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Role of *Brassica juncea* (L.) Czern. (var. Vaibhav) in the phytoextraction of Ni from soil amended with fly ash: Selection of extractant for metal bioavailability

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Abstract

A pot experiment was carried out to study the potential of the plant of *Brassica juncea* for the phytoextraction of metal from fly ash amended soil and to study correlation between different pool of metals (total, DTPA, $CaCl_2$ and NH_4NO_3) and metal accumulated in the plant in order to assess better extractant for plant available metals. The results of total metal analysis in the soil revealed the presence of Cr, which was found below detection limit (BDL) in the plants. The fly ash (FA) amendments and soil samples were extracted with different extractants and the level of metal vary from one extractant to another. The regression analysis between total and extractable metals showed better regression for all the tested metals except Mn ($R^2 = 0.001$) in DTPA extraction. Correlation coefficient between metal accumulation by the plant tissues and different pool of metals showed better correlation with DTPA in case of Fe, Zn and Ni, whereas, Cu was significantly correlated with NH_4NO_3 and other metals (Pb, Mn) with $CaCl_2$. The soil analysis results revealed that the mobility and plant availability of metals (Fe, Mn, Zn, Ni) within the profiles of amended soils was influenced by the change in pH, however, Pb and Cu was not affected. The metal accumulation in total plant tissues was found in the order of Fe > Ni > Zn > Mn > Cu > Pb and its translocation was found more in upper part. The plants grown on soil amended with 25%FA have shown significant increase in plant biomass, shoot and plant height, whereas, no significant effect was observed in root length. The cluster analysis showed 10%FA behave differently on the basis of physico-chemical properties and metal behavior. Thus, it may be concluded that *B. juncea* can be used for phytoextraction of metals, especially Ni in fly ash amendment soil.

Keywords: Fly ash; Metals; DTPA; $CaCl_2$; NH_4NO_3; *Brassica juncea*

1. Introduction

The rapid increase in the capacity of the power generation in India has resulted in the production of huge amount of coal fly ash. As per the available estimate, the production of coal ash in India including both fly ash and bottom ash is likely to touch 140 million tonnes per annum by 2020 [1]. In view of high cost of disposal and environmental pollution, the gainful utilization of coal ash to the maximum extent is of vital importance. Fly ash can be used as a soil additive in agriculture because of its capacity to supply small amounts of nutrients (Ca, Na, K, Mg, B, S, Mo) to the plants, which promote growth of the plants and alleviate the condition of nutrient deficiency in soils. In addition, it may also improve the physico-chemical properties of the soil such as pH, texture and water holding capacity. Factors against fly ash disposal in agricultural soils are especially the content of potentially toxic elements (Ni, Pb, Cd, B, Se, Al, etc.), high salinity and reduced solubility of some nutrients due to high pH of fly ash [2].

In environmental studies, the total metals' concentration is not the actual total value but refers to the fraction of metals in the soil, which is removed by strong extractant such as nitric acid and perchloric acid. It includes readily exchangeable ions along with more strongly bound forms within the solid phase of the soil that is not bioavailable. Thus, the total metal concentrations do not necessarily reflect metal level in the soil solutions; however, the plants exposed to the metals via the soil solutions [3]. In case of plants, the level of free metal ions in the soil solution may be most important parameter in view of the toxicity as free ions

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are more toxic than complexed metals. Further, the importance of metal in soil solutions is emphasized due to the mobility of metals. Soil properties such as pH and organic matter content are known to influence the bioavailability of metals in soils. Thus, there is a need to use simple methods to predict bioavailable metals concentration instead of total metal concentration in the soil. The extraction procedures are the most widely accepted and used method in soil science to assess the availability of the metal to the plants [4].

The total metal present in the soil is not available to the plant grown therein. The extractable metals (DTPA, $CaCl_2$ and NH_4NO_3) can be used as an indicator of bioavailability and toxicity of the heavy metals [5,6]. These procedures, using a single extractant like DTPA, EDTA, $CaCl_2$, $NaNO_3$ and NH_4NO_3 provide a relative empirical method for evaluating the potential availability of soil pollutants for plant uptake [7]. The amount of heavy metals extracted by such methods gives an idea of the size of a pool that might be depleted by a plant during the growth period; however, the extent of extracting methods depends on the soil tested. Thus, it is necessary to have available sufficient information about the behavior of the different extraction procedures when they are applied to different soil type and number of heavy metals.

Most of the plants growing on contaminated soils have been shown to accumulate significantly high amount of metals [8–12]. However there are "excluder plants" that have a low uptake of trace elements, by active exclusion in the roots, even at high external concentration in the soil solution [13]. *Brassica* species are known to accumulate high concentration of metals [14–17]. The presence of elevated levels of heavy metals in the growth medium suppresses metabolism and translocation of reserve material from the reserve tissues in the growing regions and their subsequent utilization there. Thus, heavy metals at supra-optimal concentrations affect growth, development and yield [12,18–20].

