Bioaccumulation of Heavy Metals in *Pisum sativum* L. Growing in Fly Ash Amended Soil

Sudarshana Sharma[1], *Parmanand Sharma[2], Poonam Mehrotra [3]
[1] Department of Biochemistry, Bundelkahnd University, Jhansi, India
[2] School of Environmental Science, Jawaharlal Nehru University, New Delhi, India
[3] Department of Botany, Bundelkahnd University, Jhansi, India
pnsjnu@gmail.com; Sudarshana77@yahoo.com; mpunu@yahoo.co.uk

Abstract: Presently, the crisis of enormous amounts of fly ash has been sorted out by using it significantly in stabilization and escalating crop growth. In present study pot-culture experiment was performed to observe the influence of fly ash amendments on the growth and accretion of heavy metal in pea plants. Fly ash utilized for this study with high alkalinity and metals was poor in N, P and humus comparable to garden soil. Fly ash and soil were mixed in different ratios i.e. 0, 5, 10, 15, 20 and 25% and used to fill earthen pots (2Kg/pot). Seven days old seedlings were transplanted (3 individual/ pot) in them at glass house. 25 ± 2^0C temperature and moisture at 50% of water holding capacity was maintained throughout the experiment. The results revealed that there was a significant increase in chlorophyll, carotenoids, proteins, biomass and overall growth of target plant up to 10% fly ash amendment. Whereas, phenols and ascorbic acid concentrations were maximum at 25% fly ash amendment. The heavy metals in growth media and plant were significantly augmented and found beneath the permissible limits up to 10% fly ash addition only. Pea seeds demonstrated fascinating results they were harboring the metal concentration in all amendments under permissible range and were safe to consume. Translocation factor was calculated and results illustrated that toxic heavy metals like Cd, Ni and Pb retained in the below ground while micronutrients like Cu, Zn and Fe translocated to above ground parts. Hence, it is evident that pea plants may be a good metal accumulator plant species that could use for restoration of waste land having high alkalinity and low nutrient values. [Journal of American Science 2010;6(6):43-50]. (ISSN: 1545-1003).

Key words: Crop yield; Heavy metals; Bioaccumulation; Translocation factor

1 INTRODUCTION:

Today thermal power plants, based on coal combustion, are major producers of both electricity and fly ash. Fly ash, a finely divided residue, regarded as an amorphous ferroaluminosilicate mineral, containing the naturally elements i.e. similar to that of soil except humus and nitrogen (Wong and Wong, 1986).

In India, about 79% of the electricity is generated by coal based thermal power plants (Singh and Siddiqui, 2003), leads into 110 million tones fly ash per year (Jamwal, 2003) and it will surpass 140 million tones by year 2020 (Kalra et al., 1997). Currently the disposal of such massive amounts of fly ash; disposed off either by dry (dump in landfills and fly ash basins) or wet methods (artificial lagoons/ pond ash) has been become a major environmental quandary. All methods ultimately lead to the dumping of fly ash on open land causing deterioration of soil and environment (Jala and Goyal, 2006). On the other hand profitable uses of the fly ash have been also reported for various uses, raw material for building making and in agricultural land reclamation, quarry restoration, (Kriesel et al., 1994) a vast potential for use in agronomy as an amendment and etc.

Today, the steadily increasing input of xenobiotics into environment has elicited growing concerns regarding the impact of fly ash on several ecological aspects. In recent years, all metals present in fly ash viz. Zn, Cu, Cd, Hg and Fe have received much attention in ecotoxicological researches (Lee et al., 2006 and Tiwari et al., 2008). Although, some of them are considered to be essential elements except Hg, and As having no role in biological systems (Pahlsson, 1989). To overcome from this serious ecological threat, (Vajpayee et al., 2000) phytoremediation may be used to clean and revegetate fly ash landfills by various suitable plantation to check the dispersal of fly ash arising into the atmosphere and to develop a bioaesthetic environment. Because earlier Cha et al., (1999) observed that addition of alkaline fly ash (pH over 9.0), may diminish soil acidity to a level and may amplify the availability of trace metals, SO_2^{-4} and other nutrients except availability of nitrogen (N) in soil did not affected by the fly ash addition (Lee et al., 2006).

