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RESEARCH ARTICLE

PHYTOEXTRACTION OF HEAVY METAL ZINC (Zn) FROM A CONTAMINATED SOIL USING DIFFERENT AMENDMENTS AND DIFFERENT SPECIES OF BRASSICA

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ABSTRACT

Phytoremediation of heavy metal contaminated soil is an emerging technology that aims to extract of inactivate metals in soils. Chemical immobilization of heavy metals by the application of ameliorants like lime, farm yard manure (FYM), phosphates and combination of FYM+$CaCO_3$ etc. Among these, soil excavation is the only method for the total removal of heavy metals from contaminated soil. Two approaches have been proposed for phytoextraction of heavy metals, namely continuous or natural phytoextraction and chemically enhanced phytoextraction. The first is based on the use of hyper-accumulator plants with exceptional metal-acumulating capability. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to heavy metals. Phytoremediation is the use of crop plants to absorb and remove metal contaminants from the soil. Some crop plants tend to concentrate a specific metal of the heavy metals and allow its removal and safe disposal at the time of harvest. The metal extractability and accumulating ability of crop species is also influenced by the addition or presence of soil amendments and complexing agents.

Key words: Phytoextraction, heavy metals, Phosphate, Farm yard manure

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INTRODUCTION

The alarmingly increasing urbanization and industrialization countrywide is generating enormous amount of inorganic and organic wastes posing to serious problem of safe disposal. Nearly 450 cities in India generate around 1200 tonnes of sewage sludge every day, although there exsits a potential to produce 4000 tonnes of sludge per day (Kaul *et al.,* 1989). Sewage sludge contains variable amounts of heavy metals like Pb, Cr, Ni, Cd *etc.* as well as essential plant nutrients like N, P, K, S and Zn *etc.* sewage sludge is generally disposed off or applied in agricultural lands as a source of plant nutrients. Long term application of sewage sludge has been reported to elevate concentrations of heavy metal in soil under peri-urban agriculture around Delhi (Bansal *et al.,* 1992), Calcutta (Adhikari *et al.,* 1993) and Ludhiana (Arora and Chhibba *et al.,* 1992). These metals once mixed into agricultural soils, do not leach appreciably and get accumulated in surface plough layer by adsorption or precipitation phenomenon (Leeper, 1978; Sakal *et al.,* 1992). Thus, a limiting factor in the long term and indiscriminate use of sewage sludge on agricultural lands is the likelihood of excessive accumulation of heavy metals such as Zn, Cd, Pb, Cr, and Ni, in the soil and resultant Phytotoxicity. Pollution of the environment with toxic metals has increased dramatically since the onset of the industrial revolution. Because they are rich sources of plant nutrients, sewage effluents and sludge are commonly used (often untreated) by the farmers for irrigating soils around industrial units and metropolitan cities of India at the cost of heavy metal contamination. However, these sewage effluents carry appreciable amounts of trace toxic metals (Brar *et al.,* 2000; Pescod, 1992; Yadav *et al.,* 2002). Recently, Rattan *et al.,* (2005) reported a significant build up of Zn, Cu, Fe, Ni and Pb in sewage-irrigated soils, as well as an accumulation of heavy metals in vegetable and field crops grown on such soil. Excessive metal concentration in contaminated soils might result in decreased soil microbial activity and soil fertility (over soil quality), yield loss (McGrath, Chaudri, and Giller, 1995) and possible contamination of the food chain (Hann and Lubbers, 1983). Although the cleanup of contaminated sites is necessary, often the application of environmental remediation strategies is very expensive and intrusive (McGrath *et al.,* 1995). Thus, development of a low –cost and environmentally friendly strategy is needed. Recently, the value of metal-accumulating plants for environmental cleanup has been vigorously pursued (Brown *et al.,* 1995; Salt *et al.,* 1995), giving birth to the philosophy of "phytoextraction" within a

