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Phytoextraction of Nickel and Cobalt by Hyperaccumulator *Alyssum* Species Grown on Nickel-Contaminated Soils

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Several *Alyssum* species native to Mediterranean serpentine soils hyperaccumulate nickel. These species can potentially be used to remediate Ni-contaminated soils. However, the ability of these species to phytoextract Ni from nonserpentine Ni-contaminated soils is unknown. Two Ni hyperaccumulator species, *Alyssum murale* and *Alyssum corsicum,* were grown for 120 days on two nonserpentine Ni-contaminated soils in a greenhouse experiment. Soils were amended to provide a range of values for three soil factors: soil pH, available phosphorus, and exchangeable Ca/Mg ratio. Both species hyperaccumulated Ni, but not Co, from both soils. Ni uptake was reduced at lower soil pH and increased at higher soil pH. Neither P fertilization nor adjustment of the exchangeable Ca/Mg ratio significantly affected phytoextraction of Ni or Co. There was no difference between the two species in the amount of Ni phytoextracted, but *A. corsicum* phytoextracted more Co than *A. murale.* Higher amounts of both metals were phytoextracted from the loam than from the organic soil. Further research is needed to better understand the unusual effect of soil pH adjustment on Ni uptake by these hyperaccumulator species.

Introduction

Phytoextraction employs metal hyperaccumulating plant species to transport high quantities of metals from soil into the harvestable parts of roots and aboveground shoots (*1*, *2*). Ni hyperaccumulation was defined by Brooks et al. (*3*) in 1977 as the accumulation of at least 1000 mg kg^{-1} Ni in the dry biomass of plants grown on a natural substrate. Subsequently, hyperaccumulators of other heavy metals were identified. Metal concentrations achieved by naturally occurring hyperaccumulating plant species can be more than 100 times those that occur in nonaccumulator plants growing in the same substrates. The extraordinary ability of hyperaccumulator plants to tolerate and accumulate heavy metals has stimulated research into possible uses of phytoextraction (*4*−*9*). Phytoextraction can be carried out either on metal-contaminated soils or on low-grade ores or naturally metal-rich soils that cannot be economically utilized by traditional mining technology (*10*). In the former case, phytoextraction is a type of phytoremediation, while the term "phytomining" has been applied to the latter case in which the economic value of the recovered metal is the primary motive.

Emissions from Ni refineries and smelters have increased Ni and cobalt (Co) concentrations in nearby soils. Downwind from a Ni refinery that operated from 1918 to 1984 in Port Colborne, ON, Canada, the concentration of Ni in the 0−5-cm layer of an untilled muck soil studied by Frank et al. (*11*) ranged from 800 to over 6000 mg kg^{-1}, well above the soil remediation generic phytotoxicity guideline of 200 mg kg^{-1} set by the Ontario Ministry of the Environment (*12*). Soil concentrations of Co, while above background levels, were below the MOE remediation generic guideline of 50 mg kg^{-1}. Vegetable crops grown on this soil showed visual symptoms of Ni phytotoxicity, and yields of several Ni-sensitive vegetable crops were substantially reduced, apparently due to the presence of the soil contaminants (*11*). In cases such as this, remediation will be necessary to meet regulatory standards.

Over the past 4 yr, research conducted cooperatively by Viridian Environmental L.L.C., the USDA-ARS, and other research institutions has led to the development of a commercially feasible phytomining technology that may provide a tool for cleaning up Ni-contaminated soils (*13*). The technology employs two Ni-hyperaccumulating species, *Alyssum murale* and *Alyssum corsicum*, to phytomine Ni from serpentine soils that are naturally rich in Ni. Both species have also been reported to hyperaccumulate Co under certain conditions. These species evolved on serpentine soils, and their ability to phytoextract Ni from nonserpentine soils is unknown. In this study, we test their phytoextraction performance on two nonserpentine Ni-contaminated soils from Port Colborne, ON. Compared to typical serpentine soils, the test soils are higher in organic matter, available phosphate, and exchangeable calcium, and they are lower in iron and manganese hydrous oxides, soil pH, and exchangeable magnesium.

