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Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L.

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Abstract

Soluble root exudates were collected from three plants (*Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L.), grown under axenic and hydroponic conditions, in order to study their metal-solubilizing ability for Cd and other cations (Cu, Fe, Mn, Ni, Zn). *Nicotiana* spp. and *Zea mays* L. root exudates differed markedly in C/N ratio, sugars *vs.* amino acids ratio and organic acids content. Metals from two soils were extracted with either root exudate solutions, containing equal amounts of organic carbon, or distilled water as control. In the presence or absence of root exudates, the solubility of Fe and Mn was much higher than of the four other metals tested. Root exudates increased the solubilities of Mn and Cu, whereas those of Ni and Zn were not affected. Root exudates of *Nicotiana* spp. enhanced the solubility of Cd. The extent of Cd extraction by root exudates (*N. tabacum* L. > *N. rustica* L. > *Zea mays* L.) was similar to the order of Cd bioavailability to these three plants when grown on soil. An increase in Cd solubility in the rhizosphere of apical root zones due to root exudates is likely to be an important cause of the relatively high Cd accumulation in *Nicotiana* spp.

Introduction

Cadmium (Cd) bioavailability to *Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L., grown on soil with and without cadmium nitrate was studied by Mench *et al.* (1989a). Irrespective of the Cd level in the soil (*i.e.* 0.4 and 5.4 mg Cd/kg soil DW), the highest Cd concentrations were found in the leaves of *N. tabacum* L. *Nicotiana* spp. were characterized as leaf accumulators, while *Z. mays* L. is primarily a root accumulator: more than 50% of total Cd taken up by *Z. mays* L. was retained in the roots at both soil Cd levels. In order to explain the Cd bioavailability, investigation of the metal behaviour in the rhizosphere of these three plants is a necessary step.

Rhizospheric interactions play a key role in micronutrient acquisition (Curl and Truelove, 1986; Dommergues and Krupa, 1978; Marschner *et al.*, 1986; Rovira *et al.*, 1983). As a result, concentrations of Cu, Zn, Mn and Co in the soil solution increase when plants are present (Linehan *et al.*, 1989). Root exudates may influence nutrient solubility and uptake indirectly through their effects on microbial activity, rhizosphere physical properties and root growth patterns, and directly by acidification, chelation, precipitation and oxidation-reduction reactions (Uren and Reisenauer, 1988). Studies of direct interaction between root exudates and metals have mainly used graminaceous spp. exudates produced from sterile or non-sterile hydroponic and field cultures (Awad *et al.*, 1988; Godo and

Reisenauer, 1980; Mench *et al.*, 1988; Morel *et al.*, 1987; Takagi *et al.*, 1988; Treeby *et al.*, 1989; Zhang *et al.*, 1989). They showed that root exudates mobilize or bind metals (*i.e.* Fe, Mn, Zn, Cu, Cd, Pb) to an extent that depends on the ligand, the solid phase and the metal involved. *Z. mays* L. exudates are able to bind Cd (Mench *et al.*, 1985; Mench *et al.*, 1987; Morel *et al.*, 1986). There is increasing evidence that soluble root exudates increase the solubility of metals in the rhizosphere depending on plant species and cultivars (Bromfield, 1958; Jolley and Brown, 1989; Kawaï *et al.*, 1988; Römheld and Marschner, 1989). However, to our knowledge, interactions between metals, especially Cd, and *Nicotiana* spp. root exudates have not yet been investigated. Therefore, the present work was conducted in order to study the potential solubilizing properties of soluble root exudates from the three plant species (*Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L.) grown under axenic conditions. Mobilization of Cd and other metals (Cu, Fe, Mn, Ni, Zn) from 2 soils, one treated with sewage sludge and the other amended with cadmium nitrate salt, was studied.

