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Use of Single Extraction Methods to Predict Bioavailability of Heavy Metals in Polluted Soils to Rice

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Human exposure to toxic heavy metals via dietary intake is of increasing concern. Heavy-metal pollution of a rice production system can pose a threat to human health. Thus, it was necessary to develop a suitable extraction procedure that would represent the content of metal available to rice plants (Oryza sativa L.). The aim of this study was to predict, on the basis of single extraction procedures of soil heavy metals, the accumulation of heavy metals (cadmium, lead, copper, and zinc) in rice plants. Six extracting agents [Mehlich 1, Mehlich 3, EDTA (ethylenediaminetetraacetic acid), DTPA-TEA (diethylenetriaminepentaacetic acid-triethanolamine), ammonium acetate (NH₄OAc), and calcium chloride (CaCl₂)] were tested to evaluate the bioavailability of heavy metals from paddy soils contaminated with lead-zinc mine tailings to rice. The extraction capacity of the metals was found to be of the order EDTA > Mehlich 3 > Mehlich 1 > DTPA-TEA > NH₄OAc > CaCl₂. The correlation analysis between metals extracted with different extractants and concentrations of the metals in the grain and stalk of the plant showed positive correlations with all metals. The greatest values of correlation coefficients were determined between the NH₄OAc- and CaCl₂-soluble fractions of soil and contents in plants in all four metals studied. Therefore, NH₄OAc and CaCl₂ were the most suitable extractants for predicting bioavailability of heavy metals in the polluted soils to rice. The results suggested that uptake of heavy metals by rice was mostly from exchangeable and water-soluble fractions of the metals in the soils. Soil-extractable metals were more significantly correlated with metal accumulation in the stalk than in the grain. The pH had more significant influence on availability of heavy metals in the soils than total content of metals and other soil properties. The bioavailability of metals for rice plants would be high in acidic soils.

Keywords Bioavailability, contaminated soil, metal uptake, single extraction method, rice

Introduction

Heavy-metal contamination of agricultural soils has become a common phenomenon as a result of emissions from the rapidly expanding industrial areas, mining, the misuse of chemical fertilizers, and the land application of sewage waste. Intake of heavy metals

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via the environment–crop system has been considered to be the predominant pathway of human exposure to environmentally toxic heavy metals. The main sources of heavy metals from the environment to plants are air, water, and the soil (Hamilton 1995; Hope 1995; Kabata-Pendias and Pendias 1992; Wenzel and Jockwer 1999). The bioavailability of heavy metals to plants is controlled by many factors associated with soil and climatic conditions, plant genotype, and agronomic management, including active/passive transfer processes, sequestration and speciation, redox states, the type of plant root system, and the response of plants to elements in relation to seasonal cycles (Kabata-Pendias and Pendias 1992; Lindsay 1979). Contaminants in bulk soil might be transferred into soil solution, making them available to roots (Wenzel and Jockwer 1999). In polluted areas, the transfer of heavy metals from soils to plants is of great concern (Rodriguez, Tome, and Lozano 2002). When bioavailability of heavy metals in soils is evaluated, total concentration and available species of the metals should be considered (De Vries et al. 2005). Analysis of total heavy-metal content provides information on the accumulation of heavy metals in soils. However, total content cannot indicate the bioavailability and mobility of these metals in soils, because not all the forms of metals present in soil are available to plants. Therefore, it is of utmost important to predict bioavailable metals rather than the total metals in order to assess toxic effects and to study biogeochemical pathways. Several methods have been used to evaluate bioavailability of trace elements in soils, which are based mainly on extractions by various solutions: (i) mineral acids at various concentrations, (ii) chelating agents, for example, ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid–triethanolamine (DTPA–TEA), (iii) buffered salts, for example, ammonium acetate (NH_4OAc), (iv) neutral salts, for example, calcium chloride (CaCl_2) and ammonium nitrate (NH_4NO_3), and (v) other extractants proposed for routine soil testing. The use of various single extractants for the prediction of bioavailability of metals from soils has been reported; however, suitability and prediction precision of the various extractions varied with soil types and plants (Adriano 2001; Alloway 1995; Alvarez et al. 2006; Feng et al. 2005; Gupta and Sinha 2007; Iyengar, Martens, and Miller 1981; Meers et al. 2007; Mehlich 1984; Mendoza et al. 2006; Young et al. 2005; Zhang, Zhou, and Huang 2006).

