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Comparative evaluation of phytoremediation of metal contaminated soil of firing range by four different plant species

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Abstract The phytoremediation potential of *Helianthus annuus*, *Zea maize*, *Brassica campestris* and *Pisum sativum* was studied for the soil of firing range contaminated with selected metals i.e. Cd, Cu, Co, Ni, Cr and Pb. The seedlings of the selected plants germinated in a mixture of sand and alluvial soil were transferred to the pots containing the soil of firing ranges and allowed to grow to the stage of reproductive growth. Subsequently they were harvested and then analyzed for selected metals by using AAS. Among the studied plants, *P. sativum* exhibited highest removal efficiency (i.e. 96.23%) and bioconcentration factor for Pb thereby evidencing it to be Pb hyperaccumulator from the soil of firing ranges. *Z. maize* appreciably reduced the levels of all the selected metals in the soil but the highest phytoextraction capacity was shown for Pb i.e. 66.36%, which was enhanced to approximately 74% on EDTA application. *H. annuus* represented the highest removal potential for Cd i.e. 56.03% which was further increased on EDTA application. Thus it proved to be an accumulator of Cd after EDTA application. It was therefore concluded that different plants possess different phytoremediation potentials under given set of conditions.

1. Introduction

Firing ranges are used mostly for routine practices of the armed forces with small arms and ammunitions and typically comprise of a series of metal targets placed in front of an impact berm equipped with bullet traps. The bullet moves through the target and strikes the impact berm, penetrating and smearing it. Pb is used as a chief component in bullet construction along with other metals such as antimony, arsenic and Ni. On firing, these heavy metals sputter out in the form of fine and coarse particulate and get deposited on nearby soil, thereby polluting it heavily. Mostly, these metals concentrate in the immediate vicinity of target area and the degree of contamination of target area decreases rapidly with depth (Dermatas et al., 2004).

The degree of contamination of the soil of firing ranges has also been found to increase with the increase in period of firing

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history. There is thus a dire need to remove the toxic metals from these contaminated areas in order to control the hazardous effects arising thereof due to leaching to nearby agricultural soils and groundwater. Recently phytoextraction has emerged as a cost effective technique to remediate the metal contaminated soils.

The ideal plant species for phytoextraction are those possessing the ability to accumulate and tolerate high concentrations of metals in harvestable tissue, and exhibit a rapid growth rate (Brennan and Shellay, 1999; Lee et al., 2002). Metal accumulation by the plants is governed by their growth rate and ability to translocate metals to the above ground tissue (Keller, 2004; Selvam and Wong, 2008). Large variety of plant species have been tested for their phytoextraction capacities for various metals i.e. various *Brassica* sp., clover (*Trifolium pratense* L.), panikum (*Panicum antidotal*), *Salix populas*, and *Nicotiana* sp. (Abdel-Sabour and Al-Salama, 2007; Grispen et al., 2006; Purakayastha et al., 2008; Reinhard et al., 2008).

Agronomic crops like *Cucurbita pepo*, *Amaranthus* sp., *Raphanus sativus oleiformis* and *Zea maize* have successfully been used as metal accumulators as well as translocators (Aggarwal and Goyal, 2007; Eleni et al., 2005). *Brassica napus* and *R. sativus* grown on multi contaminated soils have proved to be better to reclaim a marginally polluted soil (Marchiol et al., 2004). *Brassica juncea* (L.) Czern. showed the strongest ability to accumulate Pb in roots and then to transport it to the shoots from soils containing sulfates and phosphates as fertilizers (Kumar et al., 1995). Similarly, among the various varieties of grasses, vetiver grass was found to best tolerate the Pb contaminated soils (Annie et al., 2007; Wilde et al., 2005).

Phytoextraction duration is the main cost factor for phytoextraction. The phytoextraction duration of a specific heavy metal polluted soil is estimated by determining a linear relationship between the adsorbed heavy metal contents in the soil and the heavy metal contents in the plant shoots (Japenga et al., 2007). In most of the cases the efficient metal uptake by remediation plants is limited by low phytoavailability of the targeted metals. That is why numerous chelants like EDTA have been used to enhance the bioavailability of the metals in soil as high as 100 times by forming soluble M-chelant complexes. But these chelants may also enhance the risk of metal leaching from soil to groundwater (Blaylock, 1997; Houston, 2007).

The risk of metal leaching from the soil may be reduced by using suitable chelates in appropriate doses (Komarek et al., 2007; Sundar et al., 2007; Turgut et al., 2004). Optimum phytoextraction dose for EDTA was found to be 10 mM for 7 days for soils highly contaminated with Pb (Hovsepyan and Greipsson, 2004; Liu et al., 2008). In the poor soil, two applications of EDTA were more effective than once (Lina et al., 2009). The chelant application at different stages of plant growth generate different results. The EDTA application before seed germination significantly reduced *Helianthus annuus* seedling emergence and dry weight. Soil available Pb and Pb concentrations in plant biomass were found to increase with EDTA concentration but the actual amount of phytoextracted Pb decreased at high EDTA concentrations due to severe growth depression (Sinegani and Khalilikhah, 2008). Depending on the nature and type of Pb-contaminated soil being remediated, the bioavailability and uptake of Pb by coffeeweed were enhanced by amending the soil with suitable chelates especially after the plants have reached maximum biomass (Miller et al., 2008).

