

Chemical fractions and bioavailability of nickel in alluvial soils

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ABSTRACT

The present study was undertaken to sequentially fractionate nickel (Ni) in soils of divergent physicochemical characteristics and evaluate the contribution of different fractions towards plant uptake. For this, fifteen bulk surface (0–15 cm) soil samples were collected from the cultivated fields of northwestern Indo-Gangetic alluvial plains. A pot experiment was conducted with these soils to assess the contribution of soil Ni fractions to plant uptake using soybean as test crop. Results showed that residual Ni was the most dominant fraction in soil constituting 3.19–63.6% of total Ni. The water soluble plus exchangeable Ni accounted for only 0.70–4.04% of total soil Ni. Organically bound Ni varied from 1.60–6.85% of total Ni; these values are relatively lower as compared to those reported for temperate soils. Correlation studies showed that the free iron oxide (Fe_2O_3) and soil organic carbon correlated with various fractions of Ni in soil. Water soluble plus exchangeable and organically bound are the dominant fractions which contributed positively and manganese oxide (MnO_2) bound and residual fractions contributed negatively towards the phytoavailability of Ni in soil.

Keywords: pollutant; soil properties; *Glycine max*; plant Ni content

Nickel (Ni) plays a critical role in both crop production under intensive agriculture, and also as a pollutant in agricultural lands continuously receiving Ni-rich sewage sludge and industrial effluents. Positive effects of Ni at low concentration were reported in several crops, but as soon as the concentration increases beyond a limit, it causes toxic effects to the plants (Brown et al. 1987, Hasinur et al. 2005). Rather than the total content, chemically reactive form of Ni determines its mobility and bioavailability in soils (Mostafa et al. 2006). Quantification of these chemical forms also helps in predicting their potential movement and consequential contamination of the water resources (Jalali and Arfania 2011). Soil properties viz. texture (clay content), pH, organic matter, and Fe-Mn oxides were found among the most important soil properties and components influencing the mobility and bioavailability, solid-solution equilibrium of Ni operating in the soil and its distribution and transformation in the solid phase (Ma and Rao 1997, Barman et al. 2013). As indicated by sequential fractionation studies, 35–94% of the total Ni residues in the inert residual fraction associated with primary minerals

(Ma and Rao 1997, Mostafa et al. 2006, Chao et al. 2007); exchangeable and oxide-bound fractions constitute the major non-residual fraction of Ni. Soil pH and organic matter content exerted influence on the distribution of Ni in different fractions. Such studies were mostly confined to Ni-polluted soils characterized by high organic matter content. Studies on relative contribution of different soil Ni fractions towards its uptake by plants are very limited and conflicting (Chao et al. 2007). Hence, the present investigation was undertaken on sequential fractionation of Ni in low organic matter Inceptisols of northwestern Indo-Gangetic alluvial (having wide variations in the available Ni content) plains with an ultimate objective of studying the influence of soil properties on different fractions of Ni and evaluating the contribution of different fractions of Ni to plant uptake.

MATERIAL AND METHODS

Location and collection of soil samples. Fifteen bulk surface soil samples (0–15 cm) with diverse

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physical and chemical properties were collected from cultivated fields of different locations in and around Delhi. Four of these soil samples were collected from Indian Agricultural Research Institute (IARI) fields, which were under intensive cultivation with fresh irrigation water for more than seven decades. Five samples were collected from farmers' fields that have been receiving sewage effluents for last thirty years under Keshopur Effluent Irrigation Scheme of the Government of National Capital Territory Delhi, India. Rattan et al. (2005) reported a significant build-up of heavy metals particularly Zn, Cu and Ni in these soils due to sewage irrigation. Risk assessment for intake of these metals indicated that vegetable crops grown on these sewage-irrigated soil can be consumed safely by humans. One sample was collected from farmers' field located at Madanpur Khadar, which has been receiving irrigation through sewage effluent from the Okhla Sewage Treatment Plant, Delhi for last fifty years. Four soil samples were collected from the Yamuna river bank. Vegetables grown on these soils were suitable for human consumption as far as metal content in edible portion is concerned. One soil sample was collected from field adjoining Atlas Cycle factory, Sonapat, Haryana which had been receiving irrigation through effluents of the factory for fifteen years. Mathavan (2006) reported that plants (onion, lettuce and radish) grown on this soil suffered from metal toxicity.