Rice is a main food crop in the world and has been increasingly exposed to solid and industrial wastewater irrigation and point-source pollution by smelter and chemical enterprises. Pollution of heavy metals in a rice production system can pose a potential long-term threat to human health. The risks of their potential entry into the food chain need to be carefully considered. Heavy-metal uptake and accumulations in rice are dependent on both rice varieties and site conditions. Significant differences were determined among rice genotypes in heavy-metal uptake and accumulation in grains; thus, the possibility of developing rice cultivars with low grain accumulation of toxic heavy metals is considered an efficient approach for producing safe yields from rice cultivated in slightly or moderately contaminated soils (He et al. 2006; Cattani, Romani, and Boccelli 2008; Shi, Li, and Pan 2009). Increased concentrations of heavy metals in agricultural soils can increase uptake and accumulation of the metals in rice (Li et al. 2005). Inherent differences in soil chemical and physical properties can also change metal bioavailability and mobility and thus affect uptake of heavy metals in rice (Li et al. 2005). In addition, the application of amendments, such as lime, could decrease the metal bioavailability in soils/ Amendment addition thus appears to be a promising technique to reduce the bioavailability of soil metals and minimize metal concentrations in the produced rice (Kumar et al. 1998).

The soils (paddy soils) used for rice production are often flooded during the growth season, and the chemical forms of heavy metals and soil–plant transfer pathways of the

metals in the paddy soils may be different from those of other soils. Therefore, extractants suitable for the prediction of bioavailability of metals in a rice–paddy soil system could be different from other plant–soil systems. In this article, we aim to assess the potential of six widely employed single extractants, namely Mehlich 1, Mehlich 3, EDTA, DTPA–TEA, NH_4OAc , and CaCl_2 , for the prediction of bioavailability of metals from paddy soils contaminated with lead–zinc mine tailings to rice plants. Emphasis is put on selecting the best extraction procedure to estimate the content of metals in soil available to plants during the growing season.

Materials and Methods

Samples

The present study was conducted in Dongguan, Shangyu County, located at the northern part of Zhejiang Province in southeastern China. The sampling area was located at a rice production area near an abandoned lead–zinc mine and was polluted by containments of lead–zinc mine tailings for more than 100 years. It is about 10 m above sea level. Geologically, the area is covered by lacustrine sediment with low background of heavy metals. There is a humid subtropical climate with a mean annual rainfall of 1,400 mm and an average annual temperature of 16.4 °C. Representative soil and rice plant samples were taken in November 2007 from an environmentally contaminated rice production region (2.5 km²) in the town. The region was divided into 53 sectors (each of 4–6 ha). Both soil and plant samples were taken from each sector, at which intense rice cultivation occurred. For plant samples, stalk and grain with hull were simultaneously collected. Each sample was composed of a mixture of 10 samples taken from 10 places within each sector. The soil samples were air-dried at room temperature, and subsamples from each soil were ground to <2 mm and <0.149 mm, respectively, for physical and chemical analyses. The rice samples were first washed thoroughly with tap water to remove dust adhered to the samples. Then, the samples were washed three times with deionized water. Afterward, the samples were dried to a constant weight at 60 °C, thoroughly ground in a stainless steel mill to a fine powder (<1 mm), mixed, and made uniform to obtain representative samples. They were subsequently analyzed for the content of metals.

Extraction of Available Heavy Metals

Six different extraction methods were used to extract soil labile metals: (i) 0.01 mol L⁻¹ CaCl_2 -extractable metals [1:10 ratio of soil to 0.01 mol L⁻¹ CaCl_2 ; 60 min reaction time] (Sparks 1996), extracting only water-soluble metals; (ii) Mehlich 1–extractable metals [1:4 ratio of soil to 0.05 mol L⁻¹ hydrochloric acid (HCl) + 0.0125 mol L⁻¹ sulfuric acid (H_2SO_4); 5 min reaction time] (Sparks 1996), including water-soluble, exchangeable, and partially carbonate, oxide-bound metals; (iii) DTPA–TEA–extractable metals [1:2 ratio of soil to DTPA–TEA extraction solution; 120 min reaction time] (Sparks 1996), including water-soluble, exchangeable, and partially organic bound metals; (iv) 1 mol L⁻¹ NH_4OAc -extractable metals [1:4 ratio of soil to 1 mol L⁻¹ NH_4OAc extraction solution; 60 min reaction time] (Sparks 1996), including water-soluble and exchangeable metals; (v) Mehlich 3–extractable metals (1:10 ratio of soil to Mehlich 3 extraction solution [0.2 mol L⁻¹ acetic acid (CH_3COOH) + 0.25 mol L⁻¹ NH_4NO_3 + 0.015 mol L⁻¹ ammonium fluoride (NH_4F) + 0.013 mol L⁻¹ HNO_3 + 0.001 mol L⁻¹ EDTA, pH2.0];

