International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 3690-3696
© 2021 IJCS
Received: 08-10-2020
Accepted: 17-11-2020

Quantitative assessment of nickel phytoremediation potential of some *Brassica* species in nickel enriched soil as influenced by organic amendments

AK Indoria, RS Siyag, K Sammi Reddy, K Srinivas and KL Sharma

AK Indoria
1. Department of Soil Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India
2. ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India

RS Siyag
Department of Soil Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

K Sammi Reddy
ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India

K Srinivas
ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India

KL Sharma
ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India

Corresponding Author:
AK Indoria
1. Department of Soil Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India
2. ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India

DOI: https://doi.org/10.22271/chemi.2021.v9.i1az.11820

Abstract

Screen-house experiments were conducted to evaluate the nickel phytoremediation potential of *Brassica juncea, Brassica napus and Eruca sativa* in nickel enriched (i.e. 0, 150, 300, 450 and 600 mg/kg soil) soils using 3% farmyard manure-amended (w/w), 3% sewage sludge-amended (w/w) and un-amended soil. The results of the study revealed that the mean stem yields of *Brassica* species was higher by about 12% and 25% in the sewage sludge-amended and farmyard manure-amended soils, respectively as compared to un-amended soils. The corresponding differences in the mean leaf and seed yields were about 19 and 35%, and 13 and 28%, respectively in the sewage sludge-amended and farmyard manure-amended soils over un-amended soils. In the sewage sludge- amended soil, the mean Ni content was higher by about 10% in the stem and 4% each in both leaf and seed over un-amended soil. Addition of farmyard manure (farmyard manure-amended soil) resulted in decrease in mean Ni concentration by about 23.4, 33.1 and 32.3% in stem, leaf and seed, respectively over un-amended soil. Among the species, the order of mean nickel concentration was: *Brassica napus*>*Brassica juncea*>*Eruca sativa*. Whereas, the order of nickel concentration in different plant parts was: seed > leaf > stem. The mean Ni removal by stem, leaf and seed were 25.1, 27.9, and 26.8% less in the farmyard manure-amended soil in comparison to the sewage sludge-amended soil, respectively. The order of Ni removal rate was: stem>leaf > seed. Overall, the total removal of Ni by *Brassica juncea* (stem, leaf and seed) was higher by 1.2 times of *Brassica napus* and 2.4 times of *Eruca sativa*.

Keywords: Farmyard manure, nickel, *Brassica* species, phytoremediation, sewage sludge

Introduction

The excessive concentration of heavy metals not only decreases the yield potential of the crop but also increases the health hazards in animal and humans, if they are consumed beyond the permissible level [1]. It is reported that excess Ni concentration in plants has a negative effect on photosynthesis, respiration, enzymatic activities and membrane functions in different plant species [2]. Although, nickel is considered as an essential micro-nutrient for some plant species at very low concentration [3]. In agricultural soils, the major anthropogenic source of nickel contamination is Ni plating industries, combustion of fossil fuel and residual oil, Ni mining, lead production, Cu-Ni batteries production, steel and iron industries, municipal sewage sludge, phosphatic fertilizer, cement production, wood combustion, motor transport and electroplating. Power plants and trash incinerators also releases Ni into the air are deposited on the soil surface by precipitation reactions [4].

The removal of nickel by chemical and physical process (*viz*. chemical dissolution process, chemical precipitation, membrane filtration, adsorption by suitable adsorbent, photo-catalysis, electro-dialysis etc.) have significant disadvantages such as incomplete removal, high-energy requirements and high operational cost, and may have negative impact on environmental aspects. Contrary to this, phytoremediation technology (by using the different suitable plant species) is a cost effective, aesthetic, farmer friendly and lesser side effect on environment. Furthermore, addition of organic amendments such as sewage sludge and farmyard manure (as a source of organic matter) besides improving the crop growth [5] also modifies the bio-availability of Ni by affecting its complexation/immobilization/retention processes [6].

Addition of these amendments also improves various soil properties (ECs, pH, nutrient status, bulk density, soil water characteristic, porosity etc.) and mitigates the negative impact of nickel on plant. These amendments in turn affect the removal capacity of nickel from soil [7]. Some published reports suggested that *Brassica* species are capable of accumulating and tolerating high levels of some heavy metals in soils [8]. Moreover, various *Brassica* species are grown in different part of the country in considerable area and can be used for phytoremediation purposes. The present study was conducted with the objectives i) to study the phytoremediation potential of *Brassica* species for nickel removal, and (ii) to determine that which of the plant parts (leaf, stem and seed) of species are potential remover of Ni and could be effective for recycling of the Ni.

