Variation in heavy metal uptake by crop plants

Hans Bergmann, Klaus-Dieter Voigt, Bernd Machelett, Gerhard Gramss

Friedrich-Schiller-University, Institute of Nutrition, Dornburger Strasse 25,
D-07743 Jena, Germany

Abstract. Relative to heavy metal (HM) excluder plants (French bean, lupin, maize, cereals), HM sequestering crops (buckwheat, beet root species) accumulate up to the 18-fold concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn as a sum in their shoot tissue. Soil amendment with nitrogen increased HM uptake further. This is a novel treatment to make continuous phytoextraction technologies more efficient without increasing the soil's leaching rate.

Introduction

Uranium mining in East Germany left numerous rocky mine tailings and heavy metal (HM) containing overburden and ore processing deposits. Their restricted use in agriculture or their re-vegetation with the goal to stabilize and reduce the pool of HM implies the selection of plant species and a careful substrate management. Phytostabilization may be facilitated when plant covers of perennial herbs or woody plants are able to form arbuscular or ectomycorrhizal associations, respectively, or foster a root-associated bacterial flora. Microorganisms bind HM to their surfaces and extramatrical polysaccharides and proteins, or form intracellular metal complexes with proteins, peptides, and oxalates which are no longer accessible or toxic to the plant (Berthelsen et al. 2001; Van der Lelie 1998). This will less apply to annual crops whose element concentrations in root and shoot correlate frequently with those in the soil solution (Schönbuchner et al. 2002; Tyler and Olsson 2001). Its composition in a given soil is determined by variables such as pH (Sumner et al. 1991), microbial activity, the presence of complexing agents (Cotton et al. 1987; Hayes 1991), and high concentrations of Ca, K, and Mg cations which precipitate HM and metal-humic complexes from solution, and reduce the metal content in the plant (Gramss et al. 2003a).

Table 1. Organic U soil amendments and their acronyms.

Amendment	Applied at seeding	6 wk prior to seeding	2 wk prior to harvesting
Molasses 42 % (0.42 % W/DW)	M1	M2	M3
Casein (0.44 %)	C1	C2	
Sucrose (1 %)		S1	
Sucrose (0.5 %)			S2
Rape shoot homogenate (6.2 %)	R		
Aminoethanol (0.03 %)			AE

All of these soil parameters can be controlled by application of organic-C- or N-rich compounds. Amendment with sucrose initiated the microbial production of metal-chelating carboxylic acids under low pH conditions. Their mineralization turned pH then to alkalinity and solubilized humic substances. Soil amendment with casein increased pH in the phase of NH_3 release to end in soil acidification and the precipitation of humic substances in the nitrification stage (Gramss et al. 2003b; Voigt et al. 2004). With the temporary formation of the exchange cation NH_4^+, Ca^{2+} was released from the soil matrix. Its coagulating effect removed humic and fulvic acids, the dominant ligands of HM, from the soil solution to give rise to the formation of metal complexes with Cl^- and oxoanions of C, S, and P (Cotton et al. 1987; Gramss et al. 2005), which may be more plant-available. Beside the preferred free cation, sound roots seem also to take up complete sulphate and chloro complexes of Cd (McLaughlin et al. 1998) as well as ligands such as citrate, histidine, EDTA (Bell et al. 2003), and humic molecules < 3500 Da (Nardi et al. 2002). It has not yet been shown whether these compounds enter sound root cells as complete metal chelates.

In this study, seventeen crop plants were grown on uranium mine dump soil with the goal to monitor their excluder or sequestrator properties by determining the degree of HM accumulation in shoot and root, and to stimulate HM uptake by soil treatments with N, organic C, or the biocatalyst, 2-aminoethanol. With the estimation of the natural or manipulable HM accumulation potential, technologically controllable plants for a restricted crop production on moderately contaminated soils, for phytoextraction, and for phytostabilization (immobilization) treatments shall be recommended.

