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HEAVY METAL UPTAKE BY *SCIRPUS LITTORALIS* SCHRAD. FROM FLY ASH DOSED AND METAL SPIKED SOILS

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Abstract. *Scirpus littoralis* is a wetland plant commonly found in Yamuna flood plains of Delhi, India. The ability of *Scirpus littoralis* to take up and translocate five metals- Mn, Ni, Cu, Zn and Pb from fly ash dosed and metal spiked soils were studied under waterlogged and field conditions for 90 days. *Scirpus littoralis* accumulated Mn, Ni, Cu, Zn and Pb upto a maximum of 494.92, 56.37, 144.98, 207.95 and 93.08 ppm dry wt., respectively in below ground organs (BO) in 90 days time. The metal content ratios BO/soil (B/S) were higher than shoot/soil ratios (T/S) for all the metals, the highest being for Ni. Metal ratios BO/water (B/W) were also higher than shoot/water (T/W) ratios but the B/W ratio was maximum for Zn. The changes in nutrient status (N, P) in soil water and plants were also studied at interval of 30 days. The Pearson's correlation between metal uptake and N, P uptake were calculated. All the metals except Ni showed negative correlation with nitrogen but they were all non-significant. However, P uptake showed positive correlations with all the metals and all were significant at 1% confidence limit.

Keywords: accumulation, heavy metal, nutrients, phytoremediation, *Scirpus littoralis*, uptake, wetland

1. Introduction

Pollution in the biosphere with toxic metals has accelerated dramatically over the years (Nriagu, 1979). The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides and sewage. Another potential source of heavy metals is fly ash, produced due to the burning of coal in power plants. The disposal of fly ash from coal fired power generation and its possible impacts on the environment is a worldwide problem. Toxic metal contamination of soil, aqueous waste streams, wetlands and groundwater are still in need of an effective and affordable technological solution. There are different technologies to remove heavy metals from aquatic and terrestrial systems. Phytoremediation is one of them. As defined by Cunningham and Berti (1993) phytoremediation is the use of vascular plants to remove pollutants from the environment or to render them harmless. Constructed wetlands, reed beds and floating plant systems have been used for many years for the treatment of wastewater. Natural and constructed wetlands can be used for removing heavy metals for both on-site and off-site treatment. Wetland plants are also effective phytoaccumulators of heavy metals and can be efficiently used for phytoremediation. Heavy metal uptake potential has been widely studied for different wetland plant species all over the world, such as *Salvania natanas* (Sen and Mondal, 1989; Zayed *et al.*, 1998), *Lemna polyrrhiza* (Sharma and Gaur, 1995), *Ceratophylum demersum* L., *Spirodela polyrrhiza* (L.) Schleid, *Bacopa monnieri*, *Hygrorrhiza aristata* (Rai *et al.*, 1995), *Eichornia crassipes* (Vesk *et al.*, 1999, Mehra *et al.*, 2000), *Typha latifolia* and *Phragmites australis* (Ye *et al.*, 2001; Batty et al., 2004). There have been very few studies on wetland plants in fly ash rich soils. In 1983 Babcock *et al.*, studied the uptake of As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn by *Typha latifolia* growing in coal ash basin on the Savannah River Plant. The plant shoot was able to accumulate the above mentioned metals upto 0.11, 0.24, 7.04, 76.6, 769, 3.20, 2.38, 1.42 and 17.9 mg/kg dry wt respectively. Another study by Cordes *et al.*, (2002) showed that *Eichornia crassipes* accumulated higher concentration of Cd, Cu, Ni and Zn from pulverized fly ash slurries of fly ash obtained from Indraprastha Power Station, New Delhi, India and Ratcliffe-on-Soar Power Station in the U.K.

Removal of heavy metals by wetland vegetation can be greatly enhanced by the judicious selection of appropriate wetland species. The basis of species selection is the type of elements to be remediated, the geographical location, environmental conditions, and the known accumulation capacities of the species (Zayed *et al.*, 1998). For this reason, it is important to develop knowledge about the abilities of different wetland species. In view of this, it was of interest to study the locally available *Scirpus littoralis* (Schrad), which is abundantly found in Yamuna flood plains of Delhi to see the heavy metal uptake potential of this plant. The heavy metal uptake potential of this particular species has so far not been studied in India. *Scirpus littoralis* is an annual rhizomatous herb of moist sites. The clums are triangular, solid in cross section. Leafs are absent and are reduced to sheaths. The inflorescence is composed of many flowered spikelets and they are clustered.

The primary objective of this study were: (a) To asses the uptake potential of a few environmentally important heavy metals viz. Mn, Pb, Zn, Ni, Cu by *Scirpus littoralis* in different types of metal contaminated soil. (b) To determine the relation between heavy metal uptake and nutrient uptake of the plant, if there is any. (c) To calculate the accumulation factors for the selected plant and metals.

2. Materials and Methods

2.1. SAMPLING

The plants were collected from Bhalswa Lake, which is a natural freshwater wetland on the northern outskirts of Delhi and visibly an uncontaminated site. It is located in the floodplain of river Yamuna that flows about 8 to 9 km east of the lake in a north-south direction. In the northern end of the lake the area is relatively undisturbed and dominated by *Scirpus littoralis*. Here the plants occur in pure strands. Young plants were used for the present experiments.

Fly ash was collected from the bank of the fly ash pond behind Pragati Maidan and beside Ring Road, Delhi. The fly ash produced from the nearby Indraprastha Power Station and Rajghat Power Station are made into a slurry with water and then dumped in this fly ash pond.

