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Comparison of the chemical changes in the rhizosphere of the nickel hyperaccumulator *Alyssum murale* with the non-accumulator *Raphanus sativus*

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Abstract

Changes in pH and redox potential were studied in the rhizosphere soil of a nickel hyperaccumulator plant (*Alyssum murale*) and of a crop plant, radish (*Raphanus sativus*). Differences in rhizosphere pH and reducing activity were found between the lateral and the main roots of both species, but the pH changes in the rhizosphere were similar in both species. Changes in pH were associated with the relative uptakes of cations and anions; whether the concentrations of heavy metals in the growth medium did not have any effect on the rhizosphere pH. The source of nitrogen (ammonium or nitrate) was the major factor determining the pH of the rhizosphere of both species. The redox potential of the rhizosphere was influenced by both the N-source and the concentrations of heavy metals. When heavy metals were not present in the growth medium, and nitrate was the N-source, the reducing capacity of *A. murale* roots was enhanced. However, the reducing activity of *A. murale* was always smaller than that of radish. Therefore, the mechanism of metal solubilization by the hyperaccumulator plant does not involve either the reduction of pH in the rhizosphere or the release of reductants from roots. The acidification and reducing activity of the roots of *A. murale* was always smaller than that of *R. sativus*.

Introduction

Addition of organic materials which are contaminated with heavy metals, like sewage sludge, can lead to a build-up of metals in soils. Accumulation of heavy metals in compost is, in conjunction with ammonia volatilization, an important problem associated with the composting of organic wastes. Due to the decomposition of the organic material during the composting process, the concentrations of heavy metals in the resulting end product is usually greater than in the original material. Thus composts produced from organic wastes can also pollute soils to which they are added.

Extremely high uptake (hyperaccumulation) of potentially phytotoxic metallic elements is a phenomenon which has been found in a wide range of plant families for cadmium, cobalt, copper, lead, manganese, nickel and zinc (for review see Baker and Brooks, 1989). Accumulator plants are almost invariably species endemic to metal-enriched soils where few other metal-tolerant plants are able to grow and where extreme metal uptake is most probably a corollary of their mechanisms of metal tolerance (Baker, 1987).

It has been suggested for many years that Ni may play an essential role in metabolic processes of higher plants (Brown et al., 1987). In 1975 it was found that Ni is a component of the enzime urease in beans (Dixon et al., 1975). Claims were made that Ni was essential for all legumes and possibly all other higher plants (Eskew et al., 1984), but tests for essentiality in non-legumes were not reported until 1987 (Brown et al., 1987). The latter authors state that Ni deficiency is unlikely in conventional experiments with culture

solutions because the levels of Ni present as contaminants in nutrient salts and water will be enough to satisfy the plant's requirements. By implications it is unlikely that deficiency would occur in plants grown in soil, because the small amounts necessary should be available. The concentration of Ni in the leaves of hyperaccumulator plants exceed greatly the mean levels of Ni in "normal" plants, e.g. grasses range from around 0.1 to 1.7 mg kg^{-1} (dw), clovers from 1.2 to 2.7 mg kg^{-1} (dw), vegetables from 0.2 to 3.7 mg kg^{-1} (dw) and the Ni content of all cereals averaged 0.5 mg kg^{-1} (dw) (Kabata-Pendias and Pendias, 1992). Generally the range of excessive or toxic amounts of Ni in most plant species is from 10 to 100 mg kg^{-1} (dw) (Kabata-Pendias and Pendias, 1992). Hyperaccumulation of nickel, defined as concentrations > 1000 $\mu g\ g^{-1}$ (0.1 %) Ni in the leaf dry matter of plants, is a phenomenon found only in relatively few species restricted to ultramafic (serpentine) soils (Baker and Brooks, 1989; Reeves, 1992). Of the 243 nickel-hyperaccumulating taxa reported to date 76 are in the family Brassicaceae; these are largely confined to the genera *Alyssum* (48 taxa) and *Thlaspi* (23 taxa). Some *Alyssum* and *Thlaspi* species can accumulate up to 3 % Ni in leaf dry matter (Reeves, 1992). Other species of the latter genus have been shown to accumulate >2 % Zn and up to 0.5 % Pb and 0.1 % Cd from lead/zinc mineralized soils. According to Brooks et al. (1981) the tolerance of Ni hyperaccumulators is mainly achieved by complexation of Ni with malic and malonic acids in the stem and leaves. However, the ecological significance of metal hyperaccumulation and the mechanisms involved in the mobilization and accumulation metals from the rhizosphere soil are largely unknown (Boyd and Martens, 1992). However, the phenomenon has presented possibilities for exploitation in the bioremediation of metal-polluted soils and organic substrates (Baker et al., 1991; McGrath et al., 1993).

