RELATIONS BETWEEN VARIOUSLY AVAILABLE FRACTIONS OF TRACE METALS IN THE SOIL AND THEIR ACTUAL PLANT-UPTAKE

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Summary

In a pot experiment, availabilities of Cd, Cr, Ni, Pb, and Zn added to the soil as metal nitrates or as enrichment of sewage sludge were evaluated by comparing concentrations of their total potentially available, presumably plant-available and directly plant-available forms in the soil. At excessively increasing soil contamination, the plant-available concentrations increased more than the total soil contents, thus the relative availabilities of the metals increased. This was reflected in the amounts taken up by the young maize test plants and in the plant/soil transfer factors. Transfer factors calculated for the "plant-available" soil metal contents depended less on the contamination level than those based on total soil metal contents.

1. Introduction

The idea of using the bioavailable fraction of soil contaminants in risk assessment, instead of the total contents, gains growing acceptance among environmental experts. However, the bioavailable fraction of trace metals as assessed by routine chemical extraction methods, cannot be equal even theoretically to the amounts taken up by the living organisms: the former quantity is determined by the laws of chemistry while the latter is influenced by the laws of physiology (Sillanpää, 1982). Thus, "bioavailability" should be evaluated in comparison with bioaccumulation data and with toxicity symptoms in various living organisms. Such an approach is presented in this paper.

2. Material and methods

A pot experiment was conducted under natural climatic conditions but with controlled water-supply, on samples of the ploughed layer of a rust-brown forest soil (particles <0.02 mm 19 %, particles <0.002 mm 13 %, pH_{KCl} 5.14, organic matter content 1.1 %, cation exchange capacity 8.5 $cmol_c$/kg soil).

Cadmium, Cr, Ni, Pb, and Zn were added to the soil either as a solution containing a mixture of their nitrates (5 Me), or as municipal sewage sludge (SS) spiked and pre-incubated for one week with each of these metal nitrates separately (SS + Me) or with their mixture (SS + 5 Me). The resulting metal loadings in the soil corresponded to 1, 3, 10, 30, and 100 times the elemental concentrations (L) entering the soil from a usual application practice of municipal sewage sludge onto agricultural lands, as specified by the Hung. Techn. Directive (1990). Pollution rates thus based on the regulations were different for the five metals: values of L were 0.125 mg Cd, 8.33 mg Cr and Pb, 1.67 mg Ni, and 25 mg Zn per kg soil. In the SS treatments always the same amount of sludge (4%) was added to the soil. After one month incubation at ambient temperature, maize (*Zea mays* L. cv. Favea) was grown in the pots for 6 weeks, until the 6-7-leaf-stage.

Bioavailabilities of the five metals were evaluated by comparing concentrations of the total potentially available (2 M HNO_3-extractable at 1:20 soil:extractant ratio and 100 C°; Andersson, 1976), presumably plant-available (acidic [NH_4-acetate + EDTA]-, i.e. [AAAc + EDTA]-extractable, at 1:20 soil:extractant ratio; Lakanen and Erviö, 1971), and directly plant-available (in the soil solution at natural soil water contents; Csillag et al., 1999) forms of the metals. Plant metal concentrations were determined after wet digestion (cc. HNO_3 + H_2O_2) of the plant parts. Metal contents in the soil extracts, in soil solutions and in the plant parts were measured by ICP-AES. Dry matter accumulation in the plant parts was regarded as indicator of toxicity.

Single metal treatments were made in duplicates and multiple metal treatments in triplicates. The results are averages of measurements from separate pots.

3. Results and discussion

Metal concentrations extracted by 2M HNO_3 were similar in the control uncontaminated soil and in samples treated only with the original municipal SS with no metal addition (Table 1.). The sludge itself contained the selected metals in amounts similar to or lower than the levels permitted in sludges for agricultural use (Hung. Techn. Dir., 1990), and most of the metals might have been strongly attached to sludge components. Soil metal concentrations increased fairly proportionally with increasing metal enrichment of the sludge, reflecting also the ratios between the application rates of the metals at the excessive contamination of 30 and 100 L (Zn>Cr=Pb>Ni>Cd).