Materials and methods

Collection of root exudates

Maize and tobacco were chosen as test plants due to their differences in Cd uptake (Mench *et al.*, 1989a). Root exudates were collected from maize (*Z. mays* L., cv. INRA 260) and tobacco (*N. tabacum* L., cv. PBD6; *N. rustica* L., cv. Brasilia) plants. Axenic hydroponic cultures were used in order to obtain compounds of plant origin not contaminated by microorganisms or soil components. The procedure and equipment for maize described previously (Morel *et al.*, 1986) was adapted to tobacco using a series of 60 × 300 mm glass tubes containing 300 mL of Hoagland No. 2 nutrient solution adjusted to pH 6 (Hewitt, 1966). Plants were supported inside with a plastic net. Each tube was connected at the bottom to a hydrophobe filter (Millex FG 45 mm ϕ Millipore), which allowed aeration of the nutrient solution, and closed at the top by cotton wool. The tubes were autoclaved at 120° C for 30 min. The maize seeds were surface-sterilized by washing with 95% ethanol and immersing for 45 min in 10% H_2O_2 (v/v). Six maize seeds were transferred aseptically into each tube and plants were grown for 15 days under phytotronic conditions (18 h photoperiod with day/night temperature 25/16°C, 75% relative humidity, light intensity 210 μmol m^{-2} s^{-1} in the range 400–700 nm). The tobacco seeds were obtained from ITB-SEITA (Bergerac, France). They were surface-sterilized by immersion first in 95% ethanol for 1 min, then in 18% H_2O_2 (v/v) for 35 min, and thereafter grown for 2 weeks in sterile petri dishes (sterile sand and an aliquot of Hoagland No. 2 sterile nutrient solution as medium). Two axenic seedlings were then placed in each tube and incubated under the previously described phytotronic conditions for 2 months.

Two days before the collection, the sterility of each tube was checked by plating a sample of the nutrient solution on a yeast-peptone-glucose-agar medium (LPGA).

Root exudates with the lowest content of nutrient salts were required for characterization of organic compounds and use as extractants. Therefore, the following collection procedure was used. Firstly, the nutrient solution of axenic tubes was removed in a laminar flow type cabinet and replaced by sterile double-distilled water. Then, tubes were put for 24 h under phytotronic conditions. Thereafter, tube solutions were collected and filtered at 0.45 μm (Millipore). The filtrate was assumed to contain soluble root exudates.

Determination of root exudate composition

Exudates were concentrated under vacuum at 40° C. Aliquots were analyzed for the following total element contents: carbon, nitrogen (Kjeldahl), reducing sugars (Shields and Burnett, 1960 – anthron with glucose as standard), protein (Biorad kit protein assay, bovine serum albumine as standard), free amino acids and ammonium (Biotronik LC-5001 analyzer, physiological program run with lithium buffers, detection at 570 and 440 nm after post-column derivation with ninhydrin). An aliquot was passed onto an

anion exchanger Amberlite IRA-400 (Merck, 20–50 mesh ASTM). Anionic fractions were eluted with 2*N* formic acid and dried under vacuum. Free organic acids were separated by HPLC on a Aminex column 50 W × 4 with 25 m*M* HCl as mobile phase (0.65 mL min^{-1}) at room temperature and detected at 210 nm. Semi-quantitative results were obtained because only identified organic acids were integrated (*i.e.* citric, oxalic, tartaric, isocitric, malic, fumaric, succinic). The blank concentrations of the investigated metals in the used root exudates solutions were:

Z. mays L. Fe = 3.8, Cu = 0.3, Zn = 0.09, Mn = 0.4 μMol,
N. tabacum L. Fe = 1.8, Cu = 0.2, Zn = 0.15, Mn = 2.2 μMol,
N. rustica L. Fe = 5.5, Cu = 0.5, Zn = 0.50, Mn = 1.9 μMol.

Soils

Two soils were used. Soil A was a coarse, sandy, acid soil from a long-term field experiment (0–0.20 m depth, INRA, Bordeaux, France). This soil had been cropped continuously since 1974 with maize (cv. INRA 260). Soil samples were taken from 3 treatments: C = control, SS1 = sewage sludge 50 t DW ha^{-1}, SS2 = sewage sludge 300 t DW ha^{-1}. Soil B was taken from the 0–0.20 m horizon of an acid sandy-clay soil, typical of the Bergerac area (France) and usually cultivated with tobacco; this soil had been previously enriched with $Cd(NO_3)_2$ (Mench *et al.*, 1989). The main characteristics of the soils are displayed in Table 1.