An understanding of the mechanisms by which hyperaccumulator plants solubilize and absorb heavy metals is important as it may help to pinpoint ways of improving the efficiency of removal of metals from compost or soil. Factors of major importance to the solubilization of metallic elements by plants are: 1) root-induced changes in pH of the rhizosphere, 2) increased reducing capacity of the roots, and 3) the amount and composition of root exudates (Marschner et al., 1987). Increases and decreases of the rhizosphere pH can be due to the differences in the balance of cation/anion uptake as a consequence of the nitrogen source (nitrate or ammonium, respectively), and the nutritional status (e.g. Fe or P deficiency) (Dinkelaker et al., 1989; Marschner et al., 1986). Absorption of P, Fe, Zn, and heavy metals by plants can be affected by changes in rhizosphere pH. Acidification of soil increases the concentration of Fe, Mn, Zn and Co in the soil solution (Sanders, 1983), as well as that of inorganic phosphates (Grinsted et al., 1982). Hydrous Mn and Fe oxides are thought to control the availability of heavy metals in soils by providing sites for their sorption. Reduction of these oxides would be expected to result in a release of these adsorbed heavy metals. The aim of this paper was to study pH and redox changes in the rhizosphere of plants growing in heavy metal-contaminated substrates, as two potential mechanisms of heavy metal solubilization prior to absorption by hyperaccumulactor plants. The influence of the N-source and the presence of heavy metals on these changes were also tested.

Materials and methods

Experiments in soil and compost

A soil and a compost with high heavy metal contents were used as substrates in this experiment. The soil had received additions of sewage sludge from 1942–1961, which resulted in the elevated concentrations of heavy metals (Table 1). The compost was made from a mixture of 60 % sewage sludge highly contaminated with heavy metals and 40 % city refuse. In order to improve the physico-chemical properties and reduce the salinity of the compost, a 50 % (v/v) compost:peat mixture (equivalent to 10:3 w/w) was prepared. Minirhizotrons with a 6 × 3 cm base, 6 × 7 cm top and 12 cm high were filled with the two substrates (soil or compost + peat). Fertilizer was added to supply 175 mg N kg^{-1} soil as nitrate, 265 mg K kg^{-1} and 50 mg Mg kg^{-1}, and the soil and compost+peat were kept at 60 % of their water holding capacity by adding deionized water. Radish (*Raphanus sativus* L. cv. French Breakfast) and *Alyssum murale* Waldstein and Kitaibel (Brassicaceae) were grown in separate rhizotrons (one plant per rhizotron) and all treatments were replicated three times. Radish has been used to estimate the bioavailability of heavy metals in soils (Davies and Houghton, 1984), and *A. murale* has been shown to be a hyperaccumulator of nickel (Baker and Brooks, 1989). Radish was grown twice: in the first experiment 330 g of soil or 130 g of compost+peat were added to minirhizotrons; in the second experiment the amount of soil and compost+peat were reduced (293 and 122 g

Table 1. Chemical analysis of the growth media

	Soil	Compost
pH (H_2O)	7.0	7.2
pH (KCl)	6.6	7.1
Electrical conductivity (S m^{-1})	0.006	0.298
Organic C (%)	19.0	163.7
Total N (g kg^{-1})	1.3	11.3
NH_4-N (mg kg^{-1})	4	160
NO_3-N (mg kg^{-1})	13	497
Total P (g kg^{-1})	1.8	16.3
K (g kg^{-1})	2.2	3.0
Ca (g kg^{-1})	3.9	147.9
Mg (g kg^{-1})	1.3	6.0
Na (g kg^{-1})	0.2	2.0
Fe (g kg^{-1})	22.0	33.2
Zn (mg kg^{-1})	285	4848
Cu (mg kg^{-1})	85	536
Ni (mg kg^{-1})	24	402
Cd (mg kg^{-1})	6	12
Cr (mg kg^{-1})	100	853
Pb (mg kg^{-1})	93	812
Water holding capacity (g kg^{-1})	480	1233[a]

[a]Mixture compost+peat 50 % v/v.

respectively). Plants of *A. murale* were grown only in the second experiment. Seed of the latter was obtained from an extensive population growing on serpentine (ultramafic) soil at Panórama, Thessaloniki, N. Greece. The experiments were carried out in a temperature-controlled glasshouse, with 20–15°C day/night and 14 h day length.

