Availability of Heavy Metals in Soils and their Uptake by Vegetable Species[+]

Sigrid Dieckmann, Olaf Melzer and Ludwig Bender[*)]

Fachhochschule Osnabrück - University of Applied Sciences, Department of Agricultural Sciences, Oldenburger Landstraße 24, D-49090 Osnabrück

[*)] Corresponding author; Fax: + (49) 541 5170; e-mail: l.bender@fh-osnabrueck.de

Abstract

Head lettuce, bush beans and celery were grown in subsequent years in an experimental field on anthropogenously uncontaminated or heavy metal contaminated soils at a mean pH of 6.3 ± 0.1. The contaminated plots were made up by amending or replacing the upper 20 cm soil layer with heavy metal contaminated alluvial top soil. Contamination includes Cd, Zn, Pb and Cu. Phytoavailable fractions of these elements were extracted with 1 M ammonium nitrate. Cd, Zn, Pb and Cu were also determined in the leaves of all three species as well as in bean pods and celery bulbs. The results show that plant uptake of Cd and Zn increased with increasing soil contamination while the uptake of Pb was low. No dependence of Cu uptake on total soil Cu content could be seen within the given contamination range. The relationship between Cd, Zn, Pb and Cu in plants and ammonium nitrate soil extracts was determined by twofactorial linear regression where r was approximately 0.8 for Zn, 0.7 for Cd and 0.5 for Pb whereas no relationship was found for Cu.
For the pot experiments carried out in a greenhouse two different soils (loamy sand, silt loam) were amended with 5 and 10 % metallurgical slag and adjusted to pH levels around 7 and 5 while controls did not contain slag. Phytoavailable heavy metal fractions were extracted from soil samples using ammonium nitrate or Calcium chloride + DTPA (CAT). Spinach was grown on these soils and the concentrations of Cd, Zn, Pb and Cu in the shoots correlated with their concentration in the soil extracts. The uptake of these elements by the plants increased with increasing slag amendment and decreasing pH. Strong depression of growth was observed at a pH around 5 in all treatments of the lighter and in the slag treatments of the heavier soil. In the slag treatments this was accompanied by increased endogenous Cd and Zn concentrations. The plant content of Cd, Zn and Pb correlated better with the ammonium nitrate extractable soil fraction of these elements than in the field experiments ($r > 0.9$). Correlations based on the CAT extractable fraction of Cd and Zn yielded values for r around 0.5 while it was only 0.1 for Pb. As in the field trials correlations for Cu were very poor with both extraction methods.

[+] This paper contains in part data from the undergraduate thesis work of the following students: Lars Schade, Andrea Kampf, Ralf Krämer, Ursula Veenker, Kirsten Homola, Axel Milius, Kathrin Eichholz and Dagmar Lehmann.

Introduction

The uptake of toxic heavy metals from contaminated soils by food and forage plants comprises a prominent path for such elements to enter the food chain and will finally be ingested by humans. Ingestion and eventual accumulation of toxic heavy metals pose a threat to human health and should, therefore, be minimized (Kloke, 1988; Traulsen and Schönhard, 1995; Delschen and Rück, 1997). It was also reported that the uptake of heavy metals by crop plants may vary in different cultivars of a particular species (Lübben, 1991; Metz and Kloke, 1998).
The total content of a given heavy metal in soils is considered impractical for the prognostication of its uptake by plants because only certain fractions of an element are phytoavailable (Brümmer et al., 1986; Brümmer and Hornburg, 1989; Birke and Werner, 1991). Thus, it has been suggested that soil heavy metal fractions available to plants are determined using salt solutions, e.g. 0.1 M calcium chloride (Köster and Merkel, 1982), 1 M ammonium acetate (Dües, 1989) or 1 M ammonium nitrate (Prüeß, 1992). The ammonium nitrate extractable fraction is considered mobil and readily available to plants whereas the ammonium acetate extract is assumed to include a fraction which can easily replenish the available fraction (Zeien, 1995). The so called CAT method, which employes a mix of 0.01 M calcium chloride and 0.002 M DTPA (Diethylen-triamine-pentaacetate) was suggested for the determination of macro- and micronutrients in horticultural substrates (Alt and Peters, 1993; Alt et al., 1994). Also, availability is influenced by a variety of parameters, of which soil pH is certainly the most important. Depending on the particular metal, decreasing pH increases the unspecifically adsorbed (available) fraction to various degrees while the specifically adsorbed (unavailable) fraction decreases accordingly. While e.g. Cd and Zn become increasingly available starting at pH 6.5 or 6, respectively, and are thus classified as rather mobil and more readily available elements, the corresponding pH for Cu is 5 - 4.5 and for Pb 4 –3.5 which classifies them as rather immobil and unavailable at least within the pH range of most arable and grassland soils (Hornburg et al., 1995). Slags from metallurgical or waste incineration plants etc. are frequently used in landscape construction or hydraulic engineering. They reportedly contain heavy metals in chemically very inert binding forms (Lahl, 1994; Khorasani, 1999). However, if exposed to environmental influences or if mixed with soils, chemical processes such as pH changes may alter the chemical binding of metals and eventually increase their mobility and bioavailability.

In the field experiments reported here we studied the uptake of Cd, Zn, Pb and Cu by a number of vegetable species in field experiments on soils of comparable pH but different degrees of heavy metal contamination. In these experiments four cultivars of each vegetable species were tested also for differences in the uptake of the heavy metals mentioned above.
In pot experiments carried out in a greenhouse spinach was grown at two pH levels on two different soils amended with varied amounts of metallurgical slag or whithout slag. The correlation between soil heavy metal concentrations extractable with ammonium nitrate or CAT and the uptake by the vegetable species tested was assessed by twofactorial linear regression. Consequences on growth and heavy metal accumulation as well as the usfulness of these extractants for the prognosis of heavy metal transfer to plants are discussed.

Material and methods

Soils

The experimental field comprises 48 plots 1 x 2 m in size which are rimmed by concrete liners. The soil is classified as loamy sand (4 % clay, 30 % silt, 66 % sand) with 2 % organic matter and a pH of 6.2. 16 plots were left unchanged and designated *Soil 1*. Another 16 plots were made up by removing the top soil to a depth of 20 cm and replacing it by a 3 : 1 mix of original top soil as described above and alluvial top soil contaminated with Cd, Zn, Pb and Cu. The mixing resulted in a sandy loam (9 % clay, 35 % silt, 56 % sand) with 3.5 % organic matter and a pH of 6.4 which was designated *Soil 2*. In the remaining16 plots the top soil was replaced by an unmixed charge of the same alluvium (silt loam, 20 % clay, 58 % silt, 22 % sand) with 10 % organic matter and a pH of 6.3, designated *Soil 3*. Plots were arranged in randomized order. Using the described technique of soil replacement it could be avoided to amend the plots with heavy metal salt solutions which would have caused unrealistically high availability of metal ions until a natural equilibrium between specifically and unspecifically adsorbed fractions was attained.