Materials and Methods

A pot experiment was conducted in screen-house at Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana), India. The bulk surface soil (0-15 cm) sample of a sandy loam soil was collected from Research Farm of University. The sewage sludge and farmyard manure for study were collected from Municipal Sewage Plant, Okhla, New Delhi and Animal Research Farm of Chaudhary Charan Singh Haryana Agriculture University, Hisar, respectively. The experimental soil was sandy loam (63% sand, 19% silt, 18% clay) with pH 8.3 (1:2 soil to water solution), EC 0.4 dS m^{-1} (1:2 soil to water solution), organic carbon 0.34%, CEC 12.9 cmol P^+ kg^{-1}, $CaCO_3$0.3%, total Ni 11.4 mg kg^{-1} and DTPA Ni 0.95 mg kg^{-1}. The sewage sludge used in the experiment was having pH of6.9 (1: 2 soil to water solution), EC 1.8 dS m^{-1} (1:2 soil to water solution), organic carbon 11.8%, CEC 31.9 C mol P^+ kg^{-1}, $CaCO_3$0.2%, total Ni 54.0 mg kg^{-1} and DTPA Ni 19.6 mg kg^{-1}. The farmyard manure contained 28.4% organic carbon and 4.0 mg kg^{-1}total Ni. These samples (soil, sewage sludge and farmyard manure) were air dried, ground to pass through a 2 mm stainless steel sieve, separately. The processed soil samples were divided into three portions. In one portion of soil, 3% sewage sludge (w/w basis) was thoroughly mixed. In second portion, 3% of farmyard manure (w/w basis) was thoroughly mixed. The third portion of soil was kept as such without mixing any amendment. To attain the homogeneity in the mixture of soil-sewage sludge, soil-farmyard manure and un-amended soils (control), 4 wetting-drying cycles were given. Five kg of the processed soil + amendment mixtures were placed in forty-five polyethylene lined earthen pots having 25 cm internal diameter as per the treatments. To maintain desired level of Ni in the potted soils, appropriate volumes of nickel chloride ($NiCl_2$) solutions were added. A basal dose of N, P, K, Mn, Fe, Zn, Cu and S @ 50, 50, 60, 10, 10, 5, 5 and 20 mg kg^{-1} soil was applied in solution form to each pot. After addition of Ni and nutrient solutions, the contents of each pot were again given three wetting-drying and mixing cycles to attain homogeneity.

In each pot, four plants were retained to grow up to maturity. The pots were irrigated with de-ionized water as and when required. Second dose of nitrogen was applied @ 25 mg N kg^{-1} soil in solution form at pod initiation stage. The leaves shed by plants grown in different pots before maturity were collected treatment-wise. The harvested plant material was thoroughly washed with 0.1N HCl followed by with distilled water and finally with double distilled water. The washed plant material was placed in paper bags, air dried and then oven dried at 65±2 ^{0}C to constant weight. After separating the seeds, the dry weights of all the components *viz*. leaf, stem and seed were recorded. The samples were ground in a stainless-steel grinder, mixed and stored in polythene bags for chemical analysis. Total nutrients and other elements in the sewage sludge and farmyard manure were determined, using procedures as outlined by Lim and Jackson [9]and Richard [10]. DTPA extractable Ni was determined, using the procedure given by Lindsay and Norvell [11]. Other soil physico-chemical properties were analyzed using methods described by Jackson [12]. The pot experiment was conducted in completely randomized design (CRD) with three replications and data are subjected to statistical analysis by using the SPSS statistical package.

Results

Visual nickel toxicity symptoms

In the un-amended as well as sewage sludge amended and farmyard manure-amended soils, there was normal seed germination of all the three species even at the highest level of added Ni (i.e., 600 mg Ni kg^{-1} soil), but at this level, plants survived only upto 10-15 days after germination and later died.Similarly, at 450 mg Ni kg^{-1} soil, both *Brassica napus* and *Eruca sativa* survived upto 20-55 days after germination with very little growth and later died irrespective of amendments treatments. It was interesting to note that the *Brassica juncea* species, however, survived at this level of added Ni (Ni_{450}) and could grow up to maturity stage. At 300 mg Ni kg^{-1} soil, clear chlorosis symptoms were observed on the leaves of all the species after 42-48 days of germination. The severity of chlorosis decreased in the sewage sludge-amended and farmyard manure-amended soils. The overall growth of all the species was stunted with increasing levels of Ni in soils. At or above 300 mg Ni kg^{-1} soil, the plants showed poor branching, delayed flowering and poor siliqua and seed development in all the three species.