Table 1. Main characteristics of the soils (0–0.20 m depth)

	Soil A[b]			Soil B
	C	SS1	SS2	
Sand (%)	83	82	81	72
Silt (%)	13	14	15	17
Clay (%)	4	4	4	10
Organic matter (%)	2.3	1.7	2.2	1.5
C (‰)	13	10	13	9
N (‰)	0.9	0.7	0.9	1.1
pH (water)	6.0	6.4	6.8	5.3
CEC (meq 100 g^{-1} DW)	3.5	2.7	2.9	5.6
Aqua regia extract[a]				
Cd[c]	3.6	25	93	3.6
Ni[c]	8.9	70	234	9.6
Cu[c]	6.4	17	44	11
Zn[c]	13.8	43	137	43
Mn[c]	27	42	71	485
Fe[c]	1 853	2 526	3 410	15 957

[a] 12*N* HCl + 12*N* HNO_3 (75%:25% v/v).
[b] C = control; SS1 = sewage sludge 50 t DM ha^{-1} and SS2 = sewage sludge 300 t DM ha^{-1}.
[c] In mg kg^{-1} DW.

Extraction of metals from soil

The soils were air-dried and passed through a 2-mm sieve. Root exudates were adjusted to pH 5.5 with HNO_3 (2*N*). Metals from soil were extracted with either root exudate solutions containing equal amounts of organic carbon, or double-distilled water (as control). Two grams of the soil sample to be tested and either 40 mL of a root exudate solution containing 300 μg C or 40 mL of double-distilled water were transferred to a 100-mL polyethylene flask. Flasks were shaken at room temperature (approximately 25° C) for 3 hours. The content of each flask was filtered through no. 4 filter paper (ash-free) or a Millipore filter (0.45 μm); 2 ml of HNO_3 (12*N*) were added to the filtrate in order to reach about 5% (v/v). Each extraction was replicated 3 times and each experiment repeated twice.

For comparison, other soil extractions were conducted in the same way (2 g soil DW, 300 μg C in 40 mL), but with organic C in the form of glucose, glycine, citric acid or with glucose plus metal salts [$FeSO_4$, $CuSO_4$, $ZnSO_4 4H_2O$, $MnSO_4$ $7H_2O$) at levels according to the blank concentration of the investigated metals in the used root exudate solutions: *Z. mays* L. (Solution A), *N. tabacum* L. (Solution B) and *N. rustica* L. (Solution C).

Metal determination

Metals in the filtrates and in root exudates were measured by either flame (Varian SpectrA-A20) or electrothermic (Varian SpectrA-A30) atomic absorption spectrometry depending on the concentration in the solutions.

Results

The main characteristics of root exudates used for soil extraction are shown in Table 2. This composition reflects the concentrated root exudates, which are not directly related to the root weight of the 3 species. However, total soluble carbon released by each plant during the 24-h period in double-distilled water represents about 1.1% of the total weight of carbon in the plant for *Zea mays* L., 1.1% for *N. tabacum* L. and 1.2% for *N. rustica* L. *Nicotiana* spp. and *Z. mays* L. root exudates differ particularly in C/N ratio, sugars *vs.* amino acids ratio and organic acids content (*i.e. N. tabacum* L. 52%, *N. rustica* L. 37%, *Z. mays* L. 23%, expressed in percent total carbon).

The ability to extract metals from soil samples differs markedly among types of solution, soils, and cations. In the presence or absence of root exudates, the solubility of Fe and Mn is much higher than that of the four other metals tested (Figs. 1 and 2). However, dominance of either Fe or Mn depends on the content of these metals in the soil sample: soil B has a higher Mn/Fe ratio than soil A and therefore more Mn is found in solution. Compared with water, root exudates generally increase the solubilities of Mn and Cu, whereas those of Ni and Zn are not affected. The solubilities of Mn, Cu, Cd and Fe in root exudate extracts vary among plant species.

