



## **Influence of Soil Bioamendments on the Availability of Nickel and Phytoextraction Capability of Marigold from the Contaminated Soil**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author VS carried out the experimental works of the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SM designed and supervised the study. Authors AB and AK managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Phytoremediation is an emerging technology involved in heavy metal remediation processes. It is evident from the several researches that the bioamendments greatly influences various biochemical processes and thus enhance the bioavailability of metals in the contaminated soil. It should favour greater absorption (removal) of metals by plants. Therefore, a pot experiments was conducted to examine the bioavailability and subsequent uptake of Ni by marigold during the application of different biomendments such as Farmyard manure (FYM), Composted poultry manure, Pressmud compost and Prosopis wood biochar. The result of pot experiment has shown the potential of bioamendments in enhancing the bioavailability of Ni in soil. The bioamendment application also

enhanced the Ni content in plants. A significant positive correlations were obtained between Ni uptake by plants and water soluble and exchangeable Ni in soil. This explains the role of bioavailability in plant uptake of Ni. The bioconcentration factor (BCF) and enrichment factor (EF) were less than one but the translocation factor (TF) was greater than one in plants grown on soil amended with bioamendments. Marigold showed greater potential in tolerating and accumulating higher concentration Ni and therefore it could be integrated along with bioamendments for phytoextraction of Ni from contaminated soil.

*Keywords: Nickel; phytoremediation; phytoextraction; bioamendments; marigold.*

## 1. INTRODUCTION

Phytoremediation is the major mechanism through which metal is absorbed by plants and accumulated in their biomass. Then the plants may be harvested and metals recovered by burning/ incinerating the plant biomass. The major criteria for selecting plant species for phytoremediation are hyperaccumulating, fast growing species with high biomass. Phytoremediation technology is environmentally friendly and low cost-effective; zinnia may be practicable alternative for protecting the contaminated soil from leaching lead [1]. Three important processes take place during the extraction of metals from soil such as solubilisation of metals in soil matrix, root uptake of metals and translocation of metals in the shoot parts of plants. Usually Ni is stable in the soil and cannot readily be taken up by the plants. Bioavailability of Ni is the prime factor that decides the phytoextraction process. Bioavailable concentration of a metal may be defined as fraction of metal in soils that can be readily absorbed by plants and interferes in the biochemical processes. The science technology industrial revolution and agricultural technology have no doubt improved our life style, which could not be imagined a decade ago; but this advancement has simultaneously polluted the natural environment [2-4]. Plant absorption of Ni and other metals is influenced by soil pH, Cation exchange capacity, soil organic matter, lime and concentration of micro- and macro-nutrients in soils. The soil organic matter is the most important as it could influence other soil physical and chemical properties which ultimately determine phytoavailability of Ni and other metals. Humic acid, fulvic acid and humin are the most stable compounds resulting from biochemical decomposition of OM [5]. The soil organic matter may (a) inhibit metal sorption through formation of complexes with metals or competition with metals at the surface for sorption sites, (b) enhance metal sorption as ligands form stable complexes with metal ions

which has strong affinity for adsorption onto soil surface and /or (c) may have no or little effect on sorption of metals due to weak complexation [6, 7,8]. To increase the bioavailable fraction of Ni, manipulating soil properties such as pH, organic matter content, CEC etc is necessary. Therefore, in the current study, the effect of bioamendments on bioavailability of Ni in contaminated soil and subsequent uptake (removal) by marigold plants was examined by conducting pot experiment.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Characterization of Soil and Organic Amendments

The effect of different organic amendments on the bioavailability of Ni in soil was studied by conducting a pot experiment with marigold. Bulk soil samples were collected from Ni contaminated area nearby Nanjundapuram, Coimbatore where large number of industries including electroplating industries exist. After air drying for three days, the soil was passed through 2 mm sieve to achieve a homogenized sample. Different organic amendments such as FYM, composted poultry manure, pressmud compost, prosopis wood biochar were collected. Some important characteristics of the experimental soil [9] and organic amendments [10] were determined as per and presented in Tables 1 & 2.