The present study thus aims at developing the remediation strategies for metal contaminated soils of firing ranges by using four different plant species i.e. *H. annuus*, *Z. maize*, *Brassica campestris* and *Pisum sativum*. The effect of EDTA application on phytoextraction potential of these plants for selected metals was also investigated.

2. Experimental methodology

2.1. Soil sampling

Soil sample was collected from 0 to 3 cm deep top layer of soil of the firing range with the help of plastic spade after removing leaves, grass and other large external objects in polythene bags and stored in refrigerator at 4 °C to minimize bacterial activity. In order to avoid any discrepancy in metal analysis, the use of metal containers for collecting, mixing and storage was avoided. The firing range was operational for more than 20 years and served as a training facility for Rangers Headquarters Lahore (Radojevic and Bashkin, 1999). The pH of soil sample was determined by preparing a 1:2 soil–water solution, that was found to be 5.8.

2.2. Quality control and quality assurance

All the glassware used during the present experimentation were of high quality, acid resistant pyrex glass. The analytical grade reagents with a certified purity of 99% and stock metal standard solution (1000 ppm) for AAS analysis were procured from E. Merck (Germany). Working standards were prepared by appropriate dilutions of stock standard solutions with double distilled water.

HITACHI AAS Z-5000 system equipped with Zeeman background correction facility was used under optimum analytical conditions for the estimation of metals. The standard calibration method was adopted for the quantification of results and triplicate samples were run to insure the precision of quantitative results.

2.3. Green house experiment

Seeds of four different plants i.e. *H. annuus*, *B. campestris*, *Z. maize* and *P. sativum* were germinated in a mixture of sand and alluvial soil. After 3–4 weeks, the seedlings were transferred to the pots containing soil (1 kg) collected from firing ranges. Plants were grown in pots in two groups i.e. control and experimental ones. The experimental pots were applied with an EDTA dose after the plants attained maximum biomass, while the control group was given no EDTA application.

Sodium salt of EDTA was applied to the plants in amounts of 1.0 g/kg of soil in the form of water solution in a single dose after the plants attained maximum biomass. The above-ground tissues were harvested 15 days after chelate amendment by cutting the stem 1 cm above the soil surface. These harvested plants were then washed with deionized water and air dried to a constant weight. Subsequently, the plants were ground in a ball mill.

2.4. Heavy metal determination

In order to estimate the metal content of plants by AAS, the plant tissues were harvested, washed thoroughly with deionized water and subsequently air dried. Accurately weighed 0.5 g of dried, and finely ground plant samples were digested with $HClO_4$, HNO_3 and H_2SO_4 for about 15 min in a Kjeldahl digestion flask. The contents were then boiled for a few minutes and filtered (Bell et al., 1991). The solution thus obtained was aspirated onto AAS.

The soil samples were analyzed before the seedlings were planted into the pots, and after harvest. The air dried samples were passed through a sieve of 2 mm and then analyzed by the method developed by US-EPA for estimation of the available metals in the soils (Edgell, 1988). In brief, a 2.0 g portion of air dried and sieved soil was digested with a mixture of HNO_3 and 30% H_2O_2 for 30 min and subsequently heated with conc. HCl for 15 min without boiling. The contents were then cooled, filtered and diluted up to 50 mL with distilled water (Radojevic and Bashkin, 1999).

2.5. Statistical data treatment

In order to understand the relationship between total metal levels in soil and their uptake by the plants, the statistical analysis of the metal data obtained by AAS was carried out. It involved the extraction of basic statistical parameters essential to determine the spread and distribution of measured data i.e. mean, median, standard deviation, kurtosis, skewness, etc. Correlation coefficient matrix was used to establish the correlation pattern of various metal pairs in the soil and plant biomass.

3. Results and discussion

The firing ranges are highly contaminated by heavy metals such as Pb due to intensive firing activities. These heavy metals will not only devastate the nearby fertile lands but may also percolate into the groundwater thereby threatening its quality. The present investigation thus deals with not only the determination of pollution status of soil of firing range but also its phytoremediation by using four different plant species i.e. *H. annuus*, *B. campestris*, *Z. maize* and *P. sativum*. The change in phytoremediation efficiency was also determined in the presence of EDTA, one of the most commonly used chelants.

The quality of soil of firing range in terms of total metal contents is presented in Table 1. The soil was found to be highly contaminated with Pb as characterized by its highest levels i.e. 1331 mg/kg. Cu was the metal that was present at second highest concentration of 84.5 mg/kg. Cd another hazardous heavy metal was present at the level of 7.25 mg/kg. Other metals i.e. Co, Ni, and Cr were present at mean levels of 0.65, 2.83, and 0.950 mg/kg respectively. Thus the order of mean levels of metals was found to be: Pb > Cu > Cd > Ni > Cr > Co. The soil of firing range thus exhibited a multi metal flux, that represented a quite serious situation.

Table 1 Concentration of metals (mg/kg) in firing range soil.

Firing range	Cu	Cd	Pb	Co	Ni	Cr
Mean (n = 3)	84.50	7.250	1331	0.650	2.830	0.950
Standard error	0.462	0.040	20.785	0.133	0.087	0.075

3.1. Distribution of metals in soil samples of various pots

Table 2 represents the basic statistics corresponding to the metal levels in soil samples collected from different pots i.e. *Z. maize*, *H. annuus*, *P. sativum*, and *B. campestris*. The soil samples collected from *Z. maize* pot after harvest exhibited the highest mean concentration for Pb which was found to be present at 447.7 mg/kg. The standard deviation recorded for these samples was 59.90. Cu was present at second highest mean level of 69.58 mg/L. Cd and Ni secured third and fourth positions with respect to mean levels of 4.0 and 1.998 mg/kg respectively. Cr was found to be present at 0.907 mg/kg mean level and Co was present at the least mean level of 0.375 mg/kg.

