHb) complex, which is more potent than oxygen, impairing the body's ability to supply oxygen to tissues (USEPA, 2011). Concentrations higher than 10% can result in lightheadedness, dyspnea, confusion, headaches, nausea/vomiting, exhaustion, chest discomfort, and loss of consciousness (Soeroso *et al.,* 2020). Short-term exposure to CO has been linked to cardiovascular disease mortality and morbidity based on an epidemiological study (Tian *et al.,* 2018). At long-term and high exposure levels, death resulting from asphyxia may occur; at lesser levels, this may result in myocardial ischemia and rhythm disruption (Liu *et al.,* 2018). In China, short-term CO exposure in adults is associated with years of life lost (YLL). The findings showed that an increase of 1 milligram per cubic meter (mg/m^3) in CO concentrations was linked to a 2.08% increase in daily YLL from non-accidental causes, including coronary heart diseases, respiratory diseases, chronic obstructive pulmonary diseases, and cardiovascular diseases (Wang *et al.,* 2021). $PM_{2.5}$ is a specific type of airborne particle with an aerodynamic diameter of less than 2.5 µm and is known for its adverse effects on the environment and human health. Inhalation of $PM_{2.5}$ may result in serious health problems affecting vital organs such as the hearts and brain; this is common among children who are frequently exposed to this pollutant (Khanna *et al.,* 2018). Several epidemiology studies showed a connection between $PM_{2.5}$ and death rates, as well as cardiovascular and respiratory disorders (Li *et al.,* 2017). On-road transportation sources in New York City alone account for approximately 320 deaths per year, which is attributed to $PM_{2.5}$ exposure in adults and school children (Kheirbek *et al.,* 2016). Higher wind speeds facilitate the diffusion of $PM_{2.5}$ in the air, decreasing its concentration. In a study conducted in Nantong, China, it was determined that wind speed between 2 and 4 meters per second (m/s) could reduce the concentration of $PM_{2.5}$ in the air (Xu *et al.,* 2020). In western China, $PM_{2.5}$ and CO concentrations are lowest in the summer and greatest in the winter (Yang *et al.,* 2019). Similar to Beijing, China, high CO concentrations have been associated with a rise in transportation vehicles in the area and high $PM_{2.5}$ concentrations in Henan due to increased humidity and low wind speeds direction (Liu *et al.,* 2022). According to air quality simulation results, despite traffic limitations, CO concentrations did not decrease in most parts of China due to heavy traffic emissions (Zou *et al.,* 2023). In a study conducted in Bogota, it was found that shorter trip periods and lower exposure concentrations in the cabin led to reduced inhalation doses of $PM_{2.5}$ and CO among TransMiCable users (Ricardo *et al.,* 2023). In Cambodia, the housing, transportation, and waste sectors are identified as the major contributors to the country's total national $PM_{2.5}$ and CO emissions, which are projected to increase by 50 to 150% by 2030, primarily due to the rising transportation emissions. However, reducing these emissions has been seen to prevent over 900 premature deaths (Sokharavuth *et al.,* 2022). In Gilgit-Baltistan, high transportation emissions led to the highest concentrations of $PM_{2.5}$ and CO during summer, negatively affecting children (Hussain *et al.,* 2023). In Viadana, Italy, there was a significant increase of 30% in cases of childhood pneumonia, with $PM_{2.5}$ as the contributing factor (Panunzi *et al.,* 2023). Meanwhile, in Turkey, the concentration of $PM_{2.5}$ in Sanliurfa Elementary School was measured at 31.8 µg/m^3, which posed significant short-term risk of asthma symptoms (10.9%, 2.4%–19.6%) and long-term prevalence of bronchitis (19.5%, 2.2%–38.8%) in children with asthma. Those children will have a high risk of illness as they are more easily exposed to polluted environments such as pollution (Sahin *et al.,* 2022). In China, a research examining the health risks of adults found short-term exposure to $PM_{2.5}$ is associated with increased insulin resistance and poor glucose homeostasis, with every 10 g/m^3 increase in the 3-day $PM_{2.5}$ moving average was associated with an increase in Fasting Insulins (FINS) (95% CI: 2.35, 6.05), HOMA-B (95% CI: 3.66, 8.26), and HOMA-IR (95% CI: 1.41, 4.64) (Peng *et al.,* 2022). Exposure to $PM_{2.5}$ was also found to reduce liver function by 17.06 (95% CI: 31.53, 2.58) for every 10 µg/m^3 increase in $PM_{2.5}$ (2-day lag) (Xu *et al.*, 2022). Furthermore, in a study conducted in Wuhan, China, short-term exposure to CO, and $PM2_{.5}$ was determined to have negatively impacted the liver function of people residing in the city's urban areas (Qiu *et al*., 2021). Due to increasing urbanization and economic growth, motor vehicle usage has risen, significantly increasing $PM_{2.5}$ in emerging nations such as Indonesia. In fact, the yearly average $PM_{2.5}$ level in Indonesia has exceeded the WHO's recommended range by 17 µg/m^3 since 2022, highlighting its negative impact on public health (Greenstone *et al*., 2022). In a study conducted in Batam city, $PM_{2.5}$ levels were determined to be as high as 45 ug/m^3 and CO levels to be 35.83 ppm, which might have resulted in the increasing respiratory problems and cardiovascular diseases among the public (Yodi *et al.,* 2019). In Malaysia, to achieve an annual reduction in the overall pollutant emission intensity, the school has constructed and maintained a smart house and a sizable solar parking lot with a capacity of 10 MW, which employs solar- and wind-powered electricity and promotes the use of bicycles and electric scooters (Naderipour *et al.,* 2021). The city of South Tangerang, with its high residential density, industrial areas, and rapid trade growth, and also known as an education center for school children, has been heavily affected by air pollution caused by the increased number of transportation vehicles. In fact, it has been found to be the most polluted city in Indonesia, with high levels of CO and $PM_{2.5}$. The rise in motorized transportation in the area has been identified as one of the leading contributors of air pollution, with data from 2017 to 2018 showing a direct correlation between air pollution, transportation, industrial activities, and human health disturbance disorders (Listyarini *et al.,* 2020). Thus, in this current study, we aimed to assess the levels of $PM_{2.5}$ and CO in the ambient air and evaluate any of their potential effects on health and to then apply probabilistic approaches to determine how communities and school children are affected by these emitted pollutants. This study has been carried out in seven districts of South Tangerang city, Indonesia, in 2023.

MATERIALS AND METHODS

Study method

This analytic observational research of cross-sectional study design was conducted using a Monte Carlo simulation (MCS) statistical model through the health risk assessment approach. Health risk analysis is used to estimate human health risks, both carcinogenic, and non-carcinogenic. CO and $PM_{2.5}$ concentrations were measured from February to March 2023 at 32 points in 7 sub-districts, namely, North Serpong, Serpong, Pondok Aren, Ciputat, East Ciputat, Pamulang, and Setu. Meteorological data such as temperature, humidity, wind direction, and wind speed at the same sites were also collected. Respondents were residents who live along the road and school children whose schools were located

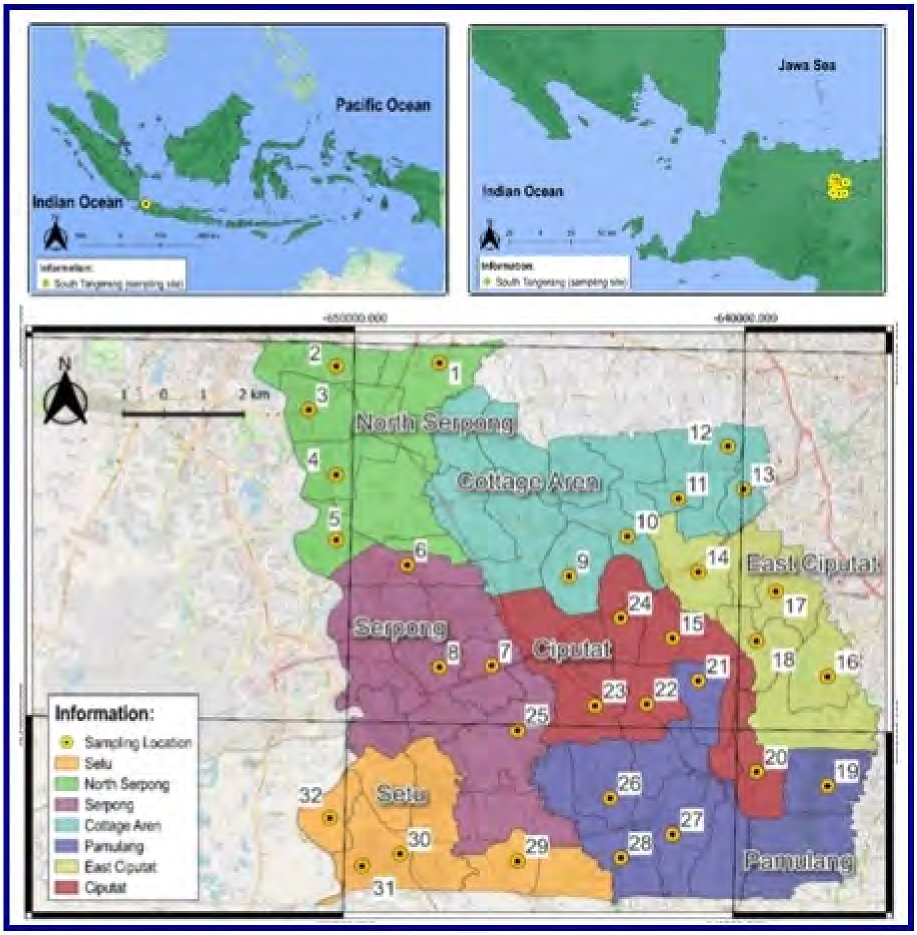

Fig. 1: Geographical location of the study area and the locations of the CO and PM$_{2.5}$ samples in 7 sub-districts of South Tangerang, Indonesia

close to this region. The inclusion criteria for adults were aged 26–45 years old and worked or lived in the main road area for at least 3 years; two respondents were taken per environmental sample point. Exclusion criteria were those who withdrew during the interview. Meanwhile, the inclusion criteria for children were aged 9–15 years and minimum grade of 4–6 in elementary school; at least 4 respondents were taken per school at 14 elementary schools located in the 7 sub-districts.

Study area

The study area situated in the province of Banten, Indonesia, specifically in South Tangerang city, comprised seven sub-districts, namely, North Serpong, Serpong, Pondok Aren, Ciputat, East Ciputat, Pamulang, and Setu. According to satellite imagery, the area's coordinates are 106°38' to 106°47' east longitude and 6°13' to 6°22' south latitude with a population of 1.3 million, as shown in Fig. 1. In total, 32 samples were collected, and measured from 32 points.

Sampling process

For a period of 32 days (January to February 2023), CO, and PM $_{2.5}$ levels were measured every day, and these data were combined with meteorological data including temperature, relative humidity, wind direction, and speed for analysis. Sampling of CO and PM$_{2.5}$ levels was carried out directly using a CO meter for CO samples and a high volume air sampler for $PM_{2.5}$. This study focused on seven households in each district that were located in close proximity to a major road in the southeast. The air quality in this area was influenced by various factors, such as emissions from heavy-duty trucks that distributed industrial products and raw materials, as well as from vehicles on major road. Yellow and white dusts are frequently found on the terraces of homes in the study area, particularly in the mornings, even in the absence of a blowing wind carrying dust. This study focused on a densely populated neighborhood that is home to street vendors, which comprised an industrial zone where factories operate continuously, as shown in Fig. 1.

Data analysis

The United States Environmental Protection Agency (USEPA) has employed human health risk analysis to calculate the health risk of CO and $PM_{2.5}$ exposure for those living near the pollutant point sources including along the main road or dwelling areas. Since inhalation is the primary route for CO and $PM_{2.5}$, this route was assessed by applying formulation of hazard quotient (HQ) and target hazard quotients (THQ) to calculate the magnitude of individual health risks.

Non-carcinogenic human health risk assessment

The non-carcinogenic risk ratio for CO and $PM_{2.5}$ was determined for two age ranges, that is, elementary school students between the ages of 10–16 and adults older than 16 years old. The estimated daily intake of CO and $PM_{2.5}$ was calculated through inhalation as it is considered as the primary exposure route. Therefore, the health risk analysis focused on this pathway. The non-carcinogenic risk analysis equation for the inhalation route is shown in Eqs. 1, 2, and 3 (Edlund *et al.*, 2021; Rauf *et al.*, 2021; 2022; Azhdarpoor *et al.*, 2019; USEPA., 1989).

$$ADD_{inh} = \frac{C \times Inh_{rate} \times EF \times ED \times ET}{BW \times AT} \quad (1)$$

$$HQ = \frac{ADD_{inh}}{RfC} \quad (2)$$

$$THQ = \frac{EF \times ED \times IR \times C}{RfC \times BW \times AT} \times 10^{-3} \quad (3)$$

where

ADD: Acceptable daily dose of CO and $PM_{2.5}$ microgram per kilogram/day (µg/kg/d)

HQ: Hazard quotient

C: Ambient of CO and $PM_{2.5}$ concentration (µg/m^3).

Inhrate: Inhalation rate of people for CO, (Ministry of Health of Indonesia Republic) set default value 0.83 m^3/day (adult) and 0.5 m^3/day (children) (Ministry of Health, 2012)

Inhrate: Inhalation rate of people for $PM_{2.5}$, USEPA default value 14.9 m^3/day (adult) and 9 m^3/day (children) (USEPA, 1989)

EF: CO and $PM_{2.5}$ residential exposure frequency for 350 days/year (USEPA, 1991)

ED: Exposure duration, USEPA default value for adult is 24 years old and is 6 years old (Rauf *et al.*, 2022)

BW: Body weight for adult (63.01 kg) and children (34.55 kg) (Rauf *et al.*, 2022; Mallongi, *et al*., 2023)

A: Average time (ED x 365 days/years for non-carcinogenic risk estimation)

RfC: Reference concentration for CO is 46.3 µg/kg/d, and $PM_{2.5}$ inhalation is 10 µg/kg/d (Novirsa and Achmadi., 2012). When HQ value is greater than 1, it means that chronic exposure to CO and $PM_{2.5}$ is not safe for the general population. Future health effects can be non-carcinogenically dangerous to the population, or the risk is minor when the HQ value is lower than 1.

The Monte Carlo simulation

The association between CO, $PM_{2.5}$, and the likelihood of human health risk was determined by examining the distribution of the major variables. The cumulative distribution is expressed in the 5th and 95th percentiles if HQ or THQ < 1 is stating the safe exposure limit for CO, $PM_{2.5}$. Additionally, using Eq. 4, a sensitivity test was conducted to ascertain the element that has the most impact on THQ value in order to create an effective risk management strategy for the population (Millard, *et al*., 1998; Mallongi, *et al.,* 2023).

$$Y = h(X) = h(X_1, X_2, \ldots, X_n) \quad (4)$$

The results were presented as both a probability risk (uncertainty) graph and a variable sensitivity graph. The MCS was conducted using Oracle Crystal Ball software version 11.1.12 rev, which is an add-in for Microsoft Excel 2019. Microsoft was used to compute the risk and the average CO and $PM_{2.5}$ concentrations. The Monte Carlo approach used in the simulation allows for the propagation of uncertainty associated with the input distributions (Ferson., 1996). A collection of spreadsheet-based programs associated with Microsoft Excel called Crystal Ball was used to run the MCS in order to determine any uncertainties in calculating the health quotient. The sensitivity analysis approach was used to evaluate the relative importance of variance in each input parameter to the overall variance in risk (Stroeve *et al*., 2009). This approach provided insights into which input parameters had a greater influence on the uncertainty of the outcome (Soleimani *et al.*, 2020).

RESULTS AND DISCUSSION

Meteorological data

Several variables can influence air pollution, with wind direction, and speed being key contributors to its spread. These elements can cause the pollution to disperse in both horizontal and vertical directions. South Tangerang city is situated adjacent to DKI Jakarta; it has a relatively flat topography, with an average slope of 0%–3% and an elevation range of 0–25 dpl. Meteorological data, including temperature, humidity, wind speed, and direction, are obtained from the online database of the Meteorology, Climatology, and Geophysics Agency of Indonesia (BMKG). The temperature in the studied area ranged from 20°C to 42°C, with an average of 28°C. Relative humidity varied between 61% and 96%, with an average of 73%, while wind speeds ranged from 0.11 m/s to 4 m/s, with an average of 2 m/s. An overview of wind speed is shown in Fig. 2.

The wind rose plot is utilized to analyze wind

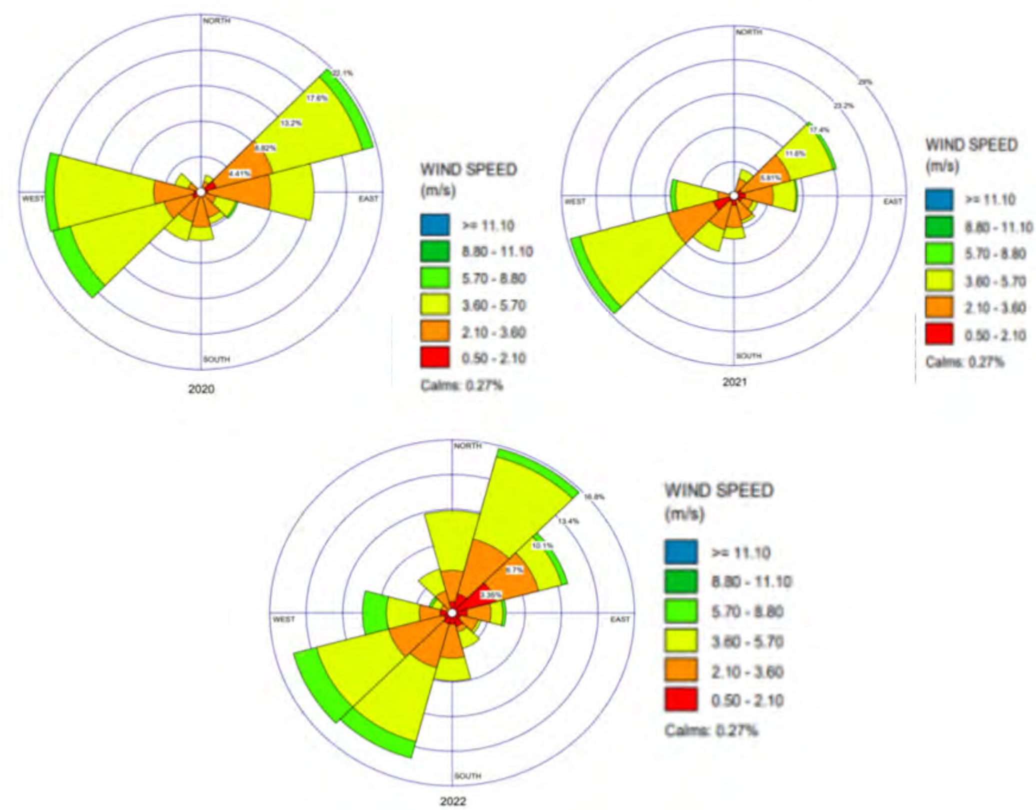

Fig. 2: Annual wind conditions in South Tangerang city in 3 years from 2020 to 2022

conditions using WRPLOT 8.0.2, with a visualization of 106° for a period of 3 years (2020–2022). The wind pattern is quite consistent, but in 2021, the highest frequency direction is southwest, while for 2020, and 2022, the highest frequency direction is northeast. Wind direction is known to affect the concentration of pollutants, particularly when blowing toward highways. The average wind speed is also consistent from 2020 to 2023, with moderate speeds of 50.3%, 43%, and 41.6% in 2020, 2021, and 2022, respectively. Meteorological factors play a critical role in determining air quality. In metropolitan areas of China, for example, moderate wind speeds contribute to the increased distribution and constant concentration of pollutants (Yang *et al*., 2020). Similarly, in the main railroad area of Atlanta, Georgia, changing wind conditions can increase CO concentrations by 1.1–1.2 (Brantley *et al*., 2019). Implementing roadside vegetation or urban woods can help mitigate air pollution levels. In California, USA, roadside vegetation has potentially reduced the average downwind pollutant concentrations by up to 50% (Deshmukh *et al*., 2018).

Measurement of CO and $PM_{2.5}$ level

Direct measurements were made all day, starting in the morning, and it was discovered that the concentrations of CO and $PM_{2.5}$ varied at each point. The spatial distributions are shown in Fig. 3, while Tables 1, and 2 highlight the differences in terms of CO and $PM_{2.5}$ concentrations between South Tangerang and other nations. This study found that the average concentrations of CO and $PM_{2.5}$ in the examined area were higher than the WHO air quality recommendation (AQG). For short-term (24-hour average) and long-term (annual average) exposures, the WHO-AQGs for CO, and $PM_{2.5}$ are 15 µg/m³ and 5 µg/m³, respectively (WHO, 2021). The concentration levels of CO and $PM_{2.5}$ found in the investigation were below Indonesian air quality standards but exceeded the WHO AQG levels (Government Regulations, 2021). The highest CO and $PM_{2.5}$ were found in the southeastern portion of the industrial area, specifically in Serua Indah, Ciputat district, with levels of 6729 µg/m³ and 116 µg/m³, respectively. This area is primarily occupied by traders who own businesses along the streets and is situated on a flat alluvial plain. The distribution of CO and $PM_{2.5}$ from various vehicles and small-scale industrial activities may have contributed to the increased levels of these pollutants in homes and some schools in the community. Tables 1 and 2 show the concentration differences of CO and $PM_{2.5}$ between South Tangerang and other nations. The adults' population faced the highest HQ risk for CO and PM2.5 with scores of 22,589 and 1.7, respectively. The highest and lowest THQ risks were measured at 8.992, and 1.386.

The highest and lowest risks of HQ for children with CO were 3.013 and 0.614, respectively. The highest HQ level of $PM_{2.5}$ was 0.180. In addition, the highest value of target hazard quotient (THQ) for CO of children was 7.370, whereas the lowest was 1.522 unit less. The highest level of THQ of PM 2.5 was 0.311 and the lowest was 0.037 unit less, respectively. According

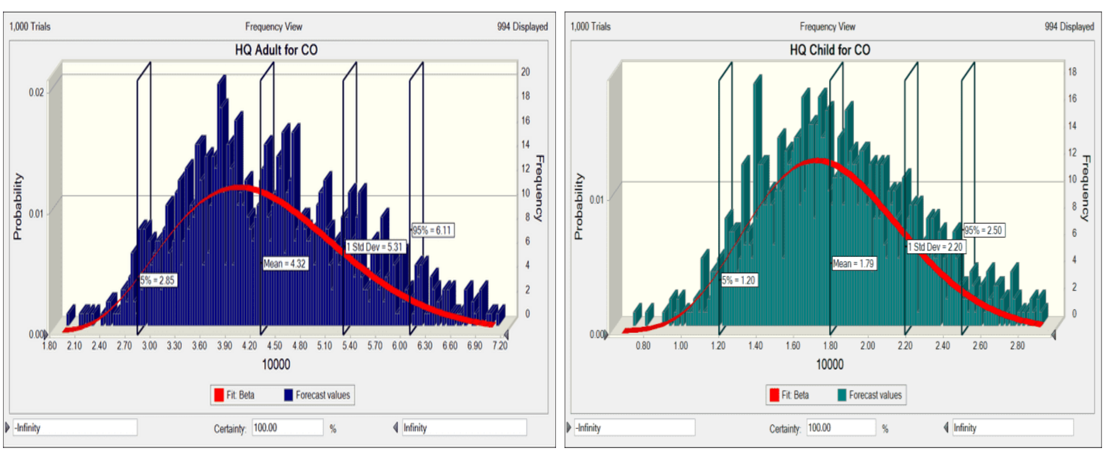

Fig. 3: The HQ adults and children for CO risk exposure

Table 1: Pollutant concentration, HQ, and THQ in adults in South Tangerang city, Indonesia

Sampling site	Measured concentration (µg/m³)		HQ		THQ		Coordinates	
	CO	$PM_{2.5}$	CO	$PM_{2.5}$	CO	$PM_{2.5}$	Latitude	Longitude
1	4010	31	8.481	0.151	5.359	0.093	106° 40' 26,015" E	6° 14' 40,078" S
2	2979	10	1.498	0.012	3.981	0.030	106° 39' 33,268" E	6° 14' 14,446" S
3	3093	11	2.038	0.017	4.133	0.033	106° 38' 58,610" E	6° 14' 51,864" S
4	2406	12	7.107	0.082	3.215	0.036	106° 39' 10,541" E	6° 15' 58,356" S
5	4563	55	6.780	0.188	6.098	0.165	106° 39' 16,027" E	6° 16' 58,282" S
6	2510	60	4.442	0.244	3.354	0.180	106° 39' 22,417" E	6° 16' 57,972" S
7	4557	38	5.508	0.106	6.090	0.114	106° 40' 38,600" E	6° 17' 19,064" S
8	1078	27	0.795	0.046	1.441	0.081	106° 41' 15,216" E	6° 18' 19,757" S
9	3594	38	4.756	0.116	4.803	0.114	106° 42' 52,344" E	6° 16' 26,155" S
10	5020	74	22.589	1.718	6.708	0.348	106° 43' 23,862" E	6° 16' 34,828" S
11	4663	32	3.835	0.061	6.231	0.096	106° 43' 34,277" E	6° 16' 17,810" S
12	3780	27	2.530	0.042	5.051	0.081	106° 44' 29,717" E	6° 16' 16,943" S
13	3551	22	3.527	0.050	4.745	0.066	106° 44' 52,359" E	6° 16' 14,914" S
14	4764	37	3.959	0.071	6.366	0.111	106° 44' 40,499" E	6° 16' 31,296" S
15	1089	22.8	1.196	0.056	1.455	0.068	106° 44' 2,076" E	6° 17' 27,917" S
16	2154	27	3.824	0.110	2.878	0.081	106° 45' 54,180" E	6° 17' 50,925" S
17	3666	19	3.852	1.046	4.899	0.057	106° 45' 33,739" E	6° 17' 48,095" S
18	3208	22	3.253	0.051	4.287	0.066	106° 45' 24,343" E	6° 18' 17,338" S
19	1834	34	1.270	0.054	2.451	0.102	106° 45' 57,366" E	6° 20' 15,133" S
20	1360	92	0.844	0.131	1.817	0.276	106° 44' 47,274" E	6° 19' 21,068" S
21	1922	96	2.857	0.328	2.568	0.288	106° 44' 16,213" E	6° 19' 4,116" S
22	6729	166	12.546	0.317	8.992	0.222	106° 43' 38,474" E	6° 18' 42,264" S
23	3551	26	2.425	0.041	4.745	0.078	106° 43' 5,405" E	6° 18' 33,610" S
24	1037	17	3.018	0.114	1.386	0.051	106° 43' 5,956" E	6° 18' 10,534" S
25	4552	38	14.396	0.276	6.083	0.114	106° 42' 13,036" E	6° 18' 56,419" S
26	2458	27	1.892	0.048	3.285	0.081	106° 42' 31,828" E	6° 19' 25,831" S
27	3817	29	1.622	0.028	5.101	0.087	106° 43' 58,008" E	6° 20' 36,582" S
28	2347	18	5.782	0.102	3.136	0.054	106° 42' 45,072" E	6° 20' 39,959" S
29	2510	60	4.604	0.253	3.354	0.180	106° 41' 45,467" E	6° 20' 49,078" S
30	1278	35	0.554	0.035	1.708	0.105	106° 40' 57,230" E	6° 21' 16,067" S
31	1468	98	1.224	0.188	1.962	0.294	106° 40' 16,241" E	6° 20' 49,056" S
32	4383	68	4.294	0.153	5.857	0.204	106° 39' 24,332" E	6° 20' 56,328" S
WHO, 2021 Standard	4000	15	1.0	1.0	1.0	1.0		

to a prior study, this region has the greatest total suspended particulate linked to metals (Rauf *et al.*, 2021; Rauf *et al.*, 2022). Furthermore, anthropogenic activities, weather, soil resuspension from roads, and other factors impact $PM_{2.5}$ pollution (Hua *et al.*, 2020; Shahri *et al.*, 2019; Sun *et al.*, 2019; Zhang *et al.*, 2022).

CO and PM2.5 concentration due to transportation mobile

Generally, from Monday to Friday, the concentrations of both CO and $PM_{2.5}$ pollutants are quite high as compared to Saturday and Sunday. In this study, the highest concentrations of CO pollutants were recorded at points 22, 14, and 11, with readings of 6729, 4764, and 4663 µg/m³, respectively. This was attributed to the increased traffic flow because workers and students used the same road, thereby leading to traffic congestion and, consequently, high concentrations of CO gas and $PM_{2.5}$. However, the lowest concentrations were recorded at points 24, 8, and 15, with readings of 1037, 1078, and 1089 µg/m³, respectively, which was attributed to fewer vehicles on the road and less crowded areas. The highest concentrations for $PM_{2.5}$ were at stations 22, 31, and 21, with concentrations of 166, 98, and 96

Table 2: Pollutant concentration, HQ, and THQ in children in South Tangerang city, Indonesia

Sampling site (Not all site collected)	Measured concentration (µg/m³)		HQ		THQ		Coordinate	
	CO	$PM_{2.5}$	CO	$PM_{2.5}$	CO	$PM_{2.5}$	Latitude	Longitude
3	3093	11	2.096	0.017	4.541	0.037	106° 38' 58,610" E	6° 14' 51,864" S
4	2406	12	1.167	0.013	3.532	0.041	106° 39' 10,541" E	6° 15' 58,356" S
6	2510	60	1.917	0.105	3.685	0.203	106° 39' 22,417" E	6° 16' 57,972" S
9	3594	38	2.646	0.064	7.370	0.128	106° 42' 52,344" E	6° 16' 26,155" S
13	3551	22	2.405	0.034	5.213	0.074	106° 44' 52,359" E	6° 16' 14,914" S
16	2154	27	1.837	0.053	3.162	0.091	106° 45' 54,180" E	6° 17' 50,925" S
18	3208	22	3.013	0.048	4.710	0.074	106° 45' 24,343" E	6° 18' 17,338" S
20	1360	92	1.160	0.180	1.997	0.311	106° 44' 47,274" E	6° 19' 21,068" S
21	1922	96	1.302	0.150	2.822	0.324	106° 44' 16,213" E	6° 19' 4,116" S
24	1037	17	0.614	0.023	1.522	0.057	106° 43' 5,956" E	6° 18' 10,534" S
25	4552	38	2.741	0.053	6.683	0.128	106° 42' 13,036" E	6° 18' 56,419" S
28	2347	18	2.349	0.041	3.446	0.061	106° 42' 45,072" E	6° 20' 39,959" S
29	2510	60	1.944	0.107	3.685	0.203	106° 41' 45,467" E	6° 20' 49,078" S
30	1278	35	0.879	0.055	1.876	0.118	106° 40' 57,230" E	6° 21' 16,067" S

µg/m³, respectively, while the lowest were recorded at three stations, namely, 2, 3, and 4 with 10, 11, and 12 µg/m³. During school hours and lunch breaks, as well as due to parked cars reducing road capacity on both sides of the road, the concentration of CO, and $PM_{2.5}$ pollutants are noted to be elevated from Monday through Friday, specifically during the day. Private cars are the primary vehicles parked in the area, often used for picking up school children and for dining purposes, as there are several eateries nearby. This congestion leads to increased emissions of CO and $PM_{2.5}$ from moving vehicles. Additionally, on weekdays, and school days in the afternoon, the concentration of CO, and $PM2_{.5}$ increases due to the higher traffic volume during after-work hours. Ambient air measurements show high levels of CO and $PM_{2.5}$ pollutants in the air, caused by multiple sources of pollution. The data indicate that traffic-related sources are the sole cause of the high levels of CO pollution. However, the accuracy of the calculations can be affected by various factors such as data collection, wind direction, and speed, humidity level, and atmospheric stability. In certain circumstances, these factors may not align with the area's conditions, resulting in higher calculated results. For example, when wind speed measurements are taken during low wind speeds, this may cause an increase in the concentration of CO and $PM_{2.5.}$

Health risk assessment of CO and $PM_{2.5}$ using HQ and THQ

The information provided pertains to quantifying health risks and potential health impacts resulting from reduced air pollution quality. This is accomplished by formulating a health risks assessment using the HQ and THQ indicators, as shown in Tables 1 and 2. The HQ and THQ values indicate the risks magnitude whether it is greater or less than 1, indicating a risk, or no risk.. It should be noted that this differs from the relative risk values obtained through a systematic review and meta-analysis of large, international epidemiological studies (Di Vaio *et al.*, 2018; Manisalidis *et al.*, 2020; Rajagopalan *et al.*, 2018; Turner *et al.*, 2020). This study evaluated the relationship between CO and $PM_{2.5}$ and the potential health risks. The toxicological risk was quantified by calculating the risk quotient for individuals who received an average dosage of CO and $PM_{2.5}$. Figs. 3 and 4 show the results of the health risk analysis conducted using the 5th and 95th percentiles. The findings indicated that children were at a higher health risk (HQ = 0.171) as compared to adults (HQ = 0.011) in South Tangerang. However, the health risk presented by exposure to CO and $PM_{2.5}$ in the ambient air was found to be lower than the USEPA limits, where HQ >1. Exposure to toxic chemicals associated with CO and $PM_{2.5}$

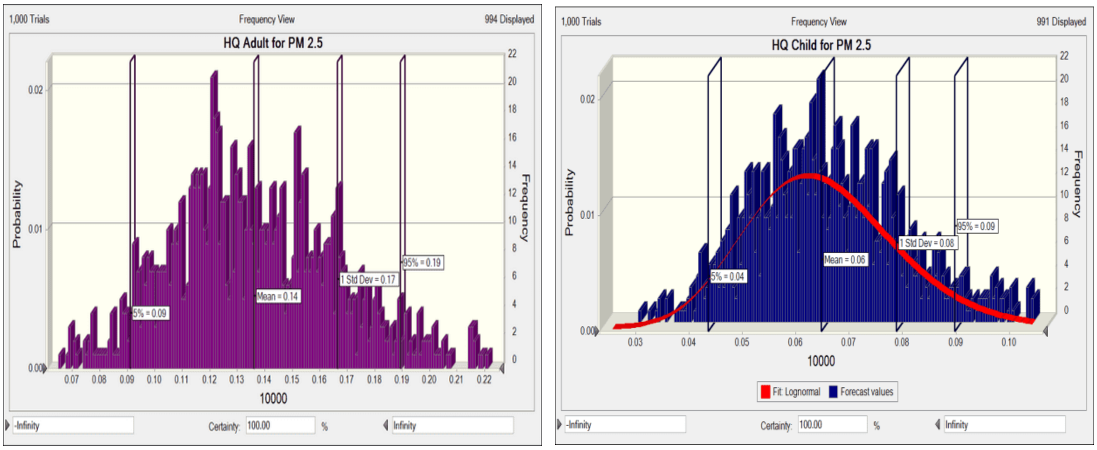

Fig. 4: The HQ adults and children for $PM_{2.5}$ risk exposure

could lead to health problems in both the short term and long term. Children's regular activities may make them more vulnerable to certain health issues than adults, such as an increase in the incidence (number new cases) of some diseases, such as; children malignancies, asthma, birth defects, neuro-developmental disorders and obesity has been observed. (De Oliveira *et al.,* 2012; Mallongi *et al.,* 2020). The production of pollutants such as CO and $PM_{2.5}$ is noted to be greater in regions with high levels of metal and gas air pollution (Rauf *et al*., 2021; Rauf *et al.,* 2022). Several factors, such as anthropogenic activities, weather, and soil resuspension from roads, may contribute to CO, and $PM2_{.5}$ pollution (Nkhama *et al.,* 2017; Shahri *et al.,* 2019).

Health risks assessment using Monte Carlo simulation

The MCS can be performed using the Crystal Ball software environment, assuming the model's behavior appears "reasonable." It is crucial to specify the number of simulation steps when commencing the simulation. The number of simulation steps was set to 10,000 in the analyzed company, meaning 10,000 values were generated within the simulation for each risk factor and criterion quantity (Iqbal *et al.,* 2020; Mallongi *et al.,* 2023). According to preliminary studies, Monte Carlo is a mathematical-statistical theory used in estimating HQ to account for the probabilities of uncertainties. In estimating the variation of HQ across various age groups, a probabilistic approach is adopted through the Monte Carlo technique, using 10,000 repetitions in addition to point estimation. The exposed group to pollutant emissions resulting from traffic exposure is the subject of the HQ estimation. The air pollutant concentration is significantly influenced by traffic density, mode of transportation, and trip distance (Guimaraes *et al.*, 2018; Han *et al.,* 2023; Laskowski *et al.,* 2018). MCS yields a frequency histogram of the criteria variable and automatically normalizes the probability distribution, which allows for the calculation of various statistical information. The number/probability distribution provides an overview of potential values and their likelihood, which is deemed significant for risk analysis. During the simulation, Crystal Ball calculates the sensitivity by determining the rank correlation coefficients between assumptions and forecasts (Wu *et al.,* 2021). Correlation coefficients serve as a valuable metric for quantifying the degree to which projections and assumptions fluctuate concurrently. A strong correlation coefficient between a forecast and an assumption indicates that the assumption has a significant impact on the forecast, both due to its uncertainty and its model sensitivity. Positive coefficients suggest that the assumption and forecast are positively related, while negative coefficients imply the opposite. This means that negative coefficients can result in more accurate risk assessments by reducing uncertainties and increasing precision in concentration measurements. Therefore, among all relevant factors, reducing the number of vehicles appears to be the most effective strategy to mitigate health risks among children and adults (Fallahzadeh and Ghadirian, 2018).

Uncertainty analysis using Monte Carlo simulation

Uncertainty analysis in this study was carried out using the Monte Carlo method for both CO and $PM_{2.5}$ risks. MCS model is known to be a sophisticated, advanced, and dependable method that generates an accurate and precise point estimate of risk. It calculates the likelihood of a health or ecological effect based on the relationship between exposure frequency and health or ecological impact, taking into account the level of exposure observed, and it also describes the element of uncertainty associated with the predicted risk. MCS was utilized in this investigation as a probability distribution to estimate risk or exposure and to ensure the assessment of elements linked to the uncertainty surrounding the anticipated risk. Oracle software, specifically Crystal Ball version 11.1.2, was employed in Microsoft Excel 2019 to conduct the simulation study.

