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Wang, Q. R., Liu, X. M., Cui, Y. S., Dong, Y. T., & Christie, P. (2002). Responses of legume and non-legume crop species to heavy metals in soils with multiple metal contamination. *Journal of Environmental Science and Health Part A Toxic/Hazardous Substances and Environmental Engi*, 37(4), 611-621.

Published in:

Journal of Environmental Science and Health Part A Toxic/Hazardous Substances and Environmental Engi

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RESPONSES OF LEGUME AND NON-LEGUME CROP SPECIES TO HEAVY METALS IN SOILS WITH MULTIPLE METAL CONTAMINATION

Qing-Ren Wang,^{1,*} Xiu-Mei Liu,² Yan-Shan Cui,¹
Yi-Ting Dong,¹ and Peter Christie³

¹Research Centre for Eco-Environmental Sciences,
Chinese Academy of Sciences, 18 Shuangqing Road,
P.O. Box: 2871, Beijing 100085, China

²College of Land Resource and Environmental Sciences, Shandong
Agricultural University, Tai'an, Shandong Province 271018, China

³Agricultural and Environmental Science Department,
Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK

ABSTRACT

Field and glasshouse investigations were conducted on the responses of two legumes (field pea and fodder vetch) and three non-leguminous crops (maize, wheat and rapeseed) to the heavy metals Cd, Cr, Zn, Pb, Cu and Mn in soil with multiple metal contamination. In general, the results indicate that the two legumes and wheat were more susceptible to soil metals than were rapeseed and maize. The dry matter yields of field pea, wheat, fodder vetch, rapeseed and maize decreased by up to 169, 123, 113, 93 and 68%, respectively, in metal-contaminated soil. Among the crops, maize had the highest concentrations of Mn, Zn and Cd, rapeseed had the highest concentrations of Cr, the concentration of Cu was highest in fodder vetch, and wheat was the highest accumulator of Pb. The bioconcentration factors (BCF) of the metals decreased as the soil metal loading rates increased except for Cr in fodder vetch and Cd in

*Corresponding author. Current address: 18905 SW 280 ST, Tropical Research and Education Center, Univ. of Florida, Homestead, FL, 33031-3314, U.S.A.
E-mail: QRWANG@mail.ifas.ufl.edu

wheat, whose BCF increased as the metal loading rate increased. Significant linear correlations were found between plant and soil metal concentrations. Patterns of metal distribution in plant parts varied with different crops and metals, with more Cd and Cu accumulating in the grain of wheat than of maize, suggesting that growing wheat would represent a higher risk of food contamination than growing maize in Cd- or Cu-contaminated soil. The results suggest that on sites with multiple metal contamination, growing maize and rapeseed would be safer than growing wheat or legumes. However, maize could perhaps be used for phytoremediation of lightly contaminated soils, providing that the crop residues were safely disposed of.

Key Words: Bioconcentration factors; Legumes; Non-legumes; Soil heavy metals

INTRODUCTION

Heavy metal contamination of soils and crops has become an important issue worldwide because of concern about the human food chain and environmental protection. Potentially fatal diseases can develop through excessive accumulation of dietary heavy metals such as Cd, Pb and Cr in the human body (1–3). A number of strategies for remediation of polluted soils have been developed to minimize contamination of food, including phytoremediation, a technique based on the relatively high capacity of some plant species to accumulate heavy metals from soil. This technique may have considerable potential because of its low cost and low technical requirements compared with conventional physicochemical remediation methods (4,5). However, different plant species vary in their sensitivity and response to heavy metals, and this can be used in the diagnosis of soil metal contamination. It may also be useful in agricultural practice to select appropriate crops in order to minimize the contamination of food grown in polluted soil. For example, our previous work showed that at the same site, weeping willow (*Salix babylonica* L.), amorpha (*Amorpha fruticosa* L.) and privet (*Ligustrum lucidum*) had much higher concentrations of Mn, while tree of heaven (*Ailanthus altissima* Mill) and amorpha absorbed more Cr and Zn than chinara (*Platanus acerifolia* Wild), Chinese pine (*Pinus tabulaeformis* Carr.) or cottonrose hibiscus (*Hibiscus mutabilis*). One individual Polygonum (*Polygonum hydropiper*) plant had $>1000 \text{ mg kg}^{-1}$ of Zn in its shoots (6), indicating that it is a hyperaccumulator of Zn and may have some potential for soil phytoremediation. Because China has a very large human population with a correspondingly large demand for food, ecological strategies including the careful selection of crop species and varieties in farming systems or rotations may be a useful approach to the amelioration of metal-contaminated soils (4,7–9). Thus, it is necessary to investigate the sensitivity