Effective phytoextraction requires both plant genetic ability and the development of optimal agronomic management practices (*8*). It has been well-documented that modifying soil pH or soil fertility may affect the efficiency of phytoextraction of heavy metals such as Zn, Cd, Ni, and Co (*6*, *14*−*17*). Soil pH is the major factor expected to influence the speciation and total soluble concentrations of Ni and Co in soil (*18*). Other factors that may affect phytoavailability of soil Ni and Co include soil physical and chemical properties, organic matter content, phosphate availability, cation-exchange capacity, presence of hydrous oxides of Fe and Mn, and ion interactions (*19*). The present experiments were planned to study three soil factors—soil pH, phosphate level, and exchangeable Ca/Mg ratio—that may affect Ni and Co uptake by *Alyssum* hyperaccumulator species grown on Ni-contaminated soils.

Materials and Methods

The study was conducted in a greenhouse and was divided into three sub-experiments to address the three soil factors being studied: pH, P fertilization, and Ca/Mg ratio. The experimental design was randomized complete block with four replications. Each of the three sub-experiments employed a 2 × 2 × 4 factorial treatment structure with the factors being plant species, soil type, and level of soil amendment, respectively. The three sub-experiments shared a common fertilized control, and there was also an unfertilized control for each plant species−soil type combination.

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TABLE 1. Soil Properties of the Ni-Contaminated Agricultural Soils Used in the Study

soil series	soil texture	bulk density (g cm^{-3})	% organic matter	pH	total Ni (mg kg^{-1})	DTPA-extractable Ni (mg kg^{-1})	total Co (mg kg^{-1})	DTPA-extractable Co (mg kg^{-1})	exchangeable Ca/Mg	Bray-1 phosphorus (mg kg^{-1})
Welland	loam	0.88	17	5.5	2570	591	37	3.1	6.3	18
Quarry	organic	0.35	72	6.1	1720	523	24	1.0	5.6	74

Two Ni-hyperaccumulating *Alyssum* species were used. Seeds of *A. murale* (accession AGK 10) were collected in Bulgaria, and seeds of *A. corsicum* (accession ECUSG 48) were collected in Turkey. Two Ni-contaminated agricultural soils were collected near a historic Ni refinery in Port Colborne, ON, Canada. Both soils were contaminated by emissions of particulate from the refinery, which operated from 1918 to 1984. The soils were a Welland loam (Orthic Humic Gleysol) and a Quarry organic soil (Terric Mesisol). Selected soil characteristics are given in Table 1. Soils were collected from the top 15 cm of the A horizon and shipped to Beltsville, MD, in plastic buckets. Soils were partially air-dried, sieved through a 5-mm stainless steel screen, and homogenized in a soil-mixing machine before amendments were added.

The soil amendment treatments included an unfertilized control, a fertilized control, three treatments with adjusted soil pH (two lower and one higher than the fertilized control), three treatments with a range of phosphorus fertilizer levels, and three treatments with a range of magnesium fertilizer levels. Soil amendments were added on an areal basis (kg ha^{-1}) because of large differences in bulk density between soils. The fertilized control contained 150 kg of N ha^{-1} as NH_4NO_3, 100 kg of P ha^{-1} as $Ca(H_2PO_4)_2 \cdot 2H_2O$, 150 kg of K ha^{-1} as a 1:1 (by weight) mixture of KCl and K_2SO_4, 1 kg of B ha^{-1} as H_3BO_3, and 1000 kg of $CaSO_4 \cdot 2H_2O$ ha^{-1}. Phosphorus fertilizer levels studied were 0, 100, 250, and 500 kg of P ha^{-1}. Magnesium fertilizer levels studied were 0, 3600, and 7200 kg of $MgSO_4 \cdot 7H_2O$ ha^{-1}, and these treatments did not receive added $CaSO_4 \cdot 2H_2O$. At the beginning of the experiment, adjusted pH of the Welland soil ranged from 5.0 to 5.8 and that of the Quarry soil ranged from 5.2 to 6.2. Soil pH was adjusted using 1.6 M HNO_3 or powdered $CaCO_3$. Acidified soils were leached with 3 vol of deionized water to remove excess soluble salts prior to fertilizer addition. Soil samples were collected both before and after treatments were applied and at the end of the experiment, when the soil in each pot was removed and mixed before sampling. Soil samples were air-dried and crushed with a stainless steel rolling pin prior to chemical analysis. Soil pH was measured in a 1:2 soil:water slurry. Bray-1-extractable soil P, a measure of readily available P, was determined according to Kuo (*20*). Exchangeable cations were measured according to the method of Helmke and Sparks (*21*). Diethylenetriaminepentaacetate (DTPA) extraction was carried out using a modification of the method of Lindsay and Norvell (*22*). For measurement of total metals in soil, soils were digested in nitric acid according to U.S. EPA Method 3050 (*23*). Soil extracts and digests were analyzed using either flame atomic absorption (AA) spectrometry or inductively coupled plasma (ICP) atomic emission spectrometry.

