



RESEARCH ARTICLE

PHYTOREMEDIATION OF HEAVY METAL NICKEL (NI) CONTAMINATED SOIL USING DIFFERENT AMENDMENTS AND DIFFERENT SPECIES OF BRASSICA

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Received 25th January, 2018; Accepted 24th February, 2018; Published Online 30th March, 2018

ABSTRACT

Remediation refers to processes or methods for treating contaminants in soil or water such that they are contained, removed, degraded, or rendered less harmful (Pierzinsk *et al.*, 2000). Use of chemical amendments is one approach for immobilization of the natural or added metals. Lime, FYM and phosphatic fertilizers have been used extensively for detoxification of the dreaded heavy metals in soil plant system. Chemical immobilization of heavy metals by the application of ameliorants like lime, farm yard manure (FYM), phosphates, iron oxides, manganese oxides, zeolites etc. among these, soil excavation is the only method for the total removal of heavy metals from contaminated soil. However, it cannot be recommended as a viable practice, since it is prohibitively expensive. The phytoremediation also takes a long period to remove a substantial quantity of heavy metals from contaminated soils. Thus, a logical and rational remediation process appears to be to render the metals immobile by using different amendments. Use of plants which are hyper accumulators of metals like *Brassica Species* for phytoremediation of polluted soil.

Key words: Remediation, heavy metals, Phosphate

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Citation: Kushmander Singh, Neeraj Kumar, Sangeeta Agarwal and Singh, J.P., 2018. "Phytoremediation of heavy metal nickel (ni) contaminated soil using different amendments and different species of Brassica" *International Journal of Current Research in Life Sciences*, 7, (03), xxxxxxxxxx.

INTRODUCTION

Phytoremediation of heavy metal contaminated soil is an emerging technology that aims to extract or inactivate metals in soils (Mc Grath, 1998; Salt *et al.*, 1998). Two approaches have been proposed for phytoextraction of heavy metals, namely continuous or natural phytoextraction and chemically enhanced phytoextraction (Salt *et al.*, 1998). The first is based on the use of hyper-accumulator plants with exceptional metal-accumulating 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 (Baker *et al.*, 2000). On the other hand, many hyper-accumulator plants tend to be slow growing and produce low biomass, with the exception of some Ni hyper accumulator species. With the planting materials currently available, years or decades are needed to clean up a contaminated site. Another problem with the continuous phytoextraction of metals from soils is related to the fact that some metals such as Pb are largely immobile in soil and their extraction rate is

limited by solubility and diffusion to root surface. Several approaches are currently being used for the prevention, control and remediation of soil contaminated with toxic metals. These include, (a) land filling- excavation, transport and deposition of contaminated soil in a permitted land fill site (USEPA, 1991), (b) chemical immobilization of heavy metals by the application of ameliorants like lime, farm yard manure (FYM), phosphates, manganese oxides *etc.* (c) leaching- using acid solutions or complexing leachants (EDTA *etc.*) to desorb and leach metals from a part of soil drawn from the contaminated area followed by the return of the soil residues to the site; (d) bioremediation – use of micro-organisms to degrade pollutants in site (since the heavy metals can not be chemically degraded, application of microbial remediation to the in site removal of heavy metals from the contaminated substrates is limited mainly to their immobilization by precipitation or reduction); and (e) Phytoremediation - use of specially selected and engineered metal-accumulating plants for environment clean-up either by phytoextraction or by phytostabilization. Among these, soil excavation the only method for the total removal of heavy metals from contaminated soil. However, it is not recommended as a viable practice, since it is prohibitively expensive. Thus, chemical immobilization of heavy metals by the application of ameliorants (lime, phosphates, FYM *etc.*),

