Bull. Environ. Contam. Toxicol. (2005) 74:715–724

DOI: 10.1007/s00128-005-0641-z

Environmental Contamination and Toxicology

Nickel Phytoremediation Potential of Broad Bean, *Vicia faba* L., and Its Biochemical Responses

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Received: 16 July 2004/Accepted: 11 January 2005

Nickel is a heavy metal ubiquitously distributed in nature (Boyle and Robinson 1988). It is found in different concentrations in all types of soils of diverse climatic regions of the globe (Nriagu 1980). Naturally derived soils from serpentine rocks are rich in nickel but due to various industrial and anthropogenic activities such as mining and refining of nickel ores, burning of fossil fuels and residual oil, coal mine spoils, sewage sludge, production of Ni-Cd batteries etc., other areas have also become prone to Ni toxicity. Average yearly emissions of Ni were $55{,}650 \times 10^3$ kg in the atmosphere, 113×10^6 kg in aquatic ecosystems and 416.5×10^6 kg in soils in 1983-84 (Nriagu and Pacyna 1988).

Nickel has been classified among essential micronutrients (Welch 1995) and it remains associated with some metallo-enzymes, however, it is toxic at supraoptimal concentrations to plants (Schickler and Caspi 1999; Rao and Sresty 2000; Pandey and Sharma 2002). In plants it causes chlorosis, yellowing and necrosis of leaves, deformation of plant parts, stunted growth, generation of free radicals and lipid peroxidation, and disturbs enzymatic activities (Sheoran et al. 1990; Halliwell and Gutteridge 1999). However, there are plants like *Sebertia acuminata* (Jaffre et al. 1996) and *Alyssum lesbiacum* (Krämer et al. 1997), which hyperaccumulate Ni. In the present study, *Vicia faba* L. var. Sadabahar (Family Fabaceae, Subfamily Papillionaceae), a hardy, erect, annual plant, has been selected to evaluate its response to various concentrations of Ni. Metal accumulation and various physico-chemical and biochemical parameters have been studied.

MATERIALS AND METHODS

The stock solution (1000 μM) of nickel was made using the salt, nickel chloride ($NiCl_2.6H_2O$, Merck). Various concentrations (1, 10, 50, 100 and 200 μM) of nickel were made by diluting the stock solution with 10% modified Hoagland medium (Hoagland and Arnon, 1950). The seeds were soaked overnight in the above five different concentrations of nickel. The seeds soaked in nutrient medium without metal served as control. Three soaked seeds of each concentration were transferred in plastic glasses containing 350 g of acid washed sand maintained with different concentrations of nickel. Treatments were performed in three replicates. One with nutrient solution without metal served as control. Treated and untreated seeds of *V. faba* were observed for initiation of germination and percentage of germination was calculated. The plants cultured in different concentrations of Ni were supplied with 10% Hoagland solution at

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regular interval of five days. Plants were harvested after 20 and 40 days treatment and used for the determination of various parameters. For biomass estimation plants were oven dried and weighed on dry weight basis using a Sartorious electronic balance. Biomass was determined for the whole plant and roots and shoots separately.

Plants were also treated with effluent of Hindustan Aeronautics Limited (HAL), Lucknow, which is manufacturing systems and accessories for various aircraft and engines. Soaked seeds were transferred to plastic glasses containing 350 g of acid washed sand as usual and treated with different dilutions (25, 50, 75, 100%) of HAL effluent made with 10% Hoagland solution. A glass having only 10% Hoagland solution served as control. Seed germination was observed and percentage of germination was calculated. The plants were harvested after 40 days to study various parameters. HAL effluent was analysed in laboratory, however, a few parameters were determined at spot with the help of Century Portable Water Analysis Kit (CMK, 731).