The pH of the rhizosphere of intact plants was measured during the growing period (see below). Plants were harvested after 89 days in the first experiment and 57 days in the second. Leaves and hypocotyls of radish were separated, and plant material weighed, before and after washing and drying at 80°C. Chemical analysis was performed after grinding the dry material.

Experiments in solution culture

In a further experiment, plants were grown in solution culture to study whether the N-source or heavy metal concentration in the growth medium changed the pH or redox potential in the rhizosphere.

Four treatments were used: NH_4-N or NO_3-N, in combination with two levels of heavy metals. The full-strength nutrient solution had the following composition (m*M*): 0.4 KH_2PO_4, 0.4 K_2SO_4, 1.2 $CaCl_2$, 0.6 $MgSO_4$, 20×10^{-3} FeEDTA, 0.04×10^{-3} $MnSO_4$, 2.0×10^{-3} H_3BO_3, 0.04×10^{-3} $CuSO_4$, 1.2×10^{-3} Na_2MoO_4, 1.2×10^{-3} $CoCl_2$, adjusted to pH 6.0 with dilute NaOH. In the treatment with NO_3-N, 0.4 m*M* $Ca(NO_3)_2$ was present, and in the NH_4-N treatment this was substituted by 0.4 m*M* $(NH_4)_2SO_4$. In the treatment with heavy metals 0.8, 0.1 and 0.1 mg l^{-1} of Zn, Cd and Ni were added to the nutrient solution in sulphate form (12, 0.88 and 1.7 μM respectively), or not added in the treatment without heavy metals.

Thirteen-day-old seedlings of radish (*R. sativus*) were grown in the nutrient solution for 4 days. Because *A. murale* grew slower than radish, 24-day-old seedlings of *A. murale* were grown for 8 days in a half strength nutrient solution, and then for 7 days at full concentration. The nutrient solutions were replaced every 2-days and the treatment with NH_4-N was tested for the absence of NO_3-N.

After harvest, roots of intact plants were carefully washed with deionized water and submerged into a fluid agar medium in Petri dishes and cooled to obtain a solid substrate. Agar (0.75 %) was prepared with each of the different nutrient solutions referred to above. To assess pH changes, bromocresol purple indicator (final concentration 0.006 %) was mixed into the agar medium and adjusted with NaOH to pH 6.5 (red-purple) for treatments with NH_4-N, and to 6.0 (red) when NO_3 was the N-source. To make the reducing processes at the root surface visible, agar was mixed with Fe (III) (FeEDTA, 0.1 m*M*) and BPDS (4, 7, diphenyl -1, 10 phenantrolinedisulfonic acid) (0.3 m*M*), which forms a red coloured complex with Fe(II). The reducing activity was also tested in a system that depended on the reduction of MnO_2 to Mn(II). To prepare this, an agar medium without Fe was mixed with $KMnO_4$ (1 m*M*), kept at 50°C and the MnO_2 formed was dispersed in the agar (Marschner et al., 1982). The agar media were sealed with transparent plastic film to avoid water loss, and covered with black plastic sheets to protect the roots from light, but the shoots were illuminated.