HQ and sensitivity level of CO and $PM_{2.5}$ for adults and children

MCS results revealed that the probability of cancer risk occurrence at the 5th and 95th percentiles among adults were 2.85 and 6.11 for CO, indicating a medium risk. Meanwhile, the probability for $PM_{2.5}$ was 0.09 and 0.19, indicating low risks. The percentiles for children were 1.20 and 2.50 for CO as well as 0.04 and 0.09 for $PM_{2.5}$, respectively, thereby indicating a low probability of non-cancer risks. This is because children spend less time outside and more time inside their school rooms than adults. These results suggest that the South Tangerang regency population is at a medium risk of non-cancer health issues due to CO exposure in adults, whereas children have a lower probability of being at risk for both CO and $PM_{2.5}$. The greater the CO value, the greater the potential health risk for both adults and children.

According to Figs. 5 and 6, the sensitivity chart for adults shows that exposure frequency (fE) has the highest impact on health problems (20.8%), followed by CO concentration (C) (16.5%), time exposure (tE) (21.6%), duration time (Dt) (20.9%), and inhalation rate (20.2%). For children, CO concentration (C) is determined as the most significant factor (19.6%), followed by R (15.8%), Fe, Dt, and tE with 18.8, 21.8, and 21.1%. This indicates that reducing exposure time and monitoring vehicle emissions near residential areas are important to minimize the negative effects of CO pollution. In addition, exposure frequency (fE) is the primary contributor to $PM_{2.5}$ risks for adults (23%), followed by C, tE, Dt, and R with percentages of 18.8, 16.6, 21.4, and 20.3%, respectively. The information presented shows that as the frequency of discrete exposure occurrences increases, the risk of inhabitants being affected by air pollution also rises. Therefore, to

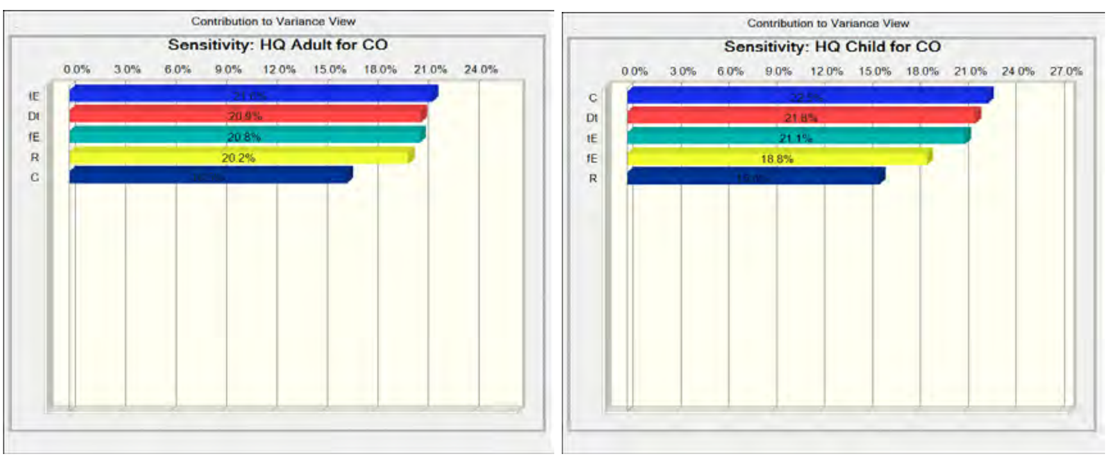

Fig. 5: The analysis of sensitivity percentage of HQ adults and children for CO

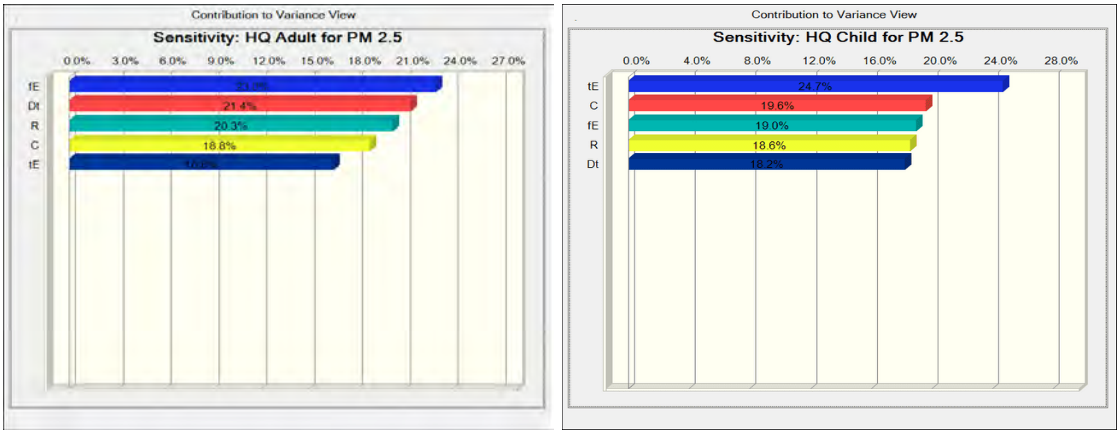

Fig. 6: Sensitivity percentage of HQ adults and children for $PM_{2.5}$

lessen their exposure to air pollution containing dangerous compounds, both kids, and adults should limit their activities and utilize safety gear. Sensitivity analysis also revealed that body weight (BW) made a negligible contribution, suggesting that it could be disregarded as a significant factor in this context. Similar to the results of Guimaraes research to reduce activities and shorten trips. Use protective equipment when using transportation in the Rio de Janeiro metropolitan area (Guimaraes *et al.*, 2018).

CONCLUSION

In conclusion, of these 32 stations, the level of CO concentration exceeded the WHO standard of 4000 µg/m³ in 9 stations. However, only three stations had $PM_{2.5}$ concentration lower than WHO standard (15 µg/m³), which means that there is high $PM_{2.5}$ pollutant concentration in the 32 stations. A significant percentage of the calculation values of HQ risk for CO of adults exceeded the standard or >1 state at risk, with only three lower than 1 and still safe. Most HQ values for PM2.5 were lower than 1, indicating safety, while all other 29 stations exceeded the WHO standard and were at risk. These findings were consistent with THQ calculation results for both CO and $PM_{2.5}$ at the 32 stations.

In adults, the likelihood of cancer risk incidence at the 5th and 95th percentiles was 2.85 and 6.11 for CO, indicating medium risks. The corresponding values for $PM_{2.5}$ were 0.09 and 0.19, indicating moderate risks. In contrast, for children, the percentiles for CO were 1.20 and 2.50, whereas for $PM_{2.5}$, the percentiles were 0.04 and 0.09, respectively, indicating a minimal likelihood for non-cancer risk occurrence. This may be due to the fact that children spend more time indoors, such as in classrooms, resulting in shorter exposure times as compared to adults. The exposure frequency (fE), concentration (C) of CO (with 16.5%), time exposure (tE) (with 21.6%), duration time (Dt) (with 20.9), and inhaling rate (with 20.2%) were also found to be important factors in the adults' increased health problems. However, in children, the most significant factor was CO concentration at 19.6%, followed by R at 15.8%, Fe, Dt, and tE at 18.8%, 21.8%, and 21.1%, respectively. This suggested that the concentration of CO pollution had the greatest impact on the prevalence of harmful health effects in both adults and children. The risk to occupants increases as exposure events occur more frequently. Therefore, both adults and children should limit their activities and utilize safety gear to reduce their exposure to air pollution containing hazardous compounds such as CO and $PM_{2.5}$.

AUTHOR CONTRIBUTIONS

E. Ernyasih performed the literature review, methodology, and experimental design, analyzed and interpreted the data, and prepared the manuscript text and manuscript edition. A. Mallongi performed the simulation model, experiments, and literature review, compiled the data, and helped in manuscript preparation. A. Daud helped in the literature review, compiled the data, and helped in manuscript preparation. S. Palutturi performed some of the remaining experiments and managed data curation and validation. R. Thaha reviewed the draft and interpreted the results and the model simulation. E. Ibrahim made a presentation draft review and data curation. W. Al-Moudhun helped in the literature review and manuscript preparation.

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CONFLICT OF INTEREST

The authors declare no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication, and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celcius
μg/m³	Microgram per cubic meter
μg/kg/day	Microgram per kilogram/day
ADDinh	Acceptable daily dose inhalation
AT	Averaging time
BW	Body weight
C	Concentration
CI	Confidence interval
CO	Carbon monoxide
COHb	Carboxyhemoglobin
Dt	Duration time
dpl	above sea level
ED	Exposure duration
EDI	Estimated daily intake
EF	Exposure frequency
Eq	Equation
ET	Exposure time
fE	Frequency of exposure
FINS	Fasting Insulins
Fig.	Figure
FIR	Food ingestion rate (300 grams/ person/day)
HI	Hazard index
HOMA-B	Homeostatic model assessment of beta cell function
HOMA-IR	Homeostatic model assessment insulin resistance
HQ	Hazard quotient
Inhrate	Inhalation rate
IR	Intake rate

MCS	Monte Carlo simulations
mg/m³	Miligram per cubic meter
m/s	meters per second
NO_2	Nitrogen dioxide
$PM_{2.5}$	Particulate matter 2.5
PM10	Particulate matter 10
THQ	Target hazard quotients
TSP	Total Suspended Particulate
tE	Time exposure
USEPA	United States Environmental Protection Agency
R	Rate
RfC	Reference concentration
VOC	Volatile organics compound
WHO	World Health Organization
YLL	Years of life lost

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CASE STUDY

Consequences of changing regional integration on environmental development, agricultural markets, and food security

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ABSTRACT

BACKGROUND AND OBJECTIVES: Geopolitical risks have made significant changes in the integration ties between the countries of Central Asia, which has affected the sustainable development of the agro-industrial complex and food security. The adoption of urgent measures to improve food security, on the one hand, is a necessary condition for the development of the Republic of Kazakhstan, but on the other hand, it can lead to a decrease in the level of environmental security in the country if the possible consequences in the reintegration process are not considered. Therefore, this article aimed to examine the sustainable agriculture practices implemented in Kazakhstan's agro-industrial complex to improve food security and reduce the environmental impact of agriculture.
METHODS: To collect information, a mixed research strategy combining qualitative and quantitative methods was used. The collection of information was carried out in the summer of 2022 and consisted of three stages. In the first stage, statistical information was collected; in the second stage, a correlation analysis was carried out; and in the third stage, a survey of 40 experts was conducted.
FINDINGS: The data obtained indicate that the efficiency of Kazakhstan's agricultural production strongly and directly depends on the level of the country's integration into the united Eurasian Economic Union market. Due to the geographical features of Kazakhstan's location, it is necessary to develop areas that are primarily focused on the internal capabilities of the country. Internal changes will help the industry increase international competitiveness and efficiency while promoting sustainable development and ensuring food security and environmental safety.
CONCLUSION: The article determined that it is necessary to prioritize developing multilateral partnerships to address transportation and logistics challenges in the export and import of agricultural products, and reduce dependence on the import of seeds, breeding products, fodder, and agricultural machinery. However, to effectively promote these areas, it is necessary to improve food security through the introduction of sustainable agriculture practices, such as crop diversification, conservation agriculture, integrated pest management, and drip irrigation.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
46	6	6

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INTRODUCTION

Global upheavals related to the persistence of crises in the global financial and economic system, the coronavirus disease 2019 (COVID-19) pandemic, military conflicts, and sanctions have shown the vulnerability of many agricultural and food systems and exacerbated the decline of food security in the world (Kryshtanovych *et al.,* 2022). It can be argued that representatives of particularly developed countries of the world are actively predicting a humanitarian catastrophe that must be averted (WFP and FAO, 2022). The Eurasian continent, particularly its central region, is currently experiencing a state of reintegration. Due to their geographical location, countries need to look for ways to improve food security by intensively developing the agro-industrial complex while avoiding damaging environmental safety in the region (Beilin *et al.,* 2019). The Eurasian Economic Union (EAEU) is the integration association that includes five member countries of the post-Soviet space (Armenia, Belarus, Kazakhstan, Kyrgyzstan, and Russia) and has significant potential to become a powerful subject of the Eurasian continent (Ametoglo *et al.,* 2018). One of the main objectives of the EAEU is to support the principles of sustainable development, which are based on three main principles: economic, social, and environmental sustainability (Liu *et al.,* 2023). Considering the focus of the article, the main attention is drawn to the topic of environmental sustainability. Environmental sustainability aims to develop uniform environmental standards, encourage the use of renewable energy, improve energy efficiency, and develop sustainable agriculture (Alekseenkova *et al.,* 2017). The development of sustainable agriculture helps ensure food security (Li *et al.,* 2021), conserve biodiversity, and reduce the impact of agriculture on the environment (Khoruzhy *et al.,* 2022). These initiatives can be implemented through regional integration, trade, socially responsible development, environmental protection, and cooperation in various fields. Therefore, the consequences of the aggravation of international conflicts and economic restrictions that occurred in 2022 started to affect the integration ties between partner countries (Bezpalov *et al.,* 2023). Practical interactions between EAEU countries and neighboring territories are seeking new ways to function in the current environment (Makhmutova *et al.,* 2019). The agricultural sector is seeing a reorientation of participants, causing a redistribution of cash flows toward new food markets and marketing channels for agricultural products and fertilizers (Ng'andu *et al.,* 2021). This disruption may negatively impact not only Kazakhstan's sustainable development, but also EAEU countries in general (Marrella *et al.,* 2021). One of the main reasons for such changes is the reduction in investment from Russian businesses in Kazakhstan's agricultural sector, as well as a decline in access to Russian technology and equipment (Suslov *et al.,* 2018). This has hindered the growth and modernization of Kazakhstan's agricultural sector, which is crucial for ensuring food security in the country (Leka *et al.,* 2022). The most acute argument that can be highlighted in the sphere of the provision of agricultural food is the attitude toward the discriminatory decision of the Russian Federation in the EAEU on the temporary ban on the export of grain (wheat, corn, rye, and barley) to the EAEU (DGRF, 2022). Preliminary data suggest that the decision had a negative impact on business in Kazakhstan (Tsirkulik, 2022). The country's leadership claims that it is because of these actions, in particular, that up to 70 percent (%) of the mills in Kazakhstan have been forced to stop or continue operating without a reserve (Kalinkina, 2022). Another instance of a conflict situation, the closure of the transport corridor for Kazakhstan's imports of foreign equipment, seeds, and spare parts through Russia, may severely impair the agricultural industry of Kazakhstan (Sarabekov, 2022). The situation in which EAEU countries find themselves due to the introduction of ten packages of sanctions against Russia and Belarus by the United States, the EU, and the United Kingdom in response to the special military operation in Ukraine can be described as complex, jeopardizing the existence of the EAEU in its former form, which cannot affect the development of the agricultural products market (Wang *et al.,* 2021). Yet, from another perspective, the consequences of the armed conflict in Ukraine (Sutyrin *et al.,* 2018) give reason to conclude that for EAEU member states, the partnership can be very promising (Zhavoronok *et al.,* 2022). The escalation of the conflict has exacerbated trade, production, price, and logistics risks. According to preliminary estimates by the Food and Agriculture Organization (FAO), the conflict will result in 20–30% of the areas under winter grains, corn, and sunflowers in Ukraine either not being planted or remaining unharvested during the 2022/23 season (FAO, 2022). This state of affairs encourages the enhancement of cooperation within the EAEU regional integration framework, as well as with other countries, which will allow loading production facilities, creating additional jobs, and expanding the geography of exports of agricultural products to many countries of the world (Pyzhikov, 2022). EAEU member states have additional opportunities to advance their own agricultural production, which will not only strengthen their food security, but will also increase the export potential of the agro-industrial complex (Nechaev *et al*., 2021). For example, the reduction in exports from Ukraine and Russia provides an opportunity for Kazakhstan to strengthen its agricultural export market and revise its markets by establishing new regional integration. Regional integration systems are a relatively new subject of research, although they have been contributing to geo-economic development for many years. Regional integration is a multidimensional process that can take the form of coordination, cooperation, convergence, and deep integration initiatives and extends not only to economic and trade issues but also to political, social, cultural, and environmental aspects (Lavery and Schmid, 2021). The purpose of this study is to study the development of Kazakhstan's agro-industrial complex by implementing sustainable agriculture practices to improve food security while reducing the impact of agriculture on environmental safety. This study was performed in the Almaty region of Kazakhstan in 2022.

MATERIALS AND METHODS

This was a joint study between Turan University, Narxoz University, Al-Farabi Kazakh National University, Kazakh Research Institute of Economy of Agro-Industrial Complex and Rural Development, Almaty Academy of Economics and Statistics, and the University of International Business. The researchers working in the universities collaborated to design and conduct the study. Involvement in the data collection and analysis process allowed them to apply a consistent mixed-method approach that incorporated both qualitative and quantitative methods. The study included a sectoral analysis of the development of the market of agricultural products of the EAEU, a correlation analysis of the influence of specific factors on the gross regional product of agriculture of Kazakhstan, and an expert evaluation of motivational factors and barriers to the development of production, which informed policies related to agricultural development and investment. The researchers' expertise in the fields of agriculture and economics played a critical role in the success of the study. Their in-depth knowledge of the subject matter and the statistical tools used in the analysis helped ensure the accuracy and reliability of the results. The researchers' ability to work collaboratively and share their knowledge and skills was instrumental in developing a robust and comprehensive analysis of the agricultural market in the EAEU.

This study has been conducted in three stages:

Stage 1 was prepared an information database of statistical data that included official sources of statistics. The statistical information used in the survey was obtained from a variety of official sources of statistics, such as the FAOSTAT, UN Comtrade, WTO, IFOAM, the Ministry of National Economy of the Republic of Kazakhstan, the Ministry of Agriculture of the Republic of Kazakhstan, and the Ministry of Trade and Integration of the Republic of Kazakhstan. These data were used in the correlation analysis to assess the impact of integration factors on the development of Kazakhstan's national agricultural market within the EAEU.

Stage 2 involved a correlation analysis. To assess the impact of integration factors on the development of the national agricultural market of Kazakhstan within the EAEU, a correlation analysis was carried out using the "data analysis" tool in the Excel environment. In studying the impact of integration factors on the development of Kazakhstan's national agricultural market within the EAEU, it is important to consider both dependent and independent variables. The dependent variable was the value of the gross value added (GVA) of agriculture as one of the most important factors characterizing the economic efficiency of the industry. In the context of regional integration, it is particularly relevant to assess the GVA of agriculture in each country of the regional organization. This allows for a comparison of the relative importance of agriculture in the economies of different countries, and can inform policies related to agricultural development and investment. Additionally, it can help identify countries with the potential for growth in their agricultural sectors, as well as countries that may require additional support to strengthen their agricultural industries. As the dependent variable in the analysis, the GVA of agriculture measures the economic output generated by the agricultural industry in Kazakhstan. It is an important indicator of an industry's overall economic efficiency and productivity. The independent variables in the analysis are the integration factors that characterize the level of integration of the country into the common agricultural market of the EAEU (Table 1).

Kazakhstan's exports to EAEU countries, as well as its exports outside EAEU countries, contribute to the overall economic growth of the country. According to the World Bank, agriculture is a key sector in Kazakhstan's economic development. Kazakhstan's share in agricultural production in the EAEU, along with its share of the volume of investment in the agricultural sector of the EAEU countries, indicates the country's position and importance in the regional agricultural sector. As a member of the EAEU, Kazakhstan benefits from various trade agreements and partnerships that enable it to boost its agricultural production and exports. The agricultural price index is an important parameter that reflects the overall performance of the agricultural sector. According to the FAO, the agricultural price index is an indicator of changes in international prices. A high agricultural price index is beneficial for the agricultural sector as it indicates higher prices for agricultural products and higher revenue for farmers. Understanding the impact of dependent and independent variables on the development of the national agricultural market of Kazakhstan within the EAEU is critical for making informed decisions regarding the development of the sustainable agricultural sector in the region.

Stage 3 included the administration of an expert survey. At this stage, based on the purposive sampling method (Jinji *et al.,* 2022), a group of 40 experts was formed. A detailed questionnaire consisting of semi-formalized questions was developed for feedback. The respondents included in the sample and registered in the web panels were sent invitations to participate in the online survey. The survey was administered online using WRI Survey Monkey subscription services. Responses were received from 34 experts out of the sample, including 27 agricultural producers operating in Kazakhstan and two importers and five exporters of agricultural products. To obtain quantitative information from the questionnaire, the method of scaled assessment was applied. Thus, some of the questions in the questionnaire involved an assessment on a five-point scale. The use of a five-point scale is a common method for gathering quantitative data in agricultural market research surveys (Dutbayev *et al.,* 2023). This type of scale provides a standardized way of measuring the opinions of survey participants, making it easier to analyze and compare responses. The use of a scale also helps to reduce response bias, as participants are forced to make decisions based on a predefined set of options. It allows researchers to obtain a more precise measurement of the importance of various factors related to the development of the agricultural industry. In this survey, experts were asked to assess

Table 1: Description of independent variables and sources of information

Independent variables of the model	Interpretation	Information base
Kazakhstan's exports to EAEU countries, million United States Dollars (USD mln.)	Characterizes the export potential of the country in trade with EAEU countries	EEUF (2022)
Kazakhstan's exports outside EAEU countries (USD mln.)	Characterizes the total value of goods exported by Kazakhstan to countries outside the EAEU	
Kazakhstan's share in agricultural production in the EAEU (%)	Allows considering the country's contribution to the production of agricultural products in the EAEU market	
Kazakhstan's share in the volume of investment in the agricultural sector of the EAEU countries (%)	Allows considering the country's investment contribution to the development of agriculture within the EAEU	
Agricultural price index	Allows monitoring changes in sales prices of major agricultural products through all sales channels	

the importance of various factors using a scale where:

1 – Very low importance of the factor
2 – Rather low importance of the factor
3 – Moderate importance of the factor
4 – Rather high importance of the factor
5 – Very high importance of the factor

The data obtained in the form of cross-tables were exported to Excel. After processing the responses, the possible solutions were ranked based on their importance. The data obtained were then summarized in tables of the main factors and barriers for the development of the agro-industrial complex for opportunities to increase food security and reduce risks.

RESULTS AND DISCUSSION

Analysis of the development of the agricultural market of the EAEU

The growth of agricultural production in Kazakhstan is significant not only for the country itself, but also for its potential vendors. Kazakhstan's main trading partners in the agricultural sector are countries such as Russia, Belarus, Uzbekistan, and Kyrgyzstan. Over the past five years, the volume of agricultural production in Kazakhstan has increased by 38%, exceeding the same figures of the EAEU member states. At the same time, Kazakhstan's share in total production increased from 11% to 12.8% (Fig. 1).

Over the five-year period, Kazakhstan has shown the highest growth rate among the EAEU countries in the number of cattle (+21%), sheep and goats (+13.79%), and poultry (+17.4%). Herewith, Kazakhstan's share of the total number of EAEU livestock increased from 21.4% to 25.3% for cattle, from 37% to 42.6% for sheep and goats, and from 5.7% to 7% for poultry. The volume of agricultural production in Kazakhstan in monetary terms increased in the considered period from 12,553 million USD in 2017 to 17,318 million USD in 2021. Kazakhstan shows the greatest growth rate of agricultural production among the EAEU countries, being well above the average for the integration union (Table 2).

Kazakhstan has significantly increasing its contribution to the volume of foreign trade in food products and agricultural raw materials among EAEU member states. Kazakhstan has more than doubled its production of livestock and poultry for slaughter in five years, raising its share of the EAEU total from 7.9% to 10.7%. Milk production increased by 12.6%, with Kazakhstan's share in total Eurasian milk production going up from 12.1% to 12.7%. During the period under review, there was an increase in the gross yield

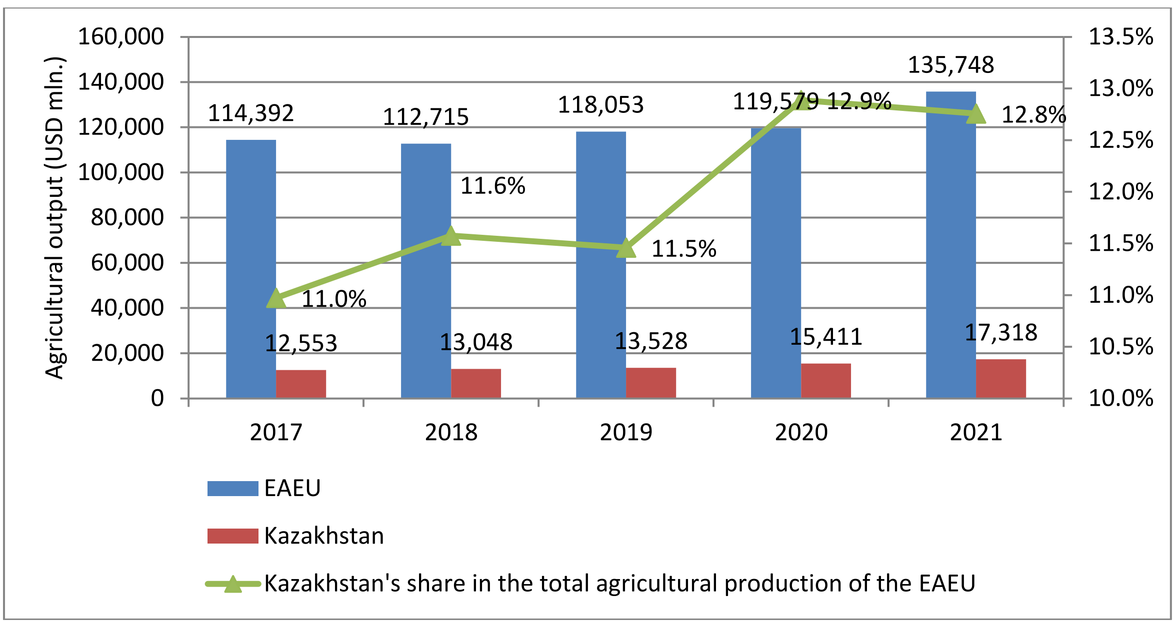

Fig. 1: Dynamics of agricultural production in the EAEU and Kazakhstan, USD million

Table 2: The volume of agricultural production in 2017-2021, USD million (EEUF, 2022)

EAEU countries	2017	2018	2019	2020	2021	Change between 2021 and 2017	
						USD mln.	%
EAEU	114,392	112,715	118,053	119,579	135,748	21,356	18.67%
Armenia	1,882	1,928	1,853	1,774	1,940	58	3.07%
Belarus	9,333	9,236	9,881	9,248	9,843	510	5.47%
Kazakhstan	12,553	13,048	13,528	15,411	17,318	4,765	37.96%
Kyrgyzstan	3,028	2,977	3,166	3,226	3,832	804	26.55%
Russia	87,596	85,526	89,625	89,920	102,815	15,219	17.37%

Table 3: Gross harvest volumes and Kazakhstan's share in the total production of the EAEU

Crop produce group	Gross harvest, thousand tons		Change in gross harvest over five years		Share in total gross output of the EAEU (%)	
	2017	2021	Thousands of tons (ths. tn)	%	2017	2021
Cereals	20,585	16,376.0	-4,209.0	-20.4%	12.4%	11.2%
Sunflower seed	903	1,032.0	129.0	14.3%	7.9%	6.2%
Sugar beet	463	332.0	-131.0	-28.3%	0.8%	0.7%
Potatoes	3,551	4,032.0	481.0	13.5%	10.6%	14.0%
Vegetables	3,791	4,768	977.0	25.8%	17.8%	22.0%
Fruits and berries	255	356	101.0	39.6%	6.40%	6.40%

in the physical terms of most types of crop products in Kazakhstan: sunflower seeds, potatoes, vegetables, fruits, and berries (Table 3).

The lower production of various agricultural products in Table 3, despite the overall increase in the volume of agricultural production in Kazakhstan between 2017–2021 (Table 1), can be attributed to the negative dynamics of the gross harvest rates. However, certain sectors, such as milk production and production of livestock and poultry for production of more resistant crops, had a positive impact and helped contribute to the overall growth in the volume of agricultural production, as shown in Table 1. Kazakhstan occupies a leading position among EAEU countries in terms of growth rates in the production of cereals, sunflower seeds, vegetables, fruits, and berries. Meanwhile, the negative dynamics of the rates of the gross harvest of sugar beet, cereals, and leguminous crops in Kazakhstan should be noted: a decrease of 28.3% and 20.4%, respectively. Among the main reasons for the decline in grain production in Kazakhstan are low yields due to difficult agro-climatic conditions (Fig. 2).

Over the five years, a decrease in the yield of grain and leguminous crops was observed in all EAEU countries. The most significant drops occurred in Armenia (from 20 to 12.9 quintals per hectare [q/ha] [-35.5%]), in Kyrgyzstan (from 30 to 22.5 q/ha [-25%]), and in Kazakhstan (from 13 to 10.4 q/ha). In the mutual trade of EAEU countries, agricultural products and food products account for more than 18% of the commodity structure, while in trade with foreign partners, their share amounts to 10%. Overall, EAEU countries demonstrate significant growth rates in agricultural exports to third parties. In five years, this figure increased by 73.4%, exceeding the same indicator of such a large integration association as the EU. In particular, exports of meat and edible meat byproducts from the EAEU increased 4.8 times, exports of dairy products and eggs rose 2.5 times, exports of fats and oils of animal and vegetable origin became 2.5 times higher, exports of cereals grew 1.5 times, etc. Comparatively, according to Eurostat, the growth rate for exports of meat and meat offal of such a large world integration association as the EU was only 11%, 12.6% for exports of dairy products and eggs, and 19.8% for fats and oils (Eurostat, 2022). The volume of foreign trade turnover of agricultural products and food products in Kazakhstan in value terms increased by 33.4%, while the volume of mutual trade between Kazakhstan and other EAEU countries increased by 37.9%. The export

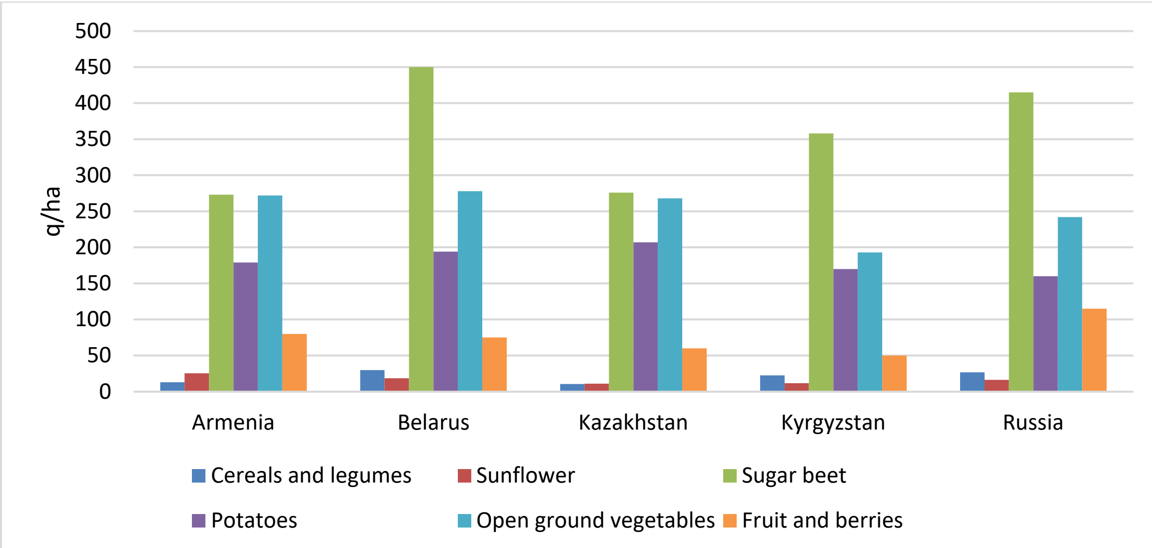

Fig. 2: Agricultural crop yields in the EAEU countries in 2021, q/ha

of agricultural raw materials and food products from Kazakhstan to the foreign market in value terms rose by 1,119.5 million USD (+58.2%). The growth in exports has led to an increase in the income of domestic agricultural producers and has created new opportunities for investment, as well as influenced the development of state programs to support the agricultural industry (SPDAICRK, 2018). The increase in exports has also led to the development of new infrastructure, such as transportation networks (Polukhina, 2021), storage facilities (SPDAICRK, 2018), and processing plants (OIRPMRK, 2019), which have improved the efficiency and competitiveness of the sector. At the same time, Kazakhstan's share in the total volume of exports of agricultural products of EAEU countries decreased from 9.4% to 8.5%. The most export-oriented goods in Kazakhstan are cereals (wheat and barley), sunflower seeds, sunflower oil, cheeses, grapes, berries, stone fruits, and pasta. Although the increase in Kazakhstan's average agricultural prices for wheat is more modest compared to other EAEU countries (+15.6%), it has reached 223.7 thousand USD per ton, which is 40% higher than Belarusian prices and 15.4% higher than Russian prices. Compared to other EAEU countries, the most competitive productions of Kazakhstan in terms of prices are the production of vegetables (onions, cabbage, and carrots), eggs, and pork in live weight (EAEU, 2022). EAEU countries' competitiveness indices by the price of agricultural production are presented in Table 4.

Despite the restrained growth, in comparison with other EAEU countries, of the average agricultural prices for wheat (+15.6%), this indicator in Kazakhstan has reached 223.7 thousand USD per ton, which is 40% higher compared to Belarus and 15.4% higher than the prices of Russian agricultural producers. Also, a significant increase in the value of rye production (+121.2%) was observed in 2021. Meanwhile, it should be noted that the growth dynamics of the average prices of agricultural producers in Kazakhstan for such products as barley, oats, corn grain, rice, potatoes, onions, cabbage, carrots, and pork are significantly lower than in other EAEU member countries. In general, the efficiency of agricultural production in Kazakhstan is inferior to that in Armenia and Russia (Fig. 3).

Each dollar of agricultural production in Kazakhstan accounts for 0.57 USD of the GDP. Meanwhile, in 2021, the same indicator reached 0.81 USD in Armenia and 0.66 USD in Russia.

Assessment of the impact of integration factors on the results of the development of Kazakhstan's agricultural industry

Initial data for the assessment of the integration factors affecting the gross value added of Kazakhstan's

Table 4: Competitiveness indices of EAEU countries by price of agricultural products

Types of products	Armenia	Belarus	Kazakhstan	Kyrgyzstan	Russia
Wheat	0.00	1.00	0.62	0.18	0.81
Barley	0.00	1.00	0.79	0.22	0.80
Potatoes	0.00	1.00	0.77	0.34	0.64
Onions	0.00	0.60	1.00	0.61	0.70
Cabbage	0.00	0.53	1.00	0.62	0.39
Carrots	0.00	0.97	1.00	0.63	0.98
Pome fruits	0.36	1.00	0.12	0.18	0.00
Stone fruits	0.96	1.00	0.00	0.95	0.35
Raw milk	0.70	0.84	0.76	1.00	0.00
Butter	0.00	0.75	0.59	1.00	0.34
Cattle meat in live weight	0.00	1.00	0.81	0.61	0.85
Pork meat in live weight	0.00	1.00	0.97	0.83	0.94
Poultry meat	0.00	1.00	0.88	0.71	0.88
Pasta products	0.56	1.00	0.28	0.89	0.00
Eggs	0.00	0.95	1.00	0.44	0.89

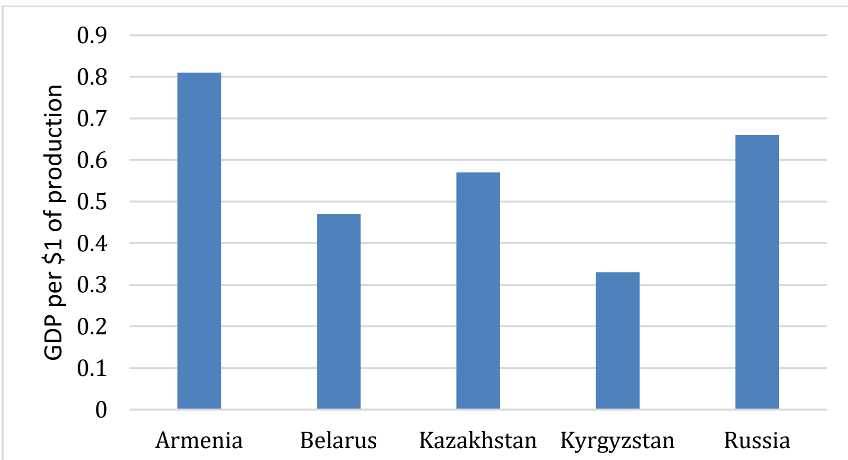

Fig. 3: Efficiency of agricultural production of EAEU countries in 2021

agricultural industry can be found in Table 5.

Kazakhstan accounts for 12.8% of agricultural production in the EAEU. Over five years, the country has taken a leading position in terms of the growth rate of cattle, poultry, grain, sunflower seeds, vegetables, fruits, and berries, as well as significantly increasing its contribution to the volume of foreign trade in food products and agricultural raw materials by EAEU countries. Among the EAEU member states, Kazakhstan is the largest exporter of live farm animals and products from the flour and cereal industries. Kazakhstan takes second place after Russia in terms of grain and oilseed exports. However, the level of the country's price competitiveness in the production of wheat, for example, is significantly inferior to Belarusian and Russian products. Also, the efficiency of agricultural production in Kazakhstan is at an average level and is currently inferior to that of Armenia and Russia. The analysis indicates that the GVA of Kazakhstan's agricultural sector has a strong positive correlation with factors such as the country's share in total agricultural production and total investment in the agricultural sector of the EAEU countries (Table 6).