and responses of different crops to elevated concentrations of heavy metals. This paper presents some results from studies on the sensitivity and responses to various heavy metals by different crop species, and some correlations between soil and plant heavy metal concentrations, and discusses the potential for lowering the contamination of food produced on contaminated soils.

MATERIALS AND METHODS

Field Investigation and Site Description

A metal ore (Pb/Zn) is currently being mined in an area within Liaoning province (40° 42'N, 123° 31'E). Very large amounts of tailings have been discharged from the mine into a giant dam continuously for over 40 years. There is some leakage from the dam and edible crops (maize, wheat and soybean) are still grown in fields adjacent to the dam. There are no plants growing directly on the tailings because the wastewater slurry from the mining process is discharged into the dam more or less continuously.

Soil and crop samples were taken from farmland in the area for analysis of metal concentrations. Wheat and maize were sampled close to maturity and the samples were separated into shoots, roots and grains. They were oven dried at 70°C for 48 h and milled before chemical analysis was carried out to examine the correlations between soil and plant metal concentrations.

Pot Experiment

A pot experiment was conducted in a glasshouse with supplementary illumination and temperature control. Two legumes, field pea (*Vicia sativa* L.) and fodder vetch (*Vicia villosa* Roth), and three non-legume crops, maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rapeseed (*Brassica napus* L.) were set up in triplicate. The plants were grown in a substrate consisting of tailings sludge mixed with soil in a ratio of 3:1 by weight. The growth substrate had a pH (in water) of 6.3 and 6.2% organic C, 0.29% Kjeldahl N, 0.059% total P and 0.51% total K. Each pot contained 260 g dry substrate containing the following 'total' (conc. HNO₃/HClO₄-extractable) concentrations of heavy metals: Mn 590, Cu 58, Zn 1982, Cd 1.2, Cr 366 and Pb 249 mg kg⁻¹, corresponding to the following concentrations of 'available' (1 M NH₄OAc-extractable) metals: Mn 3.5, Cu 0.4, Zn 0.6, Cd 0.3, Cr 0.3 and Pb 0.4 mg kg⁻¹. The pots were supplemented with six application rates of a mixture of these six heavy metals in aqueous solution (25 ml pot⁻¹) after the three-leaf stage of plant growth. Cadmium (as CdCl₂) was applied up to rates of 1, 2, 4, 6, 8 and 10 mg kg⁻¹ substrate including 1 M NH₄OAc-extractable Cd. Similarly, Pb (as Pb(NO₃)₂), Zn (as ZnSO₄), Mn (as MnSO₄), Cr (as CrCl₃) and Cu (as CuSO₄) were applied up to rates of 10, 20, 40, 60, 80 and

100 mg kg⁻¹. In order to avoid yield responses to applied N (as Pb(NO₃)₂), all pots except those receiving the highest application of metals were amended with the appropriate amount of NH₄NO₃ to bring their N application up to the same rate. Hewitt nutrient solution containing traces of micro-nutrients was used throughout the experiment. Control pots received no supplementary heavy metal applications. The controls and six application rates of metal mixtures are designated in an index system as rates 0, 1, 2, 3, 4, 5 and 6.

