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Heavy metal uptake and its effect on macronutrients, chlorophyll, protein, and peroxidase activity of *Paspalum distichum* grown on sludge-dosed soils

Heavy metal uptake and its effect on *P. distichum*

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Abstract This study assessed the heavy metal (Cr, Mn, Ni, Cu, Zn, and Pb) uptake and its effect on biochemical parameters in *Paspalum distichum*, a wetland plant. Sludge collected from Bhalswa waste dump, New Delhi, was used as heavy metal source and dosed in different proportions viz. 20%, 40%, 60%, and 80% to the garden soil. The plants accumulated metals mostly in belowground organs. The metal accumulation followed the order: Cr>Mn>Cu>Zn>Ni>Pb. The range of heavy metal concentration in tissue of belowground organs after 180 days of growth was 1,778.65–4,288.01 ppm Cr, 828.11–1,360 ppm Mn, 236.52–330.07 ppm Ni, 155.79–282.35 ppm Cu, 27.05–91.16 ppm Zn, and 27.09–50.87 ppm Pb. The biochemical parameters viz. chlorophyll and protein contents and peroxidase (POD) activity exhibited no considerable adverse effect indicating the plants' tolerance towards heavy metals. The high POD activity and synthesis of new protein bands at high sludge-dosed plants were also in support of this fact.

Keywords Wetland plant · Sludge · Metals · Chlorophyll · Protein · POD activity

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Introduction

Everyday substantial amounts of aqueous effluents contaminated with various types of potential toxic elements and heavy metals are produced due to several anthropogenic activities like mining, smelting, metal pickling, rolling industries, and fly ash. These polluted discharges pose a tremendous hazard to human and wildlife. Not only effluents contaminated with heavy metals pose a threat to the environment but also soils and sludge contaminated with metalliferous wastes which are

difficult to reclaim and have tremendous pollution potential. High toxic metal concentration has potential to restrict plant growth.

Therefore, to avoid the harmful effects of heavy metals in plants and animals, it is necessary to remediate heavy metal contaminated water and soils. Conventional physical and chemical processes for the treatment of heavy metal contaminated wastewater and soil are not only expensive but also are insufficiently effective. The cost-effective alternative is to treat the wastes with specialized plants which can effectively take up metal and control pollution. This technique is known as phytoremediation. As defined by Cunningham et al. (1995), phytoremediation is the use of vascular plants to remove pollutants from the environment or to render them harmless. Contaminants are then removed by harvesting the plant for subsequent volume reduction (i.e., ashing) and storage. Many wetland plants have been found to be effective phytoaccumulators of heavy metals and can be efficiently used for phytoremediation (Groudeva et al. 2001; Bhattacharya et al. 2006). However, wetland plants vary greatly in their potential for metal uptake and continuous exposure to plants to high concentration of metal may lead to phytotoxicity (Qian et al. 1999; Deng et al. 2004). As a result, physiological and biochemical changes can take place in the plant system. For example, a decrease in chlorophyll and protein content and an increase in peroxidase (POD) activity with increasing metal concentration has been observed in several aquatic plants (Gupta and Chandra 1996; Sinha et al. 1996; Gallego et al. 1996; Moustakas et al. 1997). Some studies also reported that increase in metal concentration in the culture medium under laboratory conditions resulted in a decrease in nutrients (NPK) in test plants (Gupta and Chandra 1996). In order to understand the metal accumulation potential of a particular plant, the toxic effects of metals on various biochemical and nutrient parameters must also be understood. The present study was aimed at assessing the exposure to heavy metals on *Paspalum distichum*, which is a common wetland grass and is common in and around Delhi.

Materials and methods

Plant collection

Plants were collected from an uncontaminated wetland in Sultanpur National Park (India).

Sludge collection

Metal contaminated sludge was used as a metal source for the plants. It was collected from Bhalswa waste dump situated on the northern outskirts of Delhi (India), where the wastes from Wazirpur Industrial Area are dumped. Wazirpur has metal pickling, rolling, and finishing industries so it had high metal content. At the waste dump, the industrial waste was mixed with municipal waste, so the organic matter and nutrient content was also high.

Preparation of sludge-dosed soils

After collection, the sludge samples were passed through 2-mm sieve and ground properly before mixing with soil. Garden soil was used as uncontaminated soil source. The garden soil and manure were also sieved through 2-mm sieve. Farmyard manure was added in the garden soil in 1:4 ratio. Metal-contaminated sludge was then dosed in different proportions viz. 20% (20S), 40% (40S), 60% (60S), and 80% (80S) in garden soil–manure mixture and mixed properly to obtain a homogeneous mixture. The garden soil–manure mixture without any sludge served as control (0S).

Plantation

P. distichum was planted in cement tubs measuring 40 × 40 × 35 cm. Soil depth in every tub was 30 cm. There were different tubs for different harvests. All treatments had three replicates. The plants collected were grown in garden soil–manure mixture, and new plantlets that emerged from these plants were then used for the experiment. Two plantlets were planted in each tub. All the tubs and buckets were watered frequently by tap water to maintain waterlogged condition.