Soil analysis. The soil samples were air-dried, ground and passed through 2 mm sieve. The processed soil samples were mixed thoroughly for ensuring homogenization; finally homogenized

samples were used for characterization of initial soil properties, greenhouse experiment and chemical analysis. Mechanical composition of soil was determined by hydrometer method (Jackson 1973). Soil pH was determined in 1:2 (soil:water) suspension using the combined electrode of digital pH meter. The electrical conductivity (EC) was determined in the supernatant liquid of the same extracts with the help of EC meter (Eutech Instruments; model:pc510). Soil organic carbon (SOC) content in soil was determined by wet oxidation method (Jackson 1973). The cation exchange capacity (CEC) of the soil samples was determined by ammonium acetate method as described by Jackson (1973). For extraction of free Fe_2O_3 and Al_2O_3 in soil 2 g of soil sample, 2 g of sodium dithionite and 20 g of sodium citrate were taken in 150 mL conical flask. Then distilled water was added up to 118 mL and shaken overnight and final volume was made up to 250 mL (Page 1982). Iron content in the extracts was determined with the help of atomic absorption spectrophotometer with flame atomization (AAS) (Analytic Jena) and Al content was estimated colorimetrically using aluminon method (Page 1982). Soil sample was extracted with 0.05 mol/L DTPA – 0.01 mol/L CaCl_2 (Lindsay and Norvell 1978), and available Ni in the extracts was determined by AAS with flame atomization. Soils were sequentially extracted with various chemical reagents according to fractionation scheme of Iwasaki and Yoshikawa (1990) (Table 1). Nickel content in the extracts of different fractions was estimated using AAS with flame and flameless atomization depending upon the concentration

Table 1. Details of sequential fractionation procedure

Fraction	Reagent used	Soil:solution	Shaking
WSPEXNi	0.05 mol/L $\text{Ca}(\text{NO}_3)_2$	1:40	16 h
PBDNi	0.05 mol/L $\text{Pb}(\text{NO}_3)_2$ + 0.01 mol/L $\text{Ca}(\text{NO}_3)_2$	1:40	16 h
ACSNi	0.44 mol/L CH_3COOH + 0.01 mol/L $\text{Ca}(\text{NO}_3)_2$	1:40	8 h
MNOBNi	0.01 mol/L $\text{NH}_2\text{OH} \cdot \text{HCl}$ + 0.1 mol/L HNO_3	1:40	30 min
ORBNi	0.1 mol/L $\text{K}_4\text{P}_2\text{O}_7$	1:40	24 h
AMFBNi	0.175 mol/L $(\text{NH}_4)_2\text{C}_2\text{O}_4$ + 0.1 mol/L $\text{H}_2\text{C}_2\text{O}_4$	1:40	4 h
CRFBNi	0.1 mol/L $\text{H}_2\text{C}_2\text{O}_4$ + 0.1 mol/L ascorbic acid	1:40	30 min in boiling water bath

WSPEXNi – water soluble plus exchangeable; PBDNi – lead displaceable; ACSNi – acid soluble; MNOBNi – Mn-oxide bound Ni; ORBNi – organically bound Ni; AMFBNi – amorphous Fe-oxide bound Ni; CRFBNi – crystalline Fe-oxide bound Ni

of Ni. Total Ni content in soil was determined by digesting soil a mixture of H_2SO_4 , HClO_4 (60%) and HF (40%) (Datta et al. 2002). Nickel content in the extract was estimated using AAS with flame atomization.

Greenhouse experiment. A pot experiment was conducted using all fifteen experimental soils to study the contribution of different Ni fractions towards its uptake by soybean (*Glycine max* cv. PS 22). Four kg of soil was filled in the plastic pots. A uniform basal dose of 20, 17.5 and 22.5 mg/kg of N, P and K was added to the soil of each pot through urea, diammonium phosphate and muriate of potash, respectively. Each treatment combination was replicated twice in a completely randomized design using 30 pots. The soil in each pot was irrigated with deionised water and incubated for one week. Fifteen seeds were sown in each pot. After two weeks of planting, a uniform population of six plants/pot was maintained. The plants were harvested at flowering stage, i.e., after 55 days of planting. The plants were harvested in flowering stage for model purposes in spite of the fact that plants are harvested at maturity stage in real conditions. After harvesting, aboveground parts of the plants were washed with deionised water and dried in hot air oven at 60–70°C. Dried plant samples were ground and digested with diacid mixture ($\text{HNO}_3\text{:HClO}_4$ 10:4) (Page 1982) and Ni content in the digests was determined by AAS with flame and flameless atomization.

Statistical analysis. Regression analysis was carried out to study the relationship of various Ni fractions with soil properties. In addition, regression and partial correlation analyses were also carried out to assess the contribution of various Ni fractions towards plant uptake.

