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Chemical fractionation and translocation of heavy metals in *Canna indica* L. grown on industrial waste amended soil

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ABSTRACT

A pot experiment was carried out to assess the effect of different amendments of industrial sludge on the growth of *Canna indica* L. as well as the translocation potential of heavy metals of this plant. The accumulation of metals (Cr, Fe, Cd, Cu, Ni, Zn, Mn and Pb) in different parts of *C. indica* L. grown on industrial sludge-amended soil increased with time and increasing doses of sludge amendments. Sequential extraction method was followed to estimate the different fractions of heavy metals in sludge-amended soils collected from different periods of this study. The results showed that Mn, Zn, Cd, Cr and Pb were mostly associated with Fe–Mn oxide fraction in all amendments, whereas, Ni was mostly found in residual (RES) fraction. Cu and Fe were found to be higher in organically bounded form (OM) and RES fraction. The metal concentration in *C. indica* L. after 90 days of experiment started, was in the order of Fe > Cr > Mn > Zn > Ni > Cu > Cd > Pb and the metal translocation was found lesser in shoot. With the increasing percentage of sludge amendments in soil the metal concentrations increased in different parts of plants. Overall, the plant *C. indica* L. was found to be well adapted in industrial sludge amendments and it may be recommended that this plant was found suitable for phytoremediation of most of the studied metals.

1. Introduction

Metals in soils are usually a common area of research in recent era. The association of metals in different forms in soil is now an area of research in plant–soil interaction. Metals are usually associated with various chemical and biological components of soils depending on their origin and input of anthropogenic activities. The industrial sludge is considered as the potential source of toxic heavy metals which creates a major problem of disposal. There is an increase in sludge application in agriculture generated from wastewater treatment plants due to the availability of both macronutrients and micronutrients [1]. The characterization of the industrial sludge is essential to minimize the risk of heavy metals on the growth of plants [2]. Many authors studied that heavy metal accumulation in different parts of plants differed with different plant species, percentage of waste and types of wastes [3–9]. The use of plant species for phytoremediation is a potential strategy to remove the heavy metals from the contaminated habitat [10,11]. It is well known that most of the microelements are not all indispensable for the growth of plants [12], beyond certain threshold concentration some of the elements show toxic effect on plants [13,14].

Disposal of industrial sludge is now a major concern in the point of heavy metal contamination in land. So, remediation of heavy metals from soil approaches phytoextraction through metal accumulation and translocation [10,15] from soil to root and root to shoot. The sequential extraction procedure is very important study to estimate the different forms of metals in the sludge-amended soil which, help to evaluate the possible mobility and bioavailability of toxic metals in plants [16] grown on sludge-amended soil. So, among the different procedures of metal fractionation the most important one is five steps fractionation study of Tessier et al. [17] which is commonly applied for metal fractionation of many studies of different sludge-amended soils [16,18,22].

The present study was carried out to estimate metal fractions in different amendments of industrial sludge and to evaluate the translocation potential of metals in different parts of plants, *Canna indica* L. This plant is very commonly used in garden ornamentation with low maintenance cost. In view of above, the main aim of our study is to observe the potential of this plant for phytoremediation of metal contaminated industrial sludge-amended soil.

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2. Materials and methods

2.1 Experimental design

Industrial sludge was collected from Wazirpur industrial area, New Delhi (India), where the main polluting industries are electroplating, pickling–rolling and textiles. The sludge was collected from an open dumping ground and roadside dumps in accordance with the standard methods and then air dried in field laboratory. The uncontaminated garden soil of School of Environmental Sciences (SES), Jawaharlal Nehru University (JNU) was used for further experimental studies. Both the soil and sludge samples were smashed and sieve through 2-mm mesh size separately. The various amendments of sludge (10%, 20% and 30%) were prepared using the SES garden soil as control, each in three replicates. The sludge amended and control soils were kept in earthen pots (5 kg/pot). The young plants, from family Cannaceae, *C. indica* L. (about 3-week old) were collected from JNU nursery of almost equal length for the present experiment. The four plants were planted in each of the pot with sludge amended and control soil. Distilled water was given to the experimental plants to maintain 60% moisture of WHC throughout the experiment. The plants were grown for a period of 3 months (April–July 2007) at JNU garden in semi-controlled condition where temperature varied from 30 °C to 40 °C. The plants and soils were harvested at an interval of 1 month. Three plants from each amendment were collected in each month (one plant from each pot). Grab soil samples were taken from root area. Plant and soil samples were taken in separate plastic bags to the laboratory. The sludge-amended soil samples were air dried in an ambient room temperature and then roll to break up the large aggregates followed by sieving through 2-mm mesh size.