The plants were grown for 6 months as the plants matured in this time period. The soil and plant samples were collected in plastic bags and stored properly for chemical analysis. Plant parameters were analyzed at every 30-day interval, and the heavy metal content was measured after 180 days of growth.

All the treatments were exposed to the natural weather conditions.

After harvest, the plants were removed carefully and washed first with tap water and then with double-distilled water to remove all soil and organic matter particles from the roots and plant surface. Plants were divided into belowground organs (BO), i.e., roots including rhizomes and aerial parts (shoots and inflorescence; referred as shoots in the text) and leaves and oven-dried at 80°C for 24 h. For chlorophyll, protein contents, and peroxidase activity, fresh plant tissues were used. Dry plant parts were ground in a glass pestle mortar and stored for chemical analysis. Total metal, nitrogen, phosphorus, and potassium were estimated in dried plant samples. Soil and plant samples were analyzed following standard methods. Total nitrogen and total phosphorous concentrations in the soil and plants were determined using acid digestion method (Anderson and Ingram 1989). The sequential extraction scheme of Tessier et al. (1979) with certain modifications was followed for fractionation of metals in exchangeable fraction (1 M $MgCl_2$), carbonates bound (1 M sodium acetate (pH 5) at 20°C), iron and manganese oxides bound (0.04 M hydroxylammonium hydrochloride *v*/*v* 25% acetic acid) at 96°C, organically bound (0.02 M HNO_3,30% H_2O_2(pH 2) 3.2 M ammonium acetate in 20% (*v*/*v*) HNO_3 and residue (by Method of Agemian and Chau 1976). After each step, the leachate was separated from the solution by centrifugation (5,000 rpm). The samples were then washed with distilled water and dried completely at 40°C before proceeding to the next step.

Plant samples were analyzed for metal, nitrogen, phosphorus, potassium (Bhargava and Raghupati 1993), chlorophyll (Arnon 1949), protein contents (Lowry et al. 1951), and peroxidase activity (Chance and Machly 1955). Metals viz., Cr, Mn, Zn, Cu, Ni, and Pb were analyzed in atomic absorption spectrophotometer (Shimadzu model AA6800) after digestion using air–acetylene gas mixture as fuel.

Electrophoresis of proteins in polyacrylamide gel was carried out in sodium dodecyl sulfate (SDS). Polyacrylamide gels were formed by polymerizing acrylamide with a cross linking agent (bis-acrylamide) in the presence of catalyst (persulfate ion) and chain initiator (TEMED). Solutions were degassed by evacuation prior to polymerization. The samples after mixing with equal volume of sample buffer were loaded in the wells, and the gel was run initially with 60 V and then to 120 V. The plates were then disassembled and the gel was stained with silver stain. With respect to the marker (20, 26, 36, 47, 85, 118 KD) the molecular weights of the protein bands in the samples were estimated.

The data were statistically analyzed using SPSS Version 10.

Results and discussion

Sludge characteristics

The sludge collected showed almost neutral pH. It also contained good amount of nitrogen, phosphorus, potassium, and organic carbon. The total metal concentrations were very high, especially for Cr and Mn (Table 1). Most of the total amount (31.87% to 98.77%) of all the six metals (Cr, Mn, Ni, Cu, Zn, and Pb) occurred in the residual form. The concentrations of different fractions in the control and sludge-dosed soils were generally in the order: Residual>>Fe–Mn oxide>organic bound>carbonate bound>exchangeable. However, the carbonate-bound fraction of Cr and Ni was less than its exchangeable fraction, and in case of Cu, its organic bound fraction was more than the Fe–Mn oxide-bound fraction.

Most available form to plant is the exchangeable fraction. Although carbonate-bound fraction become available at lower pH conditions, the pH of the sludge amended soil of this study was

Table 1 Characteristics of the sludge used for the study

Metals concentration (ppm)	Exchangeable	Carbonate fraction	Fe–Mn oxide fraction	Organic matter bound	Residual	Other parameters	
Cr	5.5 ± 1.01	3.4 ± 0.91	401.22 ± 11.98	151.01 ± 8.33	8581.32 ± 31.02	pH	7.01 ± 0.37
Mn	20.10 ± 2.87	72.10 ± 5.03	550.09 ± 2.09	81.55 ± 8.06	2522.09 ± 12.74	EC (μS/cm)	1450 ± 12.22
Ni	2.40 ± 0.76	1.80 ± 0.26	50.21 ± 9.33	38.41 ± 3.18	289.21 ± 8.12	Organic matter (%)	2.98 ± 0.03
Cu	4.99 ± 0.89	9.25 ± 1.32	167.39 ± 12.04	192.86 ± 14.77	799.22 ± 8.04	Total N (mg/g)	5.51 ± 1.01
Zn	6.09 ± 0.87	15.82 ± 1.27	110.32 ± 15.49	40.14 ± 6.87	310.70 ± 9.03	Total P (mg/g)	2.89 ± 0.52
Pb	5.38 ± 0.52	13.44 ± 1.02	210.50 ± 8.35	14.04 ± 1.07	220.57 ± 9.05	Total K (mg/g)	4.95 ± 1.13