Analytical methods

Analyses of the soil and compost were performed as follows: pH in 1:10 water and KCl suspensions, electrical conductivity in 1:10 water extracts, total nitrogen and organic carbon in a Carlo-Erba automatic microanaliser (Navarro et al., 1991), inorganic nitrogen in 2 *M* KCl extracts. Total P, K, Ca, Mg, Na

and heavy metals were determined by inductively-coupled plasma emission spectrometer after digestion with HCl/HNO_3 for soil and compost, or with $HNO_3/HClO_4$ for plant material (Zhao et al., 1994). The buffering capacity of the soil or compost+peat mixture was determined by measuring the pH after addition of 50 mL KCl and either HCl or KOH solutions (0.1 *M*) to 5 g samples and 4 days equilibration (Hartikainen, 1986). The rhizosphere pH was assessed in the minirhizotrons with the pH indicator bromocresol purple in agar and also measured using selective microelectrodes. Fine agar films (1–2 mm) were prepared by mixing 0.75% agar and 0.006 % bromocresol purple adjusted to pH 6.0 with NaOH (Marschner et al., 1982), pouring the agar into moulds at 40°C, and allowing them to solidify by cooling. The agar film was then placed on the rhizosphere exposed by the removable side wall of the minirhizotron, and pH changes were observed by changes in colour of the indicator. The pH in the different zones of the rhizosphere and in the bulk soil was measured with proton-selective microelectrodes (tip diameter 10–50 μm). Double-barrelled proton-selective microelectrodes were prepared by the method of Miller and Smith (1992), with a proton-selective mixture (Fluka 95297; Chao et al., 1988) in the designated ion-selective barrel. The proton-selective cocktail (62 %) was mixed by weight with 28 % high-molecular-weight polyvinylchloride and 10 % nitrocellulose all dissolved in approximately 4 volumes of tetrahydrofuran (Miller and Smith, 1992). A salt bridge made from a plastic tube filled with saturated KCl in agar was inserted into the bulk soil 2–5 cm away from the root area to be measured. Electrical contact with the soil was made via a porous glass frit insert into the end of the salt bridge tubing. Before and after making each rhizosphere measurement the proton-selective microelectrodes were calibrated in pH buffer solution (4 to 8) using the buffers phosphate, MES, MOPSO or TES depending on the pH (Reid and Smith, 1988). pH measurements were made at 0 to 0.5 mm from the main and lateral roots, and in the bulk soil. The results were only accepted when the pH calibration did not significantly change before and after the measurement. Measurements near the root tips were difficult due to the tendency of the growing tip to protrude from the soil-side wall interface.

Results

Production and composition of plants

In both experiments, greater leaf production was observed in radish grown in compost than for those grown in soil (Table 2), although the reverse was true for *A. murale* because this plant was very difficult to grow in the compost + peat mixture. Only one plant grew in the second experiment (three replicates in the other treatments), and so the data are not presented. Preliminary germination tests showed that the poor growth of *A. murale* in compost was not due to the presence of inhibitory substances. The physical characteristics of the compost seemed to be the main difficulty.

Higher concentrations of Zn, Cu, Ni and Cd were present in leaves than in hypocotyls of radish (Table 3). Increased Zn and Cu concentrations were found in plants grown in compost compared with those in soil, but only slight differences were found for Ni and Cd. In the second experiment, reducing the amount of substrate (soil or compost+peat) placed in the minirhizotron caused an increase in most of the nutrient and heavy metal concentrations in radish.

The concentrations of Zn and Ni in *A. murale* were greater than those of radish. The concentration of Ni in the hyperaccumulator plant was within the range of the phytotoxic Ni concentrations of non-accumulator plants (10 to 100 mg kg^{-1} dw, Kabata-Pendias and Pendias, 1992), but the values in radish where lower that the cited range. Although the Ni concentration in *A. murale* was not very large in comparison with the 2000 - 6000 $\mu g\ g^{-1}$ referred to by Morrison et al. (1980), it was nearly 10 times that in radish leaves, and Zn was 1.6 times greater. It is important to note that the total Ni content of the soil was much lower than those soils reported by Morrison et al. (1980) (900 $\mu g\ g^{-1}$). The total Ni uptake by *A. murale* (Table 4) was 4.6 times the average of radish (whole plant, hypocotyl included), despite the smaller biomass of the *A. murale*.

pH changes in the rhizosphere-soil

Changes in colour of the pH indicator placed on the rhizosphere of radish growing in compost+peat occurred very quickly: the dark purple colour observed firstly near the roots quickly extended to the bulk medium, due to the pH of the compost. This indicated a pH of 7.0–7.5 both next to the roots and in the bulk compost + peat, with no difference between these zones.