RESULTS AND DISCUSSION

Nickel fractions in soil. The characteristics of experimental soils showed significant variations in respect of physical and chemical properties (Table 2). They also differed widely with respect to distribution of Ni into different fractions (Table 3). The residual Ni (RESNi) was the dominant fraction as compared to the other fractions. Proportion of RESNi in these soils was low as compared to the values reported in literature (Ma and Rao 1997, Mostafa et al. 2006, Chao et al. 2007). This is at-

Table 2. Physical and chemical properties of experimental soils

Location ¹	Mechanical composition (%)		Textural class ²	pH	EC ($\mu\text{S}/\text{cm}$)	CEC (mmol_+/ kg)	Organic carbon	Free (%)		DTPA extractable Ni (mg/kg)
	clay	silt						Fe_2O_3	Al_2O_3	
IARI farm, New Delhi (4)	26.5 ± 4.16	15.0 ± 2.65	58.5 ± 4.30	sandy loam (1), sandy clay loam (3)	8.07 ± 0.06	370 ± 130	137 ± 22.7	0.46 ± 0.07	0.32 ± 0.04	0.06 ± 0.004
Keshopur, Delhi (5)	15.0 ± 1.81	24.3 ± 11.6	60.6 ± 12.3	silt loam (1), sandy loam (4)	7.43 ± 0.23	1740 ± 660	158 ± 13.8	0.74 ± 0.07	0.17 ± 0.01	0.04 ± 0.005
Madanpur, Delhi (5)	7.62 ± 2.45	33.0 ± 13.2	59.3 ± 12.4	silt loam (2), sandy loam (2), sand (1)	7.89 ± 0.16	760 ± 180	103 ± 17.2	0.37 ± 0.20	0.14 ± 0.03	0.24 ± 0.18
Sonepat, Haryana (1)	18.8	30.5	50.6	loam (1)	8.03	500	122	0.62	1.64	0.13
Overall (15)	15.9 ± 2.39	25.1 ± 5.76	58.9 ± 5.51	sandy loam (7), silt loam (3), sandy clay loam (3), loam (1), sand (1)	7.79 ± 0.11	9700 ± 260	130 ± 10.3	0.53 ± 0.08	0.30 ± 0.10	0.12 ± 0.06
										1.39 ± 0.65

¹Figures in parentheses indicate number of soil samples collected from respective locations; ²Figures in parentheses indicate number of soil samples belonging to respective textural classes. EC – electrical conductivity; CEC – cation exchange capacity

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Table 3. Descriptive statistics on distribution of nickel into different fractions (mg/kg) in experimental soils

	WSPEXNi	PBDNi	ACSNi	MNOBNi	ORBNI	AMFBNi	CRFBNi	RESNi	Total
Range	0.07–7.70	0.47–84.8	0.02–120	0.53–170	0.23–75.1	0.003–225	0.001–46.2	0.27–367	7.57–1096
Mean	1.10	9.32	12.6	13.5	5.92	16.1	5.65	38.7	102
Standard deviation	1.91	21.0	29.9	43.3	19.1	57.8	11.4	91.4	275
Median	0.37	3.30	4.26	2.03	0.89	0.85	3.05	18.1	30.4

WSPEXNi – water soluble plus exchangeable; PBDNi – lead displaceable; ACSNi – acid soluble; MNOBNi – Mn-oxide bound Ni; ORBNi – organically bound Ni; AMFBNi – amorphous Fe-oxide bound Ni; CRFBNi – crystalline Fe-oxide bound Ni; RESNi – residual Ni

tributed to very low clay content of these soils. In soil No. 11 (collected from Madanpur Khadar, which has been receiving irrigation through sewage effluent from the Okhla Sewage Treatment Plant, Delhi), although its clay content is relatively low, proportion of residual Ni fraction was far high as compared to soils 12, 13 and 14 (collected from the Yamuna river bank). This may be related to the unusually high organic carbon content of this soil, where Ni might have been associated with recalcitrant fraction of organic matter, which was not accessible to potassium pyrophosphate. Potassium pyrophosphate disperses organic matter by breaking the organic matter-cation complexes (Shuman 1991). Hence this reagent is less efficient than H_2O_2 , where complete oxidation of organic matter can be achieved using H_2O_2 . Wang et al. (1997) reported that large proportion of RESNi could be attributed to the fact that Ni has the highest crystal field stabilization energy of the common divalent metals, and thus has a high potential to be enriched in clay minerals. Water soluble plus exchangeable Ni (WSPEXNi) consti-