neutral, but at the rhizosphere zone, acidic root exudates or change in soil physicochemical condition can mobilize the metals from this fraction. Under reducing conditions, Fe–Mn oxide-bound fraction can be released, whereas the organically bound metals may be released by the decomposition of organic matters by microorganisms (Tessier et al. 1979). The total metal concentration in sludge for the six metals studied were 9,142.45 ± 13.08 ppm for Cr, 3,245.93 ± 8.09 ppm for Mn, 382.03 ± 6.05 ppm for Ni, 1,173.71 ± 10.07 ppm for Cu, 483.07 ± 4.95 ppm for Zn, and 463.93 ± 6.08 ppm for Pb. The metal concentrations are quite high, but the present study failed to compare the results with Indian standards for soil as the Ministry of Environment and Forest has not yet established any standard for heavy metal content in polluted soil or sludge. The sludge-containing heavy metals are treated as hazardous waste in India. So, the heavy metal contents were compared with the permissible limits of heavy metals in agricultural soil in India. The limit for Cr, Mn, and Ni were not available; limits for Cu, Zn, and Pb were 135–270, 300, and 250 ppm, respectively (Awashthi 2000). As per State Environmental Protection Administration of China, 1995 the standard limit for Cr, Cu, Zn, and Pb were 250, 100, 300, and 350 ppm, respectively (Islam et al. 2009). Standards for Mn and Cr were not available. For Mn and Cr, standards given by Thailand Government were compared, which were 1,800 and 300 ppm, respectively. It was found after comparison with various standards that the heavy metal content of the sludge used in the present study was quite high.

Garden soil–manure mixture characteristics

The pH, electrical conductivity (μS/cm), organic matter (%), total N, P, K content (mg/g) of the garden soil–manure was 7.68 ± 0.1, 200 ± 10, 2.48 ± 0.31, 1.86 ± 0.41, 0.736 ± 0.21, 1.93 ± .32, respectively. Sand, silt, and clay composition of the same were 68%, 21.9%, and 10.1%, respectively.

Metal accumulation

Tissue concentration of all the metals in all the organs of the treated plants showed an increase with increasing sludge levels after 180 days of growth. However, growth was not hindered by the higher sludge dose. Higher tissue concentration was observed in belowground organs and compared to shoots (Fig. 1a–c). Among the six metals studied, the plant accumulated Cr in maximum concentration followed by Mn, Cu, Zn, Ni, and Pb. The ranges of belowground organ concentration of Cr, Mn, Cu, Zn, Ni, and Pb in sludge-dosed plants were 1,778.65–4,288.01, 828.11–1,360, 236.52–330.07, 155.79–282.35, 27.05–91.16, and 27.09–50.87 ppm, respectively. Similarly, for shoot, the ranges were 41.97–261.35, 68.87–70, 46–68.57, 16.88–27.54, 7.44–13.70, and 2.98–5.19 and