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broader concept of phytoremediation (Kumar *et al.,* 1995). Heavy metal contamination of soils represents a serious environmental issue. In China, heavy metal pollution of soils is dramatically increasing under the influence of rapid developments in industry and agriculture (Liu *et al.,* 2016). According to the report in the Soil Pollution Condition Investigation Gazette in 2014 (The Ministry of Environmental Protection *et al.,* 2014), agricultural soils in China are mainly polluted with Pb, Cd, Cu, Zn, As, Cr, Hg, and Ni, representing significant risks for agricultural product safety and human health (Monterroso *et al.,* 2014, Zhao *et al.,* 2015). In addition, acid rain and soil acidification, which are long-term environmental probl ems in southern China, can aggravate the transportation and bioavailability of heavy metals in the soil (Alghanmi *et al.,* 2015). Therefore, a number of studies have focused on heavy metal control and remedy in soils, either under laboratory conditions or in situ, and indicated a good effect on different types of contaminated soil restoration when appropriate measures are taken (Koptsik *et al.,* 2014). However, as control areas in farmland are generally larger than the contaminated sites, the selection of appropriate techniques is difficult and restricted, because the usage value of land resources needs to be protected and the remediation cost needs to be controlled. Biochar, as a low-cost and environmentally-friendly material, has been used for treating heavy metal pollution in soil (Puga *et al*., 2016, Wang *et al.,* 2013 and Chen *et al.,* 2015).

MATERIALS AND METHODS

Incubation Experiment: The soil incubation experiment was conducted in plastic pot of 4 kg capacity. The experiment had five treatments, comprising of control, FYM, SSP, $CaCO_3$ and FYM+ $CaCO_3$ .The soil experiment was conducted in pots to study the application of Zn as well as different amendments in metal contaminated soil. A basal dose of 45 *N* and 25 K_2O (mg kg^{-1}Soil) was added in the form of urea and muriate of potash. After incorporation of basal nutrients in solution form, metal was applied at the rate of 0 and 20 Zn (mg kg^{-1} soil) in the form of hydrated salts of concerned metals viz. $ZnSO_4.7H_2O$,. Then the soil was thoroughly mixed with different amendments viz. control, FYM (1%), SSP (332 mg kg^{-1} soil), $CaCO_3$ (5%) 50g kg^{-1} soil and FYM (1%) +$CaCO_3$ (5%). Deionized water was added to bring the soil to field capacity and the soil was incubated for one week. Each treatment was replicated thrice.

Soil sampling: Soil samples from the incubation experiment from submerged soil conditions were drawn at 6 and 12 months after treatment application. The soil samples were first dried in air in oven at 70ºC till constant weight, DTPA and diacid extractable heavy metal elements in soils was estimated.

DTPA extractable heavy metals: Zn in soil were determined by DTPA. Soil was extracted with DTPA solution for available Zn as outlined by Lindsay and Norvell (1978). Extracting solution consists of 0.005 *M* DTPA, 0.01 *M* $CaCl_2.H_2O$ and 0.1 *M* triethanolamine (TEA) and the pH was adjusted to 7.2 $\pm$ 0.05. To extraction, to 10 g air dried soil in polythene bottle, 20 ml of extractant was added and the contents were shaken for 1-2 hours. After filtration the extracts were analyzed for Zn with flame Atomic Absorption Spectrophotometer (AAS).

Diacid extractable heavy metals: Soil sample were digested with diacid mixture (hydrofluoric and perchloric acids) in a platinum crucible and subsequently the contents were dissolved in 6 N HCl as per the procedure of Jackson (1967). Zn contents in the digests were determined with flame Atomic Absorption Spectrophotometer (AAS).

Pot culture experiment: Four kg of processed soil was filled in each of the plastic pots (Capacity, 5 kg) and required amount of amendments and added metals, according amount to the treatments, was added and mixed in pots . Water was added to each pot and kept standing 1 cm. above the soil surface to have the submerged soil moisture condition up to 12 months period and pots soil contents were mixed thoroughly with wooden stick.

Pot culture experiments with *Brassica species*: Pot culture experiments with *Brassica speices* were conducted in sets of 5 kg capacity , pots in the net house at the department of chemistry, N.R.E.C. College, Khurja (Bulandshar), U.P.

Crop: Different *species of Brassica*-

1. *Brassica carinata* (Ethiopian mustard)
2. *Brassica napus* (Gobi sarson)

Replication: Three

Experimental Design: Completely Randomized Design (Factorial).