Although it is scarce in N and P it was observed that the response of plants to macro- and micro-nutrients of fly ash may vary from advantageous to detrimental in various concentrations (Singh et al., 1997). Earlier Adrino et al., (1980) stated that successful revegetation of alkaline fly ash is limited by: (1) phytotoxicity due to Boron and Al; (2) restriction of root growth due to the fine particle size of the ash; (3) nutrient deficiency and; diminution in free living and symbiotic N fixing microorganisms. Therefore, disposal and utilization of fly ash is required careful assessment

So, keeping above views, the present study was designed with following objectives: to evaluate the effect of fly ash amended soil on heavy metals accretion, growth, biomass and some biochemical responses of pea plants. This study is vital because it deals with pea plant which is a leguminous plant (could compensate the N deficiency of fly ash) and also an imperative crop of north India.

MATERIAL AND METHODS:

2.1. Collection of samples and preparation

Unweathered fly ash sample was collected from a fly ash landfill of Parichcha thermal power plant, Jhansi, India. Fly ash and garden soil samples were stored in a transparent poly bags and fresh samples were used for pH (electrode), Electrical Conductivity (electrode), Moisture Content (%) and Water Holding Capacity (WHC), Organic carbon and CEC analyses. All the analyses were done by the standard methods given by Jackson (1958).

The processed fly ash and soil were mixed in six proportions i.e. 0, 5, 10, 15, 20 and 25% and used to fill earthen pots (2Kg/pot) one week before to planting. Pea seeds (*Pisum sativum* L.) were collected from National Seed Corporation, New Delhi (NSC) and seven days old seedlings were planted, 3 per pot, by pressed into soils to a depth of about 1cm under glass house condition (25 ± 2^0C temperature, moisture at 50% of water holding capacity and humidity 40-50% throughout the experiment).

2.2 Soil and plant analysis

Soil samples were analyzed at two diverse stages during the experiment i.e. at the initial stage (seedling establishment stage) of experiment and at culmination of plants. Analyses were done for the potential bioavailable heavy metals by DTPA method (Lindsay and Norvell (1978) and total heavy metal concentration (EPA 3050 method) in both fly ash and soil. This method has been adopted by the USEPA as a standard method and recovers almost 100% of the metals from samples.

At culmination, plants were aloof from pots and plant components were alienated and washed with running tap water for few minutes. Plants were kept in oven at 70^0C till constant weight, ground and sieved (through 0.1mm sieve). Heavy metals in plant material (dried samples) were estimated by using method of Allen et al., (1986) with Atomic Absorption Spectrophotometer (Model AA 6800, Perkin–Elmer, Inc., Norwalk, CT, USA).

For chlorophyll, carotenoid, total phenols and ascorbic acid content in fresh leaves were quantified following Machlachlan and Zalik, (1963); Duxbury and Yentsch, (1956); Bray and Thorpe (1954) and Keller and Schwager (1977) respectively. Foliar protein contents were precise by method of Lowry et al., (1951) using bovine serum albumin (BSA) as standard.

2.3 Translocation factor (TF)

The accretion of metals in the plant parts was determined as *f* factor, also known as transfer coefficient (Smith, 1996).

2.4 Statistical analysis:

All the data were analyzed using one way ANOVA test using GPIS software (1.13) (Graphpad, California, USA) and different correlations and regressions has been done for statistical analysis of data by using SPSS version 11.5.

RESULTS:

3.1 Physicochemical properties of fly ash and soil

The physico-chemical properties of fly ash depend on the nature of parent coal, conditions of combustion, type of emission control devices, storage and handling methods.