2.2 Physico-chemical analysis of different amendments

Physico-chemical parameter of sludge and sludge-amended soils, i.e. pH, electrical conductivity (EC), moisture content (MC), cation-exchange capacity (CEC), total N (TN) and available inorganic phosphorus (IP) were analyzed following standard procedure [19]. Organic carbon (OC) was analyzed by Black method [20]. Total metals of soil and sludge-amended soil were analyzed by Agemian and Chau [21] method. All the parameters were carried out in triplicate for better results.

2.3 Sequential extraction of different amendments

The sequential extraction of soil and sludge-amended soil was done by Tessier method [17] after each harvest from all amendments in triplicates. Through this procedure five fractions of each metal were analyzed, i.e. exchangeable (EXC), carbonate bound (CAR), iron–manganese bound (Fe–Mn), organic bound (ORG) and residual (RES) fraction.

2.4 Metal accumulation in plants

Plants were uprooted carefully from the pots and washed through tap water followed by de-ionized water to remove all soil and organic particles. Then root and shoot length were measured separately. Different plant parts separated and cut in small pieces and dried in oven at 80 °C temperature for 48 h. The heavy metals of plants were analyzed by wet digestion method [22]. All the analysis was carried out in triplicates. AA-6800 model (SHIMADZU) was used to analyze all heavy metals of both plant and soil samples.

2.5 Statistical analysis

Correlation was performed in between different physico-chemical parameters of soils as well as between metals in different parts of plants, principal component analysis (PCA) was performed by using SPSS, version 10. PCA of heavy metals was calculated based on the correlation matrix. Varimax normalization was used with rotation method in this analysis. Principal component analysis was performed for eight heavy metals as variables (Fe, Mn, Zn, Pb, Ni, Cr, Cd and Cu) of all harvested soil samples to visualize the relationship between soil metal availability and plant uptake. There are eight columns (eight heavy metals) and twenty rows (different metal fractions in different days of harvesting). This method focuses on clustering up the factors. It produces factors that have high correlations with one smaller set of variables and little or no correlation with another set of variables. The heavy metals, which showed a close correlation, were identified and set for further analysis.

3. Results and discussion

3.1 Physico-chemical parameters

The physico-chemical analysis of control soil and different amendments of sludge before growing the plants *C. indica* L. showed significant variation of all parameters (Table 1). pH decreased from 8.2 to 6.9 with an increase in sludge amendments of 30%. EC, CEC and MC also increased with increasing percentage of sludge amendments, whereas, OC decreased with sludge amendments. The TN in sludge was quite higher (0.23%) as compared with the control soil (0.05%). So, sludge application increased the TN concentration in soil at the initial day of plant growth. Whereas, IP was low in sludge (46.3 $\mu g\,g^{-1}$), as compared to the control soil (62.5 $\mu g\,g^{-1}$). Bose and Bhattacharyya [9] showed that pure sludge (100%) is highly acidic with higher concentrations of DTPA extractable metals and total metals (Zn, Cu, Mn, Ni, Cd, Cr, Pb and Fe) in it.

Sludge application increased the total metal concentration in soils (Table 1). Fe was the most abundant metal both in the soil and sludge. In control soil, abundance of total metals was followed by Fe, Mn, Zn, Pb, Ni, Cr, Cd and Cu, whereas in case of sludge-amended soil the abundance was as follows: Fe > Mn > Cr > Pb > Zn > Ni > Cu > Cd.

The metal availability and uptake is mainly dependent on the physico-chemical characteristics of soil like, pH, OC, organic Matter, CEC, etc. [23]. The effect of organic matter on heavy metal solubility also depends to a great extent upon the degree of humification of their organically bounded form (OM) and their effect upon soil pH [24]. Hsiao et al. [25] showed that *Brassica juncea* extracted efficiently Ni and Cr from mine waste amended soil. The physico-chemical characteristics of soil are known to control the fate of the metals and largely influence the plant–soil interaction through rhizospheric processes.