In view of hyper-accumulating potential of the plant, the study is planned to assess the metal accumulation of the plant of Indian mustard (*Brassica juncea* L. Czern, var. Vaibhav) in fly ash amended soil and to study correlation between different pool of metals (total, DTPA, $CaCl_2$ and NH_4NO_3) and metal accumulated in the plant in order to assess better extractant for plant available metals.

2. Materials and methods

Unweathered fly ash (FA) were collected randomly from dumping sites of National Thermal Power Plant, Unchhahar, Raibarelli, (U.P.), India, in large plastic bags and brought to the field laboratory. The garden sub soil (GS) was collected from National Botanical Research Institute, Lucknow (India). The FA and GS were air dried for 7 days and then passed through a 2 mm sieve before making various amendments (manually) for experimental studies. For convenience, the amendments are denoted as 10%FA (10%FA + 90% soil), 25%FA (25%FA + 75% soil), 50%FA (50%FA + 50% soil), 75%FA (75%FA + 25% soil), 100%FA (100%FA) and garden soil served as control.

2.1. Reagents

The analytical grade reagents were used. Certified aqueous standards of the elements (Sigma) were used to prepare standard curve for AAS. All the standards, reagents solution and samples were stored in polyethylene containers previously cleaned with 4 M HNO_3 and rinsed with double distilled water (ddw).

2.2. Physico-chemical analysis of different amendments

Physico-chemical analyses were carried out in triplicate on ground dry samples of fly ash and their different amendments with garden soil before the growth of the plants of *B. juncea.* The pH of the different amendments was measured in 1:2.5 soil–water suspension using Orion pH meter (Model 420); electrical conductivity (EC) was measured using Orion Conductivity Meter (Model 150). Organic carbon (OC) and organic matter (OM), cation exchanges capacity (CEC) by estimated by the method of Kalra and Maynard [21].

2.3. Single extraction of different amendments and estimation of metals' content

DTPA extractable fraction was obtained by mechanical shaking of 4 g of lyophilized sample with 40 ml of 0.5 M DTPA, 0.01 M $CaCl_2$, 0.1 M TEA (triethanol amine) buffered at pH 7.3 for 2 h [22]. For $CaCl_2$ extraction, 5 g soil with 50 ml of 0.01 mol l^{-1} $CaCl_2$ solution was mechanically shaken for 2 h [23]. In case of NH_4NO_3 extraction [24], 10 g of soil was added in 50 ml of 1 M NH_4NO_3 and shaken for 2 h at room temperature. For the estimation of total metals' content in the soil, 0.50 g soil + 10 ml double distilled water + 5 ml HCl + 4 ml HF + 1 ml HNO_3 was used and digested in Microwave Digestion System MDS 2000 in closed Teflon vessels. All the analyses were carried out in triplicates. GBC Avanta Σ Atomic Absorption Spectrophotometer (AAS) was used for the estimation of metals.

2.4. Experimental design

Seeds of *B. juncea* L. Czern. (var. Vaibhav) were obtained from Chandra Shekhar Azad Agricultural University, Kanpur. All the seed were sterilized with 0.1% mercuric chloride for 5 min to avoid fungal contamination, washed with redistilled water for three times and soaked in water overnight. The soaked seeds were evenly sown in pots (12″ in diameter), which were filled with different amendments (10, 25, 50, 75 and 100%) of fly ash (15 kg), along with one set of control (GS), each in three replicate and kept under natural conditions. Ten seeds were sown in each pot to a depth of 0.5 cm and watered daily till seed germination. When the seeds were developing five or six leaves, they were thinned out to retain four uniform ones per pot and allowed to grow. The plants were irrigated with tap water at regular intervals avoiding leakage of water from the pots. Plants were well developed at 90 days and harvested plants were used for the determination of growth parameters and metal accumulation.

Table 1
Certified and observed values of elements

Code nos. (elements)	Certified values	Observed values[a]
BND 1101.02		
Zn	99.69 ± 0.94	101.34 ± 0.87
Fe	100.12 ± 0.78	99.32 ± 0.44
Cu	99.95 ± 0.84	101.43 ± 0.51
BND 102.03		
Pb	2.01 ± 0.02	2.05 ± 0.03
BND 402.02		
Cr	2.00 ± 0.02	1.98 ± 0.03
BND 1001.02		
Ni	1.00 ± 0.02	0.99 ± 0.01

[a] $n = 10$.

2.5. Metal accumulation in plants

Fully grown plants were harvested after 90 days from different amendments and repeatedly washed with double distilled water. The metal content in different parts of the plant was determined in oven dried (80 °C) samples by digesting in 70% HNO_3 using Microwave Digestion System (MDS 2000).