Z. mays L. root exudates solubilize mainly Cu or Mn, depending on the Cu/Mn ratio in soil samples (Figs. 1 and 2). In soil A, where Mn content is lower than in soil B (Table 1), Cu concentration in extracts increases more than Mn concentration. Concentrations of Fe and Cd in extracts are not significantly affected by *Z. mays*

Table 2. Composition of root exudates used for soil extraction

	pH	C	N	C/N	Proteins[a]	Sugars[b]	Amino-[c] acids	Organic[d] acids
		($mg\ dm^{-3}$)			($mg\ dm^{-3}$)			
N tabacum L.	7.0	15.75	6.4	2.5	0.35	6.7	1.1	21
N rustica L.	6.2	18.75	6.4	2.9	0.50	4.5	1.3	18
Zea mays L.	7.8	94.12	7.0	13.4	1.65	110	7.4	57

[a]proteins: bovine serum albumin.
[b]sugars: glucose.
[c]amino acids: aspartic acid.
[d]organic acids: citric acid.

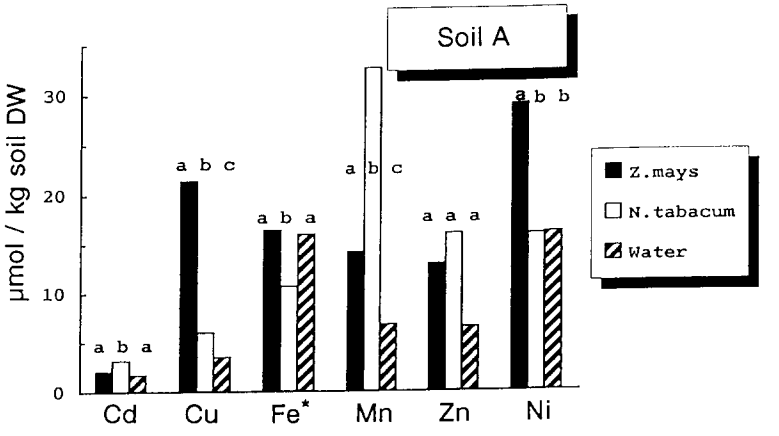

Fig. 1. Metal concentrations in the extracts of the control treatment of soil A (Table 1). Concentration of root exudates: 150 μg C g^{-1} soil DW. For each metal, treatments with the same letter above the column are not significantly different at the 5% level (Newman-Keuls test). Fe* represents the concentration of Fe divided by 10.

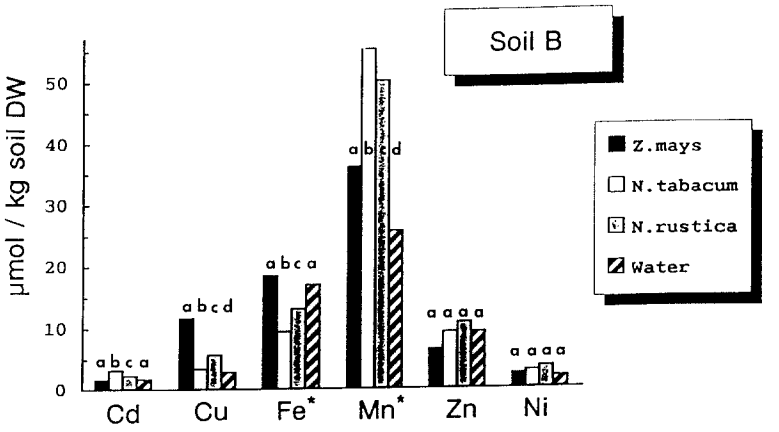

Fig. 2. Metal concentrations in the extracts of soil B (Table 1). Concentration of root exudates: 150 μg C g^{-1} soil DW. For each metal, treatments with the same letter above the column are not significantly different at the 5% level (Newman-Keuls test). Fe* and Mn* represent the concentrations of Fe and Mn divided by 10.