Plants were grown in a greenhouse equipped with supplemental high-intensity sodium and incandescent lights capable of supplying 400 μmol m^{-2} s^{-1} of photosynthetically active radiation, with a photoperiod of 16 h. Temperature was maintained at 28 °C during the day and 20 °C at night. Plants were watered with deionized water. Seeds were planted in flats of peat-based potting medium and transplanted after 5 weeks into 18 cm diameter black plastic pots containing 3.5 L of the test soil. Six seedlings were transplanted into each pot, and they were thinned to five plants per pot 2 weeks later. Plants received additional N and K fertilizer at 4-week intervals. Plants were harvested for measurement of shoot biomass and elemental composition after 60 and 120 days of growth in the pots. Two randomly selected plants were harvested from each pot at the first harvest, and the remaining three plants were harvested at the end of the experiment. Plants were harvested by clipping the stems just above the soil surface. All plants harvested from a pot at one time were combined to make one sample. Harvested shoots were washed in deionized water, oven-dried at 65 °C, weighed, and ground in a stainless steel Wiley mill. Ground plant tissue was weighed into Pyrex beakers and ashed in a muffle furnace at 450 °C for 16 h. The ash was digested with nitric acid using a modification of AOAC Method 985 (*24*). Plant shoot concentrations of Ni, Co, Zn, Cu, Mn, Fe, Mg, Ca, K, and P were determined using ICP spectrometry with yttrium as an internal standard.

To determine the effect of soil amendments, statistical analysis was performed separately on data from the three sub-experiments (soil pH, P fertilization, and soil Ca/Mg ratio). To determine the main effects of species and soil type, data from all 11 soil amendment treatments were pooled. Shoot elemental concentration and biomass data as well as calculated values for the amount of phytoextracted metal were subjected to three-way analysis of variance. Treatment and interaction means were separated by the Duncan−Waller *K* ratio *t*-test after it was determined that there was a significant ($P < 0.05$) treatment effect.

Results

Soil Analyses. The level of plant-available (DTPA-extractable) Ni measured immediately before and after soil acidification and leaching was unchanged, but as expected, by the end of the experiment all measures of extractable Ni and Co were highest at the most acidic pH and lowest in the highest pH treatment. The ratio of exchangeable Ca to exchangeable Mg (∼6 in the untreated soils) was lowered by the magnesium treatments to ∼2 in the Welland soil and ∼3 in the Quarry soil, but it did not approach the extremely low levels (∼0.2) that occur in the serpentine soils to which *Alyssum* is adapted.

Plant Growth. Both *Alyssum* species grew well on all of the soils and soil treatments. In the first few weeks, there was some necrosis and loss of leaves in plants in all treatments of the Welland soil that had a pH below 5.4, but it disappeared later in the experiment. This temporary necrosis could have been due to manganese toxicity as shoot manganese concentration in the affected plants at the 60-day harvest was as high as 212 mg kg^{-1}. In contrast, a permanent mild chlorosis that developed in *A. corsicum* on the high pH treatment of the Quarry soil was attributed to manganese deficiency. Shoot Mn concentration in the affected plants at the 120-day harvest was as low as 8 mg kg^{-1}.

Soil pH Effects. Altering soil pH had little effect on shoot yield, but it had a marked effect on shoot Ni concentration and, consequently, on the amount of phytoextracted Ni (Figure 1, Table 2). Ni concentration was increased by raising soil pH and decreased by lowering it. For *A. murale* grown on Welland soil for 120 days in the greenhouse, mean shoot Ni concentrations ranged from 11300 mg kg^{-1} at the highest pH to 4340 mg kg^{-1} at the lowest. On Quarry muck, shoot