3.2 Sequential extraction

Sequential extraction procedures have usually been used to study metal mobility and availability in soils. There are many studies [2,26] on sequential extraction of metals from sewage sludge; however, no work has so far been carried out on extraction of metals from acidic industrial sludge.

The metals in industrial sludge and sludge-amended soil were fractioned (Table 2) in five fractions (EXC, CAR, Fe–Mn, ORG and RES). The results showed that the maximum level of Cr, Cd, Cu, Zn, Mn and Pb was bounded with Fe–Mn fraction, where as Ni was found mainly in residual fraction. Fe was mostly found in organic and residual fractions. Same results were observed in both control soil and sludge-amended soils at the initial day of plant growth. Soil pH is an important factor to control the availability of metal in

Table 1
Physico-chemical properties of soil (control), sludge-amended soil and sludge collected from Wazirpur industrial area (mean ± S.D.)

Parameters	Sludge amendments				
	Control soil	10%S	20%S	30%S	100%S
pH	8.2 ± 0.2	7.3 ± 0.1	7.1 ± 0.3	6.9 ± 0.2	3.8 ± 0.1
EC (μS cm^{-1})	0.12 ± 0.005	0.12 ± .007	0.14 ± 0.005	0.15 ± 0.005	1.87 ± 0.006
CEC (mequiv. 100^{-1}g)	9.8 ± 1.2	12.2 ± 1.0	15.6 ± 0.3	15.8 ± 0.3	14.2 ± 0.4
OC (%)	0.38 ± 0.04	0.36 ± 0.03	0.34 ± 0.03	0.33 ± 0.02	3.1 ± 0.02
MC (%)	2.2 ± 0.1	2.5 ± 0.2	2.5 ± 0.1	2.7 ± 0.2	3.2 ± 0.2
TN (%)	0.05 ± 00	0.07 ± 0.00	0.09 ± 0.00	0.11 ± 0.01	0.23 ± 0.04
IP (μg g^{-1})	62.5 ± 2.4	60.4 ± 1.8	66.8 ± 1.2	63.7 ± 1.1	46.3 ± 1.6
Total Cr (μg g^{-1})	42.1 ± 2.5	692.6 ± 5.6	832.5 ± 7.4	1134.85 ± 10.5	15209 ± 229.4
Total Fe (μg g^{-1})	24,198 ± 112.85	31,954 ± 257.93	32,160 ± 367.85	32,684 ± 288.53	125,989 ± 489.47
Total Cd (μg g^{-1})	37.8 ± 2.7	40.35 ± 3.5	39.6 ± 2.9	44.5 ± 4.1	43.8 ± 3.6
Total Cu(μg g^{-1})	15.63 ± 2.64	152.83 ± 7.5	247.38 ± 10.5	316.52 ± 13.8	1298.5 ± 113.9
Total Ni (μg g^{-1})	59.58 ± 4.5	217.42 ± 11.83	318.77 ± 10.8	498.68 ± 18.5	1991.9 ± 153.9
Total Zn (μg g^{-1})	142.9 ± 8.4	158.5 ± 7.93	191.7 ± 8.45	238.3 ± 7.35	428.5 ± 8.6
Total Mn (μg g^{-1})	402.6 ± 11.85	2411 ± 175.7	4720.8 ± 143.95	6411.5 ± 185.74	21556.9 ± 132.5
Total Pb (μg g^{-1})	95.7 ± 4.2	134.57 ± 7.47	141.84 ± 6.7	174.6 ± 5.6	441.54 ± 5.7

S: sludge amendment.

soil. Low pH enhanced the availability of metals like Zn, Cu, Mn, Ni, Cd, Cr, Fe and Pb in industrial sludge-amended soil [9].

Copper strongly bound to OM would be released slowly over time as the OM of the sludge is decomposed [27], whereas, Cd and Zn are not bound as strongly to OM. In present study Fe is associated with OM. Walter and Cuevas [28] showed that Cr and Cd was found maximum in Fe–Mn oxide fraction of the soil amended with sewage sludge, which is also found in the present study. There are various reports [16,26,29] on sewage sludge showing maximum amount of Ni in RES fraction that is similar to the present study. The residual fraction is not to expect to release in short period of time, so this is very stable form of all fractions [30].

