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Research Article

BIOREMEDIATION OF PAPER AND PULP MILL EFFLUENT WITH THE HELP OF *PHRAGMITES KARKA AND POTAMOGETON PECTINATUS*

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ABSTRACT

Paper mill effluents were collected and analyzed for physic-chemical characteristics. The toxicity of effluents on chlorophyll-a content and biomass of *Phragmites karka* and *Potamogeton pectinatus* were determined by exposing these to different concentrations of paper mill effluents at different intervals. Chlorophyll-a content and biomass of both the macrophytes decreased when exposed to the paper mill effluent. Extent of reduction was in proportion to the concentration of effluent and duration of exposure. The metal (Cu, Fe, Zn and Mn) content of effluent decreased with exposure to the macrophytes for 168 h. reduction in metal contents of the effluent is probably due to uptake of these metals by the aquatic macrophytes.

INTRODUCTION

Pulp and paper industry has generated great concern about the hazardous pollutants continuously released into water bodies (Singh *et al.*, 1996), though its growth is an index of social, cultural, technical, industrial and economic development of a nation. The global population uses approximately more than 300 million tons of paper and its products per annum and the estimates show that the figure is likely to increase in the coming years. India ranks 20th among the paper producing countries of the world. A typical paper mill depending on the process consumes between 60,000 to 100,000 gallons of water per day, more than 75% of which is discharges into nearby field, rivers and other environmental components. Besides this, the daily pollution load contributed by Indian pulp and paper industries has been estimated to be equivalent to that generated by 7.12 million people. The large pulp and paper mills equipped with soda recovery, discharge about 270 to 450 liters effluent per Kg paper containing 40-50 g lignin per Kg bleached paper produced, whilst, the small paper mills without soda recovery discharge nearly 300 to 400 liters of black liquor effluent containing 200 to 250 g lignin per Kg of paper manufactured. (Nikhileswar, 1992; Chakravarthi *et al.*, 1995; Garg and Modi, 1999; Singhal *et al.*, 2005).

Paper industry plays a significant role in economic development of a nation. It discharges effluent with high organic and inorganic pollution (Chakravarthi *et al.*, 1996). Owing to high biological oxygen demand (BOD) and chemical oxygen demand (COD) values, it becomes a major source of pollution of the water resources. Improperly treated effluents disturb the natural equilibrium of aquatic ecosystem and poses threat to aquatic flora and fauna. Aquatic macrophytes have a potential to purify waste water and were effective in removing heavy metals (Nasu *et al.*, 1984; Wolverton, 1987; Brix and Schierup, 1989; Rai *et al.*, 1995). Attempts have been made to study the effect of environmental factors and other variables on chlorophyll content and biomass of some aquatic macrophytes (Billore and Mall, 1975; Pipeva, 1987; Nirmal Kumar, 1994; Srivastava and Pandey, 1998).

The present study was designed to determine the impact of paper mill effluents on the chlorophyll-a content and biomass of *Phragmites karka* and *Potamogeton pectinatus.*

MATERIAL AND METHODS

Phragmites karka and *Potamogeton pectinatus* were collected from natural ponds near the university campus, Faizabad. The stock was maintained in the aquarium under laboratory conditions. Same sized young and healthy specimens of the

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macrophytes were selected for this experiment. *P. karka* and *P. pectinatus* were acclimatized with 16 h/day white fluorescent light (115µ mole/m2/s, intensity) at 28±2.0^{0}C in 5% Hoagland's solution for three weeks.

The paper mill effluents were collected from the main discharge outlet of the Yash paper mill, Faizabad. The physic-chemical characteristics of effluents were analyzed by the standard methods (APHA, 1995).

Four concentrations (25%, 50%, 75% and 100%) of the effluent were used for treatment. The control was maintained separately. The plants were harvested at 24, 72, 120 and 168 h of exposure washed with deionized water. Chlorophyll-a was estimated by the method of Arnon (1999). Biomass was determined on dry weight basis by drying the freshly harvested plants in an oven at 105^{0}C for 24h. Chlorophyll-a was expressed as mg/g fresh weight (fw). The effluent before and exposure (168 h) was digested with HNO_3:$HClO_4$ (3:1 v/v). Metal (Cu, Fe, Zn and Mn) content was estimated using Perkin Elmer 2380 Atomic Absorption Spectrophotometer. Each experiment was replicated three times and expressed as mean ± SEM. Each experiment was laid out in a completely randomized design (CRD). Data were subjected to analysis of variance.

RESULT AND DISCUSSION

Physic-chemical analysis of the paper mill effluents revealed that the effluent samples were dark brown with pungent smell and pH 7.68.