Materials and Methods

The common crops, Avena sativa (cv. Flämingstern); Beta vulgaris (Rote Kugel); B. vulgaris rapaceae (Eckdorot); Brassica chinensis (Granaat); B. napus (Liratop); B. rapa (Goldball); Cannabis sativa (Lipko); Fagopyrum esculentum (Sobano); Helianthus annuus (Capital); Hypericum perforatum; Lupinus albus (Ares); Nicotiana tabacum; Phacelia tanacetifolia; Phaseolus vulgaris (Juvina); Sinapis alba

(Asta); Triticum aestivum (Monopol); and Zea mays (Mentor) were exposed to 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}. The soil 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. Pot cultures (10 cm in diameter, 0.5 L) of U soil (sieve 5 mm) were seeded to 3-6 plants each and incubated, after seedling emergence, for 28 to 42 d in growth chambers at a 22° C / 16 h and 16° C / 8 h day-night regime and a light intensity of 550 µmoles m^{-2} s^{-1}. Plants were also grown on soils organically amended at different stages of the experiment (Table 1). For the determination of metal contents in root and shoot (ICP-AES and ICP-MS after pressurized microwave digestion), and the concentrations of soluble elements in soils (aqueous extracts 10 mL g^{-1} soil) compare Gramss et al. (2004).

Results and Discussion

Variability in heavy metal uptake by common crops

Concentrations of HM in shoots of 17 common crops from U soil varied extremely (Table 2). Of the most critical elements, shoot concentrations of As and Cd, but not of Pb, exceeded the tolerable limits. The commonly accepted concentrations in Cr, Cu, Ni, and Zn (Table 2) were observed at least by some of the crops. Agriculture on HM contaminated soil includes therefore the *in-situ* selection of crop plants with excluder properties, which are denoted by a restricted HM transfer to the target biomass and in particular to grain and oil seed. Cereals with a common Cd load around 0.035 mg kg^{-1} fresh wt (Schachtschabel et al. 1998 p. 318) came to 0.08 to 0.29 mg kg^{-1} in 15 spring barley, and to 0.13 to 0.39 mg kg^{-1} in 20 winter wheat cultivars (decreed limits 0.1 and 0.2 mg kg^{-1} fresh wt, respectively) on soils with a natural Cd contamination in the Saxonian Mulde district (Klose 2004, 2005). Similarly, Cd concentrations in radish could be halved by the selection of the optimum excluder cultivar to indicate the importance of plant breeding (Table 3). But also sparing nitrogen fertilization reduced the transfer of HM from root to shoot (Gramss et al. 2005).

Table 2. Concentrations (mg kg^{-1} DW ± SD) of HM in shoots of seedlings potted for 28 to 42 d on U soil in quadruplicate cultures. Adapted from Voigt et al. (2004).