3.3 Metal accumulation in plants

The accumulations of metals in different parts of *C. indica* L. were shown in Fig. 1. The accumulation of all metals increased with both increasing percentage of sludge amendments as well as with the time. Overall the total metal accumulation (sum of the metal in the root and shoot) in plants was in the order of Fe > Cr > Mn > Zn > Ni > Cu > Cd > Pb. Translocation of metals was restricted from root to shoot. From Fig. 1, it was clear that the concentrations of metals were almost double in root than shoot. Variability of all studied metals in different parts of *C. indica* L. may also be due to

Table 2
Metals extracted (μg g^{-1})[a] from different amendments of sludge just before the growth of *Canna indica* L.

Different fractions	Metals							
	Cr	Fe	Cd	Cu	Ni	Zn	Mn	Pb
Control soil								
EXE	4.42 ± 0.23	65.32 ± 2.35	0.92 ± 0.00	0.92 ± 0.00	1.18 ± 0.00	16.73 ± 0.12	5.63 ± 0.06	17.32 ± 0.08
CAR	1.27 ± 0.00	2156.3 ± 15.42	1.63 ± 0.00	0.88 ± 0.00	3.84 ± 0.11	12.52 ± 0.11	39.54 ± 0.73	6.73 ± 0.03
Fe–Mn	20.3 ± 0.13	3428.9 ± 16.84	22.84 ± 0.7	5.37 ± 0.12	2.84 ± 0.00	67.83 ± 2.1	263.73 ± 7.84	46.73 ± 0.86
ORG	5.27 ± 0.07	10267.5 ± 34.52	2.41 ± 0.00	4.28 ± 0.11	3.64 ± 0.13	9.32 ± 0.42	29.43 ± 0.95	4.63 ± 0.00
RES	7.53 ± 0.07	8185.4 ± 21.63	7.82 ± 0.1	2.41 ± 0.00	50.16 ± 1.9	24.52 ± 0.54	56.73 ± 1.1	15.35 ± 0.93
10%S								
EXE	5.67 ± 0.27	77.82 ± 1.43	1.03 ± 0.00	9.21 ± 0.05	1.92 ± 0.00	16.54 ± 0.05	7.47 ± 0.05	16.29 ± 0.02
CAR	52.18 ± 1.2	24.63 ± 0.69	1.35 ± 0.00	12.2 ± 0.03	6.35 ± 0.02	15.22 ± ± 0.04	149.03 ± 3.2	10.02 ± 0.04
Fe–Mn	528.82 ± 5.2	4619.17 ± 17.32	24.1 ± 0.04	24.68 ± 0.07	8.92 ± 0.03	88.25 ± 1.89	1511.65 ± 5.21	71.28 ± 1.46
ORG	30.21 ± 0.84	15302.18 ± 34.84	2.32 ± 0.00	48.28 ± 1.1	10.28 ± 0.03	11.62 ± 0.02	241.84 ± 2.7	5.93 ± 0.01
