Is an adjusted rhizosphere-based method valid for field assessment of metal phytoavailability? Application to non-contaminated soils

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An adjusted-RHIZO method was the most promising approach for predicting metal phytoavailability to wheat under field conditions.

Abstract

Previously recommended rhizosphere-based method (RHIZO) applied to moist rhizosphere soils was integrated with moist bulk soils, and termed adjusted-RHIZO method (A-RHIZO). The A-RHIZO and RHIZO methods were systematically compared with EDTA, DTPA, CaCl₂ and the first step of the Community Bureau of Reference (BCR1) methods for assessing metal phytoavailability under field conditions. Results suggested that moist bulk soils are equally suited or even better than rhizosphere soils to estimate metal phytoavailability. The A-RHIZO method was preferred to other methods for predicting the phytoavailability of Ni, Cu, Zn, Cd, Pb and Mn to wheat roots with correlation coefficients of 0.730 ($P < 0.001$), 0.854 ($P < 0.001$), 0.887 ($P < 0.001$), 0.739 ($P < 0.001$), 0.725 ($P < 0.001$) and 0.469 ($P < 0.05$), respectively. When including soil properties, other extraction methods were also able to predict phytoavailability reasonably well for some metals. Soil pH, organic matter and Fe–Mn oxide contents, and cation-exchange capacity mostly influenced the extraction and phytoavailability of metals.

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1. Introduction

Over the past decades much work has been directed towards the estimation of metal phytoavailability in soils. Researchers have made many attempts to predict metal phytoavailability by correlating data on metal accumulation in plants with the metal contents found in various soil pools, i.e., soluble, salt exchangeable, oxide-bound or organic-bound, as determined by different sequential extractions or single extraction procedures (Shtangeeva, 2004). Single extraction methods using selective chemical extractants, such as strong chelating agents or mild neutral salts, are commonly used (Beckett, 1989). Among these extractants, EDTA (0.1–1 M) and DTPA (0.005 M), either as

sodium or ammonium salts, have been proposed because of their ability to form very stable, water soluble and well-defined complexes with a wide range of metal cations (Haq et al., 1980; Chaignon et al., 2003). A 0.01 M CaCl₂ extractant was also proposed because of the similarity between this extractant and the concentration, composition and pH of soil background electrolytes (Houba et al., 1996). Mench et al. (1997) reported that both EDTA and CaCl₂ extractable Cd was positively correlated with Cd contents in wheat roots and seeds. The Community Bureau of Reference of the European Community (now the Measurements and Testing Programme of the European Community) proposed a three-stage sequential extraction procedure (Quevauviller et al., 1993) to establish a common sequential extraction scheme. Unfortunately, conclusions obtained by many studies were inconsistent and made comparisons difficult (Qian et al., 1996; Sauvé et al., 1996; Shan et al., 2003).

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Ideally, a good method for predicting metal phytoavailability should mimic the root–soil interactions as closely as possible. Low-molecular-weight organic acids (LMWOAs) are important root exudates and microbial metabolites in soils and can influence metal solubility in soils and therefore uptake by plants due to their chelating and/or complexing properties (Shtangeeva, 2004). LMWOAs also directly affect acidification, chelation and oxidation–reduction reactions in the rhizosphere and can exert indirect effects on microbial activity, rhizosphere physicochemical characteristics and root growth dynamics. For example, Jones et al. (1996) indicated that organic acids mediated iron dissolution in the rhizosphere. Metals can be easily released from soils in the presence of LMWOAs (Qin et al., 2004). Correlation analysis indicated that LMWOAs extractable metals had high correlations with the phytoavailability of Al and Fe (Chen et al., 2006). Since chemical reactions may take place during the air-drying of wet soils, it is recommended that moist soils be used when estimating metal phytoavailability (Wang et al., 2001). Considering the influence of microorganisms and LMWOAs on the rhizosphere soil, Shan et al. (2003) recommended the use of moist rhizosphere soil in combination with a mixture of 0.01 M LMWOAs as an extractant. This technique was called a rhizosphere-based method (RHIZO).

In laboratory based experiments, Feng et al. (2005a,b) found that the RHIZO method was suitable for both acidic, neutral and near alkaline soils and claimed that the RHIZO method was therefore a more generally applicable method than those traditionally used to predict metal phytoavailability to wheat and barley. However, the question whether the RHIZO method would be successful under field conditions needed to be answered. The RHIZO method emphasized the use of moist rhizosphere soils. However, the collection of rhizosphere soils is labor-intensive and even unrealistic sometimes. Further study is needed to test whether field moist bulk soils can be used instead of rhizosphere soils for the prediction of metal phytoavailability and therefore make the RHIZO method more generally practicable. Thus an adjusted-RHIZO method (A-RHIZO) was the subject of this study, in which the original RHIZO method was applied to moist bulk soils in combination with a mixture of 0.01 M LMWOAs as an extractant.