2.2. EXPERIMENTAL DESIGN

Five cement tanks measuring $60 \times 60 \times 60$ cm were set up in the garden of our School. In all the tanks the farmyard manure was mixed in ∼1:4 proportion with the soil, fly ash or fly ash plus soil mixtures. One tank was filled with garden soil and farmyard manure, to serve as the control. Fly ash was mixed in three different proportions in three different tanks. Approximately, 1/4th of one tank was filled up with fly ash and 3/4th with mixture of garden soil and farmyard manure (FA1). Second tank was filled with equal amounts of fly ash and farmyard manure plus garden soil mixture (FA2). In the third tank no soil was added, it was filled up with fly ash and manure only (FA3). Apart from the control tank and three fly ash dosed tanks the fifth tank had a metal mixture spiked, to see how the plants behave towards high metal concentration. No fly ash was added in this tank. The spiked metals were Pb as lead nitrate, Zn as zinc sulfate, Ni as nickel sulfate, Mn as manganese chloride, and Cu as copper sulfate of 1000 ppm concentration each (Spiked). The metal mixture was spiked in the garden soil mixed with manure. Here also the manure-soil proportion was 1:4.

Ten young plants (about three weeks old) were planted in each of the six tanks. All the tanks were kept waterlogged. The water level was maintained approximately at 5 cm above the soil surface by watering them frequently with distilled water. The plants were grown for a period of four months (September to December 2003). They were harvested at an interval of one month. Three plants were harvested in each month. Grab soil samples were taken from the root area. Water samples from the top of the soil surface were taken in acid washed plastic bottles. Soil and plant samples were taken in separate plastic bags to the laboratory.

2.3. SOIL ANALYSIS

Soil samples were spread out and air-dried at ambient room temperature. The soil samples were gently rolled to break up large soil aggregates and sieved through 2 mm diameter sieves. Then they were digested for total metals (Mn, Cu, Ni, Pb, Zn) following Agemian and Chau (1976) method. Soil pH, total N and P, nitrate and available inorganic phosphorus were measured following standard procedures (Hess, 1972). The organic matter content in soil was determined following Black, 1947 method.

2.4. WATER ANALYSIS

pH, dissolved heavy metals, total N and P, nitrate, inorganic available P in water were analysed following APHA, 1989 methods.

2.5. PLANT ANALYSIS

The growth of the plants (shoot length and number of offshoots) was measured at the start of the experiment (during the plantation of species) and during each harvest. At each harvest, the plants were removed carefully and washed first with tap water then with double distilled water to remove all soil and organic matter particles from the roots and plant surface. Roots including rhizomes referred as below ground organs (BO), aerial parts (shoots and inflorescence) referred as shoots in the text were separated and kept in oven at 80 °C for 24 h. After drying BO and aerial parts were weighed on digital balance, Mettler 240. Then they were grinded and stored for chemical analysis. Metal analysis (Mn, Cu, Zn, Pb, and Ni) of the plant samples were carried out by acid digestion [conc. HNO_3+conc. $HClO_4$ (9:4)] followed by measurement of total metal using AAS. P content was measured using this digest by molybdenum blue method and plant nitrogen content was measured by Kjeldahl method (Bhargava *et al.*, 1993).

All the heavy metals, viz., Mn, Ni, Cu, Zn, Pb were measured by AAS (Philips 9200X) and the nutrient estimation was done by spectrophotometer (Hitachi U-2000).

Statistics were estimated in SPSS version 11.

3. Results and Discussion

3.1. PH, ORGANIC MATTER AND NUTRIENT STATUS IN SOIL

The pH of the tank soils and water was alkaline throughout the three-month experiment. The range of pH in soil was 7.5 to 8.2 whereas in water the range was 8.1 to 9.0.

The organic matter of the soil decreased with time upto 60 days whereas it increased in the last 30 days due to plant litter deposition (Table I). The Pearson's correlation between the soil organic matter content and soil heavy metal content were all non-significant at 5% significance level. Soil nitrate showed little variation over the study period in 30 days intervals, because in wetland conditions denitrification takes place at a greater rate. Total nitrogen decreased with time upto 60 days, then in the last 30 days it increased in the control tank and in all the tanks. The increase in soil total nitrogen in the last 30 days may be due to plant litter deposition. Soil available inorganic phosphorous and total phosphorus mostly decreased with time in all the treatment tanks and in the control tank as well (Table I). Incase of

TABLE I

Soil organic matter and nutrient status (values ± standard deviation)

