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Phytoremediation for Defending Heavy Metal Stress in Weed Flora

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Abstract

Degradation of natural resources is perhaps one of the gravest lapses mankind has ever made in its journey of progress and development. Land, air and water resources are worst affected due to anthropogenic interventions. Heavy metal contamination is of special concern due to widespread reports emanating both from India and abroad about various diseases and disorders observed both in human and livestock due to metal toxicity. The use of specially selected and engineered metal accumulating plants for environmental clean up is an emerging frontline technology called 'Phytoremediation' which describes a system wherein plants alone or in association with soil organisms can remove or transform contaminants into harmless and often valuable forms. Excessive heavy metal accumulation can be toxic to most plants leading to reduction in seed germination, root growth and biomass production; inhibition of chlorophyll biosynthesis as well as disturbance in cellular metabolism and chromosome distortion. For studying the heavy metals load of sewage and sludge and their effect on crop quality in relation to non applied sites, solid sludge and leafy vegetable plants such as *Amaranthus spp*., *Ipomoea spp*, *Basella spp.* (Pui), *Spinacia oleracea* (Palak), were collected from seven STPs viz. Howrah, Garulia, Bhatpara, Nabadwip, Srirampur, Kona, Chandannager, and from the Periurban areas viz., Nadia/Chakdaha/Ektapur(N/C/E),Nadia/Chakdaha/Pumlia(N/C/P),Nadia/Chakdaha/Sikarpur(N/C/S), Nadia/Chakdaha/Tatla(N/C/T). The results suggest that leafy vegetables like *Amaranthus* have more scavenging capacity for Cd and Pb, while *Spinacia oleracea* has more scavenging capacity for Cr. Therefore, if these plants are cultivated in sites abundant with above heavy metals, these would scavenge the heavy metal toxicity from the soil.

Highlights

- Heavy metal contamination is of special concern due to widespread reports emanating both from India and abroad about various diseases and disorders observed both in human and livestock due to metal toxicity.
- *Amaranthus viridis (red)* is the best accumulator of Pb and Cd, but the best Cr accumulator is *Spinachea oleracea*. Phytoremediation test showed that the amounts of heavy metals decreased in polluted soils under the effect of these weeds plants.
- These weeds can be utilized as effective accumulators for phytoremediation of heavy metal-polluted soils.based on the total numbers of bands, banding pattern, intensity of bands, specific presence or absence of bands.

Keywords: *Amaranthus*, Anthropogenic, Heavy metal, Phytoremediation, Sludge, Weeds

Phytoremediation is the use of plants to partially or substantially remediate selected contaminants in contaminated soil, sludge, sediment, ground water, surface water and waste water. It utilizes a variety of plant biological processes and the physical characteristics of plants to aid in site remediation. Phytoremediation has also been called green remediation, botano-remediation, agro remediation and vegetative remediation. Phytoremediation is a continuum of processes, with different processes occurring at differing degrees for different conditions, media, contaminants and plants. The primary considerations for ground-water contamination are the depth to the ground water and the depth to the contaminated zone. About 30 billion liters of wastewater is generated in India producing 1200 tons of sludge a day. Sewage is the main point-source pollutant on a global scale (Gijzen, 2002). Between 90 - 95% of the sewage produced in the world is released into the environment without any treatment (Barton *et al*., 1994). In India, about 40% sewage and domestic waste water is disposed off on land that contaminate the soil and aqueous stream with large quantities of toxic metals (Seaward and Richardson, 1990) and the remaining is drawn into different water bodies with or without little treatment. Concern over the possible built up of heavy metals in ground water resulting due to heavy land application of sewage sludge, industrial and city effluents have prompted research on the fate of these chemicals in soils. On one hand, the flow of metals to ground water as their ultimate sink is continuously taking place through geogenic or anthropogenic causes which are difficult to control, and on the other hand , the obvious question which comes to our mind is how to get rid of this metal load from ground water. The issue of decontaminating ground water from metal is even more difficult to address due to strong sorption properties of metals with soil colloids and consequent long residence time of these metals in soil. The use of specially selected and engineered metal accumulating plants for environmental clean up is an emerging frontline technology called 'Phytoremediation' which describes a system wherein plants in association with soil organism can remove or transform contaminants into harmless and often valuable and adsorb pollutant, mainly metals, from water bodies and aqueous waste streams (Rakshit *et al*., 2009; Pal *et al*., 2013). The objective of this study was to investigate the heavy metal scavenging capacity of weed flora such as *Amaranthus spp*., *Ipomoea spp*., *Basella spp*. (Pui), *Cynodon spp*. and *Spinacia oleracea* (Palak).