### 2.2 Pot Culture Experiment

Pot experiment was conducted to examine the effect of bioamendments on Ni bioavailability in soil with marigold since it is a flower crop and thus the accumulated Ni might not be entered into the food chain. It was conducted under green house set up. Syntax pots with a capacity of 2 kg were used. Two kg of soil was weighed and transferred into each pot. The nickel content of the soil is 300 mg kg<sup>-1</sup>. The bioamendments

such as FYM, composted poultry manure, pressmud compost and prosopis wood biochar were added as per the treatment schedule and uniformly mixed. The rate of application of bioamendments was based on Tamil Nadu Agricultural University recommendation. The pots were incubated at field capacity moisture ( $0.37 \text{ g g}^{-1}$ ). The Marigold seedlings were transplanted after three days incubation of bioamendment addition. Periodically known amount of water was added uniformly to all pots to compensate moisture loss. At fortnight intervals, soil samples were removed from each pot and analysed for bioavailable fractions of Ni. The plants were grown up to active vegetation stage and harvested to examine the Ni accumulation in the plants. Soil and plant samples were collected at the end of the experiment for various analyses. The experiment was carried out using completely randomized design and the results were statistically scrutinized using AGRES software.

### 2.3 Treatment Details

- T<sub>1</sub> : Control - Soil\* (No amendments)  
 T<sub>2</sub> : Soil\* + FYM ( $12.5 \text{ t ha}^{-1}$ )  
 T<sub>3</sub> : Soil\*+ Composted poultry manure ( $5 \text{ t ha}^{-1}$ )  
 T<sub>4</sub> : Soil\*+ Pressmud Compost ( $5 \text{ t ha}^{-1}$ )  
 T<sub>5</sub> : Soil\*+ Prosopis wood biochar ( $5 \text{ t ha}^{-1}$ )

### 2.4 Fractionation of Nickel

A method described by Noble and Hughes [11] was employed to determine different species of Ni in the soil as outlined below.

#### 2.4.1 Step 1 (Water soluble fraction)

One gram of air-dried soil sample was weighed in a 50 ml polypropylene centrifuge tube and added 25 ml of double distilled water. It was shaken in an end-over-end shaker for 2 hrs at  $25 \pm 2^\circ\text{C}$ . Then centrifuged the tubes at 8000 rpm for 10 minutes and filtered through Whatman No.40 filter paper. The soluble Ni in the water extract was determined using an Atomic Absorption Spectrophotometer with air- acetylene flame of Perkin Elmer 200 [12].

#### 2.4.2 Step 2 (Exchangeable fraction)

To the residue from step 1, 25 ml of 0.5 M  $\text{KNO}_3$  was added and shaken it for 16 hrs. The centrifugation, filtration and measurement were followed as in step 1.

#### 2.4.3 Step 3 (Organic fraction)

Added 25 ml of 0.5 M NaOH to the soil remaining after the exchangeable fractions (step 2) and shaken it for 16 hrs. The centrifugation, filtration and measurement were followed as in step 1.

#### 2.4.4 Step 4 (Organic plus iron-oxide bound fraction)

The residue from step 3 was shaken with 0.05 M  $\text{Na}_2\text{EDTA}$  for 6 hrs and followed centrifugation, filtration and measurement as in step 1.

#### 2.4.5 Step 5 (Residual fraction)

The soil residue from step 4 was transferred to a 150 ml conical flask using a jet of water and dried in an oven. Then added 10 ml concentrated

**Table 1. Initial characteristics of experimental soil**

S. No	Parameters	Values
1.	Soil series	Kilaiyur series
2.	USDA classification	<i>Typic Haplusterts</i>
3.	Bulk density ( $\text{Mg m}^{-3}$ )	1.44
4.	pH (1:2.5 water)	8.49
5.	EC ( $\text{dS m}^{-1}$ )	1.11
6.	Organic carbon (%)	0.45
7.	Cation exchange capacity ( $\text{cmol (p}^+) \text{ kg}^{-1}$ )	19.2
8.	$\text{KMnO}_4\text{-N}$ ( $\text{kg ha}^{-1}$ )	216
9.	$\text{NaHCO}_3\text{-P}$ ( $\text{kg ha}^{-1}$ )	18.0
10.	$\text{NH}_4\text{OAc- K}$ ( $\text{kg ha}^{-1}$ )	321
11.	Exchangeable Na ( $\text{mg kg}^{-1}$ )	295
12.	Exchangeable Ca ( $\text{mg kg}^{-1}$ )	3154
13.	Exchangeable Mg ( $\text{mg kg}^{-1}$ )	2457
14.	Total Nickel ( $\text{mg kg}^{-1}$ )	300

**Table 2. Some important characteristics of bioamendments**

Parameters	FYM	Composted poultry manure	Pressmud compost	Prosopis wood biochar
pH (1:5 H <sub>2</sub> O)	8.25	6.88	7.85	7.64
EC (dSm <sup>-1</sup> )	0.69	1.44	1.57	1.82
Organic Carbon (%)	16.54	21.07	14.80	3.62
Total N (%)	0.57	1.71	1.13	0.50
Total P (%)	0.26	0.66	0.73	0.14
Total K (%)	0.21	0.67	1.95	0.39
Total Na (%)	0.060	0.287	0.160	0.073

HNO<sub>3</sub> and digested the contents at 110°C. After digestion, the contents were diluted and filtered before taking measurements.