In the study the garden soil used for experiments was slightly alkaline pH (7.6 ± 0.04) with sufficient amount of N, P and organic carbon (Table 1). Soils metals were followed the trend Fe>Zn>Cu>Pb>Ni>Cd respectively. Whereas, fly ash illustrated a high value of pH (10.68 ± 0.02) and heavy metals (Table-1). The trend of metals in fly ash was as followed Fe>Ni>Pb>Zn>Cu>Cd. Fly ash was containing insufficient quantity of nutrients like N (0.04 ± 0.01), P (0.03 ± 0.02) and also organic carbon (0.39 ± 0.05). But occurrence of other micronutrients viz. Cu, Zn, Fe in a high enormity makes it apposite to use as manure to augment crop productivity.

3.2 Plant biomass production

Results exhibited a highly significant ($p<0.001$) rise in the growth of plants at lower ratio i.e. 5- 10% compare to control (Table 2). This may be explained by soil's high buffering capacity to the alkalinity of fly ash. At high application rates (20 and 25%), a significant diminution ($p<0.001$%) was perceived in length and weight of root and shoot. It might be due to compactness of particles which, probably served as physical barrier to root elongations.

Similar pattern for the protein, cholorophyll and carotenoid content were noticed in plants and were depicted in Table 3. At 5-10% fly ash ratios ascorbic acid and phenols demonstrated a non-significant ($p>0.05$) augmentation. But at 20 and 25% fly ash treatment a significant augmentation were noticed.

The regression (r) analysis between soil available metal concentration at culmination to root, shoot biomass and weight of seeds were represented in Table 4. Results explained that roots and shoot length did not influenced by Cu and Cd availability in soil ($p<0.001$) while seeds weight and metal concentrations were significantly correlated. Root and shoot length were highly correlated ($p<0.001$) with the availability of Zn, Pb, Ni and Fe in soil at culmination. Seeds demonstrated a different pattern and it was noticed that Cu, Cd, Pb and Ni were positively and significantly correlated with weight of seed while Fe and Zn availability explained a non significant ($p<0.001$) correlation.

3.3 Plant elemental uptake

With increasing percent of fly ash, metal concentration in plants increased in each treatment. Cu, Zn and Ni demonstrated that their concentration traversed permissible limits at 20 and 25% of fly ash addition, although Cd and Pb concentration didn't reach up to phytotoxic level in any ratio but phtotoxicity symptoms were noticed at 20 and 25% of fly ash addition viz. decline in growth parameters.

Fig 1 portrayed the concentration of metals in roots, shoots, and seeds of pea plants. Micronutrients concentration were fallen within the permissible range for roots and shoots i.e. 20-100 and 100-400 µg/g for Cu and Zn respectively at 5-10% ratios (Kabata and Pendias, 2000). But they become elevated then acceptable limit at 20 and 25% fly ash amendments. In case of seeds metals were found to be within the dietary limit i.e. 20 and 50µg/g/day respectively at all ratios and hence safe to consume (Pahlsson, 1989).

Fly ash amendments raise the Cd, Pb and Ni availability in soil and hence its uptake by the pea plants (Fig 1). They were retained by the roots and not transferred to the shoots and seeds. This study was consistent with the Kabata and Pendias, 2000 and Cd and Pb concentrations were observed within 5-30 and 30-300 µg/g respectively. Up to 15% fly ash amendment it was observed that Ni concentration was beneath or within the limit (10-100 µg/g).

Seeds obtained from all treatments demonstrated that Cd, Pb and Ni concentrations were beneath the limit for daily intake of food i.e. 3-10, 25-85 and 250µg/g/day respectively (MacNicol and Beckett, 1985).

Table 5 illustrated the linear regression coefficient values (r) between soil available metals at maturity to metal concentration in root, shoot and seed of pea plant. All the metals showed an extremely significant relation ($p>0.001$) with uptake and availability of metals in all ratios at culmination.