Plant	As	Ba	Cd	Co	Cr	Cu	Mn	Ni	Pb	U	Zn	Sum[a]
Cannabis	4.58	16.1	0.73	0.33	2.93	7.45	66.3	3.70	1.13	0.35	34.7	55.2
Sativa	±1.61	±2.61	±0.8	±0.17	±0.6	±1.34	±5.58	±0.1	±0.87	±0.09	±8.58	±8.93
Phaseolus	5.03	11.3	1.00	0.50	1.30	5.93	48.1	6.93	1.08	1.03	48.7	69.9
vulgaris	±1	±2.2	±0.61	±0.31	±0.38	±0.98	±3.4	±2.04	±0.36	±0.46	±16.4	±16.6
Triticum	8.58	16.0	1.13	0.40	6.37	7.53	37.4	3.78	1.48	1.03	47.3	76.2
aestivum	±1.72	±2.95	±0.26	±0.2	±0.66	±2.75	±9.65	±1.4	±0.78	±0.45	±18.4	±18.8
Lupinus	2.45	5.13	0.38	0.73	1.28	8.58	567	8.30	1.20	1.87	81.3	104
albus	±0.62	±1.76	±0.09	±0.22	±0.82	±1.76	±164	±3.68	±0.68	±1.25	±9	±9.95
Zea	1.83	3.98	2.03	0.20	0.80	9.13	42.0	1.60	0.70	0.10	96.0	112
mays	±1.01	±0.8	±0.71	±0.18	±0.38	±2.94	±13.2	±1.16	±0.42	±0	±20.5	±20.8
Avena	6.45	2.18	2.40	0.20	1.50	11.1	42.0	8.53	0.83	0.40	86.0	117
sativa	±0.92	±0.67	±0.85	±0.07	±0.14	±0.95	±14.4	±0.98	±0.65	±0.23	±28.6	±28.7
Hypericum	7.03	4.03	22.6	0.50	0.57	13.8	39.7	1.90	0.77	1.37	74.0	121
perforatum	±2.13	±1.06	±3.40	±0.22	±0.13	±4.14	±6.79	±0.66	±0.48	±0.88	±34.3	±34.7
Nicotiana	12.1	15.6	22.3	0.53	0.60	14.3	37.6	2.93	0.97	1.20	95.0	148
tabacum	±0.73	±3.59	±5.51	±0.13	±0.17	±4.46	±6.06	±0.34	±0.48	±0.70	±13.1	±15.0
Phacelia	31.6	93.3	3.70	0.50	4.37	8.40	47.7	7.77	0.60	0.67	121	178
tanacetif.	±9.79	±22	±0.67	±0.08	±2	±3.21	±4.18	±1.8	±0.22	±0.21	±25.3	±27.5
Brassica	3.60	12.9	4.87	0.57	2.97	15.6	22.6	4.50	0.40	0.20	177	209
chinensis	±0.51	±4.71	±0.62	±0.24	±0.66	±1.8	±3.75	±0.45	±0.08	±0	±23.8	±23.9
Brassica	1.90	10.2	4.93	0.78	0.80	13.8	62.0	3.23	0.95	0.53	184	209
napus	±0.59	±3.15	±1.4	±0.46	±0.47	±4.42	±14.3	±0.17	±0.68	±0.46	±55.3	±55.5
Helianthus	8.27	13.1	5.27	0.50	1.68	8.08	74.7	5.37	0.63	0.40	221	250
annuus	±2.87	±3.29	±0.69	±0.18	±0.21	±0.89	±11	±0.74	±0.18	±0.21	±24	±24.2
Brassica	6.35	27.6	7.80	1.43	1.53	31.1	92.2	6.05	2.63	2.73	257	312
rapa	±3.6	±5.59	±2.21	±0.61	±1.01	±11.1	±29.5	±2.35	±2.18	±1.64	±88.9	±89.7
Sinapis	2.18	11.9	3.90	0.38	1.65	11.6	22.6	2.93	1.28	0.60	294	318
alba	±0.05	±3.26	±1.34	±0.13	±0.48	±4.6	±2.58	±0.29	±1.18	±0.41	±128	±128
Fagopyrum	4.23	18.8	15.2	0.78	6.30	35.3	81.4	11.7	1.20	0.43	318	392
esculentum	±1.09	±2.93	±3.17	±0.19	±1.13	±5.19	±6.09	±1.68	±0.33	±0.46	±57	±57.4
Beta	7.17	70.5	25.5	1.48	1.40	29.0	199	7.47	1.68	1.00	841	913
vulgaris	±2.45	±5.08	±2.04	±0.39	±0.81	±4.57	±18.5	±3.12	±0.8	±0.33	±77.5	±77.7
B. vulgaris	4.90	50.3	26.5	0.83	1.90	25.5	237	6.93	1.98	0.75	926	994
rapaceae	±0.97	±10.7	±1.62	±0.25	±1.31	±7.05	±28.1	±1.02	±0.9	±0.35	±106	±106
Mean shoot conc.	6.96	22.5	8.84	0.63	2.23	15.1	101	5.51	1.15	0.86	230	269
Mean root conc.[b]	159	36.4	30.6	7.38	4.78	174	60.8	32.0	12.2	62.0	663	1075

[a] Sum of As, Cd, Cr, Cu, Ni, Pb, and Zn concentrations. [b] Individual data not shown.
Shaded type, outstanding natural HM accumulation potential.
Tolerable concentrations of HM (Schachtschabel et al. 1998 p. 318): As, 0.01-1; Cd, 0.05-0.4; Cr, 0.1-1; Cu, 2-20; Ni, 0.1-3; Pb, 0.1-6; Zn, 10-100 mg kg^{-1} DW.