Furthermore, the analysis reveals a strong relationship between the GVA of Kazakhstan's agricultural industry and the country's exports to the foreign market and the EAEU countries. Thus,

Table 5: Data for assessing the impact of integration factors on gross value added in Kazakhstan's agricultural industry

Year	GVA of agriculture (USD mln.)	Exports outside EAEU countries (USD mln.)	Share in agricultural production in the EAEU (%)	Investment in the agricultural sector of the EAEU countries (%)	Agricultural price index
2017	7,534.6	1,924.5	11.0	7.4	104.7
2018	7,883.4	2,507.5	11.6	7.1	102.8
2019	8,113.8	2,643.8	11.5	8.1	114.6
2020	9,223.6	2,697.0	12.9	9.1	113.9
2021	9,867.4	3,049.4	12.8	10.9	115.6

Table 6: Results of correlation analysis of the GVA of Kazakhstan's agricultural industry and integration factors

Variable	GVA of agriculture (USD mln.)	Exports outside EAEU countries (USD mln.)	Exports to EAEU countries (USD mln.)	Share in agricultural production in the EAEU (%)	Investment in the agricultural sector of the EAEU countries (%)	Agricultural price index
GVA of agriculture (USD mln.)	1.00					
Exports outside EAEU countries (USD mln.)	0.86	1				
Exports to EAEU countries (USD mln.)	0.83	0.98	1			
Share in agricultural production in the EAEU (%)	0.95	0.82	0.83	1		
Investment in the agricultural sector of the EAEU countries (%)	0.96	0.79	0.74	0.83	1	
Agricultural price index	0.77	0.75	0.80	0.67	0.80	1

to increase the industry's GVA, Kazakhstan needs to raise the volume of agricultural production and exports and enhance investment in the development of the industry.

Analysis of factors and barriers to the development of the agro-industrial complex for opportunities to increase food security and reduce risks

According to the results of an expert survey, the main risks of reducing food security associated with the Russian–Ukrainian conflict for Kazakh agricultural producers are higher prices for raw materials, disruptions in logistics chains, and a decrease in the price competitiveness of Kazakh products (Fig. 4).

Meanwhile, some Kazakh experts believe that the limitations associated with anti-Russian sanctions are conducive to the development of the national economy and an impetus for expanding the volume of domestic agricultural production. Most experts (73.5%) argued that the present conditions call for an enhancement of cooperation and mutual trade within the EAEU. In turn, only 8.8% of the experts asserted that it is necessary to loosen ties within the EAEU and move to other, non-sanctioned markets. Most of the surveyed experts believe that the sanctions related to the Russian–Ukrainian conflict offer a set of opportunities for the development of the national market for agricultural products. The most notable possibilities indicated by the experts were the chances of increasing exports to foreign markets and expanding the volume of mutual trade with EAEU countries (Fig. 5).

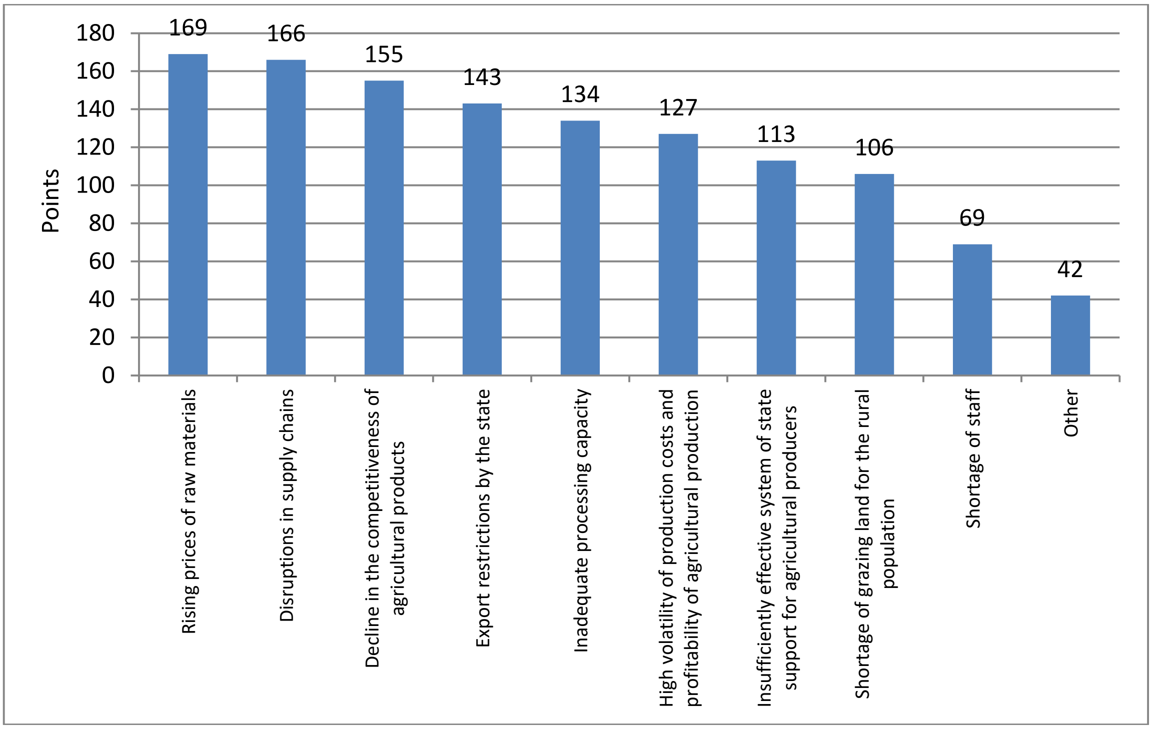

Fig. 4: The main risks of agricultural production in Kazakhstan associated with special operations in Ukraine

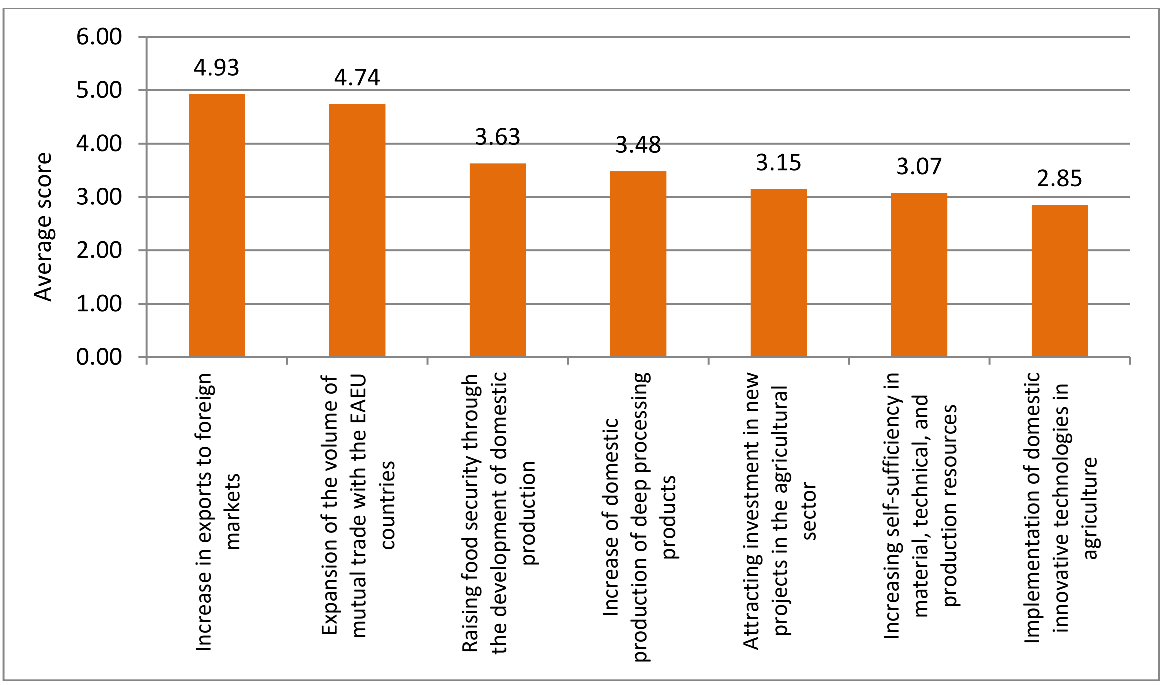

Fig. 5: Opportunities for the development of Kazakhstan's agricultural industry, average

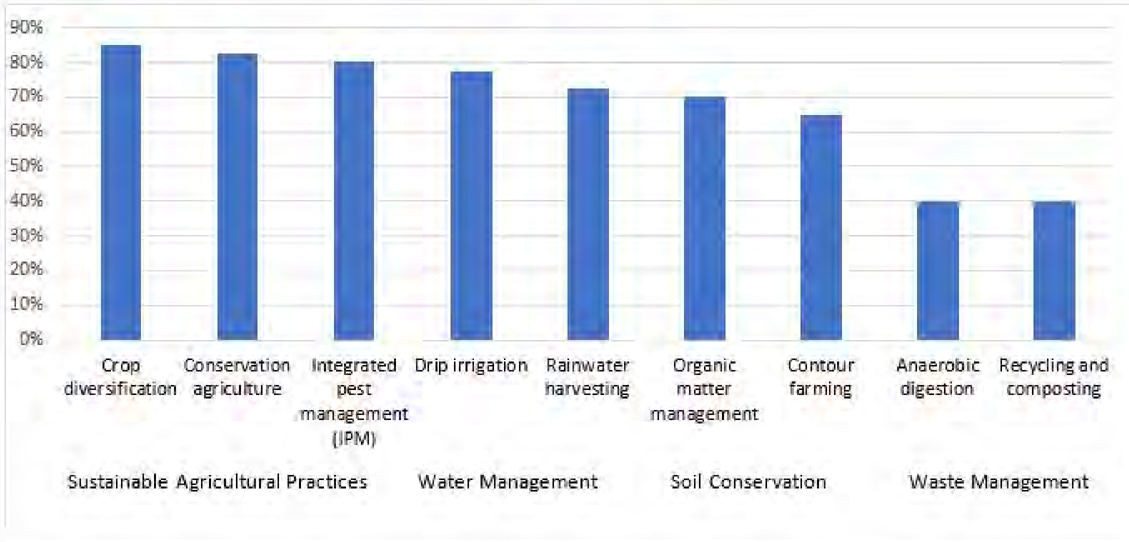

Fig. 6: Expert opinion on the risks of reducing the level of environmental safety

Also, in the experts' opinions, the sanctions imposed by Western countries will give an impetus to reinforce integration within the EAEU and contribute to the development of domestic agricultural production and deep processing products. However, according to experts, the intensive development of the agro-industrial complex in Kazakhstan can reduce the level of environmental safety. Therefore, experts believe that it is necessary to monitor and orient producers to use sustainable agricultural practices, water management, soil conservation, and waste management, which in turn include some measures (Fig. 6).

Based on the results of the study, to overcome the main risks of the agro-industrial complex to ensure food security and environmental safety, the following measures are recommended:

1) Creation of alternative routes for the import and export of goods bypassing the territory of Russia and expanding trade partners

What may become a promising logistics project for Kazakhstan is the creation of the Trans-Caspian Transport Route (TCTR). This can help diversify the country's trade relationships and reduce dependence on any one country. This multimodal route, which utilizes rail and sea transport, was established in 2017 and currently runs through the Caspian Sea, Azerbaijan, Georgia, and Turkey toward the Black Sea and on to Europe. The path across the Caspian Sea will allow Kazakhstan to solve logistical problems arising from anti-Russian sanctions. Also, through the development of logistics chains, China has recently become one of the largest trading partners in Kazakhstan. Both countries have set a goal to increase their bilateral trade volume to $35 billion by 2030 (Silk Road Briefing, 2023). China is interested in importing agricultural products from Kazakhstan, increasing the volume of rail transport, and enhancing inter-regional cooperation and partnership (World of NAN, 2023).

2) Improve the technological level of the agricultural industry through the creation and development of domestic breeding and genetic resources.

Experts note that Kazakh varieties currently occupy only 5% of the 25 million hectares of arable land, while foreign varieties account for 95% (OISPMRK, 2022). Breeding in the country largely relies on the scientific heritage of the Soviet period. There is a shortage of specialists and scientist breeders, and the issues of royalties and copyrights are acute (Burna-Asefi, 2022). Furthermore, state support for scientific and educational organizations engaged in research and innovative development in the field of breeding and genetics is quite poorly developed in Kazakhstan. It is necessary to improve current legislation, create conditions for the establishment of new seed farms, and foresee the need to introduce financial instruments to address the problems highlighted. A plan for the introduction of new varieties must be developed at the level of the Ministry of Agriculture of Kazakhstan, departments of agriculture, and agricultural research organizations (Esmagulova *et al.,* 2023) and includes the development of sustainable agricultural practices (Dashkevich *et al.,* 2022). Such measures as crop diversification and conservation agriculture help to reduce the risk of crop failure, improve soil health, and provide a more stable income for farmers (KASE, 2021).

3) Development of domestic fodder bases and the production of fodder additives

Experts believe that Kazakhstan can become the largest exporter of livestock products, but a significant obstacle is the lack of a fodder base (Iurchenko, 2015). When determining the structure of sown areas, it is important to consider not only natural and climatic conditions but also the need for agricultural producers in certain fodders and the technology for their procurement. Developing rangeland management practices and introducing legumes into crop rotation can fix atmospheric nitrogen, improve soil health, and reduce the need for synthetic fertilizers (Bayazitova *et al.,* 2023).

One topical direction is the development of partnerships with both EAEU and other countries for the development of Kazakhstan's own microbiological industry to produce vitamins and amino acids, as well as an increase in the use of protein components and secondary raw materials for the food and processing industry to reduce the proportion of forage grain. It is also necessary to legislatively unify the requirements for the quality of feed and feed additives, which are essential for veterinary and sanitary expertise.

4) Development of industrial cooperation in the field of agricultural engineering

At present, due to the introduction of measures to stimulate the agricultural machine-building industry, world producers, such as Rostselmash, Petersburg Tractor Plant (Russia), Gomelselmash, MTZ (Belarus), Lovol Heavy Industry, Yto Group Corporation (China), CLAAS Gmh (Germany), and SDF Group (Italy), have localized their production on the territory of Kazakhstan. Despite the growth in the production of agricultural machinery for national assembly, the agricultural machine-building industry still faces problems associated with a high depreciation rate of machinery, its utilization, subsidized investments in the renewal of agricultural machinery and interest rates, and the transfer of field machinery in leasing. Insufficient funds lead to the maintenance of worn-out equipment in poor conditions, which has a negative impact on labor productivity and the cost of agricultural production (Kornilova *et al.,* 2022). Moreover, it is crucial to establish an effective system for waste disposal and soil conservation measures to improve environmental safety (Tatibekova *et al.,* 2022).

5) Increase the level of environmental safety in the agro-industrial complex of Kazakhstan

The agro-industrial sector handles a significant proportion of greenhouse gas emissions, water usage, and pollution; thus, improving its environmental performance is essential to mitigate these impacts. Kazakhstan has traditionally focused on wheat production, which has contributed to soil degradation and increased vulnerability to pests and diseases. As mentioned by experts, crop diversification could improve soil health and reduce the risk of crop failure due to weather and pest-related problems. Diversifying crops also provides farmers with a more stable income and a single crop, which is particularly important given the "extreme" continental climate of the country (Yesmagulova *et al.,* 2023). Conservation agriculture involves reducing tillage and using cover crops to conserve moisture, reduce erosion, and improve soil health. Integrated pest management reduces pesticide use and improves soil and water quality, while drip irrigation reduces water usage and minimizes soil erosion. All of these measures contribute to environmental sustainability and can reduce the impact of agriculture on the environment and human health (Khan *et al.,* 2018). In addition to these measures, other actions can be taken to improve the environmental safety of the agro-industrial complex. For example, promoting organic farming practices can reduce the use of agrochemicals and improve soil health. Supporting agroforestry and reforestation efforts can also contribute to improving ecological balance and enhancing biodiversity (Dambaulova *et al.,* 2022). Furthermore, investing in the research and development of sustainable agricultural practices and technologies can help to identify new and innovative ways to improve the environmental performance of the agro-industrial complex.

CONCLUSION

For Kazakhstan, the agricultural sector, rural areas, and the EAEU market are the most important components in ensuring the sustainable and inclusive growth of the Central Asian region. A year ago, researchers predicted that the EAEU could help eliminate the distortions and disproportions that have developed over the past decades, especially on the Eurasian continent. The geopolitical risks that have arisen over the past year have made significant changes in the integration ties between countries, violated food security, and may have an impact on and significantly reduce the country's environmental security. Geopolitical risks for Kazakhstan as one of the members of the EAEU include rising prices for raw materials, disruptions in supply chains, high volatility in the cost of agricultural products, and the availability of goods and materials. Currently, agricultural production in Kazakhstan is highly dependent on the import of breeding and genetic resources, plant protection products, and agricultural machinery. The risks of a transport blockade will reduce the availability of imported goods and materials for agricultural production and the volume of exports of agricultural products and food products from Kazakhstan to EU countries. This, in turn, may discourage investment in this sector and slow down the growth of the agricultural sector. To ensure food security, Kazakhstan needs to pursue a multivector integration policy capable of solving priority tasks in solving transport and logistics problems, development problems in the field of breeding and genetics, a lack of a forage base, and the development of engineering. Such a policy could lead to the international reintegration of Kazakhstan and have an impact on the sustainable development of the country's rural sector. An important conclusion of this study is that, when taking emergency measures to solve the identified problems, it is important to pay attention to the level of environmental safety. Crop diversification, conservation agriculture, integrated pest management, and drip irrigation are important measures to increase environmental safety in Kazakhstan's agro-industrial complex. The adoption of a range of measures and actions could contribute to achieving the goal of environmental safety. By improving the environmental performance of the sector, Kazakhstan could achieve sustainable economic growth, enhance food security, and contribute to the quality of life of the population. Limitations of this study include the statistics used from various countries within the EAEU (Armenia, Belarus, Kazakhstan, Kyrgyzstan, and Russia), which may lead to inaccuracies in their analysis when combined. However, it was not considered to have a significant impact on the findings. In terms of prospects for research, it is necessary to further develop each direction highlighted in the study to improve environmental safety and food security. Increasing the level of environmental safety of the agro-industrial complex of Kazakhstan through measures such as crop diversification, conservation agriculture, integrated pest management, drip irrigation, and other sustainable agriculture practices could help reduce the industry's impact on the environment while enhancing food security. Further research and development in these areas could help Kazakhstan achieve its goals of sustainable economic growth.

AUTHOR CONTRIBUTIONS

L.A. Omarbakiyev and S.M. Kantarbayeva performed the literature review, experimental design, analyzed and interpreted the data, prepared the manuscript text, and manuscript edition. A.K. Nizamdinova and S.T. Zhumasheva performed the experiments and literature review, compiled the data and manuscript preparation. G.Z. Seitkhamzina helped in the literature review and manuscript preparation. A. Saulembekova performed some of the remained experiments.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
COVID-19	Coronavirus disease 2019
EAEU	Eurasian Economic Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GVA	Gross value added
IFOAM	International Federation of Organic Agriculture Movements
Mln.	Million
q/ha	Quintals per hectare
ths. tn	Thousands of tonnes
TCTR	Trans-Caspian Transport Route
UN Comtrade	United Nations International Trade Statistics Database
USD	United States Dollars
WTO	World Trade Organization

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ORIGINAL RESEARCH ARTICLE

Farmers' motivation and obstacles in the smallest available agricultural region

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ABSTRACT

BACKGROUND AND OBJECTIVES: The high need for tourism-supporting infrastructure has impacted the increasing conversion of land functions in Denpasar City, Indonesia. The lack of agricultural areas in Denpasar City has motivated farmers to continue farming. Farmer motivation to do farming will collide with obstacles in carrying out agricultural activities in Denpasar City. The purpose of this study was to examine the motivations and obstacles faced by farmers in carrying out agricultural activities. This research is relevant to the journal scope of Sustainable Agriculture Management, Urban, and Built Environmental Management.

METHODS: This study was carried out in Denpasar City in Indonesia, where each sub-district in Denpasar City will be selected by one Subak. The selection of Subak was based on the Subak with the most farmers in each sub-district in Denpasar City. The selected subak were Pakel 1 Subak, Kerdung Subak, Temaga Subak, and Margaya Subak. The population in this study was 672 people. The respondents in this study were 87 people. The data collection method was carried out using structured and in-depth interviews using questions and points while face-to-face between the interviewer and the respondent, via a prepared questionnaire. This study uses two variables with 41 indicators. The analytical methods used to point to objectives one, two, and three are descriptive quantitative and descriptive qualitative methods. Goal four is "Build a model for dealing with obstacles to farming in urban areas." The fourth objective was analyzed descriptive qualitative based on the results of the analysis of objectives one, two, and three.

FINDINGS: The study results show that the motivation to continue farming is caused by demands to work, not having another job to get help from other parties, a healthy work environment, the ability to take lessons from previous farming experience affect product quality, and work in nature. The obstacles for farmers to continue farming are the bargaining position of farmers in selling products, market information obtained by farmers is still minimal, and market absorption of the products produced is not maximized.

CONCLUSION: Based on the results of the research, several things deserve to be concluded. The number of dependents of a farming family is more than six because there are still children of farmers already working but still being supported by their parents for their primary needs, and most of the farmers do farming in an area of 0.21 – 0.60 hectares. The motivation to keep farming is caused by the demands to work, not having another job to get help from other parties, a healthy work environment, and the ability to take lessons from the previous farming experience affecting product quality and work in nature. The obstacles for farmers to continue farming are the bargaining position of farmers in selling products, market information obtained by farmers is still minimal, and market absorption of the products produced is not maximized.

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INTRODUCTION

Denpasar City has an area of 12.778 hectares (ha), divided into four districts: Denpasar Utara, Denpasar Timur, Denpasar Selatan, and Denpasar Barat (Rahayu, *et al.*, 2018). Denpasar City is located at 8.67 South Latitude and 115.21 Eastern Longitude (Narottama, *et al.*, 2017). The climate of Denpasar City can be seen from the average rainfall rate of 244 millimeter (mm) per month, with relatively high rainfall occurring in December, while the average air temperature is around 29.8 Degrees Celcius (°C) with the lowest average around 24.3 °C. Denpasar City has total agricultural land of 3,232 ha. The establishment of Denpasar City as the capital of Bali Province and the national tourism barometer has been triggering higher needs for infrastructure development, leading to the increasing rate of land conversion. Most agricultural land in Denpasar City areas is protected beneath the traditional *subak* organizational system. A study by Zullo *et al.* (2021), said that in European Countries, in terms of both economic and food needs, analyses have demonstrated the potential agricultural productivity lost as a result of urban land conversion. The abandonment of rural communities, particularly in hilly and mountainous regions, fueled by the economic hardships that have long plagued agriculture, is another factor in the loss of productive agricultural land. Darmawan *et al.* (2023) mentioned that Bali's farming community owns Subak, an organization that has strict rules for managing the traditional rice fields there. The Pekaseh (Kelihan Subak), Petajuh (Vice Kelihan Subak), Kesinoman (Instructor), and Subak's farmer members made up the traditional organization of Subak (Suamba *et al.*, 2023). They were held to an agreement (awig-awig) that all Subak members had to follow. The Balinese agricultural tradition known as Subak local wisdom has been recognized by United Nations Educational, Science and Cultural Organization (UNESCO) as a world cultural treasure (Sriartha and Kertih, 2019). To keep Subak in good working order, Subak as a customary institution plays a significant role in social, cultural, and economic spheres (Prastyadewi, *et al.*, 2020). Darmawan, *et al.* (2021), then urges farmers to adapt sufficiently to the face-paced changing world, retaining the agricultural land conversion activities. Jambor and Szerletics (2022) conducted an agricultural study in Europe, discovering that "land grabbing" or land conversion is a global phenomenon that takes place in many regions of the world, mostly driven by several market factors. The study results of Khoshnava *et al*. (2020) state that infrastructure as the backbone of economic growth must be awakened through green competencies to rethink infrastructure strategies and economic prosperity in dealing with local and global issues. Oddly, the reduced availability of farmland in Denpasar City has not disheartened farmers from continuing to farm. Despite their faithful commitment and undying motivation, farmers would unescapably confront numerous obstacles in conducting their agricultural activities. Ranjan *et al.* (2019) said there are four themes of motivations: economic factors, social norms, perceptions of government programs, and farm characteristics. Further, In Romania, farming activities, such as selling agricultural products, can increase agricultural productivity factors, as well as increase farmers' income (Maican *et al.,* 2021). In Ghana, Bedi *et al.* (2020), said that motivational factors significantly influence farmer adoption decisions. A study by Jambor and Szerletics (2022) in Europe further reported that the farmer's motivation to innovate and work more efficiently decreased when they became increasingly dependent on subsidies as a source of income. Findings from a study in the Czech Republic by Pechrová *et al*. (2018) revealed that the typical motive of farming among 510 young farmers was the desire to sustain the legacy of farming business from their parents or other relatives and also to work in nature with animals. Young farmers and women farmers see opportunities and challenges when starting and maintaining their farms by focusing on optimizing land to achieve high economic value through the cultivation of food crops, horticulture, and ornamental plants concerning technological innovation. Environmentally friendly production processes that increase oxygen levels and reduce air pollution are needed to produce fresh and healthy food, as well as motivation to continue farming in the form of support for farmer regeneration from parents so that their children farm and meet the demand for labor in agriculture. The limited area that farmers work on is not a barrier because the results can meet the primary needs of the household. The motivators in this study are economic motivation, sociological motivation, cultivation technical motivation, and urban farming development motivation. The results of Agyei *et al*.

(2021) research state that the key factors motivating smallholder decisions to implement climate-smart agricultural practices are product harvesting and timely storage, emergency seed storage, crop rotation, weed control, appropriate pest control, and on time. Farmers in urban areas can adapt to climate change and extreme weather by implementing the planting calendar from the Ministry of Agriculture, planting new adaptive superior varieties, and applying technology in land resource management and agricultural insurance. Furthermore, the results of Jambo *et al*. (2019) research stated that motivating small farmers is needed to stimulate economic viability, improve nutrition, reduce poverty, and maintain the natural resource base of the rural economy. Intrinsic and extrinsic motivation is equally important in smallholder implementation decisions. Urban farmers have been struggling with complicated issues in running their agricultural businesses. The cultivating, processing, marketing, and product promotion activities have been greeted by obstacles. The obstacles faced by farmers should ideally be supported by "pro-farmer" policies. Policies are needed to support redesigned agricultural systems without reducing productivity (Gava *et al.,* 2020). In Namibia, Kiesel *et al.* (2022) recommend a more knowledge and place-based policy, including a multidimensional perspective adapted to agricultural management and open decentralized governance structures that engage region-specific agricultural, economic, political, and environmental knowledge. In Turkey, regional variances should be considered when formulating agricultural assistance policies to make up for income losses brought on by physical limitations (Unuvar and Dellal, 2022). Pechrová *et al*. (2018) also discovered several obstacles to agricultural business in the Czech Republic, including the capital assets to get land, administrative burden, and financing development strategy for start-ups. Despite several challenging issues served during the process of urban farming, farmers seemed to be stubbornly sustaining their agricultural business amidst the overwhelming situation. This phenomenon is pretty intriguing. Even though urban areas present miscellaneous work opportunities, farmers prefer to run their agricultural activities to do other professions. The study objectives were to describe the characteristics of farmers, analyze the motivation in sustaining agricultural activity, analyze the obstacles, and construct a model for managing the obstacles to farming in urban areas. The Subak region, which encompasses all of Denpasar City, was the site of the study. This study was carried out in Denpasar City in Indonesia in 2022.

MATERIALS AND METHODS

The study was conducted in the *Subak* area that spread over Denpasar City. Most of the available agricultural areas in Denpasar City are still under the Subak organizational system. The area of *Subak* with the highest number of farmers in each district was then picked as the study location. Several *Subak* involved in this study were Subak Pakel 1 in North Denpasar District, *Subak Kerdung* in South Denpasar District, *Subak Temaga* in East Denpasar District, and *Subak Margaya* in West Denpasar District. The study population was all members of Subak Pakel 1, Kerdung, Temaya, and Margaya, with a total of 672 farmers. Eighty-seven participants were then eventually enrolled in this study. Proportional sampling was applied to select eligible farmers in each Subak area. Data were gathered by conducting structured interview sessions directed by a questionnaire. In-depth interview sessions were also performed with the participants to complement the study data. Previous study files and documents were included to acquire information about the profile of the study location. Finally, two variables with 41 indicators were involved in this study. The quantitative and qualitative descriptive methods were enrolled to point out the first, second, and third objectives. This analysis method provides the perspectives and thoughts from the subject's viewpoint (farmers in Denpasar City) and observes them most intimately. Quantitative data were also collected to support the qualitative findings. Before being analyzed, all data obtained was carefully studied to minimize the chance of errors by following these steps: editing (the process of potential error detection), coding (creating and assigning participant's responses into certain codes), and tabulation (compiling and representing the data in rows and columns). Based on the category scale values in Tables 1 and 2, five categories were selected to determine the level of motivation and constraints of farmers in the Denpasar City area starting from 1.00-1.79 up to a value of 4.20-5.00. Values for determining categories in each indicator are obtained from tabulation results based on interviews with farmers.

Table 1: Scale value of farmers' motivation category to keep trying farming

No.	Farmer motivation	Scale value
1	Very motivating	4.20-5.00
2	Motivating	3.40-4.19
3	Simply motivating	2.60-3.39
4	Less motivating	1.80-2.59
5	Very unmotivating	1.00-1.79

Table 2: Scale value of farmer's obstacles category to keep trying farming

No.	Farmer obstacles	Scale value
1	Very inhibiting	4.20-5.00
2	Inhibiting	3.40-4.19
3	Simply inhibiting	2.60-3.39
4	Not inhibiting	1.80-2.59
5	Very uninhibited	1.00-1.79

The final objective was to construct a model for addressing obstacles to farming activities in urban areas. This objective was analyzed thoroughly using the descriptive-qualitative method based on the findings in the first, second, and third objectives. A proper model dealing with obstacles in urban area farming is expected to be created, promising better welfare for urban farmers.

RESULT AND DISCUSSION

Participant's characteristics

The typical commodity identified was the rice paddy. But in a certain period, participants also grew several crops, such as *palawija*, water spinach, spinach, rose balsam, lotus, and mungbean. Table 3 shows the cultivated land area, land ownership status, and cultivated commodity. The majority of participants, (55.17 %), cultivated in 0.21 to the 0.60-ha-land area as tenant farmers or owners. Findings showed that ten farmers were working on <0.2 ha - land area (11.49%). This situation likely happened due to their status as novice farmers. Most had just retired from their previous work due to the massive layoffs during the pandemic. Only 8.05% of participants were cultivated on more than 1 ha of land area. According to Sitinjak (2018), the family head's educational attainment, control over the land's basis, and environmental factors (agricultural land) have all impacted rural poverty. This means rice paddy farmers with small land areas could not afford their daily expenses properly. On the other hand, based on Makate *et al.* (2019) research located in Zimbabwe, richer farmers have higher odds of planting on larger land compared to their less affluent counterparts. This finding aligns with Ren *et al*. (2019). They discovered a positive correlation between the size of cultivated land with the total income among farmers in China.

Darmawan *et al*. (2021) mentioned several challenging issues in agricultural activities in Subak Sembung, Denpasar, that contribute to the total income gained by the farmers. Significant decline in farmer population, urbanization, limited land, poverty, environmental changes, uncertainty in agricultural production, and constrained resource access was recognized as complex issues encountered in conducting agricultural activities. Another study by Veveris *et al*. (2019) in Latvia and Lituania portrayed that bigger-scale agribusiness had a better financial state, and multiple streams of funds of investment produce faster agricultural production through a proper modernization process. Additionally, Nhan and Yutaka (2019) also mentioned that the greater size of rice paddy fields in Delta Mekong Vietnam had increased the product's output selling price. The higher price may parallel the higher agricultural output volume. The majority of participants (39.8%) were tenant farmers who farmed land leased from the landowners. The revenues from the land were

Table 3: Cultivated land area, land ownership status, and cultivated commodity

No.	Type of data	Number of persons (n)
1	Cultivated land area	
	≤ 0.2 ha	10
	0.21 – 0.60 ha	48
	0.61 - 1 ha	22
	≥ 1 ha	7
	Total	87
2	Land ownership status	
	Privately owned land	32
	Land leasing (Tenant Farmer)	34
	Contract	5
	Others (privately owned + leased land)	16
	Total	87
3	Cultivated commodity	
	Paddy/rice	58
	Vegetable	3
	Flower	0
	Palawija (annual commodities, cultivated particularly in the dry season)	0
	Others (intercropping commodities, paddy, vegetables)	26
	Total	87

Table 4: Age, educational background, number of dependents, experience in agribusiness, and occupation

No	Type of data	Number of persons (n)
1	Age	
	≤ 14 years old	0
	15 - 65 years old	66
	≥ 66 years old	21
	Total	87
2	Educational background	
	Never went to school/Did not complete Elementary School	13
	Elementary School	30
	Junior High School	12
	Senior High School	27
	Vocational School/University	5
	Total	87
3	Number of dependent	
	≤ 2 persons	21
	3 -5 persons	59
	6 persons ≥	7
	Total	87
4	Experience in agribusiness	
	≤ 10 years	15
	11 - 30 years	28
	31 years ≥	44
	Total	87
5	Occupation	
	Farmer as the primary occupation (had the second occupation)	12
	Farmer is the second occupation	18
	Farmer is the only occupation	57
	Total	87

split between the tenant farmer and the landowner on the basis proportion of 2:1. The tenant farmer who resided and cultivated on the land obtained the share of 2, and the landowner earned the share of 1. Tenant farmers commonly paid the cost required for agricultural production. It was discovered farmers with contract land systems. The average payment given to the landlord in this system was $US 65/0,1 ha, annually. This finding was parallel to a study conducted by (Musdalifah *et al*., 2021) that found an equal and reasonable distribution of crop profit between tenant farmers and landowners. For instance, in the agreement, tenant farmers would get a share of 2 if they consented to pay for the farming expenditures, while the landlord would get a share of 1 because they only supplied the land. In line with this finding, Dimitri *et al.* (2016) also found that urban farmers rarely cultivated their land, signifying that most of them were tenant farmers. Table 4 shows the age, educational background, number of dependents, experience in agribusiness, and occupation.

Around 24% of the participants were elderly (above 66 years of age). They were actively engaged in the land tillage process, including processes that involved physical strength. They confirmed that land tillage activities had kept them active after their retirement. Most of them previously worked in private and government-based institutions. They voluntarily worked as farmers to kill their leisure time. Agricultural activities that mostly demanded physical strength were perceived as sufficient to maintain their health and slow down their aging process. They also believed that being less active and sleeping all day would gradually weaken them. The majority of participants graduated from Elementary School (34.8%). In the past, it was a common situation that the participants were asked to follow their parents' occupation as farmers. Only five participants (5.75%) graduated from vocational school/university. Further, most of them were placed in managerial positions in the business (did not engage in agricultural physical activities). The farmer was not the primary occupation but only a side gig. Participants with more than six dependents were discovered. The high number of dependants were clarified as working children who still received support from their parent for their primary needs. The results found that the majority of participants had been working as farmers for more than 31 years. They had been introduced to farming work since graduating from elementary school. Participants were very devoted to their occupation as a farmer because it was perceived to be more uncomplicated. No certification was required as a farmer, and it was seen to be relatively undemanding compared to other occupations. Findings also reported that 65.52% of the participants were full-time farmers with no second occupation. Eighteen participants (20.69%) put farming as their second occupation. They claimed that farming was a flexible business that could be done anytime in more flexible hours (morning and afternoon). They also stressed the uncertain income gained from the farming business. Participants who placed farming as a non-primary occupation were working as a bank employee, construction worker, an employee in Village Credit Institutions, a field officer in Denpasar City, a security guard, an employee in the Department of Environment and Sanitation Service, a cleaning service, gardener, *kalian adat* (local community leader), electric meter recorders, and pemangku (local spiritual teacher/leader). Parallel with this finding, Diaz *et al*. (2022) mentioned educational background, income from the agricultural sector, and farmer-based cooperation unit in Northern Columbia as vital factors for agribusiness sustainability.

Farmer motives to constantly engaged in agricultural business in Denpasar city

Table 5 showcases several indicators that poorly motivated the farmers: desire for luxurious goods and income from the agricultural business covered the secondary household expenses. Farmers may want to purchase luxury goods; however, their financial situation may not support their desire. The majority of crop production was initially intended to be traded by the farmers. Only a few of them personally consumed and carried the crop to their residence because of limited space to preserve the crops. Farmers also stated that the revenue from crop production was saved for their future needs, especially for traditional ceremonies, such as *manusa yadnya* or *dewa yadnya* (Hinduism). *Dewa yadnya* refers to traditional ceremonies to express assurance and gratitude to the Almighty God, *Sang Hyang Widhi Wasa*, as one path for magnificent protection and forgiveness for all wrongs (Trisanti, 2021). Suardi *et al*. (2023) further mentioned that the cultural submission of Balinese farmers in preserving their tradition was one factor that contributed to the sustainability of agricultural land.

A relatively comfortable market access for the farmers was observed. The wholesalers were spotted easily on agricultural land during the harvesting seasons. They would propose certain prices to purchase crop production during those times. The indicator of farming as a second job to supplement the primary source of income moderately motivated the farmers in sustaining their agricultural business. Further, the demand to work and no side occupation were discovered to be a great drive to engage constantly in farming activities. This finding was confirmed by the high number of participants who worked as a farmer (more than 50%). Farmers could eat crops grown on their land for food, simultaneously covering their primary needs, such as staple food (rice, side dishes, vegetables), personal hygiene essentials, water, and also electricity bills. Table 6 shows that agricultural activities constructed positive relations among the farmers.