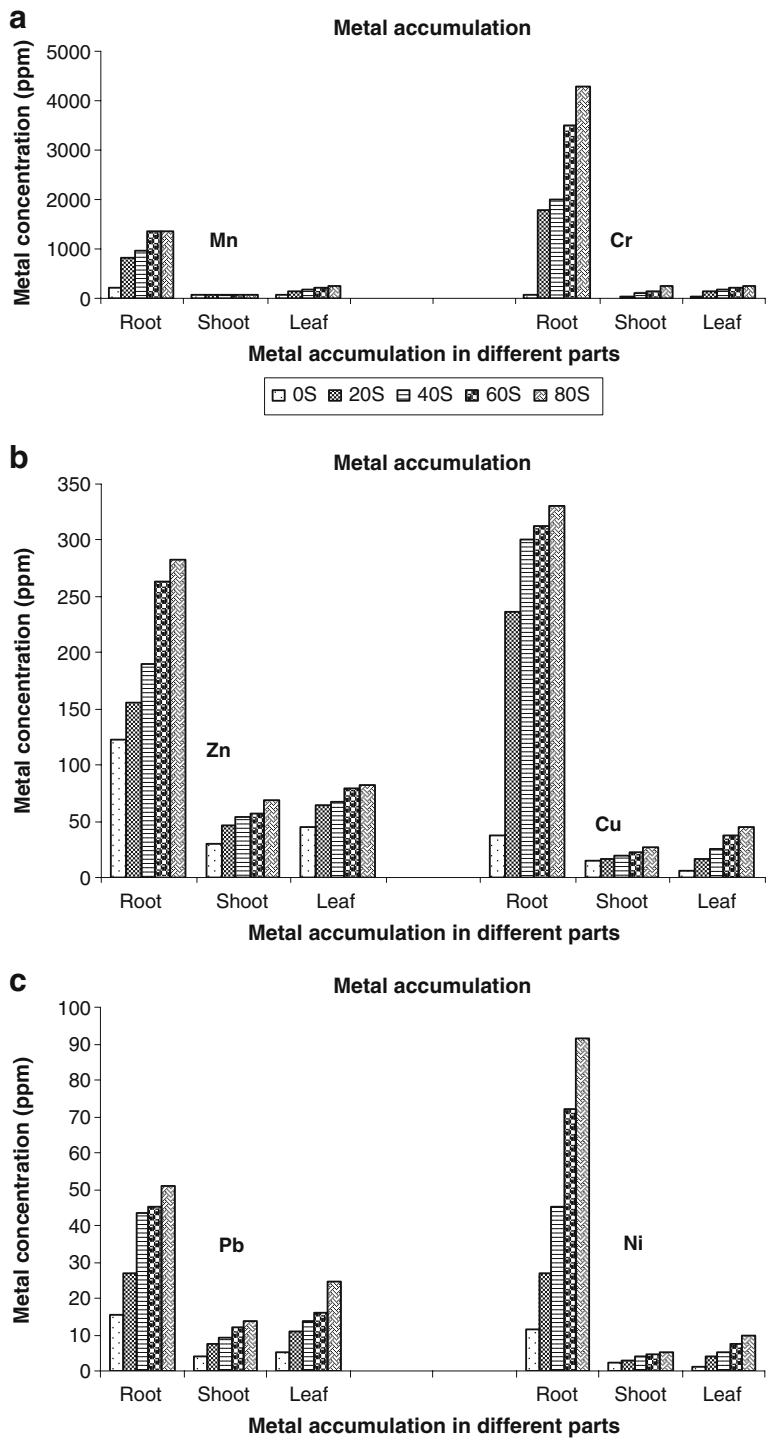

Fig. 1 **a–c** Metal accumulation in different parts of *P. distichum* grown on different sludge amended soil after 180 days of growth

for leaf, 155.9–257.21, 159.35–238.14, 64.33–82.05, 15.9–45.21, 11.09–24.35, and 3.97–9.47 ppm, respectively. These ranges corroborate with other studies on metal accumulation of wetland plants (Zayed et al. 1998; Mehra et al. 2000; Deng et al. 2004; Bhattacharya et al. 2006; Gatti et al. 2007).

From the experimental data, we can infer that the plant did not behave as hyperaccumulator, as hyperaccumulators are the plants whose shoots contain >1,000 mg/kg Ni, Pb, and Cu, or >10,000 mg/kg Zn and Mn (dry wt) when grown in metal-rich soils (Baker and Brooks 1989; Baker et al. 1994). In the present study, *P. distichum* followed exclusion strategy as it accumulated most of the metals in belowground organs and translocation to the aboveground parts was meager. Among the six metal Cr was also very poorly translocated in the aboveground plant parts. Very poor translocation of Cr to the aboveground parts might be due to the retention of most of the Cr in the vacuoles of root cells and more specifically in the protoplasmic fractions of the roots in soluble form, as shown by previous studies of Lytle et al. (1998) and Gupta and Sinha (2006) on wetland plants.

Effect of heavy metals on nutrient (N, P, K) content

The N concentration of belowground organs shoots and leaves of control plants increased gradually with time throughout the study period (Fig. 2a). However, the N concentrations in these organs increased rapidly with the increasing levels of sludge, only up to 90 or 120 days but thereafter decreased so fast that the tissue concentrations of N in plants at higher sludge levels were lower than that in the control plants after 180 days growth. Only in case of the shoots of 20S plants did the N concentration not decrease until 180 days growth.

ANOVA shows that the difference in N concentration of belowground organs, shoot, and leaf between control plants and 20S plants were not significant at $p = 0.05$. The difference in leaf and shoot N concentration between 20S and 40S plants was also not significant. However, the differences in N concentration of belowground organs of 20S and 40S, 40S and 60S, 60S and 80S plants and of the leaves of 40S and 60S and 60S and 80S plants were significant. So, it was evident from the results that the different sludge dose had effect on the nitrogen uptake of the plant.

The changes in P concentration of belowground organs, shoots, and leaves of plants grown in soils with different sludge levels (Fig. 2b) followed the same trend as N. The P concentrations increased throughout the 180 days' growth in the control plants but in plants grown on sludge-dosed soils; the P concentration increased up to 120th day and later decreased to a concentration significantly below that in the control plants. The P concentration was, however, greater with higher levels of sludge. The differences were relatively small but significant.

The data on the K concentration in the belowground organs, shoots, and leaves of *P. distichum* (Fig. 2c) show that the K concentration increased throughout the growth period at all levels of sludge and that the tissue concentrations in all plant parts increased with increasing levels of sludge. In the belowground organs, K concentration of sludge-treated plants was much higher than in the control plants, but in case of shoots and leaves, the differences between control and 20S plants were not significant. The differences in tissue concentration between different sludge levels were significant at $p = 0.05$ (F values not shown).

