EQPH-2021
V. International Symposium
Proceedings Book of the Vth International Symposium–2021
"Environmental Quality and Public Health"
May 20, 2021 Óbuda University, Budapest, Hungary
ISBN: 978-963-449-238-2. **www.iceee.hu**
TL23.

EFFECT OF DIFFERENT CHELATING AGENTS AND BIOINOCULANTS ON FRACTIONAL DISTRIBUTION OF CADMIUM AND NICKEL IN SPIKED SOIL AFTER HARVEST OF INDIAN MUSTARD (*BRASSICA JUNCEA*)

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Abstract: *Screen house experiment was conducted t o study the different forms of Cadmium (Cd) and Nickel (Ni) in the soil after harvest of Indian mustard (Brassica juncea L.) as affected by chelating agents and bioinoculants from metal enriched soil. A seven-step sequential extraction procedure was applied to evaluate the association of metals with soil constituents. The different chemical forms of fraction of Cd and Ni extracted were exchangeable + water soluble carbonate, organic matter complexed, Mn-oxide bound, occluded in amorphous and crystalline Fe-oxide and residual mineral fraction. The present study revealed that dominant Cd fraction was organic followed by carbonate, Manganese oxide, exchangeable, crystalline Fe-oxide, amorphous Fe-oxide and residual Cd fraction. Application of chelating agents increased the exchangeable fraction causing an increase in its availability in the soils. It may be ascribed that application of chelating agent caused an increase in the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, Mn-oxide, AFe-oxide and CFe-oxide bound fractions. Highest amount of exchangeable fraction was observed in EDTA treated soil. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd (greater than non-bioinoculants treated soil). Application of chelating agent (EDTA) significantly increased the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, MnOX, AFeOX, bound fractions. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd. Fractionation of soil Cd after the harvest of Brassica juncea crop showed that the application of chelating agent (EDTA) excellently improved the exchangeable pool of Cd.*

Keywords: *Bioinoculant, Cadmium, Chelating agents, Different fractions, Nickel, Indian mustard*

INTRODUCTION

Cadmium (Cd), recognized as one of the most hazardous elements, is not essential for plant growth

(Kabata-Pendias, 2000). World production of Cd increased from 11000 t in 1960 to 20200 t in 1990 (Alloway, 1995). The main source of Cd contamination is industries involved in protective plating on steel and production of various alloys, pigments, stabilizers for plastic, Ni-Cd dry batteries and other miscellaneous items including photovoltaic cells and control rods for nuclear reactors. The other sources of Cd contamination come from mining, ore dressing and smelting of Cadmium sulphide ore, which may contain up to 5 per cent Cd (Alloway, 1995).

Nickel (Ni) is considered an essential micronutrient for plant, humans, and animals. It can exist in trace amount in air, food, drinking water, and soils. Although, Ni plays an important role in the metabolism of humans and animals, its intake in excessive amount or over a prolonged period could pose health risks. Studies have shown that children living in polluted areas have hypertrophy of tonsils, enlarged lymphatic nodes, and enlarged livers. There is also evidence that soluble Ni particulate is linked to acute lung injury. The conservation of a healthy agricultural ecosystem is prerequisite for protecting the food chain from bioaccumulation of hazardous substances e.g., heavy metals. Among the pollutant elements, nickel is of great environmental concern as it is hazardous and highly carcinogenic (De et al., 1997).

Unlike organic compounds, metals cannot be degraded and their cleanup requires their immobilization and toxicity reduction or removal. In recent years, scientists and engineers have started to generate cost effective technologies which includes use of microorganisms/ biomass or live plants for cleaning of polluted areas Qiu et al., 2000),. Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long-term applicability (Richard, 1954, Su and Wong, 2004).

Microbial populations can change complexes of heavy metals to affect their availability to the plant through release of chelating agents, siderophores, acidification, phosphate solubilization and redox changes, and therefore, have potential to enhance phytoremediation processes. Application of farm yard manure (FYM) or compost or biological wastes is a common practice and traditionally followed in Indian agriculture. Integrated approach, involving, plants (hyper accumulators), suitable chemical and biological amendments, and microbial strains should be required for successful remediation of heavy metal contaminated soils. Amongst the commercial crops grown in India, Indian mustard (*Brassica juncea* L.) has been reported to produce higher biomass and accumulate significant amount of heavy metals in their tissues when induced through the addition of chelating agents (Blaylock et al., 1997, Huang and Cunningham, 1996). Additions of chelating agents have been reported to increase Cd and Ni contents in the solution phase (Gray et al., 1999, Huang and Cunningham, 1996). Hence, higher extractability of Cd and Ni due to chelating agents may lead to their increased availability to plants (Mishra, 2004).