RES	43.89 ± 1.03	11823.54 ± 21.54	7.54 ± 0.03	46.85 ± 0.95	178.27 ± 2.75	30.83 ± 0.087	431.94 ± 2.56	18.43 ± 0.03
20%S								
EXE	7.83 ± 0.03	83.28 ± 1.54	1.15 ± 0.00	9.1 ± 0.02	2.09 ± 0.00	18.36 ± 0.02	9.03 ± 0.02	17.11 ± 0.06
CAR	82.84 ± 1.55	26.26 ± 1.25	1.46 ± 0.00	11.85 ± 0.07	11.28 ± 0.06	14.32 ± 0.05	235.81 ± 2.32	9.05 ± 0.03
Fe–Mn	618.38 ± 3.5	4562.83 ± 14.6	25.73 ± 0.5	37.82 ± 0.96	15.37 ± 0.04	109.27 ± 1.43	3182.32 ± 12.52	96.58 ± 1.74
ORG	42.64 ± 0.8	14726.37 ± 31.5	2.19 ± 0.00	84.29 ± 1.9	19.28 ± 0.06	12.27 ± 0.03	467.93 ± 3.36	5.52 ± 0.02
RES	67.29 ± 1.1	12539.1 ± 33.2	7.62 ± 0.02	88.65 ± 1.74	267.38 ± 2.0	32.63 ± 0.74	779.45 ± 4.88	17.36 ± 0.04
30%S								
EXE	8.42 ± 0.04	95.28 ± 1.6	1.19 ± 0.00	8.92 ± 0.02	2.16 ± 0.00	17.35 ± 0.08	10.34 ± 0.04	15.29 ± 0.06
CAR	110.37 ± 2.2	25.18 ± 1.1	1.73 ± 0.00	11.28 ± 0.03	17.28 ± 0.6	13.52 ± ± 0.07	367.92 ± 2.18	11.36 ± 0.04
Fe–Mn	832.93 ± 4.52	4672.43 ± 11.63	27.82 ± 0.07	58.92 ± 1.42	22.85 ± 0.8	156.72 ± 1.64	4237.94 ± 3.2	120.29 ± 1.15
ORG	67.38 ± 1.74	15281.63 ± 30.48	2.37 ± 0.00	112.73 ± 1.4	31.83 ± 0.88	11.64 ± 0.02	589.03 ± 3.9	5.83 ± 0.02
RES	84.53 ± 1.52	12376.72 ± 32.95	7.71 ± 0.03	115.82 ± 1.13	428.93 ± 2.63	31.73 ± 0.6	1183.93 ± 5.73	17.11 ± 0.04
100%S								
EXE	79.65 ± 1.63	178.27 ± 1.37	2.8 ± 0.01	88.9 ± 1.36	23.3 ± 0.58	27.3 ± 0.75	30.21 ± 0.79	18.93 ± 0.2
CAR	612.8 ± 3.4	17292.75 ± 44.73	0.8 ± 0.00	110.8 ± 1.68	62.6 ± 1.1	33.6 ± 0.48	1092.2 ± 5.2	45.83 ± 0.88
Fe–Mn	7665.7 ± 20.73	11201.76 ± 32.75	22.39 ± 0.5	212.4 ± 1.95	89.5 ± 0.9	296.4 ± 1.85	14289.37 ± 32.9	319.34 ± 2.84
ORG	2328.6 ± 8.84	50152.93 ± 177.73	1.9 ± 0.00	465.8 ± 2.5	112.6 ± 1.8	19.2 ± 0.2	2196.3 ± 10.5	21.85 ± 0.5
RES	4237.4 ± 18.77	47192.8 ± 163.75	13.2 ± 0.05	457.2 ± 2.18	1742.4 ± 5.2	56.8 ± 0.95	3921.53 ± 12.62	37.5 ± 0.75

