Availability of nickel in soils for the hyperaccumulator Alyssum murale (Waldst. Kit.)

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This study was designed to investigate the potential of *Alyssum murale* to extract metals from pools in the soil that are not available to plants. Two experiments were carried out. To establish the effect of phosphorus deficiency on Ni uptake, *A. murale* was grown on a serpentine soil from Albania (total Ni concentration: 1 316 mg kg^{-1}) with increasing rates of phosphate fertilization (0–100 mg kg^{-1} P). A second experiment tested whether *A. murale* and red clover (*Trifolium pratense* L. cv. Violetta, a non-accumulating plant) had access to the same labile Ni pools in different soils. The two species were grown on three soils, the Albanian serpentine soil used in the first experiment and two agricultural soils from northeastern France (total Ni concentration: 24 and 33 mg kg^{-1}). Labile Ni had been labelled with ^{63}Ni in all soils before culture. The isotopic composition (IC_P, i.e. specific activity) of the Ni taken up by the two species was calculated. Phosphate fertilization had no significant effect on Ni uptake by *A. murale.* Plant IC_P was identical for the two plant species on each of the three soils, showing that *A. murale* takes up Ni from the same labile pool of Ni in soils as red clover. Ni uptake by *A. murale* ranged from 2% of labile Ni on the serpentine soil to 17% on one of the agricultural soils. We concluded that the accumulation of Ni by *A. murale* might not be due to the solubilization of unavailable forms of Ni in soils. Results allowed us to draw some practical conclusions on the use of *Alyssum* species in the phytoextraction of soil Ni.

Introduction

Hyperaccumulator plants have the ability to take up large amounts of metals from soils. They have received much attention since the 1990s[1–3] as potential tools for the remediation of soils contaminated with trace metals. Calculations based on field experiments have shown that a small number of successive crops of such hyperaccumulators would be sufficient to remove excess metals from soils.[1] These calculations rely on the assumption that hyperaccumulators have a specific ability to extract non-mobile forms of metals from soils. However, this has not been demonstrated so far. *Alyssum murale* (Brassicaceae) is a widespread nickel hyperaccumulator for which concentrations up to 1% of Ni have been recorded in shoots when grown on Ni-rich soils.[4]

To assess the potential of hyperaccumulators for the remediation of metal-contaminated soils, we investigated whether soil deficiency in some nutrients such as phosphorus enhances the acquisition of Ni by the hyperaccumulator *A. murale* and whether *A. murale* and red clover, a non-accumulator plant, take up Ni in the same pool of soil labile Ni.

Materials and methods

Field sampling and seed collection. A field survey was done in Albania in serpentine and metal-industry areas.[5] Two of the *Alyssum* species that were collected were Ni-hyperaccumulators: *Alyssum murale* and *Alyssum markgrafii* O.E. Schulz. *A. murale* was the most abundant species and was localized in three serpentine areas. Seeds were collected for this study. Ni content in the shoots of the plants collected in the field reached 1508 mg kg^{-1}. Seeds of red clover (*Trifolium pratense* cv. Violetta) were obtained from a French seed producer (Clause).

Soils. The A_p horizons (0–20 cm deep) of one serpentine soil from the industrial area of Prrenjas (eastern Albania) where the *A. murale* seeds had been collected[5] and of agricultural soils from Nancray (calcic cambisol) and Vannecourt (luvisol) in northeastern France were sampled. Soil samples were air-dried and sieved with a 5-mm mesh. Ni concentration in the serpentine soil (1316 mg kg^{-1}) was much higher than in the two other soils: total Ni content was 24 mg kg^{-1} for the luvisol and 33 mg kg^{-1} for the cambisol (Table 1). The two agricultural soils displayed very different values of the physico-chemical parameters that control Ni phytoavailability (e.g. pH, clay content, organic matter content).

Phosphorus fertilization. Increasing amounts of phosphorus were added to 6-kg lots of the serpentine soil: 0, 0.01, 0.1, 1, 10 and 100 mg kg^{-1} P, supplied as NaH_2PO_4. Soils were thoroughly mixed manually. Each of the treatments was also fertilized with 66.6 mg N kg^{-1} and 186.0 mg K kg^{-1} supplied as KNO_3 and fractionated into three equal additions: before sowing the seed, 30 days after seed germination, and 60 days after seed germination. There were five replicates for each treatment: *A. murale* seed was sown in five pots containing 1 kg soil each. Pots were placed in a growth chamber (16/26°C, 8 h dark/16 h light, 500 μmol photons m^{-2} s^{-1} light intensity and 75% air humidity) and their moisture content was maintained at 80% of the water-holding capacity by