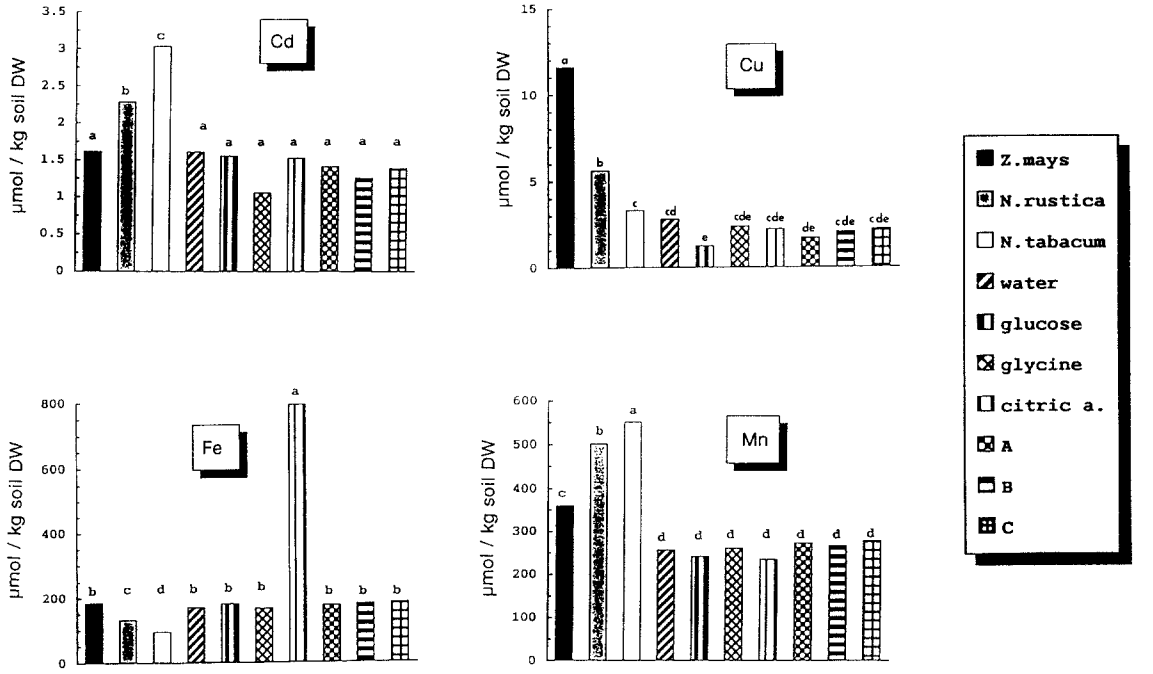

Fig. 3. Metal concentrations in the extracts of soil B (Table 1) obtained with root exudates or with one of the following solutions: glucose, citric acid, glycine, solution A, solution B, solution C (these last three were made with glucose and metal salts at levels similar to those of the blank concentrations of the investigated metals in the root exudates of *Z. mays* L., *N. tabacum* L. and *N. rustica* L., respectively). Concentration of carbon in each solution: 150 μg C g^{-1} soil DW. For each metal, treatments with the same letter above the column are not significantly different at the 5% level (Newman-Keuls test).