The wholesalers who potentially purchased their crop production were also counted as their business relations. Farmers could also establish a reciprocal connection with students or academicians that frequently targeted farmers as the subject of agricultural study. Establishing mutual partnerships, creating more harmonies, and allowing the process of exchanging thoughts and feelings were also confessed as motives to be immersed constantly in

Table 5: Economic motivation

No.	Question	Score	Category (Level of motivation)
1	Yearning for a more prosperous living state	2.79	Moderately motivated
2	Desire to own, invest and increase savings	2.74	Moderately motivated
3	The desire for luxurious goods	2.37	Poorly motivated
4	Longing for higher income	2.84	Moderately motivated
5	Desire to adequately fulfilling daily needs	3.58	Highly motivated
6	Yearning for a better life	2.95	Moderately motivated
7	The demand to work	3.89	Highly motivated
8	Personal need fulfillment	3.68	Highly motivated
9	Convenient market access	3.16	Moderately motivated
10	Income from the agricultural business covered the primary household expenses	3.58	Highly motivated
11	Income from the agricultural business covered the secondary household expenses	2.47	Poorly motivated
12	Had no side occupations, the absence of an additional stream of income	3.89	Highly motivated
13	As the second occupation, supplementing the income from the primary occupation	3.16	Moderately motivated
14	Desire to meet the daily family needs	3.74	Highly motivated
15	Crop production (agricultural products) for own personal and family use	2.47	Poorly motivated

Table 6: Social motivation

No.	Question	Score	Category
1	Building positive relationships or making friends	3.42	Highly motivated
2	Establishing mutual partnerships	3.68	Highly motivated
3	Creating more harmonies	4.00	Highly motivated
4	Establishing understanding communication, allowing the process of exchanging thoughts and feelings	4.00	Highly motivated
5	Get help from other parties	4.89	Extremely motivated
6	Healthy workplace environment	4.26	Extremely motivated

the agricultural business. The process of exchanging thoughts and ideas was typical sessions in subak, directed as a regular agenda under the *subak* organization. Farmers were asked to gather in specific sessions to communicate their thoughts with the prajuru (subak's organizational committee) and other members of the *subak*. A study by Milone and Ventura (2019) demonstrated the role of collaboration skills in the success stories of farmers in Italy. Sriartha and Kertih (2020) said that the social activities in subak are regulated through awig-awig and the principle of paras paros sarpanaya sagilik saguluk selunglung sebayantaka. In addition, Utari (2017) mentioned the interaction and intense communication during the *subak's* regular meeting sessions, which occurred between the organizational leader board of the subak with the members, primarily discussing agricultural issues and alternative strategies to manage those issues. Important collaborations are carried out between farmers and other stakeholders, such as industrialists, research institutions, consumers, and the government, to provide added value, procure seeds, post-harvest assistance, increase income, and public-private partnerships on food system issues. A collaboration platform needs to be created to share knowledge in food systems and involve banking and ventures. Farmers stated their enthusiasm for aid from officials or universities to support their agricultural work and crop production. Government policies in supporting urban farmers are in the form of subsidies and input-output price incentives, as well as legal certainty in the agricultural sector. Additional support needed includes utilizing local resources, applying appropriate technology that is easy for farmers to implement, reusing urban organic waste as a source of plant nutrition, and urban farming practices characterized by sustainable and environmentally sound concepts. National and local government institutions distributed several agricultural aids: subsidized diesel fuel, subsidized fertilizers, subsidized seeds, and subsidized tractors. Subsidized diesel fuel and subsidized fertilizers could

Table 7: Cultural motivation

No.	Question	Score	Category
1	The quality of crop production is nearly equal to the expected quality	3.05	Moderately motivated
2	The satisfaction with the previous crop production as a motive to engage persistently in agribusiness activities	3.21	Moderately motivated
3	The capacity to learn from the previous season affects the crop production quality	3.16	Moderately motivated

Table 8: Motives of urban farming development

No.	Question	Score	Category
1	Developing urban farming	3.37	Moderately motivated
2	Maintaining the existence of urban productive green spaces	3.63	Highly motivated
3	Gaining more experience in agricultural practices	3.63	Highly motivated
4	Conserving *Subak* Irrigation System	3.74	Highly motivated
5	Mandated to manage the inherited agricultural land	3.37	Moderately motivated
6	Sustaining the legacy of the farming business from parents or other relatives	3.26	Moderately motivated
7	Establishing an independent agricultural-based business	3.42	Highly motivated
8	Working in the nature	4.05	Highly motivated

be requested annually by *kelian subak* (*subak's* leader). However, the availability of these agricultural aids was very restricted. This situation was parallel with findings in a study by Varshney *et al.* (2021). In India, the government gives an assistance package (known as Pradhan Mantri Garib Kalyan Yojana (PM-GKY) to procure agricultural inputs. Farmers who benefited from the PM-GKY scheme incurred much greater costs for the purchase of seeds, fertilizer, and insecticides. The nature of the dependence of farmers on input subsidies by the government also changes their business and production patterns and, in turn, influences changes in market price balances. Therefore, it is deemed necessary to regulate the balance of sustainable inputs for farmers that guarantee business efficiency and farmers' income. Šumrada *et al.* (2020) research in Slovenia shows that there is an agricultural policy from the government in Slovenia that some countries in the EU have promoted result-based schemes (RBS) that remunerate farmers for ecological results. Asai *et al*. (2018) conducted a large-scale study in Asia, Europe, and the United States of America and reported financial and technical support as primary external factors that influence integration at the farmer level. A healthy workplace environment was also discovered to be the major motive in sustaining agribusiness activity in urban areas. Farmers felt quite qualified to manifest a healthy work environment. They had been actively clearing land that was heavily polluted by household waste carried by water. Agricultural activities were also broadly perceived as physical exercises, simple ways to balance the mind, body, and soul. The awareness of environmental sustainability, especially the irrigation system, was contrary to a study by Peltonen-Sainio, *et al*. (2020). This study was conducted in Finland and reported the farmer's ignorance of the irrigation system. Additionally, Was *et al*. (2021) found that the farmer's awareness in Poland is associated with their economic interests. Patel *et al.* (2015) underline that in India, the food sovereignty approach recognizes the importance of the economic stability of small-scale farms, local autonomy for decision-making, ecological sustainability, and the preservation of nutritional culture through the diversity of cultivated food crops. Table 7 shows the cultural motivation. The quality of crop production that closely matched the expected quality was identified as an indicator that moderately motivated the farmer.

Based on the traditional beliefs of farmers in Bali, there are two major seasons in farming, namely the Kerta and Massa seasons. Kerta occurs around the harvesting seasons from September to October (Sasih Kapat; Balinese Calendar System). During this season, it is believed that crops would be sold at the highest price. In contrast, during the Massa, from March to April (Sasih Kadasa), the crop is forecasted to be traded at the cheapest price. This situation was unique. Despite the identical quality and cultivation process, the price proposed in both seasons was somewhat different. While according to Grujic *et al.* (2021), the source of agricultural water in Central Serbia comes from various sources that can be used for irrigation. Further, Ati *et al*. (2022)

Table 9: Obstacles to maintaining urban agricultural practices

No.	Question	Score	Category
1	Strategic planning (what to produce, for whom)	1.95	Did not hinder
2	Obtaining capital at the beginning of the growing season	1.84	Did not hinder
3	Capital assets for agribusiness development	1.89	Did not hinder
4	Farmer's bargaining position	3.95	Hindered
5	Market information	2.74	Enough to hinder
6	Crop production absorption rate	2.74	Enough to hinder
7	Water resources availability	1.74	Very uninhibited
8	The profit-sharing system	2.47	Did not hinder
9	Physical strength for farming	1.47	Very uninhibited

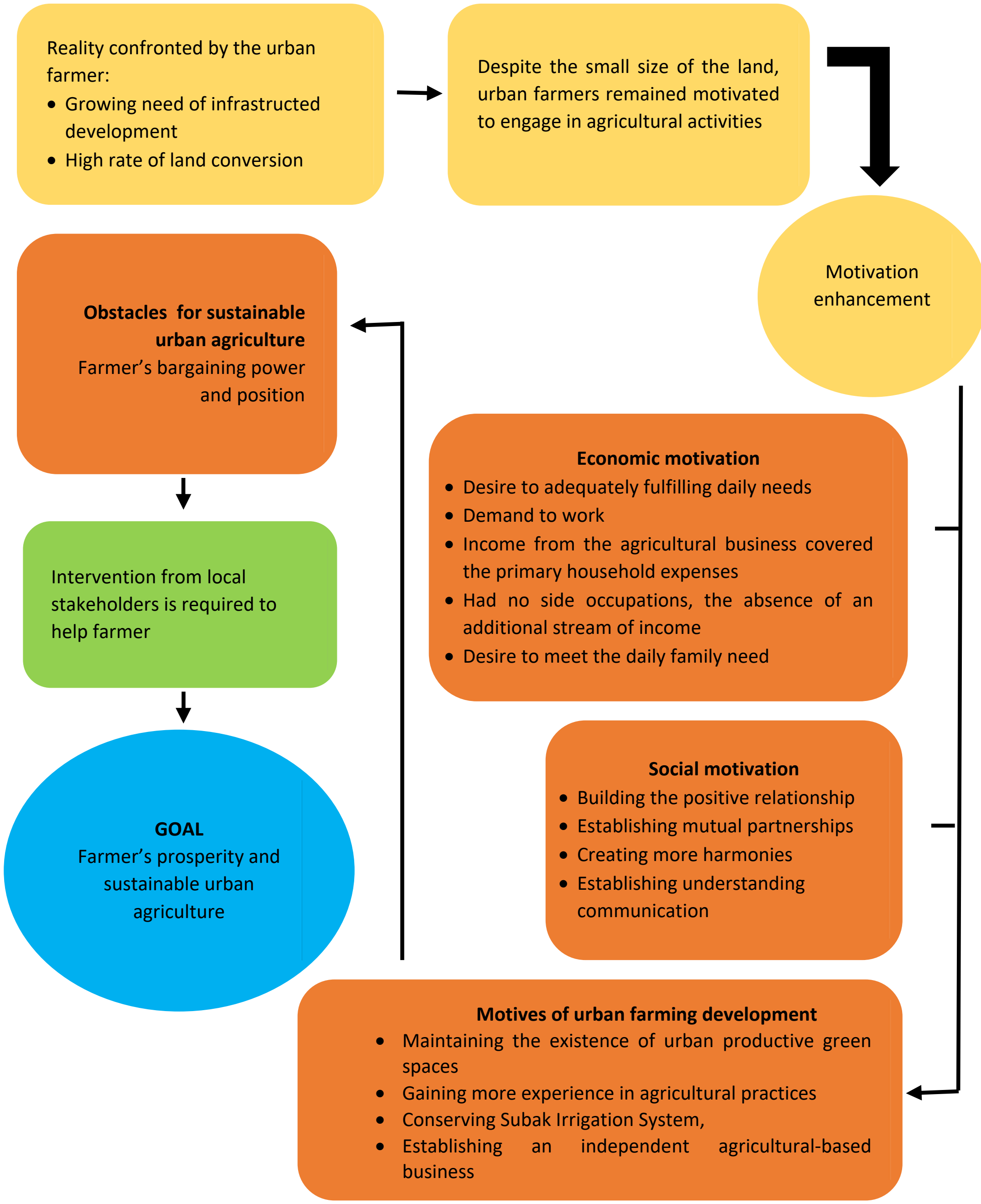

Fig. 1: Model to overcome obstacles to sustainable urban agriculture

mentioned the cultivating period was very important in rain-fed rice fields or irrigated rice fields, where the water availability was not secure. In these fields, the volume of water available for crops depended very much on the beginning, length, and end of the rainy season. Table 8 shows the motives of urban farming development.

Farmers expressed their desire for urban farming development. This motive kept them persistently maintaining their agricultural practices in Denpasar City. The inherited land from their parents and relatives also pushed them to remain engaged in agribusiness. Constant agricultural activities in the urban area, concurrently, maintain urban green spaces in the city area. The presence of a water irrigation system was expressed as one element that affected the existence of urban farming. Farmers stated their willingness to endure and sustain their farms amidst the infrastructure and residential development around their land, as long as the water source availability could be guaranteed. The establishment of independent agricultural-based businesses was parallel with their desire to produce and distribute their crop production in multiple streams of market lines. Some wholesalers distributed the local grain commodity cultivated by the urban farmers to many areas, including those located outside of Bali.

Obstacles to maintaining urban agricultural practices in Denpasar City

Table 9 shows the obstacles to maintaining urban agricultural practices. It is revealed that the rice cultivation plan has been established during the subak regular meeting. This situation made the production planning less likely to encounter obstacles because it has been well organized by Pekaseh (Subak's Leader). According to Artha (2016), the primary function of Pekaseh is to lead every Subak regular meeting, both related to the internal matters of the Subak and external issues related to institutions outside the Subak.

Slightly different from rice cultivation, the vegetable commodity was cultivated for daily food needs, especially if the land and water sources availability could be guaranteed. In West Denpasar, the majority of farmers grow vegetables, such as water spinach, spinach, and green vegetables, to earn daily income. At the beginning of the growing season, farmers obtained capital to start the season from the previous harvesting period. However, if this profit is insufficient, farmers would try to acquire funds from their relatives, friends, or Local Credit Institutions. Especially for the case of Pakistan, Ullah *et al.* (2020) said that the source of funding for farmers in Pakistan comes from banks. This institution mainly served as financial assistance official body to support the development of each traditional village or *Pekraman* village in Bali. At the same time, it was also a force to maintain Balinese customs and culture, a novel strategy to expand funding sources for the community members. Farmers lacked bargaining power and position in the market. All crops were sold to the *penebas* (wholesalers), and the prices were then subsequently set by the wholesaler. Harvested rice paddy was distributed to the market as *tebasan* (was traded while still planted in cultivation land). The vegetable commodity was distributed to small-scaled merchants, traditional markets, and cafés situated in Kedonganan. Participants were using the profit-sharing system with a proportion of 2:1. The tenant farmer who resided and cultivated on the land obtained the share of 2, and the landowner earned the share of 1. A land contract system was also identified in this study. It was perceived as a more convenient system compared to the profit-sharing system. Farmers who cultivated vegetable commodities commonly preferred land contracts to the profit-sharing system. Further, physical strength was not recognized as a prominent barrier to agricultural activities. The majority of participants declared that the physical strength required for farming activities was adjustable. The level of farming work was modified according to their physical strength and capability. Farming works were then perceived as physical exercise, allowing them to be constantly active and maintain their health status.

Model to overcome obstacles to sustainable urban agriculture

The model to overcome obstacles to sustainable urban agriculture is shown in Fig. 1. Urban farmers have been confronted with the bitter reality that the growing need for infrastructure unavoidably impacts the agricultural land conversion rate, ending with the smaller size of cultivation land.

Surprisingly, a certain number of farmers remained enthused to cultivate their crops in these congested areas. They were particularly driven by several motives: the desire to adequately fulfill daily needs, demand to work, income from the agricultural business covered the primary household expenses, had no side occupations, the absence of an additional stream of income, desire to meet the daily family need, building a positive relationship or make friends, establishing mutual partnerships, creating more harmonies, establish understanding communication, allowing the process of exchanging thoughts and feelings, maintaining the existence of urban productive green spaces, gaining more experience in agricultural practices, conserving the *Subak* Irrigation System, establishing an independent agricultural-based business, and working in nature. Farmers had been struggling with their bargaining power in the market. Intervention from local stakeholders is required to help farmers strengthen their power, pushing them to a sufficient position in the market and reap the profit from potential market lines. Obstacles in the market were in the form of access to the products produced, information, and the bargaining position of farmers. The way to overcome this is as follows: understanding the unique value proposition of the product, specific market segments, distribution channels that expand the market, good relations with consumers, marketing management with 4P (product, price, place, promotion), and optimal use of digital marketing. Local stakeholders have an influential position in providing funds/investment and granting access to credit programs for urban agribusiness development. A study conducted in Spanish by Veronica *et al*. (2021) reported motivation and competency level to construct a sustainable system became the determinants in overcoming obstacles in urban agriculture practices among farmers. Farmers make an important contribution to local food security and sustainable agriculture. In the view of farmers, local food security is increasingly fulfilled if the younger generation is involved in farming and, in turn, achieves sustainable agriculture in urban areas. Green Information Technology (GIT) is one model that can be adopted to overcome obstacles to urban agriculture to remain sustainable. Malaysia is one country that adopted GIT, which helps decision-makers and policymakers develop policies and programs for effective work and enhance green information technology (Asadi *et al*., 2021). Research conducted by Khoshnava *et al.* (2020) also added that Green Infrastructure (GI) and Green Economy (GE) have a major influence on environmental, social, and economic Sustainable Development (SD). Research conducted by Kamyab *et al*. (2020) also stated that Malaysia implements a sustainable energy system for smart cities and industries involving smart energy systems, low carbon dioxide (CO_2) emission technologies, reduction of biomass and pollution, and renewable energy. In addition, there are also several innovative products developed to support a sustainable environment. The first product is phytoremediation, a cost-effective remediation technology capable of treating sites polluted by heavy metals. According to Rezania *et al*. (2016), this environmentally friendly method has been successfully applied to artificial wetlands, which can restore natural aquatic biosystems. Research by Soni *et al*. (2022) also stated that the use of urban solid waste could be used as an alternative product of building materials for sustainable development purposes.

CONCLUSIONS

Even though farmers are faced with many obstacles encountered in the process of farming, this does not dampen the motivation of farmers to continue farming in Denpasar City. This phenomenon is interesting to study because even though there are many choices of employment opportunities, farmers prefer to continue farming. The novelty of this research is to examine the motivation of farmers from various sides. In this study, motivation is seen from the economic, sociological, and technical aspects of cultivating and developing urban agriculture. From the results of the study, it can be concluded that the number of dependents of a farming family is more than six people. This is because farmers have children who are already working but are still being supported by their parents for their primary needs, and most of the farmers do farming on an area of 0.21 – 0.60 ha. There are still farmers classified as elderly (over 66 years old) who think they can make themselves more youthful/healthy by doing farming activities. They think that when you are old, you just stay still (sleep), and your body gets tired easily. The average experience of farmers in farming is more than 31 years. Farmers think that working as a farmer does not require special requirements, and agricultural work is easier to do than other jobs. The motivation to continue farming is caused by the demand to work and not have another job. Working as a farmer can meet personal needs and primary needs, including the need for staple foods (rice, side dishes, side dishes, vegetables), the need for toiletries, the need for electricity, and water costs. The sociological motivation of farmers to continue farming is due to assistance from other parties and a healthy work environment. Farmers are very motivated when they get help from other parties, such as the government or higher education institutions, to support farming activities. A healthy work environment means farming, and farming creates a work environment close to nature and maintains environmental sustainability. Farmers consider farming activities as sports activities that make the body and soul healthy. Farmers are also motivated to develop urban agriculture. The desire of farmers to develop agriculture in urban areas so that it is more advanced causes farmers to remain enthusiastic about farming. Inheritance and mandates obtained from parents or other relatives to manage agricultural land also motivate farmers to continue farming. Continuing their farming activities in paddy fields can motivate farmers to maintain green land in urban areas. Farmers are determined, as long as there is water available for farming, even though a house might have been built on the land next to them, farmers will continue to farm their rice fields. Farmers found obstacles to king farming, namely the bargaining position of farmers in selling products, market information obtained by farmers is still minimal, and market absorption of the products produced is not maximized. Based on the research results, several things can be suggested as implications for decision-making. The local government should ideally find an alternative solution because based on field observations, farmers do not have a bargaining position in selling their crops; all crops are sold to the slashers at a price determined by the slashers. Most of the rice produced by farmers is usually sold in the form of slashes (buy directly from the land). Lembaga Perkreditan Desa (in English calls Village Credit Institutions) facilitates or creates innovative programs for farmers so that farmers can obtain sources of funds to carry out farming activities.

AUTHOR CONTRIBUTIONS

D.P. Darmawan coordinated all stages and work steps starting from the preparation of data collection, data analysis, and data interpretation to the preparation of published texts. G.M.K. Arisena conducted a literature review, analyzed, interpreted the data, funded, and wrote the first draft of the publication. A.A.A.W.S. Djelantik accompanied and carried out all stages of the work starting from the preparation of the manuscript to the preparation of the published manuscript. A.A.K. Krisnandika conducted a literature review and reviewed the first draft of the published manuscript. N.L.M.I.M. Dewi assisted the implementation of activities in the field, compiling reports, and data tabulation. N.T.L. Korri helped with the implementation of activities in the field and data tabulation. N.M.C. Sukendar compiled appropriate references, edited the manuscript according to the template, and rewrote the manuscript in English.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
$US	United State dollars
°C	Degree Celcius
CO_2	Carbon Dioxide
EU	European Union
et al.	And others
Fig.	Figure
GI	Green Infrastructure
GE	Green Economy
GIT	Green Information Technology
ha	Hectare (100 m^2)
n	Number of persons
No	Number
PM-GKY	Pradhan Mantri Garib Kalyan Yojana
RBS	Result-based schemes
SD	Sustainable Development
UNESCO	United Nations Educational, Science and Cultural Organization

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CASE STUDY

The effect of income and smallholder characteristics on cultivation, harvesting, and post-harvest management of natural rubber

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ABSTRACT

BACKGROUND AND OBJECTIVES: Indonesia is the second largest natural rubber producer, which it gets primarily from smallholders. However, smallholders are less and less competitive because of unsustainable agricultural practices, while there is huge potential for sustainable natural rubber. This study aimed to measure the effect of income and smallholder characteristics on cultivation, harvesting, and post-harvest management.
METHODS: Mixed methods with non-probability sampling were used for 100 natural rubber smallholders in Kapuas Hulu Regency, West Kalimantan, Indonesia, one of the regional centers of Indonesian natural rubber. Partial least square path modeling was used with SmartPLS software to estimate the complex cause-effect relationships of smallholder and farm factors for adopting sustainable agricultural practices.
FINDINGS: The effect of the relationship between income and smallholder characteristics on cultivation and income on post-harvest management was not significant. Age and education affected some harvesting parameters, such as tapping knives, bamboo and clean latex collection, and latex stimulus risk, and some post-harvest parameters, such as a risk of acid coagulation and storage. The worker's income source, based on off-farm, on-farm, or non-farm activities, affected harvesting parameters, including tapping knives, bamboo and clean latex collection, and latex stimulus risk.
CONCLUSION: This study offers empirical evidence for sustainable agriculture management. The acceptance of sustainable cultivation and management of rubber practices uses smallholder and farm factors as constraints since they do not form a homogenous group, and the theory of planned behavior failed to provide an effective way to explain the behaviors. Good agricultural practices must be used at all steps, including cultivation, harvesting, and post-harvest management, to prevent problems.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
69	2	6

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INTRODUCTION

Indonesia is the second largest natural rubber producer (Statista.com, 2022), and its rubber SIR 20 is the second largest commodity exported after oil palm as an essential raw material for the industry, especially in the manufacturing sector (ETRMA, 2022). It contributes 0.52 % of Indonesia's gross domestic product (GDP) or 88.61 trillion rupiahs for manufacturing (Satista.com, 2022). In 2021, Indonesia produced 3.12 million metric tons of natural rubber, making 4 billion U.S. dollars (USD), or 23.8 % of global sales (Statista.com, 2022). East Asia Pacific is the main importer of Indonesian rubber, followed by North America, the U.S., Europe, Central Asia, China, and Japan (WITS, 2020). The majority of natural rubber is produced by smallholders. Indonesian natural rubber constitutes about 80 % or 65–70 % of global production (Statista.com, 2022). Smallholders managed rubber farming as family-based agriculture with farms smaller than 10 ha (Gouyon, 2003). In 2021, dry rubber production of smallholders reached 92.81% (2.83 million tons), followed by state estates with 4.32% (0.13 million tons) and private estates with 2.87% (0.09 million tons). The majority of rubber originated from South Sumatera (29%), North Sumatra (11%), Riau (10%), Jambi (10%), and West Kalimantan (8%) (BPS, 2021). However, smallholders have faced challenges for decades, making them less competitive (Zuhdi and Anggraini, 2020). Indonesia produces 0.96 tons per hectare, way behind Thailand, Vietnam, and Malaysia, with 1.80, 1.72, and 1.51 tons per hectare, respectively (WWF, 2020). Smallholders are often forced to combine their holdings with other crops or increase land area (WWF, 2020), leading to decreased rubber exports (Fatahillah *et al.*, 2022), degraded soil (Nguyen *et al*., 2020), and loss of forest ecosystems, biodiversity, and carbon storage (Panda and Sarkar, 2020; He and Martin, 2016). Limited downstream industries can only absorb 15% of the semi-finished goods (GBGI, 2016). Natural rubber markets also remain volatile due to distinct supply-side drivers of synthetic rubber (Wagner, 2020), fluctuations in oil prices, and political changes (Raju, 2016), leading to changes in farm prices and affecting smallholders' profits in the short run (Srisuksai, 2020). Unsustainable agricultural practices are a challenge for smallholders (Inkonkoy, 2022). Most smallholders still use large amounts of chemical fertilizers (Kullawong *et al*., 2020), seedlings and unproven cultivars, outdated planting methods, few legumes cover crops, and high-frequency and low-quality tapping (Zaw and Myint, 2016). Their harvest and post-harvest methods are unsuitable (Yardha *et al*., 2022), and they lack systematic management. However, there is potential for sustainable practices in natural rubber production. Regarding environmental aspects, cultivating rubber emits little greenhouse gas (GHG), requires less fertilizer (Nguyen *et al*., 2020), and increases carbon sinks and sequestration (Pinizzotto *et al*., 2021), thus promoting soil health (Nguyen *et al*., 2020; Chotiphan *et al*., 2019), and biodiversity conservation (Wang *et al*., 2020; Lan *et al*., 2017). Mixtures of rubber trees with Eucalyptus and Acacia can enhance water use efficiency (Chotiphan *et al*., 2019), and changing the land use from the cultivation of edible crops does not threaten food availability (Kullawong *et al*., 2020). Regarding socioeconomic aspects, household incomes are increasing, with the possibility of moving households and communities out of poverty (Hauser *et al*., 2015). Sustainable agricultural practices for rubber cultivation can provide smallholders with quality assurance (Gouyon, 2003), increased productivity (Esekhade *et al*., 2021), improved livelihoods (Inkonkoy, 2022), compliance with relevant legislation (Leimona *et al*., 2015), and proper use of natural resources (Piñeiro *et al.*, 2020). Adopting sustainable practices along the value chains will strengthen strong long-term resilience to climate change (Nkeuwa *et al*., 2022; Kangogo *et al*., 2020) and markets shocks (Davis *et al*., 2021) and meet current and future societal needs (Makate *et al*., 2017). The Indonesian government tried to support smallholders in improving their rubber cultivation and post-harvest management practices through The Sustainable Natural Rubber Platform of Indonesia (SNARPI) and cooperatives or other collective organizations in processing and marketing activities for higher prices and profits (Suttipong and Koichi, 2019). However, the results depend on how and whether smallholders can get measurable outcomes, such as a higher level of technical efficiency for enhancing productivity (Kuswanto *et al.*, 2019), welfare (Danso-Abbeam and Baiyegunhi, 2020), and planning its implementation (WEF, 2016). Sustainable agricultural practices for rubber cultivation are not only a goal but a learning process (Garzón-Delvaux, 2020). Stimulating the adoption of intentions of self-identity can be a driving force to overcome the barriers of high costs and behavioral changes (Yanakittkul and Aungvaravong, 2020; Silva *et al.*, 2020). This will be encouraged by providing assistance and transferring knowledge (Barbosa Junior *et al.*, 2022), social awareness (Punzano *et al.*, 2021), perceived ease of use, and attitudes based on experience and environmental sensibilities (Saengavut and Jirasatthumb, 2021). The decision to adopt sustainable practices among smallholders has been a subject of scientific inquiry with different theoretical frameworks and is based on the interdependence of multiple factors (Kassie *et al.*, 2015). The study tried to focus on the simultaneous relationship of smallholder characteristics, such as age, education level (Coulibaly *et al.*, 2021), and income (Khanal *et al.*, 2021), and farm factors, such as experience and farming methods (Coulibaly *et al.*, 2021), as constraints for adopting rubber cultivation and management practices. Identifying the smallholder characteristics is necessary as they do not form a homogenous group (Fan and Rue, 2020) but dynamic and sensitive targets (Etana *et al.*, 2020). Education, income, and experience are key characteristics of the success potential of smallholders to cultivate and manage rubber practices (Dissanayake *et al.*, 2013), while age is linked to their ability to allocate production inputs (Kuswanto *et al.*, 2019). Smallholders' income initiates a process of using and combining inputs to acquire and assimilate information and technology (Rapsomanikis, 2015). Yet, smallholder's history, culture, traditional knowledge, economic disparity, and geographic distribution reflect their different approaches to cultivating and managing rubber (Min *et al.*, 2017). While the theory of planned behavior is commonly used to encourage the adoption of sustainable agricultural practices, it failed to provide an effective way for the intentions to explain the behaviors themselves (Norman and Conner, 2005). There are intention-behavior gaps (Nguyen *et al.*, 2019) and significant variability within the strength connection (Fan and Rue, 2020). A sustainable agricultural practice uses good agricultural practices (GAP), defined as preventing problems before they occur (Shareen, 2016). Among the three steps of GAP, cultivation addresses the risks of farm work (APO, 2016), harvesting contributes to income and employment (Abdullah *et al.*, 2021), and post-harvest ensures product quality (Coffelt *et al.*, 2009) using natural coagulation, acid coagulation, air drying and ripening, storage, and sales (Henderson, 1977), and handling the losses (UNCAPSA, 2015). Little literature has proposed mediator factors for the behavior connection in the context of GAP adoption. The current study aims to determine the effect of income and smallholder characteristics on cultivation, harvesting, and post-harvest management. It was conducted in West Kalimantan, one of the regional centers of Indonesian natural rubber production, in 2022.

MATERIALS AND METHODS

Mixed methods were used to answer research questions that could not be addressed by a singular approach (Doyle *et al.*, 2009). The study was conducted in five districts Kapuas Hulu, West Kalimantan, Indonesia: Emabaloh Hulu, Batang Lupar, Boyan Tanjung, Kalis, and Hulu Gerung (Fig. 1). The area has a very wet climate, and most of the land is covered by forest (Lusiana *et al.*, 2008).

Non-probability sampling was used as a valid and efficient non-random method (Tongco, 2007) for 100 smallholders (Purwanto and Sudargini, 2021) through a semi-structured questionnaire and an in-depth interview (Brounéus, 2011). The study was composed of three phases. First, the smallholder characteristics, including age, formal education (Coulibaly *et al.*, 2021), household income (i.e., on-farm, off-farm, non-farm activities) (Khanal *et al.*, 2021), and farm experience (Coulibaly *et al.*, 2021) were identified using quantitative descriptive with percentage (%) tabulation. Second, the characteristics of cultivation, harvesting, and post-harvest management of natural rubber (Shareen, 2016; APO, 2016) were identified based on good rubber (*Hevea brasiliensis*) cultivation guidelines from the Indonesian Ministry of Agriculture, Directorate General of Plantations (Kementan, 2014) and sustainable natural rubber production and post-harvest management (Henderson, 1977) were determined using quantitative description with percentage tabulation. The cultivation/Skillfar parameter includes pests and diseases/Y11 (i.e., pesticide usage, integrated pest management and diseases, and usage of chemical pesticides); land/Y12 (i.e., organic material, soil organisms, fertilizer, the role of forests, soil erosion control, and terracing); processed products/Y13 (i.e., costs and benefits of farming, cider cone, trees layers, products and services, use of local trees, and waste management); cultivation/Y14 (i.e., sustainable farming, optimal

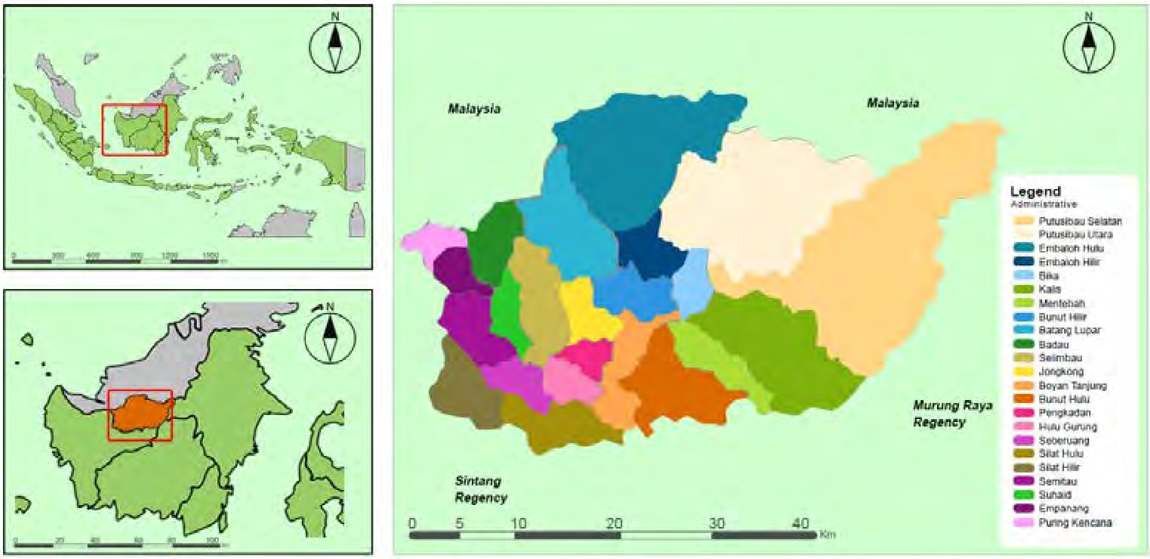

Fig. 1: Geographic location of the study area in Kapuas Hulu, West Kalimantan, Indonesia

growing conditions, optimal density, thinning for density control, pruning, and weeding); and labor/Y15 (i.e., child labor and safety, workers' rights, and community relations). The harvesting /Skillharv parameter includes time/Y21 (i.e., tapping time and frequency); step/Y22 (i.e., tapping technique); tools and materials/Y23 (i.e., tapping knife, bamboo latex collector, clean latex collection, and latex stimulus risk). Finally, the post-harvest/ Skillphar parameter includes results and sales/Y31 (i.e., quality processing, air drying, maturation, and sales) and risk/Y32 (i.e., acid coagulation and storage). Third, simultaneous effects of cultivation/Skillfar/Y1, harvesting/Skilharv/Y2, and post-harvest/Skilphar/Y3 management on household income (i.e., on-farm/X11, off-farm/X12, and non-farm/X13), and smallholder characteristics (i.e., formal education/X21, age/X22, and farm experience/X23) were studied using a partial least square (PLS) path modeling with a PLS structural equation modeling (PLS-SEM or Smart-PLS) software due to its efficiency for small sample sizes and complex models (Willaby *et al.*, 2015). PLS-SEM includes two elements: the structural/inner model and the measurement/outer model (Hair *et al.*, 2021). They include the following steps: i) reflective measurement model; ii) evaluation of formative measurement models; and iii) evaluation of the inner/structural model (Purwanto and Sudargini, 2021). In the reflective measurement model, a model is tested for the interrelationship between the variables using composite reliability (CR) > 0.70, which tends to give an estimate higher than Cronbach's alpha (α), for the internal consistency/reliability of the constructs due to the different weights of each indicator. The average variance extracted (AVE) > 0.50 is used to assess the construct validity of a measurement procedure (Chan and Lay, 2018). In formative measurement models, the content specification must relate to the scope of the latent construct using a variance inflated factor (VIF) < 10 to ensure no multicollinearity data. The accuracy of the prediction is indicated by the R-squared (R^2) and Q-squared (Q^2) values. $R^2 < 0.19$ is considered unacceptable or interpreted as the exogenous variables that cannot explain the endogenous dependent variable. $Q^2 > 0$ is relevant for a specific dependent construct (Hair *et al.*, 2021). In the inner/structural model, the significance of the relationship between the constructs/variables is assessed using a critical ratio (CR) > 1.96 or a probability *(p)* > 0.05. The f-square (f^2) indicates the effect of the size criterion on the structural level: $0.02 \leq f^2 \leq 0.15$ is small, $0.15 \leq f^2 \leq 0.35$ is moderate, and $f^2 \geq 0.35$ is a large effect. The outer loading factor indicates the estimated relationships or determines an item's absolute contribution to its assigned construct (Hair *et al.*, 2021).

RESULTS AND DISCUSSION

Smallholder characteristics

Smallholder characteristics include on-farm, off-farm, and non-farm income, education, age, and farm experience (Table 1).

As indicated in Table 1, most smallholders' income is over 25.96–45.42 USD/month from on-farm and non-farm activities and over 97.34 USD/month from off-farm activities. Most have a senior high school level or 12 years of formal education, over 45–55 years of age, and over 20 years of farm experience. Moreover, the rubber price impacts smallholders. The fluctuating rubber prices affect the product type produced, working hours, type of input (Erlina *et al.,* 2019), safety standards, use of toxic chemicals, discrimination, and child labor (Aidenvironment, 2016). The combination of biological and economic aspects of rubber production (Purnamasari *et al.*, 2002) and institutional assistance (Wang *et al.,* 2023) have been conceived to mitigate price fluctuations.

Cultivation, harvesting, and post-harvest management characteristics of natural rubber

The cultivation, harvesting, and post-harvest management characteristics of natural rubber are presented in Table 2.

Most used cultivation methods include sustainable farming, optimal growing conditions, optimal density, thinning for density control, pruning, and weeding. Most harvesting methods include tapping, and

Table 1: Natural rubber smallholder characteristics (%)

Income (USD/month):	
On-farm:	
≤5.84	1.11
>5.84-25.96	16.67
>25.96-45.42	36.67
>45.42-97.34	33.33
>97.34	12.22
Off-farm:	
≤5.84	17.78
>5.84-25.96	17.78
>25.96-45.42	7.78
>45.42-97.34	20.00
>97.34	36.67
Non-farm:	
≤5.84	3.33
>5.84-25.96	8.89
>25.96-45.42	40.00
>45.42-97.34	33.33
>97.34	14.44
Education (year):	
≤6	1.11
>6-<9	24.44
9	26.67
12	42.22
>12	5.56
Age (year):	
<25	2.22
25-35	4.44
>35-45	30.00
>45-55	52.22
>55	11.11
Farm experience (year):	
<5	2.22
5-10	3.33
>10-15	28.89
>15-20	31.11
>20	34.44

Table 2: Cultivation, harvesting, and post-harvest management characteristics of natural rubber (%)

Cultivation	
Pesticide usage, integrated pest management and diseases, and usage of chemical pesticides	71.11
Organic material, soil organisms, fertilizer, the roles of forests, healthy soil, soil erosion control, and terracing	47.78
Costs and benefits of farming, cider cone, trees layers, products and services, the use the local tree, and waste management	43.33
Sustainable farming, optimal growing conditions, optimal density, thinning for density control, pruning, and weeding	70.00
Child labor and their safety, workers' rights, and community relations	65.56
Harvesting	
Tapping time and frequency	88.89
Tapping technique	90.00
Tapping knife, bamboo latex collector, clean latex collection, and latex stimulus risk	84.44
Post-Harvest	
Quality processing, air drying, maturation, and sales	93.33
Risks of acid for coagulation, and storage	84.44

Table 3: Reflective measurement model

The variables/structural model	CR	AVE
Income (Income/X1)	0.844	0.570
Smallholder characteristics (Charac/X2)	0.799	0.643
Cultivation (Skillfarm/Y1)	0.917	0.691
Harvesting (Skillharv/Y2)	0.877	0.704
Post-harvest (Skillphar/Y3)	0.902	0.822

most post-harvest methodologies include quality processing, air drying, maturation, and sales.