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use of complexing extractant/leachants (e.g. EDTA.) and phytoremediation appears to be the potential remedial measures to alleviate the heavy metal level in soil. 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 problems 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, CaCO₃ and FYM+ CaCO₃. The soil experiment was conducted in pots to study the application of Ni as well as different amendments in metal contaminated soil. A basal dose of 45 N and 25 K₂O (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 2.5 Ni (mg kg⁻¹ soil) in the form of hydrated salts of concerned metals viz. NiSO₄.6H₂O. Then the soil was thoroughly mixed with different amendments viz. control, FYM (1%), SSP (332 mg kg⁻¹ soil), CaCO₃ (5%) 50g kg⁻¹ soil and FYM (1%) +CaCO₃ (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 70°C till constant weight, DTPA and diacid extractable heavy metal elements in soils was estimated.

DTPA extractable heavy metals: Ni in soil were determined by DTPA. Soil was extracted with DTPA solution for available Ni as outlined by Lindsay and Norvell (1978). Extracting solution consists of 0.005 M DTPA, 0.01 M CaCl₂.H₂O and 0.1 M triethanolamine (TEA) and the pH was adjusted to 7.2 ± 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 Ni 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). Ni contents in the digests were determined with flame Atomic Absorption Spectrophotometer (AAS) at the department of soils, Punjab Agricultural University, Ludhiana (Punjab).

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 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* :

Crop: Two different species of *Brassica*

- Brassica napus (Gobi sarson)
- 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 65°C 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 HNO₃ and later digested with diacid mixture of HNO₃: HClO₄ (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).

RESULT

DTPA and Diacid extractable nickel content in soil at 6 and 12 months period

Figure 1a (After 6 months) shows the Ni control as extracted by DTPA from soils treated with different amendments. In control, Ni content was 0.86 mg kg⁻¹ and the average content of Ni in pots treated with FYM, phosphate, CaCO₃ and FYM+ CaCO₃ were 1.00, 1.04, 0.67 and 0.78 respectively. Among the treatments CaCO₃ was successful in reducing the DTPA extractable Ni in metal contaminated soils followed by FYM + CaCO₃. In case of FYM and phosphate treated soils, DTPA extracted more amount of Ni compared to control. Metal application has significantly increased the DTPA extractable

Ni from 0.58 (control) to 1.16 mg kg⁻¹ in pots where the metal was applied. Extraction of Ni by DTPA as given by data in fig. 1b after 12 months follows the same trend as trend represented in the extraction in 6 month. Although amount extracted in 12 months are lesser than in 6 months. It is also clear that amendment CaCO₃ is successful in reducing the DTPA extractable Ni followed by CaCO₃+FYM. In case of FYM and SSP, DTPA extracted more amount of Ni over control. Diacid extractable Ni contents in control and amended soils are given in fig. 1a. On an average, 2.28, 2.38 and 2.74 mg kg⁻¹ of Ni were extracted by diacid from control, FYM and phosphate treated pots respectively. As in case of Zn and Cu, diacid was unsuccessful in extracting any measurable amount of Ni from either CaCO₃ or FYM+CaCO₃ treated pots. Metal application increased the diacid extractable Ni from 1.33 (control) to 1.67 mg kg⁻¹ in metal applied soils. Extraction of Ni by diacid as given by data in fig. 1b after 12 months shows that is in case of Zn and Cu, diacid is unsuccessful in extracting any measureable amount of Ni from either CaCO₃ or with combination of CaCO₃+FYM.

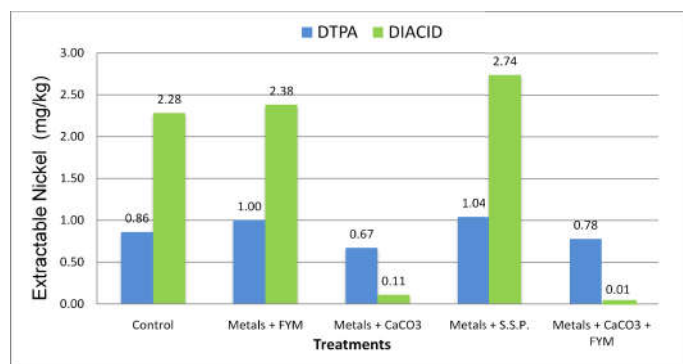


Fig. 1a. Amount of Ni extracted by different extractants from soils treated with different amendments (after 6 months period)