EXE, exchangeable form; CAR, carbonate bounded; Fe–Mn, iron–manganese bounded; ORG, organically bounded; RSE, residual form.
[a] All values are the mean of three replicates ± S.D.

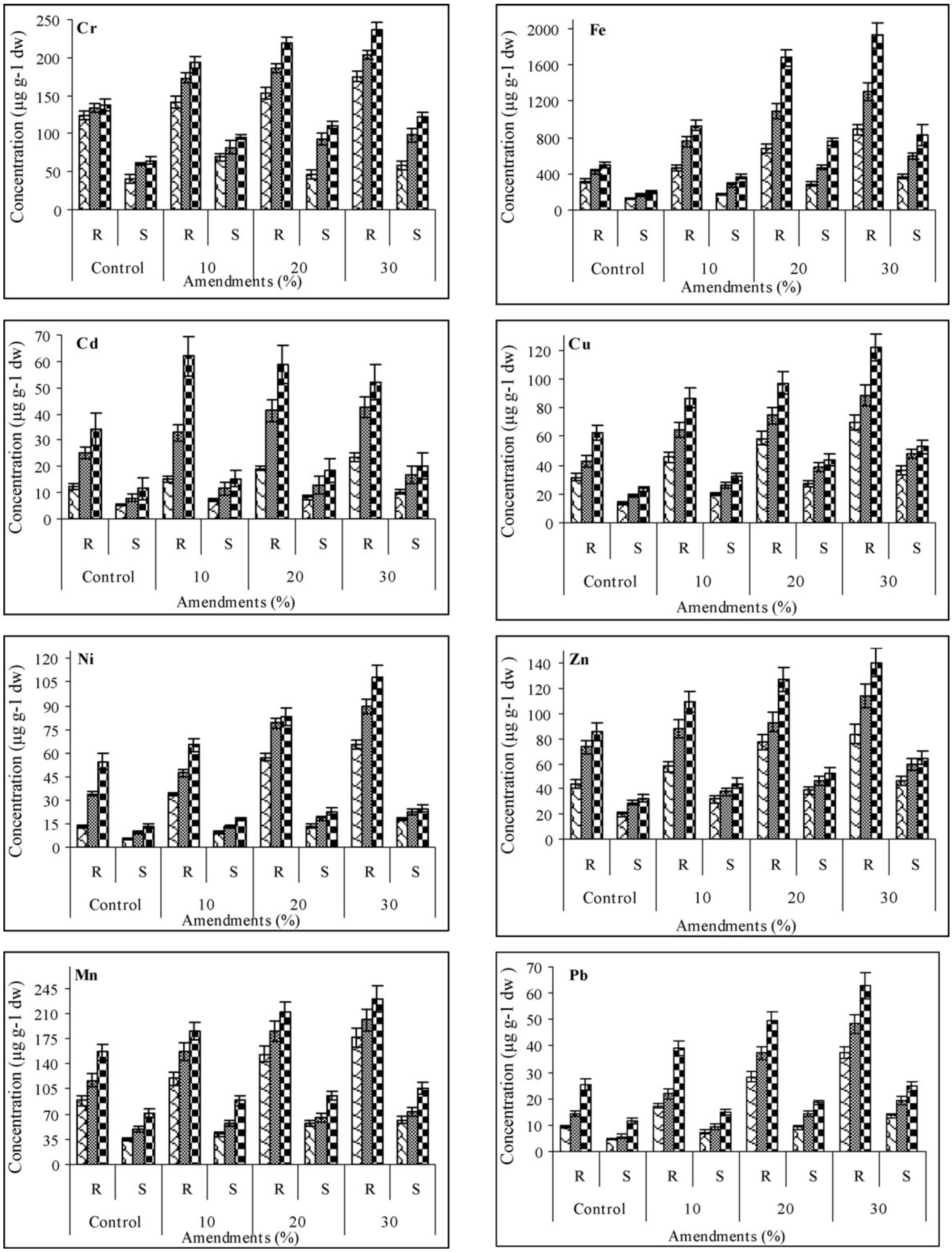

Fig. 1. Accumulation of Cr, Fe, Cd, Cu, Ni, Zn, Mn and Pb ($\mu g\,g^{-1}$ dw) in root and shoot of *Canna indica* L. grown on different amendments of sludge after different harvest (30, 60 and 90 days). All values are mean of three replicates ±S.D. R = root and S = shoot.

compartmentalization and translocation in the vascular system [31].

The root and shoot length also increased with sludge amendments (10%) as well as days (Fig. 2). But in 20% and 30% sludge amendments slightly decreased the length of both the root and shoot without showing any toxic effect. The length of root was more affected than shoot which indicated that metal accumulation was limited in below ground parts.

Normally, the accumulations of metals were restricted in upper parts [16] that were revealed in this study also. The roots of many plants (wheat, pea, *Dalbergia sissoo*, *Acacia arabica* and *Populus euroamericana*) mostly retained all heavy metals [9,32,33] which was not exceptional in this study. Kufka and Kuras [34] reported that the process of metal uptake and accumulation by different plants depend on the concentration of available metals in soils, solubility sequences, the plant species growing on these soils and soil pH, EC,

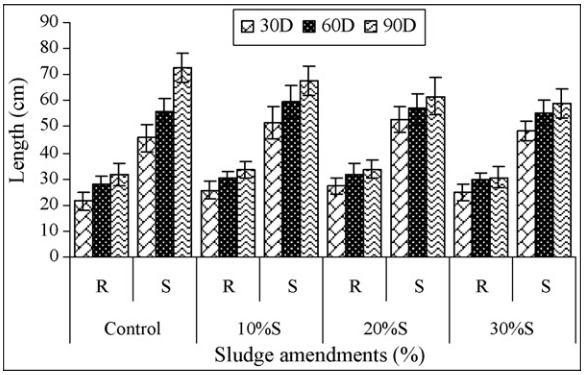

Fig. 2. Effect of root and shoot length (cm) in *C. indica* L. grown on various sludge-amended soils after different harvest (30, 60 and 90 days). All values are mean of three replicates ±S.D. R = root and S = shoot.

Table 3
Correlation coefficients (*r*) of various parameters studied in the different amendments and metals accumulation in the plants of *C. indica* L.