Growing the crop: After thorough mixing of the soil with amendments and heavy metals. Pot filling was done and the seeds of *Brassica* were sown. A handful of soil was removed from each pot, about eight to ten seeds of the respective species were spread uniformly in each pot and subsequently were covered with soil. Ten to fifteen days after germination seedling were thinned out to maintain four plants per pot.

Harvesting of *Brassica species*: The *Brassica species* in all sets of pots were allowed to grow up to full flowering stage. Irrigation was done as and when required to maintain the moisture at field capacity. All the crops (*Brassica species*) were harvesting at full blooming stage, first dried in air and then in hot air up to at 65ºC till the constant weight. Drymatter yield of each species per pot was recorded.

Plant Analysis: The oven-dried plant sample was ground with the help of a stainless steel grinder for subsequent analysis. Two-gram quantity of ground plant material was taken in 100 ml conical flasks. First predigested with HNO_3 and later digested with diacid mixture of HNO_3: $HClO_4$ (5:1) on an electric hot plate. Digested material was cooled, diluted with double distilled water and filtered through Whatman No.1 filter paper in to 100 ml volumetric flask and then the volume was made upto the mark with double distilled water. The plant digests thus obtained were analyzed for Zn using flame Atomic Absorption Spectrophotometer (AAS) at the Department of soils, Punjab Agricultural University, Ludhiana, (Punjab).

Statistical Analysis: Analysis of data generated by incubation experiment as well as pot culture experiment was followed to elucidate the effect of various amendments on availability of metals in soils. Factorial Completely Randomized Design (CRD) was used for evaluating the main effect as well as interaction effects of the three factors, species, type of soil and physiological stages of growth.

RESULTS

DTPA and diacid extractable Zinc content in soil at 6 and 12 months period

The extractability of DTPA for soil Zn as influenced by amendments and added metal is shown in table 1a (After 6 months). On an average, the content of DTPA extractable Zn was significantly increased from 18 mg kg^{-1} (control) to 23.3 mg kg^{-1}(treated) due to addition of Zn at the rate of 20 mg kg^{-1}. The content of DTPA extractable Zn were 22.2, 20.9, 21.1, 18.0 and 21.2 respectively in control, FYM, Phosphate, $CaCO_3$ and FYM+ $CaCO_3$ treated pots. These results show that $CaCO_3$ was most effective in reducing the availability of DTPA Extractable Zn in soils. Other amendments, *viz.* FYM, phosphate, and FYM+ $CaCO_3$ could not reduce the DTPA extractable Zn significantly in control as well as metal treated pots. Data presented in table 2a (After 6 months) shows that 36.4, 36.7 and 37.5 mg kg^{-1} Zn were extracted by diacid from control, FYM and Phosphate treated pots, respectively, whereas, this extractant could not extract Zn up to measurable limit (Flame AAS) from $CaCO_3$ and FYM+ $CaCO_3$ treated pots. To compare the extractability of diacid and DTPA, mean values of extractable Zn as influenced by various amendments, are presented in table 1a. Diacid extracted highest amount of Zn followed by DTPA from the soils where no amendment was applied (control). Similar trends were observed in case of FYM and Phosphate treated pots. However, extractability of diacid was practically nil in pots where $CaCO_3$ was applied either alone or in combination with FYM. Data as given in table 1b. After 12 months show the same trend although amount extracted is lesser than amount of Zn extracted in 6 months by DTPA. When extraction by diacid was studied then values given in table 2a indicate that this extractant could not extract Zn upto measurable limit. Same trend was observed after 12 months and amount Zn extracted in the presence of different amendments were lesser and not upto measurable limit (Table 2b).

Pot culture experiment

Zinc uptake in *Brassica carinata:* Data pertaining to Zn uptake by *B. carinata* as influenced by various treatments of amendments and added metals have been shown in Table 3. A personal of data displayed in indicated that Zn uptake in *B. carinata* at flowering recorded with various treatments T_2, T_3, T_4, T_5 and T_6 were significantly higher than T_1 (Control). The treatments showed a variation in the values of Zn uptake 1076.96 µg pot $^{-1}$ with T_2, 1158.56 µg pot^{-1} with T_3, 974.06 µg pot^{-1} with T_4, 1020.4 µg pot^{-1} with T_5 and 1031.63 µg pot^{-1} with T_6 as against 504.73 µg pot^{-1} observed with control (T_1). The treatments T_2, T_3, T_5 and T_6 marginal higher than T_4. When metals were added to soil (amount increased from 504.73 µg pot^{-1} to 1076.96 µg pot^{-1}). To study the effect of amendments on Zn uptake, data (T_3, T_4, T_5 and T_6) clearly indicate that the metal uptake was reduced by amendments because of immobilization of metal in soil by amendments.

