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Metal Accumulation and Ecophysiological Effects of Distillery Effluent on *Potamogeton pectinatus* L.

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With an advent of time and increased industrialization the whole world is getting worried about pollution problems day by day, which is posing concerns to civilized living. Among the liquid industrial waste sources, distillery waste poses a serious problem to our environment. There are about 285 distillery units in India producing 2.7 billion litres of alcohol and generating 40 billion litres of wastewater annually (CPCB, 1995). The waste water emanating from distilleries contains metals which could be toxic both to the flora and fauna (Srivastva et al. 2000). This has been mainly due to the nature of raw material and the processes involved in distilling alcohol. To check the environmental menaces created by such huge amount of distillery waste, there have been different treatment methods which are based on physico-chemical separation of the pollutants. Since these treatment systems are energy expensive and cost intensive, there is an urgent need of some biological treatment system, which can replace these traditional systems of treatment. Biological treatment of industrial water through aquatic macrophytes has emerged as simple, cost effective and self-sustaining alternative to the traditional method of treatment. Aquatic macrophytes have potential to purify wastewater and are effective in removing heavy metals (Nasu et al. 1983; Wolverton et al. 1975; Brix and Schierup 1989; Rai et al. 1995; Srivastava and Pandey 1998).

Aquatic plants posses tremendous potential to reduce the level of toxic metals, nutrients, BOD and to improve other physico-chemical characteristics of wastewater. However, use of these plants in designing a low cost treatment system is still at the experimental stage and is considered to be a potentially important area of environmental management. Attempts have been made to study the effect of environmental factors on chlorophyll content and biomass of some aquatic macrophytes (McGrath 1982; Guilizzoni 1991; Satyakala and Jamil 1992; Tripathi et al. 1995; Vajpayee et al. 2000).

Some industrial effluents contain metals, which may be toxic to biota. The plant *Potamogeton pectinatus* has been found to accumulate cadmium and zinc and showed tolerance / toxic response to these phytotoxic metals (Rai et al. 2003; Tripathi et al. 2003). Therefore, it was considered worthwhile to study responses and bioremediation potential of *P. pectinatus* in distillery effluent. In view of the above, this study was undertaken to assess the ability of aquatic macrophyte *P. pectinatus* L. to bioaccumulate metals (Fe, Cu, Zn, Mn) from distillery effluent and related toxicity therein.

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MATERIALS AND METHODS

The plant material *P. pectinatus* L. was collected from natural ponds at National Botanical Research Institute, Lucknow, India. The stock was maintained in the hydroponic culture under laboratory conditions. Auto ecological characteristics and biomass production of *P. pectinatus*, have been the object of several ecological studies (Van Wijk 1989). Growing plants were cultured in 250 ml flasks containing 200 ml of 5% Hoagland's solution for six weeks under laboratory conditions. Plants were provided with light intensity of 115 μmol $m^{-2}s^{-1}$ for 14 h/10 h/d at 25±2°C. Three sets of healthy plants (20 g FW in each set) were maintained in glass beakers (500 ml capacity) containing 300 ml of effluent. Equal fresh weight of plants (10 plants of average 8 cm length) were released in beakers containing different concentrations of effluent. Simultaneously control sets were also maintained using tap water.