The tube plus contents were weighed before and after extraction to calculate the volume of entrapped solution and transfer of heavy metal between extractants. The amounts of Ni extracted by each extractant were computed by using the following equation.

$$\text{Ni extracted } (\mu\text{g g}^{-1}) = C \times (E+M) - (C' \times M) / \text{weight of soil}$$

Where,

C - Concentration of heavy metal in the extraction solution

M – Mass (g) of the entrained solution carried over from previous extraction

C'- Concentration of the heavy metal in the extraction solution of proceeding step of the sequence

E – Mass (g) of the extractant

## 2.5 Plant Analysis

The plant samples (marigold) collected from the pot culture at harvest stage. The plant samples were cleaned with water and separated into roots and above ground biomass. The samples were kept in paper covers and shade dried and later oven dried (70°C). After recording the dry weight, each sample was ground in a Wiley mill and sub-samples were obtained for laboratory analysis.

### 2.5.1 Total Ni in plant parts

The plant materials (root, stem, leaves and flowers) were weighed (0.5-1 g) and digested with 10 ml of 69% concentrated HNO<sub>3</sub> of AR grade. The total Ni in the acid extract of plant materials was determined using an Atomic Absorption Spectrophotometer with air-acetylene flame of Perkin Elmer 200 [12].

### 2.5.2 Total Ni in plants

The plant samples were harvested from Ni incubated soils and oven dried. The whole powdered plant samples were analysed for Ni content and Ni was determined as described above.

## 2.6 Data Analysis

The data on various characters studied during the course of investigation were statistically analysed (AGRES) as suggested by Gomez and Gomez [13] to find out the influence of various treatment on the different parameters of soil and plants. The critical difference was worked out at five per cent probability level.

## 3. RESULTS AND DISCUSSION

### 3.1 Biotransformation and Bioavailability of Nickel in Soil

The effect of bioamendments on the biotransformation of Ni differed significantly in soils under marigold.

#### 3.1.1 Water soluble nickel

With marigold, the water soluble Ni in soil showed a slight increase initially, afterwards it was found gradually decreased up to final stage of pot experiment. The concentration of water soluble Ni varied between 0.63 and 6.73 mg kg<sup>-1</sup> (Fig. 1). The water soluble Ni was significantly found higher in the soil amended with pressmud compost (T<sub>4</sub>) and lower in the control soil at all stages (T<sub>1</sub>). The increase in water soluble Ni might be due to the solubilisation or microbial conversion of Ni in to soluble form favoured by the reduction in soil pH. Thus the soluble fraction of Ni was increased and extracted by plants throughout the period. The Ni solubilization enhanced was also due to the increase in humic and fulvic substances of bioamendments [14].

### 3.1.2 Exchangeable nickel

The exchangeable Ni in soil was found gradually decreased during initial stages and then increased rapidly in soil with marigold plants (Fig. 2). Initially, the highest concentration of exchangeable Ni ( $8.27 \text{ mg kg}^{-1}$ ) was recorded in the soil amended with composted poultry manure ( $T_3$ ) and the lowest ( $7.94 \text{ mg kg}^{-1}$ ) in the control ( $T_1$ ). At the end of 60 days of experiment the highest value ( $15.18 \text{ mg kg}^{-1}$ ) was registered in the soil amended with pressmud compost ( $T_4$ ) and the lowest value ( $12.61 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). Application of pressmud compost was found to enhance the exchangeable Ni in soil with both the crops. The enhancement of exchangeable Ni might be due to the mobilization of Ni by dissolved organic carbon present in the pressmud compost. Bioamendments could affect the bioavailability of Ni through different mechanisms. For example, Ni-organic matter association could occur both in soil solution and soil surface [15]. Both the nature and stability of soil-applied organic materials could influence metal solid-solution phase partitioning and thus alter the bioavailability of Ni. Different biochemical transformation of organic matter leads to the release of DOC and thus it increases mobility and bioavailability of metals [16].