3.4 Translocation of metals within plant body

Translocation factor for various metals from root to shoot and shoot to seed were represented in Table 6. It was noticed that translocation factors were almost less than one unit except Cu (in some cases). Ratio of root to shoot (S/R) for Cu varies 0.44-0.56, which again indicated that most of the Cu retains in the root tissues and not transferred to shoots, while ratio of shoot to seeds Se/S for Cu varies between 0.80-1.10 that shows a good translocation of Cu from shoot to seed. For Zn S/R varies between 0.53 to 0.86 and Se/S varies between 0.83 to 0.92, which again showed that pea plants tends to accumulate Zn in aerial parts than to below ground parts. Fe is also a very important metal for plant growth but translocation factor showed a different pattern for this metal. Most of the Fe remains in the shoots and not transferred to seeds (S/R 0.50 to 0.57 and Se/S is 0.22 to 0.31).

Translocation factor of S/R for Cd is more than Ni and Pb, which has almost same values but on the other hand it is also important to know that Se/S was found to lowest for Cd. It varies between 0.06-0.20 that shows most of the Cd retains by the roots and rest is restricted by shoots. Baker (1981) divides plants in three category i.e. accumulator, excluder and indicator. In accumulator plants the concentration ratio of the element in the plant to that in the soil is >1. In excluder plant metal concentrations in aerial parts are maintained low (<<1) and constant over a wide range of soil concentrations. In indicator plants the uptake and transport of metals were regulated in such a way that the ratio of the concentration of element in the plant to that in the soil is near 1.

Thus, *P. sativum* in this study was found to be accumulator for Cu, Zn and Fe while it was an excluder for Cd, Pb and Ni.

4. DISCUSSION:

According to researches, reduction in acidity by addition of a medium having pH over 9.0 is suitable for agriculture because it may increase the availability of trace metals, sulfates and other nutrients. On the other hand absence or low concentration of nitrogen, phosphorus and microorganisms makes this medium questionable.

In this study, the retarded root growth of *P. sativum*, grown in different fly ash amended soil was observed as the fly ash ratios increased. Gunse et al., (2000) reported that root growth inhibition was might be due to high contents of heavy metals like Cu, Cd, Zn, etc. which inhibited the root elongation by reducing cell division. Therefore the reduced growth of root in fly ash amended soil may be attributed to heavy metals, Boron and Al toxicity (Gunse et al., 2000). Adrino et al., (1980) stated that nutrient deficiency, reduction in free living and symbiotic N fixing microorganisms and inhibition of root growth due to the fine particle size of the ash were also responsible for retarded growth of plants.

In present study retardation of over all performance of plant attributed to the toxicity caused by high metal concentrations in higher ratios of fly ash and this high concentration of such metals affect basic photosynthetic tools. The decrease in chlorophyll content may also be ascribed due to decreases in carotenoids contents, a non-enzymatic antioxidants playing a important role in protection of chlorophyll pigments against a stress (Krupa and Baszynski, 1995). Besides it low availability of N and P contents also responsible for poor growth and development (Jala and Goyal, 2006). It is interesting to observe that the addition of fly ash to the soil did not generate any statistically significant inhibition in the production of biomass. Here growth response is in line with results of other researchers (Moliner and Street 1982).

There was no any visible injury noticed (necrosis) due to fly ash addition during the growth and development of plants during experiment. In fact upto 15%, fly ash could be used as soil ameliorant to increase crop performance. No visible symptoms of nutrient deficiency or phytotoxicity were observed in plants grown in 5-15% but at 20 and 25% of fly ash amendment heavy metal toxicity was clearly visible in the form of reduction of shoot and root length. Although Adrino et al., (1980) suggested that application of fly ash on agricultural soils should not exceed the10% rate owing the adverse effect of fly ash on soil. Therefore, this study is consistent with the findings of above mentioned scientist. Besides this Singh et al. (2008), reported that fly ash amendments affected negatively the growth of *Beta vulgaris* at all treatments (0-20%). In our study all metals were highly correlated with its availability and uptake at culmination which was consistent with the finding of Oritz and Alcaniz, (2006).