Table 1
Mean values, ranges, and variation coefficients (CV) of total and extractable heavy metals in soil samples

Extraction solution	Cd			Cu			Pb			Zn		
	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)
Total	0.11–1.49	0.39	55	17.0–132	47.6	45	21.11–436.00	95.58	70	58.5–488	128	50
Mehlich I	0.036–0.38	0.097	72	5.13–22.9	13.6	72	3.94–39.46	11.62	84	5.53–41.7	27.6	59
Mehlich III	0.040–0.56	0.12	67	4.27–29.6	15.9	62	3.68–57.22	19.63	72	9.58–56.7	31.3	58
EDTA	0.031–0.77	0.15	64	6.18–44.2	17.4	54	4.14–46.75	16.28	87	11.4–98.2	36.7	70
DTPA-TEA	0.021–0.31	0.088	65	1.27–18.6	5.65	82	2.27–21.43	5.43	75	3.28–31.5	13.4	64
NH ₄ OAc	0.011–0.23	0.058	90	0.00–5.13	0.78	128	0.11–7.66	1.66	108	0.15–18.7	3.74	117
CaCl ₂	0.011–0.083	0.018	104	0.00–0.78	0.14	99	0.00–0.63	0.094	138	0.11–3.12	0.74	84

Extractable Heavy Metals in the Soils

The contents of extractable Cd, Cu, Pb, and Zn, derived by six different extraction methods, varied greatly among the soils (Table 1). Variation coefficients (CV) of extractable heavy metals for the same extraction methods ranged from 52 to 128%. Generally, the variation coefficients changed with extraction methods in the order of NH_4Ac and $\text{CaCl}_2 >$ Mehlich 1, Mehlich 3, EDTA, and DTPA-TEA. The extraction capacity of the metals was found in the order $\text{EDTA} >$ Mehlich 3 $>$ Mehlich 1 $>$ DTPA-TEA $>$ NH_4OAc $>$ CaCl_2 .

Metal Accumulation in Rice Plant

Accumulation of metals in rice plant organs varied greatly among sampling locations. Cadmium, Cu, Pb, and Zn contents in grain ranged from 0.027 to 0.25 mg kg^{-1} , 1.29 to 8.44 mg kg^{-1} , 0.059 to 1.75 mg kg^{-1} , and 4.98 to 23.5 mg kg^{-1} , respectively (Table 2). The corresponding mean values were 0.090, 4.07, 0.36, and 9.32 mg kg^{-1} . Concentration of Cu in grain for all samples was less than the threshold level (10 mg kg^{-1}) of food quality of China (Zou, Qiu, and Zhou 2008). However, 9.4, 28.3, and 7.5% of grain samples had Cd, Pb, and Zn concentrations greater than their threshold levels (0.20, 0.40, and 20 mg kg^{-1} for Cd, Pb, and Zn, respectively) of food quality of China (Zou, Qiu, and Zhou 2008). These results indicated that concentrations of Cd, Pb, and Zn in grains in the area were a potential risk for human health. Cadmium, Cu, Pb, and Zn contents in the stalk ranged from 0.038 to 1.99 mg kg^{-1} , 2.54 to 26.8 mg kg^{-1} , 0.076 to 3.21 mg kg^{-1} , and 5.43 to 67.9 mg kg^{-1} , with their mean values of 0.38, 8.78, 0.72, and 16.9 mg kg^{-1} , respectively. The contents of heavy metals in stalks were significantly greater than those in grains.