For the pot experiments carried out in a greenhouse loamy sand as described above (Soil A) and silt loam consisting of 17 % clay, 74 % silt, 9 % sand (Soil B) were used. Portions of these soils were amended with 5 or 10 % ground metallurgical slag (particle size 0.5 to 1 mm) while a third portion of each soil remained free of slag. The soils were then adjusted to pH levels around 7 or 5, respectively, by either liming with Ca carbonate or a mixing-in of diluted sulfuric acid (0.02 to 0.5 M, depending on requirement). These preparations resulted in six different treatments for each soil designated A5/0, A5/5, A5/10 and A7/0, A7/5, A7/10 as well as B5/0, B5/5, B5/10 and B7/0, B7/5, B7/10. A or B indicate the soil, the first number shows the pH level and the second the slag amendment in % (w/w). Five pots per treatment with 12 kg soil each were set up amounting to a total of 60 pots. The actual pH ranged from 6.7 to 8.0 at the higher and from 4.9 to 5.6 at the lower end. Due to the presence of the basic slag the pH had to be checked and readjusted immediately before seeding and was also measured for variations right after harvest. It should be noted here that pH adjustment was very difficult in the beginning, however, it had stabilized acceptably after about 4 years.

Plant material

Head lettuce (Lactuca sativa, var. Capitata, Chichoriaceae, cultivar Mirian, Martina, LM 8015 and Floret), bush beans (Phaseolus vulgaris, var. nanus, Fabaceae, cultivar Modus, Pfälzer Juni, Nickel and Xavo) and celery (Apium graveolens, var. Rapaceum, Umbelliferae, cultivar Ofir, Prinz, President and Monarch) were used in the field experiments in three subsequent years. Head lettuce was harvested and analyzed in a marketable stage. Bean leaf and pod samples were taken at three developmental stages (1, anthesis; 2, first harvest of green pods three weeks after anthesis; 3, final harvest of green pods five weeks after anthesis). Celery was harvested upon development of marketable bulbs. Peeled bulb segments as well as peel and leaf samples were analyzed separately. In the pot experiments metal uptake by spinach (Spinacea oleracea, var. inermis, cultivar Laska) leaves was studied and correlated with the content of these metals in soil extracts obtained with two different salt solutions. All plant organs were thoroughly washed including a final rinse with distilled water before they were further processed.

Analytical

For the determination of the total heavy metal content of the soils 0.5 - 1 g air dried and ground soil was digested in quartz vials with 6 ml nitric acid (65 %), 2 ml hydrochloric acid (37 %) and 0.25 ml hydrogen peroxide (35 %) in a microwave digestion apparatus (MLS – Ethos plus; Mikrowellen-Laborsysteme, D-88299 Leutkirch).
Heavy metal fractions were extracted from 20 g air dried soil using 50 ml 1 M ammonium nitrate or 50 ml 0.01 M Calcium chloride + 0.002 M DTPA (pH 2.6 – 2.65; further referred to as CAT) for 1 h on a gyratory shaker.
The microwave technique and quartz vials were also used for the digestion of plant material (0.2 to 0.5 g dry matter) with 2.5 - 5 ml nitric acid (65%).
Certified soil (GBW 07404, Breitländer GmbH, D-59077 Hamm) and plant standard reference material (spinach leaf no.1570a, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA) were used to check the efficiency and precision of digestion procedures and measurements. However, no soil standard reference material certified for the extraction of element fractions with salt solutions was available. Therefore, uncertified soil samples from our own stocks with well determined contents of the elements in question were used as reference material with these analyses. Heavy metal concentration was principally measured emploing inductively-coupled plasma – optical emission spectrometry (ICP-OES, Perkin Elmer Optima 3300 XL with axial plasma viewing) while for the determinaton of lowest element concentrations graphite tube atomic absorption spectrometry (GT-AAS, Perkin Elmer SIMAA 6000) was used with a palladium-magnesium nitrate modifier to attain optimum analyte stability and atomization conditions.

Results

Field experiments

Total and ammonium nitrate extractable Cd, Zn, Pb and Cu of the three soils are given in table 1. Data derived from soil 1 are typical for anthropogeneously uncontaminated soils. The contamination of soils 2 and 3 is moderate except for the high Zn content of soil 3. It should be noted, however, that the limits set by german authorities for the application of sweage sludge (Cd 1.5, Zn 200, Pb 100 and Cu 60 mg kg^{-1}soil; AbfallKlärV, 1992) were exceeded for Zn and Pb in soil 2 and for all four elements in soil 3. Based on total contents, ammonium nitrate extractable fractions amounted to about 10 - 12 % for Cd, 5 - 6 % for Zn and 1 - 2 % for Pb and Cu at the given soil pH. Despite the increase of total Cu content ammonium nitrate extractable Cu was nearly the same in all three soils.
The content of Cd, Zn, Pb and Cu in head lettuce, beans and celery are given in table 2. Since in the bean experiment no significant differences were found at the three developmental stages investigated, only data from developmental stage 2 (first harvest of green pods) are presented. Cd and Zn increased in all three species with increasing soil contamination. The Cd content of the edible parts of lettuce and celery were about the same on soil 1, however, celery bulbs accumulated significantly more Cd than lettuce on the anthropogenously contaminated soils 2 and 3. Celery leaves contained slightly less Cd than the bulbs. While Cd uptake by beans was about one order of magnitude lower than in the other two species, the content in leaves was two to three times higher than in pods where it amounted to only about 0.1 mg kg^{-1} DW even on soil 3. Highest Zn levels were found in lettuce and celery leaves which amounted to more than 300 and 400 mg kg^{-1} DW, respectively, on soil 3. Celery bulbs contained between 20 and 30 % less Zn than leaves. Despite the high Zn accumulation on the two contaminated soils no toxicity symptoms were observed in either species. Beans, on the other

hand, exhibited Zn contents that were about an order of magnitude lower than in lettuce and celery, however, no differences could be observed between leaves and pods. The uptake of Pb by all three species was low on all soil contamination levels, as expected at the given pH. A slight increase in lettuce as well as in celery leaves and peels was observed with increasing soil contamination but no Pb could be detected in the peeled celery bulbs. It should also be noted that bean pods contained about 25 % less Pb than bean leaves. The similar amount of ammonium nitrate extractable Cu in all three soils is reflected by the Cu uptake of the plants which appeared to be independent of the total soil content. However, Cu content in plant material varied with species and also organs. Lettuce contained about 8 to10 mg kg^{-1} DW. Bean leaves exhibited approximately 17 and pods 11 mg. While celery leaves contained only 5 to 8 mg it amounted to13 mg in bulbs. Regarding the uptake of the elements under investigation no significant differences between the cultivars of either species were found.