For metal accumulation various plant parts were separated and kept in oven at 80°C for 48 h. Dried plants were weighed (1.0 g) and digested in concentrated HNO_3: $HClO_4$ (v/v, 3:1) at 80°C temperature. Digested samples were then analyzed by flame Atomic Absorption Spectrophotometer (Perkin Elmer 2380). Chlorophyll and carotenoid were estimated following the method of Arnon (1949) and Duxbury and Yentsch (1956), respectively. Protein was estimated following the method of Lowry et al. (1951). A two-way analysis of variance (ANOVA) was performed for all the data to confirm its validity.

RESULTS AND DISCUSSION

The physiochemical analysis of HAL effluent has been shown in Table 1. It contained 0.3 mg l^{-1} Ni.

Table 1. Physico-chemical characteristics of effluent from Hindustan Aeronautics Limited, Lucknow.

S.N.	Parameter	Value
1.	pH	7.5
2.	Temperature	35°C
3.	TDS	2100
4.	BOD	30
5.	COD	250
6.	Colour	Absent
7.	Odour	Unpleasant
8.	Ni	0.3
9.	Cr	0.1

All values are in mg/l except for pH and those otherwise stated

The accumulation of nickel at various concentrations and exposure duration in $NiCl_2$ treated plants has been found to be concentration and duration dependent. Nickel was not detectable in control plants. Plants accumulated up to 1942 μg Ni g^{-1} DW after 20 days at 200 μM Ni and up to 2417 μg Ni g^{-1} DW after 40 days at 200 μM Ni (Table 2).

Table 2. Nickel accumulation (μg g^{-1} DW) by *V. faba* plants exposed to various concentrations of $NiCl_2$.

Ni concentration (μM)	Treatment duration (d)	
	20	40
Control (10% Hoagland)	0.0	0.0
1.0	110±5	256±13
10.0	529±26	656±33
50.0	635±32	956±48
100.0	1256±63	1879±94
200.0	1942±97	2417±121

Values are mean ± S.D. (n=3); two way ANOVA of 20 days (F=1188.54*), 40 days (F =1200*) Ni accumulation; *= significant at $p<0.01$.

Table 3. Nickel accumulation (μg g^{-1} DW) by *V. faba* plants exposed to various concentrations of Ni enriched effluent.

Effluent concentrations (%)	Treatment duration (40 days)
Control (10% Hoagland)	0.0
25	188±6
50	226±11
75	319±21
100	440±27

Values are mean ± S.D. (n=3); two way ANOVA of Ni accumulation (F=673.91*); *= significant at $p<0.01$.

Table 4. Effect on percent seed germination in *V. faba* exposed to various concentrations of $NiCl_2$.

Ni concentration (μM)	Treatment duration (d)			
	1	3	5	7
Control(10% Hoagland)	55	77	87	99
1.0	25	69	88	99
10.0	18	62	90	90
50.0	15	56	82	87
100.0	11	42	75	82
200.0	8	31	64	77

Various concentrations of Ni did not affect the seed germination severely. The seeds seem to be having some dormancy as ~100% germination was recorded only after 7 days of incubation under normal growth condition. Maximum inhibition (22.22%) was observed at 200 μM after 7 days (Table 4). Nickel has an inhibitory effect on root, shoot and whole plant biomass of *V. faba*. The root biomass showed 43.83% inhibition at 200 μM Ni concentration after 20 days as compared to control. However, after 40 days root biomass initially increased from 2.39 g to 3.51 g at 50 μM and then it decreased to 1.276 g at 200 μM Ni showing 46.61% inhibition as compared to control (Figure 1A). At higher concentrations of Ni (>100 μM) and effluent, the root of the plant became black in colour representing severe metal toxicity. Shoot biomass showed an increase up to 100 μM Ni at 40 days similar to root biomass (Figure 1A). It