### 3.1.3 Organic nickel

In marigold, the concentration of organic Ni was gradually increased upto 30<sup>th</sup> day slightly decreased at 45<sup>th</sup> day and rapidly increased at 60<sup>th</sup> day of experimentation (Fig. 3). During early stage of marigold, the concentration of organic Ni was found high ( $20.46 \text{ mg kg}^{-1}$ ) in the treatment ( $T_5$ ) that had prosopis wood biochar and low ( $15.52 \text{ mg kg}^{-1}$ ) in the control soil. However at the end of 60 days, the highest value ( $103.13 \text{ mg kg}^{-1}$ ) was recorded in the soil amended with pressmud compost ( $T_4$ ) and the lowest value ( $26.50 \text{ mg kg}^{-1}$ ) in the control ( $T_1$ ). The increase of organic Ni is attributed to the complex formation with organic matter. Organic matter could chelate metals by coordinating with them directly and through inner-sphere complex formation with acid functional groups [17]. Organic matter could re-distribute Ni from available forms to fractions associated with organic matter in soil as well as other soil components [18].

### 3.1.4 Organic plus iron oxide bound nickel

The organic plus iron oxide bound Ni was found decreased initially but increased slightly towards the end of experiment with marigold (Fig. 4). During initial stages of marigold, the concentration of organic plus iron oxide bound

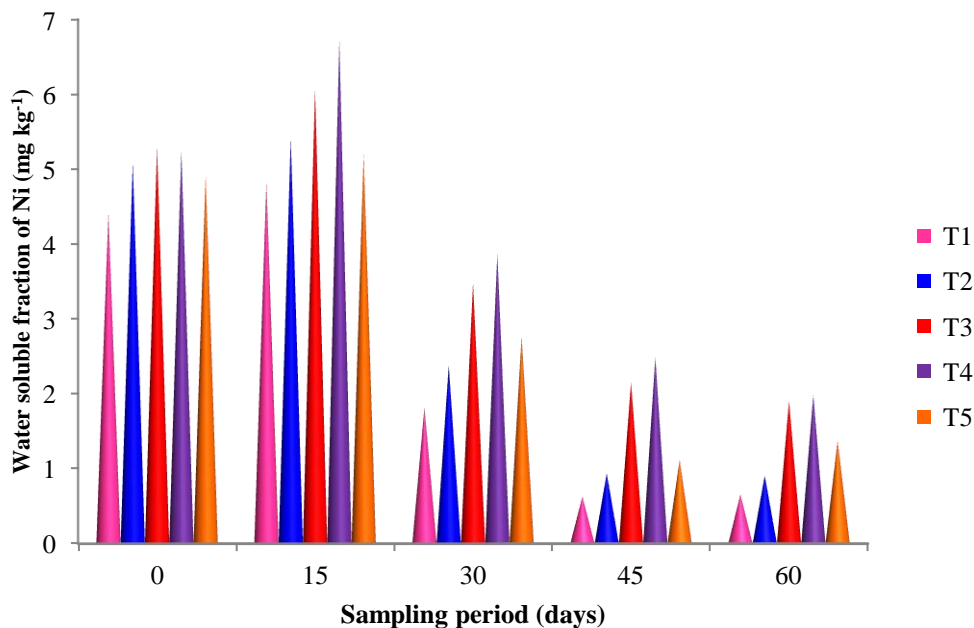


Fig. 1. Effect of bioamendments on water soluble nickel in soil with marigold

Ni was high (128.61 mg kg<sup>-1</sup>) in soil amended with pressmud compost (T<sub>4</sub>) and low (97.92 mg kg<sup>-1</sup>) in control soil. Finally it was recorded high (96.74 mg kg<sup>-1</sup>) in the treatment applied with composted poultry manure (T<sub>3</sub>) and low (58.43 mg kg<sup>-1</sup>) in the treatment applied with pressmud compost. The

increase in the concentration of organic plus iron oxide bound Ni might be due to the increased complexation of Ni with humic acid of organic matter which is insoluble in nature. Nickel may form soluble complexes with fulvic acid while humic acid forms insoluble complexes [19].

