Assessment of single extraction methods for evaluating the immobilization effect of amendments on cadmium in contaminated acidic paddy soil

Q.H. Zhu[1], D.Y. Huang[1], S.L. Liu[1], Z.C. Luo[2], H.H. Zhu[1], B. Zhou[1,3], M. Lei[1,3], Z.X. Rao[1,3], X.L. Cao[1]

[1]*Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, P.R. China*
[2]*Hunan Soil and Fertilizer Institute, Changsha, Hunan, P.R. China*
[3]*Graduate University of Chinese Academy of Sciences, Beijing, P.R. China*

ABSTRACT

Various single extractants (EDTA, DTPA, $CaCl_2$, $NaNO_3$ and NH_4NO_3) and the first step of Community Bureau of Reference (BCR1) method were used to evaluate the immobilization of Cd in contaminated acidic paddy soil by different amendments. The extractability of Cd in amended soil changed in the following order: EDTA ≈ BCR1 > DTPA > NH_4NO_3 ≈ $CaCl_2$ > $NaNO_3$. A simple correlation analysis indicated that the BCR1, $CaCl_2$, NH_4NO_3, and $NaNO_3$ methods are the best suited methods for predicting changes in the phytoavailability to rice of Cd in soil (r = 0.680 to 0.828, P < 0.001), followed by the DTPA extraction procedure (r = 0.543 to 0.666, P < 0.01). However, non-significant correlations were observed between soil EDTA-extractable Cd and Cd accumulated in rice. Accordingly, the BCR1, $CaCl_2$, NH_4NO_3, and $NaNO_3$ extraction procedures are recommended for evaluating the immobilization of Cd in contaminated acidic paddy soil.

Keywords: Cd; phytoavailability; extractant; rice; EDTA

Cadmium (Cd) is a toxic trace element with no known essential function for biological processes and high soil Cd contents are associated with high risks to human health, plants, and microorganisms. The phytoavailability, toxicity, and mobility of Cd in soil are determined by the distribution of Cd between solid and solution phases rather than by the total concentration. Various extraction solutions were used to evaluate the phytoavailability of Cd in soils, such as (a) acids (particularly mineral acids) at various concentrations; (b) chelating agents such as EDTA and DTPA; (c) unbuffered salts solutions such as $CaCl_2$, NH_4NO_3, and $NaNO_3$; and (d) other extractants proposed for routine soil testing. However, these methods are only useful under certain conditions. Linear correlations between various indicators of metal phytoavailability and metal concentration in plant parts are often used to evaluate the suitability of soil testing procedures. Feng et al. (2005a,b) evalu-

ated DTPA, EDTA, $CaCl_2$, and $NaNO_3$ as extractants for predicting the phytoavailability of Cu, Zn, Cr, and Cd in soils for growing wheat and barley. Gupta and Sinha (2007) assessed single extraction methods using DTPA, EDTA, NH_4NO_3, $CaCl_2$, and $NaNO_3$ for predicting the phytoavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown in soil contaminated with tannery wastes.

In situ remediation techniques were widely used to decrease the phytoavailability of Cd in contaminated soils through the use of amendments such as lime, phosphate, clay minerals, and organic matter (Lee et al. 2004, Li et al. 2008). Single extraction methods were also frequently used to evaluate the immobilization effects of amendments on Cd in contaminated soils. Lee et al. (2004) used EDTA and DTPA as extractants to evaluate the effects of various amendments on the phytoavailability of Cd and Pb in long term contaminated soils. Li et al.

(2008) used CaCl$_2$ extractable Cd and Cu to assess the immobilization of Cd and Cu in a variable-charge soil by different amendments. However, no single method was recognized as a universal approach for predicting changes in the phytoavailability of Cd in contaminated soil with the application of amendments. As such, the aim of the present study is to compare the single extraction methods using (1) DTPA, (2) EDTA, (3) CaCl$_2$, (4) NaNO$_3$, and (5) NH$_4$NO$_3$ as extractants and (6) the first step of the Community Bureau of Reference (BCR1) method in the context of evaluating the immobilization effect of various amendments on Cd in contaminated acidic paddy soils.