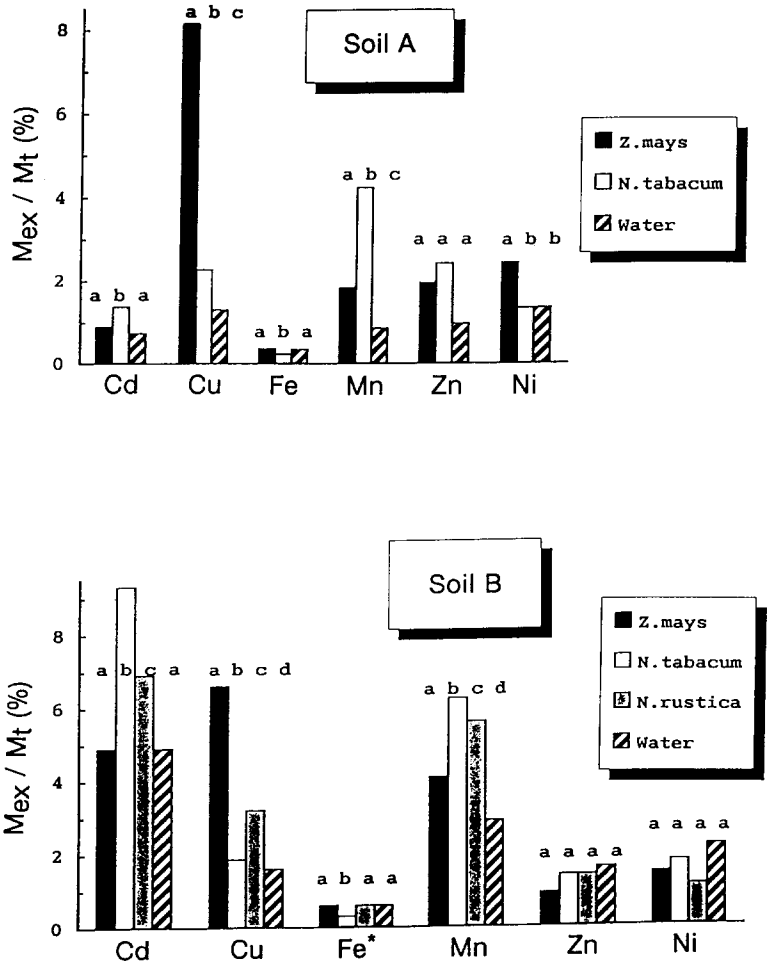

Fig. 4. Metal extracted (M_{ex}) *vs.* total metal in soil (M_t) (%). For soil A: extracts of the control treatment of soil A. Fe* represents the concentration of Fe multiplied by 10.

L. exudates. In the case of soil A, *Z. mays* L. exudates increase Ni concentration.

On the other hand, root exudates of *Nicotiana* species solubilize mainly Mn and to a lower extent Cu, irrespective of Cu/Mn ratio in the soil samples (Figs. 1 and 2). *Nicotiana* spp. root exudates increase Cd concentration in extracts. Root exudates of *N. tabacum* L. extract much more Cd than those of *N. rustica* L. Unexpectedly, *Nicotiana* spp. root exudates reduce Fe concentration; the lowest Fe concentrations are found in *N. tabacum* L. extracts.

The blank concentrations of the investigated metals in the used root exudate solutions or microbial growth during agitation might have been responsible for variation in metal solubilities. In order to assess this possibility, experiments were conducted in which root exudates were replaced by solutions with similar metal composition to each root exudates used, or by solutions with organic carbon in the form of glucose, glycine or citric acid (Fig. 3). Compared with water extracts, no differences are found in metals extracted by these additional treatments, except that citric acid increases Fe concentration.

Expressed as percentage of total metal in the soil sample, the metals in solution can be ranked in the following order (Fig. 4):

		Soil A	Soil B
Water	extract	Cu > Mn > Cd > Ni, Zn > Fe	Cd > Mn > Cu > Zn, Ni > Fe
Zea mays L.	extract	Cu > Mn > Cd > Ni, Zn > Fe	Cu > Cd > Mn > Zn, Ni > Fe
N. tabacum L.	extract	Mn > Cu > Cd > Ni, Zn > Fe	Cd > Mn > Cu > Zn, Ni > Fe
N. rustica L.	extract	not determined	Cd > Mn > Cu > Zn, Ni > Fe

Finally, extractions were conducted in the three treatments of soil A in which total metal was found to increase due to sewage sludge addition (Table 1). Data are expressed as percentages of total metal in the soil (Fig. 5). Compared with water, the rise in Cu, Cd or Mn extracted with root exudates generally decreases as total metal in soil increases. However, at the highest metal level, Cd and Mn extracted with root exudates of *N. tabacum* L. and Cu extracted with root exudates of *Z. mays* L. still exceed significantly those extracted with water.

Discussion

In both soil extracts, Mn and Cu concentrations were markedly affected by soluble root exudates of *Z. mays*. L., *N. rustica* L. and *N. tabacum* L. However, at the carbon concentration used, only root exudates of *Nicotiana* spp. increased Cd concentration.

The key role of root exudates in increasing Mn or Cu solubility has been previously reported (Bromfield, 1958; Charlanes, 1960; Nielsen, 1976; Godo and Reisenauer 1980; Jauregui and Reisenauer, 1982; Linehan *et al.*, 1989; Merckx *et al.*, 1986; Mench *et al.*, 1987; 1988; Uren, 1981), using batch systems, pot and field plants. However, available data are mainly derived from graminaceous species, and in general the effect of root exudates and microbial products could not be easily distinguished. Mobilization of Cd by *Nicotiana* spp. root exudates is worthy of attention, because many reports show a roughly linear relationship between Cd concentrations in the nutrient solution or in the soil and in plants (McGrath, 1989). *Nicotiana* spp. are particularly efficient in accumulating Cd.