The *Z. maize* plant after one harvested crop reduced the metal contents to levels that exhibited the phytoextraction potential of various metals to be as Pb: 66.36%, Cu: 17.65%, Cd: 44.83%, Ni: 29.33%, Cr: 4.526% and Co: 42.31%. Thus maximum phytoextraction capacity of *Z. maize* was exhibited for Pb. On application of EDTA, this Pb remediation was increased to 68.43% only. A significant increase in the removal efficiency of *Z. maize* was observed after EDTA application for almost all other metals with the exception of Cu. The highest removal potential for Pb may be explained on the basis of the fact that Pb–EDTA complex possess higher stability constant (log $Ks = 17.88$) than other metal–EDTA complexes under acidic and moist soil conditions (Bucheli-Witschel and Egli, 2001; Sommers and Lindsay, 1979).

The basic statistical parameters for the soil samples collected from pots of *H. annuus* also presented Pb to be present at the highest mean concentration of 680.7 mg/kg. Similar to the previous case, Cu was found to be present at second highest level of 61.66 mg/kg. The other two heavy metals i.e. Cd and Ni were present at mean levels of 3.188 and 2.083 mg/kg respectively. Cr exhibited a mean concentration of 0.684 mg/kg. Here again Co was present at the least mean concentration of 0.348 mg/kg. *H. annuus* represented the highest removal potential for Cd, i.e. 56.03%, although Pb was also appreciably reduced to the percentage of 48.86. After EDTA application, the efficiency for Pb removal was enhanced to approximately 74%. Thus it is the best hyperaccumulator of Pb from firing range soil on treatment with EDTA. The removal efficiency of *H. annuus* for Cr was significantly reduced after EDTA application.

The basic statistics corresponding to the metal data recorded for the soil samples collected from *P. sativum* pots evidenced the highest mean concentration of Cu being present at 68.25 mg/kg, whereas Pb which was present at 1st highest mean concentration in previous two cases, here secured second highest concentration of 50.22 mg/kg. Cd in these soil samples was also found to be present at levels comparable to the previous cases. The mean Cd level observed here was 3.550 mg/kg. The order for mean levels of the rest of the metals remained: Ni > Cr > Co. Among the studied plants, *P. sativum* was the one that exhibited highest removal efficiency for Pb standing at 96.23%. After EDTA application, this efficiency was reduced to 80.13%. Similar situation was also observed in the case of Ni and Cr whereby again phytoextraction potential was reduced on EDTA application, due to the fact that high solubility of Pb-EDTA complex leaches it down beyond the reach of root hairs of the plant thereby reducing plant Pb

Table 2 Basic statistics for metals in soil samples collected from plant pots without and with EDTA application ($n = 12$).

		Without EDTA						With EDTA					
		Cu	Cd	Pb	Co	Ni	Cr	Cu	Cd	Pb	Co	Ni	Cr
Zea maize	Mean	69.58	4.000	447.7	0.375	1.998	0.907	71.38	3.006	420.2	0.252	1.89	0.745
	S.D.	7.450	0.992	59.90	0.182	0.673	0.237	6.015	0.187	25.36	0.067	0.270	0.112
	Skewness	−0.639	0.030	−0.060	0.118	−0.760	0.332	−0.246	−0.616	0.542	−0.314	−1.888	0.354
Helianthus annuus	Mean	61.66	3.188	680.7	0.348	2.082	0.684	60.92	3.344	347.4	0.304	2.049	0.779
	S.D.	12.19	1.045	103.7	0.111	0.892	0.254	7.822	0.564	205.8	0.058	0.192	0.120
	Skewness	−0.056	−0.350	−0.018	0.012	0.174	−0.205	0.042	−0.107	0.305	0.156	0.032	0.068
Pisum sativum	Mean	68.25	3.550	50.22	0.210	1.770	0.499	55.63	3.085	264.5	0.343	1.81	0.798
	S.D.	6.635	0.514	2.564	0.043	0.397	0.131	8.217	0.272	15.04	0.088	0.138	0.104
	Skewness	−0.838	−1.001	−0.537	0.000	0.673	−0.028	−0.230	−0.203	0.443	−0.106	0.731	−0.180
Brassica campestris	Mean	38.64	3.483	880.4	0.494	1.536	0.39	87.88	3.268	1024	0.348	0.680	0.858
	S.D.	2.340	1.241	54.22	4.568	47.43	0.107	4.752	0.283	62.11	0.074	0.151	0.102
	Skewness	−0.001	−0.030	0.536	3.462	3.464	−0.431	−0.226	−0.267	0.928	0.193	−0.246	−0.160

uptake. Moreover, metal–EDTA complexes are too large to pass the plasma lemma lipid bilayer. It has been found that metal–EDTA uptake by plants especially Pb–EDTA takes place at breaks in the endodermis and casparian strip (Bell et al., 1991).

