Soil treatment with nitrogen facilitates continuous phytoextraction of heavy metals

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Abstract. NH_4Cl was the optimum N compound to make soil heavy metals more plant-available but less leachable, and to increase biomass production, root uptake, and translocation to the shoot of Cd, Co, Cu, Mn, Ni, and Zn in Chinese cabbage. Their total weight in the shoots of higher biomass increased to 765 %.

Introduction

Uptake of heavy metals (HM) by plants increases frequently with their concentration in the soil solution and their adequate speciation. Free metal cations and metal-mineral acid complexes were taken up by *Beta vulgaris* to the same extent (McLaughlin et al. 1998). Concentrations of dissolved elements increase in the presence of neutral (NH_3, H_2O, CH_3OH) or anionic ligands (H^-, Cl^-, CN^-, OH^-, oxoanions of C, P, S) (Cotton et al. 1987), of chelators such as humic, low-*MW* carboxylic, and amino acids (AA, Hayes 1991), and under acidifying conditions (Sumner et al. 1991) resulting from CO_2 release, nitrification, and organic acid production. Involvement of organic bases such as amines with biocatalytic properties (Bergmann et al. 1999) has not been studied. Humic substances and HM precipitate from a soil solution saturated with $Ca^{2+}>Mg^{2+}>>K^+$ (Gramss et al. 2004). Treating HM contaminated soils with synthetic (EDTA) and phytochelators (citrate, malate) increased total plant uptake and translocation to the harvestable shoot of Pb-EDTA and U by 100- to 1000-fold (Salt 2000). Chelators damaged root cell plasma membranes whose proteinaceous channels control uptake of elements (Williams et al. 2000) and allowed for the immediate access of metal chelates to the plant's transpirational flow. Daily applications of NH_4 and casein to Chinese cabbage for 36 d increased accumulation of Cd, Cu, Mn, Ni, and Zn in shoots 1.1 to 2.8 times more than in roots (Gramss et al. 2004). These elements chelate with those AA which could result from the microbial transformation of NH_4 and casein. Root uptake of metal-AA complexes was shown for graminaceous plants (Uren 2001). Metals present in the soil solution as free cations and mineral-, humic-, and fulvic-acid complexes may be released into the xylem as free cations, oxoanions, and chelates of carbocylic acids, histidine, cysteine, nicotinamine (Pich and Scholz 1996; Salt 2000), metallothioneins and phytochelatins (Shahandeh et al. 2001). Complexation of HM cations may thus repeatedly change on their way via CPx-ATPase transport proteins of root cell plasma membranes (Williams et al. 2000), symplasm, and during their release into the xylem.

In this study, Chinese cabbage potted on HM contaminated soil received daily doses of NH_4Cl, the biocatalyst 2-aminoethanol, and HM chelating cysteine and histidine, whose residues dominate the transmembrane domains of CPx-ATPase transport proteins (Williams et al. 2000). It was the goal to determine (uptake and) *in planta* formation of free AA as potential long-distance transporters of HM, the influence of AA, amines, and their mineralization products on the solubility of soil HM in competition with humic acid ligands, long-term changes in the solubility of elements in soils treated with (chelating) nitrogen compounds, and the influence of the treatments on uptake and translocation of HM to the harvestable shoot to improve phytoextraction of HM under controlled leaching conditions.

Materials and Methods

Uranium mine dump soil (U soil) from Settendorf (Germany) of pH (KCl), 7.20 ± 0.02; C_{org}, 2.50 ± 0.01 % (w/w); and organic N, 1972 ± 104 mg kg^{-1} contained (mg kg^{-1} ± SD) Al, 10025 ± 225; As, 278.2 ± 23.2; Ba, 53.0 ± 9.0; Ca, 8998 ± 598; Cd, 11.2 ± 1.2; Co, 55.4 ± 4.4; Cr, 22.0 ± 0; Cu, 564.5 ± 44.5; Fe, 19982 ± 218; K, 3503 ± 17; Li, 27.7 ± 1.7; Mg, 6349 ± 281; Mn, 1139 ± 8.9; Mo, 2.3 ± 1.3; Na, 131.1 ± 4.0; Ni, 102.1 ± 1.9; P, 866.7 ± 16.7; Pb, 114.3 ± 11.7; Sr, 78.1 ± 10.1; Ti, 86.6 ± 0.4; U, 105.8 ± 4.2; V, 30.2 ± 3.2; and Zn, 1113 ± 53.