As root exudates are not expected to change free cation concentrations in solution, the increases in metal concentrations observed in root exudate extracts are most likely due to the formation of complexed cations which disturb equilibria. For Mn also reductions should be considered. Although the metal-binding properties of *Z. mays* L. root exudates have been established in previous work (Mench *et al.*, 1985; 1987; 1988), the status of metals, inorganic or organic forms, requires further characterization. Assuming that all additional metals found in solution were complexed by root exudates, the metal solubilizing ability (MSA) of root exudates is equivalent to their metal-binding ability (MBA) described elsewhere (Mench *et al.*, 1987; Morel *et al.*, 1986). Metal concentrations in excess of those in water extract controls were used to calculate MBA values in mmol/100 g carbon exuded. Maximum values were observed in soil B extracts: *N. tabacum* L. 198, *N. rustica* L. 165 and *Zea mays* L. 75 mmol/100 g C exuded. This value for *Zea mays* L. was similar to that reported elsewhere (Mench *et al.*, 1988). However, MBA values were much lower for soil A (*N. tabacum* L. 20 and *Z. mays* L. 26 mmol/100 g C exuded) indicating an influence of the soil and its metal speciation, especially in the case of soil A, perhaps due to its low Mn content. The limits of the extraction method must be recognized: MBA values are associated with soil pH and an arbitrary carbon concentration of 150 μg C/g soil DW which might not be representative of conditions in the rhizosphere of apical root zones. For example, there are significant pH gradients from the rhizoplane to the surrounding soil (Marschner *et al.*, 1986). On the other hand, use of fresh soil samples in comparison to dried ones should be considered.

Further investigations are required to explain differences in MBA values among plant species. Obviously, the nature of root exudates is an important factor in metal extraction. Exudates of *Z. mays* L. and *Nicotiana* spp. were rather different in their C/N ratios (Table 2). Further-

