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Removal of contaminants in a paper mill effluent by *Azolla caroliniana*

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ABSTRACT: This study was focused on removal of various parameters in paper mill effluent using a method called bioremediation by *Azolla caroliniana*. The experimental investigations have been carried out using *Azolla caroliniana* for conducting the sorption study with various dilution ratios (2, 4, 6, 8, and 10), pH (3, 4, 5, 6, 7, 8 and 9) and biomass (200, 400, 600, 800 and 1000 g). The maximum removal percentage of TDS, BOD and COD in a paper mill effluent was obtained at the optimum dilution ratio of 6, pH of 8 and biomass of 800 g. The results of this study indicated that the maximum removal percentage of TDS, BOD and COD in a paper mill effluent was 82.3 %, 88.6 % and 79.1 % respectively. Also, the study focused on uptake of TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* through bioaccumulation factor and translocation factor. The results of bioaccumulation factor revealed that TDS, BOD and COD in paper mill effluent were adsorbed by *Azolla caroliniana*. The results of translocation factor revealed that the roots of *Azolla caroliniana* translocate the TDS, BOD and COD in a paper mill effluent to the shoots of *Azolla caroliniana*. From the results, this study concluded that bioremediation by *Azolla caroliniana* could be effectively used for removing TDS, BOD and COD in a paper mill effluent. This study also suggested that *Azolla caroliniana* may be used for removing various contaminants, not only from paper mill effluent, but also from any other industrial effluents.

Keywords: *Azolla caroliniana*; Bioaccumulation factor (BAF); Paper mill effluent; Process parameters; Translocation factor (TF)

INTRODUCTION

Surface water and groundwater are contaminated due to various industrial effluents when they are not discharged properly (Sivakumar, 2011; 2013). Paper mill is one of the major industries using fresh water for paper manufacturing and produces equal quantity of highly toxic effluent. There are over a thousand paper mills in and around the world to produce paper for meeting the demand. India is the 20th largest paper producing country (Kesalkar, *et al.*, 2012). In order to produce paper, paper mills normally use raw materials like wood, cellulose materials, fibers, husk of agricultural products and more than 200 chemicals at various stages of paper manufacturing. Consequently, paper mills release highly toxic effluents in dark color (Swamy, *et al.*, 2011). Contaminants produced by paper mill manufacturing generate a considerable volume of effluents containing high concentrations of organic matter, BOD, COD, TS, TDS, TSS, phenols, sulphates, calcium, magnesium, sodium and color. The generated effluent from paper mills may not be disposed as such to any media without proper treatment, but in reality, there are no proper treatment techniques followed by most of the paper mills around the world, leading to contamination in those disposal media. Soil medium is particularly affected more than water medium, because

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the affected soil medium in turn affects the growth of plants (Vinod Kumar, *et al.*, 2015). Hence, paper mill effluents must be treated and contaminants must be contained before being discharged into the environment for their acceptable limit.

In earlier stages, pysico-chemical and biological methods have been used to reduce the contaminants in paper mill effluents, which are highly efficient and reliable for treating the effluent from paper mills. The suggested treatment methods for the removal of pollutants from various industries are adsorption (Sivakumar and Shankar, 2012, Sivakumar, *et al.*, 2014a,c,d,e; Sivakumar, 2013; Shankar, 2014b), ion exchange (Salmah, 2015), chemical precipitation (Mayra, *et al.*, 2015), electro-dialysis (Sivakumar, 2014 f and g) and electro-coagulation (Akbal and Camc. 2010; Bellebia, *et al.*, 2013), membrane separation (Fazal, *et al.*, 2015), ultra-filtration (Chen, *et al.*, 2015), reverse osmosis (Ishtiyak and Chhipa, 2015) et cetera, but they produce more chemical and biological sludge, which further imposes more cost for their disposal and treatment.