Growth period	Control	FA1	FA2	FA3	Spiked
Organic matter(%)					
0 Days	5.16 ± 0.06	7.86 ± 0.04	12.87 ± 0.09	5.61 ± 0.07	6.14 ± 0.01
30 Days	2.37 ± 0.04	2.91 ± 0.04	5.42 ± 0.07	4.22 ± 0.04	3.85 ± 0.07
60 Days	1.96 ± 0.08	1.78 ± 0.04	3.96 ± 0.04	2.81 ± 0.07	3.48 ± 0.04
90 Days	6.68 ± 0.08	3.94 ± 0.04	4.18 ± 0.07	4.18 ± 0.07	5.12 ± 0.03
Nitrate(%)					
0 Days	0.0029 ± 0.00005	0.0043 ± 0.000100	0.0040 ± 0	0.0003 ± 0.00001	0.0029 ± 0.00008
30 Days	0.0005 ± 0.00005	0.0002 ± 0	0.0002 ± 0	0.0008 ± 0.00001	0.0003 ± 0
60 Days	0.0003 ± 0.00005	0.0002 ± 0.000050	0.0002 ± 0.00005	0.0002 ± 0.00001	0.0003 ± 0.00000
90 Days	0.0005 ± 0.00001	0.00029 ± 0.00001	0.0003 ± 0.00001	0.0001 ± 0	0.0003 ± 0
Total nitrogen(%)					
0 Days	0.42 ± 0.00	0.18 ± 0.00	0.61 ± 0.003	0.49 ± 0.006	0.29 ± 0.001
30 Days	0.21 ± 0.00	0.09 ± 0.003	0.09 ± 0.001	0.34 ± 0.002	0.18 ± 0.003
60 Days	0.18 ± 0.00	0.11 ± 0.08	0.08 ± 0.006	0.21 ± 0.002	0.15 ± 0.003
90 Days	0.30 ± 0.01	0.13 ± 0.001	0.11 ± 0.003	0.26 ± 0.002	0.32 ± 0.002
Inorganic available phosphorus (%)					
0 Days	0.06 ± 0.002	0.08 ± 0.002	0.12 ± 0.002	0.11 ± 0.006	0.09 ± 0.002
30 Days	0.04 ± 0.001	0.02 ± 0.002	0.09 ± 0.003	0.07 ± 0.002	0.06 ± 0.001
60 Days	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.002	0.05 ± 0.002	0.09 ± 0.002
90 Days	0.01 ± 0.002	0.01 ± 0.001	0.02 ± 0.001	0.07 ± 0.003	0.04 ± 0.002
Total phosphorus (%)					
0 Days	0.36 ± 0.006	0.31 ± 0.001	0.47 ± 0.029	0.70 ± 0.003	0.27 ± 0.003
30 Days	0.26 ± 0.005	0.08 ± 0.007	0.12 ± 0.002	0.19 ± 0.002	0.18 ± 0.001
60 Days	0.17 ± 0.002	0.12 ± 0.007	0.08 ± 0.001	0.14 ± 0.003	0.03 ± 0.001
90 Days	0.14 ± 0.029	0.16 ± 0.005	0.26 ± 0.003	0.06 ± 0.003	0.05 ± 0.011

inorganic available phosphorus FA3 and spiked tank values at 60th day were higher than the other tanks. The rate of adsorption and desorption of inorganic available phosphorus in soil under wetland conditions is controlled by soil pH, Eh, adsorptive surface area, hydrology and temperature (Reddy *et al.*, 1994). As all the treatment tanks were kept in the garden under natural conditions, the soil was exposed to diurnal and seasonal variation of temperature. This can lead to different rate of evaporation of the water which leads to different rate of phosphorus adsorption desorption and therefore the availability in different tanks. So the availability of inorganic phosphorus may change depending on the prevailing ambient conditions on a particular tank in a particular day.

3.2. pH AND NUTRIENT STATUS IN WATER

The nutrient status in water of all the tanks changed with time. Almost in all the treatment tanks the concentration of nitrate, total nitrogen, available inorganic phosphorus and total phosphorus decreased with time (Table II).

TABLE II
Water nutrient status (values ± standard deviation)

Growth period	Control	FA1	FA2	FA3	Spiked
Nitrate (mg/l)					
0 Days	0.64 ± 0.003	0.25 ± 0.003	0.21 ± 0.017	0.14 ± 0.004	0.40 ± 0.020
30 Days	0.51 ± 0.007	0.21 ± 0.002	0.31 ± 0.008	0.14 ± 0.004	0.36 ± 0.042
60 Days	0.37 ± 0.002	0.15 ± 0.005	0.25 ± 0.008	0.10 ± 0.002	0.22 ± 0.004
90 Days	0.37 ± 0.000	0.06 ± 0.006	0.14 ± 0.004	0.11 ± 0.003	0.24 ± 0.013
Total nitrogen (mg/l)					
0 Days	5.27 ± 0.026	5.23 ± 0.032	4.20 ± 0.001	5.27 ± 0.022	15.79 ± 0.005
30 Days	2.10 ± 0.004	4.73 ± 0.008	3.16 ± 0.002	3.16 ± 0.005	8.04 ± 0.001
60 Days	2.09 ± 0.013	4.21 ± 0.032	3.06 ± 0.002	2.10 ± 0.002	3.16 ± 0.001
90 Days	1.09 ± 0.006	1.05 ± 0.006	2.11 ± 0.001	1.03 ± 0.026	5.79 ± 0.006
Available inorganic phosphorus (mg/l)					
0 Days	0.21 ± 0.05	0.23 ± 0.09	0.18 ± 0.07	0.13 ± 0.08	0.14 ± 0.08
30 Days	0.12 ± 0.06	0.13 ± 0.01	0.11 ± 0.07	0.08 ± 0.05	0.07 ± 0.04
60 Days	0.03 ± 0.01	0.07 ± 0.03	0.06 ± 0.05	0.04 ± 0.00	0.03 ± 0.01
90 Days	0.01 ± 0.005	0.02 ± 0.00	0.01 ± 0.004	0.01 ± 0.00	0.05 ± 0.02
Total phosphorus (mg/lit)					
0 Days	1.03 ± 0.71	0.98 ± 0.08	1.01 ± 0.15	0.93 ± 0.12	2.91 ± 0.74
30 Days	0.73 ± 0.09	0.44 ± 0.07	0.52 ± 0.16	0.61 ± 0.05	1.60 ± 0.15
60 Days	0.31 ± 0.12	0.31 ± 0.05	0.29 ± 0.07	0.35 ± 0.15	1.01 ± 0.57
90 Days	0.09 ± 0.04	0.11 ± 0.03	0.31 ± 0.05	0.26 ± 0.08	0.71 ± 0.06