TABLE 2. Effect of Soil pH Adjustment on Phytoextraction of Ni and Co by *Alyssum* Species Grown for 120 days on Ni-Contaminated Agricultural Soil

species	soil	final soil pH	shoot yield (g pot^{-1})	shoot Ni concn (mg kg^{-1})	phytoextracted Ni (mg pot^{-1})	shoot Co concn (mg kg^{-1})	phytoextracted Co (mg pot^{-1})
A. murale	Welland loam	4.97	22.7 f[a]	4340 def	101 de	88 b	2.0 b
		5.16	27.5 ef	9250 b	254 b	120 a	3.3 a
		5.46	28.0 ef	11300 a	315 a	93 b	2.6 b
	Quarry organic	5.40	28.8 def	2180 h	61 e	7.3 d	0.2 c
		6.04	40.9 ab	4600 de	190 c	6.0 d	0.3 c
		6.32	39.9 abc	4630 de	183 c	8.5 d	0.3 c
A. corsicum	Welland loam	5.02	35.6 bcd	3980 ef	140 cd	94 b	3.3 a
		5.30	33.2 cde	5470 d	183 c	101 ab	3.4 a
		5.68	45.4 a	7270 c	325 a	50 c	2.3 b
	Quarry organic	5.49	35.7 bcd	1890 h	69 e	6.5 d	0.2 c
		5.77	41.7 ab	2390 gh	97 de	4.0 d	0.2 c
		6.08	41.2 ab	4510 de	185 c	5.3 d	0.2 c

[a] Within each column, means followed by the same letter are not significantly different ($P < 0.05$ level) according to the Duncan−Waller *K* ratio *t*-test.

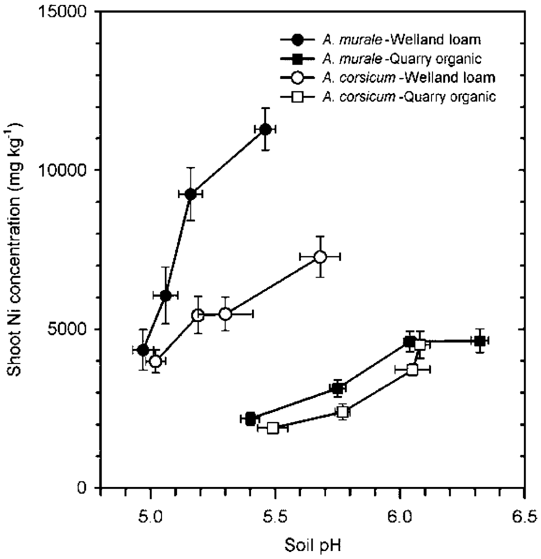

FIGURE 1. Effect of soil pH adjustment on phytoextraction of Ni by *Alyssum* species grown for 120 days on Ni-contaminated agricultural soil.

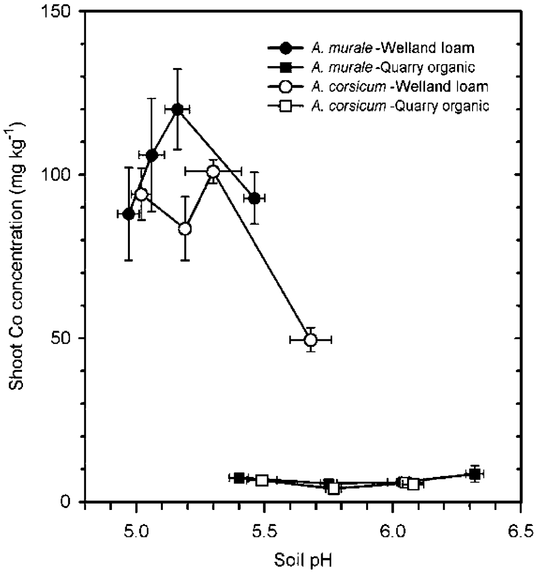

FIGURE 2. Effect of soil pH adjustment on phytoextraction of Co by *Alyssum* species grown for 120 days on Ni-contaminated agricultural soil.

Ni concentrations of *A. murale* ranged from 4630 mg kg^{-1} at the highest pH to 2180 mg kg^{-1} at the lowest. For *A. corsicum*, there was a similar effect of soil pH on Ni in shoots (Figure 1, Table 2). This result is opposite to the response seen in normal crop plants, which take up more metals at lower soil pH.