Materials and Methods

Sampling

For studying heavy metals load of sewage sludge and their effect on crop quality in relation to non applied sites, solid sludge and plants were collected from seven sewage treatment plants (STPs) viz., Howrah, Garulia, Bhatpara, Nabadwip, Srirampur, Kona, Chandannager and from the Periurban areas viz., Nadia/Chakdaha/Ektapur (N/C/E), Nadia/Chakdaha/Pumlia (N/C/P), Nadia/Chakdaha/Sikarpur (N/C/S), Nadia/Chakdaha/Tatla (N/C/T). Sludge samples were taken from heaps from various places in the pile of each plant, using an auger. The samples were generally not taken from the outer layer of the heap, as the material tended to be very dry in those places. Afterwards 6-10 individual samples were all mixed together and one average sample was compiled for analysis.

Sample preparation and storing

Sludge samples were air dried grounded and sieved through 80 mesh sieve. Then the samples were stored in refrigerator after packing in polyethylene bags. Plant samples were cleaned thoroughly with 0.01N HCl followed by distilled water and finally dried followed at 60°C and grinded.

Experimental Methods

1. Physico chemical properties of soil/sludge were determined by following methods:
   a) pH- Digital pH meter, Elico127 (Jackson, 1973)
   b) Oxidizable organic carbon- Walkley-Black (Nelson and Summers, 1982).
   c) Available N- Permanganate method (Subbia and Asija, 1956).
   d) Available K- Flame Photometer (Jackson, 1973).
2. Total heavy metal content of sludge: 0.5 g of the processed samples were digested with di-acid mixture (HNO_3:$HClO_4$, 9:4) on a hot plate (APHA, 1992). The clear solutions were filtered through Whatman No 42 filter paper and diluted to 50 ml for analysis by Atomic Absorption Spectrophotometer (GBS-902, Australia).
3. Heavy metal content in plant samples: 0.5 g of dried sample was digested with di-acid mixture (HNO_3:$HClO_4$, 9:4) on a hot plate (APHA, 1992), until the discoloration of solution.
4. Calculation of DIM value: The daily intake of metals (DIM) was calculated by the following equation:

DMI= [M]x [K]x [I]/ [W]

where, M, K, I and W represent the heavy metal concentrations in plants (mg kg^{-1}), conversion factor, daily intake of leafy vegetables and average body weight, respectively. The conversion factor used to convert fresh green leafy vegetable weight to dry weight was 0.085, as described earlier (Rattan *et al*., 2005).

Results and Discussion

Heavy metals occur in the soil as exchangeable and adsorbed forms and are found on soil colloids. They are specifically-adsorbed, bound in various chemical compounds (oxides, carbonates, phosphates, sulphides) and are structurally bound in silicates (primary and secondary minerals) (Adriano, 2001). Different factors affect the bonding of heavy metals to the soil and it is difficult to estimate the heavy metal load in the soil. The load of heavy metals in the soil can be known to some extent, by monitoring their content in the soil. The pH of all the sludge was acidic in reaction slightly varying from 5.36 to 6.85. Lowest value of pH 5.36 and 5.83 was observed in the sludge of Howrah and Bhatpara, respectively. Highest value of pH viz. 6.85 and 6.72 was observed in soil of Nadia/Chakdaha/Sikarpur and Nadia/Chakdaha/Ektapur, respectively (Table 1, Fig. 1A).