Table 2. Plant production in minirhizotron experiments (g per pot)

	Fresh weight		Dry weight	
	Leaves	Hypocotyl	Leaves	Hypocotyl
Substrate: soil				
Radish 1st experiment[a]	8.63	36.30	1.350	3.855
Radish 2nd experiment	6.68	29.85	1.462	3.299
A. murale plant	5.50[b]	–	1.628[b]	–
Substrate: compost+peat				
Radish 1st experiment	15.38	49.29	2.271	5.481
Radish 2nd experiment	18.30	23.17	2.528	2.276

[a]Experiment 1: 330 g soil or 130 g compost+peat per pot; Experiment 2: 293 g soil or 122 g compost + peat per pot.
[b]Whole plant.

Table 3. Metal concentration in the harvested plant materials (mg kg^{-1}). Means of three replicates

	Zn		Cu		Ni		Cd	
Experiments[a]	1	2	1	2	1	2	1	2
Substrate: Soil								
Radish leaves	104	233	5	9	2	4	2	4
Radish hypocotyls	78	148	3	7	2	3	1	2
A. murale plant	–	382	–	8	–	40	–	4
Substrate: Compost+peat								
Radish leaves	508	527	15	24	7	6	1	2
Radish hypocotyls	230	212	6	9	3	5	0	1

[a]Experiment 1: 330 g soil or 130 g compost+peat per pot; Experiment 2: 293 g soil or 122 g compost+peat per pot.

In the rhizosphere of radish grown in soil, the purple colour appeared next to the roots, while the reagent remained red in the bulk soil. This shows that the roots caused an increase in the rhizosphere pH to about 6.5 compared to 6.0 in bulk soil. Similar effects were observed in the rhizosphere soil of *A. murale*, and the change in colour next to the main root and roots growing close together was quicker than that next to single fine lateral roots.

The amount of acid or alkali required to reduce or increase the soil pH by 0.5 units was 21 me H^+ or OH^- kg^{-1} (Fig. 1). In contrast, a very high buffering capacity with respect to acid was found in the compost+peat mixture, 420 me H^+ kg^{-1} for 0.5 pH unit change, and although the value was greatly reduced for alkali (122 me OH^- kg^{-1}), it was still larger than for soil. This greater buffering capacity of the compost+peat mixture controlled the pH in the rhizosphere, neutralizing any proton influx/eflux that the roots could have caused, thus explaining why no net change in pH was observed.

The pH values determined in the rhizosphere soil of radish with proton-selective microelectrodes can be summarized as follows (Fig. 2, Table 5): the mean bulk soil pH was 6.05, values next to lateral roots were between the range 6.05–6.15, but the highest values (mean = 6.7) were found next to main root. Roots of *A. murale* growing in soil also increased the soil pH in the rhizosphere (Table 5), and the highest value (6.7) was found next to the main root, decreasing towards

Table 4. Total metal uptake by plants (μg per plant). Means of three replicates

	Zn		Cu		Ni		Cd	
Experiments[a]	1	2	1	2	1	2	1	2
Substrate: Soil								
Radish leaves	143	434	7	15	7	6	3	6
Radish hypocotyls	296	510	10	22	10	9	3	5
Radish whole plant	439	944	17	37	17	15	6	11
A. murale plant	–	582	–	12	–	58	–	7
Substrate: Compost+peat								
Radish leaves	1178	1218	33	52	13	14	3	3
Radish hypocotyls	957	480	31	19	18	10	1	1
Radish whole plant	2135	1698	64	71	31	24	4	4

[a]Experiment 1: 330 g soil or 130 g compost+peat per pot; Experiment 2: 293 g soil or 122 g compost+peat per pot.

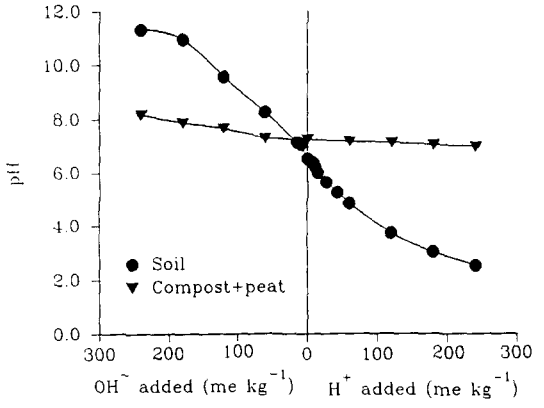

Fig. 1. Acid- and base-titration curves for the two experimental substrates, soil (●) and 50 % (v/v) compost+peat mixture (▼).