Effect of income and smallholder characteristics on cultivation, harvesting, and post-harvest management

In the reflective measurement model, the interrelationship model between the income/X1, smallholder characteristics/X2, cultivation/Y1, harvesting/Y2, and post-harvest/Y3 management displayed valid and consistent results (Table 3).

In formative measurement models, the content specification has proven related to the scope of the latent construct, and no multicollinearity data were observed (Table 4).

The accuracy of the prediction of the exogenous variables (i.e. income and smallholder characteristics) to explain the endogenous dependent variable (i.e. cultivation, harvesting, and post-harvest management) is indicated by the value of R^2 and Q^2 (Table 5).

As indicated in Table 5, income and smallholder characteristics are relevant in explaining cultivation, harvesting, and post-harvest management. In the structural/inner model, the significance of the relationship between the constructs/variables is assessed using CR value (Fig. 2).

Fig. 2 presents the non-significant effect of the relationship between income and smallholder characteristics on cultivation and income on post-harvest management.

The effect of the others relationships is significant based on f^2 values for the effect of size on the structural level (Table 6).

The size effect of the significant relationship between income and smallholder characteristics on harvesting and of smallholder characteristics on post-harvest management is small. Income is a key characteristic of the success potential of smallholders to cultivate and manage rubber plantations in the selected study area and other areas (Dissanayake *et al.*, 2013). Mentoring and introducing site-specific technology help smallholders overcome the limitations of their income and resources in rubber cultivation and management (Yardha *et al.*, 2022). The outer loading factor that indicates the estimated relationships or determines an item's absolute contribution to its assigned construct is presented in Fig. 3.

Table 4: Formative measurement model

The content specification	VIF
Income/X1:	
On-farm/X11	1.545
Off-farm/X12	1.471
Non-farm/X13	1.321
Smallholder characteristics/X2:	
Education/X21	1.160
Age/X22	1.305
Farm experience/X23	1.421
Cultivation/Y1:	
Pesticide usage, integrated pest management and diseases, and usage of chemical pesticides (Pets and Diseases/Y11)	2.721
Organic material, soil organisms, fertilizer, the roles of forests, healthy soil, soil erosion control, and terracing (Land/Y12)	3.211
Costs and benefits of farming, cider cone, trees layers, products and services, the use of local tree, and waste management (Processed products/Y13)	2.116
Sustainable farming, optimal growing conditions, optimal density, thinning for density control, pruning, and weeding (Cultivation/Y14)	2.836
Child labor and their safety, workers' rights, and community relations (Labor/Y15)	1.687
Harvesting/Y2:	
Tapping time, and frequency (Time/Y21)	1.566
Tapping technique (Step/Y22)	1.754
Tapping knife, bamboo latex collector, clean latex collection, and latex stimulus risk (Tools and Materials/Y23)	1.779
Post-Harvest/Y3:	
Quality processing, air drying, maturation, and sales (Results and Sales/Y31)	1.747
Risks of acid for coagulation, and storage (Risk/Y32)	1.747

Table 5: R^2 and Q^2 values

Endogenous dependent variable	R^2	*Adj.* R^{2*}	Q^2
Cultivation (Skillfarm/Y1)	0.188	0.167	0.149
Harvesting (Skillharv/Y2)	0.283	0.264	0.194
Post-harvest (Skillphar/Y3)	0.237	0.217	0.124
Mean	0.236	0.216	0.155

*Adjusted r-squared

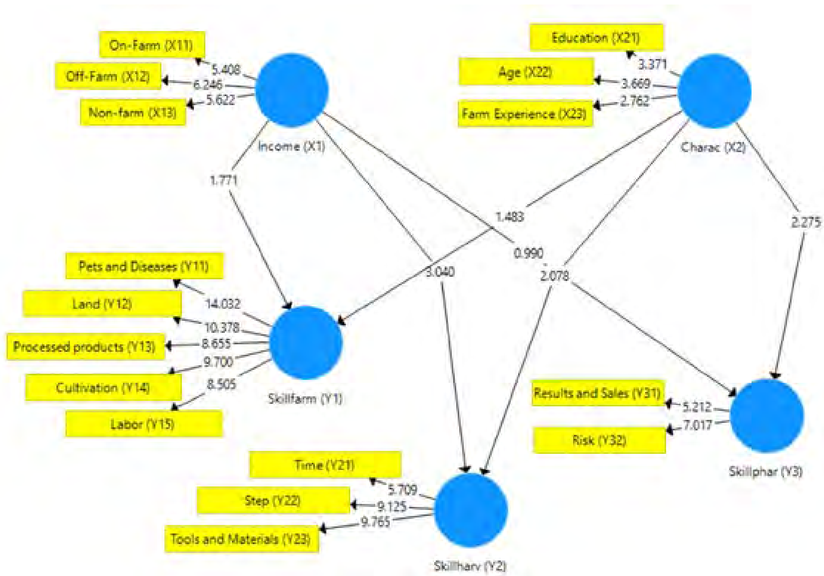

Fig. 2: *CR* value

Table 6: f^2 value

Effect of size on the structural level	Cultivation (Skillfarm/Y1)	Harvesting (Skillharv/Y2)	Post-harvest (Skillphar/Y3)
Income (Income/X1)	**0.053**	0.053	**0.054**
Smallholders characteristics (Charac/X2)	**0.047**	0.057	0.016

Note: Bold mark is insignificant

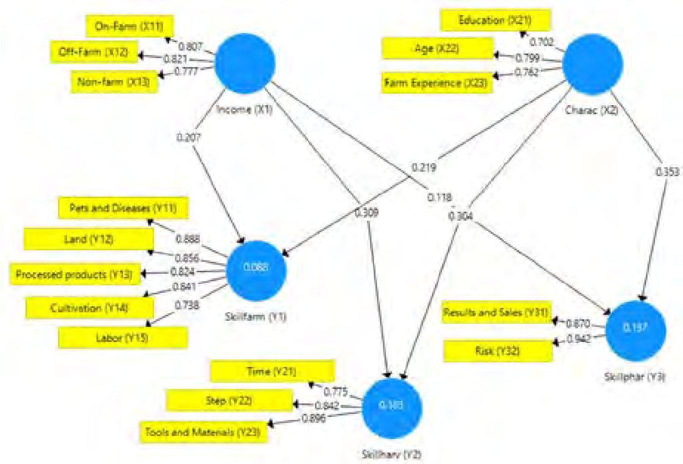

Fig. 3: Estimated coefficient

As indicated in Fig. 3, increased harvesting is more likely to depend on increasing income than smallholder characteristics, and increased income is more likely to depend on off-farm activities, followed by on-farm and non-farm activities. Increased post-harvest management depends on increased smallholder characteristics, while increased smallholder characteristics depend more on increased age than education. Increased harvesting depends more likely on increasing tapping knife, bamboo latex collector, clean latex collection, and latex stimulus risk. Finally, increased post-harvest management is likely due to increasing risks of acid coagulation and storage. Organic material, soil organisms, fertilizer, the role of forests, soil erosion control, and terracing can enhance biodiversity and soil ecology (Ogunsola *et al*., 2020). The tapping technique, a costly activity, can be improved by tapping from high left to low right (Rodrigo, 2007). Natural coagulation, formic acid coagulation, and air-drying methods are the key drivers of structuration (Noinart *et al*., 2022) for resin quality, limiting post-harvest latex losses, and increasing the time interval between harvesting and processing (Coffelt *et al*., 2009). A smallholder identity factor that is contrary to their productivity attitude can lead to non-pros-farm management behavior (Shen *et al*., 2022), the perception of which (Dixon *et al*., 2022) might mean more regulation (McGuire *et al*., 2015), fear of losing non-pecuniary benefits (Howley *et al*., 2015), place attachment (Maricchiolo *et al*., 2021), and underestimating the importance of environmental benefits (Shen *et al*., 2022; Gosling and Williams, 2010). The support of supply chain dialogue and emotional valuing of nature to target particular groups of smallholders (Barnes *et al*., 2022; Gosling and Williams, 2010) and engage in long-term supply chain relationships and co-investment with the rubber industry can create awareness and commitment while solving sustainability issues (Aidenvironment, 2016). Income changes do not necessarily reflect changes confronted by farm households (Mishra *et al*., 2002) since economic incentives alone are unlikely to encourage specific cohorts of smallholders (Howley *et al*., 2015).

CONCLUSION

Most smallholders' income is over 25.96–45.43 USD/month from on-farm and non-farm activities and over 97.36 USD/month from off-farm activities. Most have a senior high school level or 12 years of formal education, over 45–55 years of age, and over 20 years of farm experience. Most cultivation methods include sustainable farming, optimal growing conditions, optimal density, thinning for density control, pruning, and weeding. The most used harvesting technique is tapping, and the most used post-harvest management method includes quality processing, air drying, maturation, and sales. The results indicate that the effect of the relationship between income and smallholder characteristics on cultivation and income on post-harvest management is not significant. However, the effect of the relationship between income and smallholder characteristics on harvesting and of smallholder characteristics on post-harvest management is significant. Most harvesting increases are due to increased income rather than smallholder characteristics, and most income increases are due to increased off-farm activities, followed by on-farm and non-farm. Most post-harvest increases are due to increased smallholder characteristics, and most smallholder characteristics increases are due to increased age rather than education. Increasing tapping knives, bamboo latex collectors, clean latex collection, and latex stimulus risk present challenges in managing the harvesting step, while increasing risks of acid coagulation and storage present challenges in managing the post-harvest processing and marketing of natural rubber. Using organic materials, soil organisms, and fertilizers by considering healthy soil, tapping techniques, coagulation and drying methods with the support of supply chain dialogue and emotional value of nature to target particular groups of smallholders, and engaging in long-term supply chain relationships and co-investment with the rubber industry can promote pro-sustainable farm management behavior. Thus, the adoption of rubber cultivation and management methods depends on whether smallholders can provide measurable outcomes, such as a higher level of technical efficiency to enhance productivity and welfare, building partnerships in the planning and implementation of cultivation, and management with key stakeholders.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
AVE	Average variance extracted
Adj. R^2	Adjusted r-squared
α	Cronbach's alpha
CR-value	Critical ratio
CR	Composite reliability
f^2	F-square
GAP	Good agricultural practices
GDP	Gross domestic product
GHG	Greenhouse gas
p	Probability
PLS	Partial least squares
Q^2	Q-squared

R^2	R-squared
SEM-PLS	Structural equation modeling-partial least squares
USD	U.S. dollars
VIF	Variance inflated factor

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CASE STUDY

Heavy metals contamination in geothermal medicinal plant extract (*Chromolaena odorata* Linn)

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ABSTRACT

BACKGROUND AND OBJECTIVES: Medicinal plants growing in geothermal areas have been reported to possess relatively high abundance of bioactive secondary metabolites concomittant to the adaptive heat stress response. Nonetheless, the exploitation of their medicinal benefits is limited by the possible health-threatening concentrations of heavy metal contamination. *Chromolaena odorata* Linn, or also called as seurapoh, is a well-known medicinal plant but could absorb and accumulate heavy metal from the soil. Herein, this present study aimed to investigate the contents of Hg, Pb, Cd, and As in ethanolic extract of *Chromolaena odorata* Linn leaves collected from a geothermal area in Aceh of Indonesia.
METHODS: Three hot springs (Ie-Suum, Ie-Jue, and Ie-Brouk) located in the same geothermal area Seulawah Agam was selected as the sampling points, where three samples of *Chromolaena odorata* were collected in each sampling point. Extraction was carried out by means of maceration employing ethanol solvent. The contents of heavy metals in each extract were determined by priorly validated atomic absoption spectrometry and graphite furnace atomic absorption.
FINDINGS: The results revealed that arsenic (0.0482 ± 0.004 – 0.0639 ± 0.007 miligram per kilogram) and lead (0.0219 ± 0.004 – 0.0672 ± 0.006 miligram per kilogram) were found in trace levels and did not exceed Indonesian maximum safety thresholds (≤5 and ≤10 miligram per kilogram, respectively). The presence of mercury in all samples was not observable (limit of detection= 0.018 miligram per liter). Cadmium was observed in almost all samples with a concentration range of 0.0219 ± 0.005 – 1.1472 ± 0.006 miligram per kilogram exceeding the maximum threshold (0.3 miligram per liter).
CONCLUSION: Contamination of heavy metals in the ethanolic extract of geothermal *Chromolaena odorata* leaves is thought to be originated from volcanic activities. Among the heavy metals of concern, cadmium was the only one with concentration exceeding the safety limit. The presence of cadmium in the extract with high concentration could act cause its translocation to human body which eventually lead to multiple organ damages. Therefore, the extract of geothermal C. odorata leaves collected from a geothermal area should be consumed with caution for possible cadmium intoxication.

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47	0	4

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INTRODUCTION

Geothermal has provided unique ecological conditions for living organisms such as microbes and plants to live on and adapt with. Thus, there are high chance for organisms inhabiting geothermal areas to possess different characteristics from their counterparts living in other regions (Boothroyd, 2009; Rachmilevitch *et al.*, 2006).In response to geothermal heat stress, several plants have been witnessed to experience the shifting of metabolomes with increased production of phenolic acids and terpenes (Gargallo-Garriga *et al*., 2017). This phenomenon is thought to be beneficial to enhance the therapeutic effect of medicinal plants growing within geothermal areas. The study group has reported more efficacious effects of extracts of several medicinal plants collected from Mount Seulawah Agam – a volcano located in Aceh, Indonesia. Of which, the research group has investigated the antibacterial and antioxidant activities of *Calotropis gigantea* (Kemala *et al*., 2022; Ningsih *et al*., 2022) and *Vitex pinnata* (Nuraskin *et al*., 2019a; Nuraskin *et al.*, 2019b). Other than the foregoing plants, *Chromolaena odorata* Linn (also known as *seurapoh* by the locals) is commonly used as a traditional medicine to treat various diseases, including fever, diarrhea, malaria, and skin diseases (Phumthum *et al*., 2020). Scientifically, *C. odorata* Linn has been reported effective as antiulcer, anti-inflammatory, antipyretic, antimicrobial, antioxidant, analgesic, and diuretic agents (Olawale *et al*., 2022; Sabri and Yusof, 2021). In a qualitative screening, *C. odorata* from a geothermal area in Aceh was found positive containing tannins, steroids, flavonoids, terpenoids, phenols, and saponins. Previously, a preliminary study has revealed that secondary metabolites were thought to be different between those produced in geothermal and non-geothermal *C. odorata* based on chemometric classification (Abubakar *et al*., 2021). These all are indicative that *C. odorata* from geothermal area has relatively high secondary bioactive metabolites, hence higher therapeutic efficacy. Unfortunately, based on a recent investigation, hot springs manifested in Mount Seulawah Agam were contaminated with arsenic (As) in high concentration, where bioaccumulation of this heavy metal in people exposed daily to the water was observed (Irnawati *et al*., 2021). Volcanic activities are responsible to the release of heavy metals to the surrounding environment (Nagajyoti *et al*., 2010). Since *C. odorata* has a strong bioaccumulation ability (Tanhan *et al.*, 2007), its oral uptake as herbal medicine might act as the translocation route of heavy metals from environment to human body (Behera and Bhattacharya, 2016; Yap *et al*., 2010). These findings stress the importance of performing thorough assessment of heavy metal contents in the extract of *C. odorata* before used as herbal medicine. Previously, researchers have reported the heavy metal contaminations in herbal medicine commercially available and commonly consumed in China (Meng *et al*., 2022; Wang *et al*., 2019) and Pakistan (Soomro *et al.*, 2021). However, none of the published studies has investigated the geothermal medicinal plant, particularly for *C. odorata.* Herein, the contents of mercury (Hg), lead (Pb), cadmium (Cd), and As in the ethanolic extract from geothermal *C. odorata* leaves were investigated. The objective of this study was to determine the presence of heavy metal contents in the ethanolic extract of *Chromolaena odorata* Linn leaves collected from geothermal area in Aceh, Indonesia. This study was performed in Aceh Province, Indonesia in 2022.

MATERIALS AND METHODS

Study design

This study further aimed to draw a conclusion about the safety of consuming the extract based on the heavy metal content profiles. The heavy metals analyzed herein were Hg, Pb, Cd, and As, quantitatively determined using Atomic Absorption Spectrometry (AAS) or Graphite Furnace—AAS (GF-AAS). The heavy metal concentrations were then compared with the maximum thresholds observed by the Indonesian Agency for Drug and Food Control (BPOM) (Ahmad *et al*., 2022).

Plant specimens and chemicals

Plants specimen (*Chromolaena odorata* Linn) was collected from three separated hot springs namely Ie-Suum, Ie-Jue, and Ie-Brouk, located within the same geothermal area Mount Seulawah Agam in Aceh Besar Regency, Aceh Province, Indonesia. In each location, the plant specimen was collected from three different sampling points as presented in detail in Table 1. The sampling locations are not commonly exposed to anthropogenic activities, except for Ie-Suum receiving intense tourist visits. Taxonomical identification of the collected specimen was carried

Table 1: Sampling locations and sample weights of *C. odorata* Linn leaves

Location	Label	Coordinate		Leaf sample weight (kg)	
		N	E	Fresh	Dry
Ie-Suum	IS-1	5°32'51"	95°32'53"	3.00	0.61
	IS-2	5°32'52"	95°32'49"	3.45	0.64
	IS-3	5°32'54"	95°32'32"	3.95	0.68
Ie-Jue	IJ-1	5°30'23"	95°37'45"	3.35	0.64
	IJ-2	5°30'25"	95°37'42"	3.45	0.64
	IJ-3	5°30'24"	95°37'38"	3.15	0.60
Ie-Brouk	IB-1	5°51'68"	95°62'06"	3.00	0.60
	IB-2	5°51'59"	95°62'00"	3.45	0.64
	IB-3	5°51'82"	95°61'69"	3.00	0.65

out in Department of Biology, Universitas Syiah Kuala, Indonesia, with voucher number: 179/UN11.1.8/TA.00.03/2023. To determine the concentrations of heavy metals in the plant specimen, the following chemicals or materials were required: Argon gass, nitric acid (HNO_3), hydrogen peroxide (H_2O_2) 30%, perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), deionized water, and standard solutions of Hg, Pb, Cd, and As. As for the extraction, ethanol 96% solvent was used. All chemicals were analytical grade and used without pre-treatment after being procured from Merck (Selangor, Malaysia).

Preparation of the ethanolic extract from C. odorata Linn leaves

In general, the extract was prepared following the previously published study with a few modifications (Abubakar *et al*., 2021). Briefly, the leaves of *C. odorata* Linn; 200 gram (g) were air-dried for a week prior to maceration using ethanol 96% for 2x24 hour (h) at room temperature. The filtrate was separated by filtration and proceeded to rotary evaporation to obtain the concentrated extract as much as 40 g.

Pre-treatment for heavy metal determination

Before analyzed, the leaf samples were pre-treated by acidic destruction. Leaves were weighed for 1 g and placed on a glass container following with the addition of concentrated 7 mililiter (mL) HNO_3 and 1 mL H_2O_2 30%. Afterward, the mixture was heated at 80 degree Celcius (°C) on hot plate for 30 minutes (min), consequently left-cold and filtered. The 5 mL filtrate was diluted in 50 mL volumetric flask.

Calibration curve construction and validation

Calibration curves for AS and Hg were constructed based on the signal intensities recieved at 193.7 and 253.7 nanometer (nm) generated by the solution standards, respectively. Meanwhile in the case of Pb and Cd, wavelengths of 283.3 and 228.8 nm were used, respectively. Limit of detection (LoD) and limit of quantification (LoQ) were determine for each heavy metal to measure the sensitivity based on LINEST function on Microsoft Excel (Redmond, Washington, USA) (Iqhrammullah *et al*., 2021; Nisah *et al*., 2022). Quality of the calibration curve was judged based on the linearity (R^2). Precision and recovery were also calculated to validate the analytical methods (Nisah *et al*., 2022; Safitri *et al*., 2022). The relative standar deviation (RSD) was used to determine the precision, which was obtained from 6 repetitions of a particular concentration of the heavy metal. As for the recovery (%), the value was calculated based on the signal intensity generated by the spiked sample. Validities of the used methods have been presented in Table 2.

Determination of As and Hg using GF-AAS

Determinations of AS and Hg were carried out on GF-AAS (Perkin Elmer PinAAcle 900 Series AA Spectrometers, Waltham, Massachusetts, United States). Pre-treated solution (20 µL) was injected through autosampler into the furnace tube for diatomization. Concentrations of As and Hg were determined at 193.7 and 253.7 nm, respectively. Each sample was analyzed in triplicate and the data were presented as mean±standard deviation (SD).

Plant specimens and chemicals

As for Pb and Cd, their concentrations were determined using AAS Flame (Thermo Fisher Scientific iCE 3500, Waltham, Massachusetts, United States). Wavelengths of 283.3 and 228.8 nm were

Table 2: Sensitivity, precision, and accuracy of the methods used to determine the heavy metal contents

Heavy metals	LoD (mg/L)	LoQ (mg/L)	R^2	RSD (%)	Recovery (%)
Pb	0.016	0.048	0.9995	1.01	99.86
Cd	0.024	0.072	0.9990	0.99	100.11
As	0.020	0.062	0.9993	1.05	100.28
Hg	0.018	0.055	0.9994	1.07	101.77

Table 3: Geothermal soil characteristics of the sampling locations (Idroes *et al*., 2019)

Hot spring	Temperature (°C)	Elevation (m)	pH
Ie-Suum	83.63±0.075 – 86.09±0.019	70-72	6.66±0.000 –6.68±0.004
Ie-Jue	93.49±0.172 – 98.62±0.151	264-269	3.95±0.048 –5.93±0.005
Ie-Brouk	40.04±0.013 – 47.49±0.133	197-210	7.24±0.004 –7.40±0.058

employed for the determincations of atomized Pb and Cd, respectively. The analysis was carried out in triplicate for each sample, where the values obtained were presented as mean±SD.

RESULTS AND DISCUSSION

Hot spring characteristics

It is of importance to firstly understand the factors that contributing to the distribution of heavy metal. Obviously, plant species and variety are the determining factors of heavy metal uptake and accumulation. Similarly, the age of the plant is positively correlated to the heavy metal accumulation. Environmental factors are also known to affect the uptake and accumulation of heavy metals by plants including metal concentration in the soil, cation exchange capacity, soil pH, and contents of organic matters. Regardless, the most contributing factors are those related to the environment and soil characteristics (Annan *et al*., 2013). Hence, the soil characteristics surrounding the sampling locations have been presented (Table 3), which have been published in the previous work (Idroes *et al*., 2019). As for the presence of heavy metal in the geothermal area, a previous study has suggested the presence of As in geothermal soil and water of Mount Seulawah Agam (Irnawati *et al*., 2021). Other heavy metals, including Pb, Hg, and Cd, have been reported in various locations of geothermal areas (Pulungan *et al*., 2019; Zimik *et al*., 2021).

Heavy metal contaminations and their bioaccumulation

Heavy metal concentrations contained in ethanolic extract of *C. odorata* Linn leaves collected from the geothermal hot spring manifestations have been presented in Table 3. Based on the AAS method used with LoD of 0.018 mg/L for Hg, the heavy metal was not observable in all samples. In the case of As, its presence was only found in two samples collected from Ie-Jue with concentrations of 0.0482 ± 0.004 – 0.0639 ± 0.007 mg/kg. Cd was detected in all samples collected from hot springs Ie-Jue (0.8511 ± 0.005 – 1.0517 ± 0.007 mg/kg) and Ie-Brouk (0.5738 ± 0.006 – 1.1472 ± 0.006 mg/kg), while in the case of Ie-Suum, only a single sample was detected with Cd (0.0219 ± 0.005 mg/kg). Pb was quantitatively observable in all samples, where the highest concentration range was found in Ie Brouk (0.0544 ± 0.006 – 0.0672 ± 0.006 mg/kg). The concentration ranges for Pb content were 0.0219 ± 0.004 – 0.0316 ± 0.004 and 0.0329 ± 0.005 – 0.0514 ± 0.005 mg/kg for Ie-Suum and Ie-Jue, respectively.

In this present study, the presence of heavy metal contents was found in the ethanolic extract of *C. odorata* Linn leaves predominated in relatively high concentration by Cd. Hg was not observable in all samples, while only two samples were detected to contain As. Pb was found in all samples in a variety of concentrations ranging from 0.0219 ± 0.004 to 0.0672 ± 0.006 mg/kg. Of the four heavy metals assessed herein, only Cd found exceeding the maximum threshold by the BPOM. The differences in the observed heavy metal concentrations might be attributed to their solubility in ethanol, as not all species of Pb, Cd, As, or Hg are soluble in ethanol. Therefore, their concentrations are not reflective to the real concentration accumulated in the *C. odorata* Linn leaves. Regardless, the presence of heavy metals in the samples of this present study was

Table 4: Heavy metal concentrations contained in the ethanolic extract from *C. odorata* Linn leaves

Sample	Heavy metal concentration (mg/kg)			
	As	Cd	Hg	Pb
IS-1	ND	ND	ND	0.0316 ± 0.004
IS-2	ND	N	ND	0.0304 ± 0.005
IS-3	ND	0.0219 ± 0.005	ND	0.0219 ± 0.004
IJ-1	ND	0.8511 ± 0.005	ND	0.0514 ± 0.005
IJ-2	0.0639 ± 0.007	1.0360 ± 0.004	ND	0.0366 ± 0.005
IJ-3	0.0482 ± 0.004	1.0517 ± 0.007	ND	0.0329 ± 0.005
IB-1	ND	0.9482 ± 0.007	ND	0.0544 ± 0.006
IB-2	ND	0.5738 ± 0.006	ND	0.0672 ± 0.006
IB-3	ND	1.1472 ± 0.006	ND	0.0599 ± 0.004
Max. threshold	≤5	≤0.3	≤0.5	≤10

ND = Not detected

more likely occurring naturally, rather than caused by anthropogenic activities. Pb concentrations in samples collected from Ie-Suum, a place with relatively intense anthropogenic activities, were found less as compared to that of other hot springs (Ie-Jue and Ie-Brouk). As is an example of heavy metals that are commonly released through natural weathering, geochemical reactions, and volcanic eruptions (Maity *et al*., 2019). Similarly, the contamination of Cd has been associated with natural events such as volcanic eruptions and forest fires (Briffa *et al*., 2020). As weeds, *C. odorata* Linn is known to accumulate heavy metal with high survival rate against the environmental distress and has been utilized as phytoremediator for contaminated soil (Aiyesanmi *et al*., 2012; Tanhan *et al*., 2007). The uptake of heavy metals from the soil by *C. odorata* Linn have been reported in multiple studies. According to a published literature, Pb and Cd contents in the root of *C. odorata* exposed with intense vehicle traffic were found reaching as high as 3.79±0.19 and 0.01±0.00 mg/kg, respectively (Sulaiman and Hamzah, 2018). The distribution of heavy metal in *C. odorata* Linn was varied throughout the plant parts, where its shoots and roots could accumulated Cd as high as 102.3 and 1440.9 mg/kg, respectively (Tanhan *et al*., 2007). Moreover, the study also revealed that the accumulation was concentration-dependent, reaching accumulated concentrations as high as 1772.3 and 60655.7 mg/kg for Pb and Cd, respectively, confirming high heavy metal uptake capacity of this plant (Tanhan *et al*., 2007).

Health risk related to heavy metal exposure

In a previous study investigating the urinary and blood samples, accumulation of heavy metals in human body is less likely to be associated with the geothermal-sourced contamination (Nuvolone *et al*., 2022). However, accumulation through plants or animals might give a different insight on how naturally occuring heavy metal contamination could contribute to the exposure risk to humans (Ahmad *et al*., 2022). This present study is the first in reporting the heavy metal contaminations derived from geothermal sources in medicinal plant extract. Presence of heavy metal in the extract of *C. odorata* leaves found herein alarms the users of this medicinal plant regarding the possible heavy metal intoxication. Bioaccumulated heavy metal in the plant could be translocated to human and cause a series of health problems (Behera and Bhattacharya, 2016; Yap *et al*., 2010). Herein, it is found that Cd contaminated the ethanolic extract from geothermal *C. odorata* leaves at the concerning level since its concentration exceeded the maximum safety threshold. As the easiest heavy metal to be absorbed and accumulated in plants, translocation of Cd from plants to human is common (Yang *et al*., 2022). When present in human body, even at its lowest concentration, Cd could cause serious illness and even mortality. A study suggested that Cd is accumulated in immune cells, thereby modulating immune system function which triggers the systemic inflammation cascade (Hocaoğlu-Özyİğİt and Genç, 2020). Furthermore, Cd-associated immune response causes cell apoptosis and adversely alters secretion of cytokine, subset frequency of T lymphocyte, and production of selective antibodies (Wang *et al*., 2021). Organs such as lungs, kidneys, liver, gastrointestinal tract, and bones are susceptible to Cd toxicity. As the organ with the highest likelihood of being exposed to Cd after its oral intake, gastrointestinal tract has been suggested as the target of Cd toxicity (Zhao *et al*., 2006). Other than causing inflammation in the gut, Cd intoxication further progress to the dysbiosis of gut microbiome (Ba *et al*., 2017; Ninkov *et al*., 2015). Moreover, Cd exposure through inhalation could harm the upper respiratory tract, where it induced overproduction of mucus and disrupted the cilia function and squamous cells differentiation (Xiong *et al.,* 2019). When this heavy metal is absorbed and translocated to the blood stream, it could be accumulated and triggers the damages in multiple organs. In the blood stream, Cd was shown to reduce the activity of superoxide dismutase and catalase of erythrocyte which cause the imbalance of oxidative stress (Jemai *et al*., 2007). Several research has shown the acute toxicity of this heavy metal in artificial biological systems suggesting its ability to exert lethal nephortoxicity and hepatoxicity (Ibraheem *et al*., 2016; Wang *et al*., 2016). In addition, Cd accumulation has been reported to occur in bones (Tai *et al*., 2022), and consequently contributes to reduced bone mineral density, altered the expression of bone formation genes, disrupted osteoclast activities, reduced calcium absorption, and increased the risk of ostheoporosis (Ou *et al*., 2021; Reyes-Hinojosa *et al*., 2019).

CONCLUSION

Geothermal activity provides a unique activities for plants to produce different metabolite profiles as compared to those growing in area without geothermal activity. This encourage researchers and medical practitioners to utilize geothermal plants since they exert higher levels of bioactive secondary metabolites. However, geothermal activity promotes the release of toxic heavy metals from the earth's crust. Therefore, there is high possibility that the heavy metal would contaminate the plants inhabiting the geothermal areas. This is more pronounced in plants with an ability of high heavy metal uptake, including the *C. odorata*. In this present study, the contents of heavy metals in the extract of *C. odorata* collected from geothermal area were determined. The presence of As, Cd, and Pb contaminations in the ethanolic extract of geothermal *C. odorata* Linn leaves was found within the observable concentration range. It should be noted that the As, Cd, Pb, and Hg have different solubility in ethanol, so their concentrations in the extract are not reflective to their accumulated contents in the plant. The presence of these heavy metals are found to be non-dependent to the intensity of anthropogenic activities indicating their origins are from the natural occurrence. As for Hg, its concentration is thought to be below the LoD of the used analytical tool. In this case, the Hg could be present in lower concentration than the LoD so it could not be detected. Though the concentrations of the foregoing heavy metals are below the maximum safety level, their presence still deserve some concerns because of their capability to be accumulated in biological system. Cd concentrations in the extract exceeded the allowable limit, suggesting the potential harms of its consumption as herbal medicine. Cd exposure could lead to several serious pathologic conditions such as nephorpathy, pulmopathy, hepatopathy, and so on. Moreover, individuals intoxicated with Cd could experience an increased risk of ostheoporosis, reduced mineral density, and other ostheo-related problems. The presence of these heavy metals in the extract might counter the therpeutic benefits of *C. odorata* Linn. These findings also imply the possibility of heavy metal uptakes by other medicinal plants in geothermal area. A stringent quality control is therefore recommended in monitoring the content of toxic impurities in herbal products collected from geothermal area.

AUTHOR CONTRIBUTIONS

A. Abubakar contributed in the conceptualization, experiment, and original-draft writing. H. Yusuf acted as a supervisor and contributed in reviewing the final version of the manuscript. M. Syukri acted as a supervisor and contributed in reviewing the final version of the manuscript. R. Nasution acted as a supervisor and contributed in reviewing the final version of the manuscript. M. Yusuf assisted the experiment and performed formal análisis. R. Idroes performed the scientific, results validation, and reviewing the final version of the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
µL	Microliter
AAS	Atomic Absorption Spectrometry (AAS)
As	Arsenic
BPOM	Indonesian Agency for Drug and Food Control
°C	Degree celcius
Cd	Cadmium
g	Gram
GF-AAS	Graphite Furnace— Atomic Absorption Spectrometry
h	Hour
Hg	Mercury
LoD	Limit of detection
LoQ	Limit of quantification
min	Minute
mg/kg	Milligram per kilogram
mg/L	Miligram per liter
mL	Milimeter
nm	Nanometer
ND	Not detected
RSD	Relative standard deviation
SD	Standard deviation

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Podcasts

CASE STUDY

Dechlorination of selected polychlorinated biphenyl congeners using metal-impregnated pulverized shrimp shell catalyst from waste

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ABSTRACT

BACKGROUND AND OBJECTIVES: Polychlorinated biphenyls are pervasive contaminants that are receiving attention worldwide. Due to their well-known propensity to have harmful impacts on both humans and the environment, polychlorinated biphenyls have been internationally banned for use. In this study, dechlorination of five polychlorinated biphenyl congeners, 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5,5′-pentachlorobiphenyl, 2,2′,3,4,4′,5′-hexachlorobiphenyl, 2,2′,3,4,4′,5′-hexachlorobiphenyl, 2,2′,4,4′,5,5′-hexachlorobiphenyl, and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl, are evaluated. The chlorines from polychlorinated biphenyl congeners were removed using a heterogeneous catalyst synthesized via microwave-assisted impregnation of zinc metal onto pulverized shrimp shell waste.

METHODS: The five polychlorinated biphenyl congeners were dechlorinated through treatments combination of time (1–4 hours), heat (150–250 degree celsius), and catalyst proportion (1–5 percent weight/weight basis). The dechlorination trials followed the Box–Behnken experimental design and then analyzed using response surface methodology. Levels of the remaining polychlorinated biphenyl congeners were monitored by using a gas chromatograph equipped with an electron capture detector.

FINDINGS: The results of the trials demonstrated that among the five polychlorinated biphenyl congeners, only 2,2′,3,4,4′,5,5′-heptachlorobiphenyl did not respond to the provided treatments. Three congeners, namely, 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5,5′-pentachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl, showed positive response, and one congener 2,2′,3,4,4′,5′-hexachlorobiphenyl showed negative response to the provided treatments. These findings suggested that chlorine attached to the para position of the biphenyls ring was easier to remove. The efficiency calculation of total polychlorinated biphenyl concentrations after treatments was approximately 25 percent. Such a low degree of effectiveness may be caused by the catalyst becoming inactive, either chemically through the deposition of chlorines that have been removed from the biphenyl ring or mechanically by the leaching of zinc from the surface of the pulverized shrimp shell due to insufficient mechanical strength. Optimization via response surface methodology produced optimal results for dechlorination at 150 degree celcius for 2.4 hours with 5 percent additional catalyst.