Table 3. Uptake of Cd from soil (10 mg kg^{-1}Cd) by radish cultivars.

Cultivar	Cd content mg kg^{-1} biomass
Eiszapfen	23.1
Flamboyant	26.7
Mean	41.1
Cherry B	43.3
Rudi	49.5
Rodos	50
Isabell	54.3

None of the 17 crop plants examined in Table 2 displayed natural hyperaccumulation potential to the HM considered. Nevertheless, As concentrations in *Phacelia*, and Cd, Cu, and Zn levels in beet root species deserve attention when the exchangeable soil HM fractions shall be controlled by phytoextractive crops. It is the cations of Cd, Co, Cu, (Mn), Ni, and Zn, whose uptake to, and transport across the root symplasm was promoted by affinities of the CPx-ATPase transmembrane transport proteins (Williams et al. 2000), and whose translocation from root to shoot was stimulated by repeated soil treatment with nitrogen (Gramss et al. 2005). Application of the exchange cation NH_4^+ desorbed Ca^{2+} (and Mg^{2+}) from the soil matrix which, in turn, precipitated several HM and most of the dissolved humic substances, the dominant ligands of metal cations, from the soil solution to give rise to the formation of more plant-available metal complexes (e.g., McLaughlin et al. 1998). Moreover, soil application of the model substances, casein and sucrose altered greatly pH value and solubility of elements during nitrification and the formation of carboxylic acids, respectively (Gramss et al. 2003b; Voigt et al. 2004).

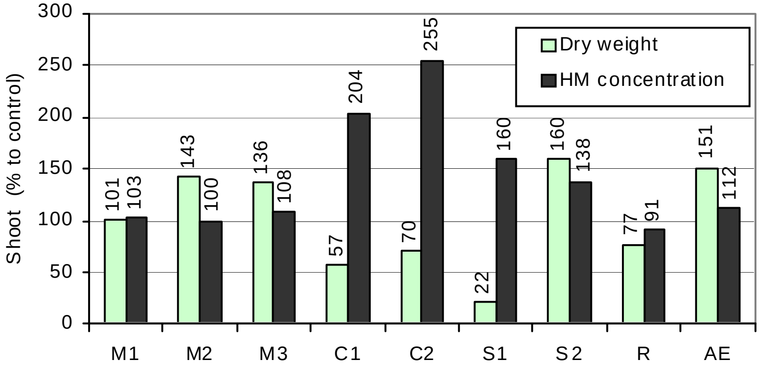

Fig 1. Influence of different soil treatments on dry wt of, and HM concentrations in, shoots of a mean of 17 crop plants, expressed in % to the water-treated controls (= 100%). Percentage values of HM comprise those of As, Cd, Cr, Cu, Ni, Pb, and Zn.

In pot trials, treatments with casein (15.6 % N, w/w), the carbohydrate-dominated molasses, sucrose, and rape shoot homogenate, and the biocatalyst, 2-aminoethanol interfered therefore with biomass production and uptake of several HM (Table 4). Elevated shoot HM concentrations were predominantly linked with NH_4/NO_3 formation from mineralizing casein applied 0 or 6 wk prior to seeding (treatments C1; C2), with microbial carboxylic-acid production from sucrose applied 6 wk prior to seeding (S1), and with the application of aminoethanol (AE) applied 2 wk prior to harvesting (individual data not shown). As a mean across all 17 crop plant species with their wide interspecific variations (data not shown), soil treatments with molasses (M2; M3), late applied sucrose (S2), and aminoethanol (AE) promoted dry wt production of aboveground biomass (Fig. 1), whereas casein (C1; C2) and early sucrose (S1) applications inhibited growth by a temporary fatty-acid toxicity (Armstrong and Armstrong 1999; Gramss et al. 2003b). Nevertheless, it were these detrimental treatments (C1; C2; S1) which promoted HM accumulation in shoots (Fig. 1). The biocatalyst, aminoethanol, stimulated HM accumulation in shoots of higher biomass although this compound is commonly applied in near-homeopathic doses (Bergmann et al. 1999). Contrary to the conditions in chelator-induced phytoextraction technologies (Salt 2000), soil treatments with N and organic C did not significantly increase water-solubility and leaching of hazardous elements in U soil (Fig. 2).