MATERIAL AND METHODS

Site description. This study was conducted in an acidic paddy soil in the Zhuzhou City, Hunan Province, China (113°01'E, 27°49'N) in 2007. Tested soil is classified as Ultisol derived from Quaternary red clay, and became contaminated by Cd via the drainage of wastewater from a nearby electroplating factory, which ceased operation in April 2006. The soil characteristics were as follows: pH, 5.34; organic carbon, 14.6 g/kg; total N, 2.45 g/kg; Olsen-P, 8.15 mg/kg; available K, 123.9 mg/kg; clay, 35.6%; silt, 40.0%; sand 24.4%; and total Cd, 2.98 mg/kg.

Experimental design. The experiment consisted of eight treatments with different amendments: CK, no amendment; L, lime [primarily Ca(OH)$_2$; pH, 12.36; total Cd 0.14 mg/kg]; P, calcium magnesium phosphate (pH, 7.36; total Cd 2.10 mg/kg); S, sepiolite (particle diameter ≤ 74 μm; pH, 9.01; total Cd not determined); H, lignite (particle diameter ≤ 1 mm; pH, 5.43; total Cd 0.28 mg/kg); and lime mixed with one of the three other amendments (LP, LS, and LH). The amounts of amendments applied were L at 150 g/m^2, P and S at 2250 g/m^2, and H at 4500 g/m^2, respectively. The plots separation, tilth, amendments application and rice seedling transplantation were the same as our previous report (Zhu et al. 2010).

Sampling. Rice was transplanted on July 22 and harvested at maturity (October 15, 2007). At the time of harvest, the rice and the soil samples were collected from each plot. Plant samples were taken as grain (separated into brown rice and hull after being dried) and straw, dried at 40°C to a constant weight, and ground to pass through a 0.3-mm sieve. Each soil sample was a composite of approximately eight cores (0–20 cm depth) collected randomly from each experimental plot. Air-dried samples were sieved through a 2-mm sieve and used to determine extracted Cd.

Analysis. Soil samples for different treatments were collected and analyzed for the extractability of Cd by single extraction methods. These methods had been widely used in recent studies, as follows:

(1) DTPA extraction method (Lindsay and Norvell 1978): 20 g of soil in 40 mL of 0.005 mol/L DTPA (analytical reagent, AR) + 0.01 mol/L CaCl$_2$ (AR) + 0.01 mol/L triethanolamine (TEA, AR), pH 7.3, shaken for 2 h.
(2) EDTA extraction method (Quevauviller et al. 1997): 5 g of soil in 25 mL of 0.05 mol/L Na$_2$-EDTA (AR), pH 7.0, shaken for 1 h.
(3) CaCl$_2$ extraction method (Novozamsky et al. 1993): 4 g of soil in 40 mL of 0.01 mol/L CaCl$_2$, pH 5.4, shaken for 3 h.
(4) NaNO$_3$ extraction method (Gupta and Aten 1993): 16 g of soil in 40 mL of 0.1 mol/L NaNO$_3$ (AR), pH 5.2, shaken for 2 h.
(5) NH$_4$NO$_3$ extraction method (Gupta and Sinha 2007): 8 g of soil in 40 mL of 1 mol/L NH$_4$NO$_3$ (AR), pH 4.8, shaken for 2 h.
(6) BCR1 method (Ure et al. 1993): 1 g of soil in 40 mL of 0.11 mol/L acetic acid (guarantee-grade reagent, GR), pH 2.8, shaken for 16 h.

Plant materials (1.000 g dried weight) were digested (open system) using a mixture of 15 mL HNO$_3$ and 3 mL HClO$_4$. To determine soil total Cd, a 1.000 g soil samples (dry weight) were digested (open system) using a mixture of 20 mL aqua regia and 3 mL HClO$_4$. Cadmium concentrations in solutions were determined by inductively coupled plasma-mass spectrometry (ICP-MS, GBC, Melbourne, Australia) and atomic absorption spectrometry (AAS, GBC, Melbourne, Australia). In all cases, the calibration method for ICP-MS was an external calibration procedure with the element indium as the internal standard. The pH of soil, phosphate, and lignite was determined in water at a solid/solution ratio of 1 to 2.5 (w/v), and the pH of the lime and sepiolite was determined in water at a solid/solution ratio of 1 to 10 (w/v).

Simple correlation analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, USA). The Pearson correlation coefficients were determined for Cd in rice in relation to the amount of Cd extracted from soils using various extraction methods.