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Table 1. Physico-chemical properties of the three soils. Physico-chemical determinations were all made according to French norms on soil quality.[12]

	Serpentine	Cambisol	Luvisol
Clay fraction (%)	24.4	33.0	19.4
Fine silt fraction (%)	17.4	34.1	21.9
Coarse silt fraction (%)	11.4	21.0	23.7
Fine sand fraction (%)	19.8	1.7	29.6
Coarse sand fraction (%)	27.0	2.8	6.0
pH (1:2.5 soil:water suspension)	8.3	7.0	5.8
pH (KCl)	7.8	6.3	–
Organic C (mg g^{-1})	9.9	42.5	16.1
Organic matter (mg g^{-1})	17.0	73.3	27.8
Total N (mg g^{-1})	0.88	4.30	1.90
Exchangeable Mg^{2+} (mg g^{-1})	0.80	0.10	0.43
Exchangeable K^{+} (mg g^{-1})	0.29	0.27	0.12
Exchangeable Ca^{2+} (mg g^{-1})	10.90	4.87	1.62
Exchangeable Na^{+} (mg g^{-1})	0.20	0.01	0.01
CEC (Metson, meq $(100\ g)^{-1}$)	134	259	110
Total Ni (mg kg^{-1})	1316.0	32.8	24.2

daily watering to weight using distilled water. Seven days after germination, seedlings were thinned to 20 per pot 15 days after germination. *A. murale* was harvested twice (10 seedlings after 90 days and the remaining five after 210 days). Plants were mineralized through acid digestion and total Ni was measured (ICP-AES). At the time of harvest, soil samples from each treatment were taken from the pots (five replicates); samples from unplanted soils (three replicates) were taken at the beginning of the culture. Samples were air-dried and Ni was extracted with DTPA.[6,7]

Isotope-labelled soils. For each of the three soils, two 5-kg lots were fertilized with 50 mg kg^{-1} N, 50 mg kg^{-1} P and 50 mg kg^{-1} K) and labile Ni was labelled with a $^{63}NiCl_2$ (in 0.5 M HCl) solution (New England Nuclear) in which the specific activity of Ni was 4.63×10^{11} Bq g^{-1}(1 Becquerel 'Bq' = 1 disintegration per second). Soils were thoroughly manually mixed to ensure homogeneity. Radioactivity in the soils was 640 kBq kg^{-1}. Soils were then placed into plastic pots containing 1 kg of soil each in which either red clover or *A. murale* were sown (six final treatments) the following day. Treatments were replicated five times. Pots were placed in a growth chamber (16/26 °C, 8 h dark/16 h light, 500 μmol photons $m^{-2}\ s^{-1}$ light intensity and 75% air humidity) and their moisture content was maintained at 80% of the water holding capacity by daily watering to weight using distilled water. Seedlings were thinned to four in each pot 14 days after germination.

Two plants from each pot were harvested after a 60-day growth period; the two remaining plants were harvested after 90 days. Plants were mineralized through acid digestion, total Ni was measured in all the treatments and radioactivity was counted by liquid scintillation spectrometry. The isotopic composition (i.e. specific activity) of Ni in plant shoots, IC_P (Bq μg^{-1} Ni), was calculated for each treatment.[7]

$$IC_P = (r_P/R_P)/Ni_P, \qquad [1]$$

where r_P is the radioactivity in plant tissues, R_P the total radioactivity added per kg of soil and Ni_P the quantity of Ni taken up by aerial plant parts per kg of soil. Isotopic compositions are calculated by giving R_P the arbitrary value of 1 Bq kg^{-1}. This allows comparison of IC values between different treatments and experiments even though the R_P value differs among experiments. Specific activity, which is the parameter generally used by researchers in such isotopic studies, is specific to experimental conditions and cannot be compared between experiments.

Soil-labile Ni, L_{Ni}, was also calculated for each treatment according to Larsen.[8]

$$L_{Ni} = (R_P/r_P)/Ni_P = 1/IC_P. \qquad [2]$$

L_{Ni} is the quantity of Ni available to plants (mg per kg soil), in which ^{63}Ni has been isotopically diluted for the period of time considered.

Statistical analysis. Analysis of variance was performed on the data from both experiments with the software STATBOXPRO™ (GRIMMER Logiciels, France). The Newman-Keuls test was used to determine the significant differences between treatments at the 5% level.