Zinc uptake in *Brassica napus:* Data pertaining to Zn uptake by *B. napus* as influenced by various treatments of amendments and added metals have been shown in Table 4. A close examination of data shown in table 4 clearly indicated that Zn uptake in *B. napus* at flowering recorded with various treatments T_2, T_3, T_4, T_5 and T_6 were significantly higher than T_1 (Control).

Table 1a. Influence of amendments and added metals on DTPA extractable zinc (mg kg^{-1}) in soil (After 6 months period).

Treatments	Added Zn, Cu and Ni (mg kg^{-1})		Mean
	0	20 Zn+10 Cu+2.5Ni	
Control	19.1	25.2	22.2
FYM	17.4	24.3	20.9
$CaCO_3$	15.4	20.6	18.0
Phosphate (SSP)	17.6	24.5	21.1
FYM + $CaCO_3$	20.6	21.9	21.2
Mean	18.0	23.3	

CD (P=0.05) Amendments (A) = 1.5; Metal addition (M) = 0.9; A X M = 2.1

Table 1b. Influence of amendments and added metals on DTPA extractable zinc (mg kg^{-1}) in soil (After 12 months period)

Treatments	Added Zn, Cu and Ni (mg kg^{-1})		Mean
	0	20 Zn+10 Cu+2.5Ni	
Control	17.2	23.0	20.1
FYM	15.2	21.1	18.1
$CaCO_3$	13.6	18.4	16.0
Phosphate (SSP)	15.4	22.4	18.9
FYM + $CaCO_3$	19.0	20.0	19.5
Mean	16.08	20.98	

CD (P=0.05) Amendments (A) = 1.5; Metal addition (M) = 0.9; A X M = 2.1

Table 2a. Influence of amendments and added metals on diacid extractable zinc (mg kg^{-1}) in soil (After 6 months period)

Treatments	Added Zn, Cu and Ni (mg kg^{-1})		Mean
	0	20 Zn+10 Cu+2.5Ni	
Control	35.5	37.3	36.4
FYM	34.8	38.6	36.7
$CaCO_3$	0.03	0.08	0.06
Phosphate (SSP)	35.8	39.4	37.5
FYM + $CaCO_3$	0.03	0.17	0.10
Mean	21.2	23.1	

CD (P=0.05) Amendments (A) = 1.30; Metal addition (M) = 0.80; A X M = 1.80

Table 2b. Influence of amendments and added metals on diacid extractable zinc (mg kg^{-1}) in soil (After 12 months period).

Treatments	Added Zn, Cu and Ni (mg kg^{-1})		Mean
	0	20 Zn+10 Cu+2.5Ni	
Control	33.0	35.0	34.0
FYM	32.6	36.2	34.4
$CaCO_3$	0.02	0.06	0.04
Phosphate (SSP)	33.3	36.9	35.1
FYM + $CaCO_3$	0.02	0.10	0.06
Mean	19.7	21.6	

CD (P=0.05) Amendments (A) = 1.30; Metal addition (M) = 0.80; A X M = 1.80

Table 3. Effect of various treatments of amendments and added metals on Zinc uptake in *Brassica carinata* (Ethiopian Mustard) at flowering:

Code no.	Treatments	Zn uptake in *B. carinata* (µg pot $^{-1}$)
T_1	Control	504.73
T_2	Metals	1076.96
T_3	Metals + FYM	1158.56
T_4	Metals + $CaCO_3$	974.06
T_5	Metals + SSP	1020.4
T_6	Metals + $CaCO_3$+FYM	1031.63

S.Em. (+)25.349
C.D. (5%)80.91

The treatments showed a variation in the values of Zn uptake 1071.8 µg pot $^{-1}$ with T_2, 1100.3 µg pot^{-1} with T_3, 1010.9 µg pot^{-1} with T_4, 918.23.4 µg pot^{-1} with T_5 and 1003.1 µg pot^{-1} with T_6 as against 507.66 µg pot^{-1} observed with control (T_1). The treatments T_2, T_3, T_4 and T_6 marginal higher than T_5. When amendments were added decrease in Zn uptake in plant was observed because of immobilization of metal in soil by the application of amendments.