Cd and Zn fractions extracted from soils using 1 M ammonium nitrate correlated fairly well with the plant content of these elements. The correlation for Pb, on the other hand, is rather poor and no relationship could be demonstrated for Cu. Since these findings were similar for all three vegetable species, only the results for lettuce are given in figure 1 as an example.

Table 1: Total and ammonium nitrate extractable heavy metal content (mg kg^{-1} DW) of the three soils used in the experimental field studies. Data are mean ± SD (n = 16).

	Cd	Zn	Pb	Cu
		Total		
Soil 1	0.36 ± 0.03	66.6 ± 6.4	22.9 ± 1.5	15.0 ± 0.6
Soil 2	1.23 ± 0.05	359.8 ± 18.0	111.1 ± 5.7	34.8 ± 1.8
Soil 3	3.91 ± 0.41	1204.6 ± 112.3	414.2 ± 42.7	77.6 ± 7.7
		Ammonium nitrate extractable		
Soil 1	0.05 ± 0.01	4.16 ± 1.17	0.30 ± 0.12	0.30 ± 0.12
Soil 2	0.15 ± 0.04	22.14 ± 7.23	2.01 ± 0.52	0.44 ± 0.11
Soil 3	0.34 ± 0.06	59.05 ± 12.18	3.21 ± 0.79	0.55 ± 0.11

Table 2: Heavy metal content (mg kg^{-1} DW; b.q., below quantification limit) of head lettuce, bush beans and celery grown on soils with increasing heavy metal levels (Soil 1 anthropogeneously uncontaminated).
Data are mean ± SD (n = 16).

	Cd	**Zn**	**Pb**	**Cu**
Head lettuce				
Soil 1	0.77 ± 0.09	144.2 ± 26.2	2.73 ± 0,58	7.4 ± 0.7
Soil 2	0.98 ± 0.18	181.0 ± 14.1	3.11 ± 0.38	7.9 ± 0.4
Soil 3	1.56 ± 0.26	329.8 ± 33.4	5.16 ± 0.54	9.9 ± 1.4
Bean leaves				
Soil 1	0.05 ± 0.02	29.8 ± 5.6	2.61 ± 0.40	16.8 ± 2.3
Soil 2	0.09 ± 0.03	48.0 ± 11.2	2.73 ± 0.40	16.8 ± 1.4
Soil 3	0.25 ± 0.05	67.0 ± 18.8	2.24 ± 0.29	16.1 ± 2.8
Bean pods				
Soil 1	0.02 ± 0.008	44.3 ± 7.2	1.81 ± 0.05	10.2 ± 1.3
Soil 2	0.04 ± 0.02	54.4 ± 6.5	1.83 ± 0.24	11.0 ± 1.0
Soil 3	0.08 ± 0.03	62.9 ± 11.4	1.88 ± 0.17	11.2 ± 1.9
Celery leaves				
Soil 1	0.41 ± 0.20	117.4 ± 38.8	0.92 ± 0.28	4.9 ± 0.7
Soil 2	0.92 ± 0.24	199.1 ± 42.2	1.61 ± 0.56	5.4 ± 0.7
Soil 3	2,08 ± 0,43	434,8 ± 56,0	1,70 ± 0,34	7,6 ± 1,4
Celery bulbs (peeled)				
Soil 1	0.63 ± 0.20	90.7 ± 15.8	b.q.	12.8 ± 2.9
Soil 2	1.32 ± 0.34	146.2 ± 18.7	b.q.	14.9 ± 2.7
Soil 3	2.90 ± 0.70	256.4 ± 37.0	b.q.	15.5 ± 2.6
Celery peels				
Soil 1	0.55 ± 0.13	95.9 ± 20.2	0.30 ± 0.15	9.4 ± 1.5
Soil 2	1.26 ± 0.23	174.5 ± 27.7	1.36 ± 0.52	11.8 ± 1.6
Soil 3	2.78 ± 0.43	318.9 ± 42.3	3.07 ± 1.30	14.3 ± 1.9