tuted 0.70–4.04% of total Ni present in the soil. However, Mostafa et al. (2006) and Lukowski and Wiater (2009) reported relatively higher proportion ($\leq 10.1\%$) of WSPEXNi in soil as compared to results of the present study. Lower SOC and clay contents might be responsible for lower CEC in these soils, which might have been responsible for poor retention of Ni as cation. The Pb-displaceable Ni (PBDNi) i.e. specifically adsorbed Ni in these soils ranged from 1.64–49.2% of total Ni in these soils. The proportion of acid soluble fraction (ACSNi) ranged from 0.15–26.5%. By and large, CH_3COOH extracted higher amounts of Ni as compared to $Pb(NO_3)_2$, which corroborates the findings of Miller et al. (1986). Organically bound Ni (ORBNI) varied from 1.60–6.85% of total Ni in these soils. Most of the experimental soils were low to medium in SOC content except soils No. 5, 9 and 11 (soils receiving sewage effluents) (Table 2) which had relatively higher SOC content. This may be the reason for low percentage of Ni in the ORBNi. The Mn-oxide bound (MNOBNi), amorphous and crystalline Fe-oxide bound (AMFBNi

Table 4. Stepwise regression equations relating soil nickel fractions with soil properties

Soil No.	Multiple regression equation	R^2	F
1	$WSPEXNi = 3.70 - 0.52 (pH) + 1.40 (OC) - 0.05 (clay \%) + 4.83 (Fe_2O_3 \%)$	0.95	51.4*
2	$PBDNi = -2.49 + 12.5 (OC) - 0.61 (clay \%) + 47.4 (Fe_2O_3 \%)$	0.98	172*
3	$ACSNi = -4.82 + 17.7 (OC) - 0.78 (clay \%) - 0.12 (silt \%) + 78.3 (Fe_2O_3 \%)$	0.99	230*
4	$MNOBNi = -78.4 + 8.61 (pH) + 26.3 (OC) - 1.29 (clay \%) - 0.10 (silt \%) + 112 (Fe_2O_3 \%)$	0.99	193*
5	$ORBNI = -32.7 + 3.21 (pH) + 7.03 (OC) + 0.49 (CEC) - 0.62 (clay \%) - 0.07 (silt \%) + 50.6 (Fe_2O_3 \%)$	0.99	130*
6	$AMFBNi = -107 + 10.8 (pH) + 19.5 (OC) + 1.52 (CEC) - 1.96 (clay \%) - 0.21 (silt \%) + 153 (Fe_2O_3 \%)$	0.99	142*
7	$CRFBNi = -1.73 + 4.20 (OC) - 0.15 (clay \%) - 0.05 (silt \%) + 29.4 (Fe_2O_3 \%)$	0.98	143*
8	$RESNi = -29.1 + 50.2 (OC) - 1.87 (clay \%) + 236 (Fe_2O_3 \%)$	0.99	241*

* $P < 0.01$; WSPEXNi – water soluble plus exchangeable; PBDNi – lead displaceable; ACSNi – acid soluble; MNOBNi – Mn-oxide bound Ni; ORBNi – organically bound Ni; AMFBNi – amorphous Fe-oxide bound Ni; CRFBNi – crystalline Fe-oxide bound Ni; RESNi – residual Ni; OC – organic carbon; CEC – cation exchange capacity

Table 5. Multiple regression equations relating nickel content of soybean (mg/kg) with soil nickel fractions

Soil No.	Multiple regression equation	R^2	F
1	Ni content in soybean = $0.6601 + 1.95 (\text{WSPEXNi}) + 0.08 (\text{PBDNi}) + 0.09 (\text{ACSNi}) - 0.71 (\text{MNOBNi}) + 2.71 (\text{ORBNi}) - 0.23 (\text{AMFBNi}) - 0.08 (\text{CRFBNi}) - 0.15 (\text{RESNi})$	0.92	8.89*

* $P < 0.01$; WSPEXNi – water soluble plus exchangeable; PBDNi – lead displaceable; ACSNi – acid soluble; MNOBNi – Mn-oxide bound Ni; ORBNi – organically bound Ni; AMFBNi – amorphous Fe-oxide bound Ni; CRFBNi – crystalline Fe-oxide bound Ni; RESNi – residual Ni

and CRFBNi) fractions of Ni in soil varied from 0.99–36.9, 0.02–20.5 and 0.01–18.1%, respectively. Total Ni content across the experimental soils ranged from 7.57–61.6 mg/kg, except one soil (soil No. 15) which, had 1096 mg/kg of total Ni. These values are well within the values reported in literature ranging from 9–139.4 mg/kg (Ma and Rao 1997). Exceptionally high Ni content in soil No. 15 (collected from field adjoining Atlas Cycle factory, Sonapat, Haryana) is due to continuous irrigation with Ni rich effluent emanating from the cycle factory.