Table 1: Physico-chemical characteristics of the soil and fly ash used in the study

Properties	Soil	Fly ash
pH	7.6±0.04	10.68±0.02
EC m mhos cm-1	0.12±0.01	5.73±0.05
CEC MEQ	4.12±0.02	0.45±0.04
Particle distribution (%)		
Sand	65.55±0.24	45.54±0.12
Silt	22.28±0.28	40.45±0.18
Clay	12.17±0.02	14.01±0.08
Total-N %	0.25±0.12	0.04±0.21
Av- N (g/Kg)	46.55±0.28	BDL
Toatl-P %	0.08±0.01	0.03±0.02
Av- P (g/Kg)	26±0.26	BDL
OC %	1.65±0.03	0.39±0.05
Cu (mg/Kg)	1.56±0.12	2.40±0.02
Zn (mg/Kg)	10.63±0.21	3.43±0.02
Cd (mg/Kg)	0.33±0.02	0.98±0.01
Pb (mg/Kg)	1.34±0.12	6.89±0.02
Ni (mg/Kg)	1.02±0.01	10.68±0.04
Fe (mg/Kg)	61.25±0.64	122.48±0.55

Notation: All the values are mean of three values (±SD); BDL: below detection limit

Table 2: Effect of fly ash amendments on crop growth and yield

Percentage of fly ash (%)	Root			Shoot			Seeds
	Length (Cm)	Fresh weight (g)	Dry weight (g)	Length (Cm)	Fresh weight(g)	Dry weight (g)	Dry weight (g/pot)
0	10.45	0.65	0.13	20.98	6.96	0.69	1.54
5	11.32[a]	0.78[c]	0.16	22.85[a]	7.65[a]	0.78[b]	1.76
10	11.98[a]	0.81[b]	0.18	24.04[a]	8.89[a]	0.89[a]	2.18

15	9.67^{a}	0.61^{ba}	0.11^{c}	21.64^{a}	7.18^{a}	0.75a	2.69^{ab}
20	7.85^{a}	0.48^{bac}	0.09^{cb}	18.12^{a}	6.72^{a}	0.66^{ab}	2.58^{b}
25	7.01^{a}	0.39^{a}	0.07^{ba}	16.01^{a}	5.43^{a}	0.60^{ba}	2.27^{c}

Notation: ^{a}‹ 0.001 ^{b}‹ 0.01 ^{c}‹ 0.05. Compare to control. Data are mean value ±SD. Means followed by the same letter are not significantly different ($p<0.05$).

Table 3: Effect of fly ash amendments on protein, photosynthetic pigments and antioxidants contents in leaves of pea plants

Percentage of fly ash (%)	Proteins (mg/g fresh leaf)	Photosynthetic pigments (mg/g fresh leaf)				Antioxidants (mg/g fresh leaf)	
		Chlorophyll a	Chlorophyll b	Total	Carotenoids	Ascorbic acid	Phenols
0	17.32	0.71	0.18	0.89	0.39	0.34	5.21
5	19.47	0.77^{c}	0.20	0.97^{a}	0.42	0.33	5.33
10	21.86^{ac}	0.80^{b}	0.22	1.02^{ac}	0.45	0.35	5.36
15	15.67^{a}	0.67^{a}	0.15^{cb}	0.82^{ba}	0.35^{b}	0.38	5.82^{a}
20	12.54^{ab}	0.59^{a}	0.13^{cba}	0.72^{a}	0.31^{cba}	0.41^{cb}	6.22^{a}
25	10.76^{a}	0.51^{a}	0.11^{ba}	0.62^{a}	0.26^{bac}	0.47^{abc}	6.65^{a}

Notation: ^{a}‹ 0.001 ^{b}‹ 0.01 ^{c}‹ 0.05. Compare to control. Data are mean value ±SD. Means followed by the same letter are not significantly different ($p<0.05$).