3.3. PLANT GROWTH PARAMETERS

Plant growth was assessed in terms of dry weight of BO and shoots; root, shoot length and number of offshoots. The weight of BO including rhizomes increased gradually during the three-month study period. However, the increase in dry weight of BO and shoots was maximum in the control tank and the spiked tank. In the control tank root dry weight and shoot dry weight increased 71.42 and 19.55 times respectively in 90 days. In FA1, FA2, FA3 and Spiked tank plants dry wt of BO increased 52, 26, 26.84 and 83.33 times respectively and shoot dry wt increased 12.36, 7.8, 12.14 and 25 times respectively (data not shown). The root, shoot length and the number of offshoots also increased with time (data not shown). Higher growth of plants in the spiked tank shows that there is likelihood of some or one of the heavy metal(s) promoting growth. This is supported by the fact that some trace metals (Cu, Co, Fe, Mo, Ni and Zn) are known to be essential for plants (Lasat, 2002). Some are proved necessary for a few species only and others are known to have stimulating effects on plant growth, but their functions are yet not recognizable (Pendias and Pendias, 1989).

3.4. N AND P CONTENT IN BELOW GROUND ORGANS AND SHOOTS

Plant nutrient uptake depends on the availability of nutrients in the soil, the N: P in the soil and on the behavior of an individual plant. Nutrient availability in wetland conditions again depends upon the pH, Eh, hydrology, temperature, prevailing soil chemistry and redox conditions in rhizosphere. If any one of the conditions varies, nutrient uptake and hence the nutrient content in plants can vary.

Since the plants were collected from the same area the average background concentration was assessed arbitrarily choosing three plants. The average nitrogen concentration in the BO at the time of planting was 0.82 ± 0.11%. Their nitrogen content increased with time. In the control tank it increased from 0.82 to 3.06%. The increase was maximum in FA1 tank where below ground organ nitrogen increased from initial concentration to 5.33%. The initial average N concentration in plant shoots was 1.61 ± 0.25%. The total nitrogen content in plant shoots increased with time. In the control tank the increase was from initial concentration to 6.5% in 90 days time period. In the fly ash dosed tanks also the trend was same. In the FA3 tank the nitrogen contents in shoots increased from initial concentration to 7% in 90 day's time. In the spiked tank however the increase in the shoots total nitrogen content was only 4.84% in 90 days time period (Table III). The average phosphorus concentration in the BO during planting condition was 0.007 ± 0.001(%). It increased with time in all the treatments. In the control tank plants it increased from 0.02 to 0.07%. Among the plants from fly ash dosed tank the accumulation was higher in plant BO in the FA2 tank than the other two fly ash dosed tanks. In the spiked tank also the total P content in BO increased from 0.01 to 0.14% in 90 days time (Table III). As the available inorganic phosphorus was

TABLE III
N and P in plant below ground organs and shoot (values ± standard deviation)

Growth period	Control	FA1	FA2	FA3	Spiked
Nitrogen in plant below ground organs (%)					
0 day	0.82 ± 0.11	NA	NA	NA	NA
30 Days	2.48 ± 0.019	1.39 ± 0.0100	1.88 ± 0.015	1.39 ± 0.010	1.49 ± 0.011
60 Days	2.30 ± 0.028	1.49 ± 0.0100	1.98 ± 0.023	2.38 ± 0.0190	2.67 ± 0.020
90 Days	3.06 ± 0.001	5.33 ± 0.0005	2.06 ± 0.063	4.82 ± 0.0200	1.78 ± 0.014
Nitrogen in plant shoots(%)					
0 Day	1.61 ± 0.25	NA	NA	NA	NA
30 Days	5.22 ± 0.018	2.87 ± 0.021	5.03 ± 0.002	2.95 ± 0.060	2.95 ± 0.055
60 Days	6.02 ± 0.001	4.82 ± 0.021	6.80 ± 0.010	3.63 ± 0.078	4.31 ± 0.026
90 Days	6.50 ± 0.011	5.34 ± 0.061	7.87 ± 0.061	7.00 ± 0.136	4.84 ± 0.051
Phosphorus in plant below ground organs (%)					
0 Day	0.007 ± 0.001	NA	NA	NA	NA
30 Days	0.02 ± 0.005	0.01 ± 0.001	0.11 ± 0.001	0.03 ± 0.002	0.01 ± 0.001
60 Days	0.04 ± 0.002	0.05 ± 0.001	0.08 ± 0.002	0.06 ± 0.001	0.03 ± 0.001
90 Days	0.07 ± 0.001	0.03 ± 0.001	0.15 ± 0.005	0.09 ± 0.002	0.14 ± 0.002
Phosphorus in plant shoots(%)					
0 Day	0.009 ± 0.001	NA	NA	NA	NA
30 Days	0.06 ± 0.003	0.010 ± 0.002	0.01 ± 0.002	0.09 ± 0.012	0.07 ± 0.004
60 Days	0.14 ± 0.001	0.04 ± 0.056	0.03 ± 0.064	0.13 ± 0.003	0.41 ± 0.009
90 Days	0.16 ± 0.004	0.18 ± 0.006	0.15 ± 0.006	0.21 ± 0.006	0.70 ± 0.008

NA: not applicable.

higher in FA3 than FA2 the plants in FA3 tank took up more phosphorus and translocated to the shoot which leads to the higher values of phosphorus in shoot of FA3 plants.

In plants both the nitrogen and phosphorous content increased with time and was found higher in shoots than in BO during each harvest. Gaudet, 1977 showed that in tropical wetlands, the growth and nutrient uptake rates of nitrogen and phosphorous are higher in younger stems. Bernad *et al.* (1988) also showed the high nutrient concentrations in young emerged stems of *Carex*.

