ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 08, pp.36833-36836, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

REMEDIATION OF HEAVY METAL (CU) CONTAMINATED SOIL USING DIFFERENT AMENDMENTS AND BRASSICA SPECIES

***Kushmander Singh, Neeraj Kumar and Kuldeep Singh**

S.S.M.V. (PG) College, Shikarpur Bulandshahr, India

ARTICLE INFO

Article History:
Received 03rd May, 2016
Received in revised form
19th June, 2016
Accepted 25th July, 2016
Published online 31st August, 2016

Key words:

Phytoremediation,
FYM, Heavy metals.

ABSTRACT

Single extraction of solid phase metals in soil using selective chemical extractants such as strong chelating agent has been used to indicate the bioavailability of heavy metals in soil. Chelating agent like DTPA is used as the common extractant to determine the bioavailability of metals in soils. Bioavailability of metals in soil is controlled by several chemical reaction like adsorption, desorption and precipitation. These reaction govern the chemical equilibrium of metal ions in solid and solution phases in soil. Use of plants which are hyper accumulators of metals like different species of *Brassica* for phytoremediation of polluted soils. Chemical immobilization of heavy metals by the application of amendments like lime, FYM, phosphate and their combination.

Citation: Kushmander Singh, Neeraj Kumar and Kuldeep Singh. 2016. "Remediation of heavy metal (cu) contaminated soil using different amendments and Brassica Species", *International Journal of Current Research*, 8, (08), 36833-36836.

INTRODUCTION

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 (Bekar *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 broader concept of phytoremediation (Kumar *et al*., 1995). The alarmingly increasing urbanization and industrialization countrywide is generating enormous amount of inorganic and organic wastes posing to serious problem of safe disposal.

****Corresponding author: Kushmander Singh***
S.S.M.V. (PG) College, Shikarpur Bulandshahr, India

MATERIALS AND METHOD

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 Cu 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 10 Cu (mg kg^{-1} soil) in the form of hydrated salts of concerned metals viz. $CuSO_4.5H_2O$. 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%). De-ionized 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: Cu in soil were determined by DTPA. Soil was extracted with DTPA solution for available Cu 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 ± 0.05.

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). Cu contents in the digests were determined with flame Atomic Absorption Spectrophotometer (AAS). For 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 Cu with flame Atomic Absorption Spectrophotometer (AAS).

Experimental design: Completely Randomized Design (Factorial).

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 the treatments and mixed in pots the last week of August, 2008. 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*:

Crop: *Two* different *species of Brassica*

- *Brassica nigra* (Banarasi rai)
- *Brassica carinata* (Ethiopian mustard)

Replication: Three

Experimental Design: Completely Randomized Design (Factorial).

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 Cu using flame Atomic Absorption Spectrophotometer (AAS).

RESULTS

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

The influence of amendments and metal application on the extractability of Cu by DTPA is shown in fig. 1a. (After 6 months). The mean content of DTPA extractable Cu was 12.6 mg kg^{-1} in control pot, the corresponding values were 13.1, 11.8, 12.6 and 12.4 mg kg^{-1} in the pots treated with FYM, $CaCO_3$, Phosphate, and FYM+ $CaCO_3$, respectively. From these values, it may be observed that the $CaCO_3$ is the most effective amendment to reduce the DTPA extractable Cu in metal contaminated soil. The effect of metal addition was significant and the average content of DTPA extractable Cu was increased from 10.5 to 14.5 mg kg^{-1} in control as compared amended soil. Data of Cu extraction by DTPA in the presence of different amendments after 12 months also follow the trend as the data in 6 months, although amounts of Cu extracted were lesser in 12 months than in 6 months (fig. 1b). The diacid extractable Cu in soils under various treatments is given in fig. 1a. From control, 20.3 mg kg^{-1} of Cu was extracted by diacid and that in FYM and phosphate treated pots were 19.0 and 18.5 mg kg^{-1}, respectively. However, the diacid failed to extract this metal up to analytically measurable limits (FAAS) from the pots treated with either $CaCO_3$ or $CaCO_3$ along with FYM. As in the case of aforesaid extractants, diacid also extracted significantly higher amount of Cu from the pots where metal was applied. The fig. 1a. indicates that there was a significant reduction in DTPA extractable Cu compared to diacid in $CaCO_3$ and FYM+$CaCO_3$ treated soils over control. The data in fig. 1b indicated that the extraction of Cu by diacid after 12 months show that diacid is not extracting the metal Cu upto measureable limit from the pots containing amendment $CaCO_3$ or combination of amendments $CaCO_3$+FYM. But diacid extracted significantly higher amount of Cu from the pots where the metal was applied.

