Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

Podcasts

ORIGINAL RESEARCH PAPER

Network meta-analysis of cadmium toxicity against Chlorella vulgaris and the role of growth stimulants and macronutrients

M. Iqhrammullah[1,2], S. Saudah[3,4], M. Monalisa[5,6], F. Fahrurrozi[1], S.A. Akbar[7,*], S.S. Lubis[8]

[1] *Research Center for Marine and Land Bioindustry National Research and Innovation Agency, North Lombok 83756, Indonesia*
[2] *Postgraduate Program of Public Health, Universitas Muhammadiyah Aceh, Banda Aceh 23123, Indonesia*
[3] *Faculty of Education and Teacher Training, Universitas Serambi Mekkah, Banda Aceh, 23245, Indonesia*
[4] *Master Program of Biology, Universitas Serambi Mekkah, Banda Aceh, 23245, Indonesia*
[5] *Department of Agribusiness, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[6] *Aceh Climate Change Initiative, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[7] *Faculty of Marine and Fisheries, Department of Aquaculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[8] *Faculty of Science and Technology, Biology Departement, Ar-Raniry State Islamic University, Banda Aceh 23111, Indonesia*

ARTICLE INFO

Article History:
Received 25 October 2023
Revised 29 January 2023
Accepted 28 February 2024

Keywords:
Bioremediation
Cadmium
Chlorella vulgaris
Growth
Heavy metals

DOI: 10.22034/gjesm.2024.04.***

ABSTRACT

BACKGROUND AND OBJECTIVES: The presence of heavy metals, specifically cadmium, in the environment poses significant threats to both ecological systems and human health. However, microalgae have shown potential in addressing this issue through their ability to absorb cadmium and produce valuable biomass, making them a promising solution for bioremediation. Among the various microalgae species, Chlorella vulgaris stands out as a suitable candidate due to its potential for biodiesel production and its capacity to effectively absorb cadmium. Therefore, the main objective of this study is to assess the toxicity of cadmium on Chlorella vulgaris cells using network meta-analysis as a methodology.
METHODS: A comprehensive search was conducted on Scopus, Scilit, Google Scholar, and Web of Science to identify relevant studies published from 1 January 1990 to 16 January 2024. Only studies that reported the cell number of Chlorella vulgaris as a result of cadmium exposure were considered for inclusion. The collected data were then subjected to Bayesian frequentist network meta-analysis, utilizing standardized mean difference and a 95 percent confidence interval as measures of effect size. Additionally, a linear regression analysis was performed to examine the dose-dependent impact of cadmium toxicity.
FINDINGS: Dose-dependent toxic effects of cadmium on Chlorella vulgaris were evident (R-square of more than 0.90), particularly at a concentration of 1 part per million, deemed as the maximum tolerable threshold. Prolonged exposure revealed a concentration-dependent reduction in cell viability, suggesting potential lifespan shortening. A comparison of growth stimulants, gibberellic acid and brassinolide (standard means differences of 1.7 and 3.8, respectively), in mitigating cadmium toxicity indicated the latter superior effectiveness in sustaining microalgal survivability. The presence of high nitrogen and low phosphorous levels was found to be significantly associated with a reduction in Chlorella vulgaris cells due to cadmium exposure.
CONCLUSION: This research has provided conclusive proof of the harmful effects of cadmium on Chlorella vulgaris through the implementation of Bayesian frequentist network meta-analysis, offering valuable insights for environmental management practices. The findings reveal concentration-dependent effects of cadmium toxicity. The survivability of Chlorella vulgaris is determined by the compositions of macronutrients nitrogen and phosphorous. Comparative analyses highlight the superior protective effect of brassinolide over gibberellic acid in mitigating cadmium toxicity. Overall, the findings highlight the potential of Chlorella vulgaris in both bioremediation of heavy metals and biomass production.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
46	6	1

*Corresponding Author:
Email: *saidaliakbar@usk.ac.id*
Phone: +62812 6585 4429
ORCID: 0000-0003-3678-1921

Note: Discussion period for this manuscript open until January 1, 2025 on GJESM website at the “Show Article”.

INTRODUCTION

Contaminations of toxic heavy metals in water bodies have been recorded in studies across countries and regions (Li *et al*. (2023) expected to cause detrimental effects on human health (Babuji *et al.*, 2023). Heavy metals are released from the effluent of various industrial sources such as mining (Ahmad *et al.*, 2022), fishing, automotive, and processing industries (Wang *et al.*, 2023). Poor wastewater treatment facilities, coupled with non-strict policies by the government, are responsible for the pollution of heavy metal being detected in multiple sites (Pratama *et al*., 2024). Pollution in aquatic environments poses a significant threat as harmful substances can spread to remote areas and build up in living organisms, potentially reaching humans through the consumption of contaminated fish (Nasir *et al*., 2021) and vegetables (Saputri *et al*., 2023). Lead (Pb), zinc (Zn), iron (Fe), and even mercury, have been reported contaminating the water, mostly in developing countries (Nisah *et al.*, 2022), but cadmium (Cd) is the most frequent (Nasir *et al*., 2021). While the toxicity level of cadmium is lower than that of mercury, exceeding the acceptable limit can still lead to negative effects on the human body. Symptoms of acute cadmium toxicity may include nausea, vomiting, abdominal pain, and respiratory issues (Genchi *et al*., 2020). Several research findings indicate that prolonged exposure to Cd may result in various health issues, encompassing both physical (Li *et al*., 2022) and psychological dimensions (Tian *et al*., 2022). Hence, technologies or strategies that do not contribute to another waste problem and eco-friendly in handling the Cd pollution is urgently required, where metal-based (Ahmad and Chiari, 2023) or bio-based materials have been used (Iqhrammullah *et al*., 2023a). Microalgae can be effectively employed for the bioremediation of heavy metal pollutants due to their ability to absorb and accumulate free Cd ions from water sources. The absorption of Cd is facilitated by transport proteins and channels present in the cell membranes of microalgae. Additionally, certain types of microalgae produce chelating agents that bind to Cd ions, resulting in the formation of stable complexes. (Manikandan *et al*., 2022). Furthermore, it produces extracellular polymeric substances that could further entrap the heavy metal ions from the water (Zhao *et al*., 2023). However, in the presence of stressors such as heavy metals, the growth of microalgae can be inhibited (Xiao *et al*., 2023). Each microalgae may have different responses towards heavy metal, and when the sensitivity is high, it is therefore unsuitable for the bioremediation of this type of contaminant (Xiao *et al*., 2023). Other than having high resistance, microalgae is expected to be capable of producing biomass with economic values. Microalgae is cultivated for its lipid content, which can be transformed into biodiesel as a renewable substitute for fossil fuels. *Chlorella vulgaris (C. vulgaris)* is the predominant microalgae species cultivated for this purpose, with a lipid content that can vary from 5 to 58 percent (%), (Ru *et al*., 2020). Moreover, living or dead cells of *C. vulgaris* have the ability to absorb heavy metals, particular Cd (Soto-Ramírez *et al*., 2021). Research on the effect of Cd on the growth of *C. vulgaris* has been conducted multiple times (Saberi *et al*., 2022; Sembada and Suyadi, 2023; Xi *et al*., 2022). The inhibitory effect on the growth of *C. vulgaris* has been observed when Cd is present in the growth medium. The presence of this heavy metal suppresses the photosynthesis pigments in the microalgae, leading to a limitation in cell division and metabolism. As a result, the growth of the microalgae is inhibited. In the case of *C. vulgaris*, the accumulation of Cd in the microalgal cell reduces the production of chlorophyll a and chlorophyll b (Wang *et al*., 2021) and carotenoids (Daliry *et al*., 2017). Increased production of endogenous antioxidants was also observed corresponding to the increase of oxidative stress concomitant to the cellular presence of Cd (Geng *et al*., 2022). As a defense mechanism, increases in biomass (including the monosaccharide and lipid contents) are observed in *C. vulgaris* exposed to Cd (Chia *et al*., 2017; Chia *et al*., 2015). Hence, the utilization of *C. vulgaris* for Cd removal from water has the potential to enhance biomass production. In various studies, the reaction of *C. vulgaris* to Cd varies based on factors such as strain, growth medium, environmental conditions, and supplements. Unlike traditional meta-analysis, the Bayesian frequentist network meta-analysis utilized in this study allows for comparison of different exposures. Previously, meta-analysis on *C. vulgaris* was carried out to see the effect of antibiotic exposure (Lu *et al*., 2023) and micro- or nano-plastics (Ge *et al*., 2024). The pooled estimates performed in the foregoing studies were the conventional pair-wise meta-analysis. This present study was the first to report the pooled estimate of the effect of Cd exposure on *C. vulgaris* growth through sophisticated statistical analysis namely Bayesian frequentist network meta-analysis. The network meta-analysis enables the assessment of various exposures at varying concentrations. It is important to note that the alterations in Cd concentration were not calculated, as the primary focus is on monitoring the growth of C. vulgaris when subjected to the heavy metal. The hypothesis of this study suggests that Cd exposure may have a negative impact on the growth of *C. vulgaris*, and that the inclusion of growth enhancers and different macronutrient compositions could potentially modify this effect. The study aims to analyze the impact of Cd exposure on the growth of *C. vulgaris* through network meta-analysis of existing data. The study was conducted in Banda Aceh, Aceh, Indonesia in 2024 using data from reports available online.