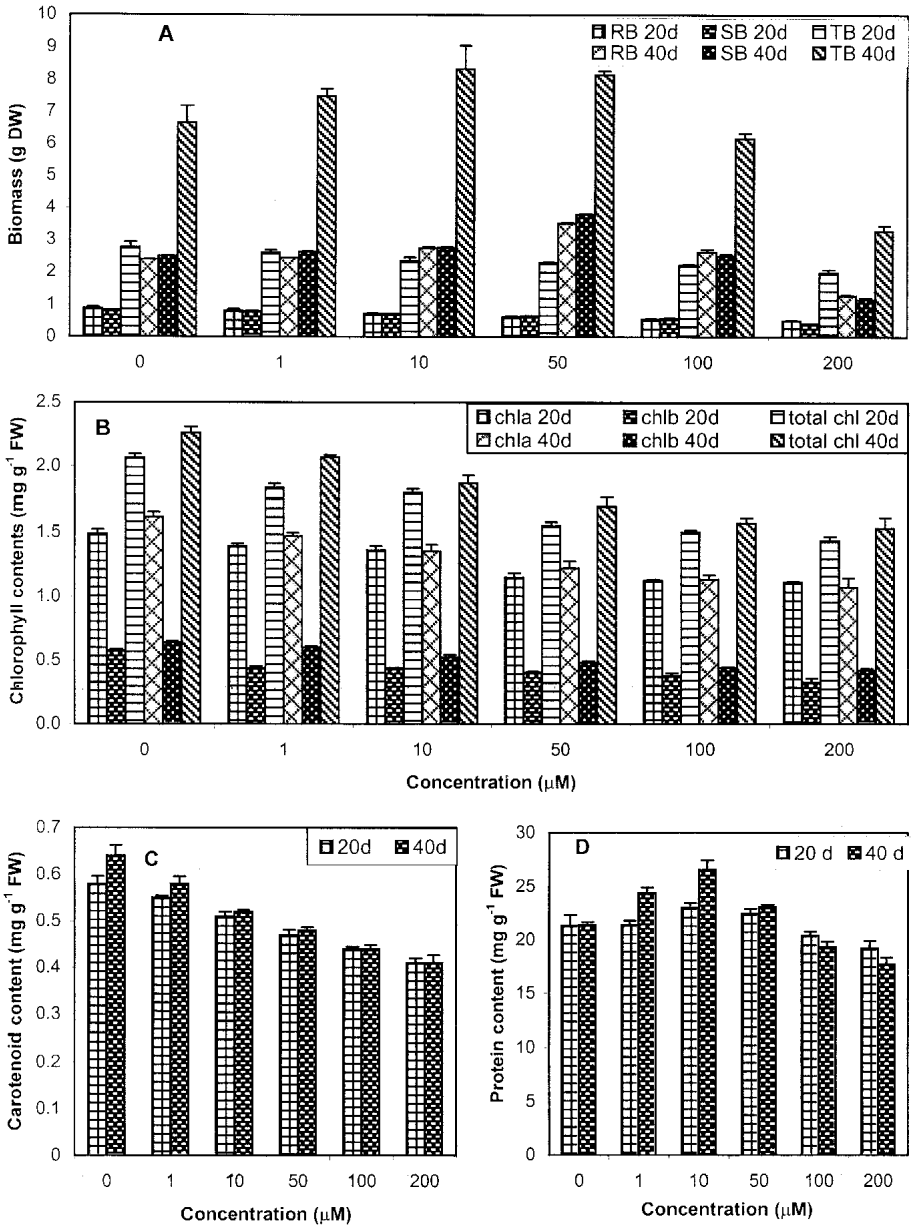

Figure 1. Effect of $NiCl_2$ treatment on Biomass (A), Chlorophyll (B), Carotenoid (C), Protein (D); Values are mean ± SD, two way ANOVA of 20 d (F= 322.48*) and 40 d (F=2215.69*) RB, 20 d (F=1995.14*) and 40 d (F=6451.15*) SB, 20 d (F=71.57*) and 40 d (F=169.04*) TB, 20 d (F=414.55*) and 40 d (F=496.59*) Chl a, 20 d (F=240.54*) and 40 d (F=798.62*) Chl b, 20 d (F=4461.01*) and 40 d (F =514.16* Total Chl, 20 d (F=748.53*) and 40 d (F=439.02*) Carotenoid and 20 d (F =92.50* and 40 d (F=487.91*) Protein; *= significant at p<0.01. RB=Root biomass, SB=Shoot biomass, TB=Total biomass

Table 5. Effect on percent seed germination in *V. faba* exposed to various concentrations of Ni enriched effluent.