2.6. Quality control and quality assurance

The standard reference material of metals (E-Merck, Germany) was used for the calibration and quality assurance for each analytical batch. Analytical data quality of metals was ensured through repeated analysis ($n = 6$) of EPA quality control samples (Lot TMA 989) for metals (Cd, Cr, Cu, Pb) in water and the results were found to be within ±2.79% of certified values. For plants, recoveries of metals from the plant tissues were found to be more than 98.5% as determined by digesting three samples each from an untreated plant with known amount of metals. The blanks were run in triplicate to check the precision of the method with each set of samples.

The reference solution of multi-elements and single element was used for calibration of analytical equipment and validation of test methods provided by National Physical Laboratory (NPL), New Delhi, India and their certified and observed values are given in Table 1.

2.7. Statistical analysis

The experiment was performed in completely randomized block design involving six amendments of fly ash with GS with three replicates. To confirm the variability of the data and validity of the results, two ways analysis of variance (ANOVA) was performed. Students' *t*-test (two tailed), regression and correlation coefficients analysis were also performed. Cluster analysis were applied on experimental data standardized through *z*-scale transformation in order to avoid misclassification due to wide difference in data dimensionality and allowed the assessment of homogenous groups for the impact of the fly ash amendments observed in this study.

3. Results and discussion

Physico-chemical properties of different amendments of fly ash and garden soil were presented in Table 2. The analysis of the results showed significant increase in pH levels with an increase in fly ash amendment ratio. This may be due to the high content of CaO and MgO in fly ash and acid-neutralizing capacities [25]. In the present study, EC decreased with an increase in fly ash amendment ratio. An increase in the level of pH by the addition of fly ash in garden soil might cause the precipitation of soluble cations in the fly ash amended soil and consequently resulted in reduction in EC. The level of CEC and OC were found to decrease with increase in fly ash ratio. Evans [26] reported that the CEC of the soil depends on soil collides and the relative charges of metal species in solution and on the soil surface.

The level of different metals (Fe, Mn, Cu, Zn, Pb, Ni, Cr and Cd) extracted with DTPA, $CaCl_2$ and NH_4NO_3 from different amendments of fly ash and garden soil is presented in Table 3. Chromium and Cd were estimated in all the samples and found below detection limit in all the three extractants. The level of DTPA extractable metals (Fe, Mn, Zn, Pb, Ni) was found significantly high in fly ash than garden soil except Cu, which was higher in the garden soil. The analysis of the results showed that the levels of all the tested metals were found to decrease with an increase in fly ash amendments ratio from 10%FA to 75%FA. In case of $CaCl_2$ extractable metals in different amendments of fly ash with garden soil, the results showed that the values of Mn and Pb decreased with an increase in

Table 2
Physico-chemical properties in different amendments of fly ash with garden soil before the sowing of seeds of *B. juncea*

Parameters	Amendments					
	GS	10%FA	25%FA	50%FA	75%FA	FA
pH (1:2)	6.3 ± 0.0	7.1 ± 0.0[a]	7.4 ± 0.0[a]	7.5 ± 0.0[a]	7.7 ± 0.0[a]	8.3 ± 0.0[a]
EC (μSM^{-1})	824.0 ± 0.0	740.7 ± 2.3[a]	725.2 ± 3.2[a]	457.0 ± 3.2[a]	445.0 ± 1.5[a]	341.0 ± 2.3[a]
CEC Cmol (p+) (kg^{-1})	39.1 ± 2.0	28.1 ± 2.1[b]	23.1 ± 1.3[c]	20.0 ± 2.1[c]	16.3 ± 0.4[c]	7.9 ± 0.9[c]
Organic carbon (%)	2.1 ± 0.0	1.8 ± 0.1[b]	1.7 ± 0.2[b]	1.6 ± 0.1[d]	1.7 ± 0.2	1.8 ± 0.2
Organic matter (%)	3.7 ± 0.0	2.8 ± 0.2[b]	2.9 ± 0.2[b]	2.7 ± 0.2[d]	2.9 ± 0.0[a]	3.1 ± 0.0[a]

All the values are mean of three replicates ± S.D., Students' *t*-test (one tailed as compared to GS).
[a] $p < 0.001$.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.02$.

Table 3
The level of extracted (DTPA, NH_4NO_3, $CaCl_2$) and total metals' concentration (mg kg^{-1} dw) in different amendments of fly ash with garden soil (w/w) before sowing of seeds of *B. juncea*