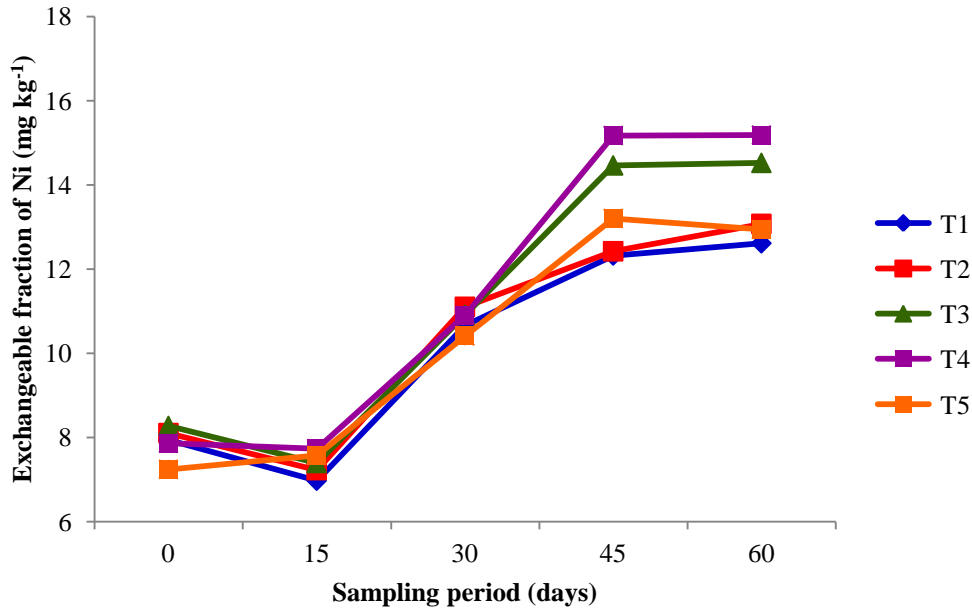


Fig. 2. Effect of bioamendments on exchangeable nickel in soil with marigold

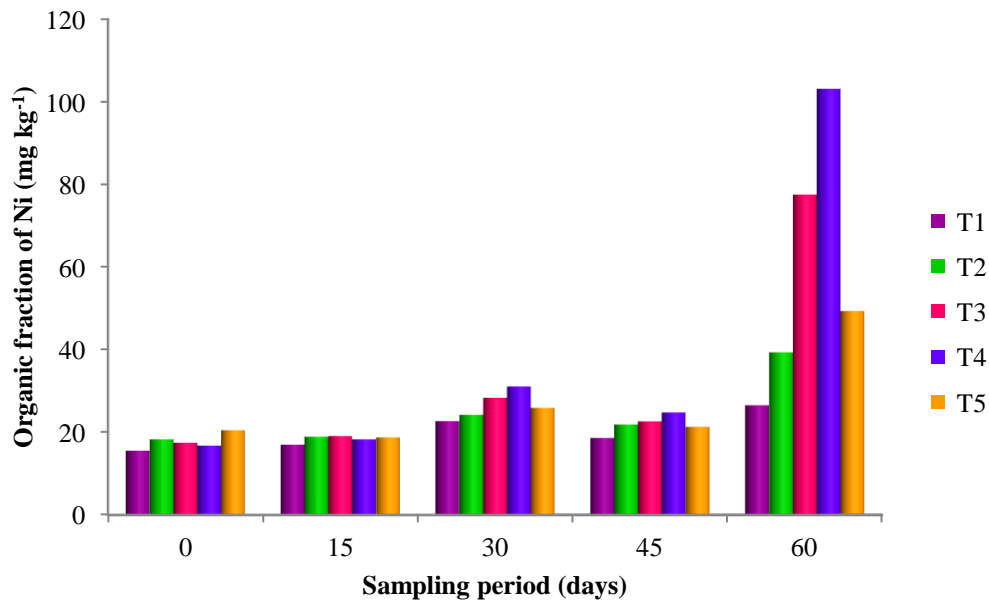
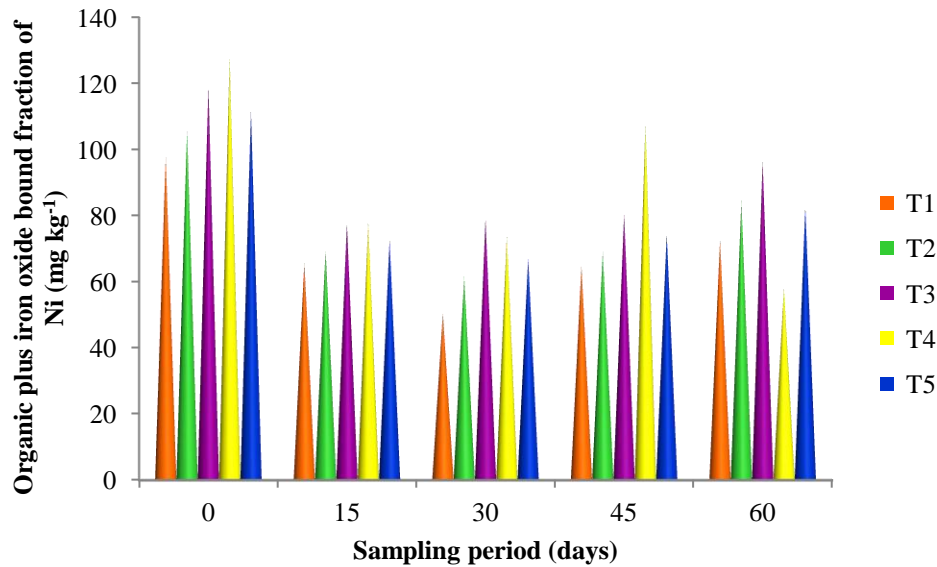


