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Effect of chelating agents on status of Nickel in soil after harvest of Indian mustard (B*rassica juncea)*

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ABSTRACT

A pot experiment was conducted with mustard in screen house at Chaudhary Charan Singh Haryana Agricultural University Hisar to evaluate the effect of chelating agents viz. CDTA, NTA, DTPA, Citric Acid and FYM on status of Ni in the sewage sludge unamended and amended soils. Application of chelating agents caused an increase in the exchangeable fraction of Ni, highlighting their utility in increasing its available pool in the soils with consequent decrease in carbonate, MnO, organic and FeO bound fractions. Maximum value of exchangeable fraction was observed in NTA treated soil and the least in control (Ni_{90}).The Exch- Ni increased from 12.76 mg kg^{-1} in control (Ni_{90}) to 17.87 mg kg^{-1} soil in NTA treated soil.It was also observed that added Ni remained in soil in available form because of the dominance of exchangeable fraction which always remains in equilibrium with the Niion in the soil solution.The mean content of exch- Ni fraction decreased significantly with increasing time in both sewage sludge unamended and amended soils. However, slightly higher content of exch- Ni was observed in sewage sludge unamended than amended soil. Highest amount of exchangeable fraction at 50 days was observed in NTA treated soil whereas CDTA was more effective at 80 days. Application of FYM also increased exchangeable and organic fraction of Ni but decreased carbonate, MnO and FeO bound fractions. Sewage sludge addition resulted in decreased in exchangeable and residual fractions and increased in organic matter bound fractions.

Keywords: Chelating agents, nickel, exchangeable fraction, FYM, sewage sludge

INTRODUCTION

Heavy metals are ubiquitous environmental contaminants in an industrialized society. A gradual increase in accumulation of heavy metals in soils of many agricultural fields owing to discharge of industrial and municipal residual wastes has caused a serious problem to the crop production and hazards to human and animal health. Nickel is present in the soil in different forms. The toxic effect of a metal is determined more by its form than by its absolute amount. The free ion Ni^{2+} is more likely to be adsorbed on the surface of soil colloids than other species, such as neutral or anionic species. The principal species of Ni in the soil solution is Ni^{2+} but the metal can also form the following complex ions: $NiCl^+$, $NiOH^+$, $NiHCO_3^+$, $NiCl_3^-$, $Ni(OH)_3^-$ and $Ni(OH_4)^{2-}$ together with organic complexes (Kabata Pendias and Pendias, 1992). Chemical extraction methods have been most widely used to assess the speciation or bound-forms of metals in contaminated soils. The ultimate aim in most cases is to assess the binding strength of toxic metals in order to evaluate their potential mobility and bioavailability. Addition of chelating agents such as Nitrilotriacetic acid (NTA), Diethylenetriaminepentaacetic acid (DTPA), Cyclohexanediaminotetraacetic acid (CDTA) and Citric acid have been reported to increase the exchangeable fraction of Ni in the solution phase (Rathore *et al*, 2019). Hence, higher extractability of Ni due to chelating agents may lead to their excessive accumulation in plants. Hence, the study was carried out to study the effect of chelating agents on distribution of Ni in soil.

MATERIALS AND METHODS

The bulk surface sample (0-15 cm) collected from the research farm, CCS Haryana Agricultural University, Hisar, Haryana was processed and used for laboratory and screen house studies. The physico-chemical properties of soil, sewage sludge and FYM are given in Table 1. The processed soil was divided into two portions. The first part treated with Ni using Ni Cl_2 at the rate of 90 mg kg^{-1} soil and second portion treated with Ni at the rate of 40 mg kg^{-1} and sewage sludge at the rate of 3 per cent.