MATERIALS AND METHODS

The current study aimed to assess how Cd exposure hinders the growth of *C. vulgaris*. Additionally, the study analyzed the impact of treatments in reducing Cd toxicity, including the use of plant growth hormones and nitrogen (N) or phosphorous (P) levels through network meta-analysis. The study was designed and conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as suggested by a previous study (Wikurendra *et al*., 2023).

Search strategy

The search was performed on 16 January 2024 using Scopus, Scilit, Google Scholar, and Web of Science per recommendation of previous study (Putra *et al*., 2023). The search was narrowed down to encompass publications from 1 January 1990 to 16 January 2024. Zotero was utilized to eliminate duplicates. The title and abstract screening process was aided by ASReview, an artificial intelligence-based software programmed in Python. Excel was employed for the full-text screening, applying the inclusion and exclusion criteria. The entire identification, screening, and selection process was conducted by two separate authors, with any discrepancies resolved through consensus.

Inclusion and exclusion criteria

The impact of Cd toxicity on *C. vulgaris* was assessed by analyzing the cell count in the treated culture in relation to the control group. *C. vulgaris* cultivated in media without Cd exposure was considered as control in this study. The study also incorporated data on the impact of Cd exposure when combined with growth stimulants and macronutrients. There were no restrictions placed on the specific types of stimulants and macronutrients used in the study. The source of Cd was from the stock solution added to the growth medium. Studies that did not include cell counting were not included in the analysis. Studies that were not in English or were not peer-reviewed were also excluded. Additionally, other factors such as the clarity of the microalgal species, study design, and recommendations from previous systematic in vitro studies (Iqhrammullah *et al*., 2023b) were taken into account during the selection process.

Data extraction

Information obtained from the chosen literature consisted of author information, publication year, and country of affiliation. Additionally, details regarding the media utilized for microalga cultivation, reactor size (measured in volume), observation time, exposure, and the range of Cd concentrations were also extracted. Graph images containing the data were obtained using ImageJ, a software for image analysis, specifically version 1.53e. To ensure consistency in presentation, all Cd concentrations were converted into parts per million (ppm). The extraction of data was carried out by two separate authors, and any discrepancies that arose were resolved through consensus.

Statistical analysis

Quantitative analysis of the collected data was performed based on Bayesian frequentist network meta-analysis using GeMTC (a package in R studio for network meta-analysis) on R studio version 2023.12.0. The pooled estimates were carried out by computing standardized mean difference (SMD) and 95% confidence interval (CI). The SMD, serving as the benchmark, was utilized to compare the impact of exposures on microalgal growth, thereby highlighting the size effect. Linear regression analysis was conducted to examine the dose-response effect of different concentrations of Cd on cell numbers, as indicated by the pooled size effects (SMDs). The results revealed a consistent decrease in cell numbers at specific Cd concentrations, suggesting that these concentrations represent the maximum tolerable level for *C. vulgaris*.

RESULTS AND DISCUSSION

Characteristics of included studies

A thorough search in the scholar database resulted in the identification of a total of 711 records. The reference manager, equipped with an automatic identification feature, successfully detected and eliminated 136 duplicate entries from the dataset. Following the application of eligibility criteria, a total of 184 full-texts were screened, resulting in the inclusion of 8 studies in the final análisis (Bajguz, 2011; Carr *et al.*, 1998; Cheng *et al.*, 2016; Chia *et al.*, 2017; Chia *et al.*, 2015; Falkowska *et al.*, 2011; Lam *et al.*, 1999; Zhang *et al.*, 2015). Exclusion in the screening process mostly because the studies reported different species than *C. vulgaris* (number [n]=39), having taxonomical nomenclature only at the genus level (n=27), the effect on growth with other parameters than cell number (n=25), evaluating the adsorption capacity on dead cells only (n=34), or did not use Cd as the heavy metal (n=9). Non-English (n=20) and non-peer-reviewed (n=22) papers were also found in the full-text screening, hence excluded. The summary of the screening process is presented in Fig. 1.