The effluent samples were collected in acid-washed plastic containers from the main discharge outlets of distillery unit of M/S K.M. Sugar Mills, Masodha, Faizabad, U.P., India. Physico-chemical parameters of effluent were determined by standard methods of APHA (1989). For metal analysis the effluent was acidified and stored in acid washed plastic containers. Effluent and plant material were digested with HNO_3 and $HClO_4$ (3:1, ratio) at 80°C. The solution was filtered through Whatman filter paper No. 44 in a volumetric flask, by adding double distilled water and final volume was made to 50 ml for metal estimation. The concentration of metals (Fe, Cu, Zn, Mn) was estimated by Perkin Elmer Atomic Absorption Spectrophotometer (AAS-2380). For the treatment 25%, 50% and 100%, effluent concentration were made with tap water and treated with equal biomass of the plant. The control was maintained separately in tap water. The plants were harvested at 7 and 14 d of exposure, and washed with distilled water. Neither the nutrient solution nor the effluent were renewed during the 14 d treatment period. Treated and untreated plant material were crushed in 5 ml of (80%, v/v) chilled acetone and the extract was centrifuged at 10,000 g for 10 min. Chlorophyll content was estimated by the method of Arnon (1949) and Machlachlan and Zalic (1963). Biomass was determined on dry weight basis by drying the freshly harvested plants in an oven at 80°C for 24 h. Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Each experiment was carried out in triplicate and repeated twice. To confirm the variability of data and validity of results all the data were subjected to an analysis of variance (ANOVA), and Duncan's Multiple Range Test (DMRT) to determine which concentration is significantly different from the control (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Physico-chemical analysis revealed characteristic of the distillery effluent. The effluent was dark brown colour having high BOD (756.66 μg ml^{-1}) and COD (2097.67μg ml^{-1}). It was slightly alkaline pH (7.4) with high sulphate content (1712.33μg ml^{-1}). The effluent was contaminated with high concentration of toxic metals like Fe, Cu, Zn, Mn i.e., 7.61, 1.13, 0.76 and 0.44 μg ml^{-1}, respectively (Table 1).

The uptake of metals (Fe, Cu, Zn and Mn) following exposure of *P. pectinatus* was studied after 7 and 14 d and data are presented in Fig. 1(A-D). The results presented in Fig. 1(A-D) shows a concentration and duration dependent accumulation of Fe in plant tissues. Maximum accumulation of Fe was recorded 689.03 μg g^{-1} DW in the plants

Table 1. Physico-chemical characteristics of distillery effluent collected from K.M. Sugar Mills Distillery Unit, Masodha, Faizabad.

Parameter	Value
Colour	Dark brown
Odour	Mollasses like
Colour intensity	8000 unit
pH	7.4±0.05
BOD	756.66±6.34
COD	2097.6±21.6
TDS	53.34±0.67
Sulphate	1712.3±2.5
Metals:	
Fe	7.61±0.13
Cu	1.13±0.04
Zn	0.76±0.03
Mn	0.44±0.005

All values are mean ±SE (n=3).
All values are in μg ml^{-1} except for those otherwise mentioned.

growing in 100% effluent after 14 d of treatment. Plants growing in tap water also showed negligible amount of Fe, which could be due to either the contamination of tap water with Fe or Fe being a constituent of various cell components. The plants growing in 25% and 50% dilution of the effluent also accumulated significant amount of Cu, Zn, Mn at 7 days which increased up to 14 d of treatment ($P<0.05$).

The metal contaminated distillery effluent had differential effect on biomass of *P. pectinatus*. The effect of different dilution of distillery effluent on dry matter production of *P. pectinatus* at different duration is shown in Table 2. By increasing the concentration of effluent in the tap water, there was a gradual reduction in biomass of plant, which was significantly affected after 14 d of treatment (DMRT $p>0.05$).

Table 2 shows the effect of different dilution of distillery effluent on chlorophyll a, b and total chlorophyll content of *P. pectinatus* at different duration. Chlorophyll a and b content following exposure of 100% effluent for 14 d were found to be 1.044 mg g^{-1} FW and 0.112 mg^{-1} FW as compared to control 2.57 mg g^{-1} FW and 0.74 mg g^{-1} FW, respectively. The effects are due to three major processes viz., (1) oxidative breakdown of chlorophyll and carotenoid (2) oxidative damage of proteins and membrane structure and (3) substitution of metal cofactors. Further, the metals have been reported to inhibit the final reduction stage in chlorophyll formation by interacting with functional -SH group of the enzyme synthesizing chlorophyll (Prasad 1995). In the present study photosynthetic pigments (chlorophyll a, b and total chlorophyll) were negatively correlated with the concentration of distillery effluent. By increasing the concentration of distillery effluent, the chlorophyll content declined in a concentration and duration dependent manner. The photosynthetic pigment degradation has routinely been observed as a response of plants exposed to various metals (Stobart et al. 1985; Rai et al. 1995; Galbego et al. 1996; Vajpayee et al. 2000). The resulting decrease in pigments causes a deficiency in light harvesting capacity (Ouzounidou 1996) and consequently decreases photosynthetic activity of the cell. The results show that these metals are potent inhibitors of the biosynthesis of chlorophyll. The major sites of

Table 2. Effect of distillery effluent on biomass and some physiological parameters of *P. pectinatus*.