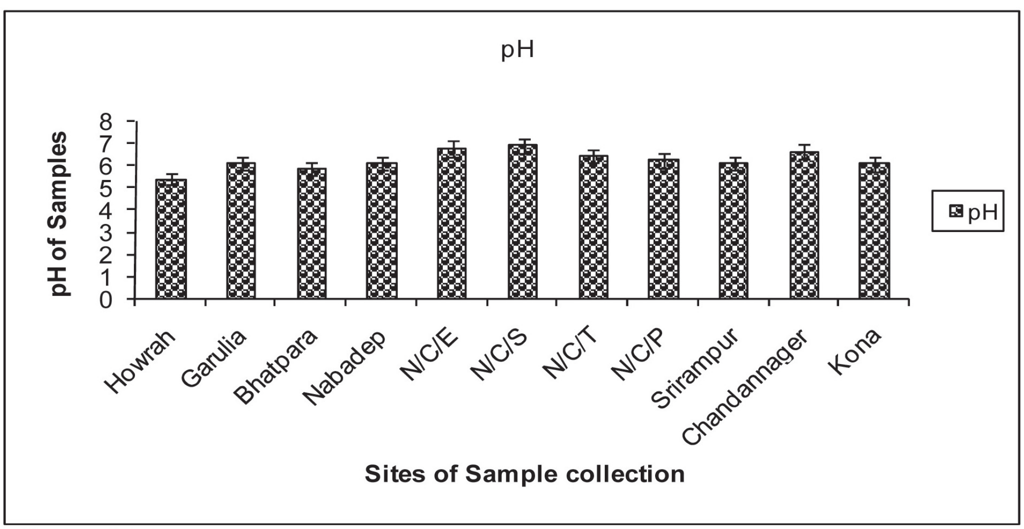
Oxidizable organic carbon: Organic carbon of all the sludge of STPs were very high ranging from 3.3 to 6.78 % , and in soils of periurban area, it ranged between 0.63 to 0.78%. Lowest value of oxidizable organic carbon viz. 0.63% and 0.65% was observed in the soils of Nadia/ Chakdaha/Tatla and Nadia/Chakdaha/Sikarpur, respectively. The Oxidizable organic carbon of sludges of different STPs

Table 1: Physico-chemical properties of soil/sludge

Site	pH	Organic Carbon (%)	Available Nitrogen (Kg/ha)	Available Potassium(Kg/ha)
Howrah	5.36	5.38	592	969.42
Garulia	6.04	3.3	248	460.65
Bhatpara	5.83	6.78	472	838.32
Nabadep	6.05	6.33	284	472.27
N/C/E	6.72	0.68	86	116.61
N/C/S	6.85	0.65	72	77.19
N/C/T	6.40	0.63	122	224.93
N/C/P	6.19	0.78	162	688.9
Srirampur	6.06	5.22	264	65.57
Chandannager	6.56	4.08	274	711.7
Kona	6.03	5.64	112	303.78
Mean Value	6.17	3.58	244.18	439.03

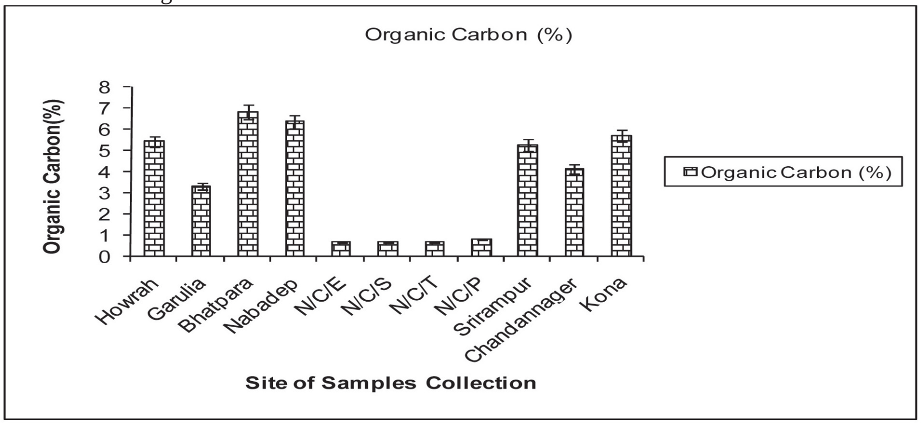
LSD (P= 0.05)