The metal data corresponding to the soil samples collected from *B. campestris* pots after harvest evidenced highest mean concentration for Pb standing at 880.4 mg/kg. The order of mean concentration level of all the metals were the same as observed for *H. annuus* and *Z. maize* pot soils with differences only in concentrations. Cu, the major constituent of bullets and ammunition was present at second highest mean concentration of 38.64 mg/kg. Ni was present at the mean level of 1.536 mg/kg. Cr exhibited the mean level of 0.39 mg/kg. Co and Cd were present at the mean levels of 1.494 and 3.483 mg/kg. Thus phytoextraction potential of *B. campestris* plants for various metals was found to be in the order Cr > Cu > Cd > Ni > Pb > Co. In fact the maximum Cr removal efficiency was insured by *B. campestris* in the absence of any application of EDTA.

On EDTA applications, the phytoextraction capacity of most of the metals was reduced. Thus solubilization does not insure bioaccumulation. Studies have evidenced that plants may not take up chelant solubilized metals because metal–chelant complex cannot penetrate the endodermis and reach xylem (Bolan and Duraisamy, 2003; Robinson et al., 2003; Tandy et al., 2005). The study also evidenced that different plants exhibit different phytoremediation potentials for different metals under a given set of conditions. This potential is governed by the nature of the metals and the nature of plant i.e. structure of its endodermis and its pore size etc. in addition to other factors. A comparison of metal levels in soil samples collected from different pots of plants is furnished in Fig. 1.

3.2. Correlation coefficient matrix

The correlation coefficient matrix for the metals present in the soil samples is presented in Table 3. The data corresponding to these soil samples from *Z. maize* pot depicted the strongest positive correlation among Cu–Pb pair with an r-value of 0.776 evidencing that in 77.6% of cases the concentration of both metals increased simultaneously. Another significant positive correlation was observed between two most hazardous heavy metals i.e. Pb and Cr with an r-value of 0.625. Co–Ni pair was found to be significantly negatively correlated (−0.897) evidencing an increase in the concentration of one metal with the decrease in the concentration of the other. Other metal pairs that were found to be significantly negatively correlated included: Cd–Pb (−0.874) and Cu–Cd (−0.638). After EDTA application, very few metal pairs were found to be significantly correlated. Cd–Cu was the only metal pair that was significantly positively correlated at an r-value of 0.514.

The correlation coefficient matrix for metal data for soil samples collected from *H. annuus* presented many metal pairs to be significantly negatively correlated and only few to be positively correlated. The strongest negative correlation was observed between Pb and Cr pair with an r-value of −0.968. Other metal pairs that were found to be significantly negatively correlated included: Cd–Cr ($r = -0.814$), Pb–Ni ($r = -0.692$) and Cd–Ni (−0.721). Cd and Pb were the metals that were most significantly positively correlated with an r-value of

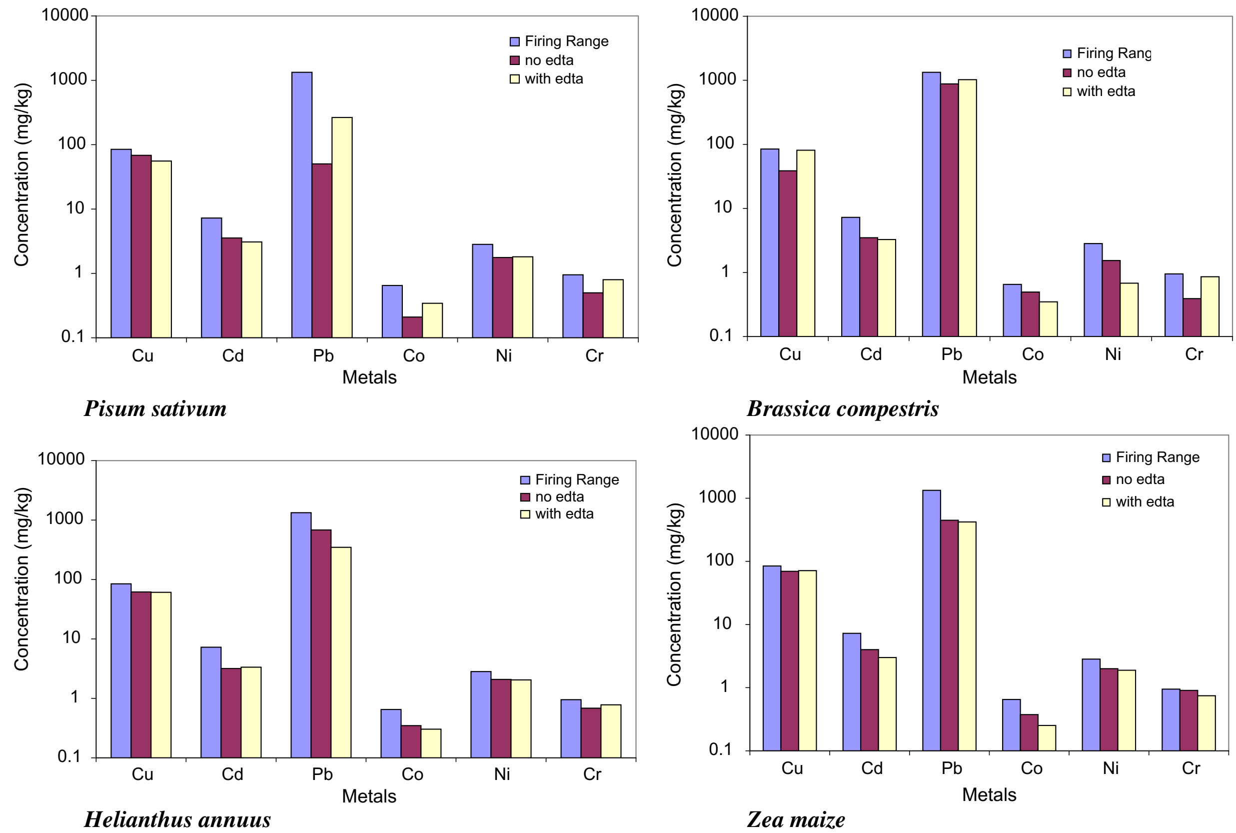

Figure 1 Comparative evaluation of metal concentrations in soil samples collected from different plants.