RESULTS AND DISCUSSION

Extraction capacity. As shown in Table 1, no significant differences were observed in EDTA

Table 1. Extractable Cd concentrations (mg/kg) in the studied soils according to different single extraction procedures

Treatments	EDTA[a]	DTPA[a]	CaCl$_2$[a]	NaNO$_3$[a]	NH$_4$NO$_3$[a]	BCR1[a]
CK	1.63 ± 0.05[a]	1.3 ± 0.03[a]	0.480 ± 0.016[a]	0.116 ± 0.011[a]	0.587 ± 0.006[a]	1.78 ± 0.08[a]
L	1.62 ± 0.05[a]	1.18 ± 0.03[ab]	0.230 ± 0.068[c]	0.045 ± 0.016[c]	0.264 ± 0.011[b]	1.50 ± 0.05[cd]
P	1.60 ± 0.02[a]	1.08 ± 0.04[bc]	0.115 ± 0.010[d]	0.013 ± 0.001[d]	0.159 ± 0.029[bc]	1.62 ± 0.08[abc]
S	1.61 ± 0.04[a]	1.06 ± 0.06[bc]	0.030 ± 0.014[de]	0.004 ± 0.002[d]	0.072 ± 0.022[c]	1.38 ± 0.04[d]
H	1.55 ± 0.08[a]	1.11 ± 0.07[bc]	0.357 ± 0.031[b]	0.072 ± 0.007[b]	0.532 ± 0.007[a]	1.66 ± 0.08[abc]
LP	1.53 ± 0.04[a]	1.02 ± 0.03[c]	0.096 ± 0.021[de]	0.011 ± 0.004[d]	0.060 ± 0.020[c]	1.51 ± 0.05[bcd]
LS	1.57 ± 0.04[a]	1.00 ± 0.04[c]	0.012 ± 0.001[e]	0.001 ± 0.000[d]	0.049 ± 0.014[c]	1.38 ± 0.05[d]
LH	1.54 ± 0.04[a]	1.13 ± 0.04[bc]	0.293 ± 0.002[bc]	0.048 ± 0.004[c]	0.277 ± 0.066[b]	1.69 ± 0.09[ab]
Mean [b]	1.58 ± 0.02[A]	1.11 ± 0.02[B]	0.202 ± 0.034[C]	0.039 ± 0.008[D]	0.250 ± 0.044[C]	1.57 ± 0.03[A]

Results are expressed as the mean concentration (± SD) of three samples; [a]within each column, the concentrations accompanied by the same lowercase letter are not significantly different ($P = 0.05$); [b]the means of the concentrations accompanied by the same capital letter are not significantly different ($P = 0.05$)

extractable Cd (EDTA-Cd) among the various treatments, whereas a significant decrease in DTPA extractable Cd (DTPA-Cd) in soils as compared with CK (except for L). With regard to the three unbuffered salt solutions extractable Cd (CaCl$_2$-Cd, NaNO$_3$-Cd, and NH$_4$NO$_3$-Cd), significantly decreased according to the following order of treatments: CK > H > LH > L > P > LP ≈ S ≈ LS. For the BCR1 extractable Cd (BCR1-Cd), significantly decreases were observed in L, S, LP, and LS treatments as compared with CK.

Overall, the extractability of Cd obtained through EDTA was similar to that obtained through BCR1, and both of these procedures yielded higher extractability than did DTPA, followed by the NH$_4$NO$_3$, CaCl$_2$, and NaNO$_3$. Similar results were reported by earlier studies (Feng et al. 2005b, Gupta and Sinha 2007). As a strong chelating reagent, EDTA could partly remove organically bound metals, carbonate bound metals, and parts of metals occluded in oxides and secondary clay minerals (Gupta and Sinha 2007, Anju and Banerjee 2011). The BCR1 procedure was reported to extract most of the calcium-carbonate-bound and mineral-fraction metals and to partially release organic-matter-bound metals (Feng et al. 2005b). However, DTPA could extract part of iron and manganese oxides from acidic soils (O'Connor 1988). Regarding the three un-buffered salt solutions, high extractability obtained with NH$_4$NO$_3$ in comparison with the other two studied reagents can be attributed to the possible complexation of these elements by NH$_3$ and to the higher salt concentration of the

NH$_4$NO$_3$ solution (Pueyo et al. 2004). Exchangeable concentrations are lower in NaNO$_3$ extraction solution rather than in CaCl$_2$ extraction solution because the monovalent cations show weak competition with the adsorption sites on organic matter (Novozamsky et al. 1993).