FYM, vermicompost, chelating agents and bioinoculants (N_2 fixer and phosphorus solubilizing bacteria) and their interaction leading to changes in different fractions of Cd and Ni in contaminated soil is to be studied for proper understanding of their role in chemically enhanced phytoextraction. Gray et al. (1999) showed that there was a wide range in the concentrations of Cd associated with individual soil fractions. The greatest concentration of Cd is associated with the organic and residual fractions, while the lowest concentration of Cd is in the exchangeable form. Fractionation of Cd in some soils indicated that a substantial portion of Cd added to the soil with P fertilizer applications ends up in the residual fraction of the soils. It is suggesting that a substantial proportion of fertilizer applied Cd in these soils has reverted to forms likely to be unavailable for plant uptake. It is reported that biosorption process for removal of the heavy metal Cd^{2+} from contaminated soil due to its economy, commercial applications and because it acts without destroying soil structure (phytoremediation). Kozáková and Kandrac (1998) studied the binding forms of selected heavy metal Cu, Pb, Cd and Ni in carbonate type soil. The present study thus was conducted to investigate an effect of chelation agents and bioinoculants on different fractions (forms) of Cadmium and Nickel in the soil after harvest of Indian mustard crop.

MATERIALS AND METHODS

i) Collection and Processing of Soil Samples:

The bulk surface sample (0-15) of a sandy loam soil was collected from the experimental area of the

Department of Soil Science, CCS Haryana Agricultural University, Hisar, Haryana. It was dried, ground to pass through a 2 mm stainless steel sieve and mixed thoroughly. The processed soil sample was used for laboratory and screen house studies. Its physico-chemical properties are given in Table (1).

Table (1): Physico-chemical characteristics of experimental soil

Properties	Contents
(A) Sand (%)	61.3
(B) Silt (%)	19.0
(C) Clay (%)	18.6
Textural class	Sandy loam
pH (1:2)	8.1
$EC_{1:2}$ (dS m^{-1})	0.4
Organic	0.36
CEC [Cmol (P^{+}) kg^{-1}]	12.7
$CaCO_3$ (%)	0.33
Available nutrients (kg ha^{-1})	
(A) Nitrigen	190.4
(B) P_2O_5	17.7
K_2O	200.6
DTPA extractable metal (mg kg^{-1})	
Fe	38.70
Mn	9.78
Zn	0.48
Cu	0.36
Cd	0.25
Ni	0.92
Pb	1.01
Total Metals (mg kg^{-1})	
Cd	3.15
Ni	11.25

ii) Collection and Processing of Farmyard manure:

The bulk sample of well decomposed farmyard manure (FYM) was taken from the manure pit of Dairy Farm, CCS Haryana Agricultural University, Hisar. The chemical composition of FYM used is given in Table (2).

Table 2: Chemical composition of experimental FYM

Properties	Content
Nitrogen (%)	1.23
Phosphorus (%)	0.61
Potassium (%)	1.90
Organic carbon (%)	29.34
C/N Ratio	24.88
Total metal (mg/kg)	
Cd	0.31
Ni	4.29

iii) Collection and Processing of Vermicompost

The bulk sample of a well decomposed Vermicompost (VC) from department of Agronomy, CCS Haryana Agricultural University, Hisar. The chemical composition of Vermicompost used is given in Table (3).

iv) Screen house Studies:

Screen house experiments were conducted to study the phytoremediation of Cadmium (cd) and nickel (Ni) by mustard (*Brassica juncea* L.) as affected by chelating agents and bioinoculants from metal enriched soil. The experimental details given below:

Table 3: Chemical composition of the experimental vermicompost

Properties	Content
Nitrogen (%)	1.75
Phosphorus (%)	0.68
Potassium (%)	0.93
Organic carbon (%)	16.18
C/N Ratio	11.40
Total metal (mg/kg)	
Cd	0.21
Ni	34

v) Imposition of Treatments:

