Research Article

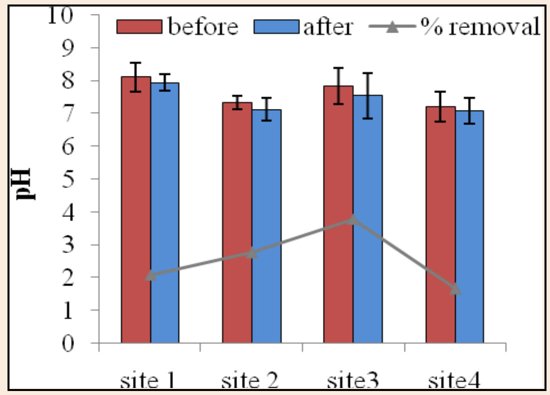
Role of *Potamogeton Pectinatus* in Phytoremediation of Metals

Meenakshi Singh[1*], Upendra Nath Rai[2], Uzma Nadeem[3] and Arun A. David[1]

[1]School of Forestry and Environment, SHIATS, Allahabad 211007, India
[2]Plant Ecology and Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow 226001, India.
[3]Department Of Chemistry, University Of Delhi, Delhi-110007, India

Abstract

The aim of the present study was to assess the phytoremediation potential of aquatic macrophyte; *Potamogeton pectinatus* in waste water. A laboratory experiment was conducted to study treatability potential of *P. pectinatus* from the water collected from different sites of Ganga River, Haridwar, India. Plants having approximately same size and weight were grown in 2 L tub filled with water samples collected from four sites of Haridwar. After 6 d of experiment a significant reduction was observed in physiochemical parameters viz., BOD, TS, TDS, PO_4-P, and NO_3-N which was 74.71%, 74%, 74.26%, 81.92% and 70.38%, respectively. The plant accumulated significant amount of metals in their tissues, which resulted in reduction of heavy metals (Fe, Cu, Zn, and Pb) from the waste water. The plants have been able to remove 70-85 % of Fe, Cu, Zn, and Pb from the waste water showing its phytoremediation potential for the metals.



***Correspondence**
Meenakshi Singh
Email: minaxi.aug88@gmail.com

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Introduction

The pollution of aquatic ecosystems with toxic metals has been attracting considerable attention over the past few decades [1]. Contamination of the aquatic environment by heavy metals has become a serious concern in developing countries. Unlike organic pollutants, heavy metals cannot be easily degraded, which enable their persistence in the nature. Heavy metals can be removed from wastewater by a range of physico-chemical methods such as precipitation, ion exchange, adsorption, electrochemical processes and membrane filtration [2]. Although these methods have diverse efficiencies, but none of them significantly reduce considerable amount of pollutant from wastes. Additionally, physico-chemical treatments are expensive, and do not meet the sustainable criteria for treatment.

Keeping this view in mind, constructed wetland (CW) treatment technology for waste water could be a great remedy. CW technology has emerged rapidly over the past few years and is now considered to be a relatively mature technology for the treatment of a wide range of effluents containing metals [3]. This technology makes use of the naturally occurring processes by which plants and their rhizospheric microbes sequester inorganic pollutants [4]. Aquatic macrophytes have been used as a main biological component of CW for the removal of heavy metals from wastewater [5, 6]. Various experiments have been performed by many authors for wastewater treatment using different aquatic plants [7, 8].

Potamogeton spp. (Potamogetonaceae), submerged macrophyte of world-wide distribution, produces large quantities of biomass and can remove such toxic metals as Cd, and Hg from wastewater [9]. *P. pectinatus* can be regarded as a pioneering, eurytopic species able to tolerate a wide range of nutrient concentrations as it quickly colonizes polluted waters, areas that have been interfered with or have become newly flooded, or environments unsuitable for other species [10]. The use of submerged aquatic macrophyte *P. pectinatus* for wastewater treatment through reduction in physiochemical characteristics of water is unexplored. *P. pectinatus*, like most submerged vascular plants, is ecologically adapted to grow with its roots in sediments that have low oxygen levels. The aim of present study was to assess treatability potential of submerged macrophytes in waste water for its use in constructed wetland.

Material and methods

Experimental setup

The water samples were collected from four sites along the river Ganga viz., Outlet of STP at Misirpur, River Ganga before mixing of sewage water, Sewage at Matrisadan, River Ganga at Matrisadan. These sites were under continuous supply of various types of effluents. The aquatic macrophyte *P. pectinatus* was collected from non-polluted sites at NBRI, Lucknow and acclimatized in 10% Hoagland solution under laboratory condition (14:10 h, light: dark cycle, at 30 ± 2^0C with 115 µ mol m^{-2} s^{-1} illumination provided through fluorescent tube lights) for 4 week. Plants with approximately same size and weight were grown separately in 2 L tub filled with 1 L water collected from different sites along Ganga, Haridwar. A control with distilled water was also maintained. The concentrations of metals in water samples were determined before the commencement of the experiment.