Correlation between extractable Cd and Cd accumulation in rice. As shown in Figure 1, the BCR1 extraction method provided the best measure of Cd phytoavailability changes in soil after the amendments were applied. The correlation coefficient (r) ranged from 0.773 to 0.828 ($P < 0.001$). Similarly, correlations between the CaCl$_2$-Cd, NaNO$_3$-Cd, and NH$_4$NO$_3$-Cd all correlated significantly ($P < 0.001$) with rice tissues Cd accumulation ($r = 0.762$ to 0.773, 0.693 to 0.725, and 0.680 to 0.742, respectively). Significant correlations were also found between DTPA-Cd and Cd concentrations in brown rice and rice straw ($r = 0.584$ and 0.543, $P < 0.01$) and between DTPA-Cd and Cd concentrations in rice hull ($r = 0.666$, $P < 0.001$). However, no significant ($P > 0.05$) correlation was observed between EDTA-Cd and Cd accumulation in rice tissues.

Nice correlation between the BCR1-Cd and Cd accumulated by plant tissues was also reported. Feng et al. (2005b) similarly found significant correlations between BCR1-Cd and Cd accumulation in wheat root in acidic, neutral, and near-alkaline soils. Chen et al. (2000) applied similar extraction procedures (0.43 mol/L acetic acid) to estimate the effect of different chemical amendments on the phytoavailability to wheat of Cd and Pb in soil.
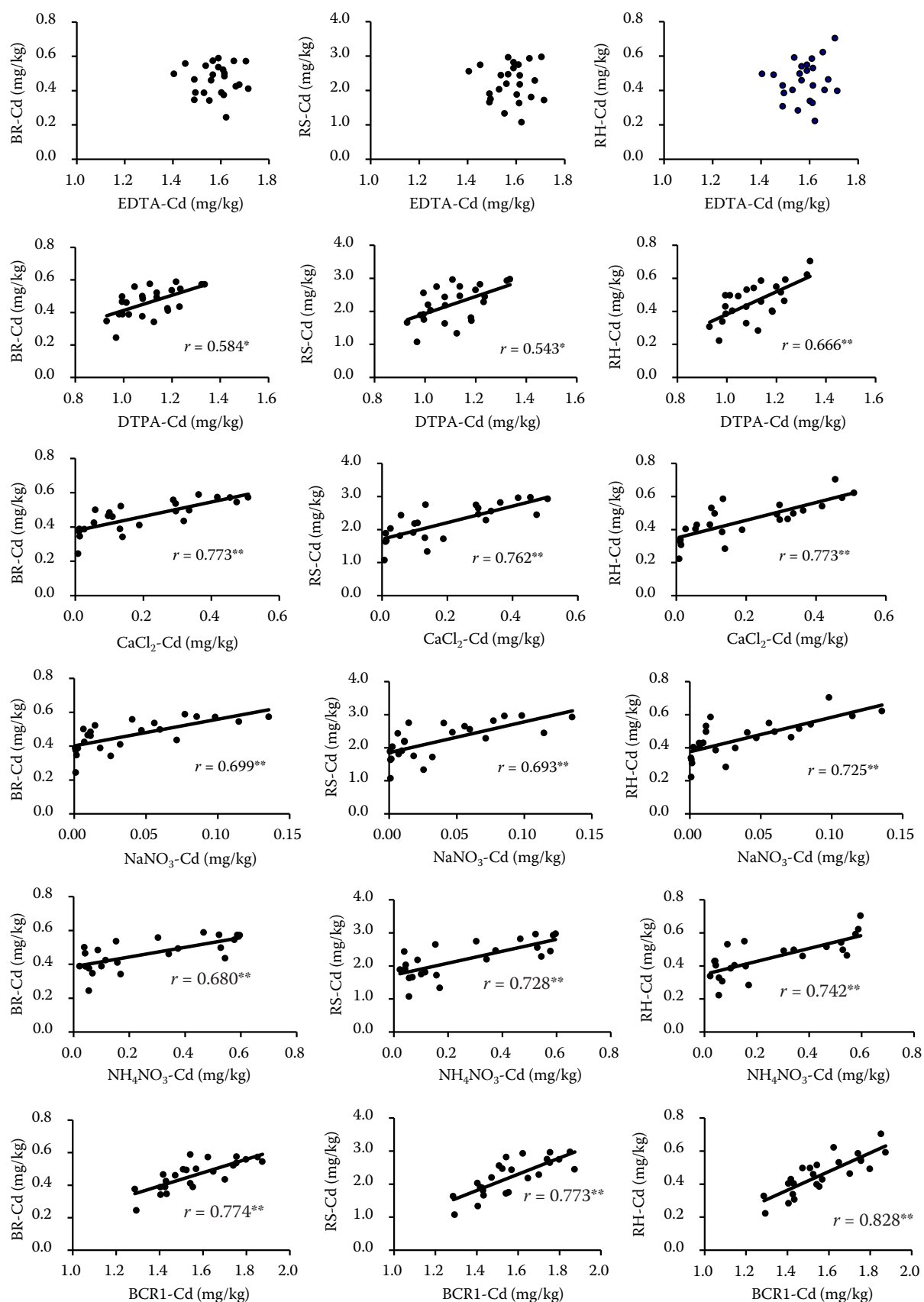
Figure 1. Relationships between extractable Cd in soils-EDTA extractable Cd (EDTA-Cd), DTPA extractable Cd (DTPA-Cd), CaCl$_2$ extractable Cd (CaCl$_2$-Cd), NaNO$_3$ extractable Cd (NaNO$_3$-Cd), NH$_4$NO$_3$ extractable Cd (NH$_4$NO$_3$-Cd), BCR1 extractable Cd (BCR1-Cd) and Cd concentrations in rice; Cd concentration in brown rice (BR-Cd), Cd concentration in rice straw (RS-Cd), and Cd concentration in rice hull (RH-Cd)