The processed soil sample was equally divided into two halves and one half was artificially spiked with Cd using $CdCl_2$ as a source of Cd and remaining half soil was artificially spiked with Ni using $NiCl_2$ as a source of Ni. These two bulk soil samples were spread evenly on polythene sheets placed over the raised platform of screen house. Pre- calculated amount of $CdCl_2$ (i.e., 100 mg Cd/250 ml distilled water) and $NiCl_2$ (i.e., 100 mg Ni/250 ml distilled water) were dissolved in distilled water. The solution so prepared were sprinkled over the uniformly spread soils at the rate of 250 ml solution per kg of soil. After sprinkling this solution, the soil samples were covered with plastic sheet for 48 hours to minimize evaporation for proper equilibration. Thereafter the covers were taken off the soils were allowed to dry to workable moisture content. Each soil sample was thus thoroughly mixed, respread uniformly over the plastic sheet and moistened to near field capacity moisture content using distilled water. This cycle was repeated thrice for proper equilibration and uniform enrichment of both soils with added Cd and Ni respectively. The Cd and Ni enriched bulk soil samples were then air dried and divided in eight equal lots. One lot was kept as such. Second lot was treated with well decomposed dry farm yard manure (FYM) @ 2% by weight. The third lot was treated with well decomposed dry vermicompost (VC) @ 2% by weight and fourth lot was kept for Ethylenediaminetetraacetic acid (EDTA) treatment @ 2mmol/kg (0.4 mmol daily for 5 days in 5 split doses) at 40 days after sowing (DAS). The remaining four lots were also treated with above said treatments. In addition to those treatments, last four lots were also treated with N_2-fixer (i.e., *Azotobacter sp.*) and phosphorus solubilizing bacteria (i.e., *Pseudomonas sp.*) as seed treatment at the time of sowing.

FYM and vermicompost @ 2% by weight will mixed uniformly in Cd-spiked and Ni-spiked soils one week before sowing.

A. Experiment No. 1: ***Effect of chelating agents and bioinoculants on different fractions of Cd by mustard (Brassica juncea L.)***

1. Bulk spiked soil with 100 mg Cd/kg soil
2. FYM / vermicompost / chelating agents treatment combinations:
   A. Control (Non-spiked soil)
   B. Cd-spiked soil (Cd_{100})
   C. Cd-spiked soil (Cd_{100}) + FYM @ 2%
   D. Cd-spiked soil (Cd_{100}) + vermicompost @ 2%
   E. Cd-spiked soil (Cd_{100}) + EDTA @ 2 mmol/kg soil at 40 DAS (Day after sowing)
3. Bioinoculants treatment combinations:
   A. Control + PSB + N2 fixer
   B. Cd-spiked soil + PSB + N_2 fixer

C. Cd-spiked soil + PSB + N_2 fixer + FYM @ 2%
D. Cd-spiked soil + PSB + N_2 fixer + vermicompost @ 2%
E. Cd-spiked soil + PSB + N_2 fixer + EDTA @ 2 mmol/kg soil at 40 DAS

4. Crop: mustard (Brassica juncea L.) variety: Laxmi
5. Number of treatments: 5+5 = 10
6. Replication: 3
7. Number of pots: 10x3 = 30
8. Experimental design: CRD
9. Harvesting stage: Two weeks after chelating agent application

B. **Experiment No. 2:** ***Effect of chelating agents and bioinoculants on different fractions of Ni by mustard (Brassica juncea L.)***

1. Bulk spiked soil with 100 mg Ni/kg soil
2. FYM / vermicompost / chelating agents treatment combinations:
   A. Control (Non-spiked soil)
   B. Ni-spiked soil (Ni100)
   C. Ni-spiked soil (Ni100) + FYM @ 2%
   D. Ni-spiked soil (Ni100) + Vermicompost @ 2%
   E. Ni-spiked soil (Ni100) + EDTA @ 2 mmol kg-1 soil at 40 DAS (Day after sowing)
3. Bioinoculants treatment combinations:
   A. Control + PSB + N_2 fixer
   B. Ni-spiked soil + PSB + N_2 fixer
   C. Ni-spiked soil + PSB + N_2 fixer + FYM @ 2%
   D. Ni-spiked soil + PSB + N_2 fixer + Vermicompost @ 2%
   E. Ni-spiked soil + PSB + N_2 fixer +EDTA @ 2 mmol kg-1 soil at 40 DAS
4. Crop: mustard (Brassica juncea L.) Variety: Laxmi
5. Number of treatments: 5 + 5 = 10
6. Replication: 3
7. Number of pots: 10x3= 30
8. Experimental design: CRD
9. Harvesting stage: Two weeks after chelating agent application