Soil pH did not have a significant effect on the very low level of Co uptake from the Quarry organic soil, but in the Welland soil, raising pH above the "as is" level by liming significantly *decreased* shoot Co concentration, which is opposite to the effect seen for Ni (Figure 2, Table 2). Shoot Ni and Co concentrations at the 60-day harvest were similar to the 120-day values.

Extent of Ni and Co Removal from Soil. In Table 3, the results for the amount of phytoextracted Ni and Co (from Table 2) are expressed as percentages of the amounts of those metals initially present in the experimental pots. Both *Alyssum* species were able to extract as much as 11% of the total Ni from the Quarry soil and as much as 5% of the total Co from the Welland soil. The amounts phytoextracted were an even higher percentage of the initial amounts of DTPA-extractable metal, which is a measure of the metal that is potentially plant-available. The amount of Ni phytoextracted by *A. murale* was equivalent to as much as 39% of the DTPA-extractable Ni and 57% of the DTPA-extractable Co. The corresponding decreases in the level of DTPA-extractable metal in the soil over the course of the experiment were 26% for Ni and 53% for Co.

Phosphorus and Magnesium Supplementation. For both soils and species, there was little to no effect of P fertilization on shoot yield, Ni concentration, or amount of phytoextracted Ni and Co (data not shown). There was a significant decrease in shoot Ni concentration only for *A. murale* on the Quarry organic soil, and the accompanying decrease in phytoextracted Ni was not significant. The highest rate of P fertilization did cause a decrease as compared to the control in shoot Co concentration for both species on the Welland soil, but the amount of phytoextracted Co was not significantly lower. For both soils and species, there was little to no effect of magnesium supplementation on shoot yield, Ni and Co concentration, and amount of phytoextracted Ni and Co (data not shown), although Mg concentration in *A. murale* shoots was low without Mg supplementation (as low as 817 and 943 mg kg^{-1} on the Quarry and Welland soils, respectively.

Soil Type and Plant Species. Both soil type and plant species significantly affected *Alyssum* shoot biomass, concentration of Ni in shoots, and amount of phytoextracted Co (Table 4). However, shoot Co concentration and amount of phytoextracted Ni were significantly affected only by soil

TABLE 3. Effect of Soil pH Adjustment on Removal of Ni and Co from Ni-Contaminated Agricultural Soil by *Alyssum* Species[a]

			amount of phytoextracted metal expressed as fraction of amount initially present in soil (%)			
species	soil	final soil pH	total Ni	total Co	DTPA-extractable Ni	DTPA-extractable Co
A. murale	Welland loam	4.97	2.1	3.5	9	40
		5.16	5.0	5.0	22	57
		5.46	6.3	3.9	27	45
	Quarry organic	5.40	3.7	1.1	13	22
		6.04	11	1.1	39	22
		6.32	10	1.3	36	25
A. corsicum	Welland loam	5.02	2.8	5.0	12	58
		5.30	3.6	4.8	15	56
		5.68	7.0	3.8	30	44
	Quarry organic	5.49	4.0	1.3	15	25
		5.77	5.5	0.7	20	13
		6.08	11	1.1	41	22

[a] The amount of phytoextracted metal is expressed as a percentage of the initial quantity of Ni and Co in the soil (either total or DTPA-extractable). Data are presented for three of the pH treatments and represent the sum of both harvests.

TABLE 4. Effect of *Alyssum* Species and Soil Type on *Alyssum* Phytoextraction Performance at 120-day Harvest[a]

factor	treatment	shoot yield (g pot^{-1})	shoot Ni concn (mg kg^{-1})	phytoextracted Ni (mg pot^{-1})	shoot Co concn (mg kg^{-1})	phytoextracted Co (mg pot^{-1})
Alyssum species	*A. murale*	30.6	6080	179	52.4	1.4
	A. corsicum	39.9	4940	189	48.7	1.6
	(*P*)	($<$0.001)	($<$0.001)	(ns[b])	(ns)	($<$0.01)
soil type	Welland	31.4	6900	210	94.9	2.8
	Quarry	39.2	4120	158	6.1	0.2
	(*P*)	($<$0.001)	($<$0.001)	($<$0.001)	($<$0.001)	($<$0.001)

[a] Averaged over all soil amendment treatments. [b] ns, difference not significant.

type. To summarize, in comparison with *A. murale*, *A. corsicum* had greater shoot yield, lower Ni concentration, the same Co concentration, and phytoextracted the same amount of Ni and more Co. *Alyssum* grown on Quarry organic soil had greater shoot yield, lower Ni concentration, and much lower Co concentration than *Alyssum* grown on Welland loam. More Ni and Co were phytoextracted from the Welland soil than from the Quarry soil.