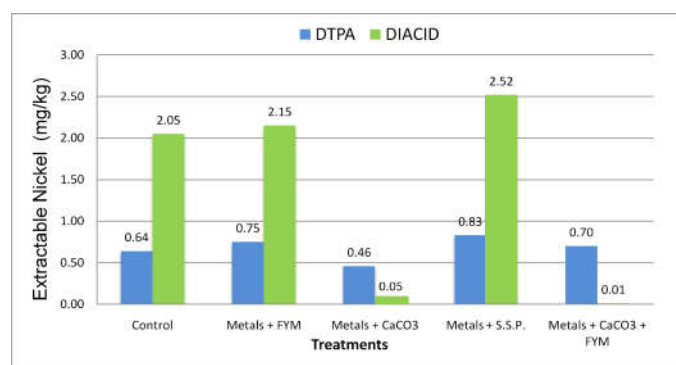


Fig. 1b. Amount of Ni extracted by different extractants from soils treated with different amendments (after 12 months period).

Pot culture experiment

Nickel uptake in *Brassica carinata*

Data pertaining to Ni 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 table 2 indicated that Ni uptake in *B. carinata* at flowering recorded with various treatments T₂, T₃, T₄, T₅ and T₆ were significantly higher than T₁ (Control). The treatments showed a variation in the values of Ni uptake 189.76 µg pot⁻¹ with T₂, 180.8 µg pot⁻¹ with T₃, 140.93 µg pot⁻¹ with T₄, 174.23 µg pot⁻¹ with T₅ and 147.66 µg pot⁻¹ with T₆ as against 102.53 µg pot⁻¹ observed

with control (T₁). The treatments T₂, T₃, T₅ and T₆ marginal higher than T₄. When amendments were added decrease in Ni 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 Nickel uptake of *B. carinata* (Ethiopian Mustard) at flowering

Code no.	Treatments	Ni uptake in <i>B. carinata</i> (µg pot ⁻¹)
T ₁	Control	102.53
T ₂	Metals	189.76
T ₃	Metals + FYM	180.8
T ₄	Metals + CaCO ₃	140.93
T ₅	Metals + SSP	174.23
T ₆	Metals + CaCO ₃ +FYM	147.66

S.Em. (±) 15.185

C.D. (5%)48.467

Table 3. Effect of various treatments of amendments and added metals on Nickel uptake of *B. napus* (Gobi Sarson) at flowering

Code no.	Treatments	Ni uptake in <i>B. napus</i> (µg pot ⁻¹)
T ₁	Control	98.86
T ₂	Metals	149.9
T ₃	Metals + FYM	178.43
T ₄	Metals + CaCO ₃	142.23
T ₅	Metals + SSP	161.8
T ₆	Metals + CaCO ₃ +FYM	151.43

S.Em. (±)9.935

C.D. (5%)31.709

Nickel uptake in *Brassica napus*

Data pertaining to Ni uptake by *Brassica napus* as influenced by various treatments of amendments and added metals have been shown in Table 3. A close examination of data shown in table 3 clearly indicated that Ni uptake in *B. napus* at flowering recorded with various treatments T₂, T₃, T₄, T₅ and T₆ were significantly higher than T₁ (Control). The treatments showed a variation in the values of Ni uptake 149.9 µg pot⁻¹ with T₂, 178.43 µg pot⁻¹ with T₃, 142.23 µg pot⁻¹ with T₄, 161.8 µg pot⁻¹ with T₅ and 151.43 µg pot⁻¹ with T₆ as against 98.86 µg pot⁻¹ observed with control (T₁). The treatments T₂, T₃, T₅ and T₆ marginal higher than T₄.