Five kg capacity earthen pots lined with polythene were used to grow the crop. The polythene lining was provided mainly to avoid leaching of soil solution and interaction of metals with the earthen pots. All, 60 pots were filled with 5 kg of thoroughly mixed air-dried soil. In each pot basal doses of N, P, K, Fe, Mn, Zn and Cu @ 50, 50, 60, 10, 5, 5 and 5 mg/kg soil through Urea, KH_2PO_4, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, and $CuSO_4.H_2O$ respectively, in solution form @ 5oo ml solution in each pot separately. After 24 hours, the nutrient treated soil from each pot was separately taken out, mixed thoroughly and refilled.

Indian mustard (*Brassica juncea* L.), variety Laxmi, was taken as the test crop. Before sowing, half of the total seeds were inoculated with microbial inoculants (*Azotobacter sp.* and *Pseudomonas sp.*). Twenty inoculated seeds of this crop were sown in each pot on 22nd Oct. 2013.

Thinning was done to 5 plants per pot on 10th day of seeding and plants were allowed to grow up to 8 weeks from the date of sowing (i.e., up to blooming stage). The pots were irrigated deionized water as and when required.

In the present study, EDTA, FYM and vermicompost were used as chelating agents. EDTA (Disodium salts) was applied at the rate of 2 mmol per kg soil (0.4mmol daily for 5 days in 5 split doses) in twelve pots filled with the Cd and Ni treated soil in 40th day of sowing.

Composite soil samples from each pot were taken before sowing and after harvesting of plants with stainless steel tube auger. These soil samples were air dried, ground, sieved and stored in polyethylene bags for their chemical analysis.

Analytical method

Soil Analysis:

The following methods were used for soil analysis:

(i) Mechanical analysis was done as per international pipette method (Piper, 1996).

(ii) pH and electrical conductivity were determined on 1:2 soil water suspension with the help of glass electrode pH meter and conductivity meter bridge, respectively.

(iii) Organic carbon was estimated by Walkley and Black's rapid titration method.

(iv) Cation Exchange Capacity was determined by Ammonium acetate method as outlined in USDA Handbook No. 60 (Richard, 1954).

(v) $CaCO_3$: was determined by rapid titration method of (Puri, 1949).

(vi) DTPA-exchangeable polyvalent transition heavy metals were extracted by using the procedure of [18] and estimated on AAS.

(vii) Total Cd and Ni was determined using by aqua regia method [7], (Conventional aqua regia digestion was performed in 250 mL glass beakers covered with watch glass. A well-mixed sample of 0.5000 g was digested in 12 mL of aqua regia on a hot plate for 3 hours at 110ºC. After evaporation to near dryness, the sample was diluted with 20 mL of 2% (v/v with H2O) nitric acid and transferred in to a 100 mL volumetric flask after filtering through Whatman No.42 paper and diluted to 100 mL with DDW) digest by atomic absorption spectrophotometer (AAS) (GBC-932 plus).

Sequential extraction of soil samples:

To study the effect of different chelating agents and bioinoculants on fractional distribution of Cd in Cd spiked and Ni in Ni spiked soil.

A seven-step sequential extraction procedure given in Table No.4 was applied to evaluate the association of metals with soil constituents. The different chemical forms of fraction of Cd and Ni extracted were exchangeable + water soluble carbonate, organic matter complexed, Mn-oxide bound, occluded in amorphous and crystalline Fe-oxide and residual mineral fraction.