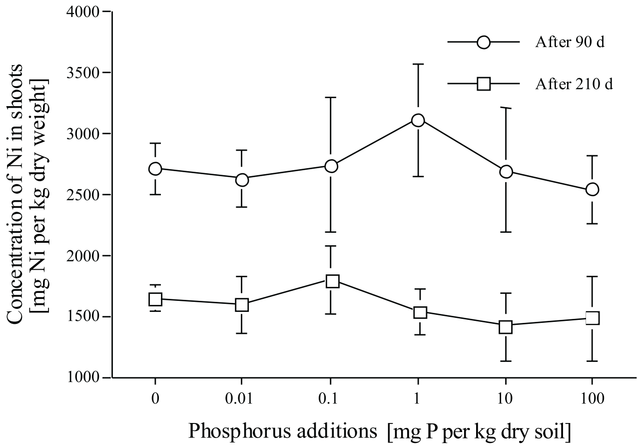

Fig. 1. Effect of phosphate fertilization on the uptake of nickel by *Alyssum murale* grown on a serpentine soil from an industrial area in Albania. Standard deviation is represented by error bars (n = 5).

Results

Effect of increasing P fertilization on Ni availability and uptake

Dry matter yield ranged from 1.3 to 2.4 g kg^{-1} of soil at first harvest and from 2.9 to 5.1 g kg^{-1} of soil at second harvest and was not significantly affected by the increase of phosphate addition to the serpentine soil. Plants reached a height of 10–15 cm after 90 days. The concentration of Ni in shoots of *A. murale* ranged from 1137 to 2137 mg kg^{-1} at first harvest (90 days) and from 1974 to 3636 mg kg^{-1} at second harvest (210 days) (Fig. 1). Again, there was no significant effect of the phosphate fertilization. Total Ni uptake by plants was higher at second harvest as a result of better development of plants and continuous accumulation of Ni during plant growth. The total cumulative uptake of

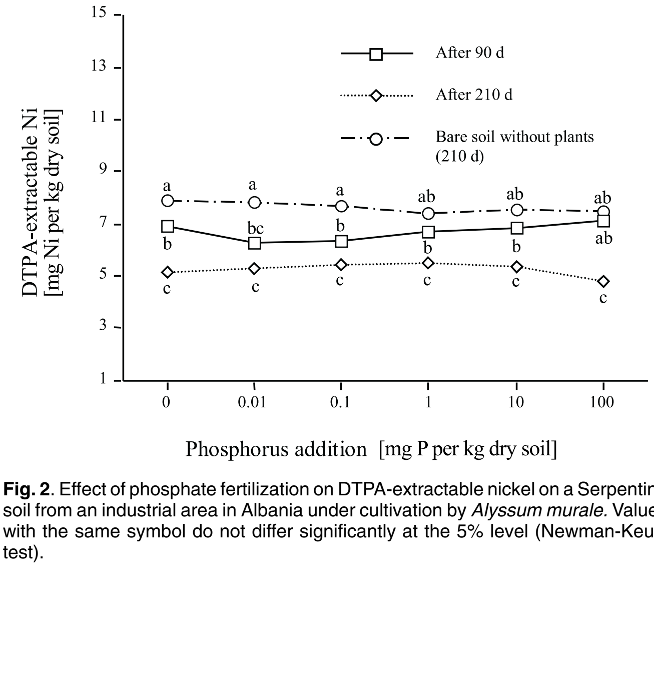

Fig. 2. Effect of phosphate fertilization on DTPA-extractable nickel on a Serpentine soil from an industrial area in Albania under cultivation by *Alyssum murale.* Values with the same symbol do not differ significantly at the 5% level (Newman-Keuls test).

Table 2. Comparison of the isotopic composition of Ni taken up by *Alyssum murale* and *Trifolium pratense* grown on three soils. Values with the same symbol do not differ significantly at the 5% level (Newman-Keuls test). In units of 10^{-2} Bq µg^{-1}.

	A. murale		*T. pratense*	
	60 days	90 days	60 days	90 days
Serpentine soil	2.1†	2.5†	1.4†	2.3†
Cambisol	48.3*	40.5*	16.0*	39.8*
Luvisol	41.5*	38.5*	29.9*	36.4*

Table 3. Uptake of stable and radioactive Ni by *Alyssum murale* and *Trifolium pratense* on three soils previously spiked with ^{63}Ni. Results are given as mean values and standard deviations are given between brackets ($n = 5$). For L_{Ni}, values with the same symbol do not differ significantly at the 5% level (Newman-Keuls test).

