RESEARCH ARTICLE

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

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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 (Pierzinka 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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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 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 lea chants (EDTA etc.) to desorb and leach metals from a part of soil drawn from the contminated 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 con 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.),
use of complexing extractant/leachants (e.g. EDTA) and phyto remediation 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 remediation 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 (Koptis 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 Ni as well as different amendments in metal contaminated soil. A basal dose of 45 N and 25 K$_2$O (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. NiSO$_4$·6H$_2$O. 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 70°C till constant weight, DTPA and diaacid 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 CaCl$_2$, H$_2$O 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 diaacid 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**

- 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-mg quantity of ground plant material was taken in 100 ml conical flasks. First predigested with HNO$_3$ and later digested with diaacid 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 up to the mark with double distilled water. The plant digests thus obtained were analyzed for Cu using flame Atomic Absorption Spectrophotometer (AAS).

**RESULT**

**DTPA and Diaacid 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, CaCO$_3$ and FYM+ CaCO$_3$ were 1.00, 1.04, 0.67 and 0.78 respectively. Among the treatments CaCO$_3$ was successful in reducing the DTPA extractable Ni in metal contaminated soils followed by FYM + CaCO$_3$. 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

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*Note:* The text above includes a comprehensive account of the experimental methods and results as presented in a research paper, focusing on the interaction between soil contamination and biochar application for heavy metal remediation.
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 CaCO\(_3\) is successful in reducing the DTPA extractable Ni followed by CaCO\(_3\)+FYM. In case of FYM and SSP, DTPA extracted more amount of Ni over control. Dicacid 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 dicacid from control, FYM and phosphate treated pots respectively. As in case of Zn and Cu, dicacid was unsuccessful in extracting any measurable amount of Ni from either CaCO\(_3\) or FYM+CaCO\(_3\) treated pots. Metal application increased the dicacid extractable Ni from 1.33 (control) to 1.67 mg kg\(^{-1}\) in metal applied soils. Extraction of Ni by dicacid as given by data in fig.1b after 12 months shows that is in case of Zn and Cu, dicacid is unsuccessful in extracting any measurable amount of Ni from either CaCO\(_3\) or with combination of CaCO\(_3\)+FYM.

The treatments showed a variation in the values of Ni uptake 189.76 µg pot\(^{-1}\) with T\(_5\), 180.8 µg pot\(^{-1}\) with T\(_3\), 140.93 µg pot\(^{-1}\) with T\(_4\), 174.23 µg pot\(^{-1}\) with T\(_5\) and 147.66 µg pot\(^{-1}\) with T\(_6\) as against 102.53 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 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

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Treatments</th>
<th>Ni uptake in B. carinata (µg pot(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(_1)</td>
<td>Control</td>
<td>102.53</td>
</tr>
<tr>
<td>T(_2)</td>
<td>Metals</td>
<td>189.76</td>
</tr>
<tr>
<td>T(_3)</td>
<td>Metals + FYM</td>
<td>180.8</td>
</tr>
<tr>
<td>T(_4)</td>
<td>Metals + CaCO(_3)</td>
<td>140.93</td>
</tr>
<tr>
<td>T(_5)</td>
<td>Metals + SSP</td>
<td>174.23</td>
</tr>
<tr>
<td>T(_6)</td>
<td>Metals + CaCO(_3)+FYM</td>
<td>147.66</td>
</tr>
</tbody>
</table>

S.E.m. (±) 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

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Treatments</th>
<th>Ni uptake in B. napus (µg pot(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(_1)</td>
<td>Control</td>
<td>98.86</td>
</tr>
<tr>
<td>T(_2)</td>
<td>Metals</td>
<td>149.9</td>
</tr>
<tr>
<td>T(_3)</td>
<td>Metals + FYM</td>
<td>178.43</td>
</tr>
<tr>
<td>T(_4)</td>
<td>Metals + CaCO(_3)</td>
<td>142.23</td>
</tr>
<tr>
<td>T(_5)</td>
<td>Metals + SSP</td>
<td>161.8</td>
</tr>
<tr>
<td>T(_6)</td>
<td>Metals + CaCO(_3)+FYM</td>
<td>151.43</td>
</tr>
</tbody>
</table>

S.E.m. (±) 19.935  
C.D. (5%) 31.709

### Nickel uptake in Brassica carinata

Data pertaining to Ni uptake by Brassica 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 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 Ni uptake 149.9 µg pot\(^{-1}\) with T\(_2\), 178.43 µg pot\(^{-1}\) with T\(_3\), 142.23 µg pot\(^{-1}\) with T\(_4\), 161.8 µg pot\(^{-1}\) with T\(_5\) and 151.43 µg pot\(^{-1}\) with T\(_6\) as against 98.86 µg pot\(^{-1}\) observed with control (T\(_1\)). The treatments T\(_2\), T\(_3\), T\(_5\) and T\(_6\) marginal higher than T\(_4\).

### 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 dicacid 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 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, CaCO\(_3\), SSP and FYM+CaCO\(_3\)) on the accumulation and uptake of heavy metals by these Brassica species. DTPA and dicacid 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, CaCO₃ and FYM+CaCO₃ were 1.00, 1.04, 0.67 and 0.78 mg kg⁻¹ respectively. Among the treatments CaCO₃ was successful in reducing the DTPA extractable Ni in metal contaminated soil followed by FYM+CaCO₃. 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 CaCO₃ is successful in reducing the DTPA extractable Ni followed by FYM+CaCO₃. 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 mgkg⁻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 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 CaCO₃ or with combination of FYM+CaCO₃.

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 CaCO₃ is added either alone or in combination with FYM+CaCO₃. 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 CaCO₃. Ascending order of uptake of Ni by B. carinata using different amendments is in the following order CaCO₃ (minimum), CaCO₃+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 CaCO₃. 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 CaCO₃ 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.

REFERENCES