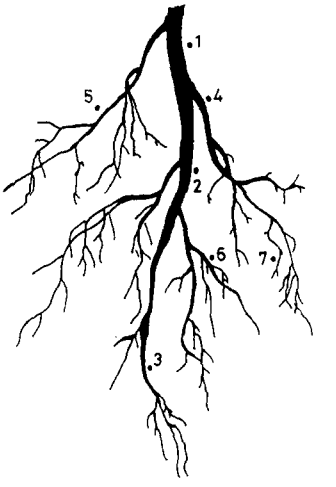

Fig. 2. Locations of points for the pH measurements with proton-selective microelectrodes in the rhizosphere. 1, 2 and 3 - main root towards the tip, 4 - lateral root near the main root, 5 - Lateral root, 6 - secondary lateral root, 7 - fine secondary root.

the root tip (6.6 to 6.5). In lateral roots the average value was 6.4, however, the smaller the root the less the pH in the rhizosphere (ranging from 6.3 to 6.1). When lateral roots grew side by side, the pH increased up to the value obtained next to the main root (6.7). The maximum pH increase in the rhizosphere soil of *A. murale* and radish (0.7 units) relates to a maximum production of 29 μe OH^- g^{-1} soil in the main root, decreasing in *A. murale* to production of 21 μe OH^- g^{-1} along the main root towards the tip (0.5 units pH change). In lateral roots the production was equivalent to 2 to 17 μe OH^- g^{-1} (pH change from 0.05 to 0.4 units).

Effect of N-source and heavy metals (solution culture experiments)

One hour after transferring the roots to the agar with bromocresol purple, a change in pH was noticed around radish roots in NO_3-N treatments with and without heavy metals, rapidly increasing from pH 6.0 to pH > 7.0 (red to purple) next to the root system (Table 6). On the other hand, in treatments with NH_4-N the agar turned yellow near the roots, indicating a decrease in pH (pH < 5.5). The same results were obtained with

Table 5. Measured pH in the rhizosphere of main and lateral roots of *A. murale* and radish grown in soil using proton-selective microelectrodes. Bulk soil pH = 6.05

Rhizosphere zones[a]	*A. murale*	Radish
1	6.70	6.70
2	6.60	6.70
3	6.50	6.70
4	6.65	6.70
5	6.40	6.25
6	6.30	6.15
7	6.10	6.05

[a]See Fgure 2 for description of rhizosphere zones.

Table 6. Visualization of the pH changes in the rhizosphere of *A. murale* and radish as a function of N-source and heavy metals. The changes were detected by bromocresol purple in agar. The pH of the agar medium was adjusted to 6.5 (red-purple) for NH_4-N treatments with and without heavy metals (±M) and to 6.0 (red) for NO_3-N treatments with and without heavy metals (±M)

Treatments[a]	Radish	*A. murale*
NH_4-N ±M	Yellow (pH < 5.5)	Yellow (pH < 5.5)
NO_3-N ±M	Purple (pH > 7.0)	Purple (pH > 7.0)

[a]See materials and methods for details.

roots of *A. murale*, but the changes were less intense. No differences were observed in treatments with or without heavy metals.

Reduction of Fe(III) was observed 18–20 hrs after placing the roots into the agar with Fe(III) + BPDS (Table 7). Radish grown in treatments with heavy metals and NO_3-N or NH_4-N showed a slightly red colour next to the roots, and darkened at the tips of lateral roots. The tips of growing roots were more active in producing reducing compounds than the older parts of the roots. However, in the NO_3-N treatment without heavy metals a smaller difference between tips and the rest of the root was found, and the general colour next to the roots was darker than in treatment with metals, indicating that the extent of reduction was more uniform along the roots when no heavy metals were added. In *A. murale* grown in the NO_3-N treatment without heavy metals the agar turned red next to the tip of some roots (mainly lateral roots) indicating reducing activity, but in the rest of the treatments the slightly pink colour observed near the roots showed that the reducing activity of the roots was very small.