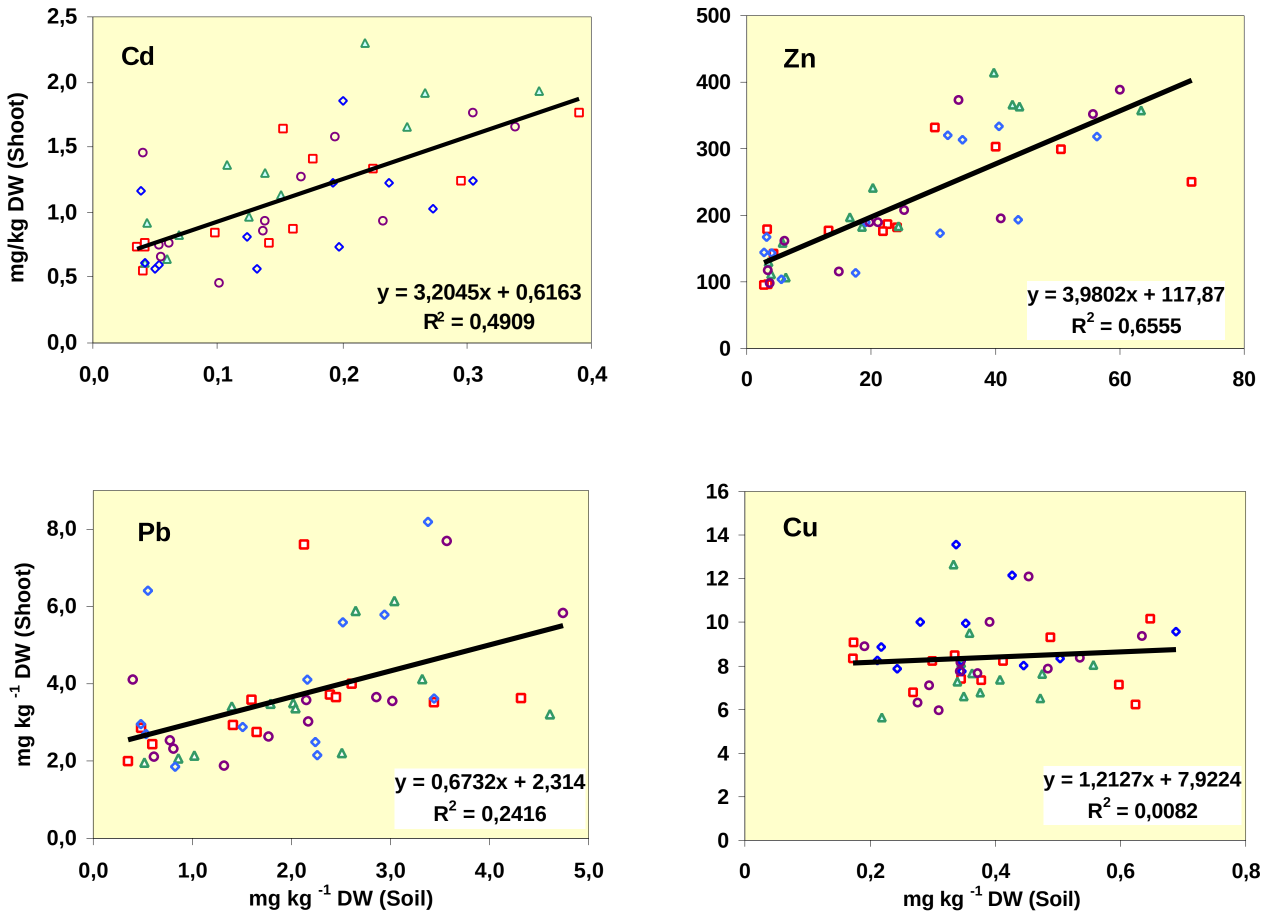

Figure 1: Relationship as determined by twofactorial linear regression between Cd, Zn, Pb and Cu in head lettuce and the ammonium nitrate extractable fraction of these elements in the three soils employed in the field experiments.(Cultivar identification: Mirian, squares; Martina, triangles; LM 8015, rhombs; Floret, circles).

Pot experiments

Total Cd, Zn, Pb and Cu content of the soils used in these experiments are shown in table 3. They are in the same order of magnitude if compared to the soils used in the field experiments. Without slag the content of the four elements was similar in both soils. Although the slag amendment and thus the heavy metal addition was the same, a somewhat lower content of these elements was measured in the slag treatments of soil A as compared to soil B, however.

The results of the ammonium nitrate and CAT extraction trials are given in table 4. Ammonium nitrate extractable Cd, Zn and Pb increased with increasing slag amendment, however, this increase was more pronounced at the lower pH level (Table 4). Around pH 5 Cd rose from about 0.03 to 0.4 mg kg^{-1} in the lighter soil A and to about 0.8 mg in the heavier

soil B, whereas around pH 7 a maximum of only 0.06 mg was found. Without slag, extractable Zn was about 1 - 2 mg kg^{-1} in both soils and pH levels. In the slag treatments it increased to about 100 mg around pH 5 whereas it reaches only 3 - 5 mg at the higher pH. Pb was low in all treatments without slag but with increasing slag amendment it amounted to about 5 - 13 mg kg^{-1} at the lower pH while only about 1 mg was measured at the higher end. This accounted for 4 – 12 % of total with Cd, 1 – 10 % with Zn and 1 – 2 % with Pb around pH 5 while at the higher pH this percentage was about an order of magnitude lower. Extractable Cu was about 0.1 – 0.3 mg kg^{-1} in all treatments accounting for approximately 0.5 - 1 % of total. As can be seen from the Cu data, extractability of this element responded only sligthly to the varied pH.
CAT extracted considerably more of the elements under investigation than did ammonium nitrate, yielding between 20 and 40 % of total Cd at both pH levels. Up to 30 % of total Zn was extracted around pH 5 and up to 20 % around pH 7. Pb recovery reached roughly 30 – 40 % of total at either pH levels. With Cu also 30 – 40 % of total was extracted around pH 5 while it still amounted to about 25 % at the higher pH.

At the lower pH Cd uptake into spinach shoots rose from about 2 mg kg^{-1} DW on uncontaminated soils to more than 20 mg on soil A and more than 40 mg at soil B due to the slag treatment. Zn uptake increased from 100 to 200 mg kg^{-1} DW to more than 3000 mg on soil A while the corresponding figures for soil B were 1200 and 1600 mg. Pb was detected in low but measurable concentrations in the shoot material. In the 10 % slag treatments it amounted to roughly 30 mg on soil A while a maximum of only 10 mg was found on soil B. At the higher pH level endogenous concentration of Cd, Zn and Pb were about one order of magnitude lower, which is also reflected in the reduction of the ammonium nitrate extractable portion of these elements at high pH. Cu concentration was in the order of 10 to 18 mg kg^{-1} DW, as found for bean leaves in the field experiment, and did not vary significantly irrespective of the treatments.
Shoot dry weight (DW) production in the various treatments are compared as a percentage of maximum yield on soil A or B, respectively (Table 5). On the lighter soil A top yield representing 100 % was obtained at pH level 7 without slag (A 7/0). At this pH dry weight production in the slag treatments was only sligthy reduced while no abnormal metal concentration was measured in the shoots. At the lower pH level dry weight yield was reduced by more than 80 %. For the slag free treatment (A 5/0) this cannot be explained by facts at hand since also no abnormal metal contents could be found. If slag was added to the soil, however, the depression of yield formation may be attributed to phytotoxic effects of high Cd and especially Zn concentration, the latter reaching a maximum of more than 3000 mg kg^{-1} DW at 10 % slag. Here not only growth reduction but also dying of more than 50 % of the plantlets shortly after emerging was observed. On soil B top yield formation (100 %) was attained at a pH around 7 and 5 % slag, with the other two treatments lagging only slightly behind. Different from soil A, only about a 30 % reduction of dry weight formation was noted on soil B without slag at the lower pH level (B 5/0). Here, the content of Cd and Zn in the shoot material reached the lower limits of potentially phytotoxic concentrations which, therefore, could have caused the growth retardation observed in this treatment. At this pH level the slag amendment caused the Cd and Zn concentration to rise to more than 40 and 1000 mg kg^{-1} DW, respectively, which may well be the reason for the yield depression of up to 90 %.

The relationship between the element fractions extractable from the soils with both extractants and the content of these elements in the spinach shoots was assessed by linear regression analysis, the results of which are depicted in figures 2 and 3. As can be seen, the correlation coefficients for Cd, Zn and Pb obtained with ammonium nitrate are better than 0.9 although the slope of the regression line for Pb was much smaller than for the others. With CAT, r falls short of that, especially for Pb and it can also be seen that the slopes are, as a result of the higher extraction yield, inferior to those obtained with ammonium nitrate. As in the field trials no relationship could be established for Cu with either extractant. It should also be noted that ammonium nitrate extraction resulted in much better correlations for Cd, Zn and Pb than in the field experiments (Figure 1).

Table 3: Total heavy metal content of the soils used in the pot experiments. Data are mean ± SD (n = 5).