The aim of this work was: (i) to compare the difference between rhizosphere and bulk soils, and to test whether moist bulk soils are equally suited than rhizosphere soils for assessing metal phytoavailability; (ii) to identify which extraction method was the most promising one for assessing the phytoavailability of soil heavy metals to wheat in field collected samples by comparing the A-RHIZO and the RHIZO methods with EDTA, DTPA, CaCl_2 and BCR1 extractions; (iii) to verify which soil properties affected metal extractability by various extractants.

2. Materials and methods

2.1. Soil and wheat samples

Twenty cultivated surface soils and the corresponding seedling wheat samples from the same field were collected from 15 different Chinese provinces

during February 2005. All the sampling sites were agriculture lands without any significant contamination. According to Hammer and Keller's (2002) method, rhizosphere soil was sampled from the surroundings of the roots (soil attached to the roots after shaking and separated from the roots by hand). Bulk soils were sampled from a location about 15 cm away from the wheat roots. Moist rhizosphere and bulk soils were separately preserved in polyethylene bags. Wheat samples were also preserved in polyethylene bags during transport to the laboratory. Wheat incorporating both shoots and roots were first rinsed thoroughly with tap water and subsequently with de-ionized water to remove any traces of residual soil. Roots were subsequently cut off and re-rinsed with ultrasonic-assisted washing with water for 20 min to remove the fine adhering soil particles. After washing, wheat shoots and roots were dried at 60 °C for 48 h. Sub-samples of the field rhizosphere and bulk soils were air-dried and small pieces of broken roots and any other extraneous materials carefully removed, prior to sieving <1 mm using a nylon fiber sieve and stored for subsequent analysis of soil characteristics. The remaining portion of field rhizosphere and bulk soils was refrigerated at –18 °C prior to measurement of soil humidity and application of the various extraction protocols. Soil organic matter (SOM) was determined using the Walkley–Black procedure (Nelson and Sommers, 1982). Soil pH was measured at soil to 0.01 M CaCl_2 solution ratio of 1:5 (w/v). Cation-exchange capacity (CEC) was determined by standard methods (Rhoades, 1982). CEC of the soils with pH < 7 was determined by the BaCl_2 method while the CEC of soils with pH > 7 was determined by the CH_3COONa method. Amorphous Fe (AmorFe) was extracted by shaking 1 g of soil in 30 ml of acidified ammonium oxalate buffer (0.175 M $(\text{NH}_4)_2\text{C}_2\text{H}_4$ + 0.1 M $\text{H}_2\text{C}_2\text{O}_4$, pH 3.0) for 2 h (Loeppert and Inskeep, 1996). Crystalline Fe (CryFe) and Mn (CryMn) were determined by the oxalate–ascorbic acid extraction method of Shuman (Shuman, 1982). All determinations were carried out in triplicate.

2.2. Determination of heavy metals in soils and plants

Total heavy metal concentrations in soil, wheat shoots and roots were determined using inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Electron Company, USA) after digestion of samples with 3 ml of HNO_3 – HClO_4 –HF (1:1:1, v/v) in PTFE vessel under high-pressure conditions (Zhang and Shan, 1997). Heavy metal concentrations in soil solution obtained from one-step extraction methods were also determined by ICP-MS. For quality control of low levels of metals in wheat roots and shoots a certified reference material (GBW 08505 tea leaves) was used throughout this work. The data determined by this method were within the certified range of each metal (data not shown), indicating that the plant metal concentrations determined in this work were reliable.

2.3. Extraction procedure

Field moist soils (both fresh rhizosphere and bulk soils) were subjected to all of the extractions. The reason for the use of moist fresh soils in all the extractions is not only to make a comparison between various methods on the same moist soil basis, but also to avoid the possible changes of soil properties during drying. All extraction procedures are described briefly below:

A-RHIZO and RHIZO: Bulk or rhizosphere soil (2 g) was extracted with an aqueous solution of LMWOAs (20 ml) in a 50 ml plastic centrifuge tube with continual shaking for 16 h. The total concentration of acetic, lactic, citric, malic, and formic acids was 0.01 M, and their molar ratio was 4:2:1:1:1 (c/c) (Feng et al., 2005a);

EDTA: Soil (2 g) was extracted with 0.05 M EDTA (20 ml) adjusted by ammonia solution to pH 7.0 in a 50 ml plastic centrifuge tube with shaking for 1 h (Wear and Evans, 1968);

DTPA: Soil (10 g) was extracted with 0.005 M DTPA + 0.01 M CaCl_2 + 0.01 M TEA adjusted to pH 7.3 (20 ml) with shaking for 2 h (Lindsay and Norvell, 1978);

CaCl_2 : Soil (2 g) was extracted with 0.01 M CaCl_2 (20 ml) in a 50 ml plastic centrifuge tube with shaking for 3 h (Novozamsky et al., 1993);

BCR1: Soil (1 g) was extracted with 0.11 M acetic acid (20 ml) in a 50 ml plastic centrifuge tube with shaking for 16 h (Ure et al., 1993).