Effluent concentration (%)	Treatment duration (d)			
	1	3	5	7
Control(10% Hoagland)	69	77	87	99
25	56	70	76	88
50	55	69	72	84
75	32	49	55	76
100	11	24	45	66

Table 6. Effect on photosynthetic area (cm^2), root and shoot length (cm) in *V. faba* exposed to various concentrations of $NiCl_2$ and Ni enriched effluent.

Ni concentration (μM)	Treatment duration (20d)		
	Photosynthetic area	Root length	Shoot length
Control (10% Hoagland)	5.40±1.68	6.26±0.50	6.23±0.85
1.0	3.78±0.27	6.53±0.85	6.46±0.06
10.0	3.38±0.10	6.69±0.43	6.56±0.32
50.0	2.92±0.02	7.20±0.26	6.43±0.38
100.0	2.73±0.12	6.27±0.74	5.66±0.15
200.0	1.99±0.41	5.83±0.21	5.23±0.25
	Treatment duration (40d)		
Control (10% Hoagland)	8.54±0.81	15.30±0.26	10.23±0.31
1.0	7.06±0.21	15.43±0.31	10.33±0.32
10.0	6.00±0.42	16.06±0.21	11.23±0.32
50.0	5.20±0.21	18.06±0.21	11.70±0.10
100.0	4.62±0.28	13.60±1.45	9.46±0.25
200.0	4.11±0.14	8.96±0.15	9.26±0.38
Effluent concentration (%)	Treatment duration (40d)		
Control (10% Hoagland)	3.13±0.11	14.86±0.32	9.30±0.52
25	2.92±0.02	3.86±0.92	8.56±0.45
50	2.70±0.06	2.70±0.43	6.20±0.45
75	0.78±0.05	1.00±0.04	4.16±0.20
100	0.31±0.03	0.90±0.03	3.46±0.35

Values are mean ± S.D. (n=3); two way ANOVA of 20 days (F=10.57*) and 40 days (F= 133.95*) PA; 20 days (F = 9.74**) and 40 days (F =114.17*) RL; 20 days (F =11.04*) and 40 days (F =294.03*) SL for $NiCl_2$ treatment and of 40 days PA (F=4657.89*); RL (F=753.34*); SL (F=1299.54*) for effluent treatment; *= significant at $p<0.01$; **=significant at $p<0.05$; PA= Photosynthetic Area; RL= Root Length; SL= Shoot Length.

was observed that the inhibition in shoot biomass at 200 μM was 49.63% after 20 days and 58.12% after 40 days as compared to control. At 200 μM Ni reduction in root and shoot length was 41.43% and 9.48%, respectively after 40 days (Table 6).

Total biomass also showed similar trend as shown by root and shoot biomass. Maximum decrease (50.53%) in total biomass was recorded at 200 μM concentration

Table 7. Effect of different concentrations of HAL effluent on chlorophyll and carotenoid contents (mg g^{-1} FW), biomass yield (g DW) and protein content (mg g^{-1} FW) of *V. faba* after 40 d treatment.