The effluent had Cu (0.367 µg/ml), Fe (5.310 µg/ml), Zn (1.084 µg/ml), Mn (0.912 µg/ml), high value of BOD (1612 ppm), COD (3364 ppm) and total dissolved solids (2130 ppm). Table 1 shows decrease metal content of the effluent with exposure to *Phragmites karka* and *Potamogeton pectinatus* for 168 h.

Both the variable (*i.e.*, concentration of effluent and duration of exposure time) resulted in a significant reduction in chlorophyll-a content and biomass of both the macrophytes (Table 2 and 3).

Minimum chlorophyll content was 0.228 mg/g fw and 0.169 mg/g fw at 100% concentration of the effluent with 168 h exposure, while the maximum values of 0.619 mg/g fw and 0.287 mg/g fw were recorded in untreated control at 24 h of exposure in *Phragmites karka* and *Potamogeton pectinatus*, respectively (Table 2 and 3).

The maximum inhibition of chlorophyll was 55.47% and 34.75% in 100% concentration at 168 h of exposure. Reduction in biomass was 9.50% and 12.04% at 100% for 168 h exposure in *Phragmites karka* and *Potamogeton pectinatus*, respectively. The decrease in chlorophyll-a content and biomass are comparable to the results of other workers (Tripathi *et al.*, 1990; Nirmal Kumar and Rana, 1994; Chaturvedi *et al.*, 1995). Tables 2 and 3 reveal that inhibition of chlorophyll content was 2.26% and 20.68% at 25% and 100% effluent concentration, respectively at 24 h in *Phragmites karka* while the inhibition was 4.18% and 12.54% in *Potamogeton pectinatus* at the same effluent concentrations and duration of exposure.

Table 1 Metal contents (µg/ml) in paper mill effluent following exposure of *Phragmites karka* and *Potamogeton pectinatus* at 168h

% conc. of effluent	*Phragmites karka*				*Potamogeton pectinatus*			
	Cu	Fe	Zn	Mn	Cu	Fe	Zn	Mn
25	0.046 ± 0.003	1.013 ± 0.05	0.258 ± 0.005	0.173 ± 0.01	0.074 ± 0.005	1.212 ± 0.07	0.271 ± 0.03	0.203 ± 0.005
50	0.095 ± 0.003	1.572 ± 0.07	0.497 ± 0.006	0.379 ± 0.02	0.102 ± 0.006	2.031 ± 0.09	0.581 ± 0.05	0.291 ± 0.005
75	0.141 ± 0.005	2.512 ± 0.07	0.713 ± 0.005	0.525 ± 0.04	0.147 ± 0.008	3.454 ± 0.15	0.812 ± 0.08	0.426 ± 0.007
100	0.173 ± 0.004	3.801 ± 0.08	0.878 ± 0.007	0.627 ± 0.04	0.163 ± 0.009	3.871 ± 0.17	0.941 ± 0.08	0.557 ± 0.008

Initially, the 100% paper mill effluent without exposure of the aquatic macrophytes, had 0.367 µg/ml Cu, 5.310 µg/ml Fe, 1.084 µg/ml Zn, 0.912 µg/ml Mn, TDS 2130 ppm, BOD 1612 ppm and COD 3364 ppm. Table showing metal contents; µg/ml (mean ± SEM) in different concentrations of the effluent exposed to macrophytes.

Table 2 Alterations in *Phragmites karka* chlorophyll (mg/g fw) and biomass (g dw) following exposure to paper mill effluents.

% Conc	Duration in hours							
	24		72		120		168	
	Chlorophyll	Biomass	Chlorophyll	Biomass	Chlorophyll	Biomass	Chlorophyll	Biomass
Control*	0.619 ± 0.002 (100)	0.912 ± 0.003 (100)	0.571 ± 0.001 (100)	0.890 ± 0.004 (100)	0.564 ± 0.003 (100)	0.878 ± 0.005 (100)	0.512 ± 0.003 (100)	0.863 ± 0.006 (100)
25	0.605 ± 0.002 (2.26)	0.906 ± 0.003 (0.66)	0.557 ± 0.002 (2.45)	0.881 ± 0.005 (1.01)	0.549 ± 0.006 (2.66)	0.849 ± 0.006 (3.30)	0.493 ± 0.003 (3.71)	0.836 ± 0.006 (3.13)
50	0.567 ± 0.002 (8.40)	0.895 ± 0.004 (1.86)	0.518 ± 0.003 (9.28)	0.865 ± 0.004 (2.81)	0.482 ± 0.004 (14.54)	0.844 ± 0.005 (3.87)	0.411 ± 0.003 (19.73)	0.809 ± 0.005 (6.26)
75	0.502 ± 0.003 (18.90)	0.889 ± 0.005 (2.52)	0.449 ± 0.004 (21.37)	0.843 ± 0.006 (5.28)	0.384 ± 0.005 (31.91)	0.811 ± 0.007 (7.63)	0.268 ± 0.004 (47.66)	0.788 ± 0.007 (8.69)
100	0.491 ± 0.003 (20.68)	0.873 ± 0.004 (4.28)	0.405 ± 0.006 (29.07)	0.834 ± 0.005 (6.29)	0.289 ± 0.003 (48.76)	0.796 ± 0.007 (9.34)	0.228 ± 0.005 (55.47)	0.781 ± 0.007 (9.50)