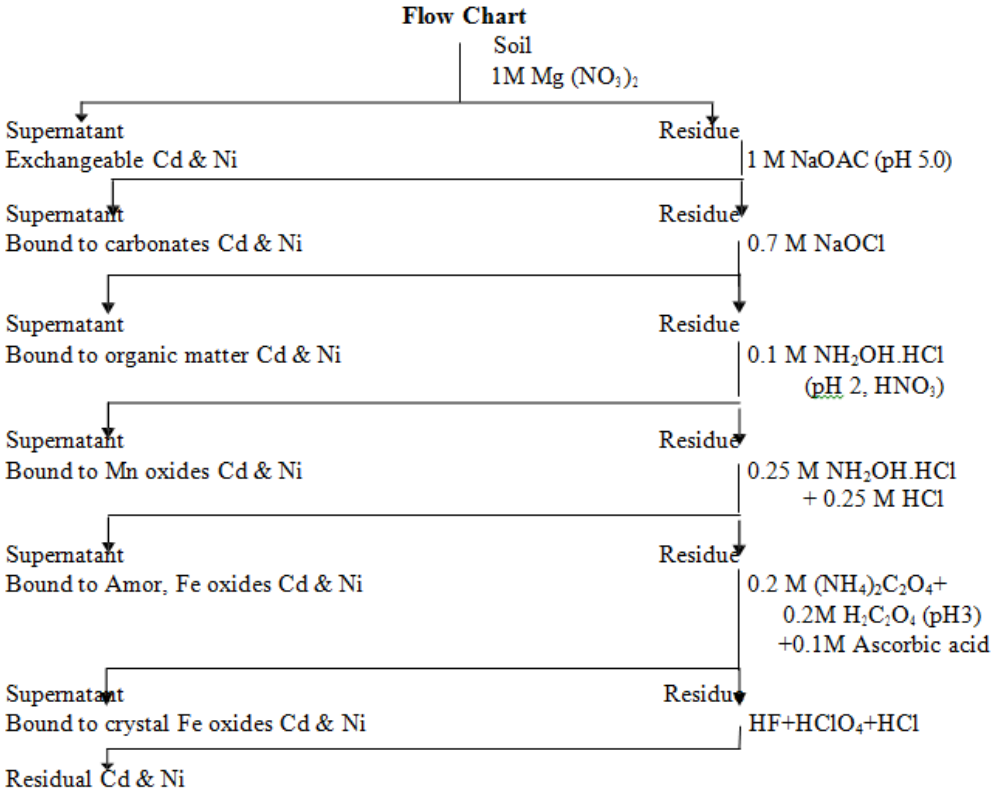


Figure (1): Flow chart of sequential extraction scheme for Cadmium and Nickel

Sequential fractionation technique was used as a measure of availability, mobility and potential

bioavailability of Cd and Ni to plants. Several methods of fractionation of heavy metals have been proposed by several workers (Chao and Zhou, 1983, Chao, 197], Gupta and Chen, 197, Shuman, 1998, Tessier et al., 1979). Here, sequential extraction procedure adopted by Singh *et al*. (1988) was used which is described in Table (4) and Figure (1). Reagents used in fractionation scheme were selected from those cited in the literature as being selective for specific chemical form in soil. All procedures were carried out in triplicate. The fractionation scheme generally starts with the weakest, least aggressive ones. Ten gram of each soil samples was taken in centrifuge tube; the extractant was added to it in required ratio. The contents of tube were centrifuged and filtered after fulfilling the essential conditions such as shaking, boiling, digestion etc. The soil residue was washed twice with double distilled water. Then five gram of soil sample was taken in centrifuge tube for determining Mn-oxide form.

Residual metal analysis:
Residual from CFeOX extraction was washed twice with double distilled water. It was subsequently dried and ground to pass through a 2 mm stainless steel sieve. A 0.5 g samples were digested with 10 ml conc. HF and 2-3 ml conc. $HClO_4$ using Teflon beaker, over stand and bath heater. Remaining material taken up in 6N HCl and volume was made to 25 ml. The procedure as described by (Tessier et al., 1979) was adopted and supernatant liquids for all chemical forms were stored in plastic bottles. The amount of Cd and Ni fractions were estimated by Atomic Absorption Spectrophotometer.

Table: 4. Sequential extraction steps for Cd and Ni fractionation

S. No.	Fractions	Reagent/Extractant	Soil (g)	Solution (ml)	Experimental condition	Reference
1	Exchangeable (EX)	1 M Mg $(NO_3)_2$	10	40	Shake 2 h at 2ºC	Shuman (1985)
2	Bound to carbonate (CARB)	1 M NaOAc (pH 5.0 CH_3COOH)	10	40	Shake 5 h at 25ºC	Tessier et al. (1979)
3	Bound to organic Matter (OM)	0.7 M NaOCl (pH 8.5)	10	20	Shake 30 min. in boiling water bath, stir occasionally	Shuman (1983)
4	Bound to Mn oxides (MnOX)	0.1 M $NH_2OH.HCl$ (pH 2, HNO_3)	5a	50	Shake 30 min.	Chao (1972)
5	Bound to Amor. Fe oxides (AFeOX)	0.25 M $NH_2OH.HCl$+0.25 M HCl	5	50	Shake 30 min. at 50ºC in water bath	Chao and Zhou (1983)
6	Bound to Crys. Fe oxides (CFeOX)	0.2 M $(NH_4)_2C_2O_4$+0.2 M $H_2C_2O_4$ (pH 3)+0.1 M Ascorbic acid	5	50	Boiling30 min. in water bath stir occasionally	Shuman (1982)
7	Residual (RES)	10 ml conc. HF + 2 ml $HClO_4$ + 6N conc. HCl in sequence	0.5	25	Sand bath, cool, boil gently and cool	Tessier et al. (1979)