Effluent concentration (%)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Control (10% Hoagland)	1.60±0.05	0.62±0.02	2.33±0.24	0.65±0.01
25	1.41±0.05	0.59±0.01	2.04±0.31	0.61±0.01
50	1.43±0.01	0.54±0.01	1.97±0.02	0.48±0.004
75	1.41±0.02	0.45±0.01	1.82±0.01	0.42±0.01
100	1.26±0.07	0.36±0.01	1.53±0.09	0.40±0.01
	Root biomass	Shoot biomass	Total biomass	Protein
Control (10% Hoagland)	2.48±0.49	3.00±0.85	6.28±0.22	22.97±1.97
25	0.44±0.02	0.73±0.03	2.03±0.02	23.29±2.65
50	0.26±0.04	0.77±0.01	2.05±0.04	18.18±0.32
75	0.10±0.01	0.34±0.04	1.37±0.08	17.09±0.12
100	0.08±0.004	0.25±0.03	1.31±0.01	15.29±0.24

Values are mean ±S.D. (n=3); two way ANOVA of Chl a (F=60.93*); Chl b (F=797.89*); Total Chl (F=14.51**); Carotenoid (F=3801*); Root Biomass (F=70.36*); Shoot Biomass (F=28.20*); Total Biomass (F=1727.50*); Protein (F=28.25*); *= significant at $p<0.01$; **=significant at $p<0.05$.

after 40 days exposure after an initial increase up to 10 μM Ni (Figure 1A). Photosynthetic area of *V. faba* was found to be highly affected by Ni concentrations showing 63.19% decrease after 20 days and 51.87% decrease after 40 days at 200 μM Ni (Table 6). Chlorophyll and carotenoid showed progressive decrease with increasing Ni concentrations. Maximum inhibition was observed at 200 μM Ni after 40 days in each case, which were 33.95% for chlorophyll a, 34.38% for chlorophyll b, 32.30% for total chlorophyll (Figure 1B) and 35.94% for carotenoids (Figure 1C). Results showed stimulatory effect for protein content up to 10 μM Ni exhibiting maximum increase of 23.93% increase after 40 days as compared to control. However higher concentrations were found to be inhibitory. Maximum decrease was 17.24% after 40 days at 200 μM Ni (Figure 1D).

Plants treated with HAL effluent accumulated appreciable amount of Ni, maximum being at 100% effluent treatment (440 μg g^{-1} DW, Table 3). Seed germination was inhibited at all treatments and maximum inhibition of 33.34% was at 100% effluent treatment after 7 days (Table 5). Various studied parameters, showed reduction at all the effluent treatments. Maximum reduction was recorded at 100% effluent treatment for all parameters, which was 93.94% for root length, 62.79% for shoot length (Table 6), 90.10% for photosynthetic area (Table 6), 96.77% for root biomass, 91.67% for shoot biomass, and 79.15% for total biomass (Table 7). Total Chlorophyll content showed maximum decrease of 26.90% and carotenoids of 38.46% at 100% effluent treatment (Table 7). Protein content (Table 7) was not affected at 25% effluent treatment but then after decreased with maximum decrease (33.43%) at 100% effluent treatment.

Earlier reports indicated a wide threshold range of 40-246 μg/ml for Ni to produce toxic symptoms or abnormalities (Kabata-Pendias and Pendias 1992; Sresty and Rao

Table 8. The summary of the responses of *V. faba* exposed to various concentrations of $NiCl_2$ and effluent based on statistical analysis of data.

Parameters	Concentrations of the Ni (μM)/ effluent (%) exhibiting first effect at different treatment duration (d)					
	$NiCl_2$ treated plants				Effluent treated plants	
	Stimulatory		Inhibitory		Stimulatory	Inhibitory
	20 d	40 d	20 d	40 d	40 d	40 d
Root biomass	-	1.0	1.0	200.0	-	25%
Shoot biomass	-	1.0	1.0	200.0	-	25%
Total biomass	-	1.0	1.0	100.0	-	25%
Chl a	-	-	1.0	1.0	-	25%
Chl b	-	-	1.0	1.0	-	25%
Total Chl	-	-	1.0	1.0	-	25%
Carotenoids	-	-	1.0	1.0	-	25%
Protein	1.0	1.0	100.0	100.0	-	50%