Present bioremediation methods using aquatic and terrestrial plants (Ingole and Bhole, 2003; Soltan and Rashed, 2003) are considered more favorable for treating various industrial effluents, because bioremediation methods have more advantages than any conventional methods in terms of less cost; more efficiency; reduced biological and chemical sludge production (Sivakumar, *et al.*, 2014i,k; Shankar, *et al.*, 2014j). There are several studies conducted on germination of plants stating that using raw paper mill effluents diminished the plant growth rate, whereas using diluted paper mill effluent improved the growth. (Vinod Kumar, *et al.*, 2015).

In this research work, aquatic plant grown in constructed wetland is used for removing contaminants from paper mill effluent (Türker, *et al.*, 2014, Sivakumar, *et al.*, 2014h,i). An artificial effluent treatment method consisting of shallow ponds called constructed wetland, sometimes called as container, is used to grow several plants, depending upon the treatment options like physical, chemical and biological options to treat industrial effluents. The constructed wetland used for the treatment is similar to that of ecological systems found in natural environment. In order to do the design and construction of wetlands for treating the effluent, it is important to know the fundamental concept on how wetlands work. Thus, this study was conducted to remove contaminants from paper mill effluent using a constructed wetland by aquatic macrophytes *Azolla caroliniana*. The uptake capacity of *Azolla caroliniana* was also determined through bioaccumulation and translocation factors. This study has been performed in Tamil Nadu, India in 2015.

MATERIALS AND METHODS

Collection of Azolla caroliniana

The aquatic macrophyte *Azolla caroliniana* was obtained from nearby local pond, Chennai, which had no connection with any nearby industrial effluent discharge points. The collected aquatic plants were washed with well water followed by distilled water and were transported to the constructed wetland. The collected *Azolla caroliniana* was then stabilized in a small plastic tank containing well water. This setup was kept for a period of 20 days. Later, the plastic tank was filled with 5 cm thickness of gravel and 15 cm thickness of local pond soil and was maintained at normal atmospheric conditions.

Collection of Paper Mill Effluent Sample

In this study, air tight bottles were used to collect five effluent samples from a paper mill located at Karur, Tamil Nadu, India. The air tight bottles were sterilized before collecting the samples from various locations. The five samples were taken to the Environmental Engineering Laboratory and were mixed together to make a homogenized effluent sample for analyzing the various contaminants in later stages. The analysis was carried out for determining the concentration of different parameters in a paper mill effluent per the standard procedure given in APPA, AWWA and WEF, 2005. The characteristics of paper mill effluent are presented in Table 1. The various parameters in the effluent from paper mills vary in wide range depending

Table 1: Physico-chemical characteristics of paper mill effluent

Sl. No.	Characteristics	Values
1	pH	8.4
2	EC	5.63μS/cm
3	Turbidity	752 NTU
4	TDS	2384 mg/L
5	BOD	1840 mg/L
6	COD	4560 mg/L
7	TOC	2756 mg/L
8	Phenol	156 mg/L
9	Calcium	358 mg/L
10	Chloride	542 mg/L
11	Alkalinity	356 mg/L
12	Sulphate	473 mg/L

on process details and working behaviors in the production step of industry.

Sorption Experiments

For sorption experiments, the *Azolla caroliniana*, were taken from the stabilized constructed wetland, then they were cleaned and introduced into the plastic tanks called experimental tanks. These plastic tanks are also similar to the plastic tank used for preserving the *Azolla caroliniana*. These experimental tanks were filled with paper mill effluent of 1000 ml. Triplicate of each experimental investigation was maintained. To reduce the various contaminants in the paper mill effluent, experimental investigations were carried out for a period of 7 days by one day interval using aquatic macrophyte *Azolla caroliniana*. The sorption study was conducted with various dilution ratios (2, 4, 6, 8, and 10), pH (3, 4, 5, 6, 7, 8 and 9) and biomass (200, 400, 600, 800 and 1000 g). The dilution ratio was obtained such that one part of effluent with various numbers of parts of well water, thus, the ratio of 2, 4, 6, 8, and 10 represents the corresponding numbers of parts of well water mixed with raw effluent of paper mills. The pH was adjusted by using 0.1 M of HCl and 0.1 M of NaOH. The concentrations of the various parameters in a paper mill effluent before and after treatment with *Azolla caroliniana* were determined by APPA, AWWA and WEF, 2005. The removal percentage of various parameters by *Azolla caroliniana* was calculated by using the below equation:

