

## Assessing nickel bioavailability in smelter-contaminated soils

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### Abstract

Metal contaminants in soil environments derived from industrial pollution have clearly established the need for research on bioavailability and potential health risks. Much research has been conducted on metal sorption in soils. However, there is still a need to better understand the availability of metal contaminants to plants and microbes. Such information will enhance both human health and decisions about remediation efforts. In this study, Welland Loam (*Typic epiaquoll*) and Quarry Muck (*Terric haplohemist*) Ni contaminated soils from Port Colborne (Canada) which had been treated and untreated with limestone, were employed in greenhouse and bioavailability studies. These soils varied in pH from 5.1 to 7.5, in organic matter content from 6% to 72%, and in total Ni from 63 to 22,000 mg/kg. Oat (*Avena sativa*), a nonhyperaccumulator, and *Alyssum murale*, a hyperaccumulating plant species, were grown on these soils in greenhouse studies for 45 and 120 days, respectively, to estimate Ni accumulation. A Ni specific bacterial biosensor was also used to determine Ni bioavailability, and the results were compared to those from the greenhouse studies and more conventional, indirect chemical extraction techniques (employing MgCl<sub>2</sub> and a Sr(NO<sub>3</sub>)<sub>2</sub>). Results from the greenhouse, chemical extraction, and biosensor studies suggested that as the pH of the soil was increased with liming, Ni bioavailability decreased. However, the phytoextraction capability of *A. murale* increased as soil pH increased, which was not the case for *A. sativa*. Furthermore, the Ni specific bacterial biosensor was successful in predicting Ni bioavailability in the soils and suggested that higher Ni bioavailabilities occur in the soils at pH values of 5.1 and 6. The combination of plant growth, chemical extraction, and bacterial biosensor approaches are recommended for assessing bioavailability of toxic metals.

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### 1. Introduction

Emissions from a Ni smelting facility in Port Colborne, Ontario, Canada have left the nearby farmland with elevated Ni levels. Extremely high soil Ni concentrations have been detrimental to the agriculture

industry in this region and have left the neighboring farmland unsuitable for growing fruits and vegetables (McLaughlin, 2002). In situ remediation is considered as the only means of cleanup due to the scale of the contamination and the cost effectiveness of the strategy. In order to restore the productivity of the elevated metal contaminated soils, the bioavailability of Ni in these soils needs to be defined to understand the fraction accessible to the food chain and to the plants used for

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phytoremediation. This study will define bioavailability as the fraction of Ni available to biological systems. The first adverse effect of soil Ni in acidic soils is phytotoxicity and toxicity to microbes. Various chemical extraction methods have been developed to indirectly assess the association of metals with various soil components (Madden, 1988; Tessier et al., 1979). Also, field and greenhouse studies can provide total metal uptake information, which defines the metal fraction available to plants and microbes (Chaney et al., 2003). The application of bacterial biosensors, obtained by placing a luciferase reporter system under the transcriptional control of bacterial heavy metal resistance operons, has been developed to quantify the metal fraction in a contaminated soil that is bioavailable to the bacteria and causes toxicity, thus inducing the metal specific resistance operon. This induction can be directly measured via the transcription of the coupled luciferase reporter system, which results in a quantitative light signal. This novel technique is, depending on the resistance operon, element specific (Corbisier et al., 1999; Tibazarwa et al., 2001). A comparison between the data obtained with the Ni specific biosensor on the Ni fraction that caused induction of the Ni-resistance operon and the fraction of Ni taken up by corn, showed that there was a linear correlation between the two data sets (Tibazarwa et al., 2001). In this paper we used a combination of the above mentioned methods as a means of risk assessment to investigate nickel bioavailability.

In situ remediation to reduce Ni bioavailability in industrially contaminated soils is very important due to the Ni's phytotoxic effects. Soil amendments have been used to alter the pH of the soil, thus altering Ni speciation and consequently its bioavailability (Kukier and Chaney, 2001). However, one of the most effective strategies is the use of hyperaccumulating plants, for example *Alyssum murale*, to phytoextract the Ni from the soil (Chaney et al., 1997; Li et al., 2003) and remove the fraction of Ni that can be taken up by these plants (McGrath, 1998; Salt et al., 1998). Brooks et al. (1977) were the first to refer to a Ni hyperaccumulator plant as one that had the ability to accumulate more than 1000 mg/kg Ni of dry weight in their shoots. The most numerous metal accumulating plants are the Ni hyperaccumulators, which currently contain 318 taxa, many of them belonging to the *Alyssum* genus (Baker et al., 2000). The species *A. murale*, a known Ni hyperaccumulator, evolved on serpentine soils and has the ability to exist on these soils, even though they have elevated Ni levels that cause strong phytotoxicity effects in other plant species under acidic soil conditions. Recently, this

species has been shown to phytoextract Ni from nonserpentine soils (Li et al., 2003). Furthermore, rhizobacteria isolated from the rhizosphere of *A. murale* contributed to an increase in Ni availability and an increase in Ni phytoextraction (Abou-Shanab et al., 2003). However, the phytoextraction performance of this species is not known for metal contaminated soils treated to circum neutral pH.

The high organic muck soils present in the Port Colborne region are ideal for vegetable production. However, this agricultural industry was adversely affected by the Ni emissions from the refinery. Therefore, much interest has been devoted to various crops, identifying toxicity symptoms and investigating losses of their marketable yields (Temple and Besessar, 1981; Frank et al., 1982; Bisessar, 1989). A very important crop that has been studied for its growth on the Port Colborne contaminated soils is *Avena sativa* (Oat) (Kukier and Chaney, 2000, 2001). Like all Gramineous plant species, Oat has an Fe chelating mechanism that unlike most other plants releases phytosiderophore and solubilizes Fe, making it available for plant uptake. Oat secretes a phytosiderophore known as avenic acid, which chelates soil Fe that is absorbed by the root of the plant in the rhizosphere (Römheld and Marschner, 1986). However, Chambers et al. (1998) concluded that phytosiderophore chelation of ferric iron was not a selective process and Ni may have the ability to compete with Fe, only to accumulate in elevated levels in the grasses. Classic symptoms of Ni toxicity in oats are interveinal chlorosis and the development of perpendicular white strips on the above ground biomass (Hunter and Vergnano, 1952). However, it has recently been noted that the classic Ni toxicity symptom of perpendicular white banding in the shoots of grasses is due to the diurnal secretion of phytosiderophores contributing to Fe deficiencies during plant development. When phytosiderophore secretion is minimal, usually during the dark hours when photosynthesis is halted, Fe cannot be taken up by plant. Therefore, perpendicular white banding appears when plant development is occurring under Fe stress deficiency (Marschner et al., 1987; Takagi et al., 1988). The ecological advantages observed in the Poaceae families of plants provide more questions on their existence at or tolerability of elevated Ni levels in contaminated soils. There is a need to explore the possibilities of *A. sativa* surviving in highly bioavailable Ni soils and determining the effects Ni has on this plant at different pH levels. Finally, this marketable plant, when grown on Ni contaminated soils, may have the ability to transfer Ni into the food chain thus identifying a health risk associated with the Port Colborne region.