Discussion

An unexpected result of this study was that Ni concentration in shoots of *A. murale* and *A. corsicum* grown on two Ni-contaminated agricultural soils was reduced at lower soil pH and increased at higher soil pH. This occurred even though plant-available Ni in the soil, as measured by levels of DTPA- and $Sr(NO_3)_2$-extractable Ni, decreased as pH increased. This unusual pattern of Ni accumulation in response to soil pH is opposite to the pattern of Ni uptake exhibited by crop plants, and it is also opposite to the pattern of accumulation of other divalent metals (e.g., Mn and Zn) by these Ni hyperaccumulator species. Shoot Mn concentration in *A. murale* grown on the Welland soil, for example, decreased from 150 mg kg^{-1} at the lowest pH to 29 mg kg^{-1} at the highest pH. In the few previous reports on the effect of soil pH on metal hyperaccumulation, only the usual response of more metal uptake at lower pH was seen. Robinson et al. (*17*), studying a naturally occurring population of the Ni hyperaccumulator *Alyssum bertolonii* on a serpentine soil in Italy, found that liming to pH 7.94 reduced Ni concentration in *A. bertolonii* in comparison with an unlimed control at pH 7.37. Two other reports, one on the Zn hyperaccumulator *Thlaspi caerulescens* (*25*) and one on the Ni hyperaccumulator *Berkheya coddii* (*26*), found that shoot metal concentration increased as soil pH was lowered to between 5.5 and 6.0 by sulfur application. Thus, the present study is the first to report that raising soil pH can increase metal concentration in a hyperaccumulator. Contrasting results obtained in previous studies may reflect use of different soils, pH range, and/or species.

Liming has been widely used to remediate metal toxicity to crop plants. Many studies have shown that application of lime to both Ni-rich serpentine and Ni-contaminated soils reduces Ni uptake by crops (*11*, *27*−*31*). Recently, Kukier and Chaney (*32*) conducted studies on the use of lime to remediate the Ni toxicity of Ni-contaminated Welland and Quarry soils obtained from the same vicinity as the soils used in this study. They reported that plant shoot Ni concentration decreased with increased soil pH in 11 crop species (oat, wheat, corn, barley, ryegrass, radish, soybean, bean, tomato, Swiss chard, and beet) grown on the Welland soil and in oat and beet grown on the Quarry soil. This finding confirms that plant-available Ni is reduced following lime application to these soils. Thus, the *Alyssum* response does not depend on unusual phytoavailability of the Ni in the industrially contaminated soils that were studied.

Factors that may be involved in the response of *Alyssum* to changes in soil pH in this study include:

(i) *Alyssum* evolved on and is adapted to serpentine soils in the eastern Mediterranean, most of which have a pH between 6 and 8.5. It is possible that the efficiency of Ni transport channels in the root is reduced at lower soil pH.

(ii) Increased solubility of other divalent cations at low pH may compete with Ni for uptake.

(iii) Both of the study soils are high in organic matter, which binds Ni fairly strongly. As pH rises, organic matter becomes more soluble, which would increase the mobility of organically bound Ni. It is possible that *Alyssum* can access organically bound Ni that is inaccessible to other plants.

(iv) The level of hydrous iron and manganese oxides in the test soils is low. These oxides are responsible for much of the increased binding of Ni to the soil matrix at higher soil pH.

(v) There is some evidence that histidine is involved in hyperaccumulation of Ni by *Alyssum* (*33*). If *Alyssum* uptake of Ni is facilitated by release of histidine or other organic acids into the rhizosphere, these compounds may be more effective at complexing with and mobilizing soil Ni at higher soil pH.

Further research on the relation of soil pH to Ni acquisition and accumulation by *Alyssum* species is necessary in order to better understand this possibly complex phenomenon. Of immediate practical concern is an extension of this study to a wider range of soil pH values. This study focused on the low end of the pH scale because of our expectation that maximum Ni uptake would occur in that region.