A. pH



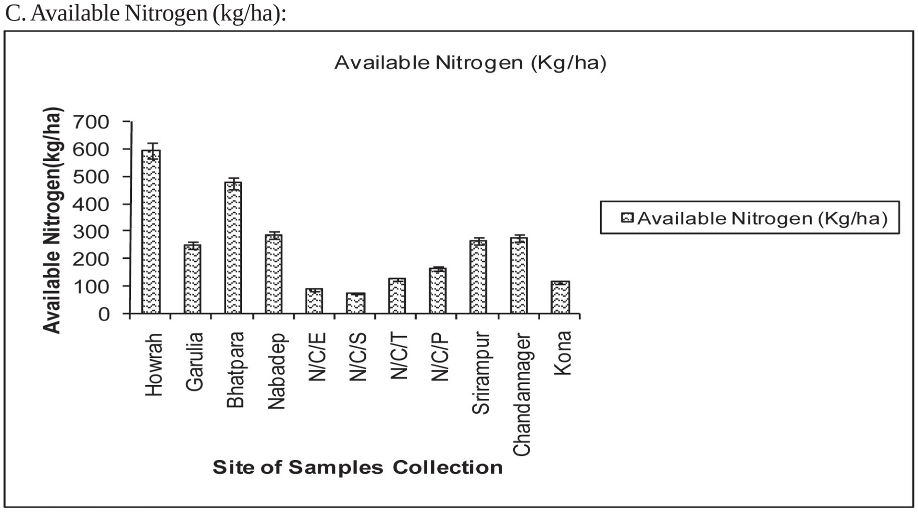
LSD (P= 0.05)

B. Oxidizable organic carbon



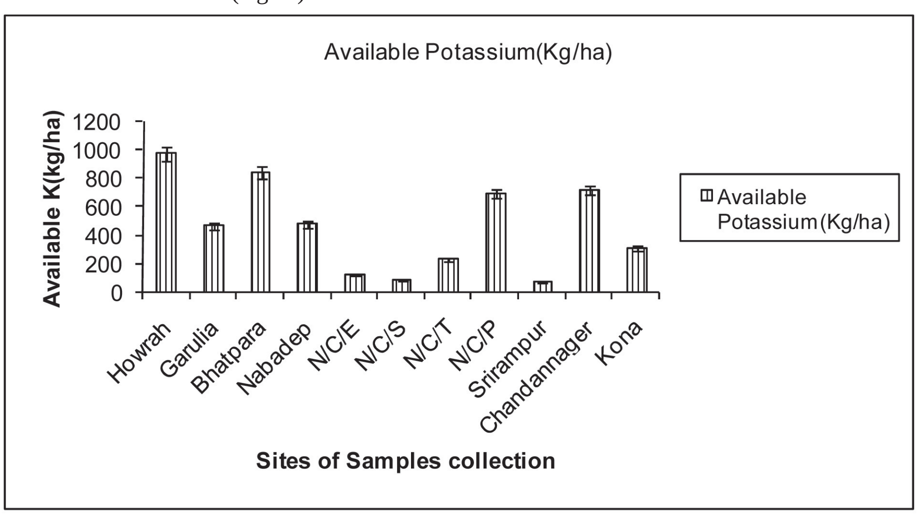
s
LSD (P= 0.05)

C. Available Nitrogen (kg/ha):



LSD (P= 0.05)

D. Available Potassium (Kg/ha):



LSD (P= 0.05)

Fig. 1: Physico chemical properties of soil/sludge

viz. Howrah, Garulia, Bhatpara, Nawadep, N/C/E, N/C/S, N/C/T, N/C/P were 5.36, 3.30, 6.78, 6.33, 0.68, 0.65, 0.63, 0.78, 5.22, 4.08, 5.64%, respectively (Table 1, Fig. 1B).

Available Nitrogen (kg ha^{-1}): Available Nitrogen of all the sludge of different STPs varied from 112 to 592 kg N. However, these values in soil of periurban area ranged from 86 to 162 kg N. The available nitrogen of sludges of different STPs viz., Howrah, Garulia, Bhatpara, Nawadep, N/C/E, N/C/S, N/C/T, N/C/P were 592, 248, 472, 284, 86, 72, 122, 162, 264, 272, 112 Kg N, respectively (Table 1, Fig.1C).