Table 3 Correlation coefficient matrix of soil from different pots of plants (n = 12).

			Cu	Cd	Pb	Co	Ni	Cr		
Zea maize	Without EDTA	Cu	1.000	0.514	0.031	0.000	0.444	0.047	Cu	With EDTA
		Cd	−0.638	1.000	−0.054	0.162	−0.107	0.234	Cd	
		Pb	0.776	−0.874	1.000	−0.033	0.081	0.389	Pb	
		Co	0.366	0.363	0.106	1.000	−0.08	−0.440	Co	
		Ni	−0.407	−0.089	−0.398	−0.897	1.000	−0.491	Ni	
		Cr	0.088	−0.590	0.625	−0.213	−0.126	1.000	Cr	
Helianthus annuus	Without EDTA	Cu	1.000	−0.287	−0.207	−0.112	0.054	0.209	Cu	With EDTA
		Cd	0.131	1.000	−0.248	0.053	0.167	0.605	Cd	
		Pb	−0.145	0.893	1.000	0.164	−0.609	−0.289	Pb	
		Co	0.689	0.543	0.173	1.000	−0.787	−0.444	Co	
		Ni	0.422	−0.721	−0.692	−0.093	1.000	0.472	Ni	
		Cr	0.036	−0.814	−0.968	−0.130	0.526	1.000	Cr	
Pisum sativum	Without EDTA	Cu	1.000	0.593	0.466	0.193	−0.038	0.082	Cu	With EDTA
		Cd	0.835	1.000	0.216	0.719	−0.137	0.156	Cd	
		Pb	0.969	0.683	1.000	0.413	0.703	0.779	Pb	
		Co	0.548	0.673	0.404	1.000	0.105	0.506	Co	
		Ni	−0.877	−0.817	−0.854	−0.310	1.000	0.635	Ni	
		Cr	0.607	0.197	0.736	0.339	−0.504	1.000	Cr	
Brassica campestris	Without EDTA	Cu	1.000	0.028	−0.04	−0.109	−0.003	−0.053	Cu	With EDTA
		Cd	0.786	1.000	0.254	0.216	−0.109	−0.286	Cd	
		Pb	0.029	0.631	1.000	0.534	−0.497	0.386	Pb	
		Co	−0.288	−0.512	−0.453	1.000	−0.275	−0.059	Co	
		Ni	0.076	0.411	0.596	−0.102	1.000	−0.128	Ni	
		Cr	0.426	0.570	0.418	−0.560	0.207	1.000	Cr	

r-Values are significant at ±0.5 at a probability of 0.05.

0.893. Cu and Co were also found to be significantly positively correlated ($r = 0.689$). After EDTA application as well, Pb–Ni pair was found to be significantly negatively correlated with $r = -0.609$. Here the strongest negative correlation was observed between Co and Ni at an r-value of −0.787, which was not present in the previous case. Cd–Cr was the only metal pair that was correlated significantly positively at an r-value of 0.605, it was found to be negatively correlated in previous case.

The correlation coefficient matrix for soil samples from *P. sativum* pots yielded mostly significant correlations. The most significant positive correlation was found between Cu and Pb at an r-value of 0.969, while Cu was significantly negatively correlated with Ni (−0.877). Cd was found to be significantly positively correlated with Pb and Co with comparable r-values of 0.683 and 0.673 respectively, while it was strongly negatively correlated with Ni at an r-value of −0.877. Ni was also found to be strongly negatively correlated with Pb at an r-value of −0.854. In 73.6% cases the concentration of Pb went in hand with concentration of Cr as evidenced by their r-value of 0.736. The correlation coefficient matrix for these metals after EDTA application presented all the significant correlations to be positive. The strongest positive correlation was observed for Cr–Pb pair at an r-value of 0.779. Cr was also significantly positively correlated with Ni (0.635). Pb and Ni were found to be significantly positively correlated with an r-value of 0.703. Similarly Cd–Co pair was found to be positively correlated with an r-value of 0.719.

In the case of *B. campestris* pots the most significant positive correlation was exhibited by Cu–Cd pair (r-value 0.786) and Cd–Pb pair (r-value 0.631). Pb–Ni and Cr–Cd metal pairs were also significantly positively correlated. Co–Cr pair was found to be significantly negatively correlated. After EDTA application, all these correlations vanished and only one significant positive correlation was observed between Co and Pb (0.534) pair.

3.3. Distribution of metals in various plants

In order to get an insight into the phytoextraction efficiency of four selected plants i.e. *H. annuus*, *Z. maize*, *P. sativum* and *B. campestris* for the removal of heavy metals, the plant material was also studied with respect to determination of metal content.