Metals	Amendments					
	GS	10%FA	25%FA	50%FA	75%FA	FA
Total metal concentration (mg kg^{-1} dw)						
Fe	25676 ± 360	21227 ± 262	30209 ± 431	29095 ± 410	26673 ± 406	18444 ± 116
Mn	221.7 ± 27.1	254.8 ± 20.6	273.7 ± 24.1	280.8 ± 14.5	273.7 ± 31.3	242.2 ± 22.9
Zn	17.3 ± 1.5	63.4 ± 3.9	65.4 ± 2.4	60.6 ± 3.1	53.0 ± 1.7	64.5 ± 5.9
Ni	18.6 ± 1.3	18.5 ± 1.2	15.2 ± 0.9	13.6 ± 0.7	12.0 ± 0.0	17.7 ± 1.5
Cu	13.1 ± 1.8	12.1 ± 1.0	11.1 ± 0.8	10.5 ± 0.5	10.2 ± 1.7	10.9 ± 0.7
Pb	4.0 ± 0.5	15.6 ± 1.0	10.8 ± 0.5	8.0 ± 0.6	6.2 ± 1.05	12.3 ± 1.0
Cr	8.5 ± 1.9	9.0 ± 2.1	8.3 ± 1.0	6.1 ± 1.8	5.0 ± 0.7	9.7 ± 0.6
DTPA extractable metals (mg kg^{-1} dw)						
Fe	15.77 ± 0.8	31.0 ± 3.1	21.5 ± 0.4	19.7 ± 1.1	18.6 ± 1.5	27.6 ± 4.1
Mn	4.0 ± 0.0	39.4 ± 3.3	24.4 ± 0.7	15.2 ± 0.2	9.1 ± 0.2	43.6 ± 2.4
Zn	3.8 ± 0.5	11.5 ± 1.1	10.4 ± 0.4	9.2 ± 0.1	9.8 ± 0.1	11.8 ± 0.1
Ni	0.6 ± 0.0	1.8 ± 0.1	1.5 ± 0.1	0.6 ± 0.16	0.5 ± 0.1	0.7 ± 0.0
Cu	4.8 ± 0.1	3.2 ± 0.0	2.1 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	2.5 ± 0.1
Pb	2.7 ± 0.1	4.3 ± 0.6	3.2 ± 0.1	2.5 ± 0.4	2.3 ± 0.0	4.0 ± 0.1
Cr	Bdl	Bdl	Bdl	Bdl	Bdl	Bdl
$CaCl_2$ extractable metals (mg kg^{-1} dw)						
Fe	740.2 ± 10.5	528.3 ± 11.9	230.1 ± 11.3	269.5 ± 19.6	239.0 ± 23.8	203.9 ± 12.0
Mn	13.0 ± 2.1	50.9 ± 2.1	42.6 ± 1.1	28.2 ± 1.1	30.4 ± 5.6	60.6 ± 10.0
Zn	5.9 ± 0.3	12.3 ± 0.5	14.3 ± 0.0	12.3 ± 1.1	14.7 ± 1.1	14.2 ± 1.6
Ni	2.3 ± 0.1	1.7 ± 0.4	2.0 ± 0.2	1.9 ± 0.4	2.0 ± 0.0	2.5 ± 0.1
Cu	2.6 ± 0.2	2.5 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	2.3 ± 0.4
Pb	2.0 ± 0.0	5.5 ± 0.0	5.0 ± 0.4	3.5 ± 0.7	2.7 ± 0.4	4.9 ± 0.3
Cr	Bdl	Bdl	Bdl	Bdl	Bdl	Bdl
NH_4NO_3 extractable metals (mg kg^{-1} dw)						
Fe	1.30 ± 0.05	3.17 ± 0.01	2.07 ± 0.03	1.70 ± 0.03	0.79 ± 0.01	1.05 ± 0.02
Mn	3.17 ± 0.01	2.61 ± 0.01	2.34 ± 0.01	1.81 ± 002	1.18 ± 0.05	0.47 ± 0.00
Zn	0.13 ± 0.01	0.20 ± 0.01	0.24 ± 0.01	0.14 ± 0.00	0.22 ± 0.01	0.41 ± 0.01
Ni	0.39 ± 0.04	0.53 ± 0.02	0.32 ± 0.01	0.26 ± 0.01	0.19 ± 0.01	0.32 ± 0.01
Cu	0.45 ± 0.01	0.72 ± 0.01	0.59 ± 0.01	0.52 ± 0.02	0.62 ± 0.03	0.63 ± 0.03
Pb	0.10 ± 0.00	1.09 ± 0.01	0.99 ± 0.01	0.44 ± 0.01	0.31 ± 0.00	1.44 ± 0.02
Cr	Bdl	Bdl	Bdl	Bdl	Bdl	Bdl

All the values are mean of three replicates ± S.D. Bdl—below detection limits; Cd is Bdl in all amendments.

fly ash ratio from 10%FA to 75%FA. Iron, Cu and Ni were found maximum in garden soil than different amendments of fly ash. In case of Zn, the level of metal was found low in garden soil than in all the fly ash amendments. In case of NH_4NO_3 extraction, the results showed decrease in the levels of metals (Fe, Mn, Ni, Pb, Cu) with an increase in fly ash concentration. No such change in Zn was observed. Pueyo et al. [6] used three extraction procedures ($CaCl_2$, $NaNO_3$, NH_4NO_3) for predicting trace metal (Cd > Zn > Cu > Cu > Pb) mobility in the contaminated soil. They found that out of three extractants, $CaCl_2$ extraction procedure seems to be most suitable method for the bioavailability of these metals. The bioavailability of metals in soils depends to a large extent on their distribution between the solid and solution phases. This distribution is, in turn, dependent on the soil processes; cation exchange, specific adsorption, precipitation and complexation. pH is generally acknowledged to be most important influencing factor on metal bioavailability in soils. Usually, pH shows an inverse relationship with solubility. Overall comparison of all the three extractants showed that the level of metal content was found low in NH_4NO_3 extraction in comparison to $CaCl_2$ and DTPA extractions.