Fig. 3. Effect of bioamendments on organic nickel in soil with marigold



**Fig. 4. Effect of bioamendments on organic plus iron oxide bound nickel in soil with marigold**

### 3.1.5 Residual nickel

In marigold grown soil, the concentration of residual Ni was increased gradually during initial stages and then decreased drastically towards end of experiment (Fig. 5). Initially, the highest concentration of residual Ni ( $121.95 \text{ mg kg}^{-1}$ ) was obtained in the soil amended with prosopis wood biochar ( $T_5$ ) and the lowest concentration ( $113.72 \text{ mg kg}^{-1}$ ) in the soil amended with pressmud compost ( $T_4$ ). However, during final stage of the experiment, relatively higher concentration of residual Ni ( $142.29 \text{ mg kg}^{-1}$ ) was recorded in the control soil ( $T_1$ ) followed by  $T_5$  ( $115.8 \text{ mg kg}^{-1}$ ) and relatively lower concentrations ( $35.41 \text{ mg kg}^{-1}$ ) was observed in soil treated with composted poultry manure amended soil ( $T_3$ ) in marigold experiment. The increase in the concentration of residual Ni might be due to various processes such as adsorption, precipitation or complexation reactions of Ni. The soil amended with prosopis wood biochar showed increased amount of residual Ni which could be attributed to strong complexation of Ni with its humified organic matter contents that has strong affinity for adsorption on to soil surface [20, 21]. Prosopis wood biochar effectively immobilized Ni due to its high cation exchange capacity also.

### 3.2 Nickel Content and Uptake by Crops

Plants can accumulate Ni usually less than 0.1 per cent of total dry weight [22].

Nickel is very mobile and can be accumulated in vegetative and reproductive parts [23]. The results from marigold experiment showed that the pressmud application resulted in the highest Ni content in plants. Plants grown on control soil had the lowest content of Ni (Table 3). Relatively higher concentration of Ni was observed in root biomass than in above ground biomass. In general the plants grown on the control soil had greater amount of Ni in root biomass than in the above ground biomass. In the above ground biomass the highest Ni content ( $103 \mu\text{g g}^{-1}$ ) was found in the plants grown on soil amended with pressmud compost ( $T_4$ ) followed by composted poultry manure ( $T_3$ ). The lowest Ni ( $3.31 \mu\text{g g}^{-1}$ ) in above ground biomass was observed in the control ( $T_1$ ). In contrast the root biomass of marigold, had significantly higher Ni content due to the application of FYM ( $T_2$ ) followed by prosopis wood biochar ( $T_5$ ) and the lowest Ni content in the root biomass was found in the plants grown on soil amended with composted poultry manure ( $T_3$ ). The uptake of Ni by marigold had shown similar effect of bioamendments. A positive correlation was obtained between Ni uptake by marigold and bioavailable fractions of Ni in soil (Figs. 6 & 7;  $R^2$  for Water soluble Ni=0.877;  $R^2$  for Exchangeable Ni=0.941). All the bioamendments significantly increased the biomass of marigold. The higher biomass production due to the application of bioamendments could be due to addition of plant nutrients particularly nitrogen released during decomposition of bioamendments [24] and

improvement in the soil physical properties. The plants grown on soil amended with pressmud compost had shown relatively higher biomass. This could be due to its higher nutrients and organic matter contents, which on mineralization can supply additional nutrients, improve buffering capacity and enhance nutrient cycling and thus can improve plant growth [24,25]. While prosopis wood biochar was found to reduce the Ni concentration in marigold, pressmud compost and composted poultry manure were found to increase Ni content and uptake by plants. This could be due to the immobilization of Ni with prosopis wood biochar in the soil compared to other amendments as evidenced from less exchangeable fractions of Ni in soil amended with prosopis wood biochar.