The objective of this research is to assess Ni bioavailability, via plant growth and a nickel specific bacterial biosensor, in contaminated Port Colborne Quarry Muck and Welland Loam soils that have been subjected to different remediation treatments. In this study, we determined Ni bioavailability associated with the smelter contaminated soils using a number of methods. The phytoremediation experiment was designed to compare the difference in uptake between hyperaccumulating, *A. murale*, and nonhyperaccumulating, *A. sativa*, plants and to establish the phytoextraction performance of *A. murale* at elevated pH values. Furthermore, the test soils were used to determine differences in Ni accumulation due to limestone treatments, organic matter, and iron and manganese oxides. Lastly, bacteria were extracted from the soils before and after the plant growth studies to determine whether Ni bioavailability and accumulation influenced the overall Ni resistance in the bacterial populations.

## 2. Materials and methods

### 2.1. Soil collection

Two soil types, a Quarry Muck (*Terric haplohemist*) and a Welland Loam (*Typic epiaquoll*) that both were Ni contaminated, were collected at various sites near the Port Colborne refinery in Ontario, Canada. The research plots where the soils were collected are managed by the United States Department of Agriculture (USDA). These test plots have been in operation since 1998. They have been used to study the effect of phytoremediation on Ni contaminated field plots and the success of limestone treatments as a remediation strategy. In the spring of 1999, the soils were field treated with limestone and then a crop of *Alyssum* was introduced and maintained until the end of August by the USDA. The soils used in this study were collected in November 2002, after two growing seasons yielding *Alyssum* and two field-applied limestone treatments. The soils collected downwind from the refinery contained elevated levels of Ni. Moderate levels of Ni were in the soils collected adjacent to the refinery. A forest was sampled downwind of the refinery and the Ni level in this soil was approximately 22,000 mg/kg of Ni. However, field plots next to this forest-contained soil had approximately 5000 mg/kg of Ni. This shows the impact large trees have on aerial deposits and how the large surface area trees affect the elevated levels of Ni. Multiple soil samples were collected with small spades at a depth of 0–15 cm at each plot. The samples were then stored in 20 gallon polyethylene tubs. The soils were incubated at room tempe-

rature for two weeks and then thoroughly mixed before any analyses were performed. The total Ni concentrations in the soils ranged from 63 to greater than 22,000 mg/kg Ni (Table 2).

### 2.2. Soil analysis

Physical and mineralogical properties of the soils were determined including bulk density, particle size analysis, and clay mineralogy of the two soil types using standard methods (Sparks, 1996). The bulk densities of the Welland Loam and Quarry Muck soils were 0.78 and 0.24 g/cm<sup>3</sup>, respectively. The particle size of the soils was determined using the Bouyoucos Hydrometer method. Clay mineralogical analyses of the less than 2 μm clay fraction were determined by X-ray diffraction. Pretreatment of the soil to remove soluble salts and carbonates, organic matter, and metal oxides followed by fractionation was conducted to obtain the <2 μm clay fraction (Sparks, 1996; Lavkulich and Wiens, 1970). Chemical property determinations included pH, soil organic matter content, cation exchange capacity, metal oxide content, total metal content, extractable metals, and sequential extraction analyses. The pH of the air-dried soils was determined using a soil to deionized water ratio of 1:2 with a 1 h equilibration time. Soil organic matter content was determined using the Loss-on-Ignition (LOI) method. The cation exchange capacity (CEC) of each soil was determined using the Compulsive Exchange Method (Sumner and Miller, 1996). This method measures CEC<sub>CE</sub> (compulsive exchange CEC) which can be defined as the capacity of the soil to retain basic cations under field conditions. Briefly, the soil was saturated with Ba<sup>2+</sup> and then exchanged by Mg<sup>2+</sup> by the addition of MgSO<sub>4</sub>. Once the ionic strength is adjusted back to that of the actual soil solution the quantity of Mg<sup>2+</sup> absorbed (=CEC) is estimated as the loss of Mg<sup>2+</sup> from the MgSO<sub>4</sub> solution added. Free iron and aluminum oxide content of the soils was determined using the sodium-citrate–bicarbonate–dithionite method (Mehra and Jackson, 1960). Total metal contents of the selected soil samples were determined using microwave assisted acid digestion of soils (US Environmental Protection Agency, 1995 method 3051). The soils were analyzed for total Ni, Cd, Co, Cu, Fe, Pb, and Zn content via Inductively Coupled Plasma Spectrometry. Extractable metal content of the soils was determined using the Mehlich III soil test procedure to identify macro- and micro-nutrient deficiencies (Mehlich, 1984). Strontium-extractable Ni was determined using 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> and a soil to solution ratio of 1:4 to determine plant-

available Ni (Helmke et al., 1997). The samples were shaken for 2 h and then filtered before analysis of the Ni concentration using atomic absorption spectrometry (AAS).

The sequential soil extraction procedure employed distinguishes five fractions (exchangeable, carbonates, Fe–Mn oxides, organic, residual) for Ni association (Tessier et al., 1979). Chemical extractions were performed on 1 g soil samples. The exchangeable fraction was extracted using 1 M  $MgCl_2$  at a pH of 7. The carbonate fraction was extracted using 1 M NaOAc at a pH of 5. The Fe–Mn oxide fraction was extracted using 0.04 M  $NH_2OH \cdot HCl$  in 25% (v/v) HOAc. The organic fraction was extracted using 0.02 M  $HNO_3$  and 30%  $H_2O_2$ . The residual fraction was extracted using 1:1 and concentrated  $HNO_3$  additions. Ni concentrations in the extracts were determined by ICP-AES.

### 2.3. Plant growth study

The growth of hyperaccumulator and nonhyperaccumulator plants in the Ni-contaminated soils was assessed in a greenhouse study. The total shoot biomass of each pot was determined as well as metal uptake by the plants. These results were correlated to the bioavailable Ni assessed by the biosensor analysis and with two alternative chemical extraction techniques. Total metal content of the plant was determined using a concentrated  $HNO_3$  and 30%  $H_2O_2$  wet digestion extraction procedure (US Environmental Protection Agency, 1995 method 3050B). Once the total metal uptake was determined it was correlated with the sequential extraction data as well as the bacterial biosensor data.