The soil was extracted with different extractants (DTPA, $CaCl_2$ and NH_4NO_3) to assess the level of the metals, which is available to plants. The analysis of data showed that the level of the metals in the extracted solution was proportional to the amount of metal in soil. The results (Table 4) of regression analysis (R^2) revealed that the amount extracted by DTPA was strongly correlated with total metals content in soil in all tested metals except Mn. Among all the metals, Zn ($R^2 = 0.919$) and Cu ($R^2 = 0.948$) extracted with DTPA showed significant correlation with total metals content in soil. Soltanpour [27] reported that very good correlations between extractable and total metals for Zn and Pb in addition to Cd for contaminated soils. However, Bhogal et al. [28] reported good correlation between total metals (Zn, Cu, Cd, Ni) with NH_4NO_3 extractable metals in the sewage sludge contaminated soil.

The information about the relationship among the different physico-chemical parameters (pH, EC, CEC, OC, OM) and metals (total, extractable) in different amendments of fly ash with

Table 4
The accumulation of metals (μg g^{-1} dw) in different parts of *B. juncea* grown in different amendments of fly ash with garden soil (w/w)

	Amendments					
	Fe	Mn	Zn	Ni	Pb	Cu
Metal accumulation (mg kg^{-1} dw) in roots						
GS	95.3 ± 18.9	3.7 ± 0.8	12.2 ± 2.7	10.4 ± 1.1	1.0 ± 0.4	4.6 ± 0.0
10%FA	133.7 ± 4.2	3.9 ± 0.3	21.6 ± 3.1	157.6 ± 16.5	8.1 ± 1.2	5.8 ± 0.0
25%FA	190.2 ± 15.5	4.6 ± 0.7	21.7 ± 7.1	153.3 ± 7.6	2.5 ± 0.5	4.1 ± 0.0
50%FA	123.3 ± 19.2	3.6 ± 0.3	38.5 ± 1.2	79.4 ± 5.8	1.5 ± 0.1	3.7 ± 0.5
75%FA	95.5 ± 13.5	3.5 ± 0.4	41.7 ± 3.0	118.8 ± 4.7	3.4 ± 0.8	5.2 ± 0.4
FA	88.5 ± 11.7	5.6 ± 1.5	37.0 ± 9.2	125.8 ± 4.8	0.8 ± 0.2	4.5 ± 0.8
Metal accumulation (mg kg^{-1} dw) in shoots						
GS	171.1 ± 15.6	13.2 ± 0.6	11.8 ± 3.0	17.8 ± 11.7	0.9 ± 0.0	5.0 ± 0.2
10%FA	233.2 ± 18.1	10.8 ± 1.7	25.7 ± 2.5	195.9 ± 13.4	5.5 ± 1.2	5.9 ± 0.2
25%FA	224.4 ± 29.1	6.4 ± 0.1	14.4 ± 3.8	129.6 ± 8.5	4.5 ± 0.0	5.8 ± 0.1
50%FA	179.1 ± 11.1	9.4 ± 3.1	8.1 ± 1.4	191.0 ± 16.9	1.9 ± 0.8	4.5 ± 0.2
75%FA	171.5 ± 15.5	10.4 ± 1.0	19.8 ± 3.2	130.3 ± 6.7	3.1 ± 0.7	5.5 ± 1.4
FA	288.5 ± 16.8	28.2 ± 3.6	23.7 ± 4.1	139.5 ± 14.5	3.2 ± 0.6	6.2 ± 0.8
Metal accumulation (mg kg^{-1} dw) in seeds						
GS	185.8 ± 27.7	15.5 ± 2.3	18.5 ± 1.8	8.6 ± 8.5	Bdl	6.3 ± 0.1
10%FA	216.8 ± 41.4	28.2 ± 2.6	55.1 ± 1.4	95.0 ± 6.0	2.9 ± 0.7	7.0 ± 0.6
25%FA	107.1 ± 15.5	24.0 ± 1.8	46.7 ± 4.6	84.5 ± 8.1	3.1 ± 0.2	4.1 ± 0.0
50%FA	95.5 ± 8.5	10.5 ± 2.7	13.4 ± 1.5	106.8 ± 13.7	2.2 ± 0.2	2.6 ± 0.1
75%FA	190.3 ± 15.4	24.4 ± 3.6	53.5 ± 7.8	101.4 ± 11.2	3.3 ± 0.0	4.7 ± 0.7
FA	569.5 ± 17.7	49.6 ± 4.2	67.1 ± 2.4	160.2 ± 9.0	3.3 ± 0.8	6.0 ± 0.6