Triplicate pot cultures (8 cm) of Chinese cabbage (*Brassica chinensis* L., cultivar Chico F1) were daily irrigated with solutions of NH_4Cl, histidine, cysteine, 2-aminoethanol (AE, all at 20 m*M* N), and sunflower seed oil suspension (10 g L^{-1}) from day 7 to 33 in a total quantity of 1.3 L kg^{-1} soil. Daily N doses amounted 1.3 m*M* referred to suspended soil. Unplanted control soils were treated in the same way. For more details and the determination of free AA in fresh shoot tissue (Amino acid analyzer LC 3000, Eppendorf, Maintal), the metal content in root and shoot (ICP-AES after pressurized microwave digestion), and the concentrations of soluble elements, humic substances (HS), and N compounds in soils (aqueous extracts 10 mL g^{-1} soil) compare Gramss et al. (2004).

Results

Mineralization products of the daily applied doses of NH_4Cl, His, Cys, and AE (14 mg N g^{-1} soil) accumulated in control soil and retained a minimum of NO_3-N in planted soil. Microbial consume of seed oil depleted N even in the control (Fig. 1).

Accordingly, shoots of Chinese cabbage attained dry weights of (g ± SD kg^{-1} soil) 7.54 ± 0.56 (control); 15.45 ± 1.25 (NH_4Cl); 12.5 ± 1.59 (His); 9.33 ± 0.65 (Cys); 10.18 ± 2.28 (AE); and 5.41 ± 0.4 (seed oil). Respective organic-N concentrations amounted (mg g^{-1} DW) 3.4 ± 0.6; 13.5 ± 0.8; 19.4 ± 2; 12.9 ± 0.8; 21.4 ± 3.4; and 7.3 ± 0.2, and corresponded with the concentrations of free AA (Fig. 2). N supply raised Arg, Gly, His, Leu, Pro, and Thr, lagging in control plants, above the detection limit of 4 μg g^{-1}. Increases were recorded for Ala (3.6 x); γ-Aba (4.2 x); Asp (7.5 x); Gln (51.6 x); Glu (4 x); and Ser (7.4 x). Concentrations of free AA in the His>Cys treatment lagged partially behind those of the NH_4 treatment.

Inorganic NH_4Cl, KNO_3, $NaNO_3$, NH_4NO_3, and $CaCl_2$ salts, sixteen of the AA found free in shoot tissue, and several organic bases in extracting fluids (20 m*M* N) showed different effects on the solubility of 22 elements and HS in U soil which compete for solution at a comparable pH (Table 1). The monovalent extractant cations $NH_4^+=K^+>Na^+$ desorbed Ca and Mg which, in turn, precipitated metal-containing HS and altered the solubility of the same cations as $CaCl_2$ extractants did. Solubilities of Al, Cr, Fe, Li, Pb, Ti, U, and V diminished to 0-28 %, whereas Ba, K, Na (76-151 %), Sr (365-1218 %), and Cd, Mn (Table 1) gained mobility. Amino, imino acids, amides and only 2 of 9 organic bases solubilized, in contrast to NH_4^+, Cu>>Co Ni>Zn>Cd>Ca Mg Mn but no other elements (Table 1). Solubility increases in the range those of metal containing HS (1 to 1.61 x) were certainly caused by HS rather than AA complexation. AA such as Arg, Lys, Ala, and β-Ala as well as most aliphatic and aromatic bases showed therefore no outstanding metal-complexing abilities in a multi-component soil solution. Hydroxy and acid AA at the respective pH value were more active and reached, apart from Co, Mn, and U, 25-100 % the solubilizing efficacy of citric acid. The level of U solubilization by citrate was only reached by the alkalinizing AE.