The greenhouse study, employed the muck and loam soils, both limed and unlimed, in triplicate arranged in a randomized block design. Soils were sieved wet, mixed thoroughly and 1500 g (dry weight) of loam soil or 800 g (dry weight) of muck soil were placed in 1.5 l plastic pots. Based on nutrient requirements for plants grown in pots, appropriate fertilizer treatments were added to all the soils used in the *A. sativa* ‘Olge’ (Oat) and *A. murale* ‘Kotodesh’ growth studies. Total amounts of macro- and micro-nutrients applied to each pot were: 154 mg N (as calcium nitrate), 291 mg P (as calcium phosphate and potassium phosphate), 231 mg K (as potassium phosphate), 22 mg Mg (as magnesium sulfate) and 1 mg B (as boric acid) (Siebielec et al., 2000). All fertilizers were added as solutions with the exception of calcium phosphate, which was added as a dry powder. Previous studies have shown that the Port Colborne soils develop a Mn deficiency when crops are introduced (Baldwin and Johnson, 1986; Brown et al., 1997; Brown and

Chaney, 1998). This is particularly true when the soils are treated with limestone to raise the pH and reduce the phytoavailability of Ni. Therefore,  $MnSO_4$  was added at a rate of 200 kg/ha of Mn to each pot (Siebielec and Chaney, 2000). The fertilizers were mixed with the soil in plastic bags and added to the pots. The pots were watered and incubated for 7 days to allow for any reactions to occur with the fertilizers and the soil. Then the soils were mixed again before planting. The plant species that were used in the experiment were *A. sativa* ‘Olge’ and *A. murale* ‘Kotodesh’. Fifteen Oat seeds were sown in each pot. Twenty days after planting, the Oats were thinned to 10 plants per pot. The *Alyssum* seeds were germinated in Pro-Mix (Premier Horticulture) for approximately 5 weeks before transplanting. Three plants were transplanted into each pot. The plants were subjected to light for 16 h each day using high pressure sodium vapor lamps as artificial lighting. Day/night temperatures were set at 25/21 °C. Pots were watered as needed using reverse osmosis filter water and saucers were used to prevent loss of the leachate.

### 2.4. Shoot biomass and total elemental analysis

The Oat plants were harvested after approximately 45 days of development. The *Alyssum* plants were harvested after 120 days of development. The shoot biomass was determined to understand the overall health of the plant with respect to treatment, pH, Ni concentration, and soil type. The plants were weighed after harvest to obtain the fresh weight, dried for 24–48 h at 65 °C, cooled in a desiccator jar, and reweighed to determine the dry weight. Dry plant shoots were prepared for total metal content analysis by acid digestion using concentrated  $HNO_3$  and 30%  $H_2O_2$  (US Environmental Protection Agency, 1995, method 3050B). Then the plant material was filtered and diluted to 50 ml with deionized  $H_2O$ . Filtrates were analyzed by ICP-AES, using Y as an internal standard, for P, K, Ca, Mg, Mn, Cu, Zn, Fe, Al, Ni, and Mo. For quality control, one NIST spinach leaf standard (SRM 1570a) was included in the analysis for every 10 samples.

### 2.5. BIOMET bacterial biosensor test

The bacterial biosensor strain *Ralstonia metallidurans* AE2515, a derivative of *R. metallidurans* CH34, was used to determine the Ni bioavailable fraction in the soil samples (for a detailed description see Tibazarwa et al., 2001). This strain has in the past been successfully used to predict nickel uptake by corn, and thus the potential transfer of this metal to the food chain (Tibazarwa et al.,

2001). Strain AE2515 contains plasmid pMOL1550 on which the *luxCDABE* luciferase reporter system is expressed under the control of the *cnrXYH* regulatory region of the *cnr* cobalt and nickel resistance operon (Tibazarwa et al., 2001). Ni is the main inducer of the *cnr* operon, and the *cnr-lux* fusion will be expressed when Ni concentrations pass the threshold that causes induction of the *cnr* operon. As the induction of the *cnr-lux* fusion is linear to the amount of bioavailable Ni, light production will directly reflect the amount of Ni bioavailable to the bacterial biosensor AE2515.

The soil, standard, and blank assays were analyzed using a Luminescan (Labsystems) luminometer at 23 °C as describe before (Corbisier et al., 1999; Tibazarwa et al., 2001). Soil suspensions were prepared by diluting 5 g of soil with 30 g of reconstitution media. The soil suspensions were then diluted 8 and 16 times and finally added to a 96-well microtiter plate, which initially contained the diluted Ni biosensor culture. The light production of the biosensor was monitored over a 12 h period every 30 min.

The maximum luminescent response of the Ni biosensor strain at a specific time for standard Ni concentrations was used to construct a calibration curve. The signal was the luminescent response of each biosensor in the calibration solutions. The noise was the luminescent response of each biosensor in the water blank solutions. Once the calibration curve was constructed, the luminescent responses of the soil samples were used to calculate the amount of bioavailable Ni in each soil sample. As a control *R. metallidurans* AE864, which shows constitutive light production, was used to monitor general toxicity and light quenching that could bias the interpretation of the Ni bioavailability test. In the absence of toxicity or matrix effects that influenced bioluminescence, strain AE864 will show a signal-to-noise ratio of 1. The Ni bioavailability data collected with strain AE2515 were only considered valid when at the same time strain AE864 showed a signal to noise ratio between 0.8 and 1.2. All experiments were repeated 3 times, and average bioavailability data plus standard deviations were calculated.

Further analysis of other metal contaminants was conducted according to the same protocol by using Cu- (*R. metallidurans* AE1269) and Zn-specific (*R. metallidurans* AE1433) biosensor strains (for a detailed description, see Corbisier et al., 1999).

### 2.6. Analysis of heavy metal resistant bacteria

A microbial study of the soils was used to determine the presence of heavy metal resistant, cultivable bacteria

before and after the plant studies. 2 g of soil was extracted with 10 ml of a 10 mM MgSO<sub>4</sub> solution for 10 min, and subsequently serial dilutions ranging from 10<sup>-2</sup> to 10<sup>-8</sup> were made in MgSO<sub>4</sub>. 100 µl of the dilutions were plated on minimal 284 gluconate medium (Mergeay et al., 1985) spiked with either 1 or 2 mM ZnSO<sub>4</sub>, NiCl<sub>2</sub>, or 0.8 mM Cu(NO<sub>3</sub>)<sub>2</sub> to determine the populations of heavy metal resistant bacteria. Semi-rich and minimally rich nonselective media were also used to determine the total populations of cultivable microorganisms. The plates were incubated at 30 °C for 72 h. Total plate counts were made to determine the total populations of cultivable and heavy metal resistant bacteria in the soils and related this to the bioavailability studies.

## 3. Results and discussion

### 3.1. Soil characterization

The two soil samples used in this study have different physiochemical properties (Tables 1 and 2). The Quarry Muck soil contained 56% organic matter while the Welland Loam contained approximately 10% (Table 1). The Welland Loam soil also contained approximately twice more clay than the Quarry Muck soil. The bulk densities of the Quarry Muck and Welland Loam soils were 0.24 and 0.78 g/cm<sup>3</sup>, respectively (Table 1). The difference in the soils' bulk densities was attributed to the much higher organic matter content of the Quarry Muck compared to the Welland Loam. The Quarry Muck soil had a much higher CEC than the Welland Loam soils, which can be also explained by the vast difference in organic matter content. The clay mineralogy of the Quarry Muck and Welland Loam soils was similar except for the presence of montmorillonite in the Quarry Muck soil. This could also attribute to the high CEC of the Quarry Muck soil.