		Cd	Zn	Pb	Cu
Soil A	0 % slag	0.36 ± 0.03	66.6 ± 6.4	22.9 ± 1.5	15.0 ± 0.6
	5 % slag	2.61 ± 0.30	567,0 ± 80.2	288.9 ± 21.1	21.3 ± 1.8
	10 % slag	4.13 ± 0.27	1099,6 ± 25.9	522.9 ± 20.1	28.4 ± 1.7
Soil B	0 % slag	0.80 ± 0.16	64.3 ± 3.7	23.2 ± 1.7	17.2 ± 0.5
	5 % slag	4.61 ± 0.81	775.0 ± 85.2	300.9 ± 44.3	29.8 ± 3.6
	10 % slag	6.93 ± 1.96	1427.5 ± 241.2	665.2 ± 114,1	37.7 ± 4.6

Table 4: Heavy metals (mg kg^{-1}) extractable with 1 M ammonium nitrate or 0.01 M calcium chloride + 0.002 M DTPA (CAT) from different soils as dependend on pH level and slag amendment. Soil designation: A, loamy sand; B, silt loam; first numeral, pH level; second numeral, slag content (% w/w). For actual pH of the various treatments see table 5. (Data are mean ± SD, n = 5).

	Cd	Zn	Pb	Cu
Ammonium nitrate				
Soil A 5/ 0	0.03 ± 0.004	2.2 ± 0.5	0.18 ± 0.02	0.20 ± 0.02
A 5/ 5	0.25 ± 0.13	33.0 ± 9.4	2.93 ± 0.81	0.25 ± 0.07
A 5/10	0.42 ± 0.11	106.8 ± 19.8	12.91 ± 1.38	0.32 ± 0.11
A 7/ 0	0.01 ± 0.001	0.9 ± 0.2	0.08 ± 0.04	0.10 ± 0.01
A 7/ 5	0.02 ± 0.004	1.6 ± 0.4	0.48 ± 0.11	0.13 ± 0.01
A 7/10	0.02 ± 0.01	2.4 ± 4.3	1.02 ± 0.51	0.16 ± 0.02
Soil B 5/ 0	0.03 ± 0.01	1.2 ± 0.4	0.04 ± 0.01	0.07 ± 0.02
B 5/ 5	0.36 ± 0.04	47.7 ± 6.0	2.47 ± 0.46	0.15 ± 0.02
B 5/10	0.83 ± 0.17	83.7 ± 15.4	4.44 ± 0.85	0.26 ± 0.07
B 7/ 0	0.01 ± 0.001	0.6 ± 0.03	0.06 ± 0.01	0.06 ± 0.01
B 7/ 5	0.06 ± 0.03	3.3 ± 1.5	0.74 ± 0.33	0.11 ± 0.01
B 7/10	0.03 ± 0.01	3.7 ± 0.5	1.09 ± 0.54	0.12 ± 0.02
CAT				
Soil A 5/ 0	0.15 ± 0.002	11.4 ± 0.7	8.6 ± 0.3	7.27 ± 0.19
A 5/ 5	0.67 ± 0.07	136.3 ± 11.7	84.5 ± 4.1	8.01 ± 0.15
A 5/10	0.87 ± 0.07	235.2 ± 16.4	144.6 ± 3.2	8.21 ± 0.08
A 7/ 0	0.13 ± 0.004	14.6 ± 0.8	12.6 ± 0.3	5.37 ± 0.09
A 7/ 5	0.67 ± 0.05	83.2 ± 17.6	98.1 ± 7.0	5.68 ± 0.48
A 7/10	1.06 ± 0.05	127.2 ± 6.5	164.9 ± 6.7	6.01 ± 0.10
B 5/ 0	0.17 ± 0.02	11.0 ± 2.4	7.9 ± 1.2	5.07 ± 0.51
B 5/ 5	1.89 ± 0.12	236.2 ± 10.3	125.5 ± 2.6	9.37 ± 0.30
B 5/10	2.70 ± 0.10	313.8 ± 15.7	155.8 ± 13.5	12.32 ± 0.26
B 7/ 0	0.14 ± 0.01	12.2 ± 1.4	9.2 ± 0.4	5.21 ± 0.23
B 7/ 5	1.52 ± 0.66	164.0 ± 52.4	141.9 ± 28.5	7.66 ± 0.95
B 7/10	1.27 ± 0.10	153.8 ± 11.1	201.8 ± 17.7	6.52 ± 0.13

Table 5: Heavy metal content (mg kg^{-1} DW) and relative dry weight production of spinach shoots grown on different soils at varied pH and slag treatments. Relative dry weight production (Rel. DW prod.) was based on treatments with highest yield formation (100 %) on soil A or B, respectively (Soil designation as in table 4). Data are mean ± SD (n = 5).

	Actual pH	Cd	Zn	Pb	Cu	Rel. DW prod. (%)
Soil A 5/ 0	5.6 ± 0.2	1.59 ± 0.14	133 ± 9	0.54 ± 0.17	12.0 ± 1.8	16
A 5/ 5	5.1 ± 0.1	14.84 ± 4.40	1433 ± 288	3.23 ± 1.06	14.8 ± 3.9	16
A 5/10	4,9 ± 0.1	21.66 ± 0.36	3292 ± 9	29.01 ± 1.71	18.5 ± 0.2	7
A 7/ 0	6.9 ± 0.1	1.23 ± 0.07	91 ± 5	0.28 ± 0.12	11.4 ± 0.6	100
A 7/ 5	7.6 ± 0.4	2.24 ± 0.23	117 ± 6	0.93 ± 0.18	11.6 ± 0.8	87
A 7/10	8.0 ± 0.4	2.75 ± 0.17	142 ± 7	1.91 ± 0.40	11.8 ± 0.7	82
Soil B 5/ 0	5.6 ± 0.3	2.42 ± 0.54	181 ± 29	0.23 ± 0.04	14.0 ± 1.1	68
B 5/ 5	5.5 ± 0.1	28.74 ± 4.18	1228 ± 103	2.99 ± 1.06	10.8 ± 1.2	18
B 5/10	5.2 ± 0.4	44.01 ± 5.98	1608 ± 158	9.17 ± 0.87	11.6 ± 0.5	8
B 7/ 0	6.7 ± 0.4	0.99 ± 0.08	107 ± 6	0.25 ± 0.08	13.4 ± 0.7	84
B 7/ 5	7.4 ± 0.2	3.44 ± 1.24	192 ± 35	1.48 ± 0.82	12.6 ± 0.5	100
B 7/10	7.9 ± 0.1	2.84 ± 0.42	176 ± 30	1.50 ± 0.28	11.7 ± 1.0	95