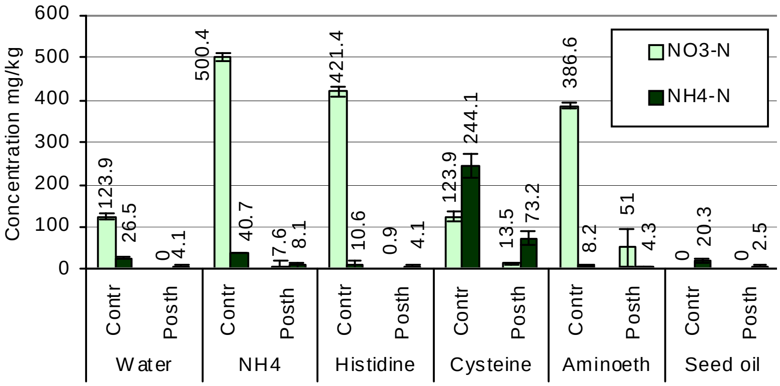

Fig. 1. Concentrations (mg kg^{-1} DW) of N in control and postharvest soils of Chinese cabbage pot cultures from different treatments. Error bars indicate confidence interval of 95%.

With the wide mineralization of the daily applied N compounds in control soil (Fig. 1), their long-term effects on the desorption of Ca and Mg and the resulting precipitation of HM and HS were widely uniform (Table 2). Uptake of Ca and Mg in planted soil ameliorated these effects. Elevated solubilizations of Cu, Ni, and Zn in the His, and Co, Mn, and Ni in the Cys treatment beyond the limits given by the levels of dissolved HS point to complexations by the corresponding AA. Means of the solubilities of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, P, Pb, Sr, Ti, U, V, and Zn in the NH_4Cl treatment divided by those in the water control amounted 70 % for control soils and 73.5 % for postharvest soils. The concentrations of dissolved HS diminished to 16 and 65 %, respectively, to indicate a reduced hazard of leaching. Immobilization was highest in the underlined elements. Solubility conditions in soils of the seed oil treatment were identical to those in the water control (data not shown).

Table 1. Quotients of the solubility of U soil elements and humic substances (HS) in N (20 m*M*) and citric acid (13.3 m*M*) containing extractants divided by the solubility in water extractant (compare Table 2 for absolute values). pH, acidity of the aqueous soil extract.