Dry biomass yield

The mean stem, leaf and seed yields (i.e., mean of species and Ni levels) were significantly higher in the sewage sludge-amended and farmyard manure-amended soils as compared to the un-amended soil (Table 1). The mean stem yields were higher by about 12% and 25% in the sewage sludge-amended and farmyard manure-amended soils, respectively over un-amended soil. The corresponding differences in the mean leaf and seed yields were about 19 and 35%, and 13 and 28%, respectively in the sewage sludge-amended and farmyard manure-amended soils over un-amended soil. The maximum decrease in the mean stem dry matter yield due to the application of 300 mg Ni kg^{-1} soil ranged between 59 to 61 per cent in the sewage sludge-amended, farmyard manure-amended and un-amended soils (Table 1). The data in Table 2 showed that Ni application significantly and progressively decreased the mean stem dry matter yield from 27.8 and 60.5 per cent in Ni_{150} and Ni_{300} treatments as compared to Ni_{0}. The corresponding decreases in leaf and seed yield were 19.8 and 53.9 per cent and 24.7 and 58.2 per cent in Ni_{150} and Ni_{300} treatments as compared to the $Ni_{0,}$ respectively. Compared to Ni_0, the maximum decreases in the mean stem dry matter yield with the application of Ni_{300} were from 51, 65.9 and 66.9 per cent in *Brassica juncea*, *Brassica napus* and *Eruca sativa*, respectively. The corresponding values in leaf and seed yield were 44.9, 60.1 and 59.7, and 51.7, 63.3 and 61.1 in *Brassica juncea*, *Brassica napus* and *Eruca sativa*, respectively. Species showed variability in their mean stem, leaf and seed dry matter yield, irrespective of soil amendments and Ni levels. The mean stem (16.94 g pot^{-1}), leaf (9.59 g pot^{-1}) and seed dry matter yield (7.76 g pot^{-1}) was the highest in *Brassica juncea*, which was followed by *Brassica napus* stem (14.37 g pot^{-1}), leaf (7.70 g pot^{-1}) and seed dry matter yield (6.73 g pot^{-1}) and *Eruca sativa* stem (9.90 g pot^{-1}), leaf (5.23 $gpot^{-1}$) and seed dry matter yield(4.37 g pot^{-1}). The total dry biomass yield was highest in *Brassica juncea*, which was followed by *Brassica napus* and *Eruca sativa*. The total dry biomass yield of *Brassica juncea* was higher by 1.2 times of *Brassica napus*and 1.8 times of that *Eruca sativa*. Overall, about 50% dry biomass yield reduction was observed in *Brassica juncea* at Ni_{300} level as compared to the Ni_0, but at the same level of nickel, about 64% dry biomass yield decrease was noticed in both *Brassica napus* and *Eruca sativa*.

Table 1: Dry biomass yield (g pot^{-1}) of different parts of *Brassica* species as affected by Ni levels, sewage sludge and farmyard manure application

Ni (mgkg^{-1} soil)	**Un-amended soil (control soil)**			**Mean**	**3% sewage Sluge-amended soil**			**Mean**	**3% farmyard manure-amended soil**			**Mean**
	B. juncea	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***	
Stem												
0	19.9	19.0	13.4	17.4	22.5	21.0	14.8	19.4	25.0	23.6	15.9	21.5
150	15.0	12.5	8.8	12.1	17.4	14.9	9.8	14.0	19.9	16.8	11.2	16.0
300	10.3	6.6	4.3	7.1	10.8	7.2	5.0	5.0	11.8	7.9	5.3	8.3
450	2.8*	-	-	-	3.6*	-	-	-	4.0*	-	-	-
Mean	15.1	12.7	8.8	12.2	16.9	14.3	9.9	13.7	18.9	16.1	10.8	15.3
$LSD_{(p=0.05)}$Soil amendments (SA)=0.48; Species (Spp)=0.48; Nickel (Ni)=0.48; SAxSpp=0.87; SAxNi=0.87; SppxNi=0.87; SAxSppxNi=Not significant												
Leaf												
0	10.6	9.0	6.6	8.7	12.3	11.0	7.2	10.1	13.3	11.7	8.0	11.0
150	8.5	6.9	4.3	6.6	10.3	8.2	5.6	8.1	11.4	9.9	6.6	9.3
300	5.9	3.6	2.0	3.8	6.2	4.1	3.1	4.5	7.8	5.0	3.7	5.5
450	1.5*	-	-	-	2.1*	-	-	-	3.2*	-	-	-
Mean	8.3	6.5	4.3	6.4	9.6	7.8	5.3	7.6	10.8	8.9	6.1	8.6
$LSD_{(p=0.05)}$ Soil amendments (SA)=0.30; Species (Spp)=0.30; Nickel (Ni)=0.30; SAxSpp=0.55; SAxNi=0.55; SppxNi=0.55; SAxSppxNi=Not significant												
Seed												
0	9.7	8.8	5.3	7.9	10.1	9.7	6.3	8.7	11.1	10.4	6.8	9.4
150	7.1	6.2	3.5	5.6	8.0	7.0	4.6	6.5	9.0	7.8	5.7	7.5
300	4.4	3.0	1.8	3.1	4.8	3.5	2.3	3.5	5.7	4.1	3.1	4.3
450	0.6*	-	-	-	1.2*	-	-	-	1.9*	-	-	-
Mean	7.04	6.0	3.5	5.5	7.6	6.7	4.4	6.3	8.6	7.4	5.2	7.1
$LSD_{(p=0.05)}$Soil amendments (SA) =0.22; Species (Spp)=0.22;Nickel (Ni)=0.22; SAxSpp=0.40; SAxNi=0.40; SppxNi=0.40; SAxSppxNi=Not significant												