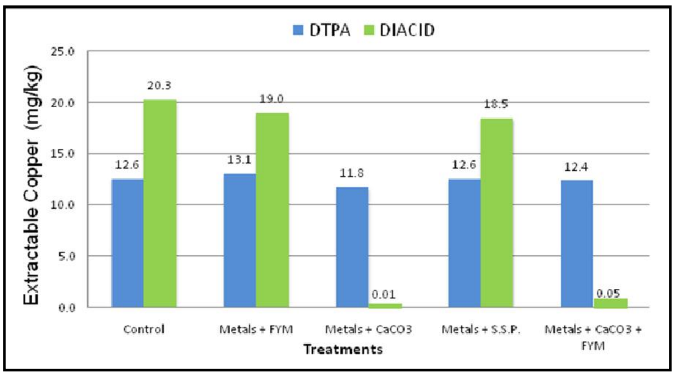

Fig. 1a. Amount of Cu extracted by different extractants from soils treated with different amendments (after 6 months period)

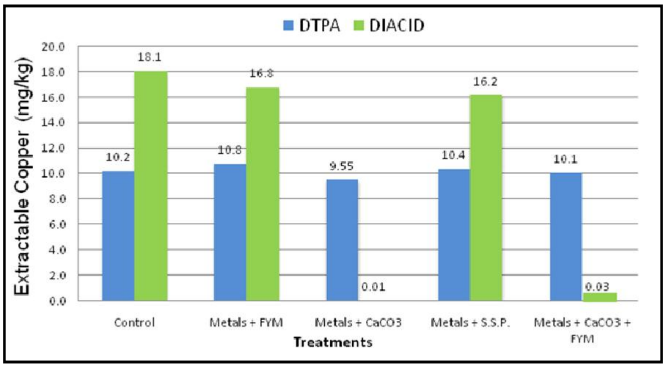

Fig. 1b. Amount of Cu extracted by different extractants from soils treated with different amendments (after 12 months period)

Put Culture Experiment

Copper uptake in *Brassica nigra*

Data pertaining to Cu uptake by *B. nigra* as influenced by various treatments of amendments and added metals have been shown in Table 1. A personal of data displayed in table 1 indicated that Cu uptake in *B. nigra* at flowering recorded with various treatments T_1, T_2, T_3, T_4, T_5 and T_6 were not significant. The treatments showed a variation in the values of Cu uptake 189.36 µg pot $^{-1}$ with T_1, 294.63 µg pot $^{-1}$ with T_2, 290.9 µg pot^{-1} with T_3, 272.2 µg pot^{-1} with T_4, 267.6 µg pot^{-1} with T_5 and 264.9 µg pot^{-1} with T_6. When amendments were added decrease in Cu uptake in plant was observed because of immobilization of metal in soil by the application of amendments.

Table 1. Effect of various treatments of amendments and added metals on Copper uptake in *Brassica nigra* (Banarasi Rai) at flowering:

Code NO.	Treatments	Cu uptake in *B. nigra* (µg pot^{-1})
T_1	Control	189.36
T_2	Zn+Cu+Ni	294.63
T_3	Zn+Cu+Ni +FYM	290.9
T_4	Zn+Cu+Ni +$CaCO_3$	272.2
T_5	Zn+Cu+Ni +SSP	267.6
T_6	Zn+Cu+Ni +$CaCO_3$+FYM	264.9
S.Em. (±)	25.49	
C.D. (5%)	N.S.	