The characteristics derived from the data extracted from the eight studies are showcased in Table 1. Studies were reported from Poland, Hong Kong, Philippines, Brazil, Singapore, and China. Media used in the experiment were varied, and the microalgae was mostly cultivated in batch reactors (Erlenmeyer). Other than Cd, some studies also evaluated the effects of the addition of gibberellic acid (GA_3), brassinolide (BSL), and other heavy metals; copper (Cu) and Pb. The lowest concentration for a single added Cd (with no combination) was 0.1 ppm, whilst the highest concentration was 7 ppm.

Network meta-analysis

A network meta-analysis was performed on 7

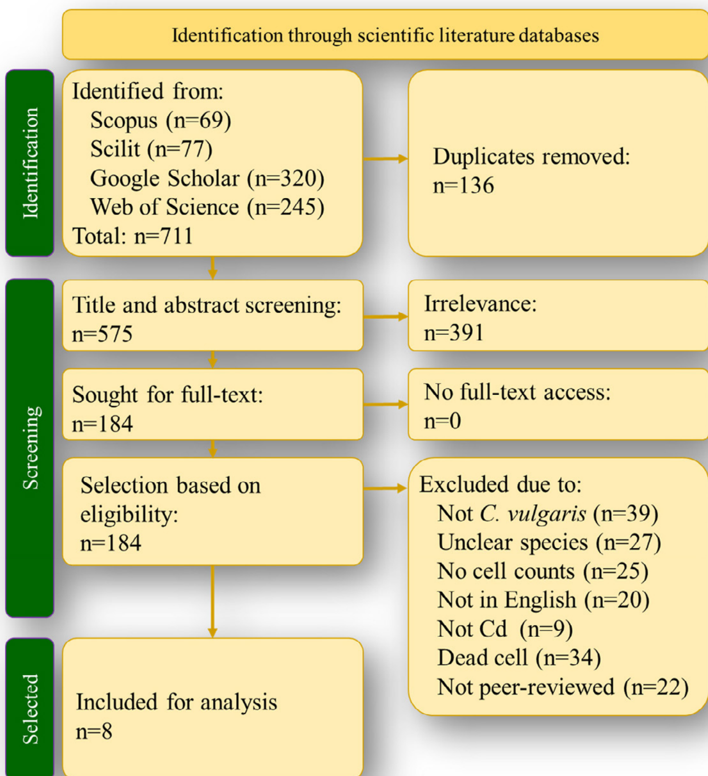

Fig. 1: Flow-chart diagram for the screening and selecting eligible studies

Table 1: Characteristics of the included studies reporting Cd toxicity against *C. vulgaris*

Country	Growth medium	Reactor size	Observation time	Exposure	Cd range (ppm)	Sources
Poland	Modified Knop's medium	250 mL	3 days	Cd, GA_3	0.01—11.2	Falkowska *et al.*, 2011
Hong Kong	Bold's basal medium	125 mL	4 days	Cd, Cu	1—3	Lam *et al.*, 1999
Poland	Modified Knop's medium	250 mL	3 days	Cd, BSL	0.1	Bajguz, 2011
Philippines	Bristol medium	250 mL	4 and 7 days	Cd	9 – 12.5	Carr *et al.*, 1998
Brazil	LC Oligo medium	250 mL	Continuous	Cd, N	0.001 – 0.01	Chia *et al.*, 2015
Singapore	Bold's basal medium	Not reported	7 days	Cd, Cu, Pb	0.1	Zhang *et al.*, 2015
China	BG11 medium	250 mL	3 and 4 days	Cd	0.5 – 7	Cheng *et al.*, 2016
Brazil	LC Oligo medium	250 mL	Continuous	Cd, P	0.001 – 0.01	Chia *et al.*, 2017

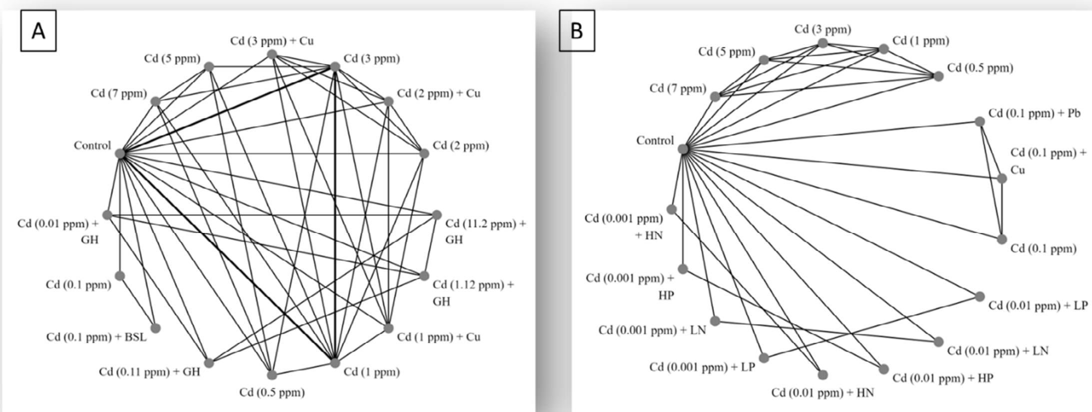

Fig. 2: Network graphs for exposures from the included studies. Observation on day 3—4 (A) and day 7 (B). The graph is constructed based on the data from the included studies (Bajguz, 2011; Carr *et al.*, 1998; Cheng *et al.*, 2016; Chia *et al.*, 2017; Chia *et al.*, 2015; Falkowska *et al.*, 2011; Lam *et al.*, 1999; Zhang *et al.*, 2015)

studies, while 1 study being excluded (Carr *et al.*, 1998). The reason for the exclusion in this state is because the data did not correspond to the dose-response principle. Moreover, the study used an extreme range of Cd concentration (9-12.5 ppm) (Carr *et al.*, 1998). The network graphs for the meta-analysis are presented in Fig. 2. The data collected from observations made on days 3 to 4 were combined due to variations in reporting across studies. Nevertheless, all studies included results from observations made on day 7, resulting in a lack of diversity in observation days for the pooled analysis. Line density indicates the frequency of variables being reported by different studies. The findings from various studies indicate that Cd exposure levels of 1 and 3 ppm have been associated with higher density. (Fig. 2a).