		Total Ni uptake (µg kg^{-1})	r_P/R_P (%)	L_{Ni} (µg kg^{-1})
Serpentine	*A. murale*	1263 (761)	1.84 (0.21)	68.6*
	T. pratense	48 (13)	0.10 (0.03)	48.5*
Cambisol	*A. murale*	195 (58)	6.93 (1.36)	2.8†
	T. pratense	11.3 (4.6)	0.32 (0.08)	3.5†
Luvisol	*A. murale*	688 (303)	17.5 (5.7)	3.9†
	T. pratense	21.3 (3.7)	0.68 (0.08)	3.1†

Ni from the soil ranged from 10.1 to 19.7 mg per kg dry soil and showed no significant effect of phosphate additions, although there was a slight increase, probably due to a very limited positive effect on biomass yield. The amount of DTPA-extractable Ni in the soil significantly decreased with time of cultivation from approximately 7.5 mg kg^{-1} at the beginning to approximately 5 mg kg^{-1} after a 210-day culture (Fig. 2).

Comparison of uptake behaviour between *A. murale* and *T. pratense*

IC_P values were statistically different between the serpentine soil and the two agricultural soils regardless of the species but were not significantly different between the two species on a given soil (Table 2). Therefore, on the three soils, red clover and *A. murale* took up Ni from the same labile pool (Table 3). Ni uptake differed strongly between the two species, from 195 μg per kg dry soil on the cambisol to 1263 μg per kg dry soil on the serpentine soil for *A. murale* and from 11 μg per kg dry soil on the cambisol to 49 μg per kg dry soil on the serpentine soil for red clover (Table 3). On the luvisol, Ni uptake by *A. murale* represented almost 3% of initial total Ni in the soil. The radioactivity taken up by the plant (r_P/R_P) represents the fraction of the labile pool that has been taken up by the plant; it ranged from 2 to 17% for *A. murale* and from 0.1 to 0.7% for red clover (Table 3). This means that 17% of labile Ni (L_{Ni}), i.e. the phytoavailable pool, was removed in 90 days by *A. murale* grown on the luvisol whereas only 2% was taken up by this species on the serpentine soil.

Discussion

Although phosphorus deficiency was suspected of enhancing Ni uptake by *A. murale*, as has been shown for other plants such as white lupin (*Lupinus albus* L.),[9] no such effect could be measured. The difference of DTPA-extractable Ni in the three soils does not explain the total amount of Ni taken up by *A.* murale. However, these results suggest that *A. murale* takes up Ni from a pool of soil Ni that can be at least partly quantified using DTPA. This is confirmed by DTPA previously having been shown to extract isotopically-exchangeable Ni, i.e. Ni from the labile pool.[7] By reducing the DTPA-extractable pool of Ni in the soil after culture, *A. murale* limited the potential for successive cultures to remove Ni from the soil.

A. murale took up Ni from the same isotopically-exchangeable pool as did red clover, a non-accumulator species. We conclude from these results that Ni uptake by *A. murale* might not result from special mechanisms of mobilization of unavailable Ni, because the behaviour of *A. murale* was not different from that of red clover. This was shown on three soils that displayed very different geochemical conditions and total concentrations of Ni. *A. murale* had a much higher uptake efficiency on the two agricultural soils. This may be due to an increased ability to take up Ni at low concentrations in the soil solution, compared to non-accumulator species. This has already been confirmed for Zn and Cd uptake by species of *Thlaspi*.[10,11] On the luvisol, the rate of uptake of Ni from the labile pool was high for both species. The low pH of this soil resulted in a relatively high labile pool for a soil with a low total Ni content. This may be partly responsible for the high uptake efficiency, especially in the case of *A. murale.* The rate of uptake of Ni by *A. murale* from the labile pool in the serpentine soil was poor. We think that the fraction of labile Ni extracted would have been greater if the plants had been allowed to grow for longer periods on the serpentine soil. In the first experiment the uptake of Ni on the serpentine soil reached 19 mg per kg dry soil).

Phytoextraction must be defined from these results as the depletion of the labile pool of Ni and not of the total Ni in the soil. Hence, phytoextraction of stable unavailable forms would be a useless and time-consuming technique as plants take up Ni exclusively from the isotopically-exchangeable pool. Phytoextraction of Ni using *A. murale* was shown to be very successful in the case of agricultural soils with a low pH. On soils with highly labile Ni content such as the serpentine soil, a high concentration of Ni adversely affects the efficiency of Ni uptake.[10,11]

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