* = Values are excluded for statistical analysis. - = Indicates 'Nil' dry biomass yield

Table 2: Stem, leaf and seed dry biomass yield (g pot^{-1}) of different *Brassica* species as affected by Ni levels (Mean of the amendment treatments)

Species	**Ni levels (mg kg^{-1}soil)**				**Mean**
	0	**150**	**300**	**450**	
Stem					
B. juncea	22.43	17.41	10.98	3.46*	16.94
B. napus	21.17	14.73	7.20	-	14.37
E. sativa	14.70	9.94	4.87	-	9.83
Mean	19.43	14.03	7.68	-	
Leaf					
B. juncea	12.05	10.08	6.64	2.25*	9.59
B. napus	10.55	8.34	4.21	-	7.70
E. sativa	7.25	5.51	2.92	-	5.23
Mean	9.95	7.98	4.59	-	
Seed					
B. juncea	10.29	8.01	4.97	1.22*	7.76
B. napus	9.63	7.02	3.53	-	6.73
E. sativa	6.14	4.59	2.39	-	4.37
Mean	8.69	6.54	3.63	-	
$LSD_{(p=0.05)}$ for stem: Species (Spp) - 0.48, Nickel (Ni) - 0.56, Spp x Ni - 0.87; for leaf: Spp - 0.30, Ni - 0.35, Spp x Ni - 0.55; for seed: Spp - 0.22, Ni - 0.26, Spp x Ni - 0.40					

* Values are excluded for statistical analysis. - = Indicates 'Nil' dry biomass yield

Nickel concentration

On an average, mean Ni concentration (i. e., mean of species and Ni levels) in different plant parts was significantly higher in the sewage sludge-amended soil over un-amended soil (Table 3). In the sewage sludge-amended soil, it was higher by about 10% in stem and 4% each in leaf and seed over un-amended soil. Addition of farmyard manure (farmyard manure-amended soil) resulted in a decreased Ni concentration by about 23, 33 and 32% in stem, leaf and seed, respectively over un-amended soil. The effect of added Ni on the mean stem, leaf and seed Ni concentration varied considerably in the un-amended, sewage sludge-amended and farmyard manure-amended soils. In un-amended soil, stem Ni concentration was significantly increased by 16.8 folds in Ni_{300} as compared to

Ni$_0$. In sewage sludge-amended and farmyard manure-amended soils, the corresponding increases were from 17.6 and 14.9 folds at Ni$_{300}$ as compared to Ni$_0$, respectively. In the un-amended soil, leaf Ni concentration was significantly increased by 18 folds at Ni$_{300}$ as compared to Ni$_0$. In the sewage sludge-amended and farmyard manure-amended soils, the corresponding increases were 17.9 and 13.4 folds at Ni$_{300}$ as compared to Ni$_0$, respectively. Similarly, seed Ni concentration significantly increased by 19.7 folds at Ni$_{300}$ as compared to the Ni$_0$ in un-amended soil. The corresponding increase in seed Ni concentration in sewage sludge-amended and farmyard manure-amended soils were 18.8 and 14.2 folds at Ni$_{300}$ as compared to the Ni$_0$, respectively. The maximum mean Ni concentration in stem, leaf and seed was observed in the sewage sludge-amended soil at Ni$_{300}$ (Table 3).

Table 3: Ni Concentration (μg g^{-1}) in different parts of *Brassica* species as affected by Ni levels, sewage sludge and farmyard manure application