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, CaCO₃, and FYM+CaCO₃) 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 pot culture experiments, each with *B. carinata* and *B. napus* used as test crops. The study is to compare the *Brassica carinata* and *Brassica napus* with respect to accumulation capacity and uptake of heavy metals (Ni) by these *Brassica species* to assess than influence of amendments (FYM, CaCO₃, SSP and FYM+CaCO₃) on the accumulation and uptake of heavy metals by these *Brassica species*. DTPA and diacid extractable Ni content in soil at 6 months in given fig. 1a show that the Ni content as extracted by DTPA from soils treated with different amendments. In control, Ni content was 0.86 mg kg⁻¹ and the average content of Ni in pots treated

with FYM, phosphate, CaCO_3 and $\text{FYM}+\text{CaCO}_3$ were 1.00, 1.04, 0.67 and 0.78 mg kg^{-1} respectively. Among the treatments CaCO_3 was successful in reducing the DTPA extractable Ni in metal contaminated soil followed by $\text{FYM}+\text{CaCO}_3$. In case of FYM and phosphate (SSP) treated soils, DTPA extracted more amount of Ni compared to control, metal application has significantly increased the DTPA extractable Ni from control 0.58 to 1.16 mg kg^{-1} in pots where the metal was applied. Extraction of Ni by DTPA after 12 months in given fig. 1b follows the same trend as trend represented in the extraction in 6 month. Although amount extracted in 12 months are lesser than in 6 months. It is also clear that amendment CaCO_3 is successful in reducing the DTPA extractable Ni followed by $\text{FYM}+\text{CaCO}_3$. In case of FYM and phosphate DTPA extracted more amount of Ni over control. Diacid extractable Ni contents in control and amended soils are given in fig. 1a (6 months) on an average, 2.28, 2.38 and 2.74 mg kg^{-1} of Ni were extracted by diacid from control, FYM and phosphate treated pots respectively. As in case of Zn and Cu diacid was unsuccessful in extracting any measurable amount of Ni from either CaCO_3 or $\text{FYM}+\text{CaCO}_3$ treated pots. Metal application increased the diacid extractable Ni from control 1.33 to 1.67 mg kg^{-1} in metal applied soils. Extraction of Ni by diacid as given by data in fig. 1b (12 months) shows that as in case of Zn and Cu, diacid is unsuccessful in extracting any measurable amount of Ni from either CaCO_3 or with combination of $\text{FYM}+\text{CaCO}_3$.

In general, the extractability of Ni with DTPA and diacid from control. FYM and phosphate treated soils followed the same order of magnitudes (fig. 1a, 1b). The extraction of Zn and Cu, diacid was not able to extract Ni up to a detectable amount from soils where CaCO_3 is added either alone or in combination with $\text{FYM}+\text{CaCO}_3$. 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- Ni uptake given in table 2 by *Brassica carinata* shows that Ni uptake is maximum with FYM and minimum with CaCO_3 . Ascending order of uptake of Ni by *B. carinata* using different amendments is in the following order CaCO_3 (minimum), CaCO_3+FYM , SSP and FYM (minimum). When *Brassica napus* was studied then following data were observed for uptake of metals using different amendment at the time of flowering- Ni uptake by *B. napus* in the presence of different amendments is given in table 3 these data indicate that uptake of Ni is maximum with FYM and minimum with CaCO_3 . When amendments were added to the soil, metal uptake in plant was reduced due to immobilization of metal in soil by amendments Comparable study of Ni uptake by two species Ni uptake with FYM is more in *B. carinata* than *B. napus* but with CaCO_3 order is reverse i.e. Ni uptake by *B. napus* is more than *B. carinata*. Same order is observed with combination of two amendments while with SSP trend is identical as with amendment FYM. With respect to uptake of Ni by five *Brassica species* following order was observed- *Brassica carinata* > *Brassica napus*.

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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