Available Potassium (Kg ha^{-1}): Available potassium of all the sludges of different STPs were very high and varied from 303.68 to 869.42 kg K/ha. However, these values in soil of periurban area ranged from 65.57 to 116.61 kg K/ha. The available potassium of sludges of different STPs viz., Howrah, Garulia, Bhatpara, Nabadep, N/C/E, N/C/S, N/C/T, N/C/P were 869.42, 460.65, 838.32, 472.27, 116.61, 77.19, 224.93, 688.9, 65.57, 711.7, 303.78 K/ha respectively (Table 1, Fig. 1D)

Comparative study of heavy metals concentration in the roots and shoots of different weeds species

Analysis of data (Table 2) showed significant correlation coefficient between the content of heavy metals in weeds parts and soil/sludge. Plants incorporated the heavy metals from the soil solution, but plants also adopted some forms of heavy metals from the atmospheric deposition through their photosynthetic organs. Linearity dependence was found between the total heavy metal content, the soils and plants for all the elements studied. This may suggest that plant absorption is controlled by the content of heavy metals in the soil/sludge solution and also by the content of heavy metals that are bioavailable. Soil properties and the form of heavy metals in the soil influence the uptake of heavy metals by plants. The concentration of Cd, Pb, Cr and Ni in the roots of *Amaranthus viridis (red), Ipomoea spp, Cynodon spp*, Palak, *Amaranthus spinosus*, *Basella spp.* (Pui) at STP ranged from 0.48 to 5.68, 9.00 to 62.5, 7.48 to 42.56 and <0.05 mg kg^{-1}, respectively, similar to other report (Rout and Das 2002). The concentration of Cd, Pb, Cr, and Ni in the shoots of *Amaranthus viridis (red), Ipomoea spp, Cynodon spp* , *Spinacia oleracea* (Palak), *Amaranthus spinosus* , *Basella alba* (Pui) at STPs ranged from 0.91to 4.50, 6.39 to 35.6, 4.82 to 22.48 and <0.05 mg kg^{-1}, respectively (Table 2, Fig. A-F) as reported by Domen (2007).

Heavy metals in soils/Sludges and their uptake by plants

Recycling of city waste for raising the vegetables crops has become a popular and profitable practice in modern agriculture. Due to the use of city wastes, heavy metal contamination in peri-urban area of surrounding Kolkata has reached to an alarming level. The total extractable concentration of four heavy metals viz. Cd, Pb, Ni, and Cr in soils/sludge of STPs ranged from 5.51 to 10.14, 195 to 826, 118 to 415, and 112 to 220, respectively (Table 3, Fig.2A-F). The upper limits of Cd, Pb, Cr and Ni in the soil during the present study were higher than the values reported for uncontaminated soil (Temmerman *et al*., 1984).

Table 2: Heavy metals content (mg/kg) in different weed samples

Sl. No	Plants	PlantParts	Cd	Pb	Cr	Ni
			Mean	Mean	Mean	Mean
1.	*Amaranthus viridis(Red)*	Root	5.68	51.7	25.1	<0.05
		Shoot	4.10	35.6	15.2	<0.05
2.	*Ipomoea spp.*	Root	4.00	9.00	28.5	<0.05
		Shoot	1.24	6.39	11.6	<0.05
3.	*Cynodon spp.*	Root	0.48	19.5	40.9	<0.05
		Shoot	0.91	31.7	7.49	<0.05
4.	*Amaranthusspinosus.*	Root	4.32	39.1	13.5	<0.05
		Shoot	4.50	31.6	11.2	<0.05
5.	*Basellaalba*(Pui)	Root	2.91	62.5	7.48	<0.05
		Shoot	2.01	21.4	4.82	<0.05
6.	*Spinachea oleracea*	Root	4.76	51.26	42.56	<0.05
	Shoot	3.84	21.45	22.48	<0.05	

LSD (P= 0.05)