All values are mean of three replicate ± S.D. ANOVA, Fe; *F* (Conc.), roots = 2.63; shoots = 2.46, seeds = 5.89*; *F* (Expo.) = roots = 0.79, shoots = 0.89, seeds = 0.04. Mn; *F* (Conc.), roots = 0.076, shots = 2.32, seeds = 0.27; *F* (Expo.); roots = 0.32, shoots = 0.011, seeds = 0.00. Zn; *F* (Conc.), roots = 21.63*, shoots = 0.00, seeds = 0.08; *F* (Expo.), roots = 0.10, shoots = 0.08, seeds = 0.00. Ni; *F* (Conc.), roots = 2.77, shoots = 0.37, seeds = 1.89; *F* (Expo.), roots = 0.51, shoots = 0.54, seeds = 0.83. Cu; *F* (Conc.), roots = 0.34, shoots = 0.10, seeds = 0.44; *F* (Expo.), roots = 0.13, shoots = 0.41, seeds = 0.26. Pb; *F* (Conc.), roots = 2.25, shoots = 1.01, seeds = 1.54; *F* (Expo.), roots = 0.34, shoots = 0.03, seeds = 1.77; *$p < 0.05$. Bdl, below detection limits; Cd and Cr are Bdl in all samples.

garden soil were analyzed using cluster analysis (Fig. 1). The cluster diagram showed two main clusters: A includes 10%FA, cluster B contain two other cluster, b1 (25%FA) and b2 (GS). These dendrograms explain the grouping of amendments of similar or nearly identical, physico-chemical properties and metal behavior. Thus, 10%FA have shown different behavior than other amendments.

The accumulation of metals, Fe, Mn, Zn, Pb, Cu and Ni in different parts of the plant grown on different amendments of fly ash was shown in Table 5. The results were analyzed on the basis of total metal accumulation in the plant (roots + shoots + seeds) which was found in the order: Fe > Ni > Zn > Mn > Cu > Pb. Interestingly, the total accumulation of toxic metals (Ni, Pb) was recorded many times higher in the plants grown on fly ash amended soil as compared to the plants grown on garden soil. The total accumulation of Fe and Mn was found almost in the same range in GS and up to 75%FA except 100%FA. The accumulation of Fe, Mn and Zn was found in the order: seeds > shoots > roots, whereas, the accumulation trend of Ni was found as shoots > seeds > roots. No particular trend was observed in case of Pb and Cu. The analysis of the results showed that upper part of the plant has shown high accumulation of metals as compared to lower part. Several authors have reported that the process of metal uptake and accumulation by different plants depend on the concentration of available metals in soils, solubility sequences and the plant species growing on these soils [29,30]. Madejon et al. [31] reported that the use of plants of *Helianthus annuus* for phytoremediation in mine spill affected area. The potential for phytoextraction is very low, however, it may be used as soil conservation. In above ground parts in the spill-affected plants, none of the major trace elements (As, Cd, Cu, Pb, Tl) reached the levels which are phytotoxic or toxic for

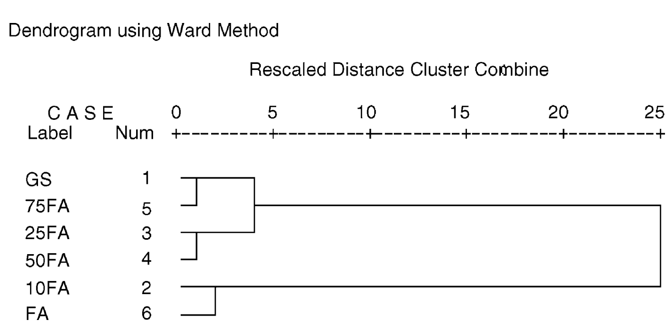

Fig. 1. Cluster analysis between physico-chemical properties and metal behavior of different amendments.

Table 5
Regression coefficient (R^2) between total metals and extractable (DTPA, $CaCl_2$ and NH_4NO_3) metals

Extractants	Fe	Mn	Zn	Cu	Pb	Ni
DTPA	0.495	0.001	0.919[b]	0.948[b]	0.818[a]	0.123
$CaCl_2$	0.003	0.015	0.817[a]	0.714	0.934[b]	0.041
NH_4NO_3	0.256	0.261	0.074	0.092	0.787	0.003

[a,b] Significant relationship at $p < 0.05$ and 0.01, respectively.