The above-results manifest that both N and P concentrations in tissues increased up to a certain growth period. This was expected as at initial growing phase, the plants take up huge amount of nutrients if nutrient supply is plenty in soils, but as the plant reaches its maturity, the uptake rate decreases. A decrease in uptake in latter stage of growth was due to the "dilution effect". According to this phenomenon due to increment of biomass, the tissue concentration gets diluted or decreases (Roongtanakiat and Chairoj 2001; Chaignon and Hinsinger 2003; Rate et al. 2004; Perez-Espinosa et al. 2005). However, the decrease may be due to the effect of heavy metal accumulation (Gupta and Chandra 1996, 1998). Srivastava and Jaiswal (1989) reported that decrease in P concentration may be also due to increased phosphatase activity under metal stress.

Effect on chlorophyll concentration

The chlorophyll concentration of the shoots (Table 2) increased with increasing sludge levels during the early stages of growth (up to 90 days), but thereafter, it decreased with the increasing level of sludge. According to ANOVA,

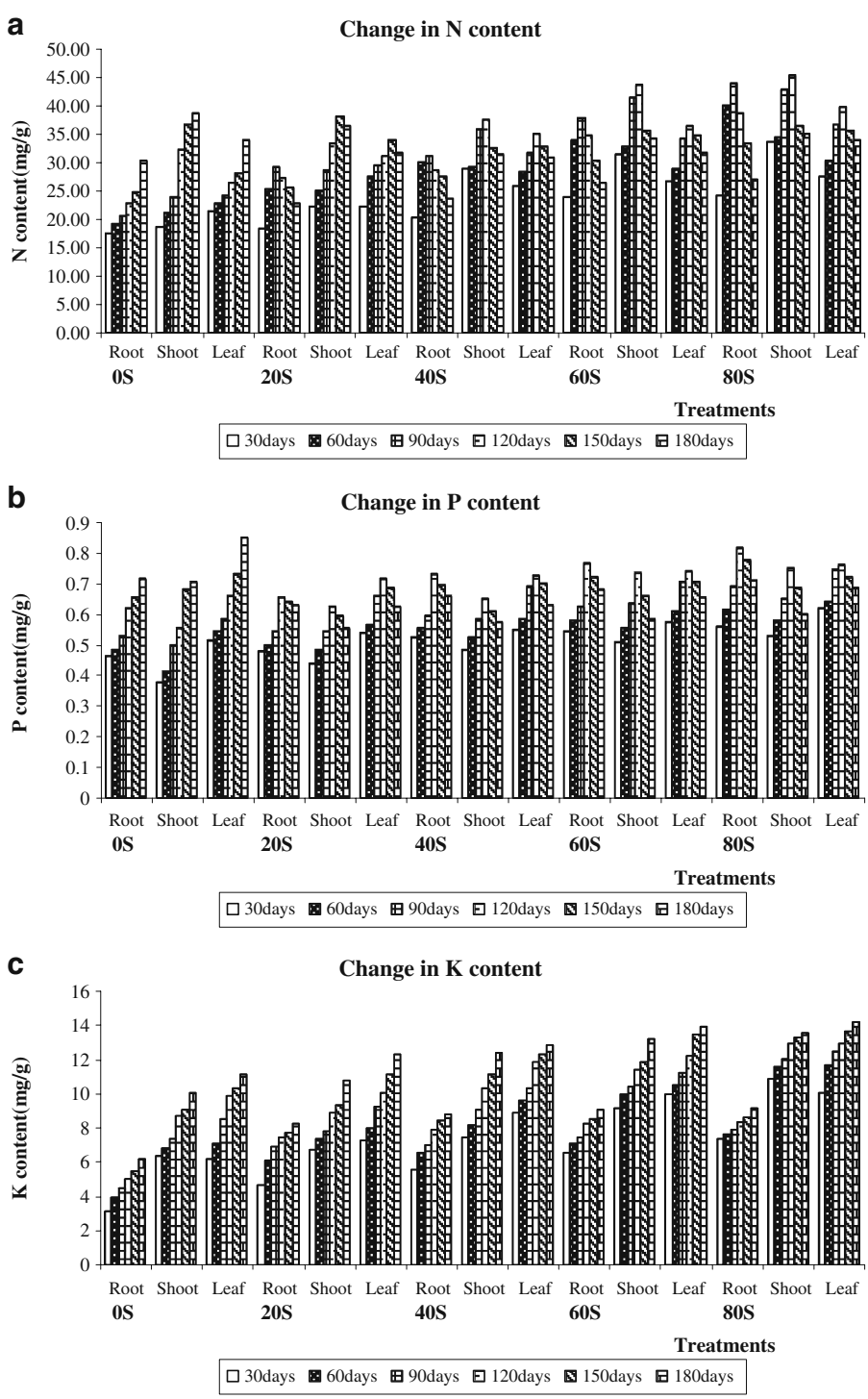

Fig. 2 Change in N, P, K content in different plant parts of *P. distichum* grown on different sludge amended soil after different harvests (30, 60, 90, 120, 150 and 180 days)

the variations in total chlorophyll concentration of shoots between different treatments were all significant at 0.05 levels, except between the 0S and 20S shoots. The chlorophyll concentration in leaves was much higher than in the shoots. It increased with both the sludge level and time up