Table 4: Linear regression coefficient values* (r) between soil available metals at maturity to length of root, shoot and wt of seeds of pea plant

Treatments	Root	Shoot	Seeds
Cu	-0.747^{NS}	-0.614^{NS}	0.777^{c}
Zn	-0.918^{a}	-0.897^{a}	0.555^{NS}
Fe	-0.847^{b}	-0. 839^{c}	0.647^{NS}
Cd	-0.676^{NS}	-0.568^{NS}	0.832^{b}
Pb	-0.760^{c}	-0.6711^{c}	0.817^{b}
Ni	-0. 912^{a}	-0.763^{c}	0.779^{c}

Notation: Significant at ^{a} highly significant ^{b} significant ^{c} less significant; ^{NS}: Non significant ($p<0.001$)

Table 5: Linear regression coefficient values* (r) between soil available metals at maturity to metal concentration in root, shoot and seed of pea plant

Treatments	Root	Shoot	Seeds
Cu	0.949^{a}	0.948^{a}	0.961^{a}
Zn	0.962^{a}	0.984^{a}	0.984^{a}
Fe	0.990^{a}	0.995^{a}	0.931^{a}
Cd	0.923^{a}	0.954^{a}	0.960^{a}
Pb	0.982^{a}	0.973^{a}	0.967^{a}
Ni	0.997^{a}	0.995^{a}	0.988^{a}

Notation: ^{a} Extremely significant ($p<0.001$)

Table 6: Translocation factors* between root to shoot (S/R) and shoot to seed (Se/S) of different heavy metals in pea plants

Percentage of fly ash (%)	Cu		Zn		Fe		Cd		Pb		Ni	
	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S
0	0.56	0.84	0.53	0.90	0.56	0.29	0.49	0.06	0.37	0.11	0.36	0.14
5	0.56	1.06	0.56	0.83	0.57	0.31	0.39	0.10	0.36	0.13	0.33	0.16

10	0.48	1.10	0.63	0.84	0.53	0.22	0.48	0.15	0.34	0.16	0.34	0.21
15	0.44	0.80	0.71	0.83	0.54	0.26	0.42	0.07	0.36	0.18	0.33	0.25
20	0.46	0.86	0.77	0.92	0.50	0.27	0.33	0.20	0.38	0.18	0.34	0.23
25	0.51	0.80	0.86	0.88	0.50	0.30	0.36	0.19	0.38	0.17	0.32	0.28

Notation: *Translocation factors the ratio of metal concentration in shoot and metal concentration in plant root.