Correlation Analysis

Bioavailability of heavy metals in soil to rice plants was evaluated by correlating the metal content in soil fractions (total content and extractable content) determined by various extraction methods with the metal content in plants, and correlation coefficients between the total content of a given metal in soil and plant ($\text{Soil}_{\text{total}} - \text{Plant}$) and extractable fraction from soil and plant ($\text{Soil}_{\text{extractable}} - \text{Plant}$) are shown in Table 3. In a majority of cases in the literature, there was no linear dependence between total soil content and plant content determined (Chojnacka et al. 2005). This study also confirmed that linear dependences between total soil content and rice plant content of Cd, Cu, Pb, and Zn was not strong, but the situation drastically changed when the plant content was correlated with the extractable fraction. It may be that not all the metal species found in the soil are available to plants. Only bioavailable forms can be absorbed. However, correlation coefficients between metal contents in plants and extractable content in soils varied greatly with extraction solution. Mean correlation coefficients of Cd, Cu, Pb, and Zn decreased in the order of NH_4OAc (0.890) $>$ CaCl_2 (0.829) $>$ DTPA-TEA (0.554) $>$ EDTA (0.443) $>$ Mehlich 3 (0.438) $>$ Mehlich 1 (0.435). Two extraction agents showed the greatest values of correlation coefficients: NH_4OAc and CaCl_2 . The DTPA-TEA can be implied to evaluate soil-plant relation. Therefore, we defined the bioavailable components as those that are extracted with NH_4OAc or CaCl_2 solution, according to the correlation coefficients described previously.

It was also found that soil pH had significant effects on the accumulation of the metals in the plants (Table 3). The accumulation of the metals in the plants increased with

Table 2
Mean values, ranges, and variation coefficients (CV) of accumulation of heavy metals in grain and stalk

Tissue	Cd			Cu			Pb			Zn		
	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)	Range (mg kg ⁻¹)	Mean (mg kg ⁻¹)	CV (%)
Grain	0.027–0.25	0.090	68	1.29–8.44	4.07	41	0.059–1.75	0.36	102	4.98–23.5	9.32	48
Stalk	0.038–1.99	0.38	125	2.54–26.8	8.78	65	0.076–3.21	0.72	93	5.43–67.9	16.9	86

Table 3

Linear correlation coefficients (r) between accumulation of the metals in plants and extractable heavy metals in soils as well as soil properties

Extraction solution	Grain					Stalk				
	Cd	Cu	Pb	Zn		Cd	Cu	Pb	Zn	
Total	0.326*	0.212	0.437**	0.3535*		0.284*	0.306*	0.374**	0.364**	
Mehlich I	0.376**	0.352*	0.367**	0.463**		0.396**	0.476**	0.482**	0.571**	
Mehlich III	0.428**	0.296*	0.472**	0.421**		0.406**	0.561**	0.413**	0.506**	
EDTA	0.363**	0.416**	0.515**	0.391**		0.426**	0.442**	0.423**	0.565**	
DTPA-TEA	0.618**	0.532**	0.466**	0.616**		0.586**	0.496**	0.409**	0.712**	
NH ₄ OAc	0.848**	0.744**	0.834**	0.957**		0.935**	0.930**	0.922**	0.948**	
CaCl ₂	0.832**	0.699**	0.796**	0.867**		0.806**	0.887**	0.892**	0.853**	
Soil properties										
Organic carbon	-0.074	-0.033	-0.098	-0.050		0.043	-0.032	-0.021	-0.040	
pH	-0.732**	-0.735**	-0.608**	-0.699**		-0.641**	-0.732**	-0.640**	-0.684**	
Clay	-0.167	-0.165	-0.071	-0.197		-0.066	-0.178	-0.054	-0.131	

Note. * and ** indicate the statistical significance at probability levels of $p < 0.05$ and $p < 0.01$, respectively.

decreasing pH. This may be due to the increase in solubility of soil heavy metals and desorption of heavy metals sorbed by soils in lower pH environments (Du Laing et al. 2007; Fernandez-Calvino et al. 2008; Rieuwerts 2007).

Prediction of Heavy Metals Accumulated in Rice

The relationship between extractable heavy metals from the soil using the following two extraction methods, NH_4OAc and CaCl_2 , were found to be the most suitable extractants for predicting bioavailability of heavy metals in polluted soils to rice (Table 3). There are significant linear relationships between metal contents in plants and NH_4OAc - and CaCl_2 -extractable fractions of metals from the soil. A statistically significant relationship between the content of a given element in the soil and plant suggested that NH_4OAc - and CaCl_2 -extractable fraction of the metals from soil can provide a simple and useful method for assessing uptake for the purposes of estimation and screening. The correlation coefficient obtained by NH_4OAc -extractable metals was generally greater than that by CaCl_2 -extractable metals. This may suggest that uptake of metals by plant mostly originated from exchangeable forms of the metals in the soils.