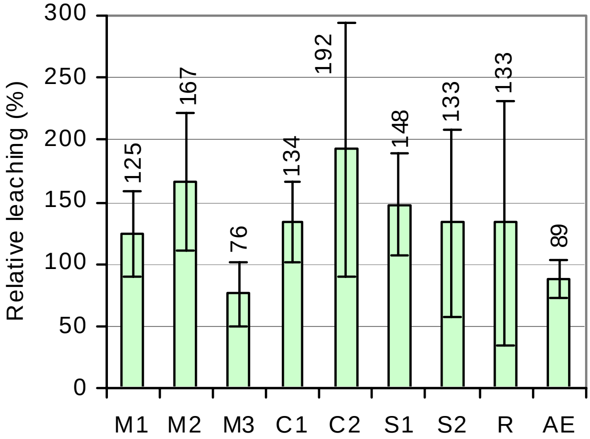

Fig.2. Influence of different soil treatments on water-solubility (potential leaching) of As, Cd, Cr, Cu, Ni, Pb, and Zn, expressed in % to the conditions in water-treated soil (= 100 %).

Table 4. Maximum concentrations (mg kg^{-1} DW) of HM in shoots of seedlings grown for 28 to 42 d on U soil, stimulated by soil treatment with N, organic C, or aminoethanol.. Blanks, moderate metal concentrations not given. Adapted from Voigt et al. (2004).

Plant	As	Ba	Cd	Co	Cr	Cu	Mn	Ni	Pb	U	Zn
Cannabis sativa					33.0 S2						
Nicotiana tabacum	50.9 C2	57.5 C2	53.5 C2			43.0 C2	2453 C2	25.9 C2	29.3 C2	13.5 C2	535 C2
Phacelia tanacetif.		191 S1				42.0 S1					
Brassica napus			23.7 C1			43.0 C2	2273 C2				954 C1
Helianthus annuus					15.5 M3		2999 C2				833 C1
Brassica rapa			20.9 C1	24.5 C2		61.0 S1	2328 C2	28.1 C2			994 C1
Sinapis alba			17.0 C1	22.1 C2		56.0 C2		41.1 C2			1733 C1
Fagopyrum esculentum				44.9 C2			3922 C2	53.2 C2			806 M1
Beta vulgaris		173 S1	37.9 S1	33.8 C2			3536 C2	47.0 C2			1395 AE
B. vulgaris rapaceae		75.9 S1	36.8 AE	62.5 C2			4709 C2	70.2 C2			1324 AE

Soil treated with AE, aminoethanol; M, melasses; C, casein; S, sucrose (see Materials). Water controls see Table 2.

Trapping dissolved heavy metal fractions by catch crops?

Several authors accept that it is impossible to quantify the plant-available (heavy) metal fraction by extracting a given soil with any chemical solution (Finck 1992). In the present study, the supernatants of U soil-water suspensions (1:10) shaken for 3 h at 25° C were partially centrifuged until the remaining clay, clay-humic, and humic particles were no longer self-depositing. A high surface area to mass ratio conferred colloidal properties (Schachtschabel et al. 1998 p. 149) to the particles up to 2 µm in size. Whereas these supernatants with their considerable metal content (HM fraction (^{a}), Table 5) could gain access to surface waters, only portions with particles < 0.45 µm and a restricted metal content (HM fraction (^{b}), Table 5) passed through soil columns.