Figs. 3a and 3b display the forest plots illustrating the magnitude of the effects of each exposure in comparison to the control. Lower size effect (SMD) indicates higher toxic effects on the microalgal growth. According to the observation day-3-4, combination of Cd 0.1 ppm and BSL yielded highest cell number, while Cd 7 ppm caused the lowest cell number. SMD of microalgal cells exposed with Cd 0.1 ppm is close to zero (0.002) suggesting that the toxic effect at such concentration is not significant. On day-7, the lowest effect of Cd toxicity was observed when the concentration was set at 0.5 ppm. Exposure of Cd 0.01 ppm with deficit level of P could drastically reduce the cell number. Interestingly, the number of cells became significantly lower in culture exposed with Cd 0.1 ppm after seven days of cultivation. Hence, the effect of introducing low concentrations of Cd to microalgal cells may not be immediately noticeable, but could manifest in subsequent days of monitoring. This implies that Cd absorption could potentially decrease the longevity of *C. vulgaris* cells, aligning with previous findings. (Priya *et al.*, 2022). The toxicity of Cd against the cell viability of *C. vulgaris* was found to be dose-dependent with correlation coefficients (R-square) higher than 0.90 in both observations (Figs. 3c and 3d). The linear equations for the effect of Cd exposure on *C. vulgaris* growth are y= 0.23 -0.72x and y=-2.76-2.27x for data obtained from day-3—4 and day-7, respectively. Following exposure to Cd, a consistent reduction in the number of *C. vulgaris* cells was noted at concentrations as low as 1 ppm. This indicates that 1 ppm represents the highest concentration that *C. vulgaris* can tolerate in terms of Cd exposure.

The influence of Cd on the quantity of *C. vulgaris* cells may be categorized according to Bayesian frequentist statistics, with the findings displayed in Fig. 4. On day 3 and 4, the highest impact was observed in cells exposed with Cd 7 ppm as suggested by the probability of being in the rank 16. The simultaneous presence of Cu and Cd resulted in a more pronounced effect compared to Cd alone at an equivalent concentration. Cultures of *C. vulgaris* supplemented with growth enhancers like GA3 and BSL exhibited a higher likelihood of ranking in the top 2 positions. A higher probability of ranking in top positions indicates that the toxicity of Cd was mitigated by the presence of stimulants. Among GA3 and BSL, the latter was more effective in reducing the

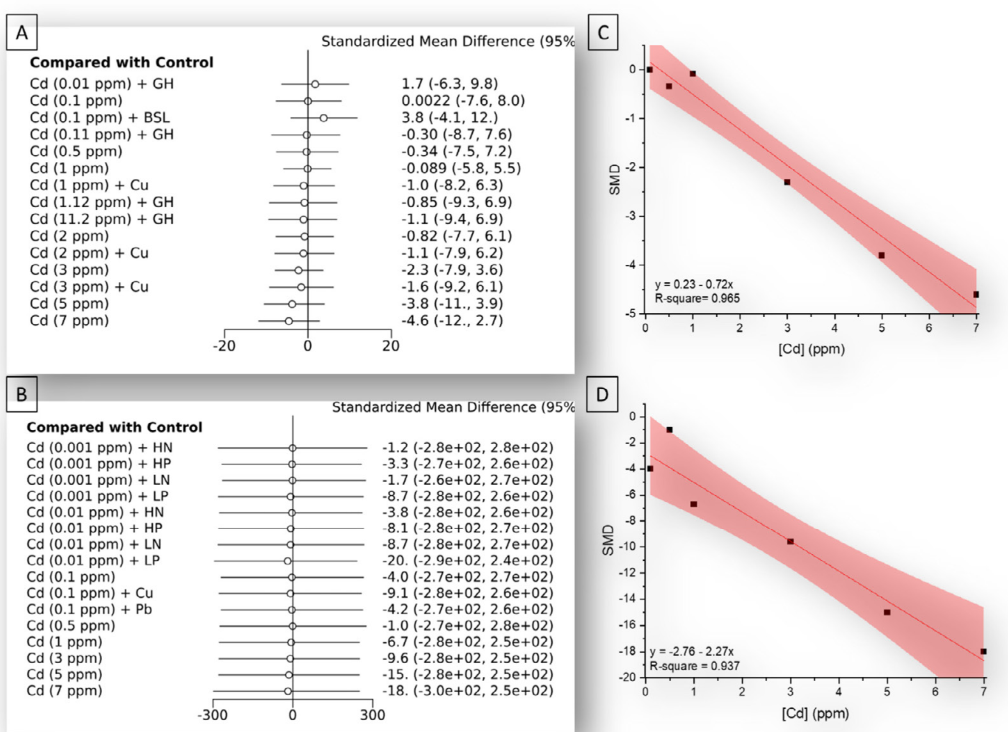

Fig. 3: Forest plot for the effect of Cd exposure on C. vulgaris cell viability as observed on day 3—4 (A) and day 7 (B). Dose-response analysis on the effect of Cd exposure based on the data from day-3—4 (C) and day-7 (D) observations

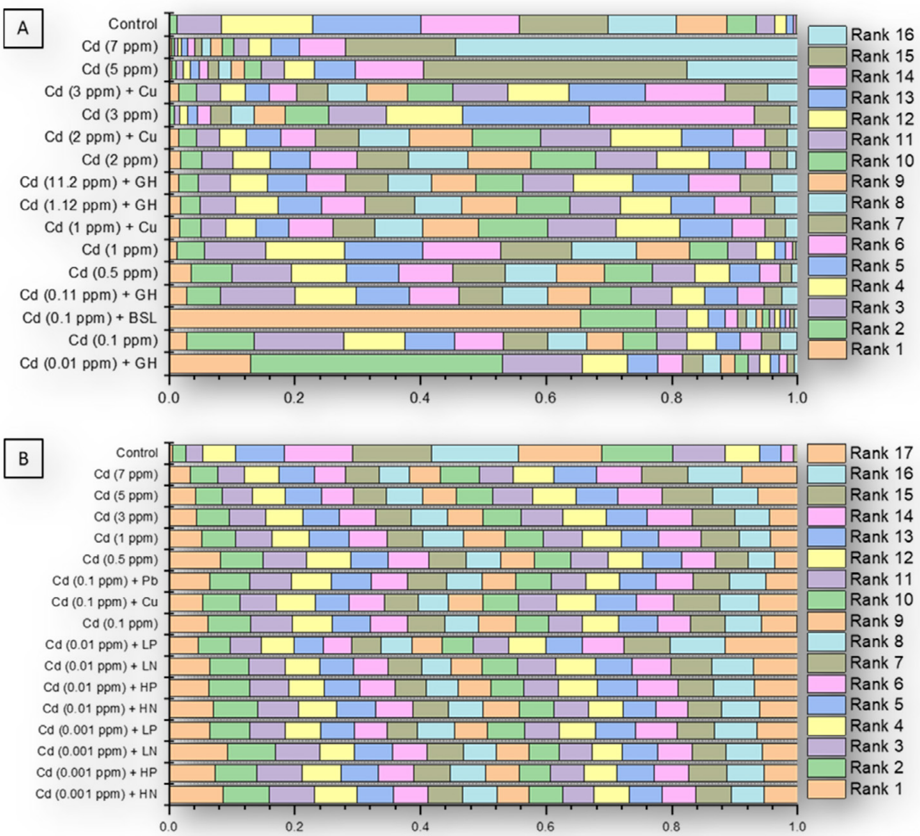

Fig. 4: Rank probabilities plots for the effect of Cd exposure on *C. vulgaris* cell viability based on the data collected on day-3—4 and day-7 observations

toxicity induced by Cd. Furthermore, on the 7th day, Cd at a concentration of 7 ppm displayed the highest level of toxicity.