Table 2 Effect of heavy metals on chlorophyll

	0S	20S	40S	60S	80S
Chlorophyll content (mg/g) in shoot					
30 days	0.225 ± 0.021	0.183 ± 0.042	0.233 ± 0.047	0.258 ± 0.042	0.288 ± 0.027
60 days	0.256 ± 0.043	0.202 ± 0.051	0.240 ± 0.058	0.262 ± 0.048	0.296 ± 0.015
90 days	0.269 ± 0.031	0.232 ± 0.087	0.234 ± 0.031	0.245 ± 0.012	0.267 ± 0.036
120 days	0.280 ± 0.008	0.261 ± 0.023	0.235 ± 0.065	0.217 ± 0.031	0.213 ± 0.058
150 days	0.288 ± 0.027	0.273 ± 0.084	0.213 ± 0.037	0.195 ± 0.049	0.185 ± 0.027
180 days	0.296 ± 0.072	0.285 ± 0.091	0.202 ± 0.028	0.180 ± 0.088	0.175 ± 0.012
F values (ANOVA)	16.19	0.068^{NS}	$1.42x10^{-5NS}$	1.24^{NS}	
Chlorophyll content (mg/g) in leaf					
30 days	2.35 ± 0.016	2.44 ± 0.032	2.49 ± 0.474	3.08 ± 0.395	3.24 ± 0.109
60 days	3.57 ± 0.033	3.87 ± 0.301	4.44 ± 0.508	4.66 ± 0.106	5.14 ± 0.121
90 days	4.25 ± 0.030	4.82 ± 0.204	4.28 ± 0.931	4.40 ± 0.031	4.91 ± 0.027
120 days	4.54 ± 0.329	4.32 ± 0.115	4.03 ± 0.652	4.13 ± 0.119	4.32 ± 0.093
150 days	4.73 ± 0.077	4.00 ± 0.110	3.83 ± 0.701	3.91 ± 0.305	4.07 ± 0.028
180 days	4.87 ± 0.063	3.57 ± 0.028	3.73 ± 0.096	3.55 ± 0.094	3.63 ± 0.047
F values (ANOVA)	0.57^{NS}	0.0507^{NS}	2.32^{NS}	12.45	

NS nonsignificant, given *F* values are for variation between the treatments

to 60 days' growth; afterwards, the concentration declined slowly in the plants grown on sludge-amended soils. The total chlorophyll concentration in sludge-grown plants remained higher than in the control plants until 90 days growth. In control plants, the leaf chlorophyll concentration increased throughout the growth period. The variation in total chlorophyll content between different treatments is all nonsignificant except 60S and 80S, which was significant. The increase in chlorophyll concentration in the early phase of growth was more rapid due to high photosynthetic activity. As the plant reached maturity, the rate of increase in chlorophyll concentration decreased. During initial days of growth, chlorophyll content increased with sludge dose, primarily because supply of metals helped in synthesis of chlorophyll. But as the metal exposure increased in higher sludge amended soils chlorophyll content decreased. Temporal decrease in chlorophyll in the plants was because the heavy metals can substitute the central Mg ion or can inhibit chlorophyll synthesis by inhibiting chlorophyll synthesizing enzyme activity (Gupta and Chandra 1996; Ewais 1997; Prasad and Strzallka 1999; Manios et al. 2003). Decrease in shoot chlorophyll content with time was also due to the production of non-chlorophyllous tissues by shoots. Finally, it can be said that the decrease in chlorophyll concentration was not so much that it can affect the plant growth, as growth was not restricted throughout the experiment.

Effect on protein concentration

Protein concentration in all the organs of control plants increased with time. In the sludge-treated plants, the protein concentration in belowground organ increased until the 90th day then decreased with time to a concentration notably below that in the control plants (Table 3). The ANOVA shows that variation in belowground organs protein concentration between 0S–20S plants and 40S–60S plants were nonsignificant but significant between 20S–40S and 60S–80S. The shoot and leaf protein concentration increased until the 120th day in the sludge-treated plants and decreased later except in 20S plants where the shoot and leaf protein concentrations increased until 150th day. The shoot and leaf protein concentrations increased with the sludge dose up to 120th day and showed little variation in the plants grown on higher sludge levels thereafter. Variation in shoot protein concentration between the different treatments was significant except between 20S and 40S plant. In case of leaves, the variation was nonsignificant between 0S and 20S and 20S and 40S plants and significant between 40S and 60S and 60S and 80S