### 3.2. Total metal content

The Ni contents of the soils were the main focus of this study. The low Ni, Muck and Loam soils had approximately 2000 mg/kg of total Ni. The high Ni, Muck soil contained approximately 3500 mg/kg of total Ni with the unlimed soil having an elevated amount of total Ni or approximately 4900 mg/kg (Table 2). The excess Ni, Muck soil had a total Ni concentration of about 22,000 mg/kg, which presented an extreme level of Ni loading when compared to the other Muck soils. The Welland Loam soils had comparable total Ni concentrations thereby providing a means for

Table 1  
Basic characteristics of the soils

Soil type	Subgroup	Bulk density (g cm <sup>-3</sup> )	Organic	Sand/silt/clay fractions	Oxide content	Mineralogy of <2 μm clay fraction
			matter %			
Organic Quarry Muck	<i>Terric haplohemist</i>	0.24	56	51/34/15	41	Kaolinite, montmorillonite, mica, goethite, quartz
Mineral Welland Silt Loam	<i>Typic epiaquoll</i>	0.78	9.8	29/35/36	54	Kaolinite, mica, goethite, quartz

identifying differences in the study related to soil characteristics (Table 2).

Aside from elevated levels of nickel in the collected soil samples, other heavy metals of interest such as Cu, Zn, and Fe were also at elevated levels (Table 2). These elements were present in the smelter contaminated soils; however, their origin is unclear and cannot be directly related to the electro-refining process of Ni.

### 3.3. Mehlich III soil test

The Mehlich III soil test method (Mehlich, 1984) was used to determine extractable P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, and Al that could be related to plant available metals (Table 3). The determination of the levels of these macro and micro elements was essential in determining a successful nutrient treatment of the soils before initiating the plant growth study. The Mehlich III soil test analyses indicated that P, K, and Mn levels were low, particularly in the Welland Loam soil. Accordingly, before implementing the plant growth study, a nutrient solution had to be added to the soils to prevent P, K, and Mn deficiencies in the plants.

### 3.4. Sequential extraction

Sequential soil extractions are useful analyses because they give a rough estimate of metal associations in soils. However, these experiments are operationally defined depending on the extractants used and do not directly identify specific metal complexes/species that are present. Table 4 shows Ni and Fe fractionation of the 11 soils used in the study. Extraction with MgCl<sub>2</sub> is an index of the Ni and Fe exchangeable fraction, which can also indicate the plant-available fraction. MgCl<sub>2</sub> exchangeable Ni fraction was compared to other Ni assessment studies such as the Sr(NO<sub>3</sub>)<sub>2</sub> extraction, plant growth study, and the bacterial biosensor test. As the pH of the soil was increased using limestone the amount of Ni extracted with MgCl<sub>2</sub> decreased, suggesting a decrease in Ni bioavailability at higher pH values. The MgCl<sub>2</sub> extractable fraction in the low Ni, unlimed Welland Loam was 303 mg/kg which was the highest amount of exchangeable Ni in any of the Loam soils (Table 4). The only other soil that had a sizable amount of exchangeable Ni associated with it was the Excess Ni, unlimed Quarry Muck. A very small exchangeable Fe

Table 2  
Chemical properties of the soils

Soil	Organic matter	Cation exchange capacity	pH	Total Ni	Total Cd <sup>a</sup>	Total Co	Total Cu	Total Fe	Total Pb	Total Zn
	%	(meq/100g)		(mg/kg)						
<i>Muck soils</i>										
Low Ni, unlimed	67.6	52.0	6.1	2006	<Det	7.25	15.5	17,308	29.3	68.4
Low Ni, limed	52.3	49.5	6.8	1756	<Det	46.8	323	19,475	57.4	150
High Ni, unlimed	71.7	49.2	5.8	4902	<Det	38.4	293	15,908	51.9	118
High Ni, limed	72.3	64.5	6.5	3516	<Det	59.9	426	20,049	63.9	164
High Ni, limed	55.9	44.0	6.9	3259	<Det	79.9	597	24,540	79.6	186
Excess Ni, unlimed	63.9	18.8	5.1	22,444	<Det	52.1	412	16,061	62.6	170
<i>Loam soils</i>										
Control	6	20.0	7.1	63.7	<Det	66.2	643	16,100	57.3	170.6
Low Ni, unlimed	11.1	17.0	6	2115	<Det	27.4	276	11,136	36.4	112
Low Ni, limed	15.8	24.0	6.7	2746	<Det	331	3021	30,523	299	480
High Ni, unlimed	9.8	18.1	7.1	4700	<Det	50.1	475	13,860	64.8	181
High Ni, limed	8.2	22.7	7.5	3468	<Det	31.5	300	13,198	37.1	101

<sup>a</sup> Total Cd was below the detection limit of the Instrument.

Table 3  
Mehlich-III extractable metal chemistry of soils (mg/kg)

Soil	P	K	Ca	Mg	Mn	Zn	Cu	Fe	B	S	Al
<i>Muck soils</i>											
Low Ni, unlimed	648	676	49,330	5221	11.9	139	194	3010	26.7	466	3730
Low Ni, limed	376	356	29,498	4846	12.6	92.4	144	1415	18.6	335	2225
High Ni, unlimed	858	1441	53,434	4241	6.6	226	366	1927	22.6	398	4580
High Ni, limed	<Det	1852	59,785	7798	8.1	257	269	2894	30.2	388	1441
High Ni, limed	670	625	30,216	5688	5.6	120	194	1203	18.3	226	1731
Excess Ni, unlimed	91.2	741	28,612	2770	81.7	270	1015	745	13.8	246	6672
<i>Loam soils</i>											
Control	33.9	195	3126	951	10.5	5.6	4.2	347	3.3	38	780
Low Ni, unlimed	17.3	131	4294	509	3.6	12.6	65.1	220	2.8	61.2	1373
Low Ni, limed	105	323	5164	1235	5.9	24.9	91	374	4.6	77.5	1383
High Ni, unlimed	188	599	4925	825	7.7	11.4	137	333	4.4	80	1840
High Ni, limed	170	388	4836	867	11.1	11.1	120	320	4.3	101	1515

fraction in both soils was extracted with  $MgCl_2$ . The sequential extraction results suggested that most of the Ni and Fe in both soils were complexed with Fe and Mn oxides. This was expected due to the high oxide content in both the Quarry Muck and the Welland Loam soils (Table 1). The Quarry Muck soils contained a larger Ni and Fe fraction associated with organic matter, as indicated by the  $HNO_3$  and 30%  $H_2O_2$  extractions, compared to the Welland Loam soils. This was expected since the Muck soils contained approximately on average 60% more organic matter than the Loam soils (Table 2).

### 3.5. Plant growth studies

#### 3.5.1. *Avena sativa*

With *A. sativa* plants, symptoms of Ni toxicity including chlorosis, interveinal chlorosis, and banded chlorosis started to develop as early as 1 week after

planting in the loam soils and as early as 4 weeks after planting in the muck soils. More drastic toxicity symptoms developed in the unlimed or lower pH soils than the limed or higher pH soils. However, most of the plants grown in both the Welland Loam and Quarry Muck soils developed some type of Ni toxicity.