3.5. METAL CONTENT IN SOIL AND WATER

Soil metal concentration decreased in all the different treatment tanks in 90 days time. The decrease in soil metal concentration in 30 days interval is shown in Table IV. The lower value of Pb in FA1 tank than in the control tank may be

TABLE IV
Metal concentrations in soil (ppm)

Growth period	Control	FA1	FA2	FA3	Spiked
Mn					
0 Day	406.03 ± 0.06	460.67 ± 0.58	520.00 ± 1.00	560.00 ± 1.00	1350.67 ± 0.58
30 Days	393.00 ± 1.00	360.00 ± 1.00	463.33 ± 1.53	316.00 ± 1.00	825.000 ± 1.00
60 Days	356.00 ± 1.00	282.00 ± 1.73	400.00 ± 1.00	324.66 ± 0.58	619.330 ± 1.15
90 Days	251.33 ± 1.15	199.33 ± 0.58	324.00 ± 1.00	314.67 ± 0.58	493.030 ± 1.00
Ni					
0 Day	64.83 ± 0.29	79.83 ± 0.76	110.33 ± 0.58	155.03 ± 0.45	1051.06 ± 0.31
30 Days	46.86 ± 0.81	44.50 ± 0.76	73.830 ± 0.29	88.86 ± 0.32	567.40 ± 0.53
60 Days	25.80 ± 0.35	27.36 ± 0.64	68.530 ± 0.50	74.83 ± 0.29	472.26 ± 0.64
90 Days	15.36 ± 0.64	11.66 ± 0.58	48.800 ± 1.06	47.90 ± 0.27	457.83 ± 0.38
Cu					
0 Day	34.01 ± 1.03	37.11 ± 0.64	58.15 ± 0.54	83.51 ± 0.65	1036.0 ± 1.26
30 Days	21.80 ± 0.52	21.80 ± 0.46	43.22 ± 0.31	56.02 ± 0.32	960.1 ± 0.99
60 Days	16.51 ± 0.81	18.00 ± 0.36	25.05 ± 0.55	45.72 ± 0.46	793 ± 1.21
90 Days	12.02 ± 0.54	11.12 ± 0.52	17.02 ± 0.62	37.26 ± 0.98	692.14 ± 0.56
Zn					
0 Day	95.12 ± 0.85	101.41 ± 0.31	115.64 ± 1.02	122.12 ± 0.25	1084.02 ± 2.15
30 Days	79.34 ± 0.75	85.11 ± 0.31	86.18 ± 0.55	102.44 ± 0.55	700.98 ± 1.89
60 Days	45.11 ± 0.65	62.66 ± 0.63	62.33 ± 0.98	84.59 ± 0.61	508.11 ± 1.49
90 Days	24.15 ± 0.86	46.56 ± 0.87	47.22 ± 0.55	48.10 ± 1.02	421.37 ± 1.01
Pb					
0 Day	60.05 ± 0.89	52.33 ± 1.23	67.26 ± 0.69	99.32 ± 0.99	1065.01 ± 0.55
30 Days	46.11 ± 0.65	39.57 ± 0.12	51.59 ± 0.31	70.21 ± 0.59	992.43 ± 1.85
60 Days	29.23 ± 1.26	22.35 ± 0.35	31.32 ± 1.20	41.01 ± 0.29	887.02 ± 2.13
90 Days	14.54 ± 0.94	15.25 ± 0.91	19.61 ± 0.79	28.73 ± 1.56	855.11 ± 0.96

ascribed to very low Pb content in fly ash as compared to garden soil mixed with farmyard manure which has been replaced by fly ash to the extent of 25% in this tank. In the control tank soil Mn, Ni, Cu, Zn and Pb concentration decreased from 406.03 to 251.33 ppm, 64.83 to 15.36 ppm, 34.01 to 12.02 ppm, 95.12 to 24.15 ppm and 60.05 to 14.54 ppm respectively. All the metals followed the same trend in all the treatment tanks. The water metal concentrations reported in the Table V are the dissolved metal concentrations. Metal concentrations in water were very low or below detectable level in all the tanks at the onset of the experiments. It increased after first 30 days, which may be attributed to release of metals due to decomposition of organic matter bound metals or due to the prevailing reducing conditions when metals got reduced and became more soluble after the initial 30 days time. Then the metal concentrations in water generally decreased with time in most of the tanks because of plant uptake.

TABLE V
Metal concentrations in water (ppm)