Effect of Cd exposure

In this current investigation, there exists an inverse relationship between the cell count and the concentration of Cd owing to its detrimental impact. The influence of concentration is particularly noticeable in the rank probability test, specifically during the observation period of day 3—4. The survival and growth of microalgae are significantly affected by the toxicity of Cd, which operates through multiple pathways. One such pathway involves the induction of an imbalance in oxidative stress by Cd, leading to an increase in the production of reactive oxygen species. Consequently, this elevation in reactive oxygen species levels can negatively impact the cellular structure and functions of microalgae. Cd induces dysfunction in the photosynthesis system by reducing the production of related pigments as reported by various studies (Geng *et al*., 2022; Wang *et al*., 2021). It has been documented that Cd can disrupt the uptake of necessary nutrients, resulting in a deficiency in essential vitamins and minerals in the body. The translocation of Cd to the cellular system of microalgae could activate the stress response mechanism including the biosynthesis of metabolites such as antioxidants, chelating agents, and other protective molecules (Rathnayake *et al*., 2021). The stress response mechanism has been reported in several studies as indicated by the increased endogenous antioxidants including catalase and superoxide dismutase (Cheng *et al*., 2016). Cd uptake can lead to changes in the metabolic pathway of microalgae. Previous research has shown that when exposed to Cd, microalgae experience a significant increase in the production of carbohydrates and protein. Additionally, an increase in lipid content has been observed in Cd-exposed *C. vulgaris*, but this is only evident when the P level is low. Another study found that the lipid productivity of C. vulgaris significantly increased when exposed to Cd at a concentration of 0.5 ppm. (Satpati and Pal, 2021). This highlights the potential of utilizing *C. vulgaris* for bioremediation of Cd and production of its biomass. A previous study reported that the living cells of *C. vulgaris* wild type could reduce the aqueous Cd concentration by more than 60% of its initial concentration. Accumulation of Cd by the *C. vulgaris* is reported to peak on day 12 of cultivation (Satpati and Pal, 2021). In a study using dead *C. vulgaris* cells, the absorption of Cd is associated with the characteristics of the cell walls (Wang *et al*., 2022). The *C. vulgaris* growth was found to be equally affected in comparison to previous studies on the impact of microplastic contaminants. However, the negative influence on growth becomes even more severe when combined with heavy metals. (Ge *et al.*, 2021).

Effects of growth stimulants on Cd toxicity

Two growth stimulants, GA3 and BSL, were compared for their protective effects against Cd toxicity. The findings of this present study suggest that BSL is superior than GA3 in maintaining the survivability of *C. vulgaris* amidst the exposure of Cd. GA3 is a phytohormone, where in microalgae, it reduces the lag phase, promotes cell division and development during the log phase, and induces the synthesis of pigments and proteins. In an alternative study, GA3 was found to exclusively impact the elongation and expansion of cells, with no indications of its influence on cell division. Apart from Cd, GA3 has been documented to possess a protective effect against Pb exposure in other studies. (Han *et al*., 2018). As for BSL, its addition to microalgal culture increases the synthesis of chlorophyll, carbohydrates, and proteins. Additionally, BSL triggers the production of additional phytohormones, specifically abscisic acid and indole-3-acetic acid, which assist microalgae in adjusting to various environmental conditions. BSL has been reported to protect microalgae from heavy metal toxicity (Talarek-Karwel *et al*., 2020). Addition of BSL is reported to sustain the carotenoid level similar to that in the microalgal cells without Cd exposure (Bajguz, 2011). By impeding the accumulation of heavy metal, BSL effectively avoids a significant reduction in the concentration of aqueous Cd. (Kour *et al*., 2021. This type of protective mechanism can be considered to be counterproductive to the aim of bioremediation. Hence, while BSL demonstrates superior performance compared to AG3 in shielding the microalgae from Cd toxicity, it is not the most optimal choice for bioremediation objectives.

Effects of nutrient compositions on Cd toxicity

In this present study, the effect of N and P compositions was compared in network meta-analysis. In microalgae, N is a macronutrient which is involved in the synthesis of proteins and nucleic acids within the cell. The sufficiency of N in the culture medium is essential for the microalgal growth, particularly in terms of the biomass productivity and biochemical composition. Macronutrients P and N are factors that could influence the production of lipid and fatty acids. An academic publication suggested that in order to achieve optimal biomass production, it is advisable to maintain a nitrogen to phosphorus ratio between 5 and 30. (Wagner *et al*., 2021). The optimal proportions of macronutrients necessary for growth are determined by the specific conditions and characteristics of the cultivation medium, which includes the concentration of heavy metals. (Su, 2021). Excessive N level on the medium could in fact inhibit the microalgal growth and affect the lipid profile. The toxic effects of Cd on *C. vulgaris* cells are linked to low levels of phosphorus, as demonstrated in this study. Interestingly, the combination of low phosphorus levels and Cd exposure can actually lead to an increase in lipid production. (Chia *et al*., 2017; Satpati and Pal, 2021). Cd uptake occurs on the algal cell walls suggesting that the heavy metal removal is favorable when more biomass is produced. In light of the results obtained from the current study, it is recommended to employ a low concentration of phosphorus when attempting to bioremediate cadmium from water sources.

Strengths, limitations, and recommendations

The current study is groundbreaking as it utilizes Bayesian frequentist network meta-analysis to assess the toxicity of Cd on microalgae at different concentrations. This study is the first of its kind to employ this approach successfully. Additionally, by utilizing network meta-analysis, the study was able to determine the size effects of various exposures, shedding light on the impact of growth stimulants and macronutrient compositions. Nonetheless, the present meta-analysis suffers from several limitations including the influence of heterogenous data derived from different laboratory settings. To reduce the influence of the heterogeneity, the meta-analysis has been performed on SMD (a less biased size effect as compared to mean difference). Moreover, due to the unavailability of a quality appraisal tool designed for microalgal research, it was not possible to determine the quality of the study included in the analysis. Regardless, the potential outlier was successfully identified based on the pooled estimate and dose-response analysis. Findings of the present study implicates the use of *C. vulgaris* in reducing the concentration of Cd, where it can be optimized if the heavy metal concentration was low (<1 ppm). Both GA3 and BSL has positive effects on the microalgal growth, but GA3 is more recommended for Cd removal purposes. Further, the presence of P should be kept in low concentration to optimize the biomass production, particularly the lipid content. Henceforth, it is advisable to conduct further research to explore the most suitable combinations of GA3 and macronutrients with Cd concentration below 1 ppm. Utilizing response surface methodology or other statistical methods could be employed to ascertain the optimal conditions.