CONCLUSION: The total amount of polychlorinated biphenyls that remained after dechlorination was not significantly impacted by the treatment combination of temperature, duration, and weight of the catalyst. However, the treatments had significant effects on the chlorine removal at the para positions of the biphenyl ring. In this case 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5,5′-pentachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl congeners have positive responses and 2,2′,3,4,4′,5′-hexachlorobiphenyl congener has a negative response. For polychlorinated biphenyl congeners having more than six chlorines, no chlorine removal was observed.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
61	5	6

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of compounds that are classified as persistent organic substances (Melymuk *et al.*, 2022). As part of persistent organic pollutants, the PCBs might travel at a very long distance due to natural phenomena such as water flow, wind force, and climate change (Carlsson *et al*. 2017; Wang 2016; Hageman *et al*. 2015, Kalleborn *et al*. 2015; Sadler and Connell 2012). PCBs could travel transboundary (Magulova, 2012) from their initial locations and arrive at or deposited in different geographical conditions (Hanedar *et al*. 2019; Jimenez *et al*. 2015). The characteristics and features of PCBs have been used in a wide range of industrial applications (Evans *et al*., 1991), either as closed or opened implementations (Dai *et al*., 2016). Examples of close applications are thermal fluids in transformers and capacitors. Open applications are carbonless duplicating paper, pesticide extenders, plasticizers, adhesives, ink, insulating cable of electricals, solenoids of discharge lamps, and flame retardants (Khalid *et al*. 2021; Erickson and Kaley, 2010; ATSDR, 2000). The expected property of PCBs for their wide applications is heat resistance. PCBs are xenobiotics (i.e., man-made products), and the substances in the environment resulted from anthropogenic activities. Numerous studies have shown that PCBs are hard to naturally degrade in the environment (Elangovan *et al*., 2019; Zhang *et al*. 2015; Alcock *et al*., 1996), present high risks to humans through food chain mechanisms (Brazova *et al*. 2012; Roveda *et al*. 2006), and may even prevent neurodevelopment of early stages of life (Marsan and Bayse, 2020; Crinnion, 2011). As a result, the use of PCBs for any application has been banned globally. The previous usage of PCBs have created accumulation problems in the environment and demonstrated potential transport into various environmental compartments that might end up in human and biota through food chain or food web mechanism (Montano *et al*., 2022; Umasangaji *et al*. 2020; Reddy *et al*.,2019). The molecular structure of PCBs comprised 10 homologs with a total of 209 congeners whose chlorine content is in the range of 19 – 71 percent (%) (Ghavami and Sajadi, 2010). The PCB congeners are identified based on a code developed by Ballschmiter and Zell (1980), which is usually abbreviated as the Ballschmiter and Zell (BZ) number. Although PCBs theoretically are composed of 209 congeners, only approximately 150 (Velazco *et al*. 2015) are found in the environmental samples and are still under consideration due to their toxic or dioxin-like characteristics (USEPA, 2010). According to the Stockholm Convention, PCBs will be totally phased out in 2028. Any remaining PCBs in signatory countries of the international convention, either those present in old stocks, still running electrical equipment, or those present in environmental matrices as pollutants must be stopped, properly managed, and then safely destroyed not later than the aforementioned year. Although the use of PCBs has been banned since the 1970s, worldwide PCB contamination problems are still present in various environmental matrices such as rivers, lakes, and harbors (Bedard, 2014); soils (Danielovic *et al*., 2015); and buildings (Herrick *et al*., 2007). Several methods have been utilized, either thermal, physico-chemical, or biological, to transform PCB congeners into safer molecules for humans and the environment. Thermal methods usually implemented incineration or plasma arc techniques that will produce carbon dioxide gas and water vapor as the final products (Seok *et al*., 2005; Western *et al*., 1995; Barton and Mordy, 1984). Thermal methods require a temperature of at least 850 degree celsius (°C) to avoid dioxin formation (Shibamoto *et al*., 2007). The plasma arc method can completely destroy PCBs; however, its implementation requires high energy, typically at 250 kilowatts (kW), to arrive at a very high temperature, that is, 25,000 °C (Barton and Mordy, 1984). The plasma arc method is a costly operated procedure. Although biological methods are considered environmentally sound techniques and low cost, these techniques proceed at slow rate kinetics, which sometimes takes months or even years (Ang *et al*., 2018; Wang and He, 2013). A combination of physical and chemical methods is developed to manage PCB contamination (Akhondi and Dadkhah, 2018; Velazco *et al*., 2015). In this study, the results of five PCB congener dechlorination treated with a catalyst developed from pulverized shrimp shell waste impregnated with zinc (Zn) metal are presented. The last three congeners shown in the Table 2 could represent hydroxylated PCB precursors or metabolites that show more toxic characteristics (Park *et al*., 2007). The five PCB congeners are typical markers in most environmental pollution (Costabeber *et al*., 2018; Ahmadkhaniha *et al*., 2013) and are thus chosen for this study. The present study aims

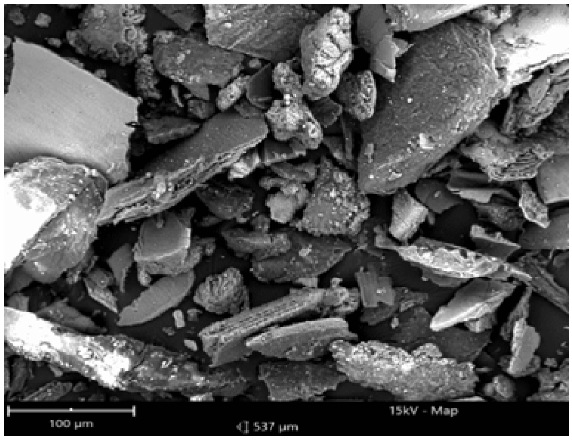


Fig. 1: Topography of pulverized shrimp shell by using SEM

Table 1: Experimental design in dechlorination PCBs using the Box–Behnken

Treatment (Factor)	Unit	Level		
		−1	0	1
Dechlorination time (A)	h	1	2.5	4
Dechlorination temperature (B)	°C	150	200	250
Catalyst weight (C)	%	1	3	5

to evaluate the performance of a heterogeneous catalyst developed from pulverized shrimp shell waste synthesized through the microwave-assisted impregnation method after heating and quenching treatments. The study was carried out in both Institut Pertanian Bogor (IPB) University and Indonesian National Research and Innovation Agency and Badan Riset dan Inovasi Nasional (BRIN) laboratory facilities from 2021 to 2022.

MATERIALS AND METHODS

Samples for PCBs treatment

Samples of transformer oil that contains PCBs were obtained from previous inventory activities carried out by the United Nations Industrial Development Organization (UNIDO) and the Ministry of Environment and Forestry Republic of Indonesia (KLHK). All the collected samples were put into dark glass bottle samples and stored in a refrigerator until use.

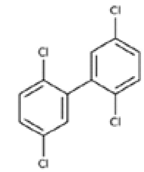
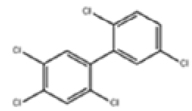
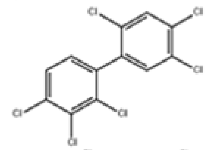
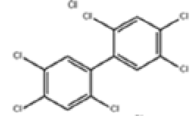
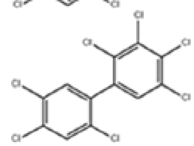
Catalyst activation

The catalyst used in this study was prepared from 90 micrometer (µm) in particle diameter of pulverized shrimp shell waste obtained from Wirontono Baru Ltd, a company located in Balaraja Regency, Banten Province, Indonesia, that produced annual 8000 tons of processed Vannamei output harvested after 90 days nursery shrimp farming. The catalyst was prepared by heating treatment for 1 h at 550 °C followed by immediate quenching using liquid nitrogen after which impregnation of zinc metal assisted with microwave at 1800 W power rate was carried out for 20 minutes (min.). The catalyst then was activated through heating at 250°C for 2 hour (h) prior to use in PCB dechlorination trials.

Catalyst characteristics

The characteristics catalyst used in this study were summarized as the following. Topography of pulverized shrimp shell by using SEM (scanning electron microscopy) showed a highly irregular morphological arrangement (Fig. 1) with a size ranging from a few to hundred microns that could be correlated with the chitin component of the pulverized shrimp shell. The pulverized shrimp shell materials are predominantly composed of calcium, carbon, and oxygen since the major component of the shrimp shell is composed of calcium carbonate (Gbnedor *et al*., 2016). The diffractogram peaks of

Table 2: PCB congeners' identity used in this study (Ballschmiter and Zell, 1980)

BZ number	IUPAC name	Chemical formula	Homolog	Molecular weight (g/mole)	Chlorine content (%)	Chemical structure
CB52	2,2′,5,5′-tetrachlorobiphenyl	$C_{12}H_6Cl_4$	Tetrachloro-biphenyls	289.9	49	
CB101	2,2′,4,5,5′-pentachlorobiphenyl	$C_{12}H_5Cl_5$	Pentachloro-biphenyl	323.9	54	
CB138	2,2′,3,4,4′,5′-hexachlorobiphenyl	$C_{12}H_4Cl_6$	Hexachloro-biphenyl	357.8	59	
CB153	2,2′,4,4′,5,5′-hexachlorobiphenyl	$C_{12}H_4Cl_6$	Hexachloro-biphenyl	357.8	59	
CB180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl	$C_{12}H_3Cl_7$	Heptachloro-biphenyl	391.8	63	

*gram per molecule (g/mole)

XRD (X-ray diffraction) at 2Θ = 29.6, 39.8, 43.5, 47.7, and 48.7) resemble calcite mineral that dominated calcium carbonate component of the pulverized shrimp shell (Gbnedor *et al*., 2017; Render *et al*., 2016). The surface area based on Brunnauer–Emmet–Teller calculation of the original pulverized shrimp shell was 1.272 square meter per gram (m^2/g) and increased to arrive at 30.43 m^2/g after heating treatment at 550°C.

PCBs dechlorination treatment

Dechlorination, that is, removal of chlorines from parent molecules, of PCBs is carried out using the Box–Behnken experimental design (Montgomery, 2017; Myers *et al*., 2016), comprising three independent factors, namely, dechlorination time (h), dechlorination temperature (°C), and catalyst proportion percent based on weight to weight ratio (w/w), as shown in Table 1. The table shows that three levels for each independent factor are set. Minimum and maximum values are set based on the state-of-the-art physicochemical destruction methods of PCBs (Akhondi and Dadkhah, 2018; Huang *et al*., 2007; Ryoo *et al*. 2007). Dechlorination time was set in the range of 1–4 h, dechlorination temperature in the range of 150 °C – 250 °C, and the proposition of catalyst weight in the range of 1% – 5%. The intermediate or mid values for each independent factor are set automatically by the Box–Behnken experimental design.

PCB congener analysis

The determination of five PCB congeners (Table 2), namely, 2,2′,5,5′-tetrachlorobiphenyl (CB52), 2,2′,4,5,5′-pentachlorobiphenyl (CB101), 2,2′,3,4,4′,5′-hexachlorobiphenyl (CB138), 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB153), and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (CB180) concentrations followed the method as described in International Standard IEC 61619: Insulating Liquids—Contamination by polychlorinated biphenyls—method of determination by capillary column gas chromatography, except for cleanup step. Instead, of using conventional glass chromatographic

Table 3: Experimental results of PCB congener dechlorination

Run order	Dechlorination temperature (°C)	Dechlorination time (h)	Catalyst weight (%)	PCB congeners (part per million, ppm)					Total PCBs (ppm)	Efficiency (%)
				CB52	CB101	CB138	CB153	CB180		
1	150	1.0	3	0.1776	0.4183	0.1025	0.4795	0.0155	2.7717	12.63
2	150	4.0	3	0.1699	0.4006	0.1304	0.5086	0.0231	2.8422	10.40
3	250	1.0	3	0.1838	0.4775	0.4588	0.5576	0.0220	3.4692	−9.56
4	250	4.0	3	0.1800	0.4192	0.0963	0.5341	0.0164	2.8847	9.06
5	150	2.5	1	0.1747	0.4118	0.1115	0.3515	0.0187	2.6593	16.27
6	150	2.5	5	0.1416	0.3288	0.0836	0.2529	0.0125	3.2254	−1.97
7	250	2.5	1	0.1696	0.3883	0.1023	0.1521	0.0150	2.4155	24.00
8	250	2.5	5	0.1762	0.4474	0.1186	0.5237	0.0168	3.0368	4.21
9	200	1.0	1	0.1769	0.4717	0.1372	0.3765	0.0237	3.3527	−5.89
10	200	1.0	5	0.1729	0.4530	0.1348	0.1839	0.0204	2.6992	14.92
11	200	4.0	1	0.1662	0.4331	0.1152	0.1670	0.0159	2.5559	19.53
12	200	4.0	5	0.1617	0.4027	0.1302	0.1675	0.1484	2.6503	16.53
13	200	2.5	3	0.1574	0.3812	0.1000	0.1513	0.0148	2.4242	23.71
14	200	2.5	3	0.1584	0.3929	0.0900	0.1563	0.0152	2.3713	25.38
15	200	2.5	3	0.1671	0.4275	0.1064	0.1766	0.0159	2.5117	20.93
16 (OIL)	-	-	-	0.2066	0.5300	0.1462	0.2172	0.0275	3.1703	0

column or solid phase extraction the Dextech unit for sample cleanup was used. Using Agilent 7890B gas chromatograph equipped with an electron capture detector, the five PCB congeners are eluted and assessed.

RESULTS AND DISCUSSION

Table 3 presents the results of the dechlorination of polychlorinated biphenyls. Fig. 2 presents the analysis of the data using the Box–Behnken response surface methodology (RSM) with Minitab Software Version 21.1. As shown in Fig. 2a, Fig. 2b, and Fig. 2d, congeners CB52, CB101, and CB153 provided positive responses respectively. Conversely, as shown in Fig. 2c negative response was obtained for CB138 congener. Contrary to the three previous PCB congeners whose levels tended to increase along with the dechlorination process, the CB138 congener tended to decrease along with the dechlorination process. Additionally, as shown in Fig. 2e CB180 is the only congener that has no response to the provided treatments. As shown in Fig. 2f, the analysis of the total congeners demonstrated that no significant effect of the given treatments was found. The results also indicated that dechlorination did not proceed for congeners that contain more than six chlorine atoms. In Table 4, the statistical analysis provided regression coefficients. As shown in Table 4 the regression equation yields an R^2 value greater than 90% for significant responses and less than 75% for inconsequential responses. The general equation of the regression is presented using Eq. 1 (Myers *et al*., 2016).

Response = constant + a (dechlorination temperature) + b (dechlorination time) + c (catalyst weight) + a^2 (dechlorination temperature)^2 + b^2 (dechlorination time)^2 + c^2 (catalyst weight)^2 + ab (dechlorination temperature) (dechlorination time) + ac (dechlorination temperature) (catalyst weight) + bc (dechlorination time) (catalyst weight) (1)

When the changes pattern of PCB congeners level and the chemical structures of the congeners were examined then it can be inferred that the dechlorination occurred to chlorine atoms attached to the biphenyl ring at 4 and 4′ positions. Thus, it is predicted that the PCB dechlorination proceeds from 2,2′,4,4′,5,5′-hexachlorobiphenyl via 2,2′,4,5,5′-pentachlorobiphenyl and then arrives at 2,2′,5,5′-tetrachlorobiphenyl, as shown in Fig. 3. The results of dechlorination trials according to the given combination treatments provided the highest efficiency of total PCB dechlorination was approximately 25% (Table 3). Optimization by implementing the RSM technique on data presented in Table 4 to obtain optimized parameter dechlorination condition provided results in dechlorination temperature 150°C, dechlorination time 2.4 h, and catalyst weight 5%, as presented in Fig. 4. Similar efficiency of dechlorination was

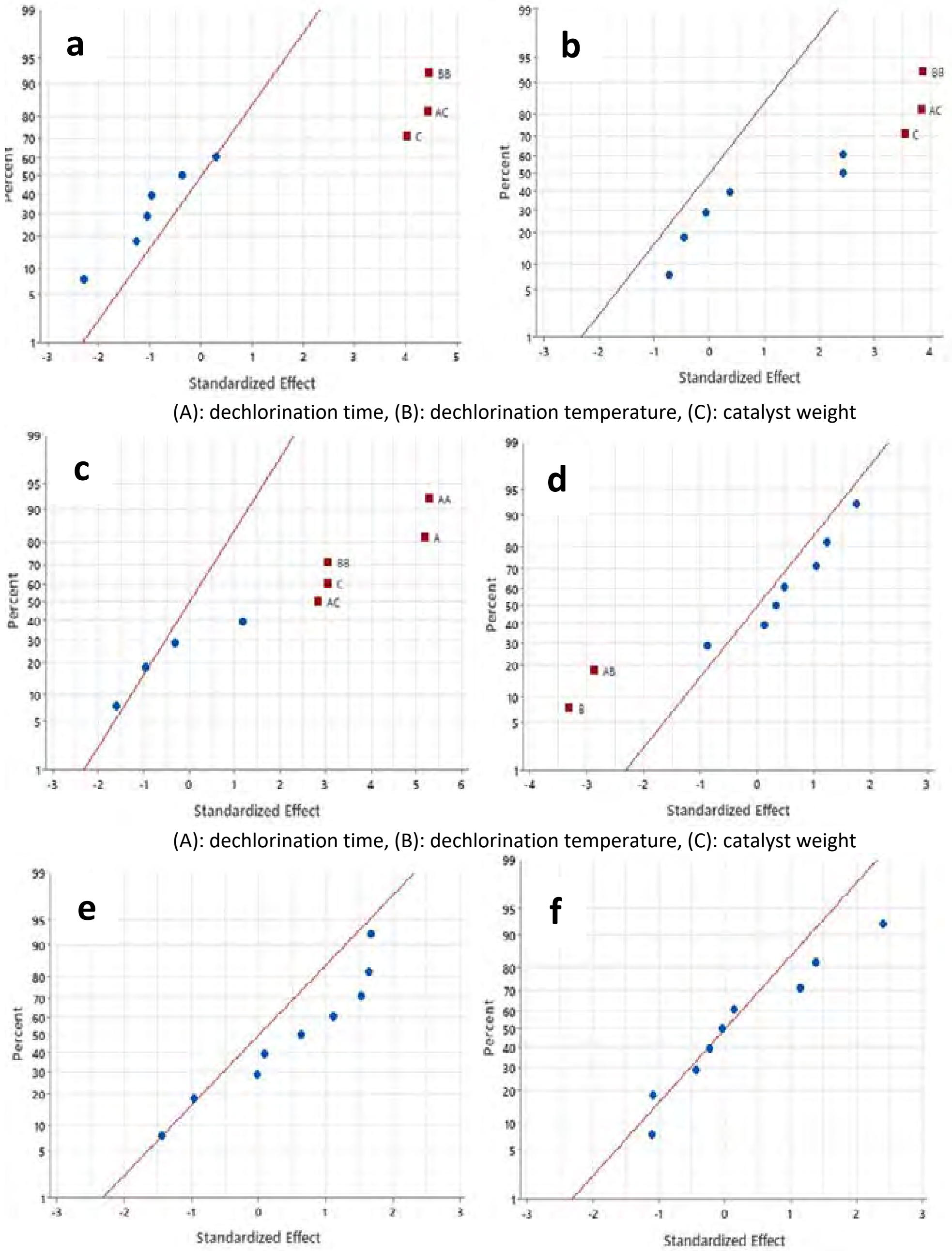


Fig. 2: Effect (at α = 0.05) of combination treatments. (A): dechlorination time, (B): dechlorination temperature, (C): catalyst weight on PCB dechlorination: (a) CB52, (b) CB101, (c) C138, (d) C153, (e) CB180, and (f) total PCBs. Dot blue color: not significant, square red color: significant

obtained using sequential combination anaerobic and aerobic microbial treatments of PCBs for a total 98-day experiment comprised of 70 days anaerobic treatment and 28 days aerobic treatment when 5 days anaerobic and 2 days aerobic cycle treatments were applied (Long *et al*., 2015). PCB dechlorination favors chlorines usually attached to meta and para positions in comparison to ortho positions (Hansen, 1999). The

Table 4: Regression coefficient and R^2 of PCB congener dichlorination

Term	Code	CB52	CB101	CB138	CB153	C180	Total
Constant		0.3294	0.231	1.377	5.69	−0.390	−6.22
Dechlorination time (h)	a	−0.00407	−0.0631	−0.384	−0.137	−0.0212	0.621
Dechlorination temperature (°C)	b	0.1305	−0.163	0.870	4.484	−0.324	−6.90
Catalyst weight (%)	c	0.02356	0.0836	0.0426	0.325	−0.0012	−0.050
Dechlorination time (h)* Dechlorination time (h)	a^2	0.00461	0.01654	0.0275	0.0583	0.00896	0.0475
Dechlorination temperature (°C)* Dechlorination temperature (°C)	b^2	0.0259	−0.0354	0.146	0.910	−0.0648	−1.299
Catalyst weight (%)* Catalyst weight (%)	c^2	−0.000483	0.00060	−0.00781	−0.0172	0.00416	−0.0073
Dechlorination temperature (°C)* Dechlorination time (h)	ab	0.00131	−0.0135	−0.1302	−0.0175	−0.0044	0.287
Dechlorination temperature (°C)* Catalyst weight (%)	bc	0.00990	0.03553	0.0111	0.1176	0.0020	−0.036
Dechlorination time (h)* Catalyst weight (%)	ac	−0.000048	−0.00098	0.0015	0.0161	0.01132	0.0322
R^2 (%)		91.84	93.59	80.21	91.05	75.30	55.04

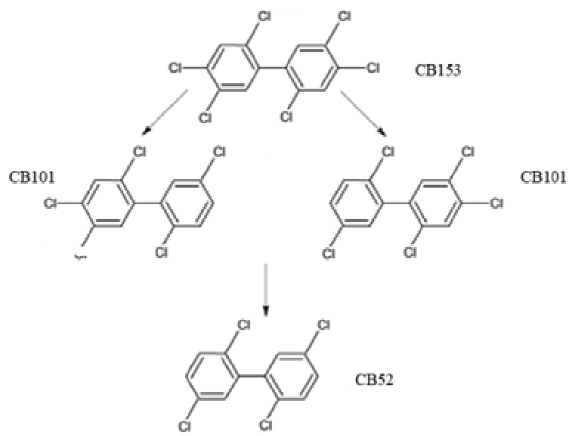


Fig. 3: Sequential dechlorination of CB153 to produce CB52

meta and para-attached chlorines readily undergo dechlorination meanwhile ortho-attached chlorines are the most difficult to remove (Shields *et al*., 2015). The order and preference of dechlorination depend on the presence or absence of adjacent chlorines as well as the position of the adjacent chlorines (Shields *et al*., 2015). The density functional theory calculation revealed that the addition of a substituent onto ortho, meta, and para positions resulted in increasing the energy distance of the highest occupied molecular orbital to the lowest unoccupied molecular orbital in that order (Ahmed and Abduljalil, 2019). The results of the calculation were in accordance with the findings of this study, that is, substituents at the para position are more prone to undergo dechlorination followed by meta and ortho positions.

In this study, the low percentage of PCBs dehalogenation efficiency might be attributed to low active sites of pulverized shrimp shell catalyst. Such low active sites may originate from several causes that include poisoning (Gurraala *et al*., 2021; Bartholomew and Argyle; 2015) onto the surface of the solid catalyst where impregnated zinc particles are expected to be centers of the active sites. The poisoning could have resulted from chlorine atoms that are detached from biphenyl rings during dechlorination being deposited and then covering the zinc atoms that should act as active sites. This

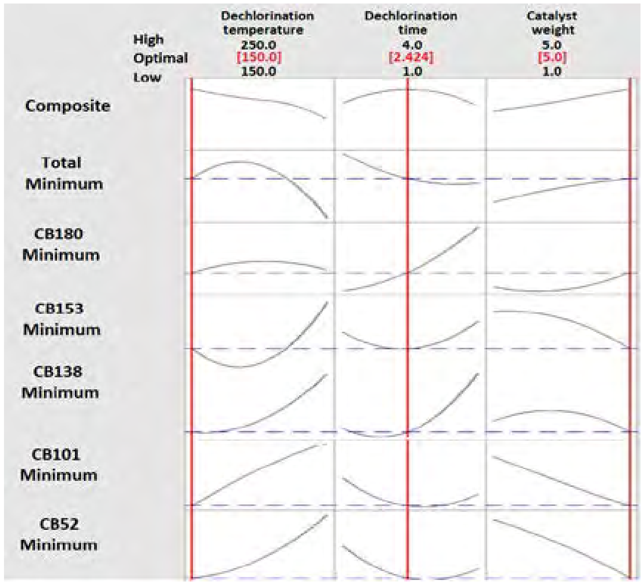


Fig. 4: Minitab output on optimization of PCB dechlorination using the RSM technique

situation may prohibit the zinc atoms from interacting with the remaining PCB particles in the mixture. Another cause of decreasing catalytic activities is related to a leaching phenomenon (Yang *et al.*, 2017; Gui *et al*. 2015) of zinc from the surface of the pulverized shrimp shell. The leaching of zinc can result from mechanical stirring during PCB dechlorination. Leaching of zinc correlates with the mechanical properties of the solid catalyst. Another aspect that should also be considered is the preparation of pulverized shrimp shells through heating in the range of 350°C–550°C followed by quenching. The purpose of the treatment was to obtain an irregular surface solid to promote the impregnation of zinc ions to the solid surface. During the heating treatment of the pulverized shrimp shell, the thermogravimetric analysis (TGA) and differential thermal analysis (DTA slope) sign records, as shown in Table 5, indicate the escape of substances from the matrices of pulverized shrimp shell. The released substances may include hydroxyl, acetyl, and acetamide groups of chitin, as shown in Fig. 5, which should play a prominent role in maintaining active centers through complexation (Nehra *et al*., 2019). The appearance of two inflection points (breakthrough) in the range of 300 °C – 400 °C and 400 °C – 550 °C as shown in Table 5 is in accordance with two exothermic peaks in another study using pure chitin (Vlaev *et al*., 2015). The aforementioned groups are expected to retain zinc ions as active centers for catalysis via coordination linkage in the solid matrix of pulverized shrimp shells. Escaping the aforementioned group from pulverized shrimp shells might cause the impregnated zinc in the pores of the solid matrix to leach.

A study of FTIR revealed that several disappearing absorption peaks were detected along with the increasing heating temperatures (Table 6). Such disappearing absorption peaks can be attributed from releasing of the adsorbed water, volatile matters, decomposition of residuals, and release of proteins and the acetyl group of chitin (Biondo *et al*., 2015). Broad absorption of infra-red that appeared between 3500 and 3200/cm (attributed from N–H groups vibration and also O–H bond stretching of a water molecule) was missing after heating at 350°C. The peak absorption at around 2920 and 2850/cm that originates from the vibration of C–H bond stretching of aliphatic hydrocarbons also disappeared after heating at 350°C. The characteristic absorption peak at 1637/cm that was assigned for the presence of carbonyl (C=O) from amide groups was also missing after heating at 350°C. Heating was continued up to 550°C demonstrating that further disappearance of peak at 1556–1540/cm, which is characteristic of amide, occurred. The rest of the peaks that appeared after heating at 550°C is the characteristic absorption

Table 5: DTA and TGA data records and slope change between temperature range

Temperature (°C)	DTA (µV)	Slope (ΔDTA/Δtemperature)	TGA (mg)	Slope (ΔTGA/Δtemperature)
25	0	0.24	30.53	0.00
50	0	−0.50	30.42	−0.03
100	0	0.34	28.89	−0.03
150	10	0.52	27.42	−0.01
200	30	0.20	26.90	−0.01
250	40	0.68	26.57	−0.02
300	50	1.92	25.49	−0.09
350	100	0.13	20.92	−0.13
400	200	−0.54	14.64	−0.03
450	170	0.71	13.05	−0.03
500	160	−0.29	11.32	−0.04
550	170	−2.72	9.23	−0.04
600	0	−1.05	7.42	−0.01
650	−25	−0.35	7.06	−0.01
700	−50	−0.18	6.75	−0.02
750	−55	−0.22	6.00	0.00
800	−60	−0.19	5.86	0.00
850	−80	−1.52	5.77	0.00

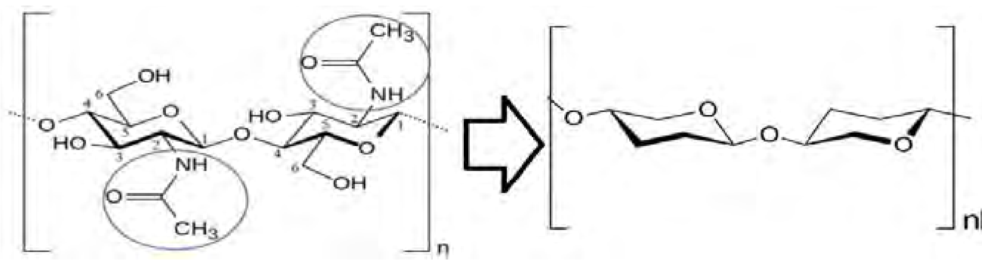


Fig. 5: Releasing of hydroxyl, acetyl, and acetamide groups of chitin

of calcite at approximately 870/cm and also calcium region (Rahman and Halfar, 2014). The study of the FTIR profile shows that the loss of several components from pulverized shrimp shells during heating as depicted by TGA and DTA records was confirmed. The findings of this study demonstrate that heating pre-treatment may affect the release of important functional groups from the pulverized shrimp shell. Such release may significantly affect the integrity of zinc impregnated onto the surface of the pulverized shrimp shell because the missing functional group capable of complexing the zinc may strongly influence the zinc atom to perform as the center of the catalysis sites. Precaution must be established to protect important functional groups that are present in the pulverized shrimp shell as such the synthesized solid heterogeneous catalyst could provide better performance in the dechlorination of PCBs. Compared with several other physicochemical methods in PCB dechlorination, this study demonstrated inferior results. For example, by using the base-catalyzed decomposition technique, chlorine removal from PCBs of arochlor 242, 1254, and 1260 mixtures obtained 99.8% efficiency when dechlorination was carried out for 4 h at 250°C (Akhondi and Dadkhah, 2018). Another method using potassium polyethylene glycol (KPEG) as a nucleophilic agent that can remove chlorine from PCBs of Sovtol-10 (containing tri to hexachlorobiphenyl) with efficiency in the range of 53%–89% (Velazco *et al*. 2015) if the dechlorination was carried out for 120 min at 90°C. The lowest efficiency was obtained when KPEG/Sovtol-10 ratio was 15, and the highest was obtained when the ratio was 30. The same technique reported no significant chlorine removal of arochlor 1016 after 14 days of evaluation but achieved 99% efficiency for arochlor 1260 in 2 days of dechlorination when the process was carried out at a temperature of 120°C (Tabaei *et al*. 1992). Irrespective of the inferior results of this study, chlorine removal of PCBs is still

Table 6: FTIR wavelength absorption characteristics of pulverized shrimp shell

Absorption (/cm)	Characteristics	Peak at temperature (°C)		
		Ambient	350	550
3500–3200	N–H groups vibration and also O–H bond stretching of water molecule	Present	Disappeared	Disappeared
2920 and 2580	Vibration of C–H bond stretching of aliphatic hydrocarbons	Present	Present	Disappeared
1637	Carbonyl (C=O) from amide groups	Present	Disappeared	Disappeared
1556–1540	Characteristics of amide	Present	Present	Disappeared
1400–800	Calcium region	Present	Present	Present
870	Characteristics of calcite	Present	Present	Present

challenging and needs further study to develop an appropriate set of parameters for chlorine removal. Additionally, the present study offered the utilization of waste materials from shrimp processing instead of nonrenewable materials for catalyst preparation. Certainly, this approach will contribute benefits to the environment, particularly in promoting waste to product approach, as well as the circular economy point of view.

CONCLUSION

The results of this study showed that dechlorination of PCB congeners at temperatures ranging from 150°C to 250°C for 1–4 h using 1–5% catalyst weight provided insignificant reduction levels, that is, 25% at best. Except for the 2,2′,3,4,4′,5,5′-heptachlorobiphenyl congener that has no effects upon the given treatments of dechlorination, the other four PCB congeners, namely, 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5,5′-pentachlorobiphenyl, 2,2′,3,4,4′,5′-hexachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl have little affected by the set dechlorination parameters. Although 2,2′,5,5′-tetrachlorobiphenyl, 2,2′,4,5,5′-pentachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl demonstrated positive (+) effect response to the given treatments of dechlorination, 2,2′,3,4,4′,5′-hexachlorobiphenyl showed negative (−) effect response to the given treatments of dechlorination. Optimization of the dechlorination process using RSM based on the given data responses provided optimized dechlorination parameters at 150°C dechlorination temperature, 2.4 h reaction time, and 5% weight/weight proportion of catalyst. The findings also revealed that the dechlorination of the PCB congeners favored chlorine attached at para positions of the biphenyl rings suggesting the transformation of 2,2′,4,4′,5,5′-hexachlorobiphenyl into 2,2′,4,5,5′-pentachlorobiphenyl to arrive at 2,2′,5,5′-tetrachlorobiphenyl. Nevertheless, the dechlorination failed to start with heptachlorobiphenyl containing seven chlorine atoms attached to the biphenyl rings. Irrespective of the low level of dechlorination of PCB congeners, that is, 25%, obtained in this study, the microwave-assisted impregnation method could be used to apply for the synthesis of a heterogeneous catalyst made from shrimp shell waste. However, heating pre-treatment of the pulverized shrimp shell waste to 550°C followed by immediate quenching has ended up with a material composed of calcium carbonate predominantly containing calcite mineral. This will have a consequence of decreasing the robust mechanical strength of the matrix to maintain zinc metal to be active as center sites for the dechlorination process. Thus, certainly, this is a drawback or limitation of the heating method at high temperatures to synthesize catalysts from pulverized shrimp shell waste. Therefore, for future trials, precautions must be anticipated to protect the important groups, such as hydroxyl, acetamide, and amide that are present in the shrimp shell from releasing during catalyst preparation as those groups play a prominent role to maintain the zinc metal at certain positions to perform as center sites for PCB dechlorination.

AUTHOR CONTRIBUTIONS

D.B. Aviantara contributed to the data collection and observation, analysis, manuscript preparation and revision. N.S. Indrasti supervised and sharpened the background, extended the discussion, and improvement recommendations. G. Hadiko contributed on interpretarion of analitycal data and statistical data, depended the discussion, and prepared the manuscript. M. Yani, the corresponding author, supervised the manuscript preparation, revised the discussion, and proofread the manuscript.

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CONFLICT OF INTEREST
The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
/cm	Wavenumber
a	Regression coefficient for dechlorination time
b	Regression coefficient for dechlorination temperature
c	Regression coefficient for catalyst weight
A	Dechlorination time
B	Dechlorination temperature
BET	Brunnauer Emmet Teller
BRIN	Badan Riset dan Inovasi Nasional
BZ	Ballschmiter and Zell
C	Carbon atom
C	Catalyst proportion
°C	Degree Celsius
CB	Chlorinated biphenyls
CB52	2,2',5,5'-tetrachlorobiphenyl
CB101	2,2',4,5,5'-pentachlorobiphenyl
CB138	2,2',3,4,4',5'-hexachlorobiphenyl
CB153	2,2',4,4',5,5'-hexachlorobiphenyl
CB180	2,2',3,4,4',5,5' -heptachlorobiphenyl
Cl	Chlorine atom
cm	Centimeter
DTA	Differential thermal analysis
ΔDTA/ Δtemperature	Delta DTA / delta temperature
DTG	Differential thermal gravimetric
ΔTGA/ Δtemperature	Delta TGA / delta temperature
Eff.	Efficiency
FTIR	Fourier Transform Infrared
GC/ECD	Gas Chromatography/Electron Capture Detector
g/mole	Gram/molecule
H	Hydrogen atom
h	hour
IEC	International Electrotechnical Commission
IPB	Institut Pertanian Bogor
KLHK	Kementerian Lingkungan Hidup dan Kehutanan (The Ministry of Environment and Forestry – the Republic of Indonesia)

KPEG	Potassium polyethylene glycol
kW	Kilowatt
μm	Micrometer
μV	Microvolt
m^2/g	Square meter per gram
Min.	Minute
N	Nitrogen
O	Oxygen
PCBs	Polychlorinated biphenyls
POPs	Persistent organic pollutants
ppm	Part per million
RSM	Response Surface Methodology
SC	Stockholm Convention
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric analysis
UNIDO	United Nations Industrial Development Organization
w/w	Weight to weight ratio
XRD	X-ray diffraction
Zn	Zinc

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CASE STUDY

Preventing water pollution using importance-performance análisis and terrain analysis

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ABSTRACT

BACKGROUND AND OBJECTIVES: The monitoring of the Brantas watershed showed a light-polluted status. This study began by identifying the priority of regional problems using importance-performance analysis. Furthermore, a hydrological analysis was conducted to determine the pollutant area of the Brantas watershed by applying terrain analysis. When terrain analysis in hydrology is combined with participatory community information, it can provide valuable insights into water pollution and help prioritize remediation efforts. Integrating local knowledge with scientific data can improve decision-making and increase the effectiveness of water management strategies.
METHODS: The methodological approach employed in this study included importance-performance analysis to determine priority problems in Batu City and terrain analysis as a hydrological analysis to determine the pollutant area in the Brantas watershed. The importance-performance analysis assessment data were obtained from 197 respondents representing the occupations of the people of Batu City. The terrain analysis data were derived from the surface elevation data in the form of a digital elevation model.
FINDINGS: According to the importance-performance analysis community assessment, urban trash management was one of the crucial yet low-rated features. The terrain analysis results demonstrated that business and industrial activities were distributed in locations with high flow accumulation values, indicating that the water pollution in Batu City was triggered by the presence of business and industrial activities in the watershed accumulation areas. Along the upstream Brantas watershed, 460 business and industrial activities were discovered. Therefore, the results of importance-performance analysis and terrain analysis had a correlation. They were also closely related to the assessment results of the contaminated Brantas watershed.
CONCLUSION: The following are some recommendations for the watershed's quality improvement: 1) cooperation among the Government, communities, and the private sector for addressing water pollution issues; 2) the development of environmentally friendly technologies in water treatment; and 3) education and outreach to communities about the importance of preserving water resources. As a city experiencing rapid urban development, environmental degradation constitutes a risk to be borne. Accordingly, Batu City must continue to develop good environmental management for the sake of nature conservation because the urban system is a unit formed by the social economy and ecological environment subsystem.