1999). In present study plant accumulated Ni in a concentration and duration dependent manner but it does not seem to reach a steady state level in the present exposure limitation. Such a high accumulation is in agreement with earlier reports (Ali et al. 1999; Jaffre et al. 1996; Baker and Brooks 1989). The first effect on various studied parameters are shown in Table 8. Various Ni concentrations inhibited biomass production at 20 days showing no stimulation. This is in conformity with earlier reports for other plants (Rao and Sresty 2000). However, it is interesting to note from the data presented in Figure 1A that the total biomass increased upto 10 μM Ni after 40 days irrespective to the decrease in content of photosynthetic pigments. Such observation could be ascribed to more fixation of atmospheric nitrogen, a base material for protein synthesis, leading to incerased biomass of the plant. Metals have been reported to enhance nitrate reductase activity and increase the total soluble proteins and total organic carbon in leguminous plants (Singh et al. 1997). The leguminous plants growing in Ni contaminated fly-ash has been shown to produce more biomass even though pigment contents showed slight decrease or increase (Rai et al. 2004). It indicates that plant can repair the damage done showing induced metal tolerance. Higher concentrations are, however, lethal for plant growth. In Radish (*Raphanus sativus*) Ni inhibits all energy requiring cellular processes and slows down seed germination (Espen et al.1997). In the present study also inhibition of germination percentage of seeds was found.

Various Ni concentrations used in these experiments drastically inhibited the photosynthetic area of the plant thus reducing photosynthetic capability leading to retarded growth. The observed changes in the pigment contents of the plant suggest similar responses of chlorophyll and carotenoid to increasing concentration of the test metal. Such a decline in photosynthetic pigments could be attributed to its possible degradation. Decline may be due to the lysis of cell wall and disruption of the thylakoid membrane. Chlorophyll and carotenoid degradation has also been reported earlier in Cu treated plants (Luna et al. 1994). Reduction may also be caused by inhibition of chlorophyll biosynthesis. Inhibition of chlorophyll biosynthesis has been earlier reported for Pb, Hg, Cd and Cr due to their effect on δ-aminolevulinic acid dehydratase (Prasad and Prasad 1987; Padmaja et al. 1990; Vajpayee et al. 2000). The

decrease in pigments causes deficiency in light harvesting capacity and consequently decreased photosynthetic activity of the cells. It was interesting to observe here that plants exposed to HAL effluent accumulated less Ni but experienced more toxicity as compared to $NiCl_2$ exposed plants where plant tolerated up 200 μM Ni concentration. This higher toxicity of effluent is due to other toxic factors including high BOD, COD, TDS and presence of other metals as well.

Protein content showed increase initially up to 10 μM in $NiCl_2$ treatment, which may be due to induction of some stress proteins (Reddy 1992; Van Assche and Clijsters 1990; Toppi and Gabbrielli 1997; Tripathi et al. 2004). The reduction in protein content at higher concentration might be due to lipid peroxidation leading to damage of membrane and inhibition of membrane proteins. Further oxidative stress caused by the metal may also denature or damage the proteins (Davies 1987). Ni toxicity may also be exerted through replacement of Mg ion in some enzymatic proteins e.g. RubisCo (Wildner and Henkel 1979).

Besides, effect on biomass, the other visible phytotoxic symptom was the darkening and stunting of roots, which is in agreement with the earlier report of root damage due to Ni toxicity in pigeonpea (Sresty and Rao 1999). In *Pinus strobus* and *Picea glauca* root growth was inhibited by Ni and Cu (Lozano and Morrison 1982), and in *Picea sitchensis* by Ni (Burton et al. 1986). Although the toxicity was expressed in terms of some morphological and physiological variables, plants were able to accumulate appreciable amount of nickel. It may thus be concluded that the plants of *V. faba* has remediation potential and some tolerance towards various Ni concentrations and could be a useful phytoremediator plant for remediation of Ni contaminated sites.

Acknowledgments. We thank the Director, National Botanical Research Institute, Lucknow for the facilities provided. SS and SM are grateful to CSIR for the award of JRF. The study was also supported by DBT grant.

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