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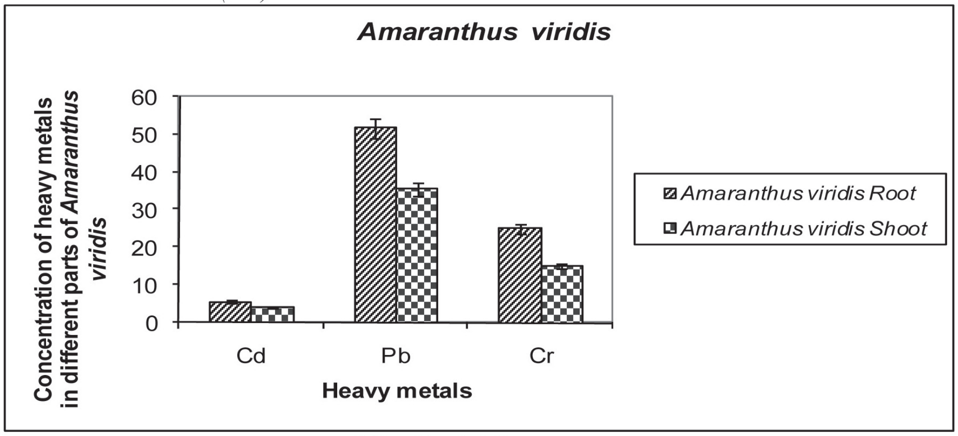
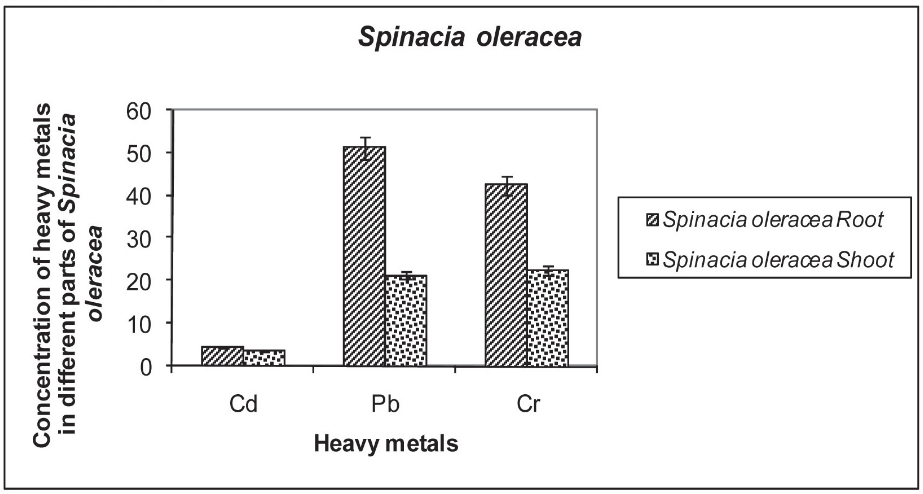
A. *Amaranthus viridis (red)*



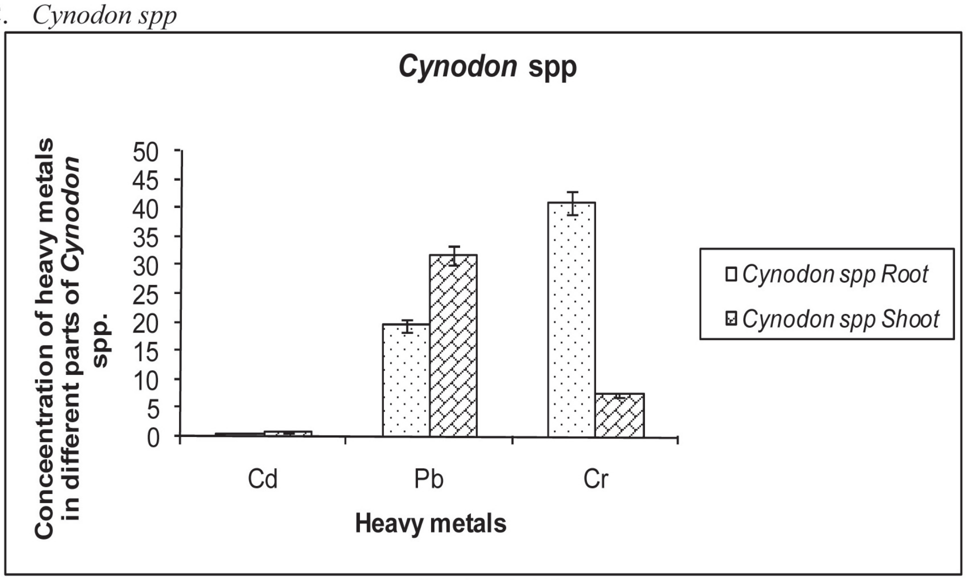
LSD (P= 0.05)