Metals	Physico-chemical parameters			
	pH	EC	CEC	OC
Cr	−0.989**	0.85*	0.968**	−0.985**
Fe	−0.905*	0.958*	0.991**	−0.991**
Cd	−0.907*	0.448	0.76	−0.748
Cu	−0.926*	0.928*	0.937*	−0.981**
Ni	−0.871*	0.967*	0.927*	−0.968*
Zn	−0.953*	0.919*	0.965*	−0.995**
Mn	−0.957*	0.914*	0.96*	−0.995**
Pb	−0.914*	0.94*	0.967*	−0.98**

* Significant at level $p < 0.05$
** Significant at level $p < 0.01$.

CEC, OC, etc. The correlation results (Table 3) defined that the low pH and OC in sludge-amended soil enhanced the metal accumulation in *C. indica* L. (negative correlation) whereas, the increase of EC and CEC also increase the metal accumulation in different parts of plants (positive correlation).

3.4 Translocation factor (TF) of metals from soil exchangeable metals to the plant

Soil to plant translocation factor is one of the key components of phytoextraction and phytoremediation. The values of TF (Table 4) for all the studied metals vary from one sludge amendments to another amendment. The TF of metals from soil to root were as follows: Cr > Ni > Mn > Cd > Cu > Fe > Zn > Pb. In all amendments the plant *C. indica* L. showed maximum TF value from root to shoot were Zn followed by Cu > Cr > Fe > Mn = Pb > Cd > Ni. The TF of all studied metals in shoot metal concentration/root metal concentration (S/R) was quite low (TF < 0.5), but the factor was much higher in root metal concentration/exchangeable metal in soil (R/Ext), i.e. TF varied from 1.3 to 19.1. According to Baker [35] and Farago and Mehra [36], *C. indica* L. comes under accumulator category as the ratio of metal concentration in the plant to that in the soil is »1. The efficiency to remove the Cr from sludge-amended soil is quite high in this plant.

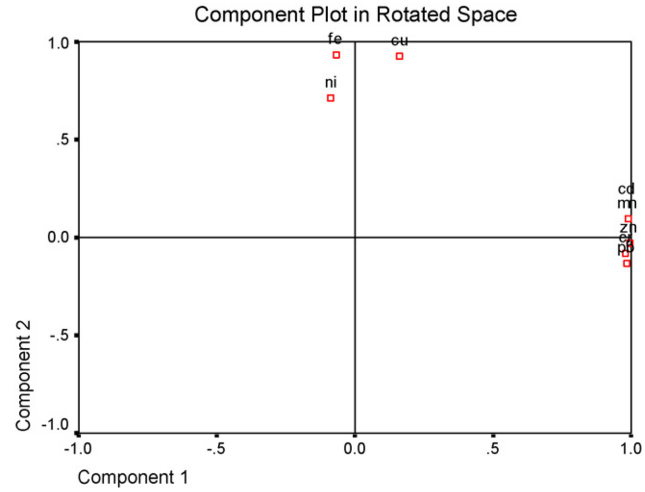

Fig. 3. Figure showing two principle components of all studied metals (different fractions) in sludge-amended soils and different parts of plants throughout the experiment.

3. 5 Principal component analysis (PCA)

The results of PCA for the metal available in soil and accumulations in the different parts of the *C. indica* L. demonstrated that two principal components (Fig. 3) were considered in the PCA analysis, accounting for 90.01% of the total variance in the complete data set. The variables of PCA were the percentage of sludge application in soil and time intervals. Elements such as Cd, Mn,

Table 4
Translocation factor (TF) of extractable metals from soil to root of plants (R/Ext) and root to shoot (S/R) in all amendments and in all harvest