Physico-chemical analysis of water

The physico-chemical parameters like pH, temp, DO, BOD, PO_4^{-3}, NO_3^-, and TSS were determined in by using standard method for examination of water and waste water APHA [11].

Estimation of chlorophyll and Biomass

The plants leaves were washed thoroughly with deionized water and used for the estimation of chlorophyll following the method of Arnon (1949). 500 mg of leaves sample were extracted in 5 ml 80 % chilled acetone and centrifuged at 10,000 rpm for 10 minutes at 4^0C and absorbance of supernatant was taken at 663 and 645 nm with the help of spectrophotometer (GBC Avanta ∑). Biomass was calculated on the fresh weight basis by weighing weight of plant at 0 d and 6 d of experiment.

Metal analysis

To determine concentration of metals Cu, Mn, Fe and Pb in water, 5 ml of water samples were digested at 80 ^{0}C with nitric acid and perchloric acid solution (3:1, v/v) [12]. Samples were allowed to cool, dissolved in 0.6% HNO_3 and filtered through Whattman filter paper No. 42. Volume of each samples were maintained up to 10 ml with 0.6% HNO_3 and analyzed by atomic absorbance spectrophotometer (GBC Avanta ∑).The detection limit of Cu, Mn, Fe and Pb were 0.001, 0.02, 0.02, and 0.06 mg/L , respectively.

Statistical analysis

The all experiments were setup in the randomized block design. To confirm the variability of data and validity of results, all data were subjected to one way analysis of variance (ANOVA) and to determine significance between treatments. For group wise comparison of means Duncan's Multiple Range Test (DMRT) was applied to see the significant level [13].

Results and discussion

Change in physico-chemical characteristics of water samples

Physico-chemical characteristics of water samples collected from different sites along Ganga river, Haridwar, has been shown in **Table 1**. It was found that DO was maximum at river Ganga before mixing of sewage ie., 3.13 mg/L. Amount of BOD, TS, TDS, PO_4-P and NO_3-N were 62.45, 180, 42.30, 6.97, and 12.9, respectively was found in sewage at Matrisadan while lower value of BOD, TS, TDS, PO_4-P and NO_3-N were observed in River Ganga at Matrisadan which were 8.17, 95.67, 80.75, 4.78 and 3.75 mg/L, respectively.

Table 1 Physico-chemical characteristics of water samples collected from different sites of Ganga River, Haridwar, India. Values are in mean ± S.D. (n=3)

Parameters	Site 1	Site 2	Site 3	Site 4
pH	8.12±0.43	7.33±0.02	7.84±0.05	7.21±0.04
Temperature (^{0}C)	18.19±0.35	19.6±0.09	19.2±0.04	18.6±0.04
DO (mg/L)	1.38±0.36	3.13±0.04	Nil	3.1±0.06
BOD (mg/L)	25.39±0.13	12.80±0.04	62.45±0.05	8.17±0.05
TSS (mg/L)	140±12.78	120±19.07	180±14	95.67±3
TDS (mg/L)	38.37±8.55	38.85±2.12	42.30±1.133	80.75±0.57
PO_4-P (mg/L)	4.78±0.7	5.78±0.06	6.97±0.06	4.78±0.08
NO_3-N (mg/L)	5.42±0.09	4.51±0.03	12.9±0.03	3.753±0.01

All values are in mg/L, otherwise mentioned.
Site 1- Outlet of STP at Misirpur; Site 2- River Ganga before mixing of sewage; Site 3-Sewage at Matrisadan; Site 4-River Ganga at Matrisadan.