Table 3 Effect of heavy metals on protein content

	0S	20S	40S	60S	80S
Protein content (mg/g) in below ground organs					
30 days	2.46 ± 0.152	2.58 ± 0.135	2.90 ± 0.214	3.83 ± 0.112	3.9 ± 0.017
60 days	2.86 ± 0.075	4.69 ± 0.089	5.13 ± 0.124	5.22 ± 0.087	5.34 ± 0.214
90 days	2.97 ± 0.057	4.92 ± 0.247	5.65 ± 0.227	5.56 ± 0.219	6.14 ± 0.086
120 days	3.03 ± 0.014	3.25 ± 0.314	4.57 ± 0.145	5.24 ± 0.128	5.81 ± 0.175
150 days	3.4 ± 0.037	3.14 ± 0.037	4.42 ± 0.038	4.52 ± 0.083	5.01 ± 0.119
180 days	4.78 ± 0.127	3.05 ± 0.020	3.38 ± 0.014	3.45 ± 0.088	3.63 ± 0.317
F values (ANOVA)	0.008^{NS}	12.88	3.17^{NS}	12.03	
Protein content (mg/g) shoot					
30 days	2.27 ± 0.012	2.85 ± 0.131	3.09 ± 0.021	3.81 ± 0.037	4.45 ± 0.011
60 days	2.98 ± 0.022	3.54 ± 0.091	3.91 ± 0.043	4.2 ± 0.073	4.83 ± 0.021
90 days	3.12 ± 0.031	3.94 ± 0.088	4.49 ± 0.038	5.44 ± 0.051	5.59 ± 0.042
120 days	3.52 ± 0.027	4.67 ± 0.105	5.22 ± 0.081	5.68 ± 0.035	6.48 ± 0.067
150 days	3.89 ± 0.101	5.04 ± 0.033	4.63 ± 0.064	4.81 ± 0.014	4.99 ± 0.034
180 days	4.21 ± 0.112	3.98 ± 0.024	3.18 ± 0.066	3.79 ± 0.022	3.92 ± 0.051
F values (ANOVA)	10.3	0.133^{NS}	21.26	11.81	
Protein content (mg/g) leaf					
30 days	3.33 ± 0.003	3.41 ± 0.011	3.8 ± 0.002	4.25 ± 0.043	4.93 ± 0.027
60 days	3.54 ± 0.009	4.42 ± 0.021	4.39 ± 0.005	5.09 ± 0.033	5.27 ± 0.024
90 days	3.73 ± 0.014	4.9 ± 0.009	4.77 ± 0.015	5.46 ± 0.042	6.18 ± 0.028
120 days	3.89 ± 0.011	5.08 ± 0.046	5.47 ± 0.011	5.91 ± 0.022	6.94 ± 0.009
150 days	4.40 ± 0.031	5.34 ± 0.083	5.22 ± 0.009	5.24 ± 0.031	6.02 ± 0.031
180 days	5.60 ± 0.022	5.13 ± 0.075	4.79 ± 0.014	5.02 ± 0.011	5.46 ± 0.019
F values (ANOVA)	5.26^{NS}	0.047^{NS}	1.53	28.34	

NS nonsignificant, given *F* values are for variation between the treatments

plants. The decrease in protein content after prolonged metal exposure may be due to increased activity of protease or other catabolic enzymes, which are activated by heavy metals and destroy proteins (Gupta and Chandra 1996; Sinha et al. 1996).

Gel electrophoresis of proteins

The protein profile of the plants and their parts was examined to find the impact of heavy metal accumulation, manifested as visible symptoms of physiological, biochemical effects, and nutritional imbalances. The plants after 180-day growth were used for protein profiling. There were significant changes in the protein profiles of belowground organs. The changes could be visibly detected from 40% sludge treatment (Fig. 3). Apart from the initial proteins present in 0% sludge treatment, high molecular weight protein of 118 KD and one band lying between 47 and 85 KD protein were induced. Maximum number of seven bands was observed in 60% and 80% sludge treatment. On the contrary, *P. distichum* shoots showed no significant variation with increasing metal

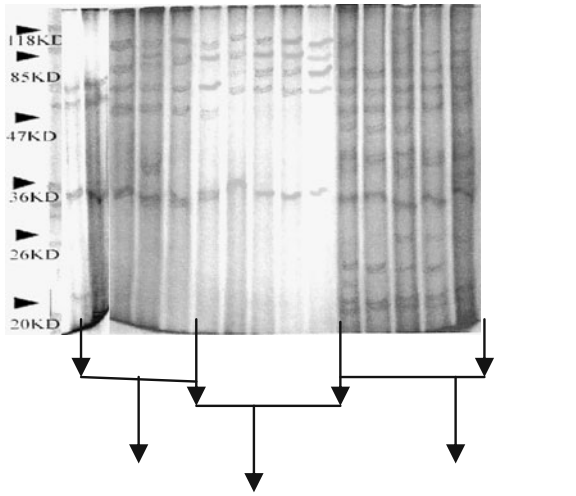

Fig. 3 Gel profile

accumulation (greater sludge treatment). As seen in lanes 6–10, there was a uniform presence of five bands in all the lanes.

Interestingly, though the *P. distichum* leaf showed a large number of protein bands, variation was very small with increasing proportion of sludge. The only exception was in 80% treatment, where five additional bands were observed, one lying between 47 and 85 KD, two bands near 47KD, one between 36 and 47 KD, and one between 20 and 26 KD.