It was necessary to keep the roots in agar+MnO_2 for 2 to 3 days to observe any change in colour. Unquestionable reduction of MnO_2 (brown) to Mn(II) (white) occurred near the roots of radish growing in NO_3-N without metals and NH_4-N with metals (Table 7). Only roots of *A. murale* growing in the NO_3-N treatment without metals caused Mn(IV) reduction. It seems that in radish roots the reduction of Mn(IV) was influenced by the N-source and the heavy metal content. Roots of *A. murale* are less actively reducing than those of radish, and in the latter the form of nitrogen may influence the effect of heavy metals or vice versa.

Discussion

Decreases in pH of the rhizosphere strongly increase the availability of metals in soils (Linehan, 1989). In our experiment, increases in rhizosphere pH with respect to the bulk soil were observed in both plant species. The pH change of the rhizosphere may be related to the excess of anions relative to cations taken up by the roots, as nitrate is often the major source of nitrogen. As Table 1 shows, NO_3-N was the main inorganic-N source in the soil and compost, and NO_3-N was also added as a fertilizer.

The largest increases in pH were found adjacent to the main root and groups of roots growing together (0.7 units), while smaller increases occurred in lateral and small roots. These results indicated that the rhizosphere is a heterogeneous system, and the activities of lateral and main roots are different. The cation/anion uptake ratio may vary between the different parts of the root system (Blanchar and Lipton, 1986) so uptake of some nutrients varies with root zone. Nitrate reduction leads to production of OH^- ions in the plant, which are largely excreted when reduction occurs in the roots. Anions of organic acids or other organic molecules are often exuded at the root apex which can affect the pH of the rhizosphere. However, neither the hyperaccumulator species nor the addition of heavy metals to the nutrient solution provoked any special changes in the rhizosphere pH. The hyperaccumulator plant did not decrease the rhizosphere pH as a mechanism of increasing the availability of heavy metals in the rhizosphere.

The agar with either Fe(III) + BPDS or MnO_2 applied to roots showed a larger reducing capacity in root tips than in the rest of the roots of radish and

Table 7. Visualization of the changes of the redox potential in the rhizosphere of *A. murale* and radish as a function of the N-source and heavy metals. Change in redox potential were detected by the reduction of Fe (III) to Fe (II) in agar with BPDS (pink to red), and Mn (IV) to Mn (II) in agar with MnO_2 (brown to white)

Treatments[a]	Fe (III) reduction		Mn (IV) reduction	
	Radish	*A. murale*	Radish[b]	*A. murale*[b]
-M NH_4-N	r++ t++	r0 t0	0	0
-M NO_3-N	r0 t0	$r0^+$ t++	++	+
+M NH_4-N	r+ t++	$r0^+$ $t0^+$	++	0
+M NO_3-N	r+ t++	$r0^+$ $t0^+$	0	0

[a]-M -treatment without heavy metals, +M - treatment with heavy metals in solution culture. See materials and methods for details.
r - roots, t - root tips, 0 - no change, 0^+ - slight change, + - moderate change and ++ - strong change in colour in the rhizosphere.
[b]Changes observed in the rhizosphere with no differences between the roots and root tips.

to a lesser extent in *A. murale*. Results for *A. murale* indicated that the reducing capacity of the roots was enhanced with NO_3-N in the absence of heavy metals, but the reducing capacity of this species was lower in all treatments than that of radish. Results from MnO_2 in agar may indicate two different reducing mechanisms in radish. When NO_3-N is present the activity of nitrate reductase is increased, but when heavy metals are present in the NH_4-N treatment the absorption of these metals by radish provokes a deficiency of Fe in the plant, which then increases the reducing capacity as a response to this deficiency.

In general, the roots of *A. murale* were less active than those of radish in both excretion of protons and reductants. When heavy metals were not present in the growth medium, the reducing capacity of the roots was enhanced and this could increase metal solubility in the rhizosphere soil. However, because the reducing activity was not greater than in the non-accumulating radish plant, this seems unlikely to be a mechanism which aids hyperaccumulation of metals by *A. murale*. The amount and composition of root exudates may play an important role in the solubilization of metals from soils prior to absorption. Further investigations should also be focussed on the capacity of root exudates to complex Ni in the rhizosphere soil.

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