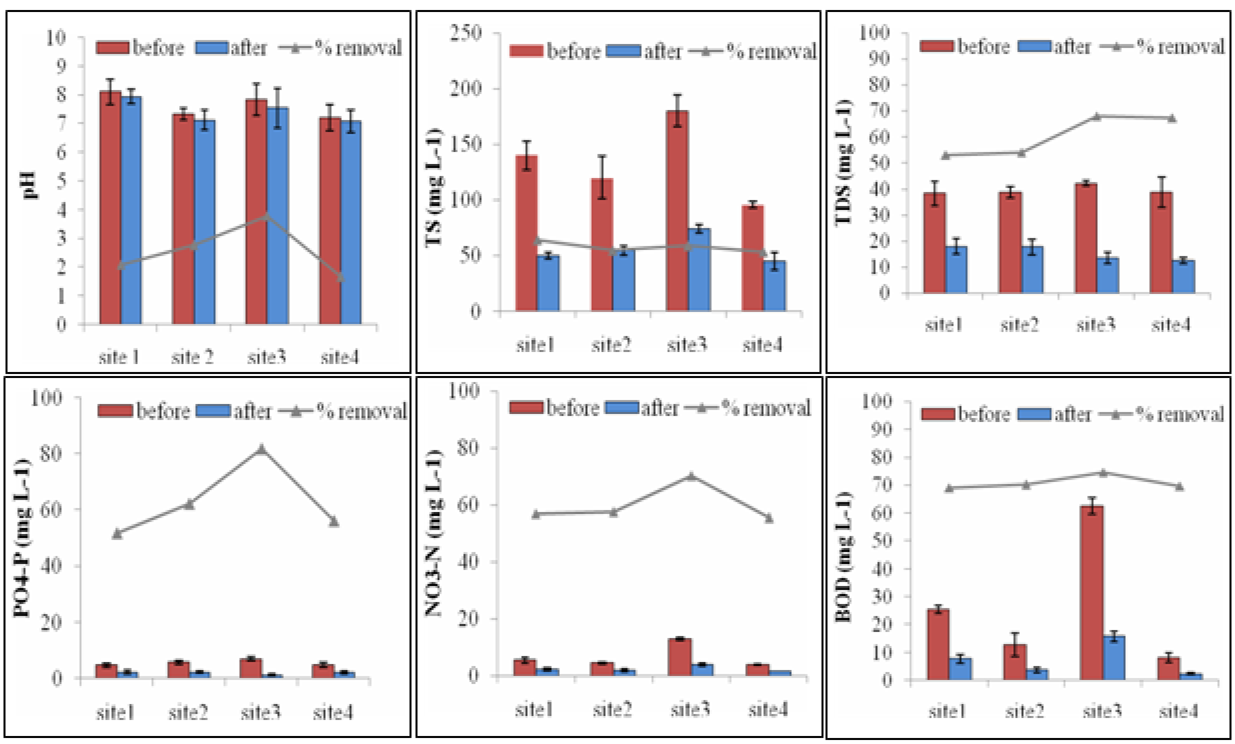

Figure 1 Change in physico-chemical characteristics and percentage removal of water samples collected from different sites of Ganga river, Haridwar. Error bars represent the standard deviation of replicate (n = 3)

The value of DO was increased at site 1 and site 2 after treatment. It is well documented that aquatic plants have hollow stem which facilitate oxygen translocation from leaves to root and release it into the rhizosphere [15] which is utilized by facultative or aerobic bacteria for the oxidation of organic carbon helps improvement in dissolve oxygen [16]. Reduction in PO_4-P, and NO_3-N was in accordance to Rai et al. (2013) showed 76% and 84% removal from sewage, respectively [8].

Table 2 Removal of metals in water samples at different site in Haridwar, India. Values are mean ± S.D. (n=3)

Sites		**Metal accumulation (mg/L)**		
		Before treatment	**After 6 d of treatment**	**Removal (%)**
Site 1	**Cu**	0.57± 0.02	0.125±0.002	78.07018
	Mn	0.902± 0.03	0.222±0.002	75.38803
	Fe	1.093± 0.47	0.311±0.004	71.5462
	Pb	0.857± 0.06	0.141±0.001	83.54726
Site 2	**Cu**	0.44± 0.02	0.1086±0.003	75.31818
	Mn	0.815± 0.1	0.223±0.013	72.63804
	Fe	0.717± 0.04	0.1968±0.021	72.5523
	Pb	0.539±0.008	0.0936±0.001	82.63451
Site 3	**Cu**	0.948±0.64	0.1786±0.002	81.16034
	Mn	1.111±0.7	0.2211±0.006	80.09901
	Fe	1.733±0.2	0.4412±0.003	74.54126
	Pb	2.065±0.4	0.3357±0.001	83.74334
Site 4	**Cu**	0.293±0.005	0.07392±0.003	74.77133
	Mn	0.618±0.003	0.1865±0.004	69.82201
	Fe	0.539±0.005	0.1569±0.002	70.89054
	Pb	0.229±0.003	0.0474±0.005	79.30131

Site1- Outlet of STP at Misirpur; Site 2- River Ganga before mixing of sewage; Site 3- Sewage at Matrisadan; Site 4- River Ganga at Matrisadan.