Relationships of Ni fractions with soil properties. SOC and Fe_2O_3 content contributed positively towards WSPEXNi while, pH and clay contributed negatively (Table 4). It is well established that solubility of metal decreases with increase in pH and retained by clay with greater affinity. Organic carbon and Fe_2O_3 contributed positively towards PBDNi, while clay content of soil showed a negative relationship. Similar relationships were also obtained with ACSNi and this could be attributed to the fact that an acidic reagent is capable of dissolving some poorly crystalline hydroxy- and carbonate metal phases. The MNOBNi showed a negative relationship with clay and silt content. Positive contribution of pH towards this fraction may be related to the fact that stability of Mn-oxide increases with increase in pH. Both ORBNi and AMFBNi showed a positive relationship with pH, SOC, CEC and free Fe_2O_3 content in the soil while, they exhibited a negative relationship with clay and silt. Crystalline Fe_2O_3 bound Ni also showed a positive relationship with SOC and free Fe_2O_3 content. In the present study, positive correlations of SOC and Fe_2O_3 were consistent with all the fractions of Ni indicating that these two constituents are the most important pools of Ni in these soils. It may be mentioned that none of the reagents used in sequential fractionation scheme are 100% selective and soil properties themselves are related to one another in a complex manner. This might have contributed in not obtaining the logical and rational relationships always between

Ni fractions and soil properties. Further studies are required to elucidate the effect of soil properties on Ni fractions by removing soil constituents like SOC, MnO, Fe_2O_3 and determining Ni content in them.

Phytoavailability of different Ni fractions. Nickel content in plant varied from 0.16–6.31 mg/kg. Regression and partial correlation analyses relating Ni content in soybean plant with soil Ni fractions indicated that water soluble plus exchangeable, PBDNi, ACSNi and ORBNi contributed positively towards Ni uptake by soybean, while, Mn and Fe-oxide bound and RESNi had negative influence on Ni content of soybean (Tables 5 and 6). Water-soluble plus exchangeable Mn-oxide bound, ORBNi and RESNi appear to be the dominant Ni fractions controlling its phytoavailability to soybean. This may be ascribed to the fact that WSPEXNi is most labile fraction of metals in soil (Mostafa et al. 2006). Kasimov et al. (1996) reported that fulvic acid at low concentration formed soluble organo-metallic complexes and this might be responsible for the enhanced mobility of Ni in soil. Positive contribution of PBDNi and ACSNi towards plant uptake can be attributed to the fact that an acidic reagent is capable of dissolving some poorly crystalline hydroxyl- and carbonate metal phases. The Pb-nitrate extraction

Table 6. Partial correlation coefficients of determination (r^2) relating nickel content in soybean (mg/kg) with soil nickel fractions

Soil nickel fraction	r^2
Water soluble plus exchangeable (WSPEXNi)	0.60
Lead displaceable (PBDNi)	0.04
Acid soluble (ACSNi)	0.06
Mn-oxide bound Ni (MNOBNi)	0.58
Organically bound Ni (ORBNi)	0.57
Amorphous Fe-oxide bound Ni (AMFBNi)	0.12
Crystalline Fe-oxide bound Ni (CRFBNi)	0.02
Residual Ni (RESNi)	0.65

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probably displaces only those metal ions bound covalently to oxidic or organic functional groups (Miller et al. 1986). The negative contribution of Mn-Fe-oxide bound and residual Ni fractions towards plant uptake indicates that Ni is held by these soil constituents tightly and is not accessible to the plant roots for absorption. Residual Ni is an index of low mobility and unavailability of Ni in soil. There is no clear-cut relationship between proportion of Ni in residual fraction and previous irrigation history of soils used in this study. Nevertheless, it appears that proportions of Ni in residual fraction are relatively lower in sewage irrigated and heavy textured soils as compared to tube well irrigated and light textured soils.

Water soluble plus exchangeable and ORBNi contributed positively towards phytoavailability of Ni. Oxide bound and RESNi could also control the phytoavailability of Ni in soil by virtue of their negative effect. Proportion of ORBNi in total soil Ni is relatively lower as compared to what has been reported in literature for the temperate soils.

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