Extractant	pH	Ca	Cd	Co	Cu	Mg	Mn	Ni	U	Zn	HS
NH_4Cl	6.85	4.76^{a}	1.38^{a}	0.41^{a}	0.62^{a}	1.66^{a}	2.17^{a}	0.77^{a}	0^{a}	0.46^{a}	0.48^{a}
KNO_3	6.67	4.70^{a}	0.47^{a}	0.32^{a}	0.49^{a}	1.72^{a}	1.26^{a}	0.42^{a}	0^{a}	0.33^{a}	0.41^{a}
$CaCl_2$ 10mM	6.52	41.9^{a}	3.24^{a}	0.80^{a}	0.40^{a}	3.33^{a}	4.96^{a}	0.88	0^{a}	0.60^{a}	0.14^{a}
Alanine^{b}	6.75	1.13^{a}	1.27	0.97	1.50^{a}	1.10^{a}	1.11^{a}	1.12^{a}	1.69^{a}	1.05	1.01
Leucine^{c}	6.99	1.21	1.00	2.84^{a}	19.7^{a}	1.11	2.19^{a}	2.33^{a}	1.60^{a}	1.39^{a}	1.08
Serine^{d}	6.95	1.33^{a}	1.29^{a}	4.99^{a}	22.2^{a}	1.11	1.53^{a}	4.67^{a}	1.03	2.06^{a}	1.14
Aminoethanol	10.0	1..57^{a}	2.23^{a}	3.17^{a}	7.16^{a}	1.24^{a}	1.08	2.04^{a}	24.5^{a}	1.64^{a}	2.47^{a}
o-Phospho-aminoethanol	6.10	5.61^{a}	4.61^{a}	1.42^{a}	1.36^{a}	1.92^{a}	6.05^{a}	1.78^{a}	1.94^{a}	2.05^{a}	0.59^{a}
Cysteine	7.26	2.22^{a}	1.68^{a}	69.0^{a}	4.85^{a}	1.31^{a}	8.38^{a}	7.72^{a}	1.40^{a}	1.67^{a}	1.61^{a}
Histidine	7.41	1.32	6.72^{a}	4.15^{a}	18.5^{a}	1.17^{a}	1.41^{a}	12.0^{a}	0.32^{a}	11.5^{a}	1.36^{a}
Aspartic acid	4.94	17.2^{a}	20.2^{a}	5.58^{a}	12.3^{a}	4.53^{a}	19.7^{a}	8.69^{a}	0.64	12.2^{a}	0.30^{a}
Glutamic acid	5.18	15.0^{a}	13.1^{a}	3.88^{a}	7.03^{a}	4.09^{a}	15.7^{a}	4.52^{a}	0.57	8.36^{a}	0.33^{a}
Citric acid$^{e\ f}$	5.16	17.1^{a}	38.7^{a}	89.4^{a}	20.1^{a}	4.34^{a}	76.7^{a}	25.4^{a}	50.8^{a}	33.2^{a}	2.24^{a}
Aspartic acid^{f}	7.04	3.81^{a}	2.43^{a}	4.64^{a}	22.6^{a}	1.67^{a}	1.60^{a}	10.4^{a}	0.82	4.83^{a}	0.98^{a}
Glutam. acid^{f}	6.74	3.30^{a}	1.37	1.09	18.2^{a}	1.50^{a}	1.42^{a}	2.81^{a}	0.57^{a}	1.08	0.86^{a}
Citric acid$^{e\ f}$	7.35	14.8^{a}	2.77^{a}	33.6^{a}	12.7^{a}	5.60^{a}	59.1^{a}	14.9^{a}	25.1^{a}	13.9^{a}	4.66^{a}

[a] Deviations from quotient 1.00 with its internal tolerance field significant at $p \leq 0.05$.

[b] Similar solubility quotients in the amino acids: Arg, Lys, β-Ala; aliphatic amines: cholinechloride, hydroxylamine, triethanolamine, urea; aromatic compounds: 2-benzylaminoethanol, salicylamide, sulfanilamide. [c] Similar solubility quotients in the amino acids: Gln, Gly, Pro, Val. [d] Similar solubility quotients in the amino acids: Asn, Thr.

[e] Reference chelator for Asp and Glu, all at 40 m*M* COOH and a comparable pH.

[f] Extractants of Asp, Glu, and citric acid neutralized with NaOH.

Amino compounds, amines and amides, with the exception of acid AA (Asp, Glu), did not increase the solubility quotients of Al, As, Ba, Cr, Fe, K, Li, Na, P, Pb, Sr, Ti, U, and V more than those of humic substances with which these U soil elements were widely linked.

Table 2. Solubility (mg kg^{-1}) of elements and humic substances (HS) in control (C) and postharvest soils (P) from Chinese cabbage cultures. pH, acidity of aqueous soil extract.

Treatment		pH	Ca	Cd	Co	Cu	Mg	Mn	Ni	U	Zn	HS
Water	C	7.06	158	0.06	0.21	3.99	113	2.42	0.91	0.41	9.88	2981
	P	7.54	124[a]	0.09	0.32[a]	3.28	114	4.79[a]	1.12	0.90	13.1	5365[a]
NH_4Cl	C	5.29	841[b]	0.16[b]	0.03[b]	3.58	346[b]	1.82[b]	0.93	0.26	9.50	473[b]
	P	6.80	229[b]	0.09	0.19	3.04	158	2.08[b]	1.15	0.46	11.7	3460[b]
	[c]C	7.00	225	0.05	0.01	4.90	228	0.43	0.45	0.27	2.68	ND
Histidine	C	6.85	446[b]	0.04[b]	0.05[b]	1.51[b]	190[b]	0.24[b]	0.31[b]	0.46	2.74[b]	1603[b]
	P	7.24	127	0.14	0.41	5.02[b]	152	5.35	1.62[b]	1.01	21.7[b]	5367
Cysteine	C	6.41	896[b]	0.29[b]	1.55[b]	4.47	325[b]	75.1[b]	2.40[b]	0.60	15.8[b]	1333[b]
	P	6.31	684[b]	0.19[b]	1.05[b]	3.79	282[b]	45.4[b]	2.21[b]	0.13	9.32	1628[b]
	[c]C	7.00	606	0.24	1.35	4.93	269	45.1	2.06	0.60	10.6	ND
Aminoethanol	C	6.87	410[b]	0.05	0.08[b]	1.96[b]	181[b]	0.74[b]	0.58[b]	0.58	4.50[b]	1918[b]
	P	7.20	120	0.11	0.39	4.07	136	4.05	1.50	1.33	18.6	5111