$$\text{Percentage Reduction: } \frac{(C_1 - C_2)}{C_1} \times 100 \quad (1)$$

Where C1 is the concentration of the parameters before treatment with *Azolla caroliniana* and C2 is the concentration of the parameters after treatment with *Azolla caroliniana*.

Bioaccumulation factor (BAF) and translocation factor (TF)

In order to validate the experiments conducted for removing TDS, BOD and COD in a paper mill effluent using *Azolla caroliniana*, bioaccumulation and translocation factors are used.

The bioaccumulation factor (BAF) is defined as the ratio of metal concentration in the roots to those in the soil or water, and is determined using Eq. (2).

$$BAF = Pplant / Pwater \quad (2)$$

Where Pplant is the concentration of parameters in the plant and Pwater is the concentration of parameters in water. BAF > 1 indicates that the plant is accumulator. The translocation factor (TF) is defined as the ratio of metal concentration in the shoots to those in the roots

$$TF = Pshoot/Proot \quad (3)$$

Where Pshoot is the concentration of parameters in the shoots of the plant to the Proot is the concentration of parameters in the roots of the plant. TF >1 indicates that the plant translocates metals effectively from the roots to the shoots.

RESULTS AND DISCUSSION

In this study, the various process parameters like dilution ratio, pH and biomass for the contact time were selected to remove TDS, BOD and COD in a paper mill effluent rather than other parameters using the constructed wetland by *Azolla caroliniana*. The selection is based on the importance of each parameter. TDS is used to indicate the total ions present in the paper mill effluent. BOD indicates the presence of organic matter present in the paper mill effluent, since paper mill effluent contains more lignin and cellulose materials. COD is used to indicate the presence of inorganic matter, since more chemicals are used for paper making at various levels in paper mills.

Effect of Dilution Ratio

Fig.1 indicates effect of dilution ratio on removal of TDS, BOD and COD from paper mill effluent. The experimental investigations were conducted against different dilution ratios from 2 to 10 (effluent 1: well water 2) with an increment of 2 by *Azolla caroliniana*. The percentage reduction of TDS, BOD and COD in paper mill effluent using *Azolla caroliniana* against the different dilution ratios with the contact time of 6 days, biomass of 200 g, and pH of 5 is presented in Fig. 1. Fig. 1 represents the results of TDS, BOD and COD on day 6, because the sixth day is the optimum contact time for which maximum removal is obtained for the parameters TDS, BOD and COD in a paper mill effluent. Hence, the results of removal percentage of TDS, BOD and COD in a paper mill effluent are not presented on the day 1, 2, 3, 4, 5 and 7.

The results of this study indicated that the removal percentage for the parameters TDS, BOD and COD in a paper mill effluent is low at the beginning of the

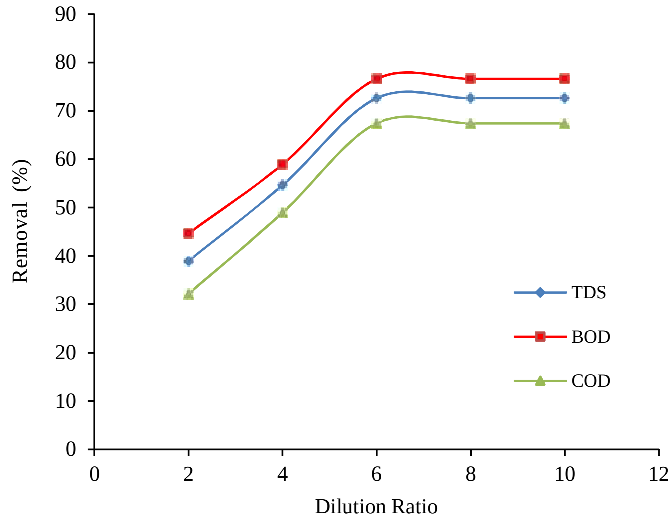

Fig. 1: Effect of dilution ratio on removal of TDS, BOD and COD from paper mill effluent