### 3.3 Bioconcentration Factor and Translocation Factor

The mobility of Ni from the polluted soil into the roots of marigold and the ability to translocate the Ni from roots to above ground parts were evaluated by computing the BCF, TF and EF as follows [26];

$$\begin{aligned} \text{BCF} &= \text{Ni in roots (mg kg}^{-1}\text{)} / \text{Ni in soil (mg kg}^{-1}\text{)} \\ \text{TF} &= \text{Ni in stover/stalks (mg kg}^{-1}\text{)} / \text{Ni in roots (mg kg}^{-1}\text{)} \\ \text{EF} &= \text{Ni in stover/stalk (mg kg}^{-1}\text{)} / \text{Ni in soil (mg kg}^{-1}\text{)} \end{aligned}$$

The ability of marigold to tolerate and accumulate Ni is useful for phytoextraction and thus for phytoremediation. Plants with both BCF and TF

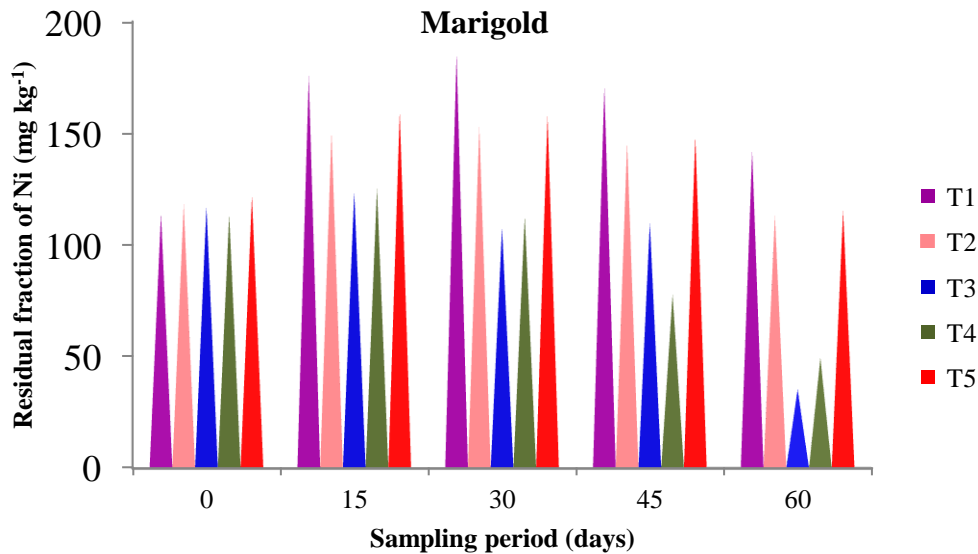


Fig. 5. Effect of bioamendments on residual nickel in soil with marigold

Table 3. Nickel accumulation in marigold

Treatments	Ni content in above ground biomass ( $\mu\text{g g}^{-1}$ )	Ni content in root biomass ( $\mu\text{g g}^{-1}$ )	Biomass of Marigold (g)	Ni uptake ( $\mu\text{g/plant}$ )
T <sub>1</sub>	3.31	84.1	7.17	635
T <sub>2</sub>	5.71	101.6	8.25	883
T <sub>3</sub>	79.1	48.9	11.26	1441
T <sub>4</sub>	103	54.9	12.37	1948
T <sub>5</sub>	24.9	87.6	9.21	1037
Mean	43.1	75.4	9.65	1189
SEd	7.18	9.16	0.59	110.15
CD (0.05)	16.00**	20.40**	1.33**	245.41**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + FYM (12.5t ha<sup>-1</sup>);

T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis wood biochar (5t ha<sup>-1</sup>)

greater than one have the potential to be used in phytoextraction. Besides, plants with BCF greater than one and TF less than one have the potential for Phytostabilisation [27]. The lesser values of BCF may suggest the restriction in soil-root transfer at this Ni concentration in the soil [28]. The hyper accumulator plant should have EF greater than 1, or TF >1. The results obtained from the pot experiments are presented in Table 4 and showed that the BCF and EF were less than one for marigold but the TF was greater than

one in plants grown on soil amended with bioamendments. Therefore, they may be considered as Ni hyperaccumulator. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator for contaminated soil [27]. However, marigold showed greater potential in tolerating and accumulating higher concentration Ni and therefore could be integrated along with bioamendments for phytoextraction and thus phytoremediation of Ni contaminated soil.