The data set corresponding to the levels of selected metals i.e. Cu, Cd, Pb, Ni, Co and Cr present in four plant species i.e. *H. annuus*, *Z. maize*, *P. sativum* and *B. campestris*, is presented in Table 4. The basic statistics for the various metals in the *Z. maize* depicted the highest mean concentration for Pb standing at 36.04 mg/kg. Cu was also present at an appreciable concentration of 21.11 mg/kg. Cd here was present at a mean concentration of 3.558 mg/kg. The other three metals i.e. Co, Ni and Cr were present at sub ppm levels with the order of mean levels being Ni > Co > Cr. After EDTA application the phytoextraction efficiency of *Z. maize* plant for Pb was enhanced to manifolds as is evident by its much higher mean level standing at 595.6 mg/kg. The phytoextraction efficiency of this plant was reduced by EDTA application for Cd, that were found to be present at a mean level of 2.776 mg/kg. Enhanced levels of Cr were observed (i.e. 2.496 mg/kg) in *Z. maize* plants after EDTA application due to increased solubilization of metal on chelation. So the order of mean metal levels found here was: Pb > Cu > Cd > Cr > Ni > Co.

Table 4 Basic statistics for metals in plant samples harvested without EDTA and with EDTA application ($n = 12$).

		Without EDTA						With EDTA					
		Cu	Cd	Pb	Co	Ni	Cr	Cu	Cd	Pb	Co	Ni	Cr
Zea maize	Mean	21.11	3.558	36.04	0.262	0.634	0.033	23.08	2.776	59.56	0.234	0.645	0.496
	S.D.	3.753	0.659	2.960	0.106	0.086	0.013	1.771	0.900	37.37	0.087	0.129	0.737
	Skewness	0.267	0.067	−0.018	1.164	0.018	0.465	0.021	0.005	0.040	0.315	0.167	−0.058
Helianthus annuus	Mean	13.17	3.055	64.96	0.274	0.646	0.208	14.57	3.503	68.38	0.263	0.596	0.164
	S.D.	1.621	0.941	6.682	0.080	0.094	0.055	1.385	0.797	2.038	0.103	0.153	0.031
	Skewness	0.257	−0.285	0.328	0.479	−0.048	−0.481	0.072	−0.024	−0.084	0.396	−0.007	−0.052
Pisum sativum	Mean	14.18	3.529	82.26	0.211	0.623	0.395	18.44	3.376	264.5	0.271	0.686	0.028
	S.D.	2.605	0.694	22.403	0.055	0.080	0.100	4.659	0.575	3.141	0.113	0.241	0.009
	Skewness	0.277	0.601	−0.247	0.120	0.423	−0.351	1.228	0.415	0.047	0.462	−0.232	0.221
Brassica campestris	Mean	14.48	2.779	27.71	0.208	0.625	0.438	18.55	2.996	32.71	0.226	0.612	1.250
	S.D.	3.157	0.742	4.653	0.065	0.151	0.114	1.383	1.119	6.064	0.070	0.090	0.275
	Skewness	0.003	−0.337	0.018	0.107	−0.138	−0.263	0.079	0.355	0.056	−0.635	−0.485	0.302

The basic statistics for the *H. annuus* plant was quite similar to previous cases, the highest concentration being recorded for Pb (64.96 mg/kg). Cu being present at a mean level of 13.17 mg/L secured second highest mean concentration. Cd in these samples was found to be present at a mean concentration of 3.055 mg/kg. Cr a very hazardous heavy metal was present at sub-ppm levels of 0.208 mg/kg, while Co was recorded at levels of 0.274 mg/kg. Ni was present at 0.646 mg/kg levels. Mostly the data was found to be negatively skewed. After EDTA application the data exhibited slightly enhanced phytoextraction efficiencies for Pb, Cu and Cd as is evident by their enhanced mean levels standing at 68.38, 14.57 and 3.503 mg/kg respectively. The mean metal levels showed slightly reduced phytoextraction efficiencies for the rest of the three metals i.e. Cr, Ni and Co after EDTA application.

The basic statistics for the *P. sativum* plant evidenced high Pb accumulating efficiency as it was found to be present at a mean level of 82.2 mg/kg. The Cu content recorded for these plants was comparable to *H. annuus*. Very little concentration of Co was present in plant mass i.e. 0.210 mg/kg. Cd in these plants was found at mean levels of 3.529 mg/kg. The order for the mean concentration of the metals was Pb > Cu > Cd > Ni > Cr > Co. The data corresponding to the basic statistics for metals in *P. sativum* plant after EDTA application yielded the highest mean level for Pb (35.44 mg/kg), which was followed by Cu being present at 18.44 mg/kg levels. Cd as in previous cases secured the third highest concentration of 3.376 mg/kg. The phytoextraction capacity recorded for Cr was quite low i.e. 0.028 mg/kg after EDTA application. The mean concentration levels recorded for Ni and Co remained 0.686 and 0.271 mg/kg. Hence the order of mean concentration levels recorded for *P. sativum* plant with EDTA application was: Pb > Cu > Cd > Ni > Cr > Co. The phytoremediation capacity of this plant for Pb and Cr was reduced on EDTA application while for rest of metals this capacity was enhanced.