CONCLUSION

Evidence on toxicity of Cd toward *C. vulgaris* cells has been established for the first time by using Bayesian frequentist network meta-analysis based on seven studies from different countries. The toxic effect of Cd on *C. vulgaris* is concentration-dependent, especially observed after the concentration was set at 1 ppm which could serve as maximum threshold of the tolerable Cd concentration. The findings of the meta-analysis indicate that short-term exposure to low concentrations of Cd may not result in noticeable toxic effects. However, prolonged observation reveals a decrease in cell viability that is dependent on the concentration of Cd, suggesting that the uptake of Cd has the potential to shorten the lifespan of *C. vulg*aris cells. Cd exposure leads to alterations in metabolic pathways, influencing the production of carbohydrates, proteins, and lipids in *C. vulgaris*. The comparative analysis of two growth stimulants, GA3 and BSL, in mitigating Cd toxicity on *C. vulgaris* indicates that BSL surpasses GA3 in sustaining microalgal survivability. Despite BSL's superior protective effect against Cd, its counterproductive role in preventing heavy metal accumulation makes it less suitable for bioremediation purposes compared to GA3. Additionally, the meta-analysis suggests that the composition of N and P determines the toxicity of Cd against *C. vulgaris* cells. Macronutrients have an impact on both microalgal growth and biochemical composition. Research indicates that Cd toxicity is more pronounced in *C. vulgaris* cultures with elevated N levels and reduced P levels. Interestingly, the presence of 0.5 ppm Cd along with low P leads to an increase in lipid content production, even though there is a notable decrease in cell count. In conclusion, this study underscores how Cd concentration affects *C. vulgaris* cells, emphasizing the roles of macronutrients N and P, and comparing the effectiveness of growth stimulants GA3 and BSL, with BSL proving more protective against Cd toxicity. To enhance future studies, it is suggested to focus on fine-tuning the concentrations of macronutrients and exploring additional growth stimulants to alleviate the toxicity caused by Cd on *C. vulgaris*.

AUTHOR CONTRIBUTIONS

M. Iqhrammullah contributed in conceptualization, methodology, investigation, and writing of the original draft. S. Saudah contributed in validation and review and revision. M. Monalisa contributed in validation and review and revision. F. Fahrurrozi contributed in supervisión. S.A. Akbar contributed in supervisión and administration. All authors have read and agreed to the final version of the submitted manuscript.

ACKNOWLEDGEMENT

M. Iqhrammullah is a visiting researcher at Indonesian Marine and Terrestrial Bioindustry, National Research and Innovation Agency (BRIN).

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.

OPEN ACCESS

PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
BSL	Brassinolide
BRIN	National Research and Innovation Agency
Cd	Cadmium
C. vulgaris	*Chlorella vulgaris*
CI	Confidence interval
Cu	Copper
Fe	Iron
GA_3	Gibberellic acid
GeMTC	A package in R studio for network meta-analysis
ImageJ	An image analysis software
n	Number
N	Nitrogen
P	Phosphorous
Pb	Lead
ppm	Part per million
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
R-square	Correlation coefficient
SMD	Standardized mean difference
Zn	Zinc

REFERENCES

Ahmad, K.; Chiari, W., (2023). Metal oxide/chitosan composite for organic pollutants removal: A comprehensive review with bibliometric analysis. Narra X., 1(2): e91 **(16 pages)**.

Ahmad, K.; Iqhrammullah, M.; Rizki, D.R.; Aulia, A.; Mairizal, A.Q.; Purnama, A.; Qanita, I.; Abdulmadjid, S.; Puspita, K., (2022). Heavy metal contamination in aquatic and terrestrial animals resulted from anthropogenic activities in Indonesia: A review. Asian J. Water Environ. Pollut., 19(4): 1-8 **(8 pages)**.

Babuji, P.; Thirumalaisamy, S.; Duraisamy, K.; Periyasamy, G., (2023). Human health risks due to exposure to water pollution: A review. Water., 15(14): 2532.

Bajguz, A., 2011. Suppression of Chlorella vulgaris growth by cadmium, lead, and copper stress and its restoration by endogenous brassinolide. Arch. Environ. Contam. Toxicol., 60(3): 406-416 **(11 pages)**.

Carr, H.P.; Carino, F.A.; Yang, M.S.; Wong, M.H., (1998). Characterization of the cadmium-binding capacity of *Chlorella vulgaris*. Bull. Environ. Contam. Toxicol., 60(3): 433-440 **(8 pages)**.

Cheng, J.; Qiu, H.; Chang, Z.; Jiang, Z.; Yin, W., (2016). The effect of cadmium on the growth and antioxidant response for freshwater algae *Chlorella vulgaris*. SpringerPlus. 5(1): 1290 **(8 pages)**.

Chia, M.A.; Lombardi, A.T.; Melao, M.d.G.G.; Parrish, C.C., (2017). Phosphorus levels determine changes in growth and biochemical composition of *Chlorella vulgaris* during cadmium stress. J. Appl. Phycol., 29: 1883-1891 **(9 pages)**.

Chia, M.A.; Lombardi, A.T.; Melão, M.d.G.G.; Parrish, C.C., (2015). Combined nitrogen limitation and cadmium stress stimulate total carbohydrates, lipids, protein and amino acid accumulation in *Chlorella vulgaris* (Trebouxiophyceae). Aquat. Toxicol., 160: 87-95 **(9 pages)**.

Daliry, S.; Hallajisani, A.; Mohammadi Roshandeh, J.; Nouri, H.; Golzary, A., (2017). Investigation of optimal condition for *Chlorella vulgaris* microalgae growth. Global J. Environ, Sci. Manage., 3(2); 217-230 **(14 pages).**

Falkowska, M.; Pietryczuk, A.; Piotrowska, A.; Bajguz, A.; Grygoruk, A.; Czerpak, R., (2011). The effect of gibberellic acid (GA_3) on growth, metal biosorption and metabolism of the green algae *Chlorella vulgaris* (Chlorophyceae) Beijerinck exposed to cadmium and lead stress. Pol. J. Environ. Stud., 20(1): 53-59 **(9 pages)**.

Ge, J.; Jin, P.; Xie, S.; Beardall, J.; Feng, Y.; Guo, C.; Ma, Z.; Gao, G., (2024). Micro- and nanoplastics interact with conventional pollutants on microalgae: Synthesis through meta-analysis. Environ. Pollut., 342: 123127 **(14 pages)**.

Genchi, G.; Sinicropi, M.S.; Lauria, G.; Carocci, A.; Catalano, A., (2020). The effects of cadmium toxicity. Int. J. Environ. Res. Public Health., 17(11): 3782 **(24 pages)**.