Seeds were sown in the pots and the seedlings were thinned to give three maize plants or five plants of the other crop species per pot. The plants grew until some of them started to exhibit toxicity symptoms and were harvested 76 days after sowing. Data presented from the pot experiment are mean values of three replicates. Means were compared using least significant difference (LSD) at the 5% level. The bioconcentration factors for the six metals in the crop plants were calculated by dividing their concentrations in the above-ground parts at harvest by their total amounts in the growth substrate. Concentrations of Cd and Cu in maize and wheat samples collected from the field site were compared with soil total concentrations by linear regression and correlation.

RESULTS

Plant Yields

There was a decreasing trend of plant yield with increasing metal loading rate (Table 1). Higher metal loadings led to a significant reduction in yield of field pea compared with controls and low metal loadings.

Table 1. Above-Ground Dry Matter Yields (g pot⁻¹) of Five Crop Species with Different Loading Rates of Six Heavy Metals Applied to the Growth Substrate*

Loading Rate	Field Pea	Fodder Vetch	Rapeseed	Maize	Wheat
0	2.96 a ^a 2.67 ab	1.56 ab 2.07 a		2.41 a 2.03 ab	0.65 a 0.57 a
2	1.50 c	1.10 b		1.66 ab	0.32 a
3	1.46 c	1.54 ab		1.73 ab	0.42 a
4	1.75 bc	1.18 b		1.53 ab	0.38 a
5	1.21 c	1.24 ab		1.35 b	0.35 a
6	1.10 c	0.73 b		1.43 ab	0.29 a

^aWithin columns, means followed by the same letter are not significantly different by LSD at the 5% level.

*Values are means of three replicates. For details on the loading rates. see text.

However, the reductions in yield of the other crop species at high metal loadings were not statistically significant.

Plant Metal Concentrations and Bioconcentration Factors

Manganese

Plant Mn concentrations increased in all crops tested as metal supply increased (Figure 1). However, the non-leguminous crops had higher Mn concentrations than the legumes. For instance, at the highest level of metal supply, maize accumulated up to 332 mg kg^{-1} , both rapeseed and wheat had about 180 mg kg^{-1} but fodder vetch and field pea contained only 64 and 107 mg kg^{-1} of Mn. The bioconcentration factor (BCF) decreased as metal supply increased, with the highest BCF occurring at the lowest metal supply for all the plants. Wheat had the lowest BCF, about 25 compared to 99 in fodder vetch and ranging from 135 to 152 in the other crops.

Chromium

All the crop species had large concentrations of Cr. For example, rapeseed had up to 7046 mg kg^{-1} , fodder vetch and wheat had over 4500 and 4200 mg kg^{-1} , but maize contained a maximum of only 1150 mg kg^{-1} (Figure 1). However, the two legumes and maize had the highest concentrations of Cr at the second to the highest metal supply but rapeseed and wheat had the highest concentrations at the maximum loading rate. BCF varied widely among plant species, e.g., field pea and rapeseed had the BCF values of more than 2300 at the lowest Cr supply, and the highest BCF in fodder vetch was 1124 at metal loading rate 5. However, the BCF in wheat increased as the metal loading rate increased.

Copper

The changes in pattern of Cu concentration and BCF were generally quite similar to those of Mn, with the highest concentrations at level 6 for most crops (except maize and wheat), but the highest BCF occurred at metal loading rate 1 for all the plant species (Figure 1).

Zinc

Comparing the two legumes, fodder vetch had higher concentrations of Zn than field pea, with concentrations reaching more than 1200 mg kg^{-1}

Figure 1. Concentrations and bioconcentration factors (BCF) of six heavy metals in (A) two legumes and (B) three non-leguminous crop plant species grown in multiple metal-contaminated mine tailings sludge spiked with different loadings of the heavy metals. (A) ◆, concentration in pea; ◇, BCF in pea; ▲, concentration in fodder vetch; △, BCF in fodder vetch. (B) ◆, concentration in rapeseed; ◇, BCF in rapeseed; ●, concentration in wheat; ○, BCF in wheat; ▲, concentration in maize; △, BCF in maize.

compared to about 800 mg kg^{-1} at the highest rate of Zn supply. However, in the non-legumes, the concentrations of Zn in maize and wheat were much higher than in rapeseed at metal rates 2 and 3, with over 1000 mg kg^{-1} in both cereals compared to $300\text{--}400 \text{ mg kg}^{-1}$ in rapeseed. The BCF values of all the crops decreased as metal application rates increased, but maize and wheat had the lowest BCF at the highest metal loading rate (Figure 1).