Classic symptoms of Ni toxicity were observed in oats grown on the low Ni, unlimed Welland Loam soil. These plants showed stunting of growth and curling of leaves, which had a characteristic needle shape, causing a reduction in aboveground biomass. Furthermore, an elevated Ni level of 146 mg/kg was found in the tissue (Table 5). The plants grown in this soil may have been subjected to Fe deficiency as suggested by the severity of chlorosis and the significant reduction in Fe concentration compared to all the other plants grown in the Welland Loam soils. Limestone alleviated the needle shaped symptoms apparent in the Welland Loam

Table 4  
Ni and Fe sequential extraction analysis (mg/kg)

Soil	Exchangeable		Carbonates		Fe–Mn oxides		Organic		Residual	
	Ni	Fe	Ni	Fe	Ni	Fe	Ni	Fe	Ni	Fe
<i>Muck soils</i>										
Low Ni, unlimed	58.2	2.5	41.6	33.8	994	7090	233	2509	142	334
Low Ni, limed	24.1	1.5	39.3	23.9	815	5193	189	2386	128	500
High Ni, unlimed	152	3.3	52.2	22.6	2337	11,539	384	3120	451	752
High Ni, limed	68.2	2.0	56.2	22.6	1492	9910	343	2967	266	488
High Ni, limed	45.2	1.6	87.8	24.5	1714	9461	374	3287	391	657
Excess Ni, unlimed	739	2.0	131	21.1	4569	27,427	234	662	751	1232
<i>Loam soils</i>										
Control	2.8	4.9	4.0	15.9	29.2	4065	9.4	285	36.1	10,894
Low Ni, unlimed	303	2.8	90.3	140	432	5462	91.9	433	942	11,819
Low Ni, limed	61.8	1.4	86.2	19.8	552	6544	86.2	384	1523	12,251
High Ni, unlimed	63.8	2.5	105	88.4	1525	7769	218	300	2493	15,672
High Ni, limed	27.2	2.7	98.1	95.2	980	5076	193	246	1980	14,271

Table 5  
The effect of soil amendments on the elemental composition of *Avena sativa* shoots

Treatment	Biomass	Ni (mg kg <sup>-1</sup> )	Mn	Fe	Zn	Cu	Mo	Al	P (g kg <sup>-1</sup> )	Mg	Ca	K
	(g pot <sup>-1</sup> )											
<i>Loam soil</i>												
Control	5.3 <sup>a</sup>	2.5 <sup>a</sup>	69.1 <sup>a</sup>	92.5 <sup>ab</sup>	46.8 <sup>b</sup>	10.7 <sup>a</sup>	0.5 <sup>a</sup>	11.3 <sup>a</sup>	3.76 <sup>a</sup>	3.92 <sup>ac</sup>	6.01 <sup>a</sup>	52.7 <sup>a</sup>
Low Ni, unlimed	0.2 <sup>b</sup>	146 <sup>b</sup>	151 <sup>b</sup>	60.8 <sup>a</sup>	53.3 <sup>ab</sup>	15.0 <sup>ac</sup>	0.1 <sup>b</sup>	0.0 <sup>b</sup>	3.84 <sup>a</sup>	7.57 <sup>b</sup>	33.3 <sup>b</sup>	20.7 <sup>b</sup>
Low Ni, limed	5.9 <sup>a</sup>	50.9 <sup>c</sup>	38.9 <sup>c</sup>	116.8 <sup>b</sup>	80.2 <sup>a</sup>	30.1 <sup>b</sup>	1.0 <sup>c</sup>	25.0 <sup>c</sup>	3.54 <sup>b</sup>	7.39 <sup>b</sup>	12.3 <sup>c</sup>	72.3 <sup>a</sup>
High Ni, unlimed	4.4 <sup>a</sup>	38.7 <sup>c</sup>	43.2 <sup>c</sup>	120.1 <sup>b</sup>	79.0 <sup>a</sup>	31.9 <sup>b</sup>	0.9 <sup>d</sup>	45.3 <sup>d</sup>	4.89 <sup>c</sup>	4.62 <sup>c</sup>	8.29 <sup>d</sup>	116 <sup>c</sup>
High Ni, limed	4.9 <sup>a</sup>	14.1 <sup>d</sup>	69.4 <sup>a</sup>	105.1 <sup>b</sup>	44.1 <sup>b</sup>	17.3 <sup>c</sup>	0.8 <sup>c</sup>	43.3 <sup>d</sup>	3.42 <sup>d</sup>	4.09 <sup>c</sup>	7.48 <sup>ad</sup>	90.4 <sup>c</sup>
<i>Muck soil</i>												
Low Ni, unlimed	6.2 <sup>ab</sup>	9.5 <sup>a</sup>	94.2 <sup>a</sup>	90.8 <sup>a</sup>	52.5 <sup>ac</sup>	15.7 <sup>a</sup>	4.5 <sup>a</sup>	14.7 <sup>a</sup>	4.74 <sup>a</sup>	4.49 <sup>a</sup>	8.88 <sup>ab</sup>	57.2 <sup>a</sup>
Low Ni, limed	5.5 <sup>a</sup>	7.4 <sup>a</sup>	34.7 <sup>b</sup>	80.1 <sup>a</sup>	41.2 <sup>a</sup>	13.9 <sup>a</sup>	2.0 <sup>b</sup>	8.1 <sup>b</sup>	3.77 <sup>b</sup>	4.31 <sup>a</sup>	7.76 <sup>a</sup>	54.7 <sup>a</sup>
High Ni, unlimed	5.9 <sup>ab</sup>	44.8 <sup>b</sup>	75.9 <sup>a</sup>	118 <sup>a</sup>	93.9 <sup>bc</sup>	24.8 <sup>b</sup>	2.9 <sup>c</sup>	15.2 <sup>a</sup>	5.59 <sup>ad</sup>	4.01 <sup>a</sup>	11.5 <sup>b</sup>	69.1 <sup>a</sup>
High Ni, limed	7.5 <sup>ab</sup>	23.1 <sup>c</sup>	48.9 <sup>ab</sup>	115 <sup>a</sup>	67.7 <sup>c</sup>	26.5 <sup>b</sup>	1.0 <sup>d</sup>	12.5 <sup>a</sup>	3.78 <sup>b</sup>	3.42 <sup>b</sup>	6.54 <sup>ac</sup>	63.2 <sup>a</sup>
High Ni, limed	7.7 <sup>b</sup>	9.4 <sup>a</sup>	49.5 <sup>ab</sup>	110 <sup>a</sup>	47.4 <sup>ac</sup>	11.5 <sup>a</sup>	1.1 <sup>c</sup>	8.4 <sup>b</sup>	3.40 <sup>c</sup>	3.30 <sup>c</sup>	5.40 <sup>c</sup>	60.1 <sup>a</sup>
Excess Ni, unlimed	0.1 <sup>c</sup>	504 <sup>d</sup>	1704 <sup>c</sup>	101 <sup>a</sup>	106 <sup>bc</sup>	28.4 <sup>b</sup>	0.6 <sup>f</sup>	0.0 <sup>c</sup>	6.80 <sup>d</sup>	8.67 <sup>d</sup>	39.3 <sup>d</sup>	24.2 <sup>b</sup>

Values are means,  $n=3$ . Means within element and soil followed by the same letter are not significantly different according to ANOVA single factor test ( $p < 0.05$ ).