Ni (mgkg^{-1}soil)	**Un-amended soil (control soil)**			**Mean**	**3% sewage sludge–amended soil**			**Mean**	**3% farmyard manure-amended soil**			**Mean**
	B. juncea	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***	
Stem												
0	2.8	3.0	2.5	2.7	3.0	3.2	2.7	3.0	2.5	2.7	2.2	2.5
150	38.6	42.1	32.6	37.8	40.6	43.5	35.3	39.8	28.3	30.2	24.1	27.5
300	47.2	52.6	38.2	46.0	52.1	60.2	44.6	52.3	39.6	42.6	27.9	36.7
450	72.7*	-	-	-	80.1*	-	-	-	59.7*	-	-	-
Mean	29.5	32.6	24.4	28.8	31.9	35.6	27.5	31.7	23.5	25.2	18.1	22.2
LSD$_{(p=0.05)}$ Soil amendments (SA)=1.43; Species (Spp)=1.43; Nickel (Ni)=1.67; SAxSpp=NS; SAxNi=2.60; SppxNi=2.60; SAxSppxNi=Not significant												
Leaf												
0	3.5	3.5	2.8	3.3	3.5	3.7	3.0	3.4	3.0	3.3	2.4	2.9
150	46.0	50.6	38.5	45.0	47.2	51.8	41.6	46.9	33.0	34.0	22.9	30.0
300	58.7	64.8	52.6	58.7	61.1	68.0	53.3	60.8	43.0	45.4	27.5	38.6
450	81.4*	-	-	-	85.3*	-	-	-	52.2*	-	-	-
Mean	36.0	39.6	31.3	35.7	37.3	41.2	32.6	37.0	26.3	27.6	17.6	23.8
LSD$_{(p=0.05)}$ Soil amendments (SA)=1.48; Species (Spp)=1.48; Nickel (Ni)=1.48; SAxSpp=NS; SAxNi=2.70; SppxNi=2.70; SAxSppxNi=Not significant												
Seed												
0	3.5	3.7	2.9	3.4	3.9	4.0	3.1	3.7	3.4	3.6	2.5	3.1
150	50.4	54.2	43.6	49.4	51.5	55.3	45.1	50.6	36.1	38.6	25.5	33.4
300	67.6	73.9	57.5	66.3	70.3	78.4	58.3	69.0	49.6	52.6	32.0	44.7
450	108*	-	-	-	111*	-	-	-	82.1*	-	-	-
Mean	40.5	43.9	34.7	39.7	41.9	45.9	35.5	41.1	29.7	31.6	20.0	27.1
LSD$_{(p=0.05)}$ Soil amendments (SA)= 1.29; Species (Spp)=1.29; Nickel (Ni)=1.29; SAxSpp=Not significant; SAxNi=2.35; SppxNi=2.35; SAxSppxNi=Not significant												

* = Values are excluded for statistical analysis. - = Indicates 'Nil' Ni concentration

Species showed the variability in their mean Ni concentration in stem, leaf and seed with added level of the nickel (Table 4). The mean stem Ni concentration significantly increased from 2.76 μg g^{-1} in Ni$_0$ to 46.29 μg g^{-1} in Ni$_{300}$, 2.96 μg g^{-1} in Ni$_0$ to 51.79 μg g^{-1} in Ni$_{300}$, and from 2.44 μg g^{-1} in Ni$_0$ to 36.91 μg g^{-1} in Ni$_{300}$ in *Brassica juncea*, *Brassica napus* and *Eruca sativa*, respectively. The magnitude of increase in mean leaf Ni concentration were from 3.32 μg g^{-1} in Ni$_0$ to 54.23 μg g^{-1} in Ni$_{300}$, 3.49 μg g^{-1}in Ni$_0$ to 59.38 μg g^{-1}in Ni$_{300}$, and from 2.70 μg g^{-1}in Ni$_0$ to 44.43 μg g^{-1}in Ni$_{300}$ in *Brassica juncea*, *Brassica napus* and *Eruca sativa*, respectively. A similar type of the trend was observed in the mean seed Ni concentration of *Brassica jucnea*, *Brassica napus* and *Eruca sativa*. Results further showed that the mean Ni concentration in different plant part of *Brassica napus* was always significantly higher than *Brassica juncea* and *Eruca sativa* at each added level of Ni, irrespective of the amendments treatment. The difference between *Brassica napus* and *Brassica juncea* ranged from 8% to 11% in stem, 5 to 12% in leaf and from 7 to 10% in seed. Between *Brassica napus* and *Eruca sativa*, it ranged from 14 to 23% in stem, 12 to 34% in leaf and 14 to 32% in seed (Table 4).

Table 4: Stem, leaf and seed Ni concentration (μg g^{-1}) of different species as affected by Ni levels (mean of amendment treatments)

Species	**Ni levels (mg kg^{-1}soil)**				**Mean**
	0	**150**	**300**	**450**	
Stem					
B. juncea	2.76	35.84	46.29	70.83*	28.30
B.napus	2.96	38.63	51.79	-	31.13
E. sativa	2.44	30.66	36.91	-	23.34
Mean	2.72	35.04	45.00	-	
Leaf					
B. juncea	3.32	42.05	54.23	72.95*	33.20
B.napus	3.49	45.47	59.38	-	36.12
E. sativa	2.70	34.33	44.43	-	27.15
Mean	3.17	40.62	52.68	-	
Seed					
B. juncea	3.57	45.99	62.47	100.79*	37.34
B.napus	3.77	49.36	68.31	-	40.48

E. sativa	2.82	38.07	49.25	-	30.05
Mean	3.39	44.47	60.01	-	
LSD$_{(p=0.05)}$ for stem: Species (Spp) - 1.43, Nickel (Ni) - 1.67, Spp x Ni - 2.60; for leaf: Spp - 1.48, Ni - 1.73, Spp x Ni - 2.70;for seed:Spp - 1.29, Ni - 1.51, Spp x Ni - 2.35.					