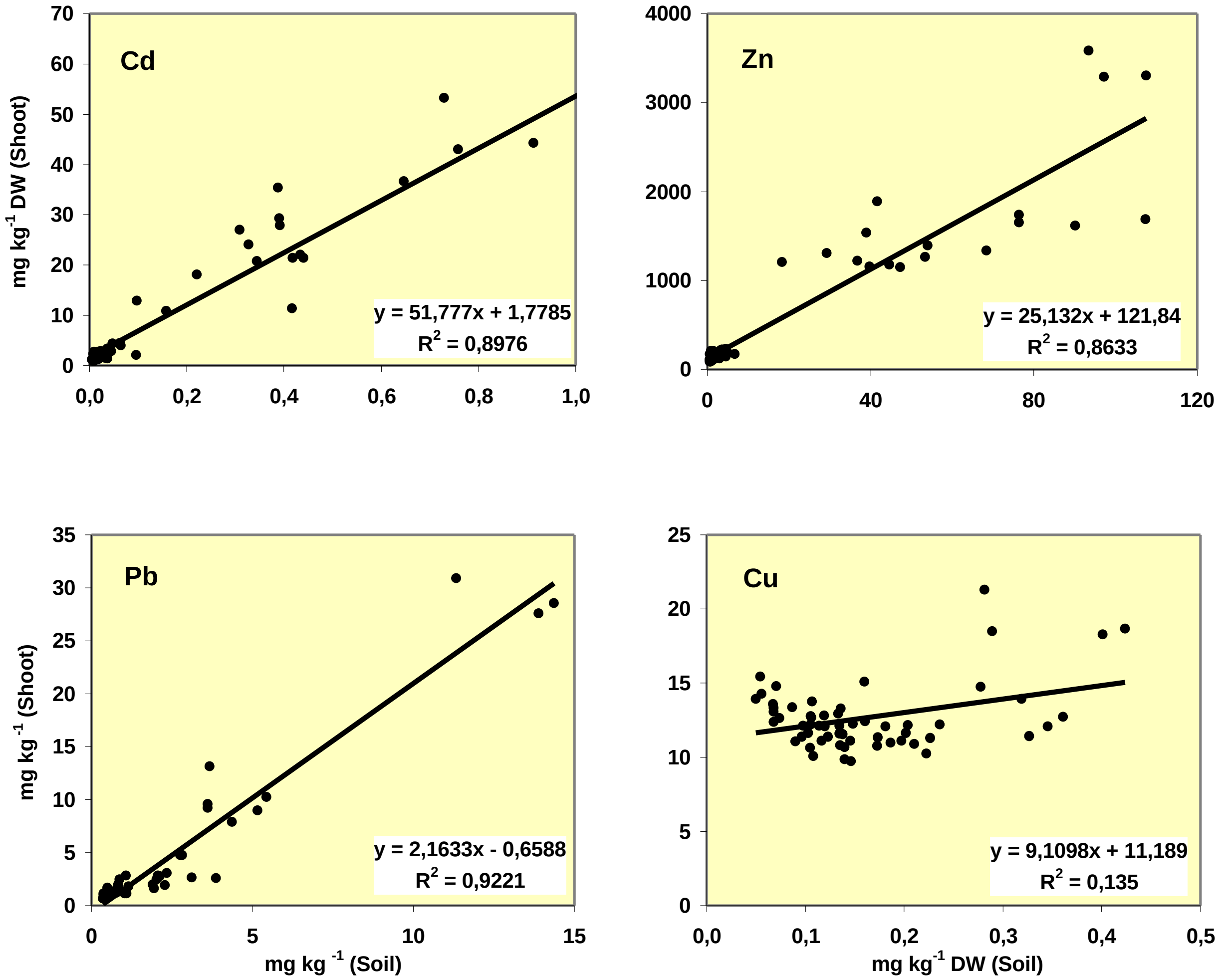

Figure 2: Relationship as determined by twofactorial linear regression between Cd, Zn, Pb and Cu in spinach shoots and the *ammonium nitrate* extractable fraction of these elements in two different soils (loamy sand, silt loam) containing 0 %, 5 % and 10 % (w/w) metallurgical slag at varied pH.

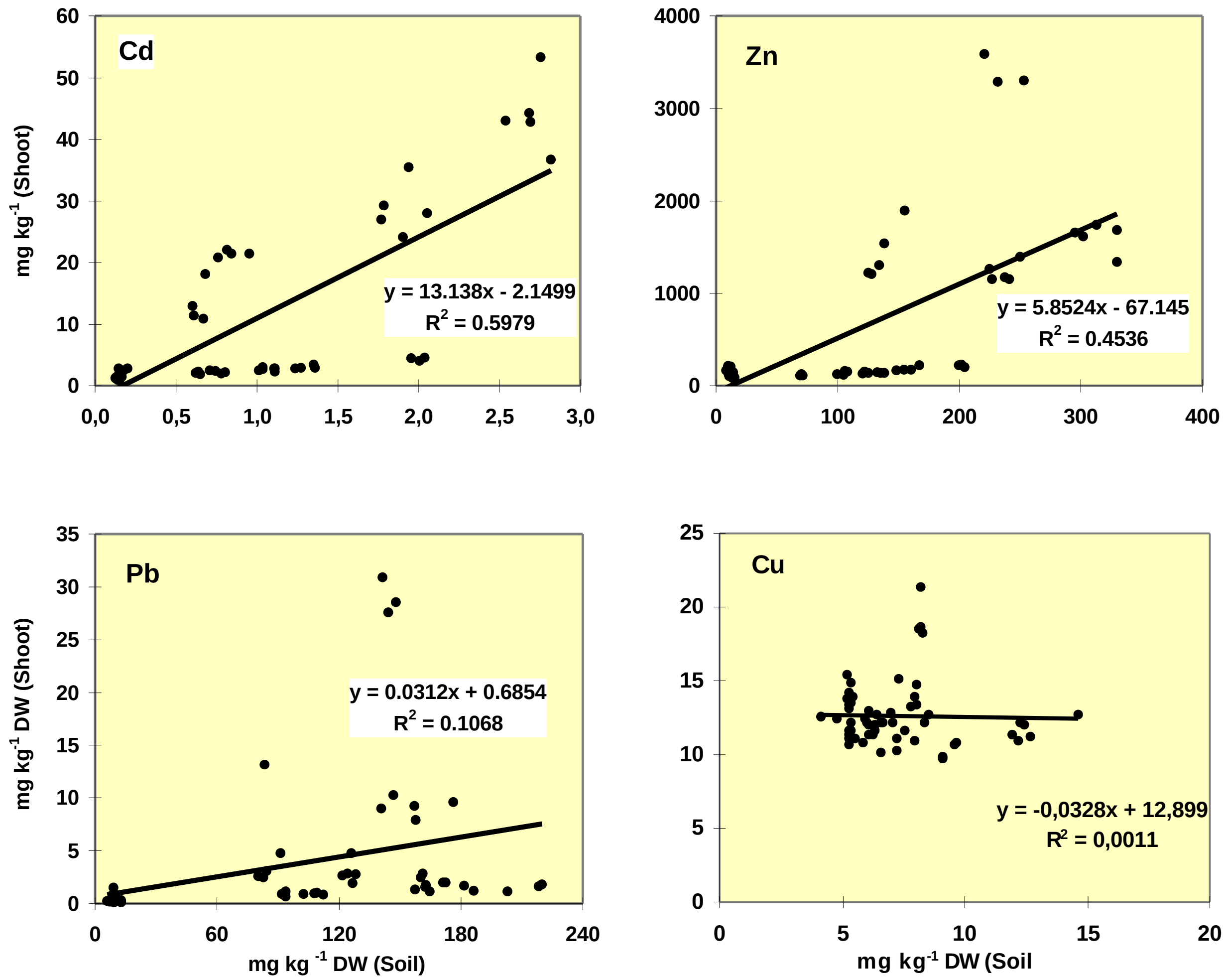

Figure 3: Relationship as determined by twofactorial linear regression between Cd, Zn, Pb and Cu in spinach shoots and the *CAT* extractable fraction of these elements in two different soils (loamy sand, silt loam) containing 0 %, 5 % and 10 % (w/w) metallurgical slag at varied pH .