RESULTS AND DISCUSSION

Fractionation studies:

Effect of chelating agents and microbial inoculants on different fractions of Cd in soil after harvest of *Brassica juncea:*
The data pertaining to different fractions of Cd as influenced by bioinoculants and chelating agents are presented in Table 5. A perusal of data in table revealed that the transformation of Cd to different fractions in Cd spiked soil is influenced differentially by different chelating agents and bioinoculants. The fraction wise description is given below:

(i) Exchangeable-Cd (Ex-Cd)
The mean value of exchangeable-Cd varies from 0.23 to 27.02 µg/g soil (Table 5). With application of EDTA a significant increase was observed in exchangeable form. The application of EDTA yields 2.19 and 2.21 times higher Ex-Cd than without EDTA in Cd_{100} and Cd_{100}+M treated soil. The organic amendments significantly decreased the exchangeable fraction of Cd (approx. 2 times lower than EDTA treated soil). Bioinoculants did not play a significant role either in increasing or in decreasing this fraction of Cd.

(ii) Carbonate bound- Cd (Carb-Cd)
It is evident from data presented in Table 5 that carbonate bound-Cd fraction was significantly higher in soil where none of the chelating agent was applied. The mean value of Carb-Cd fraction decreased from 19.51 at Cd_{100}+M to 16.05 µg/g soil in EDTA treated Cd spiked soil. The bioinoculants significantly increased Carb-Cd fraction in soil where chelating agents were not applied.

(iii) Organic bound- Cd (Org-Cd)
Organically bound-Cd fraction was significantly higher in soils where EDTA was not applied than EDTA treated soil (Table 5). The mean content of Org-Cd was 29.95 µg/g in Cd_{100}+M but decreased significantly with the addition of chelating agents. The organically bound-Cd fraction was comparatively higher than either carbonate or manganese oxide bound forms in both i.e., with or without bioinoculants soils. The Org-Cd fraction increased in organically amended soil, approximately 1.34 (without bioinoculants) 1.38 (with bioinoculants) times more than EDTA applied soil.

(iv) Manganese oxide bound-Cd (MnOX-Cd)
Data related to the effect of chelating agents and bioinoculants on the MnOX-Cd fraction are presented in Table 5. It is clear from the data that application of chelating agent caused a significant reduction in the MnOX-Cd fraction as compared to Cd_{100} where chelating agent was not applied. The MnOX-Cd fraction decreased from 15.22 in Cd_{100} to 14.75 µg/g soil in Cd_{100}+EDTA treated soil.
However, FYM and vermicompost proved useful in maintaining this pool as compared to chelating agent. Slightly lower content of MnOX-Cd fraction was observed in bioinoculants treated soil than untreated soil as the bioinoculants release some chelating agents or siderophores which make the micronutrients available for the plants for their growth.

(v) Amorphous Fe-oxide bound Cd (AFeOX-Cd)
The AFeOX-Cd fraction was higher in Cd_{100} but decreased slightly due to addition of FYM, vermicompost and EDTA (Table 5).
The mean content of AFeOX-Cd fraction was 5.44 and 5.08 µg/g in Cd_{100}+FYM and Cd_{100}+VC treatments whereas in Cd100+EDTA treatment, 4.78 µ g/ g observed. In case of bioinoculants treated soil, the values for AFeOX- Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vi) Crystalline Fe-oxide bound Cd (CFeOX-Cd)
The mean content of CFeOX-Cd fraction decreased significantly with the addition of chelating agent (EDTA) (Table 5). The CFeOX-Cd fraction (13.95 µ g/g) was higher in Cd100 but decreased slightly due to addition of vermicompost whereas the addition of FYM and EDTA caused a significant reduction in the value of CFeOX-Cd fraction.
The mean value of CFeOX-Cd fraction was 12.92 and 12.31 µg/g soil in FYM and EDTA treatments. In case of bioinoculants treated soil the values for CFeOX-Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vii) Residual-Cd (Res-Cd)
Residual fraction showed a decreasing trend with addition of organic amendments and chelating agent (Table 5). Among all, EDTA was more effective in decreasing Res-Cd fraction as compared

to others. The mean value of Res-Cd fraction decreased from 7.15 to 4.95 µg/g on the addition of EDTA.
The mean values of this fraction in FYM and vermicompost was 6.25 and 5.49 µg/g. But in case of bioinoculants treated soil, the mean content of Res-Cd fraction was reported lower than the soils which was not treated with bioinoculants.