Table 4. Effect of various treatments of amendments and added metals on Zinc uptake in *Brassica napus* (Gobi Sarson) at flowering:

Code no.	Zn uptake in *B. napus* (µg pot^{-1})
T_1	507.66
T_2	1071.8
T_3	1100.3
T_4	1010.9
T_5	918.23
T_6	1003.1

S.Em. (±)24.106
C.D. (5%)74.94

DISCUSSION

The results generated from the soil incubation study and pot culture experiments are discussed below. The first part of the discussion deals with the soil incubation experiment conducted with the aim to observe the changes occurring in DTPA and diacid extractable heavy metals (Zn,Cu and Ni) in soil at 6 and 12 months period after the addition of amendments (FYM, SSP, $CaCO_3$, and FYM+$CaCO_3$) and added metals (Zn,Cu and Ni) under as well as submerged soil moisture condition. The second part of the discussion deals with the finding emerged from the five pot culture experiments,each with *.B.juneca, B.campestris, B. carinata, B.napus* and *B.nigra* used as test crops. The study is to compare *the Brassica juneca, Brassica campestris, Brassica carinata*, *Brassica napus* and *Brassica nigra* with respect to accumulation capacity and uptake of heavy metals (Zn, Cu and Ni) by these *Brassica species* to assess than influence of amendments (FYM, $CaCO_3$, SSP and FYM+$CaCO_3$) on the accumulation and uptake of heavy metals by these *Brassica species*. DTPA extractable for soil Zn as influenced by amendments and added metals are shown in table 1a indicate that the content of DTPA extractable Zn was significantly increased from control. These results show that $CaCO_3$ was more effective in reducing the availability of DTPA extractable Zn in soils. Other amendments, *viz.* FYM, phosphate and FYM+$CaCO_3$ could not reduce the DTPA extractable Zn significantly in control as well as metal treated pots. Data presented 6 and 12 months in table 2a and 2b shows that Zn were extracted by diacid from control, FYM and phosphate treated pots, respectively whereas, this extractant could not extract Zn up to measurable limit (flame AAS) from $CaCO_3$ and combination with FYM+$CaCO_3$ treated pots. Data as given in table 1b (after 12 months) show the same trend although amount extracted is lesser than amount of Zn extracted in 6 month by DTPA. When extraction by diacid was studied the values in table 2a and 2b indicate that this extractant could not extract Zn up to measureable limit. Same trend was observed after 12 months and amount Zn extracted in the presence of different amendments were lesser and not up to measureable limit. When amendments were added to the soil, metal concentration in soil was reduced due to immobilization of metal in soil by amendments. Effect of various treatments of amendments and added metals on metals uptake in different *Brassica species* was observed at the time of flowering and following results were obtained Metals uptake by *Brassica carinata* in the presence of different amendments was studied at the time of flowering. Data given in Table 3 about uptake of Zn indicate that it is maximum in the presence of FYM while minimum in the presence of amendment $CaCO_3$. When *Brassica napus* was studied then following data were observed for uptake of metals using different amendment at the time of flowering. Zn uptake as indicated by data in table 4 show that uptake of Zn is maximum with FYM and minimum with SSP. Comparable study of Zn uptake by different *Brassica species* in the presence of different amendments as indicated that in case of amendment FYM, SSP, and combination of $CaCO_3$+FYM maximum uptake was by *Brassica carinata*. While in the presence of amendment $CaCO_3$ maximum uptake was *Brassica napus*. Ascending order of Zn uptake with amendments FYM, SSP and combination of two amendments $CaCO_3$+FYM is identical given as *B. juneca, B. campestris, B. nigra, B. napus* and *B. carinata*. Only in case of amendment $CaCO_3$ Zn uptake by *B. napus* is more than *B. carinata* remaining order being identical for *B. juneca, B. campestris* and *B. nigra*.

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