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After incubation soils were mixed thoroughly and filled in 72 earthen pots at the rate of 5 kg soil/pot. The experiment was carried out in a completely randomized design, arranged in a (2X6X6=72) factorial, two soil (sewage sludge unamended and amended), six amendment (control, four chelating agents and FYM) and six replication. Ten healthy seeds of the Indian mustard were sown in each pot. After emergence of seedlings, only four plants per pot were allowed to grow. Four chelating agents namely, CDTA, Citric acid, DTPA and NTA were applied at rate of 3 mmol kg^{-1} soil (1mmol daily for 3 days in 3 split doses) 40 DAS. The FYM at rate of 3% was uniformly mixed in portion of Ni spiked and Ni spiked sewage sludge treated soil 10 weeks before sowing. Ten days after application of chelating agents half of the plants of mustard crop (i.e. 3 replication out of 6) were harvested at 50 DAS and remaining 3 replications were harvested at 80 DAS. After harvesting of the mustard crop, soil in each pot was wetted with about one litre of distilled water, covered with plastic sheet and allowed to equilibrate for five days. Then at workable moisture content, representative soil samples (covering full depth of soil in pot) were taken with the help of a stainless steel tube augar, air dried, ground, sieved and kept in polyethylene bags for fractionation studies. Six fractions, viz.exchangeable Ni (Exch-Ni), carbonate-bound Ni (Carb-Ni), Mn oxide-bound Ni (MnO-Ni), organically complexed Ni (Org-Ni), Fe oxide-bound Ni (FeO-Ni), residual Ni (Res-Ni) were sequentially determined by adopting the method of Berti *et al* (1998). Nickel in all soil extracts was measured using atomic absorption spectrophotometer.

Table 1: Physico-chemical characteristics of the experimental soil, Sewage sludge and FYM

Properties	Content			
		Soil	Sewage sludge	FYM
Mechanical composition				
	(a) Sand (%)	69.70	-	-
	(b) Silt (%)	16.50	-	-
	(c) Clay (%)	13.80	-	-
Textural class	Sandy loam		-	-
pH (1:2)		8.10	7.2	-
$EC_{1:2}$ (dS m^{-1})		0.50	2.1	-
Organic carbon (g kg^{-1})		3.2	122.0	278.0
CEC [Cmol(P^+) kg^{-1}]		11.80	-	-
$CaCO_3$ (g kg^{-1})		4.0	2.5	-
Total Nutrients				
(a) Nitrogen (%)		0.05	1.29	1.18
(b) Phosphorus (%)		0.15	0.41	0.70
(c) Potassium (%)		1.00	0.73	2.50
C/N Ratio		-	9.46	23.55
Total metals (mg kg^{-1})				
Cd		3.2	7.2	0.6
Ni		11.3	64.2	10.9
Zn		29.7	215	45.4
Mn		145.9	360	114.9
Fe		4321.3	946	1072.4
Cu		22.1	263	15.2

RESULTS AND DISCUSSION

Exchangeable-Ni: (Exch-Ni)

It is evident from the data (Table 2) that application of chelating agents caused an increase in exchangeable–Ni fraction. Though, the overall effect of chelating agents was non significant on the amount of exch-Ni fraction but maximum value was observed in NTA treated soil. Kaur *et al.*, (2018) & Sindhu *et al.*, (2018) also reported that addition of chelating agents enhanced exchangeable pool of Ni fraction in soil. Exch-Ni fraction increased from 12.76 mg kg^{-1} in control (Ni_{90}) to 17.87 mg kg^{-1} soil in NTA treated soil. The mean content ofexch-Ni fraction decreased significantly with increasing time in both the soils. However, content of exch-Ni fraction was slightly higher in sewage sludge unamended than amended soil. It is also evident from the table 2 that added Ni remained in plant available form because of the dominance of exchangeable form which always remains in equilibrium with the Ni^{++} in the soil solution. Addition of chelating agents further increased the exchangeable fraction highlighting their significance in increasing its availability to plants. Thus, addition of chelating agents resulted in an increase in the exchangeable form of Ni and decrease in the carbonate bound, MnO bound, organic bound and FeO bound fractions. Similar results were observed by Cay *et al.* (2015); Yin *et al.* (2015); Alexander and Akoto, (2018) and Nawaj *et al.* (2019). Highest amount of exchangeable form at first stage was observed due to the NTA whereas at second stage, it was due to CDTA. Application of FYM also resulted in increased in amount of exchangeable fraction of Ni.

Table 2: Effect of chelating agents on status of Ni (mg kg^{-1}) in post harvest soil