Deviation from linearity could be explained by the complexity of the process of metal ion transfer from soil to plant, and many factors influence element transfer to plants. It is necessary to take into consideration that the content of metals in plants are influenced by a variety of factors, including plant species, type of soil, physicochemical properties, weather conditions, type of cultivation, and applied fertilizers. Also, metals are taken up by plants not only from soil but also from air deposition and water. Additionally, soil pH and other soil components can influence metal content in plants (Strobel et al. 2005). In the majority of cases, soil pH and contents of organic matter and clay showed negative correlation coefficients, which were statistically significant for pH and plant concentrations for Cd, Cu, Zn, and Pb (Table 3).

The stepwise multiple regression analyses for Cd, Cu, Pb, and Zn concentrations in plants as a function of total metal, pH, organic matter, and clay contents are listed in Table 4. The significance of coefficients, r , an evaluation of the overall prediction of bioavailability, is also included in Table 4. The stepwise multiple regression equation of metal in plants consisted of four steps and was quite similar for Cd, Cu, Pb, and Zn. The first variable entered was pH, and the second variable entered was total metal. The third and fourth variables entered were organic-matter and clay contents, respectively. The results indicate that the combination of total metal, pH, organic-matter contents, and clay contents could also be useful tools to predict heavy metals accumulated in rice plants (Table 4).

Conclusions

The presence of heavy metals in soils does not imply that they are available to plants, particularly if they are insoluble in the soil solution. Of significance is the metal concentration in the available form, because this form simulates if a given metal could be absorbed by plants. Therefore, there is a need to elaborate an extraction procedure that would enable determination of the quantity of metals available to plants during growing season. On the basis of single extraction procedures of soil heavy metals with six extracting agents, we found that both NH_4OAc and CaCl_2 were the most suitable extractants for predicting bioavailability of heavy metals from paddy soils contaminated with lead-zinc mine tailings to rice plant and could be taken as a measure of the bioavailability of a given metal to rice plant. The results also suggested that uptake of heavy metals by rice plants

Table 4

Simple regression and stepwise multiple regression equations of metals in plants as a function of extractable metals in soil by NH₄OAc and CaCl₂ or total metal and properties of the soils

Metals	NH ₄ OAc-extractable (X)		CaCl ₂ -extractable (X)		pH (X ₁), total (X ₂), OC (X ₃), and clay (X ₄)		
	Tissue	Linear regression	r	Linear regression	r	Multiple linear regression	
Cd (Y, mg kg ⁻¹)	Grain	$Y = 0.032 + 0.99 X$	0.848	$Y = 0.041 + 2.68 X$	0.832	$Y = 0.41 - 0.058X_1 + 0.089X_2 - 0.0025X_3 + 0.00086X_4$	0.798
	Stalk	$Y = -0.12 + 8.46 X$	0.935	$Y = 0.012 + 20.2 X$	0.806	$Y = 0.19 - 0.41X_1 + 0.61X_2 - 0.010X_3 + 0.021X_4$	0.705
Cu (Y, mg kg ⁻¹)	Grain	$Y = 3.11 + 1.24 X$	0.744	$Y = 2.91 + 8.05 X$	0.699	$Y = 12.8 - 1.56X_1 + 0.012X_2 - 0.023X_3 + 0.007X_4$	0.752
	Stalk	$Y = 4.62 + 5.31 X$	0.922	$Y = 3.64 + 35.4 X$	0.887	$Y = 37.8 - 5.31X_1 + 0.069X_2 - 0.093X_3 + 0.007X_4$	0.775
Pb (Y, mg kg ⁻¹)	Grain	$Y = 0.075 + 0.17 X$	0.834	$Y = 0.14 + 2.25 X$	0.796	$Y = 1.68 - 0.27X_1 + 0.0019X_2 - 0.017X_3 + 0.014X_4$	0.705
	Stalk	$Y = 0.14 + 0.35 X$	0.930	$Y = 0.24 + 4.69 X$	0.893	$Y = 2.97 - 0.55X_1 + 0.0028X_2 - 0.0038X_3 + 0.030X_4$	0.701
Zn (Y, mg kg ⁻¹)	Grain	$Y = 5.66 + 0.98 X$	0.957	$Y = 4.69 + 6.22 X$	0.867	$Y = 32.1 - 3.95X_1 + 0.022X_2 - 0.12X_3 - 0.027X_4$	0.768
	Stalk	$Y = 5.18 + 3.13 X$	0.948	$Y = 2.16 + 19.8 X$	0.853	$Y = 80.2 - 12.8X_1 + 0.076X_2 - 0.31X_3 + 0.27X_4$	0.760

was mostly from exchangeable and water-soluble fractions of the metals in the soils. The pH influenced the availability of heavy metals in the soils more than the total content of metals and other soil properties.

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