Upon the re-vegetation of uranium mine tailings, overburden soils, and ore processing deposits, plants may serve as a water-depleting mechanical barrier to the flow of contaminated surface water and a temporary sink for toxic soil solution constituents (Table 5). Both the possible 20 t ha^{-1} of aboveground biomass and 4 t ha^{-1} root tissue with the mean uptake potential of Table 2 were able to trap 3 times the Cd load of a static soil solution such as HM fraction (^{b}). In comparison to the soil's total HM content, the portion of the temporarily trapped elements remained low (Table 5). A vegetation cover reduced nevertheless percolation in fallow land with 600 mm annual rainfall from 250 to < 50 mm (unpublished).

Re-vegetation comprises the formation of sparingly soluble HM phosphates in plant tissue (e.g., Günther et al. 2002) and the slow and repeated release of the soluble HM species from decaying biomass which may facilitate their gradual and time-consuming incorporation into soil minerals and the quantitatively increasing soil organic substance (Schachtschabel et al. 1998). Fungi and bacteria of living and decaying plant material bind HM to cell surfaces, exopolysaccharides, proteins, peptides, and oxalates and reduce their plant-availability (Berthelsen et al. 2001; Van der Lelie 1998). This is counteracted by metal-chelating carboxylic and amino acids of root and microbial origin (Hayes 1991) and may be one of the reasons that planting increased the soluble HM fraction in U soil, which must not necessarily result in higher shoot HM concentrations (Gramss et al. 2004).

Table 5. Temporary accumulation (%) of the water-soluble HM fraction[b] of U soil by a vegetation cover of 20/4 metric tons ha^{-1} of dry aboveground/root biomass with the mean shoot/root uptake potential indicated in Table 2.

	As	Ba	Cd	Co	Cr	Cu	Mn	Ni	Pb	U	Zn
HM fraction[a]	5.59	0.61	0.08	0.32	0.53	5.80	4.17	1.49	0.66	0.88	13.1
mg kg^{-1} ± SD	±0.36	±0.01	±0.01	±0.02	±0.08	±0.16	±0.12	±0.04	±0.05	±0.15	±0.30
HM fraction[b]	2.76	0.17	0.04	0.14	0.09	4.70	2.59	0.91	0.27	0.13	4.63
mg kg^{-1}± SD	±0.57	±0.01	±0.01	±0.05	±0.03	±0.81	±0.21	±0.06	±0.09	±0.06	±0.74
HM fraction in shoots[c] %	2.52	134	315	4.44	25.6	3.21	39.1	6.07	4.26	6.67	49.6
HM fraction in roots[d] %	23.0	86.6	279	20.8	22.0	14.8	9.40	14.1	18.0	192	57.3
Uptake of total HM[e] %	0.25	0.70	2.33	0.06	0.19	0.15	0.11	0.18	0.05	0.24	0.44

Water-soluble HM fraction carried off by surface water ([a]), and migrating through soil columns ([b]). Binding (%) of HM fraction ([b]) by aboveground biomass ([c]), and by roots ([d]). Accumulation of the soil's total HM concentration (see Materials) by whole biomass ([e]).

Conclusions

The sum of As, Cd, Cr, Cu, Ni, Pb, and Zn concentrations in shoot tissue of 17 common crop plants varied by factor 18 when grown on a moderately HM contaminated uranium mine dump soil (Table 1). Excluder plants such as French bean, lupin, maize, and several cereals confined HM transfer to shoot tissue and potentially to the grain fruit. Sequestrator plants such as buckwheat and beet root species accumulated with Cd, Cu, (Mn), Ni, and Zn those elements to which the plant's proteinogenic amino acids showed highest affinities (Gramss et al. 2005). This mechanism did not apply to the low-concentrated Cr, Pb, and U. Soils with a specific HM contamination can be admitted to the production of excluder crops whose HM load can be further reduced by the selection of appropriate cultivars and by a restricted N fertilization. For the remediation of seriously HM contaminated soils, the phytoextractive potential of *Phacelia* (As) and *Beta* sp. (Cd, Cu, Zn) is negligible but manipulable by soil treatment with N and organic C compounds in an upcoming field of research. The phytostabilisatory effect of plant covers with their root zone microflora is multicomponent and indispensable to soil forming processes. Increases in the water-solubility of HM in planted soils do not immediately result in their increased plant-availability.

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