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INTRODUCTION

Compared to the previous year, Batu City's economic growth in 2021 showed positive movement at 4.04 percent (%). Batu City ranked eleventh out of 38 districts and cities in East Java as a region with positive economic growth (CBSBC, 2022a). However, sustainable urban development is not only based on the economic aspect but also requires coordinated development between the socio-economic and ecological environments to create prosperity for all current and future residents (Fan *et al.,* 2019; Lee *et al.,* 2021). The subject of sustainable urban development, which discusses coordinated development between socio-economic and ecological environments, has been increasingly studied in recent years (Jepson and Edwards, 2010; Rasoolimanesh *et al.,* 2011; Schluter *et al.,* 2012; Bai *et al.,* 2016; Liang and Yang, 2018; Fan *et al.,* 2019; Rasoolimanesh *et al.,* 2019; Xing *et al.,* 2019; Ahmed *et al.,* 2020; Li and Yi, 2020; Michalina *et al.,* 2021). There are many basic needs to live comfortably in a city with limited resources, one of which is clean water resources. Water resources are vital and inseparable from human life. Water also serves as a strategic economic resource and an indispensable necessity for human survival and social development (Xia *et al.,* 2011; Walter *et al.,* 2012). Meanwhile, population density and high economic activity in urban areas pose a risk to the resilience of water resources. Therefore, urban water security requires relatively high-risk management, so its governance arrangements need to be considered. In the case of urban areas, the main factors that can reduce water quality are environmental pressures, such as changes in land use and cover, and socio-economic pressures, such as population growth (Hoekstra *et al.,* 2018). Human activities can cause a decrease in water quality. In the long term, bad management can lead to a water crisis (Tan *et al.,* 2013; Wu *et al.,* 2014). The Brantas watershed is the second-largest watershed on the island of Java, located in East Java Province at 110°30' east longitude to 112°55' east longitude and 7°01' south latitude to 8°15' south latitude. The position of the Brantas watershed in Batu City, particularly upstream, functions as a buffer for water availability for people downstream. The watershed length is about 320 kilometers (km) with a watershed of ±12,000 square kilometers (km²), which covers ±25% of the area of East Java Province or ±9% of the area of Java Island. The Brantas watershed, which springs from Batu City, crosses the areas of Kediri, Kertosono, and Mojokerto. Furthermore, this watershed branches into Kali Surabaya and Kali Porong (Sidoarjo) before emptying into the Madura Strait. Kali Surabaya forms two tributaries, i.e., Kali Mas and Kali Jagir (Wonokromo). East Java Governor Regulation Number 61 of 2010 stipulates that the water quality of the Brantas watershed is categorized as Class I drinking water. However, based on the Ecosystem Services-Based Environmental Carrying Capacity and Accommodation document of 2021, 31 sampling points in the monitoring of the Brantas watershed showed a light-polluted status (ESBC, 2021). It means the upper part of the Brantas watershed no longer complies with the standards of its designation class, Class I. Changes in land use in urban areas have decreased water quality in the Brantas watershed (Wirosoedarmo *et al.,* 2016). Deforestation in the Sumber Brantas watershed covered an area of 1.59 hectares (ha) between 1997 and 2001. The area was converted into an agricultural area for seasonal crops, especially vegetables, with poor soil and water conservation conditions (Widianto *et al.*, 2010). Based on the Regulation of the Minister of Public Works and Public Housing Number 04/PRT/M/2015, the Brantas watershed area is a National Strategic Watershed Area. The importance of handling the water resources problems in the Brantas watershed becomes the basis of this study. Marselina *et al.* (2022) and Utami *et al.* (2020) successfully implemented terrain analysis in the management of the Citarum River Watershed in West Java, Indonesia. Terrain analysis is applied to identify critical points contributing to water pollution and to identify areas vulnerable to pollution. With this information, the Government and local communities can design more effective strategies to reduce water pollution, such as constructing waste treatment facilities, enforcing regulations, and implementing greening initiatives in vulnerable areas. Lee and Xue (2021) adopted the importance-performance analysis approach to sustainable city assessment. By involving various stakeholders, including participatory mapping integrated with the importance-performance and terrain analysis, effective and sustainable strategies can be effected for managing water resources. The aims of the current study include identifying the priority of regional problems using importance-performance analysis (IPA) and determining the pollutant area of the Brantas watershed using terrain analysis. When terrain analysis in hydrology is combined with participatory community information, it can provide valuable insights into water pollution and help prioritize remediation efforts. Integrating local knowledge with scientific data can improve decision-making and increase the effectiveness of water management strategies. This study was conducted in Batu City, Indonesia, in 2022.

MATERIALS AND METHODS

The geographical location of Batu City is 7° 44' 55,11" until 8° 26' 35,45" NL and 122° 17' 10,90" until 122° 57' 00,00" EL, as presented in Fig. 1.

The methodological approach used in this study was IPA analysis to determine priority problems in Batu City and terrain analysis as a hydrological analysis to determine pollutant areas in the Brantas watershed. The IPA technique was first described by Martilla and James (1977). IPA is relatively easy to use, easy to interpret, and a good tool used by researchers to investigate the level of satisfaction and importance of an aspect by assessing several attributes (Simpson *et al.,* 2020). IPA is one of the common methods applied in sustainable development research (Riviezzo *et al.,* 2009; Lee *et al.,* 2021). Using IPA enables some attributes of a sustainable city to be measured on a scale of importance and performance. Therefore, this method is suitable for adoption in research involving the attributes of sustainable development. As a tool that assesses importance and performance aspects, IPA can identify key areas where management needs to be improved (Lee *et al.,* 2021). The IPA findings can also facilitate decision-making by providing further insight into urban development performance, including the occurrence of water pollution. Assessing urban sustainability contributes to sustainable development, including the availability of sustainable clean water (Lee and Xue, 2021). IPA is a two-dimensional matrix plot with important attributes ranked on the vertical axis and performance attributes plotted on the horizontal axis (Fig. 2). According to Taplin (2012), the IPA assessment incorporates a Likert scale with five possible points. The range of importance assessment is as follows: very important, important, moderately important, slightly important, and not important. The range of performance ratings is as follows: very good, good, acceptable, poor, and very poor. The attributes used in

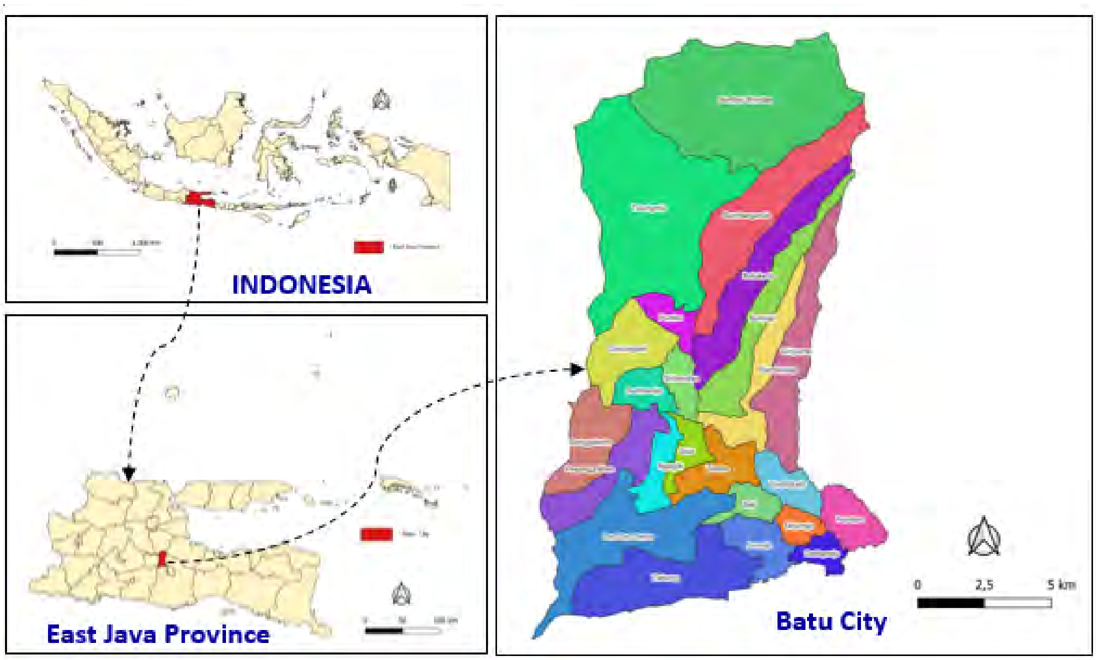

Fig. 1: Geographic location of the Batu City study area, Indonesia

this study refer to the Sustainable Development Goals (SDGs) related to sustainable urban development. The SDGs points include points 8, 9, 11, 13, and 15. Point 8 includes promoting inclusive and sustainable economic growth, productive and comprehensive employment opportunities, and decent work for all; point 9 includes building resilient infrastructure, promoting inclusive and sustainable industries, and encouraging innovation; point 11 includes making cities and settlements inclusive, safe, resilient and sustainable; point 13 includes taking urgent action to address climate change and its impacts; and point 15 includes protecting, restoring, and promoting the sustainable use of terrestrial ecosystems, managing forests sustainably, combating desertification, restoring land degradation, and stopping biodiversity loss. The IPA respondents comprised 197 people in Batu City, consisting of entrepreneurs, private employees, farmers, civil servants, teachers, students, and the unemployed. The community measured the importance and performance of the aspects related to Batu City's sustainable development through a questionnaire. Subsequently, the results of the questionnaire were interpreted into the IPA matrix.

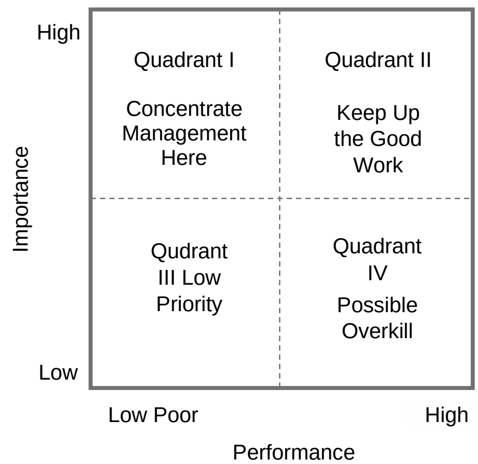

Fig. 2: Importance-Performance Analysis Matrix
(Martilla and James, 1977; Taplin, 2012; Jurdana and Frleta, 2012)

Next, the stages of finding areas to be handled and controlled regarding water pollution in the Brantas watershed were carried out by estimating the water accumulation area connected with industrial and business activities. These stages are demonstrated in Fig. 3.

Terrain analysis in hydrology is the processing of spatial data to examine the surface characteristics and water flow patterns in a given area. The steps in performing the terrain analysis in hydrology to produce flow accumulation involve data collection, data cleaning, slope and flow direction calculation, and the identification of watersheds and river networks. First, it is necessary to collect surface elevation data in the form of a digital elevation model (DEM) with appropriate resolution. DEM is a digital representation of the Earth's surface that presents elevation information at specific points. The second step is cleaning and processing the DEM to correct errors and remove noise. This step is essential for producing accurate and reliable data for further analysis. Third,

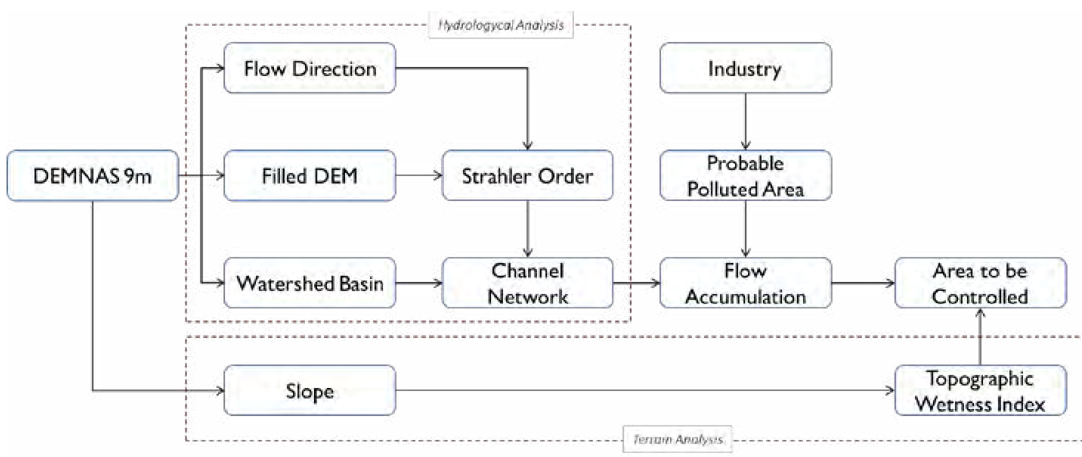

Fig. 3: Terrain analysis flow

using specialized algorithms to calculate slope and flow direction based on the DEM elevation data. The slope is the degree of surface incline, while the flow direction indicates the direction in which water will flow based on the lowest elevation in the vicinity. Fourth, flow accumulation is the number of cells along the water flow path that flows through each cell in the DEM. To calculate the flow accumulation, the flow direction must be performed earlier. Algorithms such as D8 or multiple flow direction (MFD) are used to calculate the flow accumulation. This process results in a map showing water flow patterns and areas with high flow accumulation. Fifth, from the flow accumulation results, watersheds and river networks can be identified. Areas with high flow accumulation indicate significant rivers or water channels, while watersheds cover areas that drain surface water to a specific collection point, such as a main river or lake. By understanding water flow patterns through terrain analysis, the potential for erosion, flooding, and water pollution can be measured in an area. This information is invaluable in managing water resources, land-use planning, and disaster mitigation. Terrain analysis is an analysis carried out to obtain a synthesis of the Earth's surface (Willson and Gallant, 2000). This analysis consists of tools adopting DEM to model the Earth's surface processes at various scales. DEM and its derivatives are part of a collection of digital terrain models (DTM) used in various fields to model material flow across the Earth's surface. Terrain analysis in hydrology characterizes how water and associated materials move across the landscape (Creed and Sass, 2011). This study required this analysis to determine the slope of the land and the level of wetness due to the formed topographic indentation. In addition, this analysis also found the possibility of accumulating pollutant collection from the area above it to determine how to handle it. The DEM map was obtained from the website of the Geospatial Information Agency with an accuracy of 8.5 meters (m). Furthermore, the Slope process in Qgis was carried out to find the slope level.

According to Fig. 4a, the DEM map shows that Batu City is at the lowest point in Pendem and Dadaprejo Villages, with an altitude of 580 meters above sea level (masl), and the highest point in Sumberbrantas Village, with an altitude of 3,317 masl. Fig. 4b displays that the slope ranges from 00 to 3070, where the sloping category is in the existing urban area. The highest slope is found in the Villages of Oro-Oro Ombo, Tlekung, Sumberbrantas, and Tulungrejo. The identification of the Topographical Wetness Level can be seen in Fig. 4c, where the most water accumulation is around the Brantas River and its tributaries. Administratively, this is in the villages of Pendem, Dadaprejo, Torongrejo, and Mojorejo.

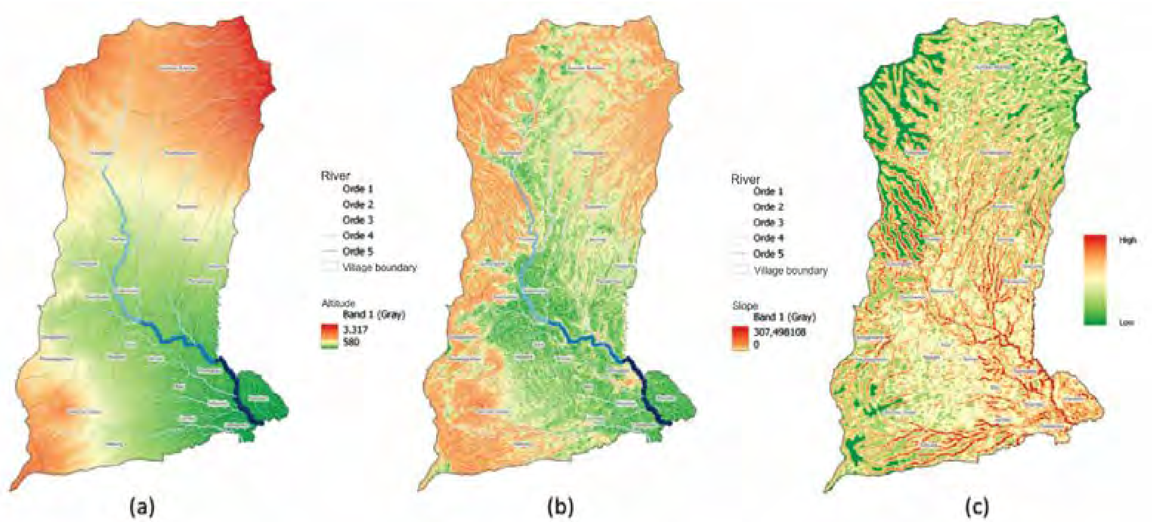

Fig. 4: Digital elevation model map: (a); Slope (b); Topographical Wetness Index (c)

RESULTS AND DISCUSSION

The characteristic of Batu City

On October 17, 2001, Batu City was officially separated as an Autonomous Region, which is no longer part of Malang Regency and includes three districts (Batu District, Bumiaji District, and Junrejo District) consisting of 19 villages and 4 urban villages. Batu City is the youngest autonomous city in East Java Province that has been experiencing rapid economic and population growth and playing a strategic role in maintaining the ecological sustainability of the Brantas watershed resources. The lands of Batu City are dominated by forests with a cold mountain climate, making this city a tourist attraction. In several tourism areas, most residents of Batu City work in the service support sector, including wholesale, retail, and business fields offering accommodation, food and beverages, arts, entertainment, and tourist attractions. The second business field that absorbs the most labor in Batu City is agriculture, forestry, hunting, and fishing (CBSBC, 2022b).

IPA analysis

Based on the analysis results, the formed IPA matrix can be seen in Fig. 5.

The analysis results indicate that economic growth (1), sustainable agricultural sector progress (4), social protection and employment programs (5), urban public transportation (11), orderly and easy licensing mechanisms (14), urban solid waste management (15), air quality yearly maintenance (17), and air temperature yearly maintenance (18) are included in quadrant I. Quadrant I shows attributes with high importance values and low performance, so the attributes in this quadrant must be the focus of management to increase the performance values. Quadrant II contains progress in education programs (6), supporting small industries (9), urban infrastructure to accommodate community needs (10), provision of green open spaces (16), disaster management (19), integration of capacity building for disaster prevention and management in schools (20), and strengthening disaster capacity in the neighborhood (21). Quadrant II covers attributes with high importance and performance values. Therefore, the quality of the management of the attributes in this quadrant is expected to be maintained so the performance remains good. Furthermore, quadrant III has both low importance and performance values. Attributes in quadrant III include informal employment in the non-agricultural sector (2), labor in the manufacturing industry sector (8), community participation in urban management planning (12), and Planning Dialogue Forum (13). Attributes in quadrant III have low management priority. Meanwhile, the contribution of the tourism sector (3) and the progress of the manufacturing industry (7) are included in quadrant IV, which has low importance but high performance. Attributes in quadrant IV are considered

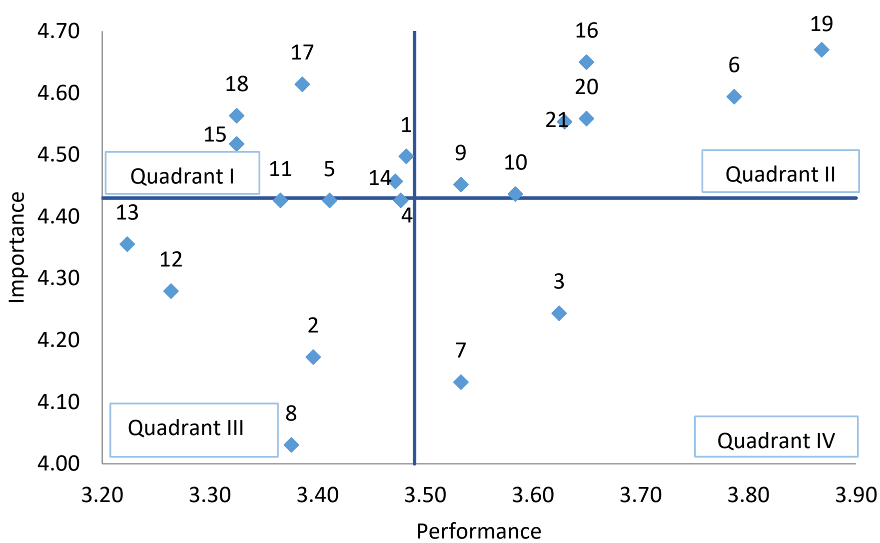

Fig. 5: IPA analysis result matrix on the sustainable development of Batu City

to have inappropriate and excessive management. Many studies have agreed that the carrying capacity of water resources is one aspect of sustainable development (Zhu *et al.,* 2002; Yang *et al.,* 2019). The community assessment analyzed in the IPA matrix (Fig. 4) shows the attributes that are considered important. However, their performance remains low and must be the management focus of the Batu City Regional Government to improve. These attributes include economic growth, sustainable agricultural sector progress, social and employment protection programs, urban public transportation, orderly and easy licensing mechanisms, urban solid waste management, air quality yearly maintenance, and air temperature yearly maintenance. Suppose it is associated with the lightly polluted condition of the Brantas watershed, among these attributes. In that case, 'management of urban solid waste' is related to this. As studied by Hoekstra *et al.* (2018), there is a strong correlation between waste management in cities and pollution in rivers and canals.

Terrain analysis

Batu City is located upstream of a large river in East Java Province, the Brantas watershed. The morphology of Batu City is influenced by the Brantas watershed. A hydrological analysis was essential to review how the accumulation of water flows from the morphological formation of the area. The hydrological analysis began with extracting DEM into watershed, flow direction, and filled DEM. The results of the filled DEM were processed by hydrological analysis in Qgis and produced river orders and channel networks. From this data, the flow accumulation area was calculated. The distribution of business and industrial activities in Batu City was concentrated along the main road from Malang City to Batu City and Pujon and from Batu City to Pacet. The overlapping concentration with Flow Accumulation (Fig. 6) and Flow Through (Fig. 7) demonstrated that business and industrial activities were found precisely in locations with high flow accumulation values. Thus, it was indicated that water pollution in Batu City was caused by business and industrial activities in the area of water flow accumulation.

The results of terrain analysis for Flow Accumulation (Fig. 6) and Flow Through (Fig. 7) showed that business and industrial activities were distributed in locations with high flow accumulation values, indicating that water pollution in Batu City was generated by the presence of business activities and industry in the watershed accumulation areas. Along the upstream Brantas watershed, 460 business and industrial activities were found. Companies and businesses operating around the Batu City watershed included real estate companies, tourism companies, flower plantations, horticultural plantations, canned mushroom industries, lodging (hotels, guesthouses, and villas), and eateries. Terrain analysis has become one of the essential approaches to preventing water pollution. In several cases, the application of this method successfully

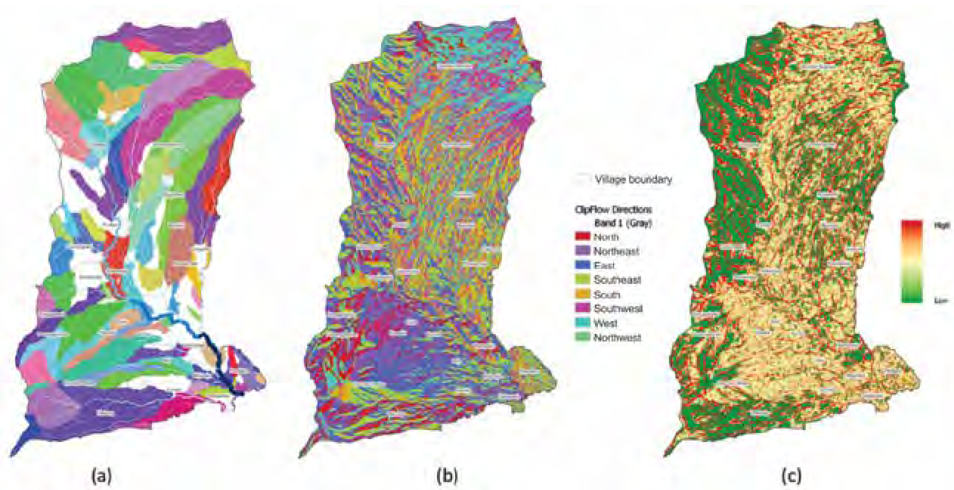

Fig. 6: Drainage watershed (a) Flow direction (b) Flow accumulation (c)

reduced the negative impacts of water pollution on the environment and human welfare. One example of the successful implementation of terrain analysis is the management of the Citarum River Watershed in West Java, Indonesia (Marselina *et al.,* 2022; Utami *et al.,* 2020). The Citarum River Watershed is one of the largest and most crucial river watersheds in Indonesia, providing clean water for over 25 million people. However, in previous years, the river experienced severe pollution due to the disposal of industrial and domestic waste. The application of terrain analysis in the management of the Citarum River Watershed includes detailed mapping and observation of the geographical, hydrological, and geological conditions in the area. This aims to identify critical points contributing to water pollution and areas vulnerable to pollution. With this information, the Government and local communities can design more effective strategies to reduce water pollution, such as constructing waste treatment facilities, enforcing regulations, and implementing greening initiatives in vulnerable areas. From the case of managing the Citarum River Watershed, it is important to note that the application of terrain analysis in water resource management is crucial for identifying critical points causing pollution and designing effective strategies to reduce the impact of pollution (Syeed *et al.,* 2023).

Water pollution of the upstream river

Compared with the data on the inventory status of the Brantas River conducted by Environmental Services in 2021, the Brantas watershed was in a mildly polluted condition with the terrain analysis results in this study (Figs. 6 and 7). Inventory data on the status of the Brantas watershed in the environmental carrying capacity and carrying capacity document in 2021 revealed that all chemical oxygen demand (COD) and biological oxygen demand (BOD) parameters, as well as several dissolved oxygen (DO), total suspended solid (TSS), phosphate (PO4), total coliform, and fecal coliform parameters have exceeded the Class I quality standard. Fig. 6 shows that industry and business activities in the Brantas watershed were mostly built in the Districts of Sumber Brantas, Tulungrejo, Punten, Sidomulyo, Sisir, Ngaglik, Pesanggrahan, Songgokerto, Beji, and Tlekung. This is in accordance with Darmawan *et al.* (2019). Total phosphate was assumed to be caused by residential waste, agricultural runoff, and plantations. Meanwhile, according to Yang *et al.* (2019), there were two sources of COD production, i.e., urban residents and industry. The study conducted by Wirosoedarmo *et al.* (2016) suggested that the change of forest land into dry land and plantations could affect the value of the Water Pollution Index occurred in the Brantas Coban Talun watershed. The

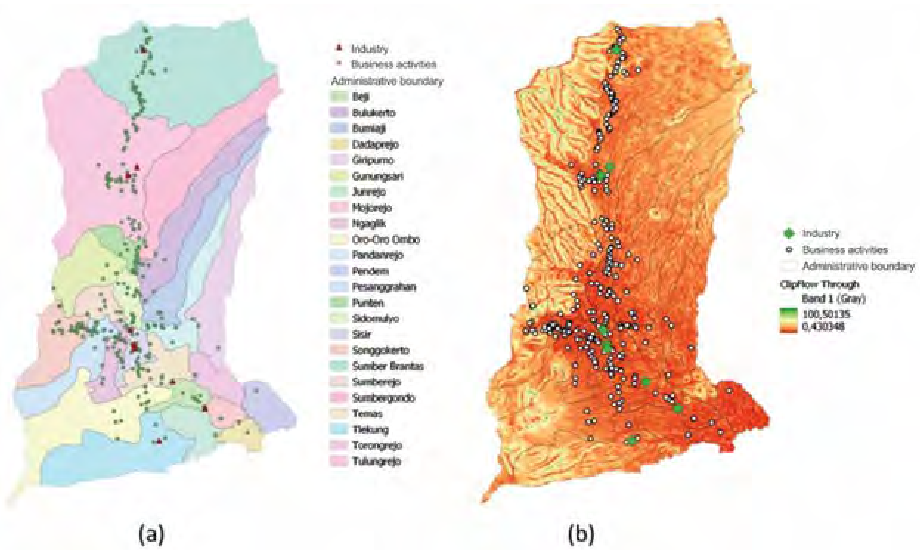

Fig. 7: Distribution of locations of business and industrial activities (a); Patching with flow through (b)

water pollution index status in 2008 met the quality standard, while in 2015, it was lightly polluted. The factors stimulating surface water pollution include agriculture, inadequate wastewater treatment, urbanization, and associated runoff and industrial activities. Pollution from nitrogen and phosphorus is a major problem caused by agriculture and wastewater stresses (Heneghan *et al.,* 2021). One activity that can potentially reduce water quality is agricultural intensification to increase agricultural production rapidly. The use of pesticides in agricultural intensification business activities is one example of a business that can reduce water quality (Lusiana *et al.,* 2017a). In turn, water pollution can contribute to water scarcity and impact the ecosystem and human health (Biswas and Tortajada, 2011). When groundwater or surface water sources are contaminated, it becomes unsuitable for use as untreated drinking water. At the same time, people experiencing poverty may not be able to afford treatment and face health risks. Communicable and non-communicable water-borne diseases have become a major source of human suffering and economic damage. In addition, poor sanitation may lead to urban water pollution. In many ways, water quality issues are much more complex than water quantity issues (Biswas and Tortajada, 2011; Falkenmark, 2011). The urban water environment is closely related to the residential environment, industrial development, and human health. As a result, the degradation and destruction of the urban water environment will result in widespread economic losses and security risks. Inventory data on industrial activities and business activities along the upstream area of the East Brantas River, as shown in Fig. 7, indicated that companies and businesses operating around the Batu City watershed consisted of real estate companies, tourism companies, flower plantations, horticultural plantations, canned mushroom industries, lodging (hotels, guesthouses, and villas), and eateries. In this regard, many studies examined that the problems of the Brantas watershed include the large proportion of land use changes to agricultural land and tourism without considering land conservation aspects (Lusiana *et al.,* 2017a), as well as the transfer of the riverbanks function designated as green areas whose function changes are not in accordance with their designation, for example, industrial or business activities (Sofyan *et al.,* 2015; Lusiana *et al.,* 2017b; Jariyah, 2018). As a city experiencing rapid urban development, environmental degradation is a risk that must be borne. Accordingly, Batu City must continue to develop good environmental management for the sake of nature conservation. The rapid development of cities has introduced many pollution-related challenges, such as the increasing need to collect and treat municipal waste (Chen, 2007). Environmental degradation is particularly challenging in rapidly developing urban areas where socio-economic development needs to consume large amounts of resources and emits many pollutants (wastewater, solid waste, and air pollutants) (Fan *et al.*, 2019). The significantly increased rate of economic growth and urban population growth creates challenges in terms of environmental pollution and overconsumption of resources (Yang *et al.,* 2013; Liu *et al.,* 2015). The population growth of Batu City was shown by the results of the 2010 population census projection. In 2021, the population of Batu City grew by 0.75%, while the population density reached 1,078 people/km^2 (CBSBC, 2022b). Meanwhile, the need for water resources will increase along with economic and population growth (Yang *et al.,* 2019). The quality of the aquatic environment reflects the sustainability of urban development and directly affects the quality of human life and the sustainable use of water resources (Kilkis, 2016). Increasing the level of urban socio-economic development is useful for increasing investment in technology for urban public pollution control infrastructure and other facilities. Meanwhile, implementing technology is effective in reducing energy consumption, reducing pollution emissions, and improving the urban environment. The attractive urban environment supports the improvement of the health situation of the urban population and attracts population inflows which help stimulate the economy and provide employment for social development. Fully socio-economically developed cities have further education, which is highly significant for encouraging people to practice a resource-saving and environmentally friendly lifestyle (Fan *et al.,* 2019). This illustrates that an urban system is a unit formed by the social economy and ecological environment subsystem (Wang *et al.,* 2011; Bai *et al.,* 2016; Fang *et al.,* 2016). As a result, several issues may be affected by the application of IPA and terrain analysis in this study to reduce water pollution. The first is identifying pollution sources. Participatory mapping with the community can help identify potential sources of pollution, such as industrial waste discharge, agricultural runoff, or untreated sewage. Integrating this information with terrain analysis can pinpoint pollution hotspots and enable targeted remediation efforts. The second is assessing vulnerability. Community participation can provide information on areas particularly vulnerable to water pollution, such as areas with high population density, poor sanitation infrastructure, or sensitive ecosystems. Overlaying this information with hydrological data from terrain analysis can help prioritize areas for intervention and protection. The third is developing effective management strategies. Collaborating with the community can help identify locally appropriate and culturally sensitive solutions for addressing water pollution. Integrating these ideas with terrain analysis data can help design effective and sustainable water management strategies that take into account local conditions and needs. The fourth is monitoring and evaluation. Engaging the community in monitoring and evaluation efforts can help track the effectiveness of implemented strategies, identify new pollution sources, and promote transparency and accountability in water management. One example of the successful integration of terrain analysis and participatory community information is the management of the Citarum River Watershed in West Java, Indonesia (Marselina et al., 2022; Utami et al., 2020). In this case, terrain analysis helped identify pollution hotspots, while community engagement facilitated the development and implementation of appropriate water management strategies. This collaborative approach resulted in improved water quality and more effective pollution control measures. In conclusion, combining terrain analysis in hydrology with participatory community information can provide valuable insights into water pollution and help prioritize remediation efforts. This integrated approach can lead to more effective and sustainable water management strategies that protect the environment and improve the well-being of communities. The following are some recomm-endations for the watershed's quality improvement. First, cooperation among the Government, comm-unities, and the private sector is essential in addressing water pollution issues. Second, the development of environmentally friendly technol-ogies in water treatment, such as biofiltration systems, can help reduce the negative impacts of water pollution on the environment and human welfare (Xu *et al.,* 2022). Third, education and outreach to communities about the importance of preserving water resources are also vital steps to prevent water pollution (Hemachandra and Sewwandi, 2023). Successfully managing the Citarum River Watershed can also serve as an example for other regions facing similar water pollution issues. The Brantas River Watershed in Batu City, which has been selected as a research area, also experiences similar problems; therefore, the successful management in Citarum can be replicated in this region. Water resource management based on terrain analysis, participatory approaches, and environ-mentally friendly technology-oriented solutions can be adapted and implemented in other areas according to their specific characteristics and local needs. For instance, developing countries facing water pollution challenges due to rapid industrial and urban growth can learn from and apply the successful approach implemented in the management of the Citarum River Watershed to address their water pollution problems (Marselina *et al.,* 2022; Utami *et al.,* 2020). In order to prevent water pollution, it is important to recognize that each region has unique characteristics and challenges. Therefore, an approach that is successful in one area may not always be suitable for implementation in another. However, by involving various stakeholders, including participatory mapping with importance-performance analysis, applying terrain analysis, and incorporating environmentally friendly technologies, effective and sustainable strategies can be effected for managing water resources. As a result, the environment will be protected, the negative impacts of water pollution will be reduced, and the welfare of communities worldwide will be improved.

CONCLUSION

In this study, IPA and terrain analysis are employed for identifying pollution sources, assessing vulnerability, developing effective management strategies, and monitoring and evaluation. The terrain analysis results reveal that business and industrial activities were distributed in locations with high accumulated flow values. Along the upstream Brantas watershed, 460 business and industrial activities have been found. Companies and businesses operating around the Batu City watershed comprise real estate companies, tourism companies, flower plantations, horticultural plantations, canned mushroom industries, lodging (hotels, guesthouses, and villas), and eateries. Therefore, it is identified that the water pollution in Batu City is caused by the presence of business and industrial activities in the accumulation area of water flows. When compared with the inventory data on the status of the Brantas River in the 2021 Environmental Carrying Capacity and Capacity document, all COD and BOD parameters and several parameters of DO, TSS, PO4, T. Coli and F. Coli have exceeded the Class I quality standard. Industry and many business activities in the Brantas watershed were built in the Districts of Sumber Brantas, Tulungrejo, Punten, Sidomulyo, Sisir, Ngaglik, Pesanggrahan, Songgokerto, Beji, and Tlekung. Regarding the lightly-polluted Brantas watershed, the results from the Community Assessment in IPA point out that 'urban solid waste handling' is one of the attributes considered important, yet the performance remains low. This study can be used as a reference for the Government, especially regarding the problems of the Brantas Watershed, which include the large proportion of changes in the function of land to agriculture and tourism without regard to the aspects of land conservation, as well as the transfer of the function of river banks designated as green areas, which are not in accordance with their designation. In turn, water pollution can impact the health of ecosystems and humans. Degradation and destruction of the urban water environment will result in widespread economic losses and security risks because the carrying capacity of water resources is an aspect of sustainable development. This should be the management focus of the Batu City Regional Government so that the performance increases. As a city experiencing rapid urban development, environmental degradation is a risk that must be borne, and accordingly, Batu City must continue to develop good environmental management for the sake of nature conservation. Therefore, enhancing the level of urban socio-economic development is beneficial for increasing investment in the technological level of urban public pollution control infrastructure and other facilities. Furthermore, increasing the level of technology is effective in reducing energy consumption, reducing pollution emissions, and improving the urban environment. In this case, the terrain analysis helps identify pollution hotspots, while community engagement facilitates the development and implementation of appropriate water management strategies. This collaborative approach results in improved water quality and more effective pollution control measures. In conclusion, combining terrain analysis in hydrology with participatory community information can provide valuable insights into water pollution and help prioritize remediation efforts. This integrated approach can lead to more effective and sustainable water management strategies that protect the environment and improve the well-being of communities. The following are some recommendations for the watershed's quality improvement: 1) cooperation among the Government, communities, and the private sector for addressing water pollution issues; 2) the development of environmentally friendly technologies in water treatment; 3) education and outreach to communities about the importance of preserving water resources.

AUTHOR CONTRIBUTIONS

The corresponding author, L. Sulistyowati, developed the idea and plan for this Project, collected the data for IPA, supervised the entire research, and reviewed and edited the manuscript. N. Andareswari collected the data for and performed IPA. F. Afrianto developed the idea and plan for this Project and performed the terrain analysis. A. Rais, M.F. Hafa, Darwiyati, and A.L. Ginting collected the data for IPA and conducted a literature review..