Table 5: Post-harvest distribution of Cd (µg/g soil) into different forms as influenced by different chelating agents and bioinoculants in Cd enriched soil

Treatment	Ex-Cd	Cab-Cd	OM-Cd	MnOX-Cd	AFeOX-Cd	CFeOX-Cd	Res-Cd
Control	0.23	0.61	0.20	082	0.47	0.59	2.05
Cd_{100}	12.32	19.36	26.65	15.22	6.10	13.95	7.15
Cd_{100}+FYM	13.85	18.22	25.45	15.91	5.44	12.92	6.25
Cd_{100}+VC	12.71	19.33	26.08	16.11	5.08	13.72	5.49
Cd_{100}+EDTA	27.02	16.05	19.37	14.75	4.78	12.31	4.95
Control+M	0.26	0.67	0.25	0.80	0.51	0.62	1.90
Cd_{100}+M	12.21	19.51	29.95	14.60	6.21	14.02	6.89
Cd_{100}+FYM+M	13.90	19.30	26.16	15.12	7.25	13.79	5.35
Cd_{100}+VC+M	12.79	19.11	29.42	15.78	8.13	14.55	5.05
Cd_{100}+EDTA+M	27.10	17.14	21.29	14.40	5.45	12.10	4.18
CD at 5%	1.24	1.30	1.81	1.08	0.43	0.96	0.41

M: Microbial inoculants VC: Vermi Compost

Effect of chelating agents and microbial inoculants on different fractions of Ni in soil after harvest of *Brassica juncea*

The data pertaining the fractions of Ni in soil after the harvest of *Brassica juncea* are presented in Table 6. The fraction wise description is given below:

(i) Exchangeable-Ni (Ex-Ni)

The mean value of exchangeable-Ni varies from 0.26 to 25.5 µ g g^{-1} soil (Table 6). With application of EDTA a significant increase was observed in exchangeable form. The application of EDTA yields 1.41 and 1.38 times higher Ex-Ni than without EDTA in Ni_{100} and Ni_{100}+M treated soil. The organic amendments significantly decreased the exchangeable fraction of Ni (approx. 2 times lower than EDTA treated soil). Bioinoculants did not play a significant role either in increasing or in decreasing this fraction of Ni

(ii) Carbonate bound- Ni (Carb-Ni)

It is evident from data presented in Table 6 that carbonate bound-Ni fraction was significantly higher in soil where none of the chelating agent was applied. The mean value of Carb-Ni fraction decreased from 15.10 at Cd_{100}+M to 13.12 µ g g^{-1} soil in EDTA treated Ni spiked soil. The bioinoculants significantly increased Carb-Ni fraction in soil where chelating agents were not applied.

(iii) Organic bound-Ni (Org-Ni)

Organically bound-Ni fraction was significantly higher in soils where EDTA was not applied than EDTA treated soil (Table 6). The mean content of Org-Ni was 8.14 µg/g in Ni_{100}+M but decreased significantly with the addition of chelating agents. The Org-Ni fraction increased in organically amended soil, approximately 1.08 (without bioinoculants)
1.21 (with bioinoculants) times more than EDTA applied soil.

(iv) Manganese oxide bound-Ni (MnOX-Ni)

Data related to the effect of chelating agents and bioinoculants on the MnOX-Ni fraction are presented in Table 6. It is clear from the data that application of chelating agent caused a significant reduction in the MnOX-Ni fraction as compared to Ni_{100} where chelating agent was not applied.

The MnOX-Cd fraction decreased from 18.50 in Ni_{100} to 17.21 µg/g soil in Ni_{100}+EDTA treated soil. Slightly lower content of MnOX-Cd fraction was observed in bioinoculants treated soil than untreated soil as the bioinoculants release some chelating agents or siderophores which make the micronutrients available for the plants for their growth.