Cadmium

In general, plant Cd concentrations increased as the application rates increased and BCF values decreased in all the crops except for wheat (Figure 1). However, maize attained a maximum of 65 mg kg^{-1} at the highest loading rate but fodder vetch had only 5 mg kg^{-1} . Although wheat showed a much lower BCF at any loading rate the plant concentration increased with increasing loading rate until a concentration of 45 mg kg^{-1} of Cd was attained.

Lead

Wheat accumulated higher concentrations of Pb than any other crop (over 380 mg kg^{-1}). Field pea contained 260 mg kg^{-1} at loading rate 4, and fodder vetch and rapeseed reached up to 200 mg kg^{-1} at the highest Pb loading. The BCF values generally decreased as Pb application rate increased (Figure 1).

Relationship Between Soil and Plant Metal Concentrations

In the field investigations, some good relationships were found between soil and plant metal concentrations. For example, Cd and Cu showed a significantly linear relationship between soil and plant parts (Table 2). Wheat had higher concentrations of Cd than maize, especially in the grains. However, higher concentrations of Cu accumulated in the wheat grain but lower concentrations in the shoots and roots compared to maize. Roots and shoots of the two crops accumulated higher concentrations of both metals compared with the grain.

DISCUSSION

Uptake of heavy metals by various crops has been studied by a number of researchers. For example, Chukwuma (10) compared the accumulation of Cd, Pb and Zn in cultivated and wild plant species in a derelict Pb/Zn mine.

Table 2. Relationship Between Soil and Crop Concentrations of Cd and Cu in Various Plant Parts of Wheat and Maize Collected from the Field Site

Crop Species	Crop Component	Regression Equation	Number of Samples	<i>r</i>
Cd				
Maize	Grain	$y = 0.0238x + 0.0526^a$	10	0.8998 ^{***}
	Shoots	$y = 0.1646x + 1.4832$	10	0.9764 ^{**}
	Roots	$y = 1.2323x + 8.9289$	10	0.8896 ^{**}
	Grain	$y = 0.0047x + 0.0032$	8	0.9213 ^{**}
	Shoots	$y = 0.0125x + 0.0188$	8	0.8786 ^{**}
	Roots	$y = 0.0346x + 6.0232$	8	0.8897 ^{**}
Cu				
Maize	Grain	$y = 0.0326x + 2.3825$	12	0.8892 ^{**}
	Shoots	$y = 0.0481x + 3.9829$	12	0.8282 ^{**}
	Roots	$y = 0.0563x + 6.0482$	12	0.9267 ^{**}
	Grain	$y = 0.1462x + 0.8998$	8	0.8918 ^{**}
	Shoots	$y = 0.2145x + 4.2178$	8	0.9206 ^{**}
	Roots	$y = 0.9173x + 6.3876$	8	0.8998 ^{**}

^a y = Metal concentration in plant component, x = Soil total metal concentration.

^{***}Correlation coefficient significant at $P < 0.01$.