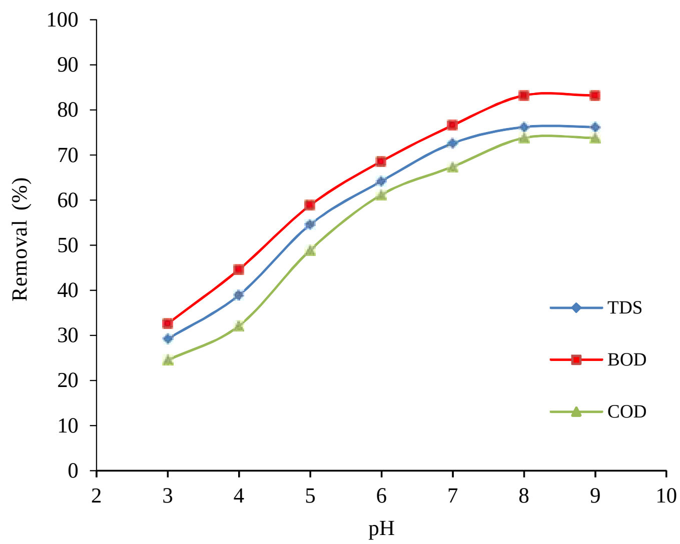

Fig. 2: Effect of pH on Removal of TDS, BOD and COD from paper mill effluent

experiment and high for increased dilution ratio. This is because, for the diluted concentration, TDS, BOD and COD in a paper mill effluent were sorbed easily by *Azolla caroliniana* than high concentration paper mill effluent. In other words, the active sites in *Azolla caroliniana* could not sorb the TDS, BOD and COD in a paper mill effluent since there is a very strong bondage between the various parameters in paper mill effluent at an elevated concentration and in later stage sorbent sites of *Azolla caroliniana* can be utilized effectively. Up to a dilution ratio of 6, the sorption of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* increased steadily. For dilution ratios of 8 and 10, the percentage removal of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* showed resembles of results obtained for a dilution ratio 6. Even sufficient contact time 7 days and sufficient dilution ratio of 8 and 10 are available, the various parameters TDS, BOD and COD in a paper mill effluent were sorbed completely on the active sites of *Azolla caroliniana* for the dilution ratio 6, and hence, there was no difference in sorption on dilution ratio 8 and 10.

Similarly, the sorption of TDS, BOD and COD in a paper mill effluent on day 7 was similar to that of day 6, indicating that the maximum removal percentage of TDS, BOD and COD in a paper mill effluent was completed on day 6 itself. Hence, an optimum dilution ratio against the maximum removal of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* is found to be 6. Further, the maximum sorption removal percentage of TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* against dilution ratio of 6 was found to be 72.6 %, 76.6 % and 67.4 % respectively (Fig. 1).