Table 6
Correlation coefficient (*r*) between metal accumulation in the plant of *B. juncea* and metals (total, DTPA, $CaCl_2$ and NH_4NO_3)

Metals	*r*-Values			
	$Soil_{total} - Plant_{Accu}$	$Soil_{DTPA} - Plant_{Accu}$	$Soil_{CaCl_2} - Plant_{Accu}$	$Soil_{NH_4NO_3} - Plant_{Accu}$
Fe	−0.818[a]	0.655	−0.450	0.348
Mn	−0.376	0.726	0.756	−0.577
Zn	0.644	0.822[a]	0.777	0.513
Cu	0.450	0.461	0.553	0.818[a]
Ni	−0.259	0.438	−0.351	−0.017
Pb	0.810	0.614	0.743	0.721

[a] Correlation coefficient significant at the level $p < 0.05$ level.

human and animal. In contrast, the accumulation of metals in above ground part was recorded more in the present study. Similar to the present findings, Gupta et al. [32] also reported that the translocation of metals was found more from roots to shoots in the plants grown on fly ash amended soil and may be significant to use the plants for phytoextraction. In the present study, maximum accumulation of Fe was recorded which is in agreement with other reports [12,30]. These authors have estimated the total Fe content in the soil, which does not provide the actual available fraction to the plants.

One of the most important applications of single extraction (DTPA, $CaCl_2$ and NH_4NO_3) of metals in the soils is to assess the bioavailability of metals. In the present study, correlation analysis (*r*) was performed in order to investigate the relationship between the extractable metals content in different amendments of the FA and the total accumulation of metal in the plant (Table 6). Significant ($p < 0.05$) negative correlation was found between total Fe content and plant accumulated metals ($r = -0.818$), non-significant positive correlation was emerged between total metals in the soil and metals (Zn, Cu, Pb) accumulation in the plants. The comparison of the results of correlation coefficient between total metal accumulation in the plant and the level of metals (total and extractable metals) in soil revealed that DTPA have shown better correlation in case of Fe, Zn and Ni, whereas, Cu ($r = 0.818, p < 0.05$) was significantly correlated with NH_4NO_3 which may be due to chelation by NH_3 [14]. Other metals (Pb, Mn) have shown better correlation with $CaCl_2$. In $CaCl_2$, the formation of chloride complexes reduces Pb-organic complexation. In case of Zn, significant positive correlation was found between DTPA extracted metals and Zn accumulation in plants ($r = 0.822, p < 0.05$). In case of Cu, the extractability with NH_4NO_3 was lower as compared to other extractants, which may probably be due to the formation of the strong amino complexes of Cu [33]. Overall analysis revealed that DTPA fraction was found better extractants for bioavailability of metals in fly ash contaminated soil.

pH was one of the important factors influencing metal accumulation. Values of regression coefficients (R^2) between total metal accumulations in the plant *B. juncea* and pH of soil/fly ash amendments are presented in Fig. 2. The value of regression coefficient showed that the mobility and plant availability of Fe, Mn, Zn and Ni within the profiles of amended soils was influenced by the change in pH, however, Pb and Cu was not affected. In contrast to the present finding acidification increases the metal absorption by plant through a reduction of metal adsorption to soil particles [34].

The growth parameters are shown in Table 7. The plant height was found to decrease in fly ash amendments in comparison to GS, maximum significant decrease was found in 100%FA as compared to GS. The root length and biomass were found to increase up to 75%FA and maximum significant decrease of 19.58 and 54.70% was recorded in root length and biomass, respectively in 100%FA in comparison to GS. However, shoot length was found to increase up to 50%FA as compared to GS followed by decrease. The significant decrease of 60.50% was recorded in the shoot length in 100%FA, compared to GS. The increase in growth parameters at initial amendment of fly ash may be due to the presence of nutrients and essential metals in

Table 7
Effect of fly ash on growth parameters plant height, root length, shoot length and biomass of the *B. juncea*

Amendments	Plant height (in.)	Root length (in.)	Shoot length (in.)	Biomass (g dw)
GS	34.5 ± 1.6	6.5 ± 0.7	19.5 ± 1.5	7.3 ± 1.4
10%FA	29.0 ± 1.4[a]	7.0 ± 0.4	23.5 ± 3.2	9.4 ± 1.4[b]
25%FA	31.0 ± 2.5[b]	6.0 ± 0.6	24.0 ± 1.5[c]	10.3 ± 2.1[b]
50%FA	30.0 ± 2.3[b]	7.0 ± 0.6	21.0 ± 1.5	9.2 ± 1.7
75%FA	28.0 ± 1.8[a]	7.2 ± 0.2	18.6 ± 1.5	8.5 ± 1.2
FA	18.7 ± 3.2[d]	6.4 ± 0.2	15.6 ± 2.1[b]	4.5 ± 1.0[a]

All the values are mean of three replicates ± S.D.; Students' *t*-test (one tailed as compared to control).
[a] $p < 0.05$.
[b] $p < 0.20$.
[c] $p < 0.10$.
[d] $p < 0.02$.