Growth period	Control	FA1	FA2	FA3	Spiked
Mn					
0 Day	-Bdl-	-Bdl-	-Bdl-	-Bdl-	0.089 ± 0.04
30 Days	8.21 ± 0.03	8.02 ± 0.06	8.21 ± 0.02	5.12 ± 0.04	8.32 ± 0.06
60 Days	5.03 ± 0.04	7.26 ± 0.06	3.85 ± 0.02	6.36 ± 0.01	9.37 ± 0.09
90 Days	6.14 ± 0.06	4.32 ± 0.07	4.89 ± 0.03	3.11 ± 0.04	8.83 ± 1.09
Ni					
0 Day	0.053 ± 0.01	0.088 ± 0.01	Bdl	Bdl	0.088 ± 0.01
30 Days	0.052 ± 0.01	0.053 ± 0.01	0.049 ± 0.01	0.053 ± 0.02	0.052 ± 0.01
60 Days	0.031 ± 0.01	0.046 ± 0.01	0.053 ± 0.01	0.028 ± 0.02	0.062 ± 0.01
90 Days	0.008 ± 0.00	0.025 ± 0.004	0.049 ± 0.003	0.011 ± 0.001	0.009 ± 0.001
Cu					
0 Day	0.08 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.003	0.08 ± 0.01
30 Days	0.17 ± 0.03	0.03 ± 0.01	0.24 ± 0.03	0.14 ± 0.007	0.25 ± 0.02
60 Days	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	0.17 ± 0.01
90 Days	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.10 ± 0.04
Zn					
0 Day	-Bdl-	-Bdl-	-Bdl-	0.015 ± 0.002	0.061 ± 0.003
30 Days	0.06 ± 0.001	0.09 ± 0.003	0.172 ± 0.003	0.285 ± 0.002	0.090 ± 0.004
60 Days	0.007 ± 0.001	0.045 ± 0.002	0.06 ± 0.002	0.009 ± 0.003	0.045 ± 0.004
90 Days	0.003 ± 0.001	0.015 ± 0.001	0.01 ± 0.005	0.06 ± 0.001	0.093 ± 0.002
Pb					
0 Day	0.02 ± 0.003	0.01 ± 0.000	0.01 ± 0.002	0.08 ± 0.003	0.01 ± 0.001
30 Days	0.28 ± 0.002	0.31 ± 0.002	0.39 ± 0.003	0.21 ± 0.004	0.50 ± 0.003
60 Days	0.31 ± 0.006	0.21 ± 0.003	0.17 ± 0.001	0.17 ± 0.001	0.23 ± 0.005
90 Days	0.16 ± 0.002	0.04 ± 0.002	0.03 ± 0.005	0.01 ± 0.003	0.08 ± 0.002

Bdl: Below detectable limit.

3.6. Metal accumulation in below ground organs and shoots

During the plantation time the average Mn, Ni, Cu, Pb, Zn concentration in the BO of three arbitrarily chosen saplings were 124.12 ± 0.92, 4.39 ± 0.65, 2.06 ± 0.35, 39.54 ± 0.34 and 7.85 ± 0.29 ppm dry wt respectively. Similarly, in shoots they were 101.28 ± 0.83, 1.98 ± 0.34, 10.76 ± 0.66, 1.89 ± 0.18 ppm dry wt respectively. The metal accumulation in both plants BO and shoots increased with time. The uptake of metals by the plant organs at the interval of 30 days is shown in Figures 1–10.

The accumulation factors were calculated to see how the plant behaved towards each metal. The accumulation factors give an idea whether the plant is behaving as an accumulator or indicator or excluder for a specific metal. The ratios, viz. BO/soil (B/S), shoot/soil (T/S), shoot/BO (T/B), below ground organ/water ratio (B/W) and shoot/ water (S/W) ratios were calculated to get an idea about the bioavailability

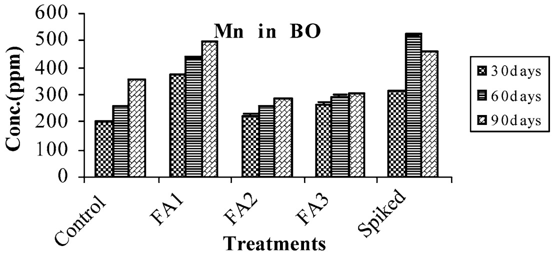

Figure 1. Changes in Mn concentration in BO at 30 days interval.

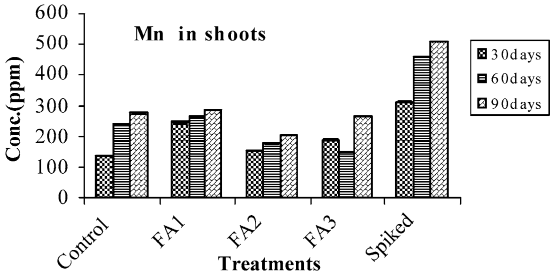

Figure 2. Changes in Mn concentration in shoots at 30 days interval.

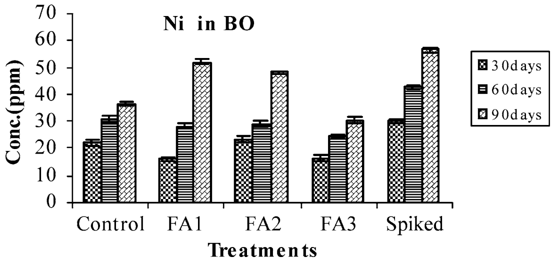

Figure 3. Changes in Ni concentration in BO at 30 days interval.

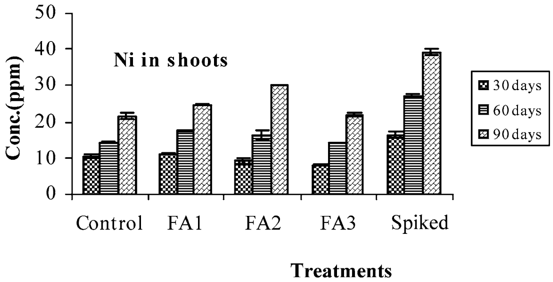

Figure 4. Changes in Ni concentration in shoots at 30 days interval.

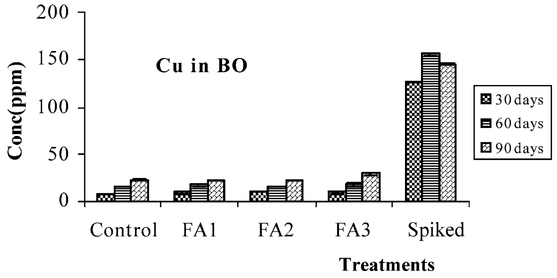

Figure 5. Changes in Cu concentration in BO at 30 days interval.