Geng, W.; Xiao, X.; Zhang, L.; Ni, W.; Li, N.; Li, Y., 2022. Response and tolerance ability of *Chlorella vulgaris* to cadmium pollution stress. Environ. Technol., 43: 4391-4401 **(11 pages)**.

Han, X.; Zeng, H.; Bartocci, P.; Fantozzi, F.; Yan, Y., (2018). Phytohormones and effects on growth and metabolites of microalgae: A review. Fermentation. 4(2): 25 **(15 pages)**.

Iqhrammullah, M.; Fahrina, A.; Chiari, W.; Ahmad, K.; Fitriani, F.; Suriaini, N.; Safitri, E.; Puspita, K., (2023a). Laccase immobilization using polymeric supports for wastewater treatment: A critical review. Macromol. Chem. Phys., 224(9): 2200461 **(19 pages)**.

Iqhrammullah, M.; Rizki, D.R.; Purnama, A.; Duta, T.F.; Harapan, H.; Idroes, R.; Ginting, B., (2023b). Antiviral molecular targets of essential oils against SARS-CoV-2: A systematic review. Sci. Pharm., 91(1): 15 **(18 pages)**.

Kour, J.; Kohli, S.K.; Khanna, K.; Bakshi, P.; Sharma, P.; Singh, A.D.; Ibrahim, M.; Devi, K.; Sharma, N.; Ohri, P., (2021). Brassinosteroid signaling, crosstalk and, physiological functions in plants under heavy metal stress. Front. Plant Sci., 12: 608061 **(19 pages)**.

Lam, P.K.S.; Wut, P.F.; Chan, A.C.W.; Wu, R.S.S., (1999). Individual and combined effects of cadmium and copper on the growth response of *Chlorella vulgaris*. Environ. Toxicol.: Int. J., 14(3): 347-353 **(7 pages)**.

Li, G.; Yan, L.; Chen, X.; Lam, S.S.; Rinklebe, J.; Yu, Q.; Yang, Y.; Peng, W.; Sonne, C., (2023). Phytoremediation of cadmium from soil, air and water. Chemosphere. 320: 138058 **(9 pages)**.

Li, Z.; Fan, Y.; Tao, C.; Yan, W.; Huang, Y.; Qian, H.; Xu, Q.; Wan, T.; Chen, Y.; Qin, Y., (2022). Association between exposure to cadmium and risk of all-cause and cause-specific mortality in the general US adults: A prospective cohort study. Chemosphere. 307(4): 136060 **(8 pages)**.

Lu, W.; Xu, C.; Liu, F.; Su, M.; Cheng, S.; Zhang, Y., (2023). Antibiotic removal efficiency by microalgae: A systematic analysis combined with meta-analysis. Process Saf. Environ. Prot., 174: 912-920 **(9 pages)**.

Manikandan, A.; Suresh Babu, P.; Shyamalagowri, S.; Kamaraj, M.; Muthukumaran, P.; Aravind, J., (2022). Emerging role of microalgae in heavy metal bioremediation. J. Basic Microbiol. 62(3-4): 330-347 **(18 pages)**.

Nasir, M.; Muchlisin, Z.A.; Saiful, S.; Suhendrayatna, S.; Munira, M.; Iqhrammullah, M., (2021). Heavy metals in the water, sediment, and fish harvested from the Krueng Sabee River Aceh Province, Indonesia. J. Ecol. Eng., 22(9): 224-231 **(8 pages)**.

Nisah, K.; Muslem, M.; Ashari, T.M.; Afkar, M.; Iqhrammullah, M., (2022). Distribution of Mercury in soil, water, and vegetable fern in a former gold mining area–evidence from Nagan Raya Regency, Aceh Province, Indonesia. J. Ecol. Eng., 23(8): 30–39 **(10 pages)**.

Pratama, Y.A.; Kadir, M.Y.A.; Rivaldi, A.; Mulya, I.C.; Amirah, S.; Iqhrammullah, M., (2024). Bibliometric analysis of the impact of environmental degradation on women and the importance of women's representation. Global J. Environ. Sci. Manage., 10(3): 1–16 **(16 pages)**.

Priya, A.; Jalil, A.; Vadivel, S.; Dutta, K.; Rajendran, S.; Fujii, M.; Soto-Moscoso, M., (2022). Heavy metal remediation from wastewater using microalgae: Recent advances and future trends. Chemosphere., 305: 135375 **(11 pages)**.

Putra, M.I.; Gusti, N.; Duta, T.F.; Alina, M.; Qanita, I.; Naufal, M.A.; Henira, N.; Tsurayya, G.; Amirah, S., (2023). Vitamin D supplementation improves foot ulcers among diabetic patients: Pooled analysis of randomized controlled trials. Narra X., 1(3): e104 **(13 pages)**.

Rathnayake, I.V.N.; Megharaj, M.; Beer, M.; Naidu, R., (2021). Medium composition affects the heavy metal tolerance of microalgae: A comparison. J. Appl. Phycol., 33: 3683-3695 **(13 pages)**.

Ru, I.T.K.; Sung, Y.Y.; Jusoh, M.; Wahid, M.E.A.; Nagappan, T., (2020). *Chlorella vulgaris*: A perspective on its potential for combining high biomass with high value bioproducts. Appl. Phycol., 1(1): 2-11 **(10 pages)**.

Saberi, A.; Taherizadeh, M.; Biuki, N.A.; Fathurrahman, L.; Lavajoo, F., (2022). Mg, Sn, Cd, Zn and Fe accumulation in unicellular green alga *Chlorella vulgaris* and its effects on growth, content of photosynthetic pigments and protein. Thai J. Agric. Sci., 55(3): 135-145 **(11 pages)**.

Saputri, P.; Harahap, D.; Lubis, S.S.; Ilhami, S., (2023). Biochemical and Fe-resistant characteristics of indigene bacteria from a high iron concentration landfill in Indonesia. Narra X 1(3): e95 **(8 pages)**.

Satpati, G.G.; Pal, R., (2021). Co-Cultivation of *Leptolyngbya tenuis* (Cyanobacteria) and *Chlorella ellipsoidea* (green alga) for biodiesel production, carbon sequestration, and cadmium accumulation. Curr. Microbiol., 78, 1466-1481 **(16 pages)**.

Sembada, A.A.; Suyadi, T.A., (2023). Phycoremediation of Cadmium using *Chlorella vulgaris* in Photobioreactor. Bioeksperimen: Jurnal Penelitian Biologi. 9(1): 1-6 **(6 pages)**.

Soto-Ramírez, R.; Lobos, M.-G.; Córdova, O.; Poirrier, P.; Chamy, R., (2021). Effect of growth conditions on cell wall composition and cadmium adsorption in *Chlorella vulgaris*: A new approach to biosorption research. J. Hazard. Mater., 411: 125059 **(12 pages)**.