(v) Amorphous Fe-oxide bound Ni (AFeOX-Ni)

The AFeOX-Cd fraction was higher in Ni_{100} but decreased slightly due to addition of FYM, vermicompost and EDTA (Table 6). The mean content of AFeOX-Ni fraction was 20.82 and 19.71 µg/g in Cd_{100}+FYM and Cd_{100}+VC treatments whereas in Cd100+EDTA treatment, 19.33 µg/g observed. The Amorphous Fe-oxide bound Ni fraction was comparatively higher than either exchangeable or manganese oxide bound forms in both i.e., with or without bioinoculants soils. In case of bioinoculants treated soil, the values for AFeOX-Cd fraction were higher than the treatments which were not inoculated with bioinoculants.

(vi) Crystalline Fe-oxide bound Ni (CFeOX-Ni)

The mean content of CFeOX-Ni fraction decreased significantly with the addition of chelating agent (EDTA) (Table 6). The CFeOX-Ni fraction (16.60 µ g g^{-1}) was higher in Ni_{100} but decreased slightly due to addition of vermicompost whereas the addition of FYM and EDTA caused a significant reduction in the value of CFeOX-Ni fraction. The mean value of CFeOX-Cd fraction was 15.62 and 14.91 µg/g soil in FYM and EDTA treatments. In case of bioinoculants treated soil, the values for CFeOX-Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vii) Residual-Ni (Res-Ni)

Residual fraction showed a decreasing trend with addition of organic amendments and chelating agent (Table 6). Among all, EDTA was more effective in decreasing Res-Ni fraction as compared to others. The mean value of Res-Ni fraction decreased from 6.40 to 4.90 µ g/g on the addition of EDTA. The mean values of this fraction in FYM and vermicompost was 5.77 and 5.12 µg/g. But in case of bioinoculants treated soil, the mean content of Res-Ni fraction was reported lower than the soils which was not treated with bioinoculants.

Table (6): post-harvest distribution of Ni (µg/g soil) into different forms as influenced by different chelating agents and bioinoculants in Ni enriched soil

Treatment	Ex-Ni	Carb- Ni	OM-Ni	MnOX- Ni	AFeOX-Ni	CFeOX-Ni	Res-Ni
Control	0.26	0.90	0.48	0.91	1.81	0.72	4.51
Ni_{100}	18.30	14.91	7.51	18.50	22.78	16.60	6.40
Ni_{100}+FYM	19.67	13.49	6.39	18.78	20.82	15.62	5.77
Ni_{100}+VC	18.87	14.70	6.75	19.05	19.71	16.33	5.12
Ni_{100}+EDTA	25.95	13.12	6.24	17.40	19.33	14.91	4.90
Control+M	0.35	0.88	0.41	0.80	1.72	0.77	4.35
Ni_{100}+M	18.76	15.10	8.14	17.80	22.95	16.85	6.00
Ni_{100}+FYM+M	19.80	13.60	7.18	18.36	21.08	15.75	5.50
Ni_{100}+VC+M	18.90	14.52	7.33	19.30	21.27	15.20	4.94
Ni_{100}+EDTA+M	26.12	13.80	6.03	17.11	19.00	15.35	4.44
CD at 5%	1.48	0.96	0.49	1.25	1.48	1.11	0.68

M: Microbial inoculants VC: Vermi Compost

CONCLUSIONS

It is evident that dominant Ni fraction was amorphous iron-oxide followed by exchangeable-Ni, crystalline iron-oxide, manganese oxide, carbonate, organic and residual Ni fraction. Application of chelating agents increased the exchangeable fraction causing an increase in its availability in the soils. It may be ascribed to the fact that EDTA targeted Ni mainly to amorphous and

exchangeable fractions whereas other fractions were less effectively solubilized. It has been suggested that levels of Nickel in Fe-Mn oxide fraction depend on how much Mn oxide is absorbed in soil because Ni^{2+} can substitute for surface manganese in mixed valence Mn oxides. Similar results were confirmed by (Ahmed, 2000).

Application of chelating agent (EDTA) significantly increased the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, MnOX, AFeOX, bound fractions. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd.

Application of chelating agents increased exchangeable fraction with a consequent decrease in organic fraction. Highest amount of exchangeable fraction was observed in EDTA treated soil. Application of FYM and vermicompost increased AFeOX fraction of Ni but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost also increased AFeOX fraction of Ni.

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