B. *Spinacea oleracea*



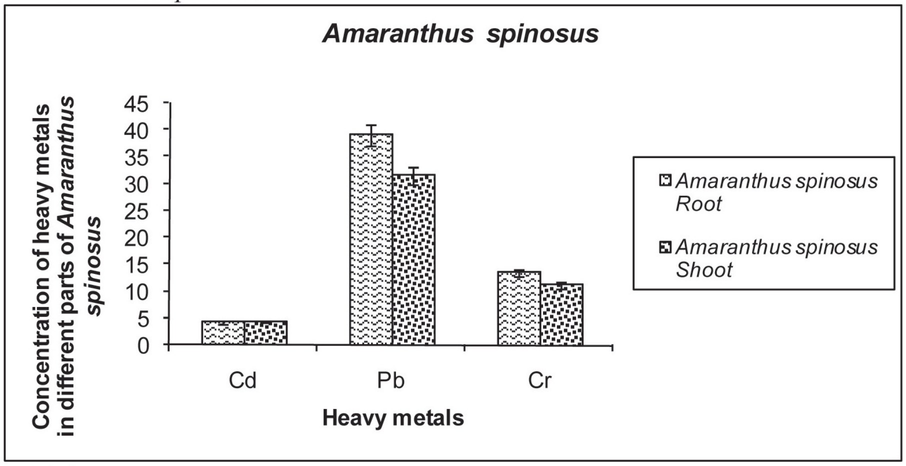
LSD (P= 0.05)

C. *Cynodon spp*



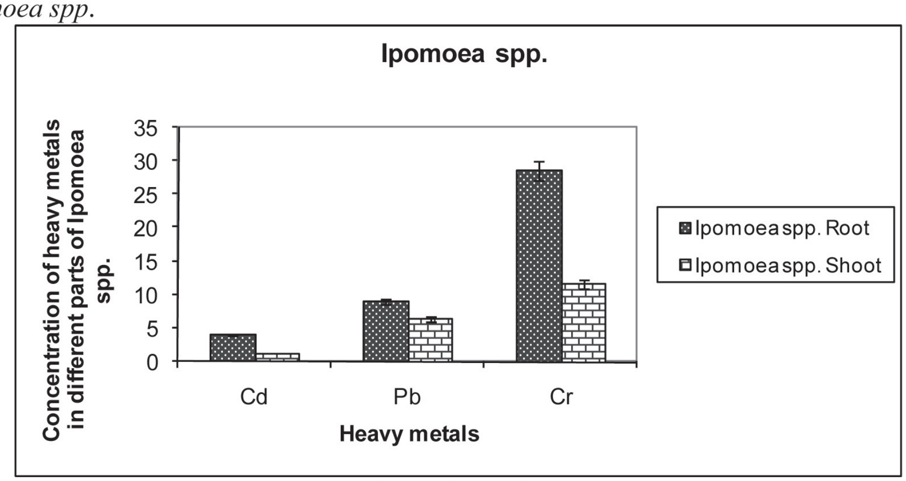
LSD (P= 0.05)

D. *Amaranthus spinosus*



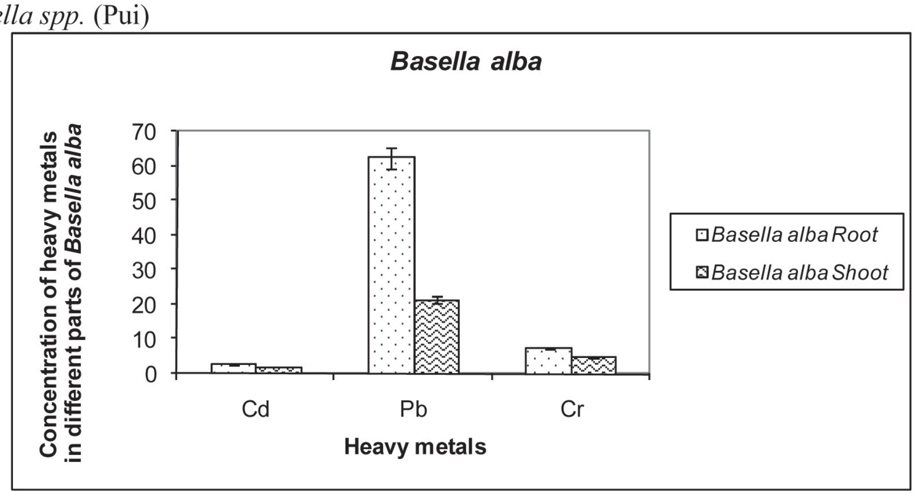
LSD (P= 0.05)