[a] Values of water-treated postharvest soil significantly ($p \leq 0.05$) different from those of water-treated control soil. [b] Values significantly ($p \leq 0.05$) different from corresponding values of water-treated control and postharvest soil, respectively. [c] Control soil values recalculated for pH 7.0 to exclude metal solubilizing effects of low pH. ND, not

Relative to water-treated cultures of Chinese cabbage, soil treatment with NH4Cl or AE resulted in equal or lower solubilities of Cd, Co, Cu, Mn, Ni, and Zn but predominantly in higher root acquisition and higher translocation rates (S/R) of these elements to the shoots (Table 3). In the His and Cys treatments, root and shoot concentrations increased with the solubilities of HM in postharvest soils. Root concentrations in the N-starved plants of the seed oil treatment were as high as those of water treated plants, whereas HM translocation to shoots was significantly inhibited. The range of root-to-shoot translocation quotients (S/R) varied thus with the N supply to, and the resulting AA formation in, Chinese cabbage. With the mineralization of AE in control and planted soils (Fig. 1) and the resulting loss in alkalinity (Table 1), the amine was no longer able to solubilize U in potted soil (Table 2) and promote its uptake by the plant. Nevertheless, in NH_4Cl-treated plants grown for 48 d the concentrations of organic N (1.9 x), Co (2.7 x), Mn (3 x), Ni (1.8 x), and U (7.85 mg g^{-1} DW) were higher than in 34-d-old plants.

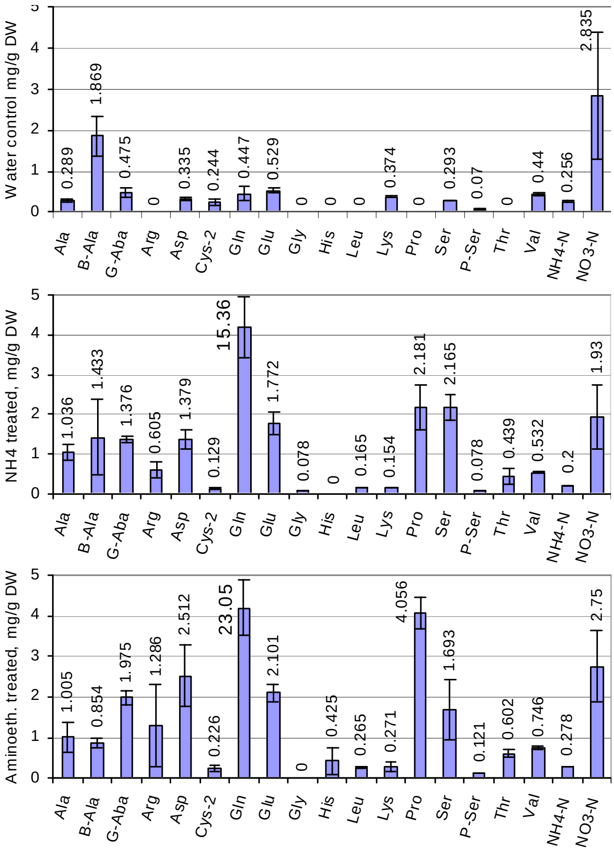

Fig. 2. Concentrations (mg g^{-1} DW) of free AA, NH_4-N, and NO_3-N in shoots of 34-d-old Chinese cabbage, irrigated daily with water, NH_4Cl, or 2-aminoethanol.