*significant correlation at $P < 0.01$, **significant correlation at $P < 0.001$

The three unbuffered salt solutions are used to estimate soil contamination and trace metal phytoavailability and are standardized or are undergoing standardisation worldwide: $CaCl_2$ in the Netherlands, $NaNO_3$ in Switzerland, and NH_4NO_3 in Germany (Pueyo et al. 2004). Meers et al. (2007) reported that $CaCl_2$-, $NaNO_3$-, and NH_4NO_3-Cd significantly correlated with Cd accumulated in leaves and total shoots of *Phaseolus vulgaris.* Li et al. (2008) found that the $CaCl_2$ extraction procedure could be used to assess the immobilization effect of amendments on Cd and Cu in contaminated acidic paddy soil. Brown et al. (2005) reported that the NH_4NO_3 extraction procedure could well predict the changes of phytoavailability for Cd and Zn caused by amendments with lime, phosphorus, red mud, cyclonic ashes, biosolids, and water-treatment residuals. Krebs et al. (1998) used the $NaNO_3$ extraction procedure to evaluate the phytoavailability of Cd to pea in soil contaminated by sludge and amended with or without lime.

The two chelating agents (DTPA and EDTA) were widely used to assess the phytoavailability of many heavy metals in soils with or without the application of amendments. For example, Lee et al. (2004) found that DTPA-Cd well predicted the accumulation of Cd by wheat in acidic soil after amendments were applied. The suitability of EDTA-extractable Cd was also observed in the evaluation of the phytoavailability of Cd in contaminated soils with the application of amendments (Chen et al. 2000). Apparently conflicting results were been reported in previous studies. Feng et al. (2005a,b) reported poor correlations between plant Cd concentrations and DTPA/EDTA-Cd from acidic soils. Similar results were also reported by Menzies et al. (2007). As strong extractants, EDTA and DTPA might extract more metals than the plant is able to take up and overestimate phytoavailability (McLaughlin et al. 2000, Menzies et al. 2007). Compared with the unbuffered salt solutions, DTPA and EDTA solutions are poor representatives of the true soil pH (Wenzel and Blum 1997, Menzies et al. 2007). Furthermore, Menzies et al. (2007) noted that these differences could result in changes to the characteristics and trace metal speciation of soils. The weaker correlations between the concentrations of Cd in rice tissues and the DTPA/EDTA-Cd in soils may be attributed, at least partially, to the difference between solution pH and soil pH.

In conclusion, the BCR1, NH_4NO_3, $CaCl_2$, and $NaNO_3$ extraction procedures are recommended for evaluating the immobilization of Cd in contaminated acidic paddy soil.

REFERENCES

Anju M., Banerjee D.K. (2011): Associations of cadmium, zinc, and lead in soils from a lead and zinc mining area as studied by single and sequential extractions. Environmental Monitoring and Assessment, *176*: 67–85.

Brown S., Christensen B., Lombi E., McLaughlin M., McGrath S., Colpaert J., Vangronsveld J. (2005): An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn in situ. Environmental Pollution, *138*: 34–45.