Effect of pH

Fig. 2 represents the effect of pH on removal of TDS, BOD and COD from paper mill effluent. The effect of pH was done by varying pH value from 3 to 9 with an increment of 1 by *Azolla caroliniana* for removing TDS, BOD and COD in a paper mill effluent. The percentage reduction of TDS, BOD and COD in a paper mill effluent using *Azolla caroliniana* for the pH with a contact time of 6 days, biomass of 200 g, and an optimum dilution ratio of 6 is presented in Fig. 2. The reduction percentage of TDS, BOD and COD from a paper mill effluent using *Azolla caroliniana* is presented in Fig. 2 for the day 6; since on day 6, maximum removal of TDS, BOD and COD in a paper mill effluent was achived by *Azolla caroliniana*. The results of the percentage removal of TDS, BOD and COD in a paper mill effluent indicated that the removal was low at the beginning and high with increased pH. This increased pH indicats that the effluent is in a slight alkaline to alkaline condition and as a results, the TDS, BOD and COD in a paper mill effluent is coupled with various cations and anions present in a paper mill effluent. Thus, TDS, BOD and COD can be easily adsorbed by *Azolla caroliniana* than in acidic nature. Up to the pH of 8, the absorption of TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* increased continuously and for pH 9, the results of the removal percentage showed that the results of pH 9 and pH 8 are similar to each other. Hence, the optimum pH found in this study for the maximum removal of TDS, BOD and COD in paper mill effluent is 8.

The sorption of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* on day 7 and for pH 9, the removal percentage of TDS, BOD and COD in paper mill effluent was not determinant even the contact time and pH were higher, the sorption was completed for the contact time day 6 and for pH 8, leads to low specific uptake for the pH of 9 and for the contact time of 7 days. Thus, the maximum sorption percentage removal of TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* against the optimum pH of 8 was found to be 76.2 %, 83.2 % and 73.8 % respectively (Fig. 2).

Effect of Azolla caroliniana biomass

Fig. 3 indicates the effect of *Azolla caroliniana* *b*iomass on removal of TDS, BOD and COD from paper mill effluent. The ability of *Azolla caroliniana b*iomass for removing TDS, BOD and COD in paper mill effluent was done by varying the *Azolla caroliniana* biomass

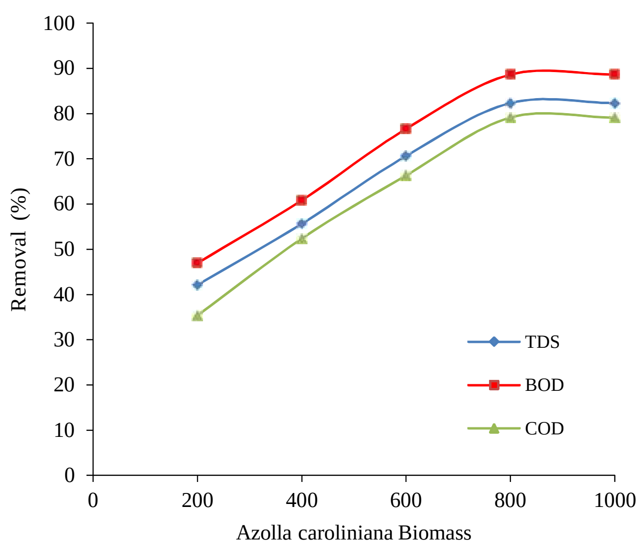

Fig. 3: Effect of *Azolla caroliniana* biomass on removal of TDS, BOD and COD from paper mill effluent

Table 2. Maximum removal of TDS, BOD and COD from paper mill effluent by *Azolla caroliniana*

Type of Solution	Parameters	$P_{Water,}$ mg/L	$P_{plant,}$ mg/Kg	$P_{shoot,}$ mg/Kg	$P_{root,}$ mg/Kg
Paper mill effluent	TDS	421.97	1962.03	1777.60	184.43
	BOD	209.76	1630.24	1509.60	120.64
	COD	953.04	3606.96	3148.88	458.08

Table 3: BAF and TF for the parameters TDS, BOD and COD from paper mill effluent by *Azolla caroliniana*

Type of Solution	Parameters	BAF	TF
Paper mill effluent	TDS	4.65	9.64
	BOD	7.77	12.51
	COD	3.78	6.87

from 200 g to 1000 g with 200 g incremental level. The percentage reduction of TDS, BOD and COD in paper mill effluent using *Azolla caroliniana* against biomass with a contact time of 6 days, optimum dilution ratio of 6, and optimum pH of 8. As similar to the effect of pH and dilution ratio, the maximum removal for the effect of biomass also obtained on day 6 and hence, the results on day 1, 2, 3, 4, 5 and 7 were not presented.