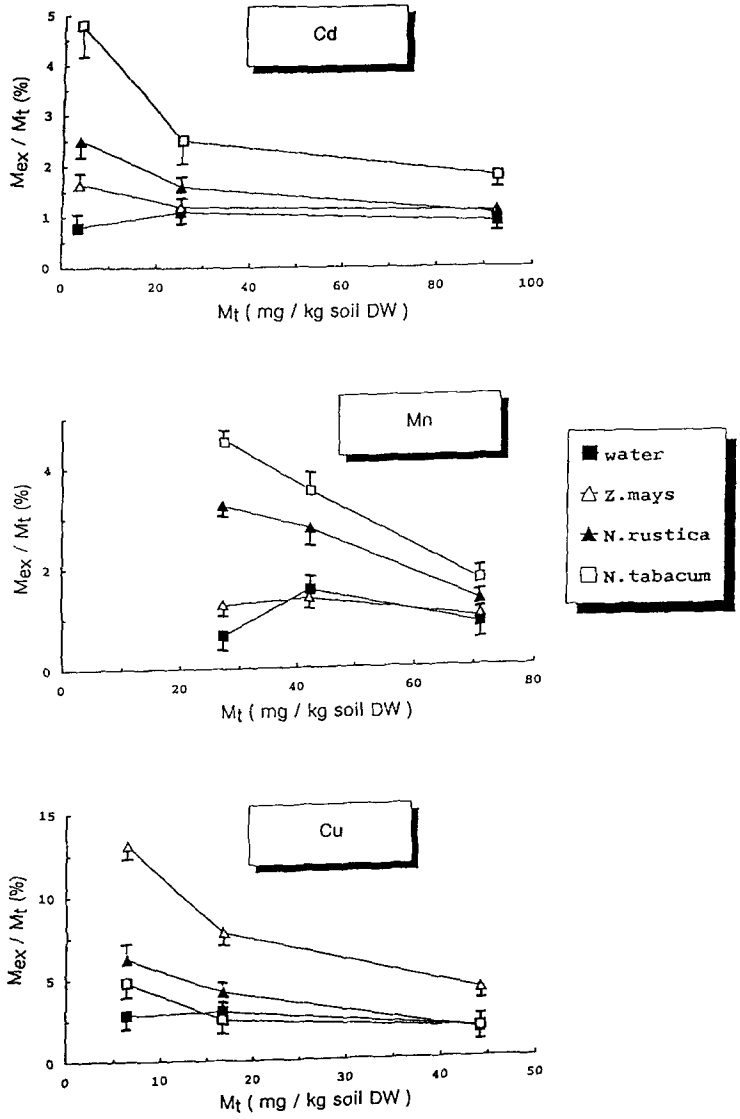

Fig. 5. Changes in metal extracted (M_{ex}) *vs.* total metal in soil (M_t) (%) in relation to total metal in soil, (⊥: standard deviation).

more, *Nicotiana* spp. exudates contained less sugars and more organic acids than those of *Z. mays* L. Amino acid contents were similar but could be different from a qualitative point of view, judging from the presence of 2'deoxymugineic acid in root exudates of *Z. mays* L. (Kawaï *et al.*, 1988).

It is likely that organic acids and, to a lesser extent, amino acids (including phytosiderophores in the case of graminaceous species), are the major components responsible for the increased extractions of Mn, Cu or Cd by root exudates. It should be noted that the MBA and organic acid ratios for *N. tabacum* L. *vs. Z. mays* L. or *N. rustica* L. are very similar (*i.e. N. tabacum* L./*Z. mays* L.: MBA = 2.6, organic acids = 2.3). Organic acids, especially malic acid, have been proposed as compounds which may enhance dissolution of sparingly soluble Mn oxides (Jauregui and Reisenauer, 1982). Our data suggest that citric acid, glycine, and glucose were not suitable as extractants simulating the actions of root exu-

dates. Other organic solutions, based on root exudate composition, should be tested.

The high MBA value of *Z. mays* L. root exudates for Cu was in agreement with the order of stability constant reported (Mench *et al.*, 1988). Moreover, an efficient binding of Cu by phytosiderophores was shown (Nomoto *et al.*, 1981). Surprisingly, Fe or Zn were not affected by *Z. mays* L. exudates in our study. However, firstly, *Z. mays* L. appeared less efficient in phytosiderophore release than other graminaceous species (Römheld and Marschner, 1990) and, secondly, results could differ if root exudates were collected from Fe- or Zn-deficient plants as in studies by Treeby *et al.* (1989) or Zhang *et al.* (1989). Therefore, it must be stressed that the MBA value of root exudates probably depends on the nutrient status of the plants.

The effect of axenic root exudates reacting with soil is supposed to approximate that of soluble root exudates (*i.e.* acid or complexing compounds) in mobilizing metals in the rhizosphere of apical root zones. This area has been claimed to be sparsely populated by microorganisms (2% coverage) when root elongation is not impaired (Uren and Reisenauer, 1988). In such cases, organo-metallic complexes due to root exudates are likely to be an important source of metal supply to the roots, because increasing metal solubilization would enhance metal supply at the root surface. Therefore, metal concentrations in soil B extracts were compared with those in the three plant species when grown on soil B. In the case of Cd and Mn, the extent of metal extraction by root exudates was in the order *N. tabacum* L. > *N. rustica* L. > *Z. mays* L., which is similar to the order of metal bioavailability to these three plants (Cd: Mench *et al.*, 1989a; Mn content in leaves, mg kg DM^{-1}: *N. tabacum* L. 654, *N. rustica* L. 501, *Z. mays* L. 260, Mench, unpublished data). On the other hand, such a correlation did not exist for Cu, because Cu in leaves of *Nicotiana* spp. was higher than or equal to that of *Z. mays* L. (mg kg DM^{-1}: *N. tabacum* L. 6.6, *N. rustica* L. 10.2, *Z. mays* L. 6.5, Mench, unpublished data), whereas *Z. mays* L. root exudates extracted more Cu than those of *Nicotiana* spp.

Consequently, the classification of a plant species on the basis of the effect of its root exudates should be considered with care and special consideration must be given to type of metal and of soil, and to macroscopic factors, such as architecture of the root system and climatic conditions (Mench *et al.*, 1989*b*).

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