soil and appeared to reduce the phytotoxic effects of the Ni. The low Ni, limed Loam soil had a statistically significant increase in aboveground biomass and Fe concentration compared to the low Ni, unlimed Loam soil and the phytotoxic effects of Ni seemed to diminish. However, the limestone treatment, which raised the pH from 6 to 6.7, did not completely alleviate Ni phytotoxicity. Interveinal chlorosis and banded chlorosis developed in the low Ni, limed Loam soil indicative of Ni toxicity and was confirmed by elemental analysis of the tissue. The high Ni, unlimed Loam soil developed symptoms of Ni toxicity, however, raising the pH from 7.1 to 7.5 with limestone alleviated Ni phytotoxicity. After the limestone treatment the Ni concentration was reduced to 14 mg/kg. The results of the tissue analysis also revealed a competition in uptake between Ni and Fe by the oat plants grown on the Welland Loam soils, which was also consistent with the visible symptoms (Table 5). The addition of limestone significantly increased Fe concentration in the shoots and diminished Ni phytotoxicity in these plants. The symptoms of stunting and extreme chlorosis observed in the plants grown on the low Ni, unlimed Loam soil could be a result of Ni toxicity and Ni toxicity-induced Fe deficiency (Kukier and Chaney, 2001).

The plants grown on the Quarry Muck soils exhibited more moderate symptoms of Ni toxicity than the Welland Loam soils. This can be ascribed to the high amount of organic matter in the Quarry Muck soils, which is more effective in complexing Ni, making it less available. The plants grown on the high Ni, unlimed Muck soil experienced significant interveinal and banded chlorosis. Results of the tissue analysis indicated

that these symptoms were due to Ni toxicity (Table 5). However, even though the soil pH was 5.8 the plants were not stunted. Furthermore, when the pH was raised by approximately one unit, chlorosis was very minimal suggesting abatement of Ni phytotoxicity in the low and high Ni, limed Muck soils. The white chlorotic banding on the plants grown in the neutral pH Muck soils was very evident after germination but became very sparse at harvest. The plants grown on the excess Ni, unlimed Muck soil exhibited the most severe Ni toxicity. These symptoms of stunting and extreme chlorosis were attributed to Ni phytotoxicity, as shown in the tissue analysis.

### 3.5.2. *Alyssum murale*

Symptoms of Ni toxicity did not develop in most of the *A. murale* plants. However, *Alyssum* plants grown in the low Ni, unlimed Loam soil and the excess Ni, unlimed Muck soil did yield different aboveground biomass results compared to all the other plants (Table 6). The plants grown on the low Ni, unlimed Loam soil were stunted compared to those grown on the low Ni, limed loam soil. The stunted plants had a total aboveground biomass of 2.8 g/pot which was significantly different than the plants grown on the low Ni, limed soil and the control soil. The *Alyssum* transplanted in the excess Ni, unlimed Muck soils did not grow and had to be harvested within 30 days. These plants developed severe yellowing which led to chlorosis. It was not possible to obtain total biomass and elemental composition after harvest due to the size of these plants.

All the *Alyssum* plants experienced purple to reddish foliar symptoms, indicative of phosphorus deficiency

Table 6  
The effect of soil amendments on the elemental composition of *Alyssum murale* shoots

Treatment	Biomass (g pot <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )	Mn	Fe	Zn	Cu	Mo	Al	P	Mg	Ca	K
									(g kg <sup>-1</sup> )			
<i>Loam soil</i>												
Control	9.1 <sup>a</sup>	353 <sup>a</sup>	88.9 <sup>a</sup>	37.3 <sup>a</sup>	42.7 <sup>a</sup>	3.12 <sup>a</sup>	1.64 <sup>a</sup>	6.40 <sup>a</sup>	2.49 <sup>a</sup>	1.00 <sup>a</sup>	18.9 <sup>a</sup>	13.7 <sup>a</sup>
Low Ni, unlimed	2.8 <sup>b</sup>	8327 <sup>bc</sup>	395 <sup>b</sup>	29.1 <sup>a</sup>	134 <sup>bc</sup>	16.8 <sup>b</sup>	1.39 <sup>b</sup>	0.16 <sup>b</sup>	3.91 <sup>b</sup>	0.85 <sup>b</sup>	29.2 <sup>ab</sup>	12.6 <sup>a</sup>
Low Ni, limed	12.4 <sup>a</sup>	6818 <sup>b</sup>	137 <sup>a</sup>	26.4 <sup>a</sup>	106 <sup>bc</sup>	6.54 <sup>c</sup>	2.56 <sup>c</sup>	3.00 <sup>c</sup>	2.33 <sup>c</sup>	1.04 <sup>c</sup>	20.5 <sup>ab</sup>	11.7 <sup>a</sup>
High Ni, unlimed	7.9 <sup>c</sup>	12,861 <sup>c</sup>	195 <sup>a</sup>	36.9 <sup>a</sup>	213 <sup>c</sup>	17.2 <sup>b</sup>	2.39 <sup>d</sup>	11.5 <sup>d</sup>	2.95 <sup>d</sup>	1.45 <sup>d</sup>	24.7 <sup>ab</sup>	14.1 <sup>a</sup>
High Ni, limed	7.4 <sup>c</sup>	7992 <sup>b</sup>	142 <sup>a</sup>	30.4 <sup>a</sup>	76.9 <sup>c</sup>	23.9 <sup>b</sup>	1.94 <sup>c</sup>	11.4 <sup>d</sup>	3.17 <sup>c</sup>	1.29 <sup>c</sup>	30.2 <sup>b</sup>	14.7 <sup>a</sup>
<i>Muck soil</i>												
Low Ni, unlimed	8.7 <sup>a</sup>	1446 <sup>a</sup>	92.9 <sup>a</sup>	23.1 <sup>a</sup>	61.5 <sup>a</sup>	2.45 <sup>a</sup>	1.15 <sup>a</sup>	11.4 <sup>a</sup>	1.64 <sup>a</sup>	0.62 <sup>a</sup>	25.4 <sup>a</sup>	8.81 <sup>a</sup>
Low Ni, limed	6.6 <sup>a</sup>	5066 <sup>b</sup>	41.6 <sup>b</sup>	24.5 <sup>a</sup>	55.6 <sup>a</sup>	2.80 <sup>b</sup>	2.05 <sup>b</sup>	3.75 <sup>b</sup>	1.69 <sup>b</sup>	0.89 <sup>b</sup>	23.7 <sup>a</sup>	10.9 <sup>a</sup>
High Ni, unlimed	6.6 <sup>a</sup>	1103 <sup>a</sup>	116 <sup>a</sup>	35.0 <sup>a</sup>	71.5 <sup>a</sup>	3.93 <sup>c</sup>	1.05 <sup>c</sup>	13.7 <sup>a</sup>	2.56 <sup>c</sup>	0.52 <sup>c</sup>	30.6 <sup>a</sup>	12.9 <sup>a</sup>
High Ni, limed	8.4 <sup>a</sup>	2442 <sup>c</sup>	76.1 <sup>a</sup>	27.0 <sup>a</sup>	57.6 <sup>a</sup>	3.27 <sup>d</sup>	0.40 <sup>d</sup>	2.68 <sup>c</sup>	2.15 <sup>d</sup>	0.68 <sup>d</sup>	25.7 <sup>a</sup>	12.5 <sup>a</sup>
High Ni, limed	8.5 <sup>a</sup>	6079 <sup>b</sup>	120 <sup>a</sup>	28.7 <sup>a</sup>	82.7 <sup>a</sup>	2.94 <sup>c</sup>	1.60 <sup>c</sup>	11.1 <sup>a</sup>	2.25 <sup>c</sup>	0.76 <sup>c</sup>	26.0 <sup>a</sup>	11.7 <sup>a</sup>
Excess Ni, unlimed						Did not grow						