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
BOD	Biological oxygen demand
CBSBC	Central Bureau of Statistics of Batu City
COD	Chemical oxygen demand
DO	Dissolved oxygen
DEM	Digital elevation model
DEMNAS	DEM data provided as national data
DTM	Digital terrain model
ESBC	Environmental Services of Batu City
ha	Hectare
IPA	Importance-Performance Analysis
km	Kilometer
MFD	Multiple flow direction
mpsl	Meter above sea level
m	Meter
PO_4	Phosphate
SDGs	Sustainable development goals
TSS	Total suspended solid

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REVIEW PAPER

Effectiveness of the voluntary disclosure of corporate information and its commitment to climate change

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ABSTRACT

BACKGROUND AND OBJECTIVES: Although governments and companies have been implementing various measures, such as technological innovation, new emissions regulations, and policies to reduce greenhouse gas emissions, it seems that global warming is not decreasing. In order to reduce greenhouse gas emissions, the commitments of companies were considered to be the key for climate change. However, since the Paris Climate Agreement, there has not been an accurate evaluation of the efforts and contributions of companies toward emission reductions. This study investigated the effectiveness of companies in Climate Action and tested its impact on greenhouse gas emissions at the country and per capita levels.
METHODS: This study focuses on companies of the countries from the main Latin American economies (Mexico, Chile, Brazil, Colombia, and Argentina) and their major trading partners (the United States of America, Canada, China, Korea, Germany, and Japan). There are 894 companies from Latin America and 3680 companies that represent their trading partners of referred countries in Climate Action. This study used two data sources, the commitment of companies from Global Climate Action and the annual greenhouse gas emissions levels of each country from an open-access data platform called Our World in Data.
FINDINGS: The findings demonstrate a significant and positive relationship between changes in greenhouse gas emissions from 2021 and 2020 and the number of companies participating in Global Climate Action (Pearson = .718*, significance = .013) and per capita (Pearson = 0.827** significance = 0.002). Correlations indicate there is a higher level of commitment to climate action but with marginal contributions to greenhouse gas emissions reduction. Previous expectations were that greater corporate involvement in climate action would reflect a link to greenhouse gas reductions, but this was not the case. Additionally, the reduction in greenhouse gas emissions during the pandemic was due to the economic slowdown and was not necessarily because of the climate action efforts of companies and governments to reduce emissions. The findings demonstrated a negative and significant correlation at the country level during the pandemic (Pearson = −0.629 significance = .038). The lack of effective results for reducing (from 2020 and 2021) greenhouse gas emissions justifies the relevance of increasing transparency and accountability for both companies and countries. The acceleration of the production system reflected in an increase in greenhouse gas emissions is not keeping pace with the commitments and the reported achievements on Global Climate Action.
CONCLUSION: This study contributed to justifying efforts for a better way to follow up international efforts to reduce greenhouse gas emissions. Transparency and accountability are key to effectively achieving greenhouse gas reductions and curbing the impending climate crisis.

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INTRODUCTION

The UN has urged action to limit global warming to 1.5°C, with businesses committing to reduce carbon dioxide (CO_2) alongside governments. Approximately 62% of greenhouse gases (GHGs) are CO_2 generated by fossil fuels, 11% is from land use, and 3% is from chemicals (EPA, 2023). The effects of GHGs on the planet are diverse, and alterations are seen in climate factors and in the various life systems on the planet. The United Nations (UN, 2023) notes that high temperatures, severe storms, increased drought, a warming and rising ocean, species loss, insufficient food, greater health risks, and poverty and displacement are expected. According to Our World in Data (2023), 73.2% of GHG emissions are due to energy consumption, which involves different industries such as iron and steel, transportation, chemicals, aviation, and others. The UN's action roadmap, the 17 Sustainable Development Goals (SDGs), proposes five targets and eight indicators for reducing, mitigating, and adapting to the climate crisis. One of the important initiatives for developing transparent data on the actions taken by companies. Global Climate Action (2023) and Our World in Data (2023) are open information portals that monitor and provide open access to data and information on the CO_2 reduction policies of companies. This paper tracks the correlations between the Global Climate Action Commitments of business players and the level of GHG emissions in Latin America (LATAM) economies and their major trading partners. It examines the effectiveness of companies' contribution to reducing GHG emissions by analyzing the commitments of companies to Global Climate Action (2023) and GHG emissions per country from Our World in Data (2023). There are several platforms that share standards, programs, guides for inventorying and monitoring GHG emissions and actions taken by companies to respond to the climate crisis. The following are some of the important ones, but this is by no means an exhaustive list: 1) World Resources Institute (WRI) develops shared programs focused on solving challenges at the intersection of environmental and human development: Cities, climate, energy, food, forests, ocean, and water (WRI, 2023). 2) World Business Council for Sustainable Development (WBCSD) is a corporate executive officer (CEO)-led community focusing on accelerating the system transformations needed for a net-zero, natural, positive, and more equitable future (WBCSD, 2023). 3) International Organization for Standardization provides guidance at the organization level for quantification and reporting of GHG emissions and removals (ISO, 2023). 4) The Intergovernmental Panel on Climate Change provides guidelines for national GHG inventories (IPCC, 2023). 5) The Greenhouse Gas Protocol provides GHG accounting standards to measure and manage emissions for GHG inventory (GGP, 2023). It was decided to use the two platforms referred to above because of the ease of comparing countries and companies that are involved in climate action classified by country. Digital platforms, like Global Climate Action, provide important advantages. For example, various global and national stakeholders can observe the performance of the region, the country, a certain sector, or a specific company. The disadvantages of this platform are that it is difficult to compare the impact of efforts from one company to another. It can also be observed that most companies include short-term commitments, whereas the results of climate action require a longer time frame. For instance, making technological changes in a plant to make it more sustainable may require more time than carrying out one-off actions with minimal environmental impact. In addition, to ensure everything reported is comparable, the way in which emissions are measured must be standardized. Despite these limitations, these platforms are valuable with scope of further improvement. Although the decision of companies to participate and share information is voluntary, the legal framework for each country can exert an important coercive effect to comply with regulations. On the contrary, such regulations should be standardized at the international level. In the case of LATAM, because of the platforms such as climate action, it is possible to observe that the tendency practice of sharing climate action initiatives and commitments are lower and exhibit a reduced tendency to report results. This is unlike other countries (trading partners with LATAM), that show a higher level of initiative and commitment and a greater practice of reporting results than its international partners. As part of the corporate information shared by global companies, a sustainability section is increasingly a requirement demanded by stakeholders. However, it is not enough to share reports on websites. Companies must adjust the information on their sustainability efforts based on global standards to provide comparative evidence of what they are doing (reported or unreported results) and to follow up on them in different timeframes.

Corporate information on climate change contributions is useful to make decisions based on this information for international agreements or trade agreements. This information can also be useful for financial investors and global companies. Companies or countries that are not interested in climate action may represent a risk to invest in them and those showing achievements may attract investment or financing.

The novelty of this study is that it demonstrates how the GHG emissions of a group of LATAM countries and their main international trading partners have not fallen. Increase in GHG emission persists despite the increased corporate participation. Their commitments and progress on climate change have also been increasing. This demonstrates that reducing climate change by reducing greenhouse gas emissions is one of the most challenging efforts for international bodies. The only thing that has been shown to reduce GHGs on the planet is, unfortunately, the pandemic, and not the intentions or commitments of governments and companies. This study aims to investigate the effectiveness of business actors in Climate Action for greenhouse gas emissions reduction at the country level from major Latin American economies and their major trading partners. This study is part of a sustainable research project related to Business and Climate Change at Tecnologico de Monterrey, Monterrey City, Mexico, during 2021 – 2023.

Business involvement in climate change

Organizations are a major source of GHG that is causing global warming (Levine and Steele, 2021). In response, the UN continues to apply pressure on business organizations to reduce CO_2 emissions under the framework of the Paris Climate Agreement (Hu *et al*., 2017) and the UN's SDGs (Qian *et al.*, 2022) to ensure significant progress in this reduction (Biró and Szalmáné Csete, 2020). Considering that the key to responding to climate change is the reduction in CO_2 emissions (82% of GHG), initiatives such as the emission trading scheme (ETS) have emerged. ETS is a market-based pollution-control strategy that provides economic incentives to reduce emissions and has the potential to reduce them by establishing a pollution limit and creating a market for each country (Hashim *et al.,* 2022). Under these circumstances, transparency and accountability are essential for these mechanisms to function on the global stage. Moreover, universities have joined the effort to reduce carbon emissions, such as by reducing GHG emissions through compost processed from food and green wastes generated at a university campus, by converting organic wastes into organic fertilizer (Kamyab *et al.,* 2015). Furthermore, Europe has committed to full decarbonization and introduced a cross-border carbon tax, which has prompted companies to respond by implementing governance processes and reducing emissions in their supply and value chains to address climate change (Carpio-Aguilar *et al.*, 2019; Sullivan and Gouldson, 2020). However, criticism of the low standards imposed by countries for their environmental regulations and the occurrence of the free rider syndrome (Maraseni and Reardon-Smith, 2019) relating to emission reduction has emerged. Therefore, several previous studies have examined the effectiveness with which companies in various industries have tackled climate change. In the corporate information shared by global companies, the sustainability section is increasingly a requirement demanded by stakeholders. However, it is not enough to share reports on their websites. Companies must adjust the information on their sustainability efforts based on global standards to provide comparative evidence of what they are doing (reported or unreported results) and strictly follow up on them in different timeframes. The initiative for climate action for companies is mainly adaptation/resilience, mitigation and equally adaptation/resilience, and mitigation. Specifically, the different sections for Climate Action commitments are emission reduction, renewable energy, energy efficiency, adaptation and resilience, and finance. These studies have highlighted the lack of knowledge about the effectiveness of companies' efforts to reduce GHG emissions and the need for more ambitious targets (Hsueh, 2022) and complete reporting. For instance, studies share findings about how adaptation strategies could help lessen vulnerability to climate change (Biró and Szalmáné Csete, 2020), shareholder protection (Giesekam *et al.*, 2021), decarbonizing of transportation sector (Gota *et al.*, 2019), innovative technologies for reductions in carbon emissions (Levine and Steele, 2021), and climate change and oil companies (Roginko, 2021). In addition, companies sought to respond through governance processes and took steps to reduce their emissions in their supply and value chains (Sullivan and Gouldson, 2020). However, very little is known about the efforts of companies to contribute to GHG reduction and whether or not their efforts are sufficiently meaningful. Companies would also be expected to set targets for achieving reductions in their emissions that are more ambitious and to avoid reporting incomplete results (Mancini *et al.*, 2022). At the international level, a harsh criticism exists of the low standards that countries impose on themselves, which takes advantage of legal loopholes in environmental matters. Similar to public goods, emission reduction suffers from the free rider syndrome (Maraseni and Reardon-Smith, 2019). Academic researchers have explored the effectiveness of the efforts of companies to tackle climate change in various industries using different approaches and their incorporation of carbon and climate reduction strategies in all areas of their value chain. In terms of green energy (Khabibrakhmanov *et al.,* 2021) recognized the importance of community demand for green energy to reduce GHG emissions and paid due attention to fundamental shifts in the balance of utilities toward less carbon-intensive fuels. Hafker (2018) discussed the role that demand for green energy had in reducing GHG emissions and described fundamental shifts in the balance of utilities toward less carbon-intensive fuels. Javadi *et al.* (2021) analyzed energy consumption in the automotive industry and demonstrated that applications of renewable energy sources could decrease carbon emission intensity. Lazarus *et al.* (2021) stated that business organizations, particularly fossil fuel producers and livestock companies, are responsible for climate change in the livestock industry. Studies have investigated the contribution in addressing the climate crisis made by industrial sectors such as energy (Maraseni and Reardon-Smith, 2019; Filho *et al.,* 2018). In addition, Rekker *et al.* (2018) underline that meeting global and national climate targets requires the serious commitments of various companies, such as fossil fuel producers, to mitigate climate change. Tunji-Olayeni *et al.* (2021) assessed the strategies for climate change mitigation used by manufacturing firms in Nigeria. Granberg (2018) maintained that local governments could design action strategies aligned with local policy environments to advance low-carbon transition. Previous studies have highlighted the importance of monitoring the commitments and achievements of companies' actions toward responding to climate change (Christiansen *et al.,* 2023; Dye *et al.,* 2021; Preudhomme and Mazzacurati, 2020). There are different approaches to academic reports regarding GHG emissions related to responsibility for the social, environmental, and economic impacts of a company's operations and corporate accountability. These include, for instance, government involvement and accountability for climate change (Abbass *et al.*, 2022); lack of accountability for a just transition in fossil fuels reduction (Bastos Lima, 2022); digital values, such as accountability, in the organization (Bianco *et al.*, 2021); connections between governance, accountability, and social and environmental issues (Denedo and Egbon, 2021); accountability for quantify GHG emissions (Foster, 2021). Additionally, responsibility and accountability have been studied from different perspectives, such as human rights violations (Jägers, 2021); lack of sustainability, environmental, social governance, transparency, and accountability (Kharas, 2021); reporting to promote business transparency and mitigation actions (Keat-Chuan-Ng and Webber, 2023); and sustainability and accountability in emerging economies and transparency (Ortiz Palafox, 2021). Education is also a relevant institution for working on climate change (Esprit, 2021; Öztürk and Pizmony-Levy, 2022). There is also a committed involvement for environmental accountability and transparency (Sautya *et al.,* 2022; Silvola and Landau, 2021; Segers *et al.*, 2022; Villiers, 2022). Efforts have been made to enable actions to reduce environmental impact and contribute to a more sustainable future. These include reporting on activities to ensure compliance with international guidelines (Obergassel *et al.*, 2021). In this regard, transparency, as a way of making information accessible to customers, would encourage green behaviors (Aguiar *et al.,* 2022; Al Sadawi and Ndiaye, 2021; Barros *et al.,* 2020; Jowers and Morales, 2017). Moreover, providing transparent information to relevant stakeholders has been part of research on climate change (Chowdhury *et al.,* 2021; Dawson *et al.,* 2022; Dyarto and Setyawan, 2021; Hori *et al.,* 2022; Iftekhar *et al.,* 2021). Moreover, there is also research focused on transparency and environmental mitigation activities in the urban context (Kim and Choi, 2022; Melnyk *et al.,* 2021). Digital technology, transparency, and accountability for climate change are also trends seen in academic research (Ahl *et al.*, 2020; Bharti and Anand, 2021; Fantke *et al.,* 2021; Kamyab *et al.,* 2022; Lodhia *et al.* 2021). In particular, how digital technology, throughout the value chain (Chowdhury *et al.,* 2021; Sanderson and Stridsland, 2022) and supply chain (Basu *et al*., 2023), can raise public awareness (Lazarus *et al.*, 2021) has been an object of interest. Furthermore, carbon disclosure can enhance the transparency and accountability of firms, leading to a reduction in firm risk exposure (Alsaifi *et al.,* 2022; García-Sánchez *et al.,* 2022; Kedward *et al.,* 2022; Strauß, 2021). In particular, there have been research approaches related to business transparency, climate governance, risk assessment, and finance (Simane and Bird, 2017; Smith and Lawrence, 2021). Moreover, there have been academic contributions related to sustainable production systems (Gill and Ramachandran, 2021; Lahtinen and Yrjölä, 2019) and lower corruption (Bhattarai and Conway, 2021). Board diversity, having more female members, should be pursued (Al-Qahtani and Elgharbawy, 2020; Jizi *et al.,* 2022; Ooi *et al.,* 2019) to increase transparency. The utilization of green energy platforms to report to its major stakeholders or trading partners is an effective tool for advancing transparency (Jang, 2020; Kumar *et al.,* 2020; Bizikova, 2022; Zebra *et al.,* 2021). Corporate accountability and transparency can serve stakeholders' interests better (Anderson *et al.*, 2020; Camilleri, 2019), as they can promote democratic and transparent governance processes (Amin-Chaudhry, 2016; Bernauer *et al.*, 2016; Gibbs and Maassen, 2021; Wiseman, 2018). One effective means of monitoring the progress of business commitment is with the use of open digital information portals, such as Global Climate Action. Although there are concerns about the accuracy and comparability of commitments and achievements using digital portals (Romijn *et al*., 2018), they still provide stakeholders with the opportunity to evaluate the actions taken by companies to reduce GHG. This promotes transparency and accountability and may encourage more companies to take steps toward tackling climate change. This article investigates the effectiveness of actions made by companies on climate action for greenhouse gas emissions reduction at the country level from major Latin American economies and their major trading partners. This study is part of a sustainable research project related to Business and Climate Change at Tecnologico de Monterrey, Monterrey City, Mexico, undertaken in 2021–2023.

Greenhouse gas emissions and corporate participation in Global Climate Action

The main LATAM economies in this study (5) were selected based on their gross domestic product (GDP) and participated in the climate action platform. The main trading partners of these economies were identified (6) based on each countries' foreign trade information. In addition, information from international financial and banking institutions that study trade relations between them was considered to select trading partners. A focus on the strongest economies is expected to show that the responsibility and commitment to GHG reduction is higher because under their current production model, these countries generate a higher rate of GHG. Both per capita and global levels of GHG were studied, as statistics differ. For example, China emits the most GHG globally, but its per capita is not the highest due to its high population density. The opposite is also true: Canada is the second highest GHG emitter, but as a country, it is not high on the list, due to its low population. In Fig. 1, it can be seen that the emissions status in major LATAM countries indicates that it decreased in 2019 and 2020, with Mexico showing the most significant reduction due to strong quarantine measures. Chile had the highest emissions, attributed to its intense economic activity, particularly in the extractive industry and lower population density. However, Fig. 1 shows a rebound in emissions in 2021, likely influenced by the economic recovery.

In Fig. 2, Brazil is the largest CO_2 emitter, followed by Mexico. The top five major economies in Latin

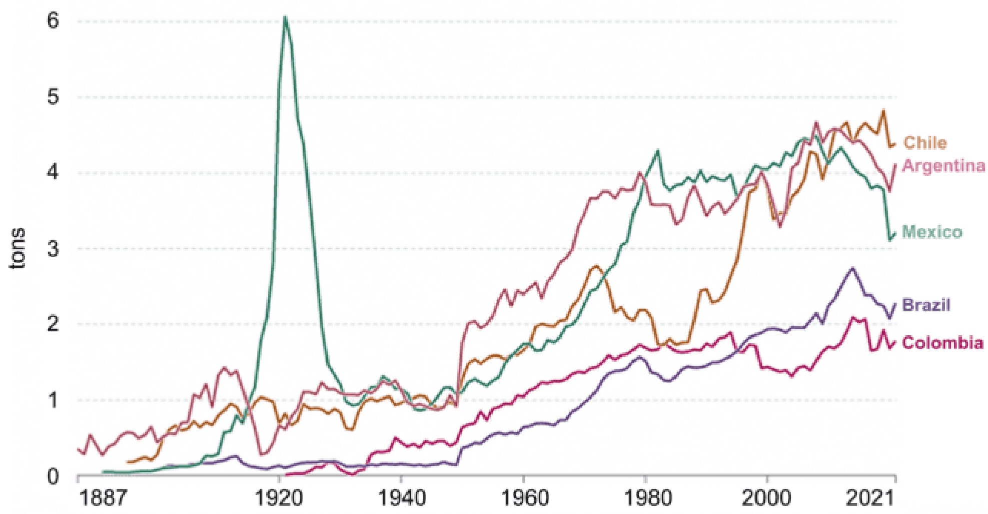

Fig. 1: Most relevant LATAM economies (Argentina, Brazil, Chile, Colombia, and Mexico) and their impact on CO_2 emissions per capita (Our World in Data, 2023)

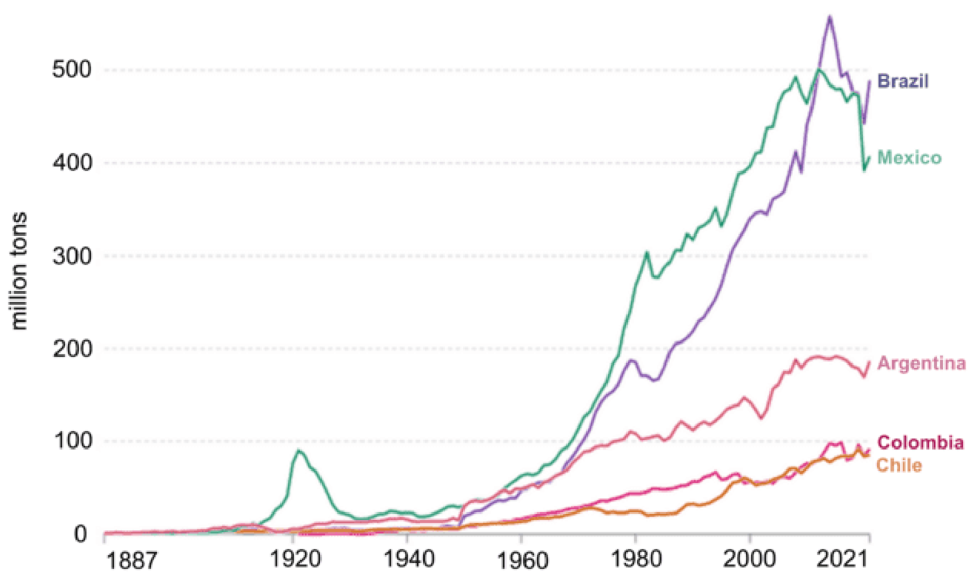

Fig. 2: Most relevant LATAM economies (Argentina, Brazil, Chile, Colombia, and Mexico) and their impact on CO_2 global emissions (Our World in Data, 2023)

America showed a decrease in CO2 emissions globally in 2020 due to the Coronavirus disease (COVID-19) effect, but there was an increase in 2021. This highlights the importance of taking climate action to reduce emissions.

In Fig. 3, the CO_2 emissions per capita of major trading partners of Latin American countries decreased during 2020, except for China, which showed a slight decrease in 2019 and an increase during the pandemic. However, like Fig. 1, this group of countries saw an increase in GHG emissions per capita during 2021.

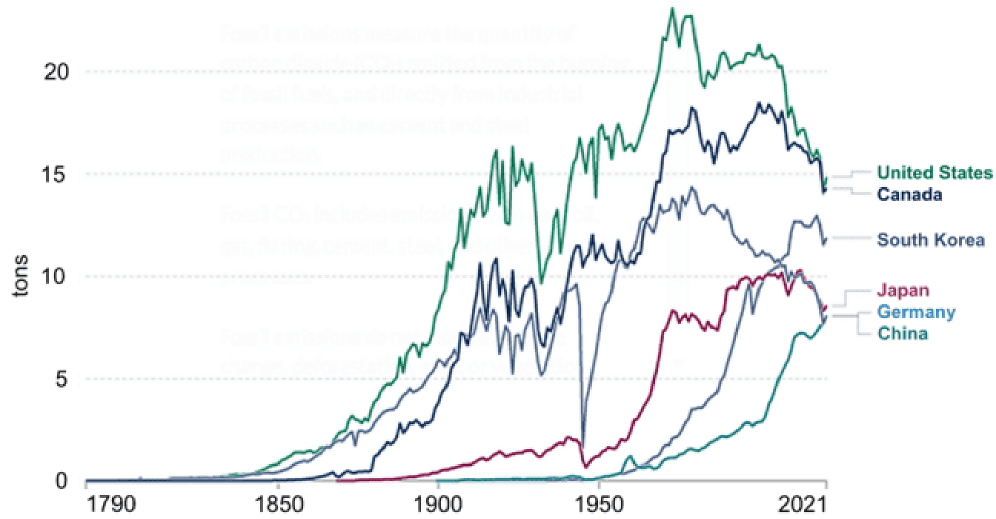

Fig. 3: Major trading partners (United States, Canada, Republic of Korea, Japan, Germany, and China) of the most relevant LATAM economies and their impact on CO_2 emissions per capita (Our World in Data, 2023)

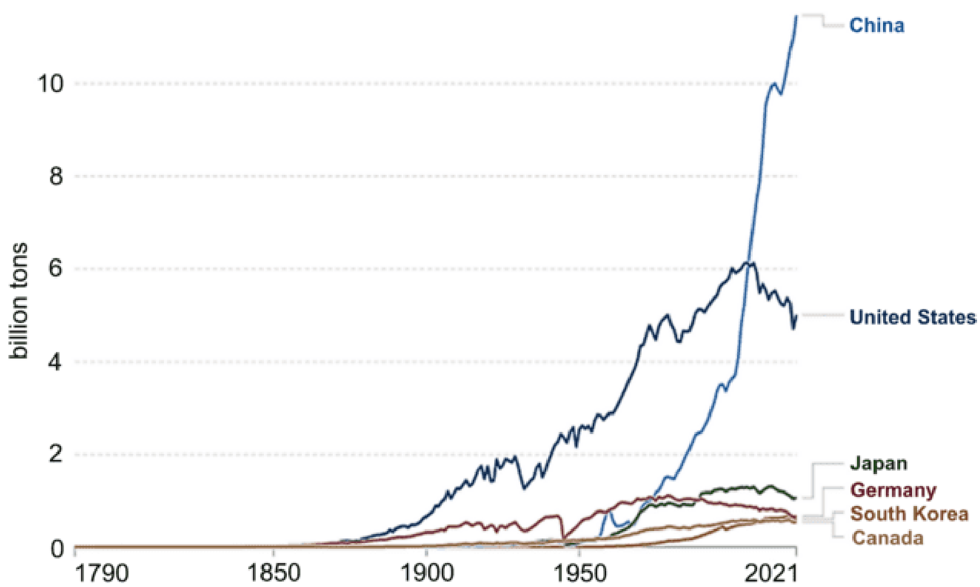

Fig. 4: Major trading partners (United States, Canada, Republic of Korea, Japan, Germany, and China) of the most relevant LATAM economies and their impact on CO_2 emissions at the country level (Our World in Data, 2023)

China has the highest CO_2 emissions among the major trading partners of LATAM economies, followed by the United States (USA) (Fig. 4). Other countries showed a slight increase in emissions in 2021 after a decrease in 2020, whereas China's emissions continued to grow due to its rapidly growing economy. Although Chinese companies joined Climate Action, they have not effectively reduced global CO_2 emissions.

The study analyzed the major LATAM economies with their international trading partners using GHG emissions per capita and by country, considering

Table 1: Companies participating in the Global Climate Action of the main LATAM economies and their major trading partners

Countries	A # Companies in GCAC* A	B # Companies in GCAI** B	C # Companies in GCAC* with reporting the progress C	D # Companies in GCAC* without reporting the progress D	E C/A (%) E	F C/D (%) F
LATAM economies						
Mexico	166	132	28	99	17%	28%
Chile	136	131	4	119	3%	3%
Brazil	354	276	68	259	19%	26%
Colombia	92	84	10	52	11%	19%
Argentina	146	144	2	125	1%	2%
Main International partners						
USA	1535	1118	522	937	34%	*56%*
Canada	304	234	71	177	23%	*40%*
China	788	186	416	365	53%	*114%*
Korea	149	50	83	74	56%	*112%*
Germany	346	270	96	184	28%	*52%*
Japan	558	211	404	254	72%	*159%*

*Global Climate Action Commitment (GCAC)
**Global Climate Action Initiatives (GCAI)

the difference in GHG emission by years (2020–2019 and 2021–2020) in correlation with companies participating in Global Climate Action and the rate of public reporting. The study hypothesized corporate participation in Global Climate Action and their progress is negatively related to GHG emission per capita and at the country level over time. The study focused on Argentina, Brazil, Chile, Colombia, and Mexico and their major international trading partners: China, South Korea, Germany, Japan, Canada, and the United States. References to the European Union were deliberately excluded unless they explicitly mentioned the country in question, as in the case of Germany. Table 1 summarizes the number of companies per country committed to Global Climate Action, such as emission reductions, the use of renewable energy, and the avoidance of CO_2 emissions. Brazil has the highest number of companies participating and reporting progress in LATAM, followed by Mexico, but both have room for improvement. In this region, the percentage of progress reporting is lower than their major trading partners, highlighting the importance of increasing accountability and focusing on results.

Meanwhile, declaring commitments and exercising discipline in reporting progress are necessary initiatives in terms of GHG reduction. The USA stands out in terms of the number of companies participating in Global Climate Action, but China, Japan, and the Republic of Korea stand out for their accountability in GHG reduction initiatives.

In Fig. 5, Japan and the Republic of Korea have low emissions, with a high proportion of companies publicly reporting on their climate actions. Their governments have declared emissions reduction targets, and companies have established measures to reduce GHG emissions, possibly due to the importance of ESG (environmental, social, and governance concerns) among investors. These countries promote information disclosure related to ESG to attract conscious investors, and their major global leading companies have high sustainability reporting rates.

Korea and Japan incentivize ESG reporting and

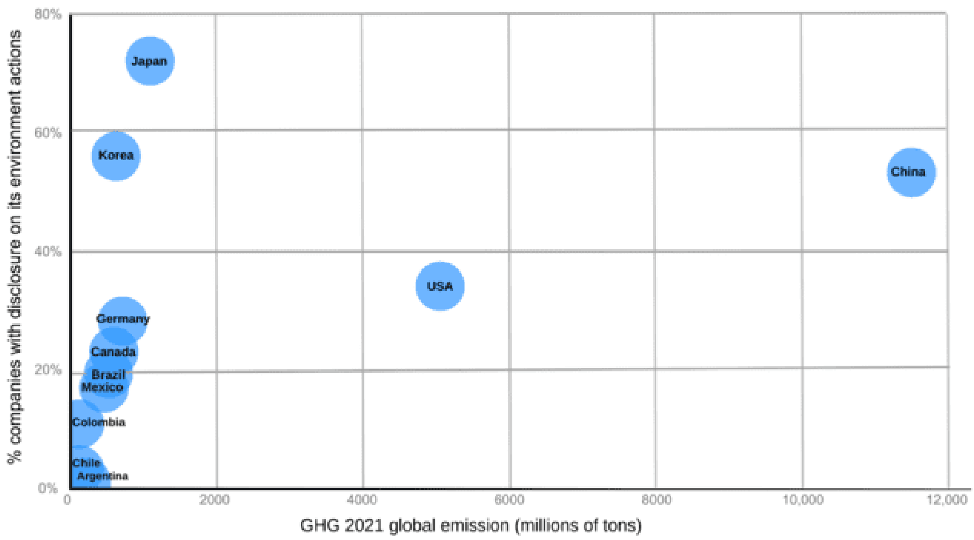

Fig. 5: GHG emission (million tons) and % of companies with public reporting on their Global Climate Actions

Table 2: GHG global and per capita of the main LATAM economies and their major trading partners

Countries	GHG2019 per capita (tons)	GHG2020 per capita (tons)	GHG2021 per capita (tons)	GHG2019 global (millions of tons)	GHG2020 global (millions of tons)	GHG2021 global (millions of tons)
			Main LATAM economies			
Mexico	3.77	3.11	3.21	472.19	391.71	407.21
Chile	4.83	4.34	4.38	91.96	83.83	85.45
Brazil	2.24	2.07	2.28	475.10	442.31	488.88
Colombia	1.92	1.68	1.78	96.44	85.53	91.70
Argentina	3.99	3.76	4.12	178.51	169.26	186.45
			Major trading partners.			
USA	15.73	14.04	14.86	5260.00	4720.00	5010.00
Canada	15.58	14.12	14.30	584.71	534.86	545.63
China	7.55	7.69	8.05	10740.00	10960.00	11470.00
Korea	12.47	11.53	11.89	646.10	597.63	616.08
Germany	8.50	7.67	8.09	707.15	639.38	674.75
Japan	8.79	8.32	8.57	1110.00	1040.00	1070.00

disclosure, with Korea also offering financial incentives for ESG-compliant companies (Sahar *et al.*, 2022; Yeo, 2021). China has also employed penalties such as lower credit ratings for companies that violate carbon emissions targets (Huld, 2022). For LATAM, this visualizes that fewer emissions reductions are seen, maybe because its economies are emerging and have only limited growth. In addition, the number of companies participating in Global Climate Action and their level of progress reporting is very low. Based on the previous discussion, more companies should join Global Climate Action with

Table 3: Pearson's correlation of the annual changes in GHG emissions by country (2020–2019 and 2021–2020) and participation in Global Climate Action

		Changes in GHG Per Capita		Changes in global GHG Emissions	
Corporate participation in climate action	Statistics	Difference GHG per capita (2020–2019)	Difference GHG per capita (2021–2020)	Difference GHG global emissions by country (2020–2019)	Difference GHG global emissions by country (2021–2020)
Number of companies participating in Global Climate Action	Pearson Correlation	−0.412	.827**	−0.629*	.718*
	Sig. (2-tailed)	0.207	0.002	0.038	0.013
	N	11	11	11	11
Rate of companies with public reporting	Pearson Correlation	0.132	0.485	0.087	.995**
	Sig. (2-tailed)	0.698	0.130	0.800	0.000
	N	11	11	11	11

concrete commitments; however, apart from their commitment to climate action, the public reporting of climate actions should be reinforced to verify the progress and effectiveness of their actions. Given the number of companies and their public reporting in Global Climate Action (2023) and GHG emissions by country, global, and per capita from 2021–2020 and 2020–2019, from Our World in Data (2023), the study analyzed the correlations between both sources. The test statistic used in this study is the Pearson correlation coefficient, which measures linear correlation between two sets of data (values from −1 to 1). Here, it describes the data on GHG emissions and the participation of companies in climate action. Although the statistic does not predict future results, it does show a trend that can be expected to continue unless drastic changes are made. For that purpose, GHG emissions by country (Table 2) from Our World in Data (2023) and data from Global Climate Action (2023) are provided (Table 1).

First, to find the correlations between the difference in GHG emissions *per capita* from 2019, 2020, and 2021 and the number of companies participating in Global Climate Action in 2023, Pearson correlation statistical test was applied. Then, the correlation between the difference in GHG emissions from 2019, 2020, and 2021 was explored to see how the public reporting progress emissions impact the GHG emissions (Table 3).

Table 3 presents a positive correlation between GHG 2021–2020 change in global emissions companies participating in climate action (Pearson = 0.718*, sig = 0.013) and reporting progress on their commitments (Pearson = 0.995*, sig = 0.000). Furthermore, the per capita level of GHG emissions 2021–2020 and the relationship with the participating companies is also significant (Pearson = 0.827** sig = 0.002). However, this contradicts what was expected, namely, a negative correlation. The correlation observed between the number of companies and the 2019–2020 emissions (Pearson = −0.629* sig = 0.038) can be attributed to the impact of the pandemic on the global economy and thus a significant reduction of GHG (Kumar *et al.*, 2022). The findings of this study are in line with previous work by Van den Berg *et al.* (2022), Taylor *et al.* (2021), and Kinley *et al.* (2021), who highlighted gaps between what stakeholders promise and what they actually deliver, as well as a lack of scientific climate information and data sharing related to corporate climate change. These issues are consistent with the results of the present study, indicating that they are ongoing challenges that require immediate attention. In addition, the current study's findings are also consistent with work by Bastos Lima (2022), Ortiz Palafox (2021), Kharas (2021), and Keat-Chuan-Ng and Webber (2023), who identified a lack of sustainability, environmental, and social governance as key issues facing organizations. They called for greater transparency and accountability in addressing these challenges. Thus, this study forms a contribution to the growing body of literature emphasizing the importance of sustainability and environmental responsibility in corporate decision-making. Companies must focus efforts to reduce GHG emissions through mitigation measures, seeking to counteract or minimize negative environmental impacts. These measures are consolidated in a mitigation plan and are part of the environmental impact assessment. A key mitigation measure is to stop using fossil energy and use alternative energy, as well as applying technology to achieve greater energy efficiency. While several authors have argued on this topic, this article has the distinction of directly analyzing the situation of the climate crisis and climate action during and after the pandemic for a group of countries. In addition to reinforcing previous discussion, this particular analysis specifically points out how the post-pandemic economic recovery threatens the planet's climate crisis more strongly, regardless of the significant contributions made by companies to climate action. For Van den Berg *et al.* (2022), accountability-driven and evidence-based evaluation are needed to assess the effectiveness of investments in adaptation and mitigation. The findings of this research support the aforementioned need. Evidence is provided to demonstrate how participating with commitments and showing progress in mitigation or adaptation to climate change is not sufficient for the climate crisis. For Kinley *et al.* (2022) leading with climate crisis requires systems focused on climate treaties' goals for data sharing and transparency and the growing engagement of stakeholders. Unfortunately, governments have failed to fully implement treaty obligations, exacerbated by the still inadequate response of the business community. The results of this research provide evidence of the need to increase the level of response from the business community to achieve greater effectiveness in addressing the climate crisis.

CONCLUSIONS

During the pandemic, GHG emissions decreased, primarily due to the economic slowdown rather than the efforts of companies to address climate change (Pearson = −0.629 sig = .038). After the pandemic, there was a statistically significant and positive correlation between the number of companies committed to GHG reduction and GHG global emissions (global Level Pearson = .718*, sig = .013 and per capita, Pearson = .827**, sig = .002). This suggests that business organizations have made only marginal contributions to reducing GHG emissions, and it raises questions about the effectiveness of the current voluntary disclosure regime for companies' climate change commitments. LATAM countries have fewer companies participating in Global Climate Action and reporting their progress (10%) than their major trading partners (44%). Initiatives and commitments are necessary for climate action but that alone is not enough. Transparency and accountability are key to effectively achieving GHG reductions and curbing the impending climate crisis. From a global perspective, it is clear that the acceleration of the production system has led to an increase in GHG emissions that has not kept pace with the commitments and reported achievements for reducing emissions. Companies must take significant actions to reduce emissions and report their results in a timely manner. These findings do not reduce the importance of initiatives like Global Climate Action but do highlight the need for more comprehensive and standardized approaches to climate change. Analyzing the information of countries and companies to adhere to such initiatives is crucial to increasing demand for countries and economic factors to address climate change.

Limitations

The size of its sample limits this article since it is based on secondary information reported by Our World in Data (2023), which presents information on GHG emissions up to 2021. Moreover, Global Climate Action (2023), this limitation represents a strength since it allows us to include the main economies of LATAM countries and their major trading partners in the analysis. Considering these limitations, the study yields interesting results that reflect the way forward for the business sector to follow the actions being taken by its trading partners. The other limitation is that the most current information on GHG emissions includes up to 2021. Also, there is no historical information on corporate participation in Global Climate Action; only the information generated to date is available.

AUTHOR CONTRIBUTIONS

F.G. Arredondo-Trapero, the first author, has conducted the literature review, gathered data from open-access platforms, and interpreted the results of the data analysis. E.M. Guerra-Leal, the second author, has analyzed the data and interpreted the results for the implications. J. Kim, the corresponding author, has drawn implications from the conclusions of the result and prepared the manuscript.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
CEO	Chief Executive Officer
CO_2	Carbon dioxide
COP26	2021 Climate Change Conference
COVID-19	Coronavirus disease
EPA	U.S. Environmental Protection Agency
ESG	Environmental, Social, and Governance
ETS	Emission Trading Scheme
GCAC	Global Climate Action Commitment
GCAI	Global Climate Action Initiatives
GDP	Gross Domestic Product
GHG	Greenhouse gas
ISO	International Organization for Standardization
IPCC	Intergovernmental Panel on Climate Change
LATAM	Latin America
SDG	Sustainable Development Goals
Sig.	Significance
UN	United Nations
USA	United States of America
WBCSD	World Business Council for Sustainable Development
WRI	World Resources Institute

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