Neither species of *Alyssum* hyperaccumulated Co—defined by Baker and Brooks (*34*) as accumulation of at least 1000 mg of Co kg^{-1} in the shoot—in this study, although Co concentrations in plants grown on the Welland soil were higher than those found in crop plants (<5 mg kg^{-1}) grown in similar Port Colborne soils (*35*). Malik et al. (*36*) reported that both *A. murale* and *A. corsicum* hyperaccumulated Co from a low-Ni sandy soil to which Co salt had been added. The relatively low accumulation of Co seen in this study can be attributed to the soils' high concentrations of organic matter, which binds Co, and of Ni, which competes with Co for plant uptake.

A number of crop plants are considered highly susceptible to limestone-induced Mn and P deficiency in high Ni soils as well as in organic soils (*32, 37*). In this study, both of the Ni-contaminated soils had adequate P supply for *Alyssum* growth without supplementation. It had been considered possible that high rates of P fertilization might lower Ni uptake by *Alyssum* because of the insolubility of nickel phosphates. In this study, the highest rate of P fertilization did cause a decrease as compared to the control in *A. murale* shoot Ni concentration on the Quarry soil and in shoot Co concentration for both species on the Welland soil, although the amounts of phytoextracted metal were not significantly lower. Plants grown on the Quarry soil had low Mn concentrations, and symptoms of Mn deficiency were apparent in *A. corsicum* growing on the limed treatments. Thus, it will be necessary to fertilize the Quarry soil with Mn, especially if liming to maximize Ni uptake.

The importance of Ca and Mg in regulating Ni uptake for *Alyssum* and nonaccumulator species has been recognized (*38*−*41*). The serpentine soils on which *Alyssum* evolved are high in Mg and low in Ca, with an extractable Ca:Mg ratio of about 0.2. The soils in this study, however, have high Ca:Mg ratios of about 6. It was considered possible that the high level of extractable Ca in these soils might reduce Ni uptake by *Alyssum*. We observed that Mg supplementation had no effect on the level of extractable Ca at the end of the experiment, nor did it affect *Alyssum* shoot yield, Ni and Co concentration, and amount of phytoextracted Ni and Co. Nevertheless, shoot Mg reached deficient levels, as low as 817 mg kg^{-1} in *A. murale*, without Mg supplementation (the normal range for Mg in plant shoots is 2500−10 000 mg kg^{-1}). Mg may be supplied to these soils by the use of dolomitic limestone.

The significant differences that were observed between the two *Alyssum* species for shoot biomass, concentration of Ni in shoots, and amount of phytoextracted Co, while indicating that genetic variations exist between these two Ni hyperaccumulator species, are of doubtful relevance to phytoextraction in the field. During this 4-month pot experiment, both species remained in the vegetative stage, in which *A. murale* has a prostrate growth habit. *A. murale* must be vernalized and enter the reproductive growth stage before it grows tall enough to be easily harvested using mechanical methods. Furthermore, both species need to be evaluated in the field under prevailing environmental conditions before their suitability for use in phytoremediation can be determined.

Phytoextraction performance of *Alyssum* on the Welland loam was consistent with that achieved in a related pot experiment in which the same *Alyssum* lines were grown on 18 serpentine soils from Oregon and Maryland. *Alyssum* grown on Quarry organic soil had greater shoot yield but lower Ni concentration and much lower Co concentration than *Alyssum* grown on Welland loam. The differences can be attributed to the Quarry soil's very high level of organic matter (72% vs 17% for the Welland soil). Soil organic matter binds strongly to heavy metals, including Ni and Co, making them less available for plant uptake. Although *Alyssum* did not accumulate high concentrations of Ni from the Quarry soil, it is remarkable that it was able to remove up to11% of the total Ni that was initially present in this soil. In the Welland soil, *Alyssum* removed as much as 5% of the total Co. The amount of phytoextracted metal was equivalent to an even larger percentage of the amount of DTPA-extractable metal initially present in the soil (up to 41% of the Ni and 58% of the Co). For many metals, the DTPA-extractable pool is considered to be a measure of the amount of potentially plant-available metal in the soil. In this study, the decrease in DTPA-extractable metal during the course of the experiment was frequently very close to the amount of metal phytoextracted. In some cases, more metal was phytoextracted than was lost to the DTPA-extractable pool, which suggests that the DTPA-extractable pool was replenished during the experiment.

The results of this study suggest that phytoremediation using *Alyssum* is a promising method for remediation of Ni-contaminated soil and that soil management will be an important component of the technology.

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