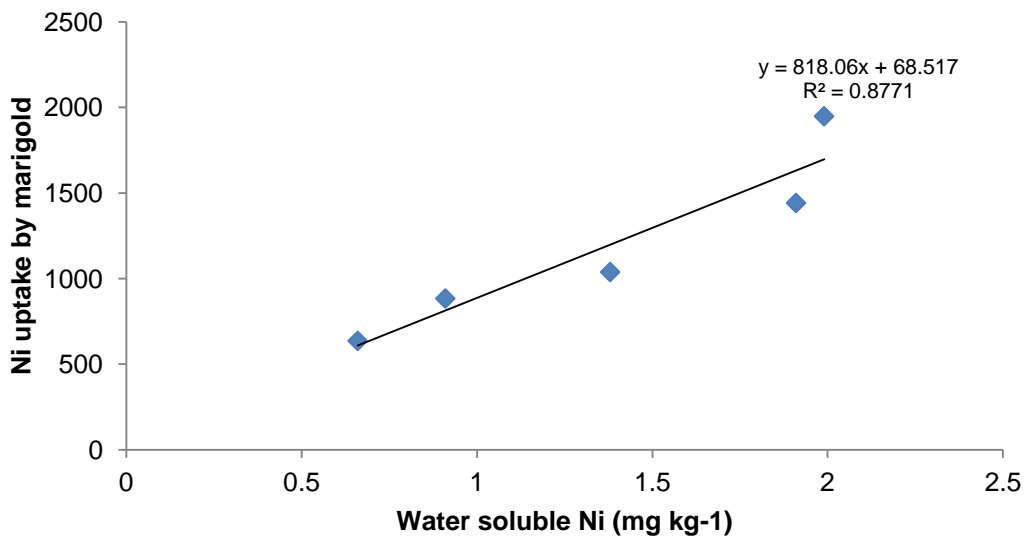


Fig. 6. Relationship between water soluble nickel in soil and nickel uptake by marigold

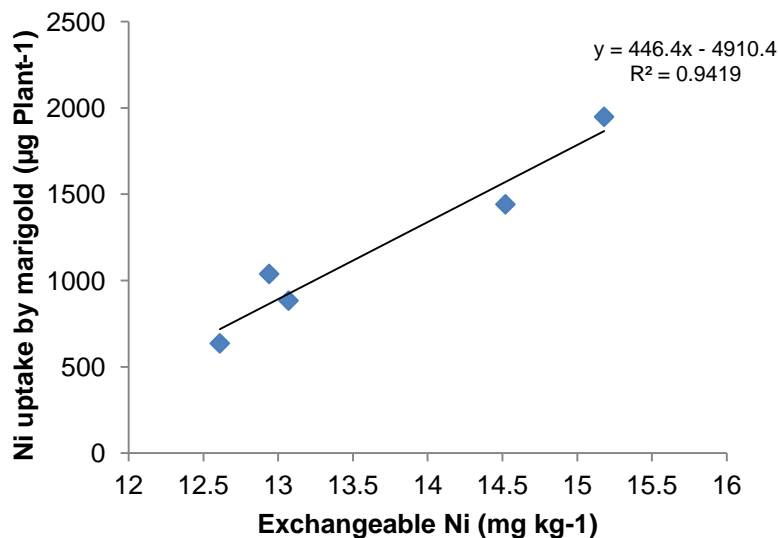


Fig. 7. Relationship between exchangeable nickel in soil and nickel uptake by marigold



**Fig. 8. Pot experiment with Marigold**

**Table 4. BCF TF and EF for marigold**

Treatments	BCF	TF	EF
T <sub>1</sub>	0.280	0.042	0.011
T <sub>2</sub>	0.339	0.056	0.019
T <sub>3</sub>	0.163	1.921	0.264
T <sub>4</sub>	0.183	1.866	0.342
T <sub>5</sub>	0.292	0.285	0.083
Mean	0.2514	0.834	0.1438

#### 4. CONCLUSION

The results of the pot experiment consistently have shown that the application of bioamendments to the Ni contaminated soil enhanced the bioavailability of Ni in the contaminated soil. After soil application, the bioamendments may form complexes with fulvic acid and humic acid fractions of SOM. While fulvic acid complex would enhance the bioavailability by mobilization of Ni ions, the humic acid complex results in immobilization of Ni and enhances the adsorption of Ni ions on soil exchange sites. The enhanced bioavailability of Ni (as evident from the increase in water soluble and exchangeable Ni) facilitates greater absorption of Ni by plants. The higher Ni content of below ground biomass of marigold provides evidence for this. It is also expected that due to the decomposition of bioamendments various organic acids are produced resulting in reduction of soil pH. Low soil pH solubilises some of the insoluble Ni precipitates and would increase the

bioavailability of Ni and thus greater removal of Ni by plants. The other benefits of bioamendments include improvement in soil physical characteristics and enhancement of soil cation exchange capacity besides adding nutrients to the soil, which favours better growth and biomass production of plants. Thus the study has demonstrated that a phytoremediation strategy could be developed by integrating bioamendments and hyperaccumulators (Marigold) for remediation of Ni contaminated soil.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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