Values are means,  $n=3$ . Means within element and soil followed by the same letter are not significantly different according to ANOVA single factor test ( $p<0.05$ ).

with the most severe symptoms appearing on the plants grown on neutral Loam soils. This helps to explain the statistically significant reduction in total biomass of the plants grown on the high Ni, Loam soils. This deficiency was due to phosphorus as the symptoms disappeared after an addition of a calcium phosphate nutrient solution.

*A. murale* plants accumulated elevated amounts of Ni in their shoots (Tables 6 and 7). *Alyssum* was able to phytoextract Ni from the soils even as the Ni became less bioavailable as defined by the chemical extractions, bacterial biosensor, and *A. sativa* growth study (Tables 6 and 7). Significantly elevated Ni quantities were evident in the shoots of the *Alyssum* plants grown on the calcareous soils. Ironically, the more acidic soils, which showed elevated Ni amounts in the *A. sativa* plants, had the least amount of Ni accumulation in the *Alyssum* plants (Tables 5 and 6). Therefore, as limestone was added to the soils, making them more calcareous, the total biomass of the plants increased and their phytoextraction performance was enhanced.

### 3.6. Effect of pH and soil type on Ni extractability

The plant bioavailable Ni fractions were determined using two chemical extraction procedures. These extractions were implemented to mimic the soil solution. A 1 M MgCl<sub>2</sub> solution was used to extract the exchangeable fraction of Ni, which was then assumed to equate to the bioavailable fraction. A good correlation was observed between this chemical extraction and the fraction that accumulated in *A. sativa* (Tables 4 and 5). Therefore, it was observed that as the pH of the soil solution was

increased the actual Ni fraction available for plant uptake decreased (Table 7). This is comparable to many other studies which have shown that as Ni contaminated soils are limed, or made calcareous, Ni uptake by various crops is significantly reduced (Crooke, 1956; Frank et al., 1982; Bisessar, 1989; Kukier and Chaney, 2001). The hyperaccumulating, *A. murale* plant species followed a different trend compared to that of *A. sativa*. As the pH of the soil increased the *Alyssum* species had the ability to accumulate more of the Ni. This observation is comparable to a recent study, which employed the same soils at pHs between 5 and 6 (Li et al., 2003). This phenomenon differs from the results of the chemical extraction techniques (Table 7). Therefore, one can conclude that Ni accumulation by the hyperaccumulator species *A. murale* was enhanced as the pH increased while the bioavailable fraction of Ni decreased.

The 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> extraction experiment was implemented because it better represented the soil solution compared to the 1 M MgCl<sub>2</sub> solution. The Sr(NO<sub>3</sub>)<sub>2</sub> procedure is very advantageous in determining plant-available Ni because it does not alter the pH of the soils and omits chloride (Kukier and Chaney, 2001). Therefore, the exchange reactions occurring during the soil extraction with 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> are a better representation of reactions occurring in the plant growth studies. The two soils that show the highest 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> extractable Ni fraction are the low Ni, unlimed Loam soil and the excess Ni, unlimed Muck soil. These Loam and Muck soils had 40 and 402 mg/kg Sr(NO<sub>3</sub>)<sub>2</sub> extractable Ni fractions, respectively (Table 7). When the Loam soil was limed nearer to neutral pH the Sr(NO<sub>3</sub>)<sub>2</sub>

Table 7  
Effect of pH on phytoextraction of *Alyssum murale* and Ni bioavailability

Soil treatment	soil pH	Total Ni in soil (mg kg <sup>-1</sup> )	Oat shoot Ni concentration (mg kg <sup>-1</sup> )	<i>Alyssum</i> shoot Ni concentration (mg kg <sup>-1</sup> )	<i>Alyssum</i> phytoextracted Ni (mg pot <sup>-1</sup> )	MgCl <sub>2</sub> extractable Ni (mg kg <sup>-1</sup> )	Sr(NO <sub>3</sub> ) <sub>2</sub> extractable Ni (mg kg <sup>-1</sup> )	Ni-Biosensor (mg Ni kg dry ground <sup>-1</sup> )
<i>Welland loam</i>								
Control	7.1	63.7	2.5	353	3.2	2.8	1.0	<Det <sup>a</sup>
Low Ni, unlimed	6	2115	146	8327	23	304	40	51 ± 2 <sup>b</sup>
Low Ni, limed	6.7	2746	51	6818	84	62	5.3	<Det
High Ni, unlimed	7.1	4700	39	11,595	92	64	3.0	<Det
High Ni, limed	7.5	3468	14	8280	61	27	1.8	<Det
<i>Quarry muck</i>								
Low Ni, unlimed	6.1	2006	9.5	1446	13	58	5.7	<Det
Low Ni, limed	6.8	1756	7.4	5066	33	24	2.9	<Det
High Ni, unlimed	5.8	4902	45	1103	7.3	152	18	<Det
High Ni, limed	6.5	3516	23	2442	21	68	4.0	<Det
High Ni, Limed	6.9	3259	9.4	6079	52	45	2.4	<Det
Excess Ni, unlimed	5.1	22,445	504	dng	dng <sup>c</sup>	740	402	484 ± 72

<sup>a</sup> Ni biosensor was below detection limit.

<sup>b</sup> S.D. were calculated based on the average of three independent experiments.

<sup>c</sup> Did not grow.

extractable Ni fraction was reduced to 5.3 mg/kg (Table 7). Furthermore, the Quarry Muck soil yielded far less extractable soil Ni than the Welland Loam soils, again suggesting a much more stable Ni complex presumably with the organic matter. In comparison with the *A. sativa* growth study, elevated Ni levels were present in plants grown on these two highly bioavailable Ni soils and as the soils were made calcareous the Ni levels were reduced (Table 5). The trends observed in the *A. sativa* plant growth study, and 10 mM Sr(NO<sub>3</sub>)<sub>2</sub>, and the 1 M MgCl<sub>2</sub> Ni extraction procedures, were analogous to each other indicating that as the pH of the soil was increased, the bioavailability of Ni decreased (Table 7). These results also justify the validity of the chemical extraction methods in determining the bioavailable Ni fraction in the soils.