	Metals															
	Cr		Fe		Cd		Cu		Ni		Zn		Mn		Pb	
	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext	S/R	R/Ext
30 days																
Control	0.33	27.98	0.37	4.86	0.43	13.38	0.42	34.38	0.40	11.25	0.46	2.61	0.39	15.85	0.48	0.54
10%S	0.49	25.00	0.38	5.96	0.48	14.78	0.43	4.92	0.27	17.57	0.55	3.50	0.35	15.97	0.43	1.06
20%S	0.30	19.50	0.43	8.10	0.43	16.77	0.47	6.43	0.23	27.38	0.50	4.21	0.37	16.97	0.32	1.66
30%S	0.33	20.82	0.42	9.33	0.43	19.76	0.53	7.78	0.27	30.23	0.55	4.84	0.35	17.07	0.36	2.46
60 days																
Control	0.45	19.91	0.40	4.97	0.32	14.81	0.43	22.40	0.27	9.17	0.39	3.12	0.42	11.61	0.40	0.56
10%S	0.47	19.48	0.36	7.96	0.35	1.15	0.41	4.13	0.28	14.33	0.43	3.90	0.37	15.79	0.43	1.09
20%S	0.50	16.06	0.43	11.93	0.31	11.51	0.51	5.42	0.23	16.79	0.50	3.28	0.35	15.71	0.38	1.44
30%S	0.48	16.16	0.46	12.15	0.39	14.73	0.54	0.93	0.25	16.89	0.52	5.28	0.37	11.64	0.40	2.54
90 days																
Control	0.47	15.69	0.39	5.61	0.34	17.12	0.38	23.19	0.24	12.92	0.38	3.23	0.46	11.86	0.46	0.83
10%S	0.49	17.79	0.39	9.31	0.25	1.92	0.37	4.63	0.27	13.51	0.41	4.00	0.48	15.71	0.38	1.58
20%S	0.51	15.44	0.45	17.59	0.31	15.51	0.45	6.60	0.27	15.07	0.41	4.09	0.45	13.46	0.37	0.89
30%S	0.51	15.21	0.43	16.88	0.39	15.72	0.43	1.19	0.23	14.03	0.46	5.68	0.46	10.16	0.39	0.94

TF, conc. in plant's aerial part/conc. in plant's root (S/R); TF, conc. in plant's root/conc. in soil extractable metals (R/Ext).

Zn, Pb and Cr were in the first principal component (PCA_1), they showed close association which explained over 61.38% of the total variance. All these metals were derived from similar sources, i.e. mixed domestic and sewage sludge. However significant loading for Fe, Cu and Ni contributed to second principal component (PCA_2) which explained over 28.63% of total variance and was considered to originate from same source, i.e. sludge. Fe and Ni showed negative loading which suggests that Fe and Ni have antagonistic effect on Cu. Further, it also suggests the similar sources for Fe, Cu and Ni, i.e. pickling, rolling and electroplating industries [32]. The trend of metal accumulation and translocation observed from root to shoot was as follows: Cu > Cr > Fe > Mn = Pb > Cd > Ni. *C. indica* L. thus accumulates the metals from sludge-amended soils and translocated it from root to shoot in same manner for all these metals, except Cu. Fe and Ni showed antagonistic effect on Cu, which is due to its highest translocation factor in *C. indica* L. As Fe is not a trace element and the concentration of Fe was highest in sludge, the absorption and translocation of Fe were relatively higher than any micronutrients and hence it falls in the middle of the PCA_1 elements.

4. Conclusion

The present study clearly specifies that the increasing percentage of sewage sludge in soil increased the concentrations of Cr, Fe, Cd, Cu, Ni, Zn, Mn and Pb in soil as compared to the control soil. The concentrations of heavy metals also amplified in *C. indica* L. plant grown on industrial sludge-amended soil due to higher uptake of heavy metals causing accumulation of heavy metals in plant tissues. The root length was affected by 20% and 30% sludge amendments in soils which indicated that all the metals were restricted in root zone.

The results obtained from sequential extraction schemes indicate that Cr, Cd, Cu, Zn, Mn and Pb were bounded with Fe–Mn fraction in different amendments while, Ni was found mainly in residual fraction. Fe was mostly found in organic and residual fractions. The TF of metals from soil to root was reasonably higher without any exception which indicated that the plant *C. indica* L. can be use for phytoremediation.

The results strongly supported that the plant *C. indica* L. accumulated higher concentrations of all metals, even in higher sludge amendments. As *C. indica* L. is an ornamental garden plant, the plant can be used to remove the heavy metals from metal contaminated soils or sludge-amended soil without any threat of bio-magnification. Phytoremediation can be reduced the amount of industrial sludge to be landfill and the harvested plants can be further utilized as bio-ore of heavy metal. In a large-scale application the harvested plants can be utilized to generate thermal energy [37]. So, it may be recommended that *C. indica* L. can be used for phytoremediation in most of the studied metals.

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