Discussion

Problems caused by heavy metal contamination of arable soils include phytotoxic effects of certain elements such as Cd and Pb and also Zn and Cu which are well known as micronutrient elements, however can cause severe phytotoxicity if critical endogenous levels are exceeded (Mengel and Kirkby, 1982). Another and even more serious problem is posed by the uptake of potentially noxious elements by food or forage plant species and their transfer to the food chain and, finally, to humans (Kloke, 1980). Therefore, critical assesment of the phyto-availability of heavy metals in contaminated soils at pH levels falling within the range commonly found in arable soils is inevitable.

The determination of phytoavailable amounts of heavy metals in soils may be used to prognosticate their potential transfer to plants. Such information may in turn be helpful in deciding on the use of contaminated soils for the production of certain crops. This is especially important if vegetables, which are directly consumed by humans, are to be grown on such soils.

In the field experiments it could be shown that Cd and Zn uptake was higher in lettuce and celery than in bush beans where the pods exhibited the lowest Cd concentration of all species and organs tested. Celery is known to take up heavy metals to a greater extent than do many other vegetable species. It was reported that celery leaves accumulate about 50 % more Cd in comparison to bulbs (Leh, 1988). This is not in accordance with our results as we measured about 30 % less Cd in the leaves than in the bulbs, however, it must be noted that Leh had found an order of magnitude higher Cd concentration in the celery material which may have caused different distribution pattern within the plant. Maximum levels of toxic heavy metals as recommended by german authorities, (BGVV, 1997) for edible plant parts (formerly known as ZEBS values) include Cd (0.1 mg kg^{-1} FW for lettuce and bean pods, 0.2 mg for celeriac bulbs) and Pb (0.8 mg kg FW^{-1} for lettuce, 0.25 mg for bean pods and celeriac bulbs). The Cd content of celeriac bulbs, if expressed on a fresh weight basis, exceeded the respective value on soil 2 and 3. Thus it must be concluded that the growing of celery cannot be recommended even on soils with moderate Cd contamination such as soil 3 and pH levels less than 6.5 to 6.8. Although the endogeneous Zn concentration of head lettuce and celery exceeded the critical level for phytotoxicity (150 to 200 mg kg^{-1} DW; Sauerbeck, 1982) about twofold on soil 3, no phytotoxic effects were noted. This implies a better than average Zn tolerance in these two species. Bush beans, on the other hand, did not accumulate critical amounts of Zn even on soil 3 indicating a better exclusion potential but also a lesser physiological requirement for Zn in comparison to the other species. Still it has to be taken into account that the given soil pH (6.3) does not favour Zn availability (Hornburg et al., 1995). Low Pb uptake by plants was expected at the given pH since the critical pH for increasing Pb availablility is well below 4 (Hornburg et al., 1995) and even celery did not exceed the assigned BGVV value. Cu content was lowest in celery leaves (5 to 7 mg kg^{-1} DW) while bulbs contained two to three times more. Head lettuce contained 30 to 50 % more Cu than celery leaves. In bean leaves a Cu content of 16 to 17 mg kg^{-1} DW was measured while pods showed only 2/3 of that concentration. These findings demonstrate nicely the different physiological Cu requirements of the three species and different organs of a given species. The observation that Cu uptake does not increase with soil contamination in either species can be explained if one looks at the ammonium nitrate extractable portion which also varies only insignificantly. Still, it remains unclear why this fraction did not increase with the increasing total Cu content.

Although cultivar dependend variations in heavy metal uptake of vegetable species have been reported (Lübben, 1991; Metz and Kloke, 1998) no significant differences between cultivars of either species were found in these studies. One of perhaps a variety of reasons may be that the soils were optimally fertilized and in particular rich in phosphorus (35 to 50 mg P kg^{-1}, CAL). In a recent paper it was demonstrated that differences between two spinach cultivars in the uptake of Cu, Zn and Cd were only found in phosphorus deficient plants (Keller and Römer, 2001) This implies that the nutritional status of plants should be considered in strategies aiming at the selection of genotypes with minimized uptake of potentially noxious heavy metals.

In the pot experiments the Cd and Zn content of the spinach shoots increased excessively at a pH level around 5 on both soils reaching more than 20 mg Cd and 3000 mg Zn on soil A 5/10 whereas more than 40 mg Cd and 1600 mg Zn were attained on soil B 5/10. The strong growth depression observed in these treatments was probably caused by the high endogeneous levels of Zn and also Cd since phytotoxic effects may be caused by the latter at plant levels above 5 to 10 mg kg^{-1} DW (Sauerbeck, 1982), however, also Pb toxicity cannot be ruled out on soil A 5/10. The more than 80 % growth depression on soil A without slag (A 5/0), on the other hand, cannot be attributed to Cd and Zn toxicity as the endogeneous concentration of these elements did not reach critical levels. Moreover, in the corresponding treatment of soil B (B 5/0) only some 30 % dry weight reduction was noted although the shoots contained considerably more Cd and Zn. Here, the Zn content had just reached potentially phytotoxic levels so that the observed growth depression may partly be due to Zn toxicity. However, some other undefined factors must have also been involved as is indicated by the largely reduced growth on soil A 5/0. It is almost needless to say that in both slag treatments the spinach Cd levels exceeded the BGVV limits (0.5 mg kg^{-1} FW) up to four times. At the higher pH level the increase of the heavy metal content in the spinach shoots due to the slag amendments was negligible. As observed with the other species in the field experiments the Cu content of the spinach shoots varies only insignificantly. This is also reflected by rather small variations of ammonium nitrate and CAT extractable Cu.