The Ni bacterial biosensor AE2515 detected bioavailable Ni fractions in two of the soils; the low Ni, unlimed Loam soil, and the excess Ni, unlimed Muck soil. These were the same soils that gave elevated extractable fractions using 1 M MgCl<sub>2</sub> and 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> (Table 7). Also, these two soils resulted in the most drastic Ni phytotoxicity symptoms in the *A. sativa* growth study and caused stunting of growth to the *A.*

*murale* (Tables 5 and 6). More importantly, extraction data obtained with 10 mM Sr(NO<sub>3</sub>)<sub>2</sub> that is used to predict the plant-available amount of Ni were very similar to the Ni bioavailability measurements obtained with the AE2515 Ni biosensor (Table 7). The extractable Ni in the unlimed loam soil was 40 mg/kg and the biosensor predicted bioavailable Ni fraction was approximately 25% more. The extractable Ni in the unlimed Muck soil was 402 mg/kg and the biosensor predicted bioavailable Ni fraction was 484 mg Ni/kg for the dry ground soil. The bioavailability of Ni in the other soils was below the detection limit of the AE2515 Ni biosensor.

The Ni biosensor strain AE2515 was accurate in predicting the bioavailable Ni fraction in the soils. However, at low dilutions the soil suspensions were toxic to both AE2515 and the constitutive control strain AE864, this due to other contaminants. As Zn and Cu were also present in elevated amounts in the smelter contaminated soils, the Cu (*R. metallidurans* AE1269) and Zn (*R. metallidurans* AE1433) specific biosensors were used to identify the bioavailable amounts of these metals. However, for both sensors the concentration of its specific metal was below detection limit.

A microbial study was employed on the rhizosphere of *A. murale* grown on the Loam soils to determine the percentage of cultivable Ni, Zn, and Cu resistant bacteria (Table 8), and to evaluate if their proportions would change with changes in heavy metal bioavailability and plant metal uptake. Selective media spiked with different concentrations of metals were used to identify changes in the microbial populations, especially as far as heavy metal resistance was concerned, before and after the plant growth. Total microbial plate counts of the soils suggested a high number of Ni resistant bacteria in the soils (Table 8). When these results were compared to Ni bioavailability, the total amount of Ni resistant bacteria increased as Ni bioavailability increased. Furthermore, as the total countable bacterial colonies increased in the soils the phytoextraction performance of *A. murale* increased suggesting that rhizobacteria increased the accumulation of Ni in *A. murale*: we observed the highest number of Ni resistant bacteria in the unlimed loam soil, which is also the soil that showed the highest Ni phytoextraction value (Table 7). This finding supports the study conducted by Abou-Shanab and others (2003) who found that isolated rhizosphere bacteria aided in the uptake of Ni by *A. murale*. Accordingly, more Ni accumulated in *A. murale* as the seeds were inoculated with the isolated bacteria (Abou-Shanab et al., 2003). The limed loam soil also identified a high number of Zn and Cu resistant bacteria. The total number of these resistant bacteria also increased as the pH of the soils was increased (Table 8).

Table 8  
Microbial ecology of the soils

Soil	Medium	Cfu/g soil
Loam control	Minimal-rich <sup>a</sup>	$2 \times 10^4$
	Semi-rich <sup>b</sup>	$2 \times 10^4$
	1 mM NiCl <sub>2</sub> spike <sup>c</sup>	$2.5 \times 10^4$
	1 mM ZnSO <sub>4</sub> spike <sup>d</sup>	0
	0.8 mM Cu(NO <sub>3</sub> ) <sub>2</sub> spike <sup>e</sup>	0
Loam unlimed	Minimal-rich	$4 \times 10^6$
	Semi-rich	$2 \times 10^6$
	1 mM NiCl <sub>2</sub> spike	$1 \times 10^5$
	1 mM ZnSO <sub>4</sub> spike	0
	1 mM Cu(NO <sub>3</sub> ) <sub>2</sub> spike	0
Loam limed	Minimal-rich	$7 \times 10^6$
	Semi-rich	$4.5 \times 10^6$
	1 mM NiCl <sub>2</sub> spike	$7 \times 10^4$
	1 mM ZnSO <sub>4</sub> spike	$5 \times 10^3$
	1 mM Cu(NO <sub>3</sub> ) <sub>2</sub> spike	$8 \times 10^2$

<sup>a</sup> Minimally rich non-selective media.

<sup>b</sup> Semi rich non-selective media.

<sup>c</sup> Ni spiked media for identification of Ni resistant bacteria.

<sup>d</sup> Zn spiked media for identification of Zn resistant bacteria.

<sup>e</sup> Cu spiked media for identification of Cu resistant bacteria.

#### 4. Conclusions

This study was successful in determining the bioavailability of Ni in smelter contaminated soils and the amelioration of these soils with added amendments. First, the *A. sativa* plant growth study showed symptoms of Ni toxicity and Ni toxicity-induced Fe deficiency. Chlorotic patterns observed in the shoots of the plants were the result of the plants' circadian rhythm and the diurnal secretion of phytosiderophores during photosynthesis (Römheld and Marschner, 1986; Marschner et al., 1987; Takagi et al., 1988). Specifically, the symptoms of Ni toxicity became less drastic with an increase in pH and organic matter. Increasing the pH of the soil is effective in ameliorating Ni phytotoxicity by converting soluble Ni into forms that are sequestered in the soil. These unavailable or insoluble forms of Ni may include partitioning to Fe and Mn oxides, formation of surface precipitate complexes, or sorption to clay mineral surfaces (Bruemmer et al., 1988; Bryce et al., 1994; Backes et al., 1995; Scheckel et al., 2000; Scheckel and Sparks, 2001). Organic matter can also have a significant impact on metal sequestration thus reducing the solubility of Ni by forming complexes with organic functional groups (Nachtegaal and Sparks, 2003).

Results from the *A. murale* growth study were comparable to those observed by Li et al. (2003). Our study identified the phytoextraction performance of *A. murale* and shows the plant's tolerance in Ni contaminated soils. This tolerance to high levels of Ni can be a result of adaptation or evolution on native serpentine soils (Baker et al., 2000). The *A. murale* growth study showed that as the pH of the smelter contaminated soils increased with limestone addition, the plants' ability to phytoextract Ni from the soils improved. This contradicted the MgCl<sub>2</sub> and Sr(NO<sub>3</sub>)<sub>2</sub> chemical extraction studies, the *A. sativa* growth study, and Ni biosensor experiment which all identified plant-available Ni and suggested that making the soils calcareous would make Ni less available for plant uptake. Reasons for the opposite effect of Ni uptake with pH changes observed with the Ni hyperaccumulator plant species *A. murale* were suggested by Li et al. (2003). However, many physiological processes occurring in this plant are still unknown. Knowledge of mechanisms of Ni uptake and accumulation from the soil/plant interface to partitioning and distribution in cellular compartments can lead to a stronger understanding of the plant's ability to hyperaccumulate Ni. In the same context, the role of rhizosphere bacteria in the Ni uptake process by *A. murale* should be further exploited, as a direct effect was observed between plant Ni uptake and Ni resistant rhizosphere bacteria. This understanding can offer reasoning behind

why *A. murale* is so successful in Ni phytoremediation of contaminated soils and offer an alternative remediation method for environmental cleanup.

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