Table 1. Total potentially available (2M HNO_3-extractable) metal content (mg/kg) in the soil treated with sewage sludge spiked with all the five metals

	Cd	Cr	Ni	Pb	Zn
control	1.27 ± 0.09	10.3 ± 0.7	13.4 ± 1.4	12.4 ± 0.5	45.9 ± 0.6
SS + 5 Me					
0 *L* (control)	1.22 ± 0.16	10.5 ± 1.4	12.0 ± 1.8	13.2 ± 2.1	55.9 ± 7.7
3 *L*	1.79 ± 0.47	34.3 ± 11.4	17.5 ± 5.1	35.7 ± 11.6	142 ± 43
10 *L*	2.32 ± 0.51	82.2 ± 15.2	28.2 ± 5.8	90.2 ± 12.7	283 ± 42
30 *L*	4.65 ± 1.07	253 ± 71	57.1 ± 12.9	270 ± 57	797 ± 151
100 *L*	13.7 ± 1.3	749 ± 103	179 ± 14	780 ± 69	2710 ± 143

Andersson (1976) showed that this extraction method released more of the total content of trace metals from soils treated for long periods with SS than from the native content of unpolluted soils. The differences between the extracted amounts in the polluted and unpolluted soils represented 82-96 % of the amounts of Cd, Cr, Cu, Ni, Pb, and Zn that accumulated in the soil during sludge application. He concluded that the method is suitable for determining the total potentially available fraction of the metals in the soil.

Table 2. Recovery of the added metals in the total potentially available metal fraction of the soil: $(c_{2M\ HNO3,\ metal\ treated\ soil} - c_{2M\ HNO3,\ control\ soil}) * 100 / c_{added}$

	Cd	Cr	Ni	Pb	Zn
SS + Me					
10 *L*	21 ± 19	66 ± 31	101 ± 21	101 ± 32	96 ± 51
30 *L*	36 ± 1	89 ± 28	97 ± 22	61 ± 34	110 ± 17
100 *L*	71 ± 23	88 ± 15	98 ± 57	151 ± 7	84 ± 1
SS + 5 Me					
10 *L*	73 ± 41	86 ± 18	95 ± 35	94 ± 15	89 ± 17
30 *L*	87 ± 29	97 ± 28	90 ± 26	103 ± 23	98 ± 20
100 *L*	99 ± 10	89 ± 12	100 ± 8	92 ± 8	106 ± 6
5 Me					
10 *L*	43 ± 4	70 ± 2	44 ± 3	76 ± 2	77 ± 3
30 *L*	68 ± 8	68 ± 5	66 ± 7	77 ± 6	83 ± 5
100 *L*	108 ± 47	78 ± 27	104 ± 45	105 ± 36	109 ± 38

In the present experiment, recovery of the metals in the metal-spiked sludge treatments was in average of all treatments 89 % and was generally similar when either all the five elements or only one single metal was used as contaminant (Table 2.). However, recovery of Cd tended to be less when applied single. Since Cd was present in much smaller amounts than the other metals, it may have been bound in the soil more strongly when no competition occurred in the single metal treatments. In the pots receiving no SS

but only the mixture of the five metal nitrates, the recoveries at the lower metal rates were smaller, reaching ~100 % only at the 100*L* loading rate. Inorganic forms of the metals are generally regarded as more available for plant-uptake than metals bound to the sorption sites in sludges (McBride, 1995). Although in our experiment the differences between the sludged and inorganic metal treatments were generally not significant, the lower recoveries observed in the 5 Me treatments need further studies..