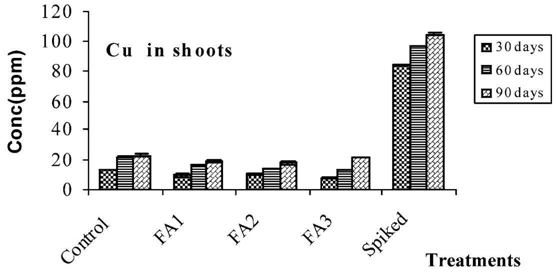

Figure 6. Changes in Cu concentration in shoots at 30 days interval.

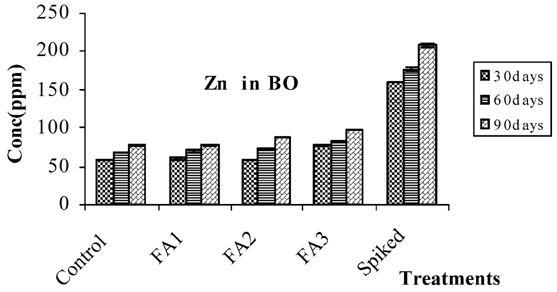

Figure 7. Changes in Zn concentration in BO at 30days interval.

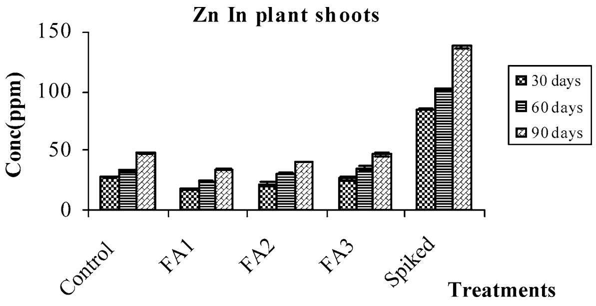

Figure 8. Changes in Zn concentration in shoots at 30 days interval.

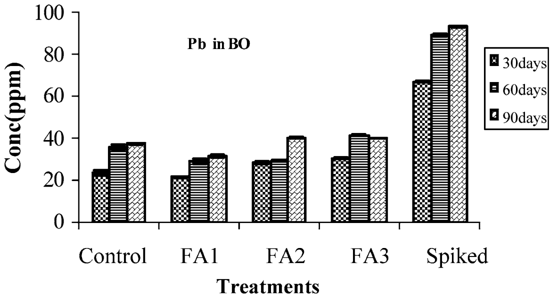

Figure 9. Changes in Pb concentration in BO at 30 days interval.

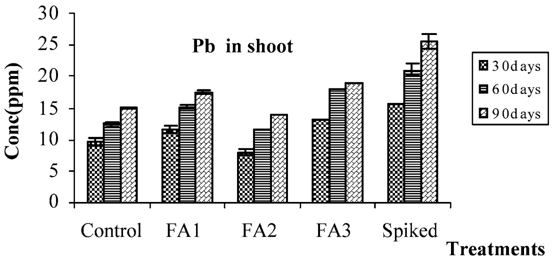

Figure 10. Changes in Pb concentration in shoots at 30 days interval.

of the metals and the part of the plant where they can accumulate. The factors and ratios given in Table VI are the average values of the ratios at different harvests and for all the treatments together.

From the results it was clear that *Scirpus littoralis* accumulated metal as the time increased. As the metal content in the BO and shoots increased the soil metal content decreased. The metal content was higher in BO than in shoots. BO of *Scirpus littoralis* accumulated upto 1601.66 ppm dry wt Mn, 56.37 ppm dry wt Ni, 314.01 ppm dry wt Cu, 207.95 ppm dry wt Zn and 244.01 ppm dry wt Pb. B/S and B/W ratios also show that the metal accumulation was more in BO than shoot (Table VI). The maximum accumulation in BO was observed in case of Pb and Zn as the B/S ratio were 1.11 and 1.17 with respect to soil but in case of water the

TABLE VI
Metal Accumulation factors (average)

Metals	B/S	T/S	T/B	B/W	T/W
Mn	0.97	0.71	0.76	56.98	42.61
Ni	0.86	0.46	0.57	1496.03	920.30
Cu	0.69	0.67	0.92	817.48	683.72
Zn	1.17	0.56	0.46	4916.71	2559.82
Pb	1.10	0.49	0.39	606.68	252.45

maximum accumulation was for Zn and Ni as the ratios were 1496.03 and 4916.71. This also shows that these metals were more bioavailable and were accumulated by the BO. Moreover, the uptake of all metals by *Scirpus littoralis* is stronger in the BO than in the shoots of this plant as shown by the mean B/S, T/S, T/B and B/W ratios. This agrees with several studies indicating that more accumulation occurs in the BO than in the shoots of the wetland plants (Mudroch, 1979; Welsh *et al.*, 1980; Taylor *et al.*, 1983; Babcock *et al.* 1983; Jain *et al.*, 1990; Sen *et al.*, 1989; Kadelec, 1995; Mungur *et al.*, 1997; Zayed *et al.*, 1998; Qian *et al.*, 1999; Sadwosky 1999; Mehra *et al.*, 1998; Cordes *et al.*, 2000, Mehra and Farago, 2000; Zurayk 2002).