The results indicated that removal percentage of TDS, BOD and COD in paper mill effluent is low by *Azolla caroliniana* at the low biomass and then increases with increased biomass. This is because the supplied low biomass is completely utilized for the removal of TDS, BOD and COD in a paper mill effluent, but still there are some portions of TDS, BOD and COD in paper mill effluent available against the less biomass condition which may be adsorbed by supplying higher *Azolla caroliniana* biomass. Up to biomass of 800 g, the sorption of TDS, BOD and COD in paper mill effluent increased steadily and for the biomass of 1000 g, the removal percentage for the parameters TDS, BOD and COD in a paper mill effluent showed the same results as that of results obtained for the biomass of 800 g. It is because, maximum removal percentage could be achieved for the biomass of 800 g and hence, there was no change in difference of removal percentage for the *Azolla caroliniana* biomass of 1000 g.

Similarly, there was no difference in removal of TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* for the day 7. Thus, an optimum biomass found in this study is 800g for removing TDS, BOD and COD in paper mill effluent by *Azolla caroliniana* at maximum level. Further, the maximum sorption percentage of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* against the biomass of 800 g was found to be 82.3 %, 88.6 % and 79.1 % respectively. As demonstrated in Figs. 1 to 3, it may be observed that the order of maximum percentage removal of TDS, BOD and COD in paper mill effluent is BOD > TDS > COD for all selected process parameters of dilution ratio, pH and biomass.

Verification of Experiment

In order to verify the experimental results for the reduction of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana*, the BAF and TF were determined. The Maximum mass removal of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana*, is represented in Table 2. From Table 2, it is noted that the maximum mass removal of TDS, BOD and COD in a paper mill effluent per unit Kg biomass of *Azolla caroliniana* is 1962.03, 1630.24 and 3606.96 mg/Kg respectively against the optimum contact time of 6 days, optimum dilution ratio of 6, optimum pH of 8 and optimum biomass of 800 g.

Similarly, the BAF and TF for the parameters TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* are presented in Table 3. From the Table 3, it may observed that the bioaccumulation factor of *Azolla caroliniana* for removing TDS, BOD and COD in a paper mill effluent is 4.65, 7.77 and 3.78 respectively and the translocation factor of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* is 9.64, 12.51 and 6.87 respectively. The BAF value is greater than 1, indicating that the TDS, BOD and COD in a paper mill effluent is accumulated into the *Azolla caroliniana*. The TF value is greater than 1, indicating that there was TDS, BOD and COD movement from the root to the shoot by *Azolla caroliniana* in a paper mill effluent.

Based on the experimental results and the BAF and TF results, the study found that *Azolla caroliniana* is used as sorbent for removing TDS, BOD and COD in a paper mill effluent.

CONCLUSION

The ability of *Azolla caroliniana* for removing TDS, BOD and COD in a paper mill effluent was studied with various dilution ratio, biomass and pH against the optimum contact time of 6 days. The maximum reduction percentage of TDS, BOD and COD in a paper mill effluent by *Azolla caroliniana* was obtained at an optimum dilution ratio of 6, biomass of 800 g and pH of 8. The results of this study indicated that the maximum percentage removal in a paper mill effluent by *Azolla caroliniana* was found to be 82.3 %, 88.6 % and 79.1 % for the parameters TDS, BOD and COD respectively. The results of bioaccumulation factor revealed that TDS, BOD and COD in a paper mill effluent are adsorbed by *Azolla caroliniana* and the results of translocation factor revealed that the root of *Azolla caroliniana* translocate Zn to the shoots of *Azolla caroliniana*. This study concluded that the aquatic macrophyte *Azolla caroliniana* might be used as a sorbents for removing TDS, BOD and COD along with other parameters in paper mill effluent. Further, this study may extend to remove any pollutants not only from paper mill effluent, but also from any industrial effluents.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this manuscript.

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