and Wahbeh (11) compared the absorption and accumulation of various heavy metals in three species of sea grasses. It has been found that wetland plant species, e.g., cootail (*Ceratophyllum demersum* L.), giant duckweed (*Spirodela polyrrhiza* (L.) Schleid.), bacopa (*Bacopa monnieri* (L.) Pennell) and wild rice (*Hygrorrhiza aristata*) accumulated appreciable amounts of Cu, Cr, Mn, Cd and Pb, but channel grass (*Vallisneria spiralls* L.) and alligator weed (*Alternanthera sessilis*) accumulated less. Previous research in our laboratory (12) on aquatic and wetland plants showed that water hyacinth and duckweed were good accumulators of Cd (462 and 14 200 mg kg⁻¹ with BCF 1225 and 2567, respectively). Water dropwort had a higher concentration of Hg (1.21 mg kg⁻¹ with BCF of 821). Other results indicated that rapeseed and peanut (*Arachis hypogaea*) displayed a high tolerance to Cd, but soybean (*Glycine max*) displayed more susceptibility. Sugarcane (*Saccharum officinarum*) tolerated as much as 100 mg kg⁻¹ of Cd without any detectable yield reduction but jute (*Corchorus olitorius*) was unable to grow at a soil Cd concentration of 25 mg kg⁻¹ (13).

The present results show that both susceptibility and response to metals differed among crop species and among various metals, and this might be related to both adaptation to the environment and genetic traits of the crops. Legume crops in China are usually grown in relatively unpolluted areas with low fertilizer applications compared with non-leguminous field crops such as maize and rapeseed. However, genetic characters are likely to be the dominant factors. The relatively high capability of rapeseed and maize to

accumulate metals suggests that these crops could be applied to remediate polluted soils in some circumstances. However, the straw and other residues in which the highest metal concentrations tend to occur should be treated properly after harvest, e.g., burnt and buried in special toxic landfill sites to prevent secondary contamination.

The concept of the bioconcentration factor (BCF) was introduced to indicate the degree to which plants accumulate metals from their substrates (14). In some circumstances, BCF is an even better criterion for identifying hyperaccumulators than plant metal concentration because the concentration of the element in the substrate is not taken into account by the latter (15). The BCF values were highest at low rates of metal supply and then decreased in most crops except fodder vetch, wheat and maize for Cr, the BCF values of which increased as metal supply increased (Figure 1). The pattern of decreasing BCF accords well with our previous work on aquatic and wetland plants (12). The changes in BCF with metal supply rates in our experiments indicate that BCF is a concentration-dependent parameter, which can be used to demonstrate large differences when there are different metal concentrations in soils or other growth substrates.

With multiple contamination with several heavy metals, plants showed different patterns of concentrations of metals and BCF values, which varied not only among crop species but also among metal forms. For example, all the crops accumulated very high concentrations of Cr. However, Cr was applied in the Cr^{3+} form in this experiment which is 10 times less toxic to plants than Cr^{6+} (16,17).

The patterns of distribution of various metals in different parts of a crop may permit the adoption of ecological engineering approaches in the prevention of food contamination and profitable utilization of polluted land. For example, in the case of Cu-, Mn- or Pb-contaminated soil, growing paddy rice would minimize the risk of food contamination because 3–9% of these metals is distributed in the edible parts of the plant. In contrast, 22–24% of Cd, Zn and Cr occur in the edible parts (6). However, the relationship between soil metal concentration and plant uptake reflects the bioavailability of heavy metals in soils and some agricultural strategies in planning cropping systems. The correlations between soil metals and their concentrations in different crop components suggests that in Cd- or Cu-contaminated soil, growing wheat would represent a higher risk than growing maize because of the differences in the accumulation of these metals in the grains of the two crops (Table 2).

CONCLUSIONS

Susceptibility to various metals differed among crop species. However, wheat and the two legumes were more sensitive than rapeseed or maize. Therefore, growing legumes or wheat in heavy metal contaminated soils is

not a wise approach. The relatively high accumulation of metals and BCF values in maize and rapeseed in plants imply that these crops, especially maize, could be used for the phytoremediation of soils that are moderately contaminated with metals providing that the residues were carefully disposed of since a relatively small proportion of Cd and Cu enters the edible parts.

ACKNOWLEDGMENTS

This research was funded by a Knowledge Innovation Project from the Chinese Academy of Sciences (KZCX2-401-1-1). The authors thank Dr. L. Zhao and Ms. J. Sun of the State Key Laboratory for Aquatic Environmental Chemistry at the Research Centre for Eco-Environmental Sciences for their help with sample analysis.

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