For all extractions, after shaking, the suspensions were centrifuged at 7000 g for 20 min and the supernatant filtered through a disposable 0.45 µm cellulose acetate membrane filter prior to metal determination. All extractions, including blanks, were carried out in triplicate, and the amount of metals extracted from moist soils was converted to dry soil weight according to the previously determined soil humidity.

2.4. Reagents and glassware

HClO₄, HNO₃ and HF were guaranteed reagent grade, and all other reagents were of analytical reagent grade (purity > 99%). All glassware and plastic containers were soaked in 5% (v/v) HNO₃ for at least 12 h and thoroughly rinsed initially with tap water and subsequently with de-ionized water before use.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., USA). Pearson correlation coefficients were determined by correlating metal contents in roots and shoots with extractable heavy metal concentrations from soils for various extraction methods. Two-factor ANOVA and a multiple comparison with Bonferroni correction was performed between extractable metal concentrations from rhizosphere and bulk soils, and also applied to identify the differences of metal extractability among various methods. Forward stepwise multiple regressions were used to derive best-fit models of metal concentrations in plants utilizing basic soil properties and soil extractable heavy metal concentrations as input parameters and were also used to identify which soil properties were primarily correlated with soil extractable heavy metals.

3. Results

3.1. Total metals in soil and wheat

The ranges and means of total metal contents in soils, wheat roots and shoots are given in Table 1. There is no significant difference in the total metal contents between the bulk and rhizosphere soils. Generally, the concentration of metals in wheat roots was significantly higher than that in wheat shoots, and the concentrations of metals in wheat roots and shoots were not significantly correlated with total metal concentrations in either the bulk or rhizosphere soils (results not shown).

3.2. Soil properties of bulk and rhizosphere soils

The relationships between CEC, pH, SOM, CryFe, AmorFe, and CryMn contents in the bulk and in the rhizosphere soils are shown in Fig. 1. In most cases, pH, CEC and SOM contents of the rhizosphere soils on average were almost equal to that of

the bulk soils, although pH values were sometimes considerably higher or lower in the rhizosphere soils compared to bulk soils. However, CryFe, AmorFe and CryMn contents of the rhizosphere soils were consistently higher than those of the bulk soils.

Furthermore, some intercorrelation was observed among soil properties. For bulk soils, CryFe ($r = 0.810$, $P < 0.001$), CryMn contents ($r = 0.535$, $P < 0.05$), -pH ($r = 0.556$, $P < 0.05$) and CEC ($r = 0.469$, $P < 0.05$) were correlated to soil AmorFe content, respectively. For the rhizosphere soils, the CryFe content, -pH and CEC were also correlated to soil AmorFe content with correlation coefficients of 0.778 ($P < 0.001$), 0.623 ($P < 0.01$), 0.535 ($P < 0.05$), respectively.

3.3. Extractable metals from soils by various extraction methods

A two-factor ANOVA was used to test the effects of extractants (LMWOAs, EDTA, DTPA, CaCl₂ or BCR1) and soil fractions (bulk and rhizosphere soil), or the interaction effect between them on the metal extractability by various extraction methods (Table 2). Extractants had significant effects on extractable metals. Significant interaction of extractant × soil fractions was obtained for Ni and Zn. Fig. 2 shows the means and standard deviations of extractable metals from 20 soils, where significant differences ($P < 0.05$) in extractable metal concentrations between soil fractions (the bulk and rhizosphere soils) were indicated with an asterisk and the extractability differences ($P < 0.05$) among various methods were lettered. CaCl₂, RHIZO, BCR1 and EDTA methods extracted more Ni from the rhizosphere soils than from the bulk soils. EDTA method also extracted more Zn from rhizosphere soils, while DTPA extracted less Zn from rhizosphere soil than those from bulk soils. The extractability orders of various methods were not consistent and dependent on the metals of interest.

3.4. Simple linear correlation analysis between extractable metals and phytoavailability

Simple linear correlation analysis was performed between metal concentrations in wheat roots and shoots, and metals extracted by different extraction methods from bulk and rhizosphere soils. The results in Table