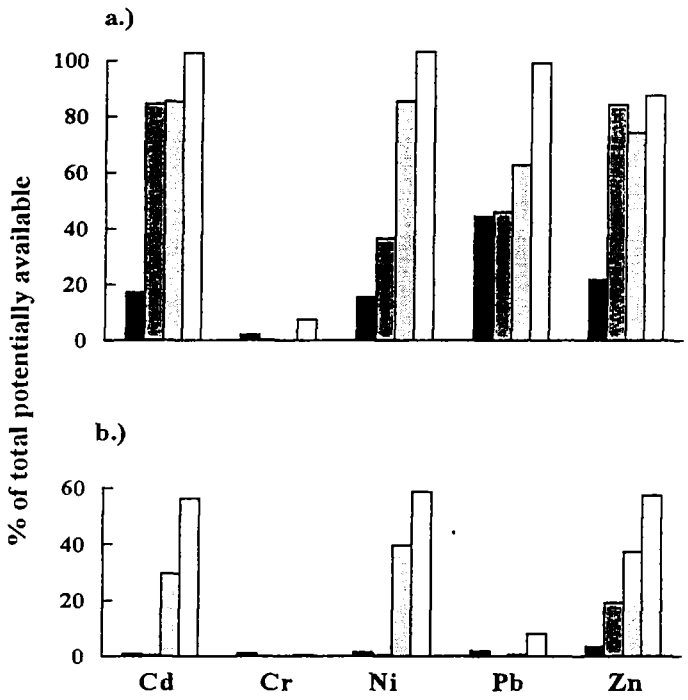

Fig.1. Plant-available soil metal contents in % of the total potentially available amounts

a.) (AAAc + EDTA)-extractable
b.) directly plant-available in the soil solution

no metal addition to sewage sludge
single metal addition (100*L*) to sewage sludge
multicomponent metal addition (100*L*) to sewage sludge
multicomponent metal addition (100*L*) without sludge

The (AAAc + EDTA) extraction method has been used in Hungary since the early 80's for determination of macro- and micronutrients or for assessing plant-availability of trace metals (cit. in Csathó, 1994). In the present experiment, extractability of the added elements was similarly high for Cd, Ni, Pb, and Zn; while that of Cr was very low (Fig.1.a.). The (AAAc + EDTA)-extractable amounts of Pb and particularly of Ni were higher when all the 5 metals were added simultaneously to the sludge as compared to the (SS + Me) treatment. A somewhat higher fraction of the soil's metal content was extracted from the inorganic treatments than from the SS-treated soil. Extractability of

the metals from the soil treated with uncontaminated sludge (SS) was much lower than from the metal-spiked sludge treatments, in agreement with the strong sorption of the metals on sludge components during sludge processing.

The centrifugation method used for separating the soil's liquid phase allowed us to obtain those fractions of the soil solution which, - on the basis of the energy relations between the soil's water potential (-20 kPa) and the suction forces exerted by plants (conventionally −1500 kPa), - may be regarded as directly available for plant-uptake (Csillag et al., 1999). Chromium and Pb concentrations in the soil solution were negligible. Availabilities of Cd, Ni, and Zn were 50-60 % of their total potentially available amounts when all the five metals were applied together, without sludge (Fig.1.b.). Presence of sewage sludge significantly decreased the recovery of the metals in the soil liquid phase, similarly as in a previous bulk experiment (Csillag et al. 1999). When the metals were applied single, their concentrations in the liquid phase were negligible, with the exception of Zn (which was applied in relatively high amount).

At excessive soil contamination, when the soil's adsorption capacity might have become saturated and the pH decreased, plant-available ([AAAc + EDTA]-extractable) concentrations of Cd, Ni, Pb, and Zn increased more than the total soil contents, i.e. their relative availabilities increased. Table 3. shows this for the (5 Me) treatments. Similar trends but smaller increases were found for the (SS + 5 Me) treatments.

Table 3. Relative availabilities of Cd, Cr, Ni, Pb, and Zn after addition of metal nitrates without sludge (soil availability, $SA = c_{AAAc + EDTA} * 100 / c_{2M\ HNO_3}$)

	Cd	Cr	Ni	Pb	Zn
0 *L* (control)	10 ± 1	0	14 ± 1	30 ± 2	0
1 *L*	22 ± 7	4.2 ± 1.4	19 ± 17	47 ± 11	25 ± 4
3 *L*	30 ± 5	4.7 ± 0.2	30 ± 5	53 ± 7	35 ± 6
10 *L*	73 ± 1	6.7 ± 0.2	65 ± 3	91 ± 1	64 ± 2
30 *L*	102 ± 11	7.0 ± 0.4	95 ± 9	104 ± 8	87 ± 7
100 *L*	112 ± 36	7.9 ± 2.4	112 ± 35	106 ± 31	93 ± 26