Treatment	50 DAS		80 DAS		Mean
	Without SS	With SS	Without SS	With SS	
			Exchangeable-Ni		
Ni_{90}	13.84	12.42	13.02	11.76	12.76
Ni_{90}+CDTA	18.36	17.38	17.21	16.45	17.35
Ni_{90}+CA	16.42	15.34	15.14	14.63	15.38
Ni_{90}+DTPA	17.43	16.82	16.46	15.64	16.59
Ni_{90}+NTA	21.00	19.62	15.89	14.95	17.87
Ni_{90}+FYM	14.63	13.84	13.84	12.83	13.79
Mean	16.95	15.90	15.26	14.38	
CD(0.05)	Soil = 0.13	Time = 0.13	Chelating agents = NS		
	SXT = 0.19	SXCA = NS	TXCA = 0.32	SXTXCA = NS	
			Carbonate bound-Ni		
Ni_{90}	18.33	15.41	16.68	15.20	16.41
Ni_{90}+CDTA	15.64	14.21	12.32	12.23	13.60
Ni_{90}+CA	15.83	14.86	15.00	13.94	14.91
Ni_{90}+DTPA	16.12	15.46	12.84	13.64	14.52
Ni_{90}+NTA	14.32	14.47	14.98	13.78	14.39
Ni_{90}+FYM	17.12	15.21	15.93	15.96	16.06
Mean	16.23	14.94	14.63	14.13	
CD(0.05)	Soil = 0.11	Time = 0.11	Chelating agents = 0.19		
	SXT = 0.15	SXCA=0.27	TXCA = 0.27	SXTXCA= 0.38	
			MnO bound-Ni		
Ni_{90}	13.92	13.53	12.36	12.23	13.01
Ni_{90}+CDTA	13.68	12.83	12.10	11.84	12.61
Ni_{90}+CA	14.63	14.13	14.19	12.89	13.96
Ni_{90}+DTPA	14.04	14.789	12.86	13.20	13.72
Ni_{90}+NTA	13.31	12.21	13.43	11.53	12.62
Ni_{90}+FYM	14.10	13.64	13.00	14.53	13.82
Mean	13.95	13.52	12.99	12.70	
CD(0.05)	Soil = 0.10	Time = 0.10	Chelating agents = NS		
	SXT = 0.14	SXCA=0.24	TXCA = 0.24	SXTXCA = 0.34	

SS = sewage sludge

Carbonate bound-Ni: (Carb-Ni)

The mean Carb-Ni fraction also decreased significantly with the addition of chelating agents. The Carb-Ni fraction was maximum in control (Ni_{90}) but it decreased significantly in chelating agents treated soil. The mean Carb-Ni fraction was 16.23 and 14.63 mg kg^{-1} in sewage sludge unamended and 14.94 and 14.13 mg kg^{-1} soil in amended soil at 50 and 80 days after sowing ofIndian mustard, respectively. Among the chelating agents, FYM was found more effective in enhancing the Carb-Ni pool in the soil. The Carb-Ni fraction decreased significantly with increasing time due to application of chelating agents.

Manganese oxide bound Ni: (MnO-Ni)

The mean MnO-Ni fraction varied from 13.01 to 13.96 mg kg^{-1} soil. Significantly low content of MnO-Ni fraction was observed at 80 days than 50 days after sowing ofIndian mustard. It decreased from 13.95 to 12.99 mg kg^{-1} in sewage sludge unamended soil and 13.52 to 12.70 mg kg^{-1} soil in sewage sludge amended soil after harvest of Indian mustard at 50 DAS and 80 DASrespectively. The maximum value of MnO-Ni fraction was observed in citric acid treated soil. It decreased slightly with increase in time and levels of sewage sludge.

Organic bound-Ni: (Org-Ni)

It is clear from the data (Table 3) that the dominant Ni fraction was organic followed by Fe, exch-Ni, carbonate, manganese oxide and reserve Ni. Sewage sludge application caused an increase in Org-Ni fraction whereas it decreased in chelating agents treated soil. Sewage sludge addition increased the mean Org-Ni fraction from 28.43 to 31.26 mg kg^{-1} at 50 and 25.75 to 28.67 mg kg^{-1} at 80 days after sowing ofIndian mustard, respectively. The mean content of Org-Ni fraction varied from 26.77 mg kg^{-1} in NTA to 30.47 mg kg^{-1} soil in chelating agents untreated soil (Ni_{90}). Nitrilotriacetic acid application caused a significant reduction in Org-Ni fraction in relatively higher amounts than other chelating agents. In general, all the chelating agents decreased significantly Org-Ni fraction as compared chelates untreated soil (Ni_{90}). The Org-Ni fraction was comparatively higher than either carbonate or manganese oxide bound fractions at both the growth stages in sewage sludge unamended and amended soils.The increase in organic matter bound fraction in the sewage sludge amended and the

Table 3: Effect of chelating agents on status of Ni (mg kg^{-1}) in post harvest soil