Error bars indicate confidence intervals of 95 %.

Ala, alanine; B-Ala, *β*-alanine; G-Aba, *γ*-amino-*n*-butyric acid; Arg, arginine; Asp, aspartic acid; Cys-2, cystine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Pro, proline; Ser, serine; P-Ser, phosphoserine; Thr, threonine; Val, valine. Not depicted: Isoleucine; *β*-aminoisobutyric acid.

Not detected: Asparagine; phenylalanine; tyrosine; taurine; hydroxyproline; *α*-aminoadipic acid; citrulline; *α*-aminobutyric acid; cystathionine; methionine; 3-methylhistidine; 1-methylhistidine; tryptophane; carnosine; ornithine. Cysteine (Cys) concentration may be twice the measured cystine value.

Total N (mg g^{-1} DW) bound to free AA: 0.767 (control); 4.780 (NH_4Cl); 7.125 (AE); 3.418 (His); 1.943 (Cys treated).

Discussion

Microbial consume of AA with their half life of 1-3 h in soil is saturated at concentrations of 1 m*M* (Jones et al. 2005). With daily applications of 1.3 mM, their mineralization products did not accumulate in planted soil (Fig. 1) and created no leaching problems. Traces of applied organic N should nevertheless rhythmically persist in soil and expose the plant to metal-AA complexes. Nitrogen supplements introduced not only metal-desorbing $Cl^->NO_3^-$ ligands (Shuman 1991) into the soil solution. The NH_4^+/Ca^{2+} Mg^{2+} couple gave rise to extensive HM precipitations and the wide neutralization of the dominating HS ligands (Table 1). With uptake of Ca and Mg, the plant ameliorated these effects which were expressed, e.g., in control soil of the NH_4Cl treatment (Table 2). In this case, the sum of solubilized cations amounted 6685 mg g^{-1} of dissolved C (Fig. 3). In the wide absence of the dominating HS ligand, cations should be complexed with Cl^- and oxoanions of C, S, and P many of which are as easily taken up by the plant as the free cation itself (McLaughlin et al. 1998). In the postharvest soil, the sum of solubilized elements (580 mg g^{-1}) was as low as those in most other treatments (Fig. 3). The surmised changes in complexation could be causal for the increased uptake and shoot trans-

Table 3. Mineral content (mg kg^{-1}) in shoot (S) and root (R) of Chinese cabbage grown on treated U soil for 34 d. S/R, quotient of shoot to root concentration divided by the corresponding quotient in the water control.

Treatment		Ca	Cd	Co	Cu	Mg	Mn	Ni	U	Zn
Water	S	12764	3.37	0.72	11.4	3117	38.7	6.29	0.94	137
control	R	9678	9.15	8.70	185	6627	132	32.1	155	466
	S/R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NH_4Cl	S	25128[a]	12.5[a]	1.93	21.2[a]	6052[a]	155[a]	11.6[a]	0.89	535[a]
	R	21978[a]	26.7[a]	16.7[a]	184	6283	242[a]	56.0[a]	40.9[a]	998[a]
	S/R	0.87[b]	1.28	1.39	1.86[c]	2.05[c]	2.19[b]	1.05	3.67	1.80[c]
Histidine	S	18727[a]	6.01[a]	1.10	15.9	5081[a]	122[a]	4.59[a]	0.15	284[a]
	R	23415[a]	16.4[a]	15.6[a]	175	5898[a]	188	60.4[a]	77.4[a]	835[a]
	S/R	0.61[c]	0.99	0.85	1.46[c]	1.83[c]	2.21[b]	0.39[c]	0.33	1.16
Cysteine	S	27523[a]	12.7[a]	8.85[a]	14.9	5182[a]	838[a]	12.4[a]	0.62	531[a]
	R	8801	26.0[a]	53.8[a]	744[a]	5840	707[a]	74.4[a]	74.0[a]	121[a]
	S/R	2.37[c]	1.33	1.98	0.32[c]	1.89[c]	4.05[c]	0.85	1.33	1.50[c]
Amino-	S	21302[a]	7.41[a]	1.35	21.9[a]	5010[a]	161[a]	6.93	0.27	377[a]
ethanol	R	21127[a]	14.3[a]	24.6[a]	274[a]	6155	514[a]	114[a]	91.7[a]	896[a]
	S/R	0.76[b]	1.41	0.66[c]	1.29	1.73[c]	1.07	0.31[c]	0.50	1.43
Sunflower	S	19406[a]	2.19[a]	0[a]	9.06	3237	12.4[a]	1.76[a]	0	116[a]
seed	R	20212[a]	12.0	9.77	197	6895	140	35.0	168	702[a]
oil	S/R	0.73[c]	0.50[b]	0[c]	0.75[c]	1.00	0.30[c]	0.26[c]	0[c]	0.56[c]
Variability of S/R quotient		3.89	2.86	∞	5.81	2.05	13.3	4.04	∞	3.21