Effluent concentration (%)	Biomass (g DW)		Physiological properties (mg g^{-1} FW)							
			Chlorophyll a		Chlorophyll b		Total chlorophyll		Protein	
	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
Control	2.66^{a} ±0.13	3.56^{a} ±0.15	2.33^{a} ±0.18	2.57^{a} ±0.16	0.72^{a} ±0.05	0.74^{a} ±0.03	2.99^{a} ±0.15	3.31^{a} ±0.15	11.89^{a} ±0.57	12.11^{NS} ±0.57
25	2.58^{ab} ±0.14	2.44^{ab} ±0.08	1.89^{ab} ±0.26	1.84^{ab} ±0.12	0.69^{ab} ±0.03	0.72^{ab} ±0.06	2.53^{ab} ±0.10	2.23^{a} ±0.30	14.70^{ab} ±0.49	17.03^{NS} ±0.88
50	2.57^{abc} ±0.26	2.16^{abc} ±0.63	1.62^{abc} ±0.10	1.51^{bc} ±0.17	0.53^{abc} ±0.04	0.39^{abc} ±0.02	2.17^{abc} ±0.03	1.74^{b} ±0.13	17.23^{b} ±0.72	22.62^{NS} ±1.12
100	2.32^{abc} ±0.02	2.08^{abc} ±0.14	1.16^{bc} ±0.15	1.04^{bc} ±0.06	0.29^{c} ±0.02	0.11^{bc} ±0.01	1.43^{bc} ±0.06	1.15^{NS} ±0.10	8.04^{NS} ±0.74	5.78^{NS} ±0.45

All values are mean ±S.E. (n=3). ANOVA ($p<0.05$), Identical superscript on values denoted significant difference ($p>0.05$) between mean of different treatment, while NS=non significant according to Duncan's Multiple Range Test (DMRT).

inhibition are (i) in the formation of the proteolytic PC halide reductase complex and (ii) the synthesis of ALA, the first characteristic precursor of the porphyrins.

The effect of different dilutions of distillery effluent on protein content of *P. pectinatus* at different treatment duration is shown in Table 2. Results presented in the table showed a differential effect of different concentration of the effluent on protein content of the plant. While an increase was found with regard to protein content of the plants up to 50% dilution of the effluent at both the treatment duration, it reduced drastically in 100% distillery effluent. Such an effect on the protein content of *P. pectinatus* could be ascribed to the synthesis of new stress protein or metallothioneins at lower concentration and their subsequent degradation at higher effluent concentration (Tripathi et al. 2003). However, higher concentrations of distillery effluent (100%) had a toxic effect on the protein content of *P. pectinatus*. A decrease in protein content in the presence of metal contaminated distillery waste may be due to the breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes which were activated and destroyed the protein. The metal accumulation potential by *P. pectinatus* was more from the concentrated medium, which showed high amelioration potential of metals (Fe, Cu, Zn, Mn) by accumulating it in its tissue. The decline of protein content under heavy metal stress in aquatic plants was reported (Jana and Chaudhuri 1982; Mazhoudi et al. 1997; Chaoui et al. 1997). Such inhibitory effects could be ascribed to cumulative effects of depleting oxygen, high sulphate contents, increased microbial activities and high metal content in the effluent.

Since plants of *P. pectinatus* were tolerant to grow in raw distillery effluent and accumulated metals in their tissues, it appears to be a promising likely candidate for treatment of distillery effluent. The submerged rooted macrophytes are of great potential in developing phytoremediation strategies of metal polluted water bodies or polluted waste, as they do not migrate. These attain equilibrium with their surroundings with in a short period (Guilizzoni 1991; Rai et al. 1995). *P. pectinatus* accumulated metals in the order Fe>Mn>Cu>Zn which agrees with the findings of earlier studies on aquatic plants (Baudo et al. 1985; Smith et al. 1989). It is apparent