Table showing chlorophyll-a content and biomass (mean ± SEM of three replicates) following exposure to varying durations and different concentrations of the effluent. Values in parentheses indicate % inhibition of chlorophyll-a content and biomass with untreated control taken as 100%.
*(p<0.01) significant when analysis of variance was applied to see whether change in duration and concentration significantly altered chlorophyll-a content and biomass, respectively.

Table 3 Alterations in *Potamogeton pectinatus* chlorophyll (mg/g fw) and biomass (g dw) following exposure to paper mill effluents

% Conc	Duration in hours							
	24		72		120		168	
	Chlorophyll	Biomass	Chlorophyll	Biomass	Chlorophyll	Biomass	Chlorophyll	Biomass
Control*	0.287 ± 0.006 (100)	0.690 ± 0.007 (100)	0.283 ± 0.004 (100)	0.681 ± 0.005 (100)	0.267 ± 0.003 (100)	0.673 ± 0.005 (100)	0.259 ± 0.004 (100)	0.681 ± 0.005 (100)
25	0.275 ± 0.004 (4.18)	0.684 ± 0.008 (0.87)	0.258 ± 0.005 (8.83)	0.668 ± 0.007 (1.91)	0.243 ± 0.001 (8.99)	0.651 ± 0.006 (3.27)	0.232 ± 0.003 (10.42)	0.643 ± 0.003 (5.58)
50	0.267 ± 0.004 (6.97)	0.680 ± 0.007 (1.45)	0.249 ± 0.005 (12.01)	0.653 ± 0.007 (4.11)	0.231 ± 0.003 (13.48)	0.631 ± 0.005 (6.24)	0.219 ± 0.001 (15.44)	0.623 ± 0.005 (8.52)
75	0.256 ± 0.006 (10.80)	0.671 ± 0.006 (2.75)	0.239 ± 0.007 (15.55)	0.642 ± 0.004 (5.73)	0.215 ± 0.002 (19.48)	0.617 ± 0.005 (8.32)	0.201 ± 0.001 (22.34)	0.614 ± 0.004 (9.84)
100	0.251 ± 0.005 (12.54)	0.662 ± 0.006 (4.06)	0.220 ± 0.007 (22.26)	0.621 ± 0.005 (8.81)	0.197 ± 0.002 (26.22)	0.598 ± 0.004 (11.14)	0.169 ± 0.002 (34.75)	0.599 ± 0.004 (12.04)

Table showing chlorophyll-a content and biomass (mean ± SEM of three replicates) following exposure to varying durations and different concentrations of the effluent. Values in parentheses indicate % inhibition of chlorophyll-a content and biomass with untreated control taken as 100%.
*($p<0.01$) significant when analysis of variance was applied to see whether change in duration and concentration significantly altered chlorophyll-a content and biomass, respectively.

The metal contents (Cu, Fe, Zn and Mn) present in the effluent samples were beyond the maximum permissible limits (WHO, 2000). A significant decrease in chlorophyll-a content and biomass of both the macrophytes may be due to absorption of above metals as well as the influence of abiotic environmental factors on the growth and development of the species. Similar results has also been reported by Baszynski *et al.*, 1981; Wong and Chang, 1991; Rai *et al.*, 1995. Inhibition of chlorophyll may be due to the effluent induced inhibition of electron transfer mechanism in photosystem-II (Srivastava and Pandey, 1999). Both the aquatic macrophytes are also sensitive to paper mill effluents and the results are comparable with those of Sinha *et al.*, 1993. The loss in biomass may be associated with the decrease in chlorophyll contents (Jeyachandran *et al.*, 1994). The results indicate that the chlorophyll-a contents and biomass of *Phragmites karka* and *Potamogeton pectinatus* are reduced by the paper mill effluents. The reduction in metal concentrations shows that these macrophytes might be able to absorb the metals from the effluent.

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