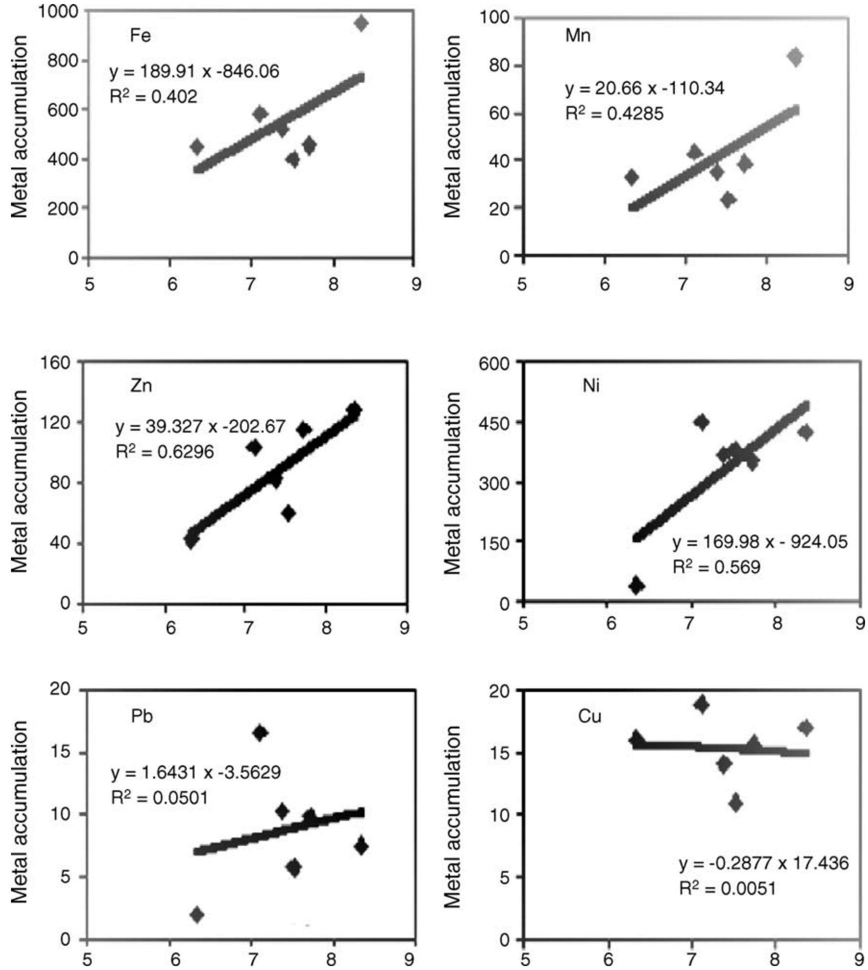

Fig. 2. Relationship between total (roots + shoots + seeds) metal accumulation (mg kg^{-1} dw) in the plant and their corresponding pH in soil/fly ash amendment.

fly ash. The present findings that the lower amendment favors the growth of the plant are also supported by Singh and Sinha [19] in the plant of *B. juncea* and sunflower [20] grown on different amendment of tannery sludge. At low concentration of Zn, the growth of *B. juncea* and *Cajanus cajan* [35] and *B. juncea* seedlings [18] was promoted followed by suppression at higher metal concentration. The plants grown on fly ash amended soil have shown an increase in growth parameters [12]. The results of the present study conform to the finding of these authors. Studies carried out with different varieties of *B. juncea* (Indian mustard) have shown that these plants were able to take up and concentrate toxic heavy metals (Pb, Cu, Zn and Ni) to a level up to several percent higher of their dried shoot biomass [14–17]. According to the results of the present study, it would be possible to extract approximately 20.95 and 0.31% of Ni and Pb, respectively as calculated on the basis of DTPA extractable metals from 10%FA. The translocation of toxic elements (Pb and Ni) was found less in comparison to essential elements in seeds. Plants can be used to remediate fly ash contaminated soils under present sets of experimental conditions instead of growing this plant for edible purposes. The prompt restoration of a dense vegetation cover is the most useful and widespread method to stabilize waste physically and to reduce effects of metal pollution.

4. Conclusion

The outcome of the present study is significant for the phytoextraction of Ni from the fly ash contaminated sites using plants of *B. juncea* without affecting its growth. Further, the results of cluster analysis showed that 10%FA have shown different behavior than other amendment. Correlation coefficient between metal accumulation by the plant tissues and different pool of metals showed better correlation with DTPA in case of Fe, Zn and Ni, whereas, Cu was significantly correlated with NH_4NO_3 and other metals (Pb, Mn) with $CaCl_2$. The regression analysis between pH of the amendments and metal accumulation in the plant showed that the mobility and plant availability of Fe, Mn, Zn and Ni within the profiles of amended soils was influenced by the change in pH, however, Pb and Cu was not affected.

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