E. Ipomoea spp.



LSD (P= 0.05)

F. Basella spp. (Pui)



LSD (P= 0.05)

Fig. 2: Distribution pattern of different heavy metals in plants parts

Table 3: Heavy metal concentration (mg/kg) in Sludges and soils Sample (Soil- Total)

Site	Cd	Pb	Cr	Ni
Howrah STP	8.28	269	415	216
Bhatpara STP	6.99	195	243	112
Srirampur STP	10.1	316	375	155.77
Nabadep STP	5.51	296	313.73	184
Chandannager STP	5.84	826	345	220
Garulia STP	6.27	218	118	218

LSD (P= 0.05)

Analysis of Daily Intake of Heavy Metals (DIM)

In order to observe the health risk of any pollutant, it is very important to estimate the level of exposure by detecting the routes of exposure to the target organisms. There are several possible pathways of exposure to humans and amongst them the food chain is the most important pathway. The daily intake of metals was estimated according to the average vegetable consumption for both adults and children (Table 4) and the DIM values for heavy metals were high based on the consumption of leafy vegetables grown in wastewater- irrigated soils. The average adult and child body weights were considered to be 55.9 and 32.7 kg, respectively, while average daily leafy vegetable intakes for adults and children were considered to be 0.345 and 0.232 kg/ person/day, respectively, as reported in the literature (Wang *et al.*, 2005). The highest intakes of Cd, Pb, Cr and Ni were from the consumption of leafy vegetables for both adults and children, grown in wastewater-irrigated soils. The findings of present study regarding DIM suggest that the consumption of plants grown in wastewater contaminated soils/sludge is high. However, there are also some other sources of metal exposure like dermal contact, dust inhalation and ingestion of metal-contaminated soils/sludge, which were not taken into account in the present study. So, further detailed studies are required to completely understand the problem and risk involved.

Conclusion

Results of the present studies indicated that six weed species namely *Amaranthus viridis (red)*, *Spinacia oleracea* (Palak), *Cynodon spp*, *Amaranthus spinosus* (White), *Ipomoea spp* and *Basella alba* (Pui) are accumulator of the studied heavy metals. *Amaranthus viridis (red)* is the best accumulator of Pb and Cd, but the best Cr accumulator is *Spinachea oleracea*. Phytoremediation test showed that the amounts of heavy metals decreased in polluted soils under the effect of these weeds plants and we conclude that these weeds can be utilized as effective accumulators for phytoremediation of heavy metal-polluted soils. The scope of phytoremediation can be extended to phytomining in order to extract heavy metals from soils or ores that are sub-economic for conventional mining.

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Table 4: Daily intake of metals (mg) for individual heavy metals in different weeds grown in wastewater-irrigated soils.

Plants	Cd		Pb		Cr		Ni	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Amaranthus viridis	0.00254	0.00293	0.0227	0.02619	0.01048	0.01209	<0.00003	<0.00003
Ipomea spp.	0.00136	0.00157	0.004	0.00462	0.01043	0.01203	<0.00003	<0.00003
Cynodon spp.	0.00036	0.00042	0.01331	0.01536	0.01258	0.01452	<0.00003	<0.00003
Amaranthus spinosus	0.00229	0.00265	0.01838	0.02121	0.00642	0.00741	<0.00003	<0.00003
Basella alba	0.00128	0.00148	0.02181	0.02517	0.0032	0.00369	<0.00003	<0.00003
Spinachea oleracea	0.00224	0.00258	0.0189	0.02181	0.01692	0.01952	<0.00003	<0.00003

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