To get an idea how the plant behaved towards different treatments and different metal concentrations in soil and water the concentration factors at the time of final harvest in plant BO and shoot with respect to water and soil in differently treated tanks were calculated. It was apparent from the results (Table VII) that plant also accumulated metal through water, T/W, B/W ratios were very high in different treatment tanks. In case of Mn, the highest accumulation was in BO of FA1 tank plants, both with respect to soil and water. The ratios were 2.48 and 114.56 respectively. However, in case of Ni, maximum accumulation with respect to soil was in the FA1 tank. The highest accumulation, however, was in BO of spiked tank plants, with respect to water, as the B/W ratio was 6263.33. In this tank the ratios with respect to soil were very less suggesting that almost all the uptake of Ni was through water. In case of Cu, maximum accumulation in plant organs with respect to water was in the spiked tank and with respect to soil, was the FA1 tank. In case of Zn, the maximum accumulation with respect to soil and water in plant organs was in FA3 and FA2 tanks respectively. In case of Pb both the B/S and B/W ratios were higher in FA1 tank plants, the highest B/W and S/W ratio was in FA3 and spiked tank respectively.

Thus, in the present study, *Scirpus littoralis* was found to be a very good accumulator of all the five metals viz., Mn, Cu, Ni, Zn and Pb especially with respect to water, as it was evident from the high accumulation factors in BO and shoot. However, the correlation between the water metal content and the BO and shoots of the plants were all non-significant for all the metals and for all the treatments. This indicates that if the metal load in water increases, it will not increase the plant metal uptake in the same proportion. Correlation between soil metal content and metal content in plant parts were also non-significant suggesting the same.

The plant nutrient contents viz, the nitrogen and phosphorous contents in plants were correlated with the metal content in the plants (Table VIII). The correlations between nitrogen content and metal content in plants were all negative except in case of Ni but all the correlations were non-significant at 1% level of significance. The metal content and phosphorous content in plants were positively correlated incase of all the metals and all the correlations were significant at 1% level of significance. This indicates phosphorous uptake enhances heavy metal uptake or vice versa. And except Ni, nitrogen uptake reduces heavy metal uptake in plants. To explain the characteristic behavior of Ni further study is needed.

TABLE VII
Metal concentration factors at the time of final harvest in below ground organs and shoots

Ratios	FA1	FA2	FA3	Spiked
Mn				
B/S	2.48	0.88	0.97	1.05
T/S	1.43	0.63	0.85	1.03
B/W	114.56	58.52	98.75	58.93
T/W	66.27	41.57	85.97	57.39
Ni				
B/S	4.47	0.98	0.44	0.12
T/S	2.1	0.62	0.32	0.1
B/W	2084.4	983.26	2750.9	6263.33
T/W	981.2	617.55	2002.73	131.42
Cu				
B/S	1.89	1.27	0.77	0.3
T/S	1.71	1.05	0.48	0.2
B/W	2111	722	718	1449.8
S/W	1905	600	450	2415.5
Zn				
B/S	1.66	1.89	2.04	0.49
T/S	0.74	0.85	0.97	0.33
B/W	5150	8829	1633.83	2236.02
S/W	2288	4002	783.66	440.55
Pb				
B/S	2.06	2.04	1.39	0.11
T/S	1.15	0.72	0.66	0.05
B/W	787.5	1340	4011	1136.5
S/W	437.25	468.66	1902	2218.27

TABLE VIII
Correlation between nutrient content and metal content in plant

Parameters correlated	Correlation
N and Mn	−0.030
N and Ni	+0.453
N and Cu	−0.228
N and Zn	−0.147
N and Pb	−0.105
P and Mn	+0.710**
P and Ni	+0.824**
P and Cu	+0.674**
P and Zn	+0.805**
P and Pb	+0.818**

** Significant at 1% confidence limit (two tailed test).

In the three-month study period, the plant did not show any significant toxicity symptoms. However, the growth of the plants between the second harvest (60 days) and third harvest (90 days) was found less compared to the growth between initial stage and the second harvest. The low growth in terms of plant dry wt and root-shoot length may be attributed to metal stress or due to the fact that the third harvest was done in the month of December and in winter season plants grow less. The BO in the third harvest also showed dark colouration in some parts, which is an indication of metal stress. But other toxicity symptoms like decolouration of stems etc were not visible in any plant growing in the six different tanks. But the plants in the control tank, spiked tank and FA1 tank showed considerable increase in plant dry weight after 90 days, whereas, in FA2 and FA3 tank plant growth was comparatively low (data not shown). In case of metal accumulation, among the three fly ash dosed tanks, Mn and Ni accumulation was higher in FA1 tank plants than FA2 and FA3 tank plants. However, Cu, Pb and Zn accumulation was more in the FA3 tank plants. Overall, metal accumulation was in appreciable quantity in all the fly ash dosed tank plants. But in the long run as the plant growth was more in FA2 tank plants this proportion may be more effective for phytoremediation purpose. Metal concentrations in control tank soils were comparatively low, in the fly ash dosed tanks moderate and in the spiked tank very high. This indicates *Scirpus littoralis* efficiently accumulated Mn, Ni, Cu, Zn and Pb in all the soil metal concentration. The T/B ratio also shows metals are less accumulated in above ground biomass, which is good from phytoremediation perspective, as metal accumulators, but not root- shoot translocators produce polluted above ground biomass, which increases the risk of exposing wildlife to the contaminated plants (Zurayk *et al.*, 2002).

4. Conclusions

The results showed that, under our experimental conditions *Scirpus littoralis* accumulated Mn, Ni, Cu, Zn, and Pb mostly in BO. As metal concentrations in shoot were usually maintained at low levels, metal tolerance in wetland plants seem to depend mainly on their metal exclusion ability. However, the higher than toxic level concentrations of metal especially Mn, indicates that internal detoxification mechanisms might also exist (Kabata-Pendias *et al.*, 1989). So it can be concluded that this particular wetland species can be successfully used for phytoremediation. However, plant soil interaction, response of the species after long exposure to metals should be taken into account while choosing suitable conditions during wetland system construction and management.

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