Plant metal concentrations increased with increasing metal application rates, and were generally higher when the metals were applied together than at single metal additions (Table 4.). Also, more metals were taken up when no SS was added to the pots, only the inorganic metal salts. These features of the metal uptake were observed for all the 5

metals, although to a lesser extent for Cr and Pb. Chromium uptake was generally low, the maximum value was < 0.6 mg/kg. Lead entered the plants in appreciable quantities, maximal Pb concentration reached 9.3 mg/kg (at the 100 *L* rate of SS + Pb treatment). The highest plant Cd, Ni, and Zn concentrations exceeded reported critical tissue levels (Kabata-Pendias and Pendias, 1992).

Table 4. Metal concentrations (mg/kg d.m.) in aerial parts of maize at the 6-7-leaf-stage

loading rate	Cd			Ni			Zn		
	+ SS		- SS	+ SS		- SS	+ SS		- SS
	Me	5 Me	5 Me	Me	5 Me	5 Me	Me	5 Me	5 Me
0 *L* (control)	0.325		0.357	1.84		2.25	51.5		31.3
1 *L*			0.424			1.32			83.0
3 *L*		0.522	0.806		1.21	3.35		93.7	210
10 *L*	2.44	1.37	2.45	1.23	3.69	16.4	194	239	984
30 *L*	7.51	9.12	-	3.91	68.1	-	1083	1430	-
100 *L*	21.6	-	-	17.0	-	-	-	-	-

No plants could emerge in the treatments indicated by (-) in Table 4. Dry matter accumulation strongly decreased (to 30% of the control) at the 100*L* rate of (SS + Cr) but not at the 100*L* rates of other single metals, and it decreased by 85 % at the 30*L* rate of (SS + 5 Me) addition. In these strongly retarded plants metal concentrations were higher than in the plants growing normally at the 30*L* application rate of single metals (Table 4., second and first columns for each metal), but the concentration differences were smaller than expected on the basis of the growth differences.

Calculation of the soil-to-plant transfer of the metals was based not only on total soil contents, like the "classic" TF calculations, but also on the plant-available contents (Table 5.). Transfer of Cr into the aerial plant parts was negligible in all treatments, the average TF related to the soil total contents was only 0.008 ± 0.007. Transfer of Pb was also universally small (in average 0.22 ± 0.21). TFs calculated for the soil's total metal content increased with increasing loading rates for Cd, Ni, and Zn; at the highest metal application rates the increase was significant. TFs related to the "plant-available" soil metal contents seemed to depend less on the metal loading rates than those related to total contents. The higher availability of inorganic metal forms as compared to the SS-bound metals clearly showed when we compared TFs for the same metal loading rates.

Table 5. Transfer factors of metal uptake (TF = $c_{plant\ dry\ matter}/c_{air\text{-}dry\ soil}$)

	for total potentially available soil contents			for (AAAc +EDTA)-available soil contents		
	Cd	Ni	Zn	Cd	Ni	Zn
SS + Me						
0 *L* (sludged control)	0.33	0.10	1.15	2.49	0.64	4.57
10 *L*	**1.47**	0.04	0.74	2.59	0.11	1.44
30 *L*	**1.74**	0.06	1.23	2.79	0.15	2.71
100 *L*	**2.13**	0.11	-	2.56	0.27	-
SS + 5 Me						
3 *L*	0.31	0.07	0.73	1.15	0.26	1.50
10 *L*	0.6	0.14	0.89	1.27	0.33	1.33
30 *L*	**2.07**	**1.26**	1.84	**2.62**	**2.17**	**3.02**
5 Me						
0 *L* (control)	0.29	0.17	0.68	3.14	1.30	4.57
1 *L*	0.38	0.11	1.26	1.78	0.46	5.07
3 *L*	0.55	0.21	1.79	1.80	0.66	5.01
10 *L*	**1.35**	**0.79**	**4.10**	1.86	1.23	6.42

values in bold are statistically significantly higher

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