Copper uptake in *Brassica carinata*

Data pertaining to Cu uptake by *B. carinata* as influenced by various treatments of amendments and added metals have been shown in Table 2. A personal of data displayed in indicated that Cu 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 Cu uptake 278.33 µg pot $^{-1}$ with T_2, 271.33 µg pot^{-1} with T_3, 241.73 µg pot^{-1} with T_4, 230.4 µg pot^{-1} with T_5 and 231.26 µg pot^{-1} with T_6 as against 186.53 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 Cu uptake in plant was observed because of immobilization of metal in soil by the application of amendments.

Table 2. Effect of various treatments of amendments and added metals on Copper uptake in *Brassica carinata* (Ethiopian Mustard) at flowering

Code NO.	Cu uptake in *B. carinata* (µg pot^{-1})
T_1	186.53
T_2	278.33
T_3	271.33
T_4	241.73
T_5	230.4
T_6	231.26
S.Em. (±)	13.072
C.D. (5%)	44.278

DISCUSSION

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 (Cu) in soil at 6 and 12 months period after the addition of amendments (FYM, SSP, $CaCO_3$, and FYM+$CaCO_3$) and added metals (Cu) under as well as submerged soil moisture condition. The second part of the discussion deals with the finding emerged from the pot culture experiments,each with *B. carinata,* and *B.nigra* used as test crops. The study is to compare *the Brassica carinata* and *Brassica nigra* with respect to accumulation capacity and uptake of heavy metals (Cu) 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 and diacid extractable Copper content in soil at 6 and 12 months period data given in fig 1a and 1b indicate that mean content of DTPA extractable Cu was 12.6 mg kg^{-1} in control pot. These values, it may be observed that the $CaCO_3$ is the more effective amendment to reduce the DTPA extractable Cu in metal contaminated soil. The effect of metal addition was significant and the average content of DTPA extractable Cu was increased from 10.5 to 14.5 mg kg^{-1} in control as compared amended soil. Data of Cu extraction by DTPA 12 months period as indicated in table 1b in the presence of different amendments after 12 months also follow the same trend as the data in 6 months, although amount of Cu extracted were lesser in 12 months than in 6 months.

The diacid extractable Cu in soils under various treatments (6 months period) are given in table 2a from control, 20.3 mg kg^{-1} of Cu was extracted by diacid and that in FYM and phosphate (SSP) treated pots were 19.0 and 18.5 mg kg^{-1}, respectively. However, the diacid failed to extract this metal up to analytically measurable limits from the pots. Treated with either $CaCO_3$ or $CaCO_3$ along with FYM as in case of aforesaid extractants, diacid also extracted significantly higher amount of Cu from the pots where metal was applied. The table 1a, 1b indicates that there was a significant reduction in DTPA extractable Cu compared to diacid in $CaCO_3$ and FYM+$CaCO_3$ treated soils over control. Effect of various treatments on uptake of different species of Brassica was studed using pot culture experiments. The study of metals uptake by *Brassica nigra* at the time of flowering using different amendments is given below. Case of Cu uptake there is no much difference in the uptake amount using different amendments although values is maximum with FYM and minimum with the combination of two amendments

$CaCO_3$+FYM (Table 1). Metals uptake by *Brassica carinata* in the presence of different amendments was studied at the time of flowering. Data on uptake of Cu by *Brassica carinata* in the presence of different amendments given in table 2 indicate that Cu uptake is maximum with FYM and minimum with SSP. Comparable study of Cu uptake as given in Table 1 and 2 shows that ascending order for *B. carinata* and *B. nigra* is identical with all amendments given as *B. carinata and B.nigra* . The total comparable study of Cu uptake maximum with *B. nigra.* When amendments were added to the soil, metal uptake in plant was reduced due to immobilization of metal in soil by amendments.

Acknowledgement

I feel immense pleasure in expressing my sincere obligation and deep sense of gratitude to my revered supervisor Dr. J.P. Singh, Reader, Department of Chemistry, N.R.E.C. College, Khurja (Bulandshahr) in helping me to finish this endeavour in time. His judicious guidance, thoughtful, criticism and constant encouragement have been really invaluable to me, not only for the period of study but also for the time ahead.

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