Chen Z.S., Lee G.J., Liu J.C. (2000): The effects of chemical remediation treatments on the extractability and speciation of cadmium and lead in contaminated soils. Chemosphere, *41*: 235–242.

Feng M.H., Shan X.Q., Zhang S., Wen B. (2005a): A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution, *137*: 231–240.

Feng M.H., Shan X.Q., Zhang S.Z., Wen B. (2005b): Comparison of a rhizosphere-based method with other one-step extraction methods for assessing the bioavailability of soil metals to wheat. Chemosphere, *59*: 939–949.

Gupta A.K., Sinha S. (2007): Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown on tannery waste contaminated soil. Journal of Hazardous Materials, *149*: 144–150.

Gupta S.K., Aten C. (1993): Comparison and evaluation of extraction media and their suitability in a simple model to predict the biological relevance of heavy metal concentrations in contaminated soils. International Journal of Environmental Analytical Chemistry, *51*: 25–46.

Krebs R., Gupta S.K., Furrer G., Schulin R. (1998): Solubility and plant uptake of metals with and without liming of sludge-amended soils. Journal of Environmental Quality, *27*: 18–23.

Lee T., Lai H., Chen Z. (2004): Effect of chemical amendments on the concentration of cadmium and lead in long-term contaminated soils. Chemosphere, *57*: 1459–1471.

Li P., Wang X., Zhang T., Zhou D., He Y. (2008): Effects of several amendments on rice growth and uptake of copper and cadmium from a contaminated soil. Journal of Environmental Sciences, *20*: 449–455.

Lindsay W.L., Norvell W.A. (1978): Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal, *42*: 421–428.

McLaughlin M.J., Hamon R.E., McLaren R.G., Speir T.W., Rogers S.L. (2000): A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. Australian Journal of Soil Research, *38*: 1037–1086.

Meers E., Samson R., Tack F.M.G., Ruttens A., Vandegehuchte M., Vangronsveld J., Verloo M.G. (2007): Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by *Phaseolus vulgaris*. Environmental and Experimental Botany, *60*: 385–396.

Menzies N.W., Donn M.J., Kopittke P.M. (2007): Evaluation of extractants for estimation of the phytoavailable trace metals in soils. Environmental Pollution, *145*: 121–130.

Novozamsky I., Lexmond T.H.M., Houba V.J.G. (1993): A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. International Journal of Environmental Analytical Chemistry, *51*: 47–58.

O'Connor G.A. (1988): Use and misuse of the DTPA soil test. Journal of Environmental Quality, *17*: 715–718.

Pueyo M., López-Sánchez J.F., Rauret G. (2004): Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb, and Zn extractability in contaminated soils. Analytica Chimica Acta, *504*: 217–226.

Quevauviller P., Rauret R., Rubio G., Lopez-Sanchez J.F., Ure A.M., Bacon H., Muntau J.R. (1997): Certified reference materials for the quality control of EDTA- and acetic acid-extractable contents of trace elements in sewage sludge amended soils (CRMs 483 and 484). Fresenius' Journal of Analytical Chemistry, *357*: 611–618.

Ure A.M., Quevauviller P., Muntau H., Griepink B. (1993): Speciation of heavy metals in soils and sediments: an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. International Journal of Environmental Analytical Chemistry, *51*: 135–151.

Wenzel W.W., Blum W.E.H. (1997): Effect of sampling, sample preparation and extraction techniques on mobile metal fractions in soils. In: Adriano D.C., Chen Z.S., Iskandar I.S. (eds.): Biogeochemistry of Trace Metals. Science Reviews, Northwood, 121–171.

Zhu Q.H., Huang D.Y., Zhu G.X., Ge T.D., Liu G.S., Zhu H.H., Liu S.L., Zhang X.N. (2010): Sepiolite is recommended for the remediation of Cd-contaminated paddy soil. Acta Agriculturae Scandinavica Section B – Soil and Plant Science, *60*: 110–116.

Corresponding authors:

Dr. Qi-Hong Zhu, Chinese Academy of Sciences, Institute of Subtropical Agriculture, Key Laboratory of Agro-ecological Processes in Subtropical Region, Changsha, Hunan Province, 410125, P.R. China
fax: + 86 731 8461 2685, e-mail: qhzhu@isa.ac.cn
Prof. Dao-You Huang, e-mail: dyhuang@isa.ac.cn