Su, Y., (2021). Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. Sci. Total Environ., 762, 144590 **(14 pages)**.

Talarek-Karwel, M.; Bajguz, A.; Piotrowska-Niczyporuk, A., (2020). 24-Epibrassinolide modulates primary metabolites, antioxidants, and phytochelatins in Acutodesmus obliquus exposed to lead stress. J. Appl. Phycol., 32, 263-276 **(14 pages)**.

Tian, X.; Xue, B.; Wang, B.; Lei, R.; Shan, X.; Niu, J.; Luo, B., (2022). Physical activity reduces the role of blood cadmium on depression: a cross-sectional analysis with NHANES data. Environ. Pollut., 304: 119211 **(8 pages)**.

Wagner, D.S.; Cazzaniga, C.; Steidl, M.; Dechesne, A.; Valverde-Perez, B.; Plosz, B.G., (2021). Optimal influent N-to-P ratio for stable microalgal cultivation in water treatment and nutrient recovery. Chemosphere. 262: 127939 **(11 pages)**.

Wang, L.; Liu, J.; Filipiak, M.; Mungunkhuyag, K.; Jedynak, P.; Burczyk, J.; Fu, P.; Malec, P., (2021). Fast and efficient cadmium biosorption by *Chlorella vulgaris* K-01 strain: The role of cell walls in metal sequestration. Algal Res., 60: 102497 **(13 pages)**.

Wang, R.; Sang, P.; Guo, Y.; Jin, P.; Cheng, Y.; Yu, H.; Xie, Y.; Yao, W.; Qian, H., (2023). Cadmium in food: Source, distribution and removal. Food Chem., 405: 134666 **(11 pages)**.

Wang, S.; Wufuer, R.; Duo, J.; Li, W.; Pan, X., (2022). Cadmium caused different toxicity to photosystem I and photosystem II of freshwater unicellular algae *Chlorella pyrenoidosa* (Chlorophyta). Toxics. 10(7): 352 **(17 pages)**.

Wikurendra, E.A.; Aulia, A.; Fauzi, M.L.; Fahmi, I.; Amri, I., (2023). Willingness-to-pay for urban green space: A meta-analysis of surveys across China. Narra X., 1(3): e105 **(13 pages)**.

Xi, Y.; Lai, J.; Lei, Y.; Ren, C.; Zhang, M.; Kong, W.; Jia, L., (2022). Biosorption of Cd~(2+) and Cr~(3+) by *Chlorella vulgaris* and its influencing factors. Microbiol. China. 49(1): 39-48 **(10 pages)**.

Xiao, X.; Li, W.; Jin, M.; Zhang, L.; Qin, L.; Geng, W., (2023). Responses and tolerance mechanisms of microalgae to heavy metal stress: A review. Mar. Environ. Res., 183: 105805 **(10 pages)**.

Zhang, W.; Tan, N.G.J.; Fu, B.; Li, S.F.Y., (2015). Metallomics and NMR-based metabolomics of *Chlorella* sp. reveal the synergistic role of copper and cadmium in multi-metal toxicity and oxidative stress. Metallomics. 7(3):426-438 **(13 pages)**.

Zhao, D.; Cheah, W.Y.; Lai, S.H.; Ng, E.-p.; Khoo, K.S.; Show, P.L.; Ling, T.C., (2023). Symbiosis of microalgae and bacteria consortium for heavy metal remediation in wastewater. J. Environ. Chem. Eng., 11(3): 109943 **(14 pages)**.

AUTHOR (S) BIOSKETCHES

Iqhrammullah, M., Dr., Assistant Professor, Research Center for Marine and Land Bioindustry National Research and Innovation Agency (BRIN), North Lombok 83756, Indonesia; Postgraduate Program of Public Health, Universitas Muhammadiyah Aceh, Banda Aceh 23123, Indonesia.

- Email: *m.iqhrammullah@gmail.com*
- ORCID: 0000-0001-8060-7088
- Web of Science ResearcherID: AAT-4629-2020
- Scopus Author ID: 57208859701
- Homepage: https://mkm.unmuha.ac.id/

Saudah, S., Dr., Assistant Professor, Faculty of Education and Teacher Training, Universitas Serambi Mekkah, Banda Aceh, 23245, Indonesia; Master Program of Biology, Universitas Serambi Mekkah, Banda Aceh, 23245, Indonesia.

- Email: *saudah@serambimekkah.ac.id*
- ORCID: 0000-0002-5655-6820
- Web of Science ResearcherID: NA
- Scopus Author ID: 57209409781
- Homepage: https://serambimekkah.ac.id/

Monalisa, M., M.Si., Assistant Professor, Department of Agribusiness, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; Aceh Climate Change Initiative, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.

- Email: *monalisa@usk.ac.id*
- ORCID: 0000-0003-0005-9558
- Web of Science ResearcherID: NA
- Scopus Author ID: NA
- Homepage: https://fsd.usk.ac.id/monalisa/

Fahrurrozi, F., Dr., Associate Professor, Research Center for Marine and Land Bioindustry National Research and Innovation Agency (BRIN), North Lombok 83756, Indonesia.

- Email: *fahrurrozi@brin.go.id*
- ORCID: 0000-0002-9230-0347
- Web of Science ResearcherID: NA
- Scopus Author ID: 58090585800
- Homepage: https://fsd.usk.ac.id/monalisa/

Akbar, S.A., Ph.D., Senior Lecturer, Faculty of Marine And Fisheris, Department of Aquaculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.

- Email: *saidaliakbar@usk.ac.id*
- ORCID: 0000-0003-3678-1921
- Web of Science ResearcherID: JXN-6103-2024
- Scopus Author ID: 57190374979
- Homepage: https://fsd.usk.ac.id/saidaliakbar/

Lubis, S.S., M.Si, Senior Lecturer, Faculty of Science and Technology, Biology Departement, Ar-Raniry State Islamic University, Banda Aceh 23111, Indonesia.

- Email: *syafrinasarilbs@ar-raniry.ac.id*
- ORCID: 0000-0002-8098-7604
- Web of Science ResearcherID: NA
- Scopus Author ID: 57225370238
- Homepage: https://ddt.ar-raniry.ac.id/profil/198004252014032001

HOW TO CITE THIS ARTICLE

Iqhrammullah, M.; Saudah, S.; Monalisa, M.; Fahrurrozi, F.; Akbar, S.A.; Lubis, S.S., (2024). Network meta-analysis of cadmium toxicity against Chlorella vulgaris and the role of growth stimulants and macronutrients. Global J. Environ. Sci. Manage., 10(4): 1-12.

DOI: 10.22034/gjesm.2024.04.***

URL: ***