[a] Values S and R significantly ($p \leq 0.05$) different from the corresponding shoot or root values of the water control. S/R quotients significantly different [b] at $p \leq 0.33$; [c] at $p \leq 0.05$ from those of the water control of 1.00 with their internal tolerance fields. Variability of S/R factor for further elements without/with the data of the seed oil treatment: As, 11.5/∞; Ba, 2.48/2.54; Cr, 11.4/∞; Li, 1.66/∞; Mo, 6.64/7.78; Na, 1.85/1.85; P, 1.59/1.59; Pb, 2.38/∞; Sr, 2.65/2.71; Ti, 2.77/2.77; U, 11.0/∞; V, 3.6/∞.

location of metals although treatments with NH_4Cl had reduced their concentration in the soil solution to a mean of 70 %. The influence of most AA and amines on solubilization and complexation of elements as well as on the physiological state of the plant should be restricted to their N content set free during nitrification, although His in the present treatment, and AA in general (Jones et al. 2005) were taken up by the plant.

The N content of free AA in shoots of Chinese cabbage correlated with the potential NO_3^- resources indicated by the control soils ($r = 0.750$; Figs. 1 and 2). Inorganic N stimulated AA formation more than did His and Cys supplements to the soil, but stimulation was surpassed by the biocatalyst AE. Although free AA are associated with long-distance transports of N and the local degradation and resynthesis of proteins in plant tissue (Ortiz-Lopez et al. 2000), their abundance could facilitate the transport of metals they preferentially complex. Plants treated with NH_4Cl showed predominantly higher concentrations of free Asp (preferring Co, Cu, Ni, Zn), Ser (Co, Cu, Ni), Thr (Cu, Ni), and Pro, Gln, and Glu (Cu) than plants from His>Cys treatments (data not shown). Total uptake and shoot translocation of heavy metals were higher, too (Table 3), although the plants lagged free His and notable Cys concentrations. This means that other high-affinity AA such as Asp, Ser, and Thr could play a role in the *in-planta* metal transport. In transmembrane domains of CPx-ATPase transport proteins presumed to carry heavy metals across plasma membranes of (root) cells, residues of Cys, His, Pro, Ser, and Met dominate whereby Leu, Phe, and Tyr residues contribute to specific affinities for Cd, Cu, Hg, and Zn (Williams et al. 2000).

The negligible translocation quotients for Ni in the His, and for Cu in the Cys treatment (Table 3) suggest a re-complexation of these elements on their way from soil solution to shoot rather than an unimpaired passage of the original metal-AA complex. Translocation of Ni to the xylem of *Alyssum montanum* L. was increased by 40-fold when Ni^{2+} was replaced by Ni-His complex (Krämer et al. 1996). On U soil, translocation of Ni to shoots of His and AE treated plants remained inhibited (Table 3) although these plants were the only to express free His in their tissue (Fig. 2). In several Ni hyperaccumulating plants, Ni was associated with citrate, malate, and malonate rather than with His (Salt 2000). The background of the dramatic uptake and translocation rates in the Cys-preferred elements, Co, and Mn (Table 3) should be re-examined in this context, as they are readily solubilized by carboxylic acids, too (Table 1), which could be formed upon the degradation of the carbon skeleton of AA (Hayes 1991).