* Values are excluded for statistical analysis. - = Indicates 'Nil' Ni concentration

Nickel removal

Different amendments treatment resulted in significant difference in the mean Ni removal (i.e., mean of species and Ni levels) by different plant parts (Table 5). Application of sewage sludge (sewage sludge-amended soil) significantly increased the mean Ni removal by stem, leaf and seed over un-amended soil. About 22.9, 24.4 and 19.8% significantly higher Ni removal was observed by stem, leaf and seed in sewage sludge-amended soil as compared to un-amended soil, respectively. In the farmyard manure-amended soil, the mean Ni removal by stem and leaf was statistically at par with the un-amended soil (although it slightly decreased by 1 to 3%), but in seed it was significantly less (about 6%) as compared to the un-amended soil. The sewage sludge-amended and farmyard manure-amended soil were significantly different from each other with respect to Ni removal pattern by different plant parts. The mean Ni removal by stem, leaf and seed were 25.1, 27.9, and 26.8% per cent less in the farmyard-amended soil in comparison to the sewage sludge-amended soil. In general, the metal uptake by different plant parts, however, followed the order: sewage sludge-amended>un-amended>farmyard manure-amended soils (Table 5). The data further showed that Ni application significantly increased the mean Ni removal by different plant parts at Ni_{150} and thereafter it decreased significantly at Ni_{300} irrespective of amendment treatment. Although, the effects of added Ni on mean Ni removal by stem, leaf and seed varied considerably in the un-amended, sewage sludge-amended and farmyard manure-amended soils. In the un-amended soil, the per cent decrease in the mean Ni removal by stem, leaf and seed at Ni_{300} was 20.6, 24.8, and 26.6% when compared with Ni_{150} treatment. In the sewage sludge-amended and farmyard manure-amended soils, the corresponding difference in the mean Ni removal at Ni_{300} and Ni_{150} treatment by stem was 28.1 and 38.5%, in leaf, 28.3 and 23% and in seed between 25.5 and 22.5 per cent, respectively. The un-amended and farmyard manure-amended soils did not differ significantly from each other in the mean Ni uptake by stem and leaf at each levels of added Ni. Although, the un-amended and farmyard manure-amended soils differed significantly from each other in their mean Ni uptake by seed at Ni_{150}, but were statistically at par at Ni_{300} (Table 5).

Table 5: Ni removal (µg pot^{-1}) by different plant parts of the *Brassica* species as affected by Ni levels, and sewage sludge and farmyard manure application

Ni (mgkg^{-1} soil)	**Un-amended soil (control soil)**			**Mean**	**3% sewage sludge soil-amended soil**			**Mean**	**3% farmyard manure-amended soil**			**Mean**
	B. juncea	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***		***B. juncea***	***B. napus***	***E. sativa***	
Stem												
0	55	57	33	48	68	67	39	58	61	64	34	54
150	578	524	284	462	706	650	345	567	563	509	269	447
300	485	345	162	331	567	432	224	407	469	356	147	324
450	201*	-	-	-	286*	-	-	-	239*	-	-	-
Mean	373	309	159	280	447	383	203	344	365	310	150	275
LSD$_{(p=0.05)}$Soil amendments (SA)=20.60; Species (Spp)=20.60; Nickel (Ni)=20.60; SAxSpp=Not significant; SAxNi=37.42; SppxNi=37.42; SAxSppxNi=Not significant												
Leaf												
0	37	31	18	29	43	41	21	35	40	38	19	32
150	392	348	164	301	487	428	233	383	376	337	150	288
300	342	230	107	227	382	278	163	274	337	228	100	222
450	123*	-	-	-	175*	-	-	-	165*	-	-	-
Mean	257	203	96	186	304	249	139	231	251	201	89.6	181
LSD$_{(p=0.05)}$ Soil amendments (SA)=14.41; Species (Spp)=14.41; Nickel (Ni)=14.41; SAxSpp=Not significant; SAxNi=26.18; SppxNi=26.18; SAxSppxNi=Not significant												
Seed												
0	33	33	15	27	39	39	19	33	37	37	17	30
150	356	336	152	281	411	388	206	335	324	303	145	257
300	295	220	104	207	339	277	132	249	285	215	98	199
450	67*	-	-	-	130*	-	-	-	154*	-	-	-
Mean	228	196	91	172	263	235	119	206	215	185	86	162
LSD$_{(p=0.05)}$ Soil amendments (SA)=8.81; Species (Spp)=8.81; Nickel (Ni)=8.81; SAxSpp=NS; SAxNi=16.01; SppxNi=16.01; SAxSppxNi=Not significant												