Treatment	50 DAS		80 DAS		Mean
	Without SS	With SS	Without SS	With SS	
			Organic bound-Ni		
Ni_{90}	28.60	33.31	28.14	31.84	30.47
Ni_{90}+CDTA	28.14	30.43	24.56	28.34	27.87
Ni_{90}+CA	28.58	31.16	24.64	28.73	28.28
Ni_{90}+DTPA	28.21	29.81	25.63	26.61	27.57
Ni_{90}+NTA	26.63	30.42	23.89	26.12	26.77
Ni_{90}+FYM	30.40	32.40	27.63	30.40	30.21
Mean	28.43	31.26	25.75	28.67	
CD (0.05)	Soil = 0.20	Time = 0.20	Chelating agents = NS		
	SXT = 0.28	SXCA = 0.48	TXCA= 0.48	SXTXCA = 0.68	
			FeO bound-Ni		
Ni_{90}	17.64	19.82	17.54	19.09	18.52
Ni_{90}+CDTA	17.12	18.84	17.02	16.60	17.40
Ni_{90}+CA	17.64	18.61	16.70	17.83	17.70
Ni_{90}+DTPA	17.32	18.14	17.42	18.94	17.96
Ni_{90}+NTA	17.21	18.56	16.31	18.40	17.62
Ni_{90}+FYM	17.40	19.94	16.50	14.54	17.10
Mean	17.39	18.99	16.92	17.57	
CD(0.05)	Soil = 0.07	Time = 0.07	Chelating agents = 0.12		
	SXT = 0.09	SXCA = 0.16	TXCA= 0.16	SXTXCA= 0.23	
			Residual-Ni		
Ni_{90}	6.42	6.92	5.62	4.24	5.80
Ni_{90}+CDTA	5.30	5.40	4.77	3.51	4.75
Ni_{90}+CA	5.32	5.63	4.82	3.94	4.93
Ni_{90}+DTPA	5.28	4.91	4.90	3.40	4.62
Ni_{90}+NTA	4.10	3.28	4.21	3.52	3.78
Ni_{90}+FYM	5.32	5.10	4.81	3.43	4.67
Mean	5.29	5.21	4.86	3.67	
CD(0.05)	Soil = 0.08	Time = 0.08	Chelating agents = 0.14		
	SXT = 0.12	SXCA = 0.20	TXCA =0.20	SXTXCA= .29	

FYM treated soils may be ascribed to the formation of organo-Ni complexes. Indoria (2004) reported that in sewage sludge treated soil most part of the total Ni in soil was in carbonate and organic matter fractions. Similar results reported be Dede *et al.* (2012).

Ferrous bound Ni (Fe-Ni)

The mean value of Fe-Ni fraction decreased significantly with time whereas it increased with the addition of sewage sludge. It decreased from 17.39 to 16.92 mg kg^{-1} in sewage sludge unamended and 18.99 to 17.57 mg kg^{-1} in amended soil at 50 and 80 days after sowing ofIndian mustard, respectively. Application of FYM increased Fe-Ni fraction from 17.40 to 19.94 mg kg^{-1} soil with an increasing sewage sludge level from 0 to 3 per cent at 50 days, respectively. The content of Fe-Ni fraction was relatively higher compared to its exch, carbonate and manganese oxide bound forms.

Reserve-cadmium (Res-Ni)

It is clear from the data (Table 3) that Res-Ni fraction decreased with increasing time as well as with the addition of chelating agents. The Res-Ni fraction in sewage sludge treated soil decreased from 5.21 to 3.67 mg kg^{-1} with an increase in the time from 50 to 80 days. Similar trend was also observed in sewage sludge unamended soil. The decrease was more pronounced in sewage sludge treated soil. Here also NTA was more effective in decreasing Res-Ni fraction in soils.

It may be concluded from the results that application of chelating agents resulted in an increase in the exchangeable forms of Ni, hence phytoextractibility by plants and consequent decrease in the carbonate bound, MnO bound, organic bound and FeO bound fractions. Dominant Ni fraction was organic followed by Fe, Exch-Ni, carbonate, manganese oxide and reserve Ni. Highest amount of exchangeable fraction at 50 days was observed in NTA treated soil whereas CDTA was more effective at 80 days. Application of FYM also increased exchangeable and organic fraction of Ni but decreased carbonate, MnO and FeO bound fractions. Application of sewage sludge in soil resulted in decrease in exchangeable, Carbonate bound, MnO bound and residual fractions and increase in organic and Fe bound fractions.

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