Increase in number of protein bands due to increase in sludge dose may be because of synthesis of new proteins like phytochelatins to combat metal stress. This can also be seen in SDS gel profile of belowground organs of plants treated with more sludge showed additional protein bands. Leaves of 80% sludge-amended plants showed five additional protein bands. Thus, it can be said that enhanced metal accumulation in leaf culminated in the synthesis of these bands, which helps the species to cope up with the stress and impaired metabolism due to increasing sludge treatment and exposure to metal levels.

Effect on POD activity

The peroxidase activity increased with time as well as with the increase in sludge dose in both belowground organs and leaf (Table 4). Similar trend was also reported by Mohan and Hosetti (1997), Macfarlane and Burchett (2001), and Pang et al. (2003). Increase in the peroxidase activity with time in plant belowground organs and leaf of control plants was not much as compared to the plants grown in sludge-dosed plants. However, peroxidase activity was more in leaves than in the belowground organs. The differences in POD activity in belowground organs and leaf between different treatments were significant at $p > 0.05$.

The increase of POD activity is related to oxidative reactions corresponding to an increase in peroxides and free radicals in the plant cells due to metal stress. These reactive oxygen species are quenched by induction of specific enzymes such as peroxidase (Hippeli and Elstner 1996; Van Loon 1986). In higher plants, induction of POD is a general response to uptake of toxic amounts of metals. Finally, an increase in POD activity can also be due to plant aging or senescence of the plants (Mohan and Hosetti 1997). In the present study, POD activity was more in leaves than belowground organs. Higher POD activity in leaves might be because they are sites of high metabolic rate processes and are exposed to various pollutants, which are taken up by roots and translocated to leaves. Consequently, the defense mechanism is quite strong in leaves (Pflugmacher et al. 1999).

Table 4 Effect of heavy metals on POD activity

	0S	20S	40S	60S	80S
POD activity (μmol H_2O_2 destroyed/g fresh wt/min) in below ground organs					
30 days	80.03 ± 3.15	120.95 ± 5.25	133.12 ± 2.49	145.62 ± 8.29	168.87 ± 4.61
60 days	93.64 ± 5.91	156.74 ± 8.63	224.72 ± 11.26	233.21 ± 7.61	250.95 ± 8.16
90 days	105.36 ± 2.84	161.15 ± 3.44	252.11 ± 7.03	260.21 ± 9.31	275.19 ± 2.77
120 days	112.19 ± 1.45	183.03 ± 6.81	271.55 ± 5.25	285.91 ± 13.7	296.22 ± 1.73
150 days	125.61 ± 4.62	201.59 ± 11.4	294.10 ± 10.09	315.66 ± 6.73	321.15 ± 2.18
180 days	141.42 ± 7.16	255.11 ± 8.57	337.20 ± 8.61	356.38 ± 13.46	380.57 ± 6.73
F values (ANOVA)	48.41	33.08	39.01	28.78	
POD activity (μmol H_2O_2 destroyed/g fresh wt/min) in leaf					
30 days	253.45 ± 1.56	333.79 ± 4.22	353.45 ± 2.49	474.29 ± 5.33	538.13 ± 10.51
60 days	268.74 ± 4.92	386.45 ± 4.65	429.93 ± 11.26	546.45 ± 2.55	633.33 ± 4.28
90 days	271.15 ± 2.08	424.49 ± 10.52	456.55 ± 7.03	582.11 ± 2.84	689.42 ± 9.52
120 days	280.11 ± 5.19	459.22 ± 6.18	494.19 ± 5.25	631.22 ± 2.53	714.46 ± 3.08
150 days	293.22 ± 2.28	488.61 ± 2.92	522.21 ± 10.09	673.91 ± 6.18	769.05 ± 11.35
180 days	305.05 ± 2.45	538.09 ± 5.61	561.09 ± 8.61	688.15 ± 10.57	793.21 ± 7.73
F values (ANOVA)	50.64	78.14	620.79	189.04	

NS nonsignificant, given *F* values are for variation between the treatments

Conclusions

Finally, it can be concluded that *P. distichum* took up considerable amounts of heavy metals from the sludge-amended soils. The plant behaved as good accumulator of Cr and Mn and moderate accumulator of Ni, Cu, Zn, and Pb. The tissue concentration of metals in plants increased with their increasing concentration in the soil. However, all metals accumulated in belowground organs in higher concentration. Thus, the plant appears to follow an exclusion strategy by restricting the metals to translocate to the aboveground parts. So, the plant can be used as a good phytostabilizer as it will reduce the bioavailability of metals by binding the metals in roots. Other studies also reported the phytostabilizing capability of other wetland plants such as *Phragmites australis*, *Typha latifolia*, *Scirpus littoralis* and *P. distichum* (Azaizeh et al. 2006; Bhattacharya et al. 2006; Shu et al. 2002).

The metabolic parameters of the plant, viz chlorophyll, protein, and POD activity and the nutrient content were not adversely affected by the accumulation of heavy metals. It is concluded that *P. distichum* has innate tolerance or has developed defense mechanisms against heavy metal toxicity. The high POD activity and synthesis of new protein bands in high sludge-dosed plants also indicate this. Finally, *P. distichum* can be used as a good phytostabilizer of metals in highly metal contaminated soils.

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