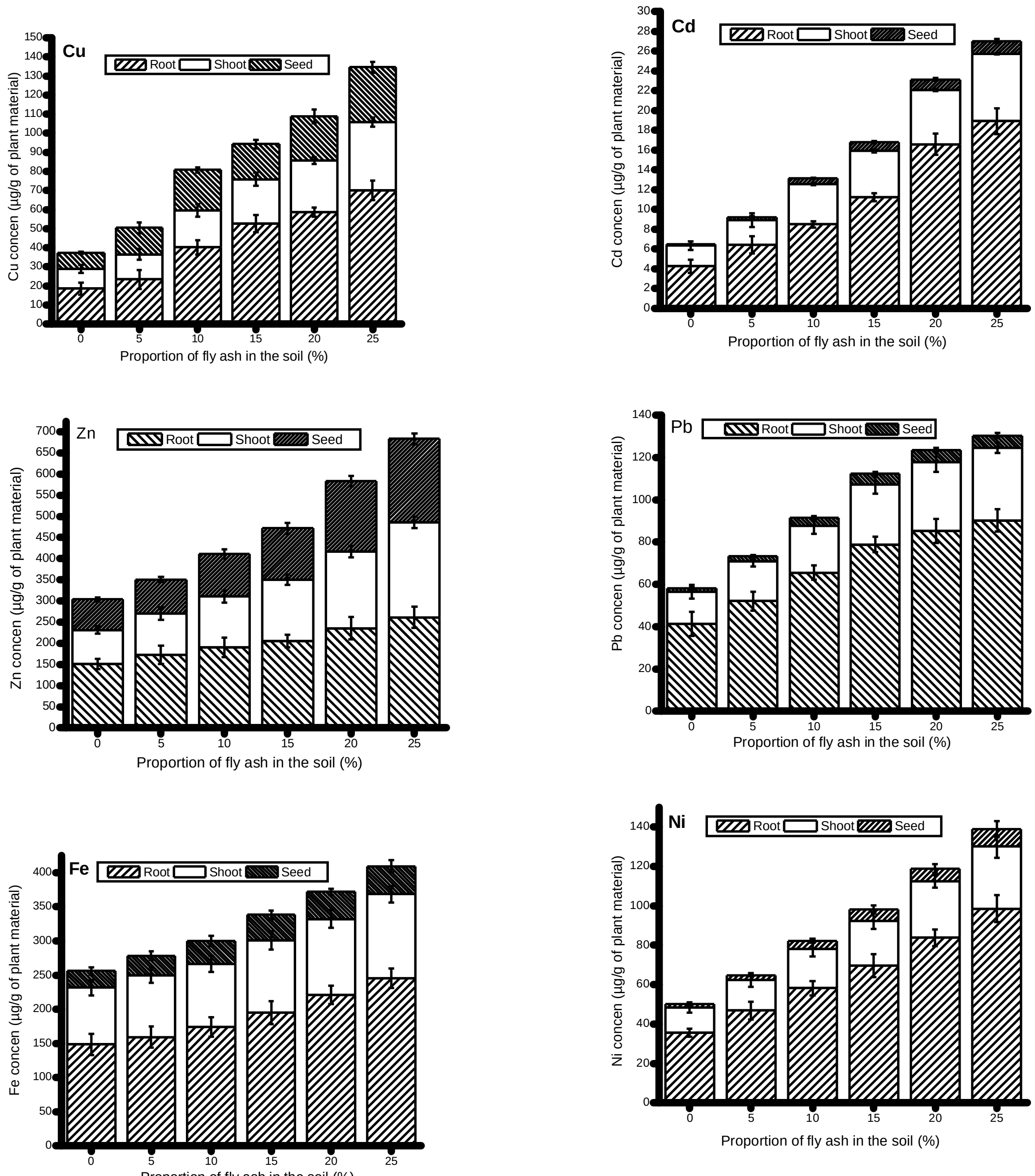

Figure 1: Showing the different metal concentration in root, shoot and seeds of pea plants at maturity. Data are mean of three replication ±SD.

5. CONCLUSIONS

It is well documented that fly ash generated by thermal power plants is similar in physicochemical properties to soil except nitrogen and of organic matter (humus). Application of fly ash to soil has been found to increase the bioavailability of heavy metals, and its low doses (up to 10%) did not cause significant increases in heavy metal concentration and could be used as soil manure. It is most interesting finding of this study that metals which transferred to aerial parts are micronutrients and important for plant growth and heavy metals which are considered to toxic, retains in the roots of pea plants only. Pea seeds showed interesting results they were harbouring the metal concentration in all amendments under permissible range and were safe to consume.

So, pea plants may be an alternative plant species for restoration of waste land having high acidity with low nutrients. However, extensive trials are prerequisite to find out a proper combination of fly ash with each soil type. But the care should be taken to access the level of metals at the time of consumption of seeds.

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Corresponding author:
Dr. Parmanand Sharma,
School of Environmental Science,
Jawaharlal Nehru University,
New Delhi, India-110067,
Phone No. - +91 9968897332,
Fax No. +91 11 26741502
E-mail: pnsjnu@gmail.com

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