* = Values are excluded for statistical analysis. - = Indicates 'Nil' uptake

Species showed variability in their mean Ni removal by stem, leaf and seed irrespective of soil amendments (Table 6). It was the highest in *Brassica juncea* followed by *Brassica napus* and *Eruca sativa*. The mean Ni removal by *Brassica napus* and *Eruca sativa* was less by 15.5 and 56.8 in stem, 19.6 and 60.0 in leaf and 12.9 and 58.1% in seed, respectively as compared to the *Brassica juncea*. Data further revealed that out of total Ni removal by *Brassica juncea*, *Brassica napus* and *Eruca sativa*, stem contributed about 43.4 to 46.6%, 43.9 to 46.1 and 43.0 to 49.3%, respectively at added level of Nickel. The contribution of leaf ranges between 28.9 to 30.3 in *Brassica juncea*, 27.2 to 29.1 in *Brassica napus* and 26.9 to 29.3 in *Eruca sativa*. Similarly, out of the total nickel removal by the plant the share of removal by seed by *Brassica juncea*, *Brassica napus* and *Eruca sativa* was 26.0 to 26.6, 26.7 to 27.6 and 23.8 to 27.0%. Thus, the total removal of Ni by *Brassica* *juncea* (stem, leaf and seed) was 1.2 times higher than *Brassica napus* and 2.4 times than *Eruca sativa*. In another words, Ni removal by *Brassica napus and Eruca sativa* was about 84% and 42% that of *Brassica juncea*.

Table 6: Nickel removal (µg pot^{-1}) by stem, leaf and seed of different species as affected by Ni levels (mean of the amendment treatments)

Species	Ni Levels (mg kg^{-1}soil)				Mean
	0	150	300	450	
Stem					
B. juncea	61.54	616.11	506.75	241.96*	394.80
B.napus	62.53	560.89	377.62	-	333.68
E. sativa	35.41	299.26	177.52	-	170.73
Mean	53.16	492.09	353.96	-	
Leaf					
B. juncea	39.96	418.33	353.92	154.52*	270.74
B.napus	36.76	371.10	245.38	-	217.75
E. sativa	19.31	182.50	123.23	-	108.34
Mean	32.01	323.98	240.84	-	
Seed					
B. juncea	36.69	363.62	306.32	116.77*	235.55
B.napus	36.26	341.96	237.32	-	205.18
E. sativa	17.12	167.55	111.34	-	98.67
Mean	30.02	291.04	218.33	-	
LSD$_{(p=0.05)}$ for stem: Species (Spp) - 20.60, Nickel (Ni) - 24.06, Spp x Ni - 37.42; for leaf: Spp - 14.41, Ni - 16.83, Spp x Ni - 26.18;for seed: Spp - 8.81, Ni - 10.29, Spp x Ni - 16.01					

* Values are excluded for statistical analysis. - = Indicates 'Nil' uptake

Discussion

The results of the present study indicate that the *Brassica juncea* was proved tolerant to the nickel levels as compared to the *Brassica napus* and *Eruca sativa* as it survived at nickel level as high as Ni$_{450}$ and reached up to maturity. The decline in dry biomass yield of different plant parts was more in *Brassica napus* and *Eruca sativa* as compared to the *Brassica juncea* indicting the higher tolerance this species to Ni as compared to the rest two species. Several other authors also reported that *Brassica juncea* is tolerant most species for Ni and many other heavy metals [20-15]. It is reported that *Brassica juncea* transgenics under heavy metals stress resulted in higher accumulation of glutathione and phyto chelatins (S-rich metal chelators), this might be making the plant more tolerant as compared to the rest of the two species because these S-rich metal chelators plays a central role in protecting plants from heavy metal, environmental and oxidative stresses [16]. Apart from this, several authors have reported that excess Ni affects nutrient absorption by roots, impairs plant metabolism, inhibits photosynthesis and transpiration, and causes ultrastructural modifications [17], resulting in decline in the dry biomass yield. The application of sewage sludge and farmyard manure significantly improved the yield components of the crop. Firstly, may be due to additional supply of plant nutrients and subsequent change in the soil physico-chemical and biological environment, and secondly due to possible decrease in toxic effect of added Ni through formation of organometallic complexion reactions. Hence, slow releases of Ni to root system seem to have alleviated to some extent its toxicity in the root zone. Although application of farmyard manure was more effective (double increase in dry biomass yield) than the application of sewage sludge sewage. The beneficial effect of sewage sludge and farmyard manure and adverse effect of Ni on crops growth have also been reported by several other researcher [18].Species shows the considerable variation in Ni concentration and its uptake by different plant parts. The maximum Ni concentration was observed in *Brassica napus*, which was followed by *Brassica juncea* and *Eruca sativa*. The relatively higher Ni (a divalent) concentration in *Brassica napus* may thus be ascribed to its relatively high root cation exchange capacity. Brar [19] reported that the root cation exchange capacity of *Brassica napus* was higher than the other *Brassica* species under study. However, Ni uptake was the greatest by *Brassica juncea*, followed by *Brassica napus* and *Eruca sativa*. Similarly, among the different plant parts, stems of the species were potential accumulator for Ni followed by leaf and seed. Obviously, considerably greater biomass production in *Brassica juncea* more than compensated for its low tissue Ni concentration to affect greater uptake; reflecting thereby its superiority over the other two species from phytoremediation point of view. Sewage sludge-amended soil resulted in an increase in the tissue Ni concentration, while in farmyard manure-amended soil resulted in a considerable decrease in the tissue Ni concentration. However, total uptake of Ni in sewage sludge-amended soil increased, while it was decreased in farmyard manure-treated soils, suggesting that the role of modifying Ni bio-availability to plant during the growing period was quite different.