Aquatic plants have capacity to accumulate substantial amount of metals in their tissue from water augments their use in developing a plant based treatment system coupled with mechanical skills [17, 18]. Translocation factor was also evaluated to check root to shoot translocation. From the **Table 3** it is clear that mobility of metals from root to shoot was low. Many plants show low mobility of heavy metals also due to the fact that there are barriers or lack of transport mechanism suitable for transport from root to shoot [19]. Maximum accumulation of metals was restricted to root. The ability of plants to tolerate and accumulate heavy metals is useful for phytoextraction and phytostabilization purpose [20]. Plants with both bioconcentration factors and translocation factors greater than one (TF and BCF> 1) have the potential to be used in phytoextraction. As reported in **Table 3**, translocation factor (TF) of *P. pectinatus* showed lower value of TF in all tested site at 6 day. Lower value of TF confirmed that *P. pectinatus* was not suitable for phytoextraction of Cu, Zn, Fe and Pb. Similar results was also observed [21]. However, lower value of translocation factor indicated that specific genera could be used as potential plant for phytostabilization [22, 23]. Rai et al. (2012) also observed lower value of TF with Cu, Cr, Pb, Zn and Mn [18].

Effect on Biomass and Chlorophyll

Biomass and chlorophyll content *in P. pectinatus* were determined after 6 d of treatment (**Table 4**). From the table it is clear that biomass was increased after 6 d of treatment. Increased in biomass content may be due to utilization of organic contaminants in the form of nutrient, for growth of the plant. Increased in biomass was reported by various author [8, 24]. Decrease in chlorophyll concentrations at 6 day of experiment showed little effect of sewage toxicity. This decrease in chlorophyll content may be due to result of inhibition of enzymes involved in chlorophyll biosynthesis [25], enzymatic degradation of these pigments [26], and peroxidation of chloroplast membranes [25].

Table 3 Accumulation and translocation factor of metals at different sites of Ganga river, Haridwar, India. Values are mean ± S.D. (n=3)

Sites	Metal accumulation (mg/g dw)				
		Cu	**Mn**	**Fe**	**Pb**
Site 1	Root	0.271 ±0.003	0.477 ±0.015	0.478 ±0.01	0.585 ±0.01
	Shoot	0.101 ±0.007	0.182 ±0.007	0.213 ±0.00	0.107 ±0.007
	TF	0.37	0.38	0.44	0.18
Site 2	Root	0.231 ±0.01	0.307 ±0.009	0.328 ±0.014	0.312 ±0.007
	Shoot	0.0704 ±0.008	0.203 ±0.007	0.146 ±0.007	0.0921 ±0.001
	TF	0.30	0.66	0.44	0.29
Site 3	Root	0.554 ±0.010	0.626 ±0.007	0.832 ±0.019	1.014 ±0.073
	Shoot	0.179 ±0.009	0.237 ±0.019	0.437 ±0.007	0.632 ±0.013
	TF	0.32	0.37	0.52	0.62
Site 4	Root	0.146 ±0.010	0.247 ±0.01	0.256 ±0.012	0.119 ±0.014
	Shoot	0.0674 ±0.002	0.111 ±0.0142	0.084 ±0.005	0.031 ±0.001
	TF	0.46	0.44	0.32	0.26

Site1- Outlet of STP at Misirpur; Site 2- River Ganga before mixing of sewage; Site 3- Sewage at Matrisadan; Site 4- River Ganga at Matrisadan.

Table 4 Chlorophyll (mg /g fw) and Biomass content (g/fw) of *P.pectinatus* at 6 d of experiment. Value are mean ± S.D. (n=3)

Parameter	Control	Site 1	Site 2	Site 3	Site 4
Chlorophyll a	0.243±0.008ab	0.219±0.012ab	0.352±0.019a	0.388±0.020a	0.262±0.006a
Chlorophyll b	0.11±0.037a	0.119±0.012a	0.268±0.024a	0.282±0.017a	0.269±0.009a
Total Chlorophyll	0.353±0.009b	0.439±0.012b	0.419±0.025a	0.45±0.086a	0.441±0.017a
Biomass	3.43±0.184c	4.61±0.208c	4.06±0.195b	4.83±0.384b	4.72±0.208b

Site 1- Outlet of STP at Misirpur; Site 2- River Ganga before mixing of sewage; Site 3-Sewage at Matrisadan; Site 4- River Ganga at Matrisadan. One way ANNOVA has done. Simmilar letter indicates no significant difference ($p \leq 0.05$).

Conclusions

From the experiment it can be concluded that *P. pectinatus* reduced significant amount of BOD, TS, TDS, PO_4-P and NO_3-N by using them (PO_4-P, NO_3-N) as nutrient for their growth which leads to increased biomass. Further lower value of translocation factor conferment its potential for phytostabilization sequestering significant amount of all tested toxic metals in roots and thus, can employed as a phytoremediator plant.

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