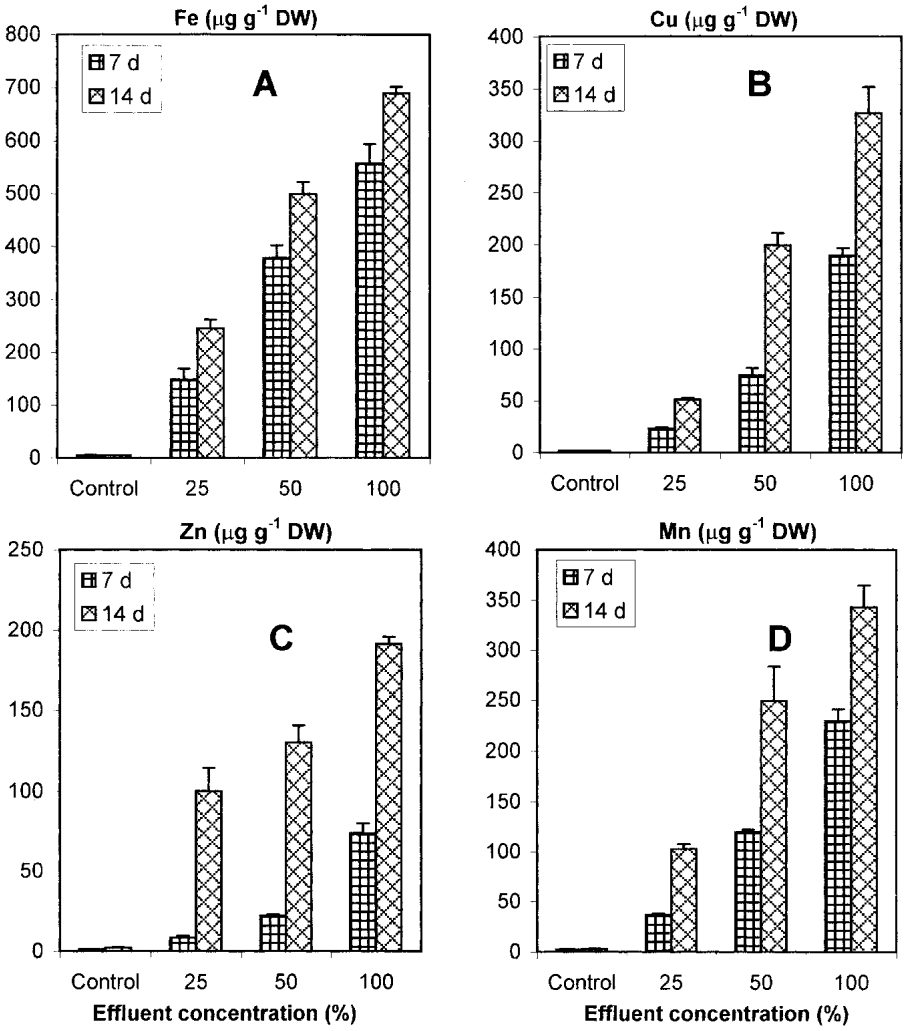

Figure 1 (A-D). Metal accumulation profile of *P. pectinatus* following exposure of different distillery effluent concentration. Two way ANOVA. For Fe F (expo.) = 1.118429* F (cont.) = 4.348357*, For Cu F (expo.) = 5.023283*, F (cont.) = 5.156003*, For Zn F (expo.) = 21.38679* F (cont,) = 4.676111, For Zn F (expo.) = 4.798513* F (cont.) = 5.353422* [*$p<0.05$].

from the present study that accumulation of the metals occurred in 7 d exposure period. However, increasing the treatment duration up to 14 d resulted in higher level of accumulation. Similar results were obtained on metal removing potential of aquatic plants (Rai et al. 1995; Srivastava and Pandey 1998). Results showed that *P. pectinatus* appears to a suitable plant for the phytoremediation of metal polluted water, as it accumulated significant amounts of Cu, Fe, Zn, Mn from different concentration of distillery effluent during a period of two weeks. The plants can be harvested easily after treatment and utilized for bio-gas production and other commercially viable products, or the plant may be incinerated and the resultant ash can be subjected to

chemical procedures for recovery of metals. Experiments are underway for safe utilization of treated plant biomass, however, field trials will be required.

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