Conclusion

Brassica juncea species was found to be tolerant to Ni levels as compared to the *Brassica napus* and *Eruca sativa*. Addition of amendments (sewage sludge and farmyard manure) increased the dry biomass yield of different species. Higher Ni concentration was recorded in *Brassica napus* followed by *Brassica juncea* and least in *Eruca sativa*. Addition of sewage sludge increased the Ni concentration and its uptake by different species, contrary to this, application of farmyard manure decreased both concentration and uptake of Ni. Stems of the species were found to be a potential accumulator of the Ni as compared to the leaf and seed. Overall, among the three species B*rassica juncea* was found to be superiorin terms of Ni removal as compared to the *Brassica napus* and *Eruca sativa*.

References

1. Salt DE *et al.* Phytoremediation: A noval strategy for the removal of toxic metals from the environment using plants. Biotechnology 1995;13:468-474.
2. Kucharski J, Boros E, Wyszkowska J. Biochemical activity of nickel-contaminated soil.Pol. J Environ. Stud 2009;18:1039-1044.

3. Eskew DL, Welch RM, Cary EE. Nickel: An essential micronutrient for legumes and possibly for all high plants. Science 1983;11:621-623.
4. Kabata-Pendias A, Pendias H. Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL, USA 2001, P1-365.
5. Mingorance MD *et al.* Stabilized municipal sewage sludge addition to improve properties of an acid mine soil for plant growth.Soils Sediments 2013;14:703-712.
6. Piccolo A. Reactivity of added humic substances towards plant available heavy metals in soils. Sci. Total Environ 1989;81/82:607-614.
7. Ramani VP, Patel BK, Patel KP, Patel KC. Effect of nickel and FYM on spinach and different forms of nickel in Fluventic Ustochrepts of middle Gujarat. J. Ind. Pollu. Contr 2007;23:325-333.
8. Nanda-Kumar PBA, Dushenkov V, Motto H, Raskin I. Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol 1995;29:1232-1238.
9. Lim CH, Jackson ML. Dissolution for total elemental analysis. In Methods of Soil Analysis (eds Page, A. L., Miller, R. H. and Keeney, D. R.) ASASSSA, Madison, Wisconsin, USA 1982, P1–12.
10. Richards LA. Diagnosis and improvement of saline and alkali soils-USDA Handbook No. 60,US Department of Agriculture, Washington D. C., USA 1954, P1-160.
11. Lindsay WL, Norvell WA. Development of a DTPA soil test for Zn, Fe, Mn and Cu. Soil Sci. Soc. Am. J 1978;42:421-428.
12. Jackson ML. Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi, India 1967, P1-498.
13. Bandera AD. Phytochemical screening and return on investment of pechay (*Brassica napus* L. subsp. *chinensis* var. Black Behi) as influenced by inorganic fertilizers (ground and foliar application) and organic fertilizer. Int. J Agric. Food Sci. 2020;2(1):24-30. DOI: 10.33545/2664844X.2020.v2.i1a.31
14. Panwar BS, Ahmed KS, Mittal SB. Phytoremediation of nickel-contaminated soils by *Brassica* species. Environ. Dev. Sustain 2002;4:1–6.
15. Indoria AK, Poonia SR. Phytoextractability of lead from soil by some oilseed crops as affected by sewage sludge and farmyard manure, Arch. Agron. Soil Sci 2005;52:667-677.
    DOI: 10.1080/03650340601036608.
16. Gasic K,Korban SS. Heavy metal stress.In Physiology and molecular biology of stress tolerance in plants, (eds Rao, M. K.W., Raghavendra, A.S., and Reddy, J. K.), Springer, Heidelberg, Germany 2006, P219-254.
17. Prasad SM, Dwivedi R, Zeeshan M. Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. Photosynthetica 2005;43:177-185.
18. Abdullah NA, Fathy SE, Samir GA, Fahd MA. Nickel uptake from enriched sewage sludge amended soil by cabbage (*Brassica oleracea* L.) through phytoremediation technology. Adv. Environ. Biol 2013;7:2311-2318.
19. Brar JS. Phytoremediation of lead and cadmium contaminated soils. Ph.D. Thesis, CCS Haryana Agricultural University, Department of Soil Science, CCS, Haryana Agricultural University, Hisar, India 1999, P1-163.3.
20. Indoria AK, Poonia SR, Sharma KL. Phytoextractability of Cd from soil by some oilseed species as affected by sewage sludge and farmyard manure. Commun. Soil Sci. Plant Anal 2013;44:3444-3455.