The basic statistical parameters for the *B. campestris* plant revealed very little Pb accumulation efficiency, the mean Pb concentration here was found to be 27.71 mg/kg. Cu exhibited here the mean concentration of 14.48 mg/kg comparable to that found in *P. sativum* and *H. annuus*. Cr and Co were present at mean levels of 0.438 and 0.208 respectively. The mean concentration order observed for various metals in *B. campestris* plant was: Pb > Cu > Cd > Ni > Cr > Co. After EDTA application the mean Pb levels were enhanced to 32.71 mg/kg while Cu levels witnessed an increase of approximately 4 mg. Cd was the metal that was present at third highest mean concentration of 2.996 mg/kg. The rest of the three metals followed the order Cr > Ni > Co being present at mean levels of 1.250, 0.612 and 0.226 mg/kg respectively. The growth of the plants was found to be normal prior to the application of EDTA and after addition of EDTA. The plant biomass was however, found to be lower as compared to the normal case. After EDTA application, the phytoextraction potential of the plant for all the metals was observed to be enhanced with the exception of Ni.

3.4. Correlation coefficient matrix of metals in plants

The data for correlation coefficient matrix of metals present in the different plants before and after EDTA application are given in Table 5. For the *Z. maize* plants one significant positive correlation was observed only between Cu and Cr pair with an *r*-value of 0.874, and Cd and Pb with an *r*-value of 0.608. Among the negatively correlated pairs, Ni and Cr were the most significant one with *r*-value of −0.829. After EDTA

Table 5 Correlation coefficient matrix of metal data for four different plant species (n = 12).

			Cu	Cd	Pb	Co	Ni	Cr		
Zea maize	Without EDTA	Cu	**1.000**	−0.316	−0.422	−0.215	−0.38	−0.497	Cu	With EDTA
		Cd	−0.432	**1.000**	−0.371	0.117	−0.476	−0.217	Cd	
		Pb	−0.305	0.608	**1.000**	−0.294	0.422	0.243	Pb	
		Co	0.250	−0.552	−0.220	**1.000**	0.118	0.182	Co	
		Ni	−0.581	0.490	−0.096	−0.460	**1.000**	0.225	Ni	
		Cr	0.874	−0.380	0.056	0.272	−0.829	**1.000**	Cr	
Helianthus annuus	Without EDTA	Cu	**1.000**	0.382	0.069	−0.342	0.217	0.404	Cu	With EDTA
		Cd	0.557	**1.000**	−0.348	0.239	−0.548	−0.213	Cd	
		Pb	−0.238	−0.588	**1.000**	0.234	0.429	0.273	Pb	
		Co	0.185	0.047	0.029	**1.000**	−0.387	−0.448	Co	
		Ni	0.543	0.099	−0.107	0.170	**1.000**	0.505	Ni	
		Cr	0.487	0.044	0.342	0.433	0.284	**1.000**	Cr	
Pisum sativum	Without EDTA	Cu	**1.000**	0.437	−0.28	0.094	−0.593	0.207	Cu	With EDTA
		Cd	−0.432	**1.000**	−0.416	−0.002	−0.459	0.433	Cd	
		Pb	−0.345	0.095	**1.000**	0.39	−0.074	−0.358	Pb	
		Co	0.212	0.362	−0.585	**1.000**	−0.246	−0.073	Co	
		Ni	−0.261	0.107	−0.553	0.030	**1.000**	−0.083	Ni	
		Cr	0.524	−0.530	−0.132	−0.364	−0.157	**1.000**	Cr	
Brassica campestris	Without EDTA	Cu	**1.000**	−0.111	−0.519	0.063	−0.027	0.318	Cu	With EDTA
		Cd	−0.180	**1.000**	0.492	0.231	−0.089	−0.143	Cd	
		Pb	0.032	0.311	**1.000**	0.401	0.309	−0.131	Pb	
		Co	−0.257	−0.428	−0.524	**1.000**	0.58	0.282	Co	
		Ni	−0.212	0.438	0.215	0.001	**1.000**	0.389	Ni	
		Cr	−0.421	0.283	−0.281	−0.102	−0.257	**1.000**	Cr	

r-Values are significant at ±0.5 at a probability of 0.05.

application all the correlations observed were quite non- significant. This was due to the fact that after EDTA applications, chelates were formed which behaved differently depending on the nature of the metal.

The correlation coefficient matrix for *H. annuus* plant presented the Cd–Cu pair to be most significantly positively correlated. Ni here was also found to be significantly correlated with Cu. The counter part data obtained after EDTA application recorded the highest positive *r*-value for Ni–Cr pair (0.505), while the highest negative *r*-value was recorded for Cd–Ni pair at an *r*-value of −0.548.

The data pertaining to the correlation coefficient matrix for various metal pairs in *P. sativum* contained mostly negative correlations which were marginally significant with the exception of Cr–Cu pair that was found to be associated positively with an *r*-value of 0.524, while the highest negative *r*-value was recorded for Pb–Co pair at an *r*-value of −0.585. Pb was also found to be negatively correlated with Ni at an *r*-value of −0.553 thereby evidencing that in 55.3% cases, the concentration of Pb decreased with a simultaneous increase in concentration of Ni. After EDTA application most of the observed correlations were found to be non significant. The only significant correlation was observed between Cu and Ni with an *r*-value of −0.593. No significant positive or negative correlation was observed in the case of *B. campestris* with the exception of Pb–Co pair that was significantly negatively correlated.

The correlation coefficient matrix for these metals after EDTA application evidenced the strongest positive correlation among the Co–Ni pair with an *r*-value of 0.580. Cu and Pb were found to be negatively correlated with an *r*-value of −0.519.