Continuous treatment with organic C such as seed oil inhibited metal translocation to shoots of the small and N-starved plants. Tyler and McBride (1982) reported inhibited translocation of Cd to shoots of French bean as a result of ion competition with Ca. Table-3 data document that neither the translocation of Cd nor of most other elements correlated negatively with Ca concentrations in root or shoot of plants from the different treatments. Factors such as plant nutritional status and soil metal speciation should therefore be involved.

Summary and Conclusions

Chinese cabbage plants potted for 34 d on U soil were daily irrigated with deionized water and metabolizable N doses applied as NH_4Cl, His, Cys, and AE, or with seed oil to create the conditions of N starvation with the goal to make soil heavy metals more plant-available but less leachable. Owing to the rapid mineralization of N supplements to NO_3^-, N-treated plants increased shoot DW up to 204 % and free-AA nitrogen to 623-929 % relative to the water control. Whereas NH_4^+ as an intermediate mineralization product liberated Ca and Mg from the soil matrix which repressed the solubility of HS and a mean of 21 elements to 70 %, the AA found free in shoot tissue solubilized mainly Cd (1-6.7x); Co (1-69x); Cu (1.5-22.6x); Mn (1.1-8.4x); Ni (1.1-12x); and Zn (1.1-11.5x) at a comparable pH by complexation, and had no effects on others of the 23 elements examined. Surprisingly, plants from NH_4Cl, His, Cys, and AE treatments showed the same uptake preferences although the solubility of these elements in NH_4Cl treated soil had been reduced and lagged AA ligands. In addition, NH_4Cl-treated plants with access to the highest soil nitrate resources combined highest root concentration and shoot translocation rates of these elements with highest concentrations of free AA. The metals taken up were apparently determined by the affinity of the AA residues in the transmembrane domain of the CPx-ATPase transport proteins of cell membranes (Williams et al. 2000). Resulting shoot concentrations of Cd, Co, Cu, Mn, Ni, and Zn for which AA were selective showed maximum increases to 377, 1229, 192, 2165, 197, and 391 %, respectively across all treatments, promoted by higher translocation rates from roots to shoots (1.05 to 4.04-fold). Uptake of U was not promoted. Hence, application of NH_4Cl increased the total accumulation of these elements in the shoots of higher biomass to 765 %. A root uptake of unsplit metal-AA complexes could not be postulated. It is therefore concluded that accumulation of AA-preferred heavy metals in shoots is best stimulated by NH_4 rather than by undegraded AA, or by biocatalytic amine compounds. NH_4 did not only trigger the formation of more plant-available metal species via

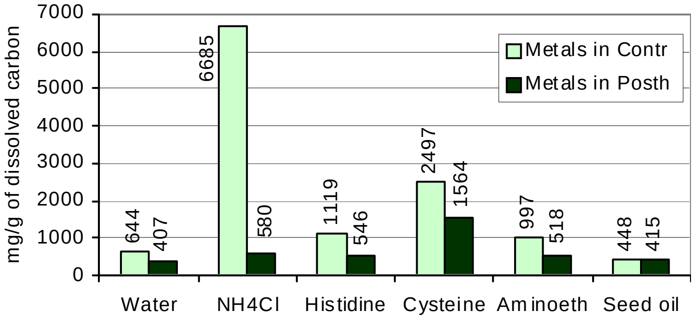

Fig. 3. Sum of the dissolved 20 cationic elements examined (without As, Mo, P, in mg g^{-1} of dissolved C) in the solution of control (Contr) and postharvest (Posth) soils of Chinese cabbage pot cultures from different treatments.

interaction with Ca^{2+}. It promoted the development of large, vigorous plants with a high content in protein and free AA, a need for trace metals in the shoot, and with the energy to re-complex soil metals for their transport through plasma membranes, symplasm, and xylem. As these advantages did not apply to the small and N-starved plants of the seed oil treatment, differences in the extent of metal translocation from the root to the shoot were inevitable. Repeated mineral-N supply at the limit of metabolizable doses was therefore a key to the improvement of continuous phytoextraction technologies.

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