The ammonium nitrate extractable fractions of Cd, Zn and Pb which decreased considerably with increasing pH correlated well with the content of these elements in the spinach shoots. Despite the much wider pH range correlation coefficients (r) were considerably better than in the field trials where only a very narrow pH range was tried. This implies that in pot experiments carried out in a more controlled greenhouse environment phytoavailability of the four elements tested can be assessed more precisely than in the field, however, it cannot be ruled out that the source of heavy metals, namely metallurgical slag as in these experiments, might influence chemical processes involved in extraction or element aquisition by plants. The high CAT extraction yield for all four elements even at the higher pH level may be a consequence of the low pH of this extractant itself and the added chelator DTPA. This extraction yield does not reflect the strongly reduced element uptake at this pH. Apparently, this is the reason why CAT soluble fractions of Cd, Zn and Pb did not correlate with uptake as good as did the ammonium nitrate soluble fraction. This is in accordance with results of Bucher and Schenk (1997) who found similar correlations employing the CAT method for the extraction of potentially phytoavailable Zn from peat substrates. While Alt and Peters (1993) reported good correlations for Cu using the CAT method on Cu deficient peat substrates, ammonium nitrate and CAT are apparrently not usefull for Cu in the soils and concentration ranges tested.

In summary it must be noted that transfer of potentially noxious heavy metals such as Cd or Zn to plants can occur in amounts that can adversely influence the nutritional value of food plants or cause yield depression even at comparably moderate soil contamination, especially if the soil pH is not strictly controlled. Ammonium nitrate extraction remains the method of choice if phytoavailable fractions of Cd and Zn are to be extracted in order to prognosticate potential transfer of these elements to plants while this method may or may not work with Pb. Still, the search for a suitable method which reliably extracts phytoavailable Cu from all soils and at all possible concentration ranges continues. Another main outcome of these studies is that heavy metal containing industrial slags which are frequently used in landscape construction or hydraulic engineering are not as chemically inert as reported (Lahl, 1994; Khorasani, 1999) if mixed with soils and thus being exposed to chemical processes occurring therein such as decreasing pH and others. Contamination of soils with such industrial waste products should, therefore, be avoided.

References

AbfallKlärV (1992): Klärschlammverordnung. BGBl I Nr. 21.

Alt, D. and I. Peters (1993): Die $CaCl_2$/DTPA-Methode zur Untersuchung gärtnerischer Erden auf Mengen- und Spurenelemente. 2. Mitteilung: Kupfer und Mangan. Agribiol. Res. 45, 215-224.

Alt, D., I. Peters and S. Gaus (1994): Die $CaCl_2$-Methode zur Untersuchung gärtnerischer Erden auf Mengen- und Spurenelemente. 7. Mitteilung: Zink. Agribiol. Res. 47, 75-83.

BGVV (1997): Richtwerte für Schadstoffe in Lebensmitteln. Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (ed.). Bundesgesundheitsblatt 40, 182-184.

Birke, C. and W. Werner (1991): Eignung chemischer Bodenextraktionsverfahren zur Prognose der Schwermetallgehalte in Pflanzen. In: Auswirkungen von Siedlungsabfällen auf Böden, Bodenorganismen und Pflanzen. Forschungszentrum Jülich (ed.), Berichte aus der ökologischen Forschung 6, 224-289.

Brümmer, G. W., J. Gerth and U. Herms (1986): Heavy metal species, mobility and availability in soils. Z. Pflanzenernähr. Bodenkd. 149, 382-398.

Brümmer, G. W. and V. Hornburg (1989): Untersuchungen zur Verfügbarkeit von Schwermetallen in Böden. Mitteilgn. Dtsch. Bodenkundl. Ges. 59, 727-732.

Bucher, A. and M. K. Schenk (1997): Characterization of phytoavailable zinc in compost-peat substrates – Comparison of methods. Z. Pflanzenernähr. Bodenk. 160, 595-601

Delschen, T. and F. Rück (1997): Eckpunkte zur Gefahrenbeurteilung von schwermetallbelasteten Böden im Hinblick auf den Pfad Boden/Pflanze. Bodenschutz 4, 114-121.

Dües, G. (1989): Bindungsformen von Schwermetallen im Boden, dargestellt auf der Basis fraktionierter Extraktion. In: Behrens, D. & J. Wiesner (eds.) Beurteilung von Schwermetallkontaminationen in Böden. Dechema, Frankfurt a. M., 143-167.

Hornburg, V., G. Welp and G. W. Brümmer (1995): Verhalten von Schwermetallen in Böden. 2. Extraktion mobiler Schwermetalle mittels $CaCl_2$ und NH_4NO_3. Z. Pflanzenernähr. Bodenk. 158, 137-145.

Kloke, A. (1980): Richtwerte `80: Orientierungsdaten für tolerierbare Gesamtgehalte einiger Elemente in Kulturböden. VDLUFA-Mitteilungen 1-3, 9-11

Kloke, A. (1988): Das „Drei-Bereiche-System" für die Bewertung von Böden mit Schadstoffbelastung. VDLUFA-Schriftenreihe, Kongressband 28, 1117-1127.

Köster,W. and D. Merkel (1982): Beziehungen zwischen den Gehalten an Zink, Cadmium, Blei und Kupfer in Böden und Pflanzen bei Anwendung unterschiedlicher Bodenuntersuchungsmethoden. Landwirtsch. Forsch., Sh. 39, 245-254.

Khorasani, R. (1999): Die Beurteilung der Verwitterungsbeständigkeit und Umweltverträglichkeit von metallurgischen Schlacken. In: Schlacken in der Metallurgie. Gesellschaft für Bergbau, Metallurgie, Rohstoff- und Umwelttechnik (ed.) GDMD-Schriftenreihe 83, 237-249.

Lahl, U. (1994): Schlackenaufbereitung durch Alterung und Laugung. Müll und Abfall 31, 86-92.

Leh, H.-O. (1988): Schwermetallgehalte verschiedener Gemüsepflanzen und Möglichkeiten zu deren Verminderung durch ackerbauliche Maßnahmen – Teilergebnisse aus Freilandversuchen. Nachrichtenbl. Deut. Pflanzenschutzd. (Braunschweig) 40, 106-112.

Lübben, S. (1991): Sortenbedingte Unterschiede bei der Aufnahme von Schwermetallen durch verschiedene Gemüsepflanzen. VDLUFA-Schriftenreihe, Kongressband 33, 605-612.

Metz, R. and A. Kloke (1998): Einfluß der Sortenwahl auf den Cadmiumtransfer Boden-Pflanze. VDLUFA-Schriftenreihe, Kongressband 49, 139-142.

Mengel, K. and E. A. Kirkby (1982): Principles of Plant Nutrition (3rd Edition). International Potash Institute, Bern, Switzerland.

Sauerbeck, D. (1982): Welche Schwermetallgehalte in Pflanzen dürfen nicht überschritten werden, um Wachstumsbeeinträchtigungen zu vermeiden. Landwirtsch. Forsch. Sh. 39, 108-129.

Traulsen, B.-D. and G. Schönhard (1995): Verfügbarkeitsstudien über Schwermetalle und Nährstoffe im Boden. In: Folgenabschätzung in der Landnutzungsforschung. Werner, A. & M. Glemnitz (eds.). ZALF-Berichte 22, 20-24.