A comparison of mean metal concentration in plant samples obtained from different pots of control (without EDTA) and experimental (with EDTA application) is presented in Fig. 2.

3.5. Bioconcentration factor

Bioconcentration factor for each plant was calculated as the ratio of concentration of metal in harvested tissue to the metal concentration in soil. Brooks' criterion for interpretation ofhyperaccumulation was used (Brooks, 1998). For the *P. sativum* plant, BCF for Cu was 0.209 without EDTA application which was increased to 0.414 after EDTA application, but for Cd it was observed to decrease after EDTA application. *P. sativum* was found to be a hyperaccumulator for Pb in the absence of EDTA, whereas EDTA application decreased its BCF. Similar results were obtained for Co. For Ni, EDTA application has almost no effect on its BCF value while for Cr, BCF was increased from 0.792 to 3.12, thereby proving *P. sativum* plant a good hyperaccumulator on addition of EDTA.

In the case of *B. campestris* an increase in BCF was observed for Cd, Co, Ni and Cr on EDTA application. BCF of *B. campestris* plant for Pb was not increased or decreased on addition of EDTA while BCF for Cu was decreased on EDTA application (i.e. from 0.374 to 0.211).

H. annuus was proved to be a hyper-accumulator for Cd after EDTA application, as shown by increase in BCF of Cd increasing from 0.958 to 1.047. BCF for Cu and Co, was increased on EDTA application. While for rest of the metals (Pb, Ni and Cr), EDTA application caused a decrease in the BCF. In case of *Z. maize*, BCF was increased for all metals on EDTA application. A significant increase in BCF was observed for Co and Cr i.e. from 0.036 to 1.087 for Co and from 0.036 to 3.345 for Cr.

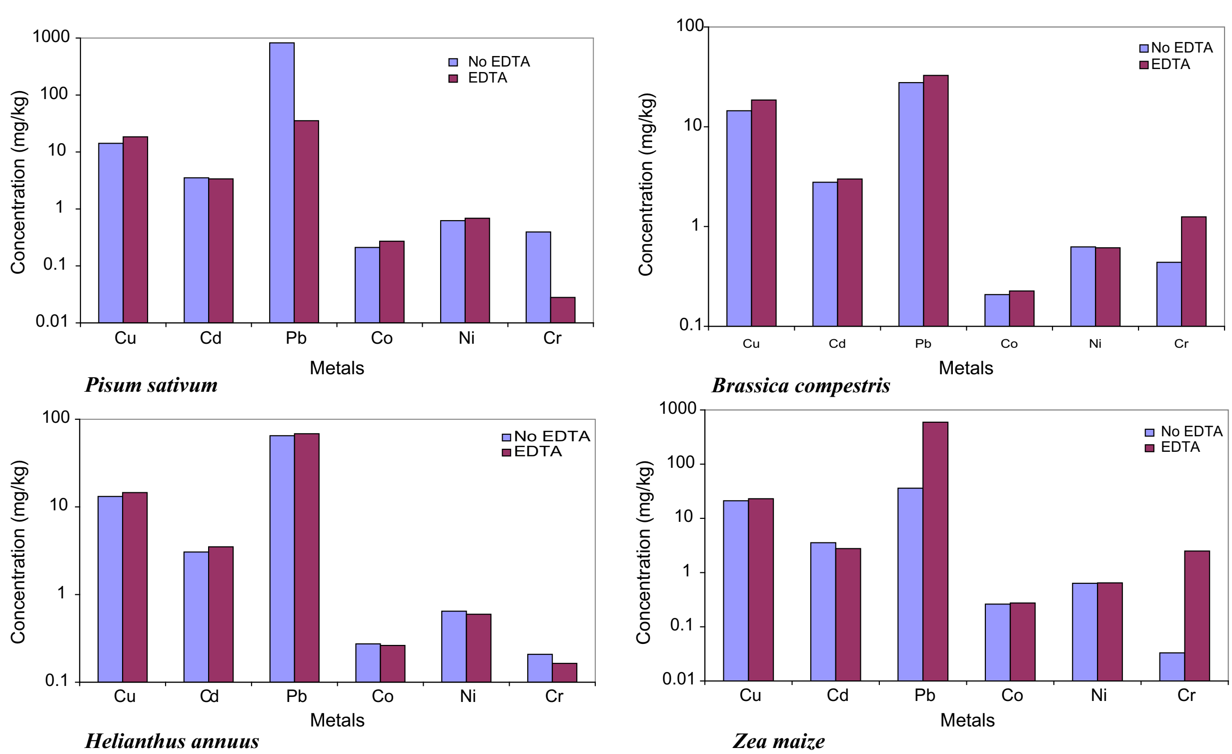

Figure 2 Comparison of metal concentrations in different plants.

4. Conclusions

The coexistence of metals in the soil poses serious threat to the various plant species grown therein, but the net flux of metals may be remediated successfully by using various hyperaccumulators. These hyperaccumulators possess different accumulation potentials for different metals. The study evidenced *Z. maize* to be a hyper-accumulator for Co and Cr after EDTA application, while *H. annuus* proved to be a hyper-accumulator for Cd under similar conditions. *B. campestris* plant exhibited hyperaccumulating properties for Cr – a very hazardous metal. Moreover, *P. sativum* was found to be a best accumulator of Pb without EDTA application. Thus these four plant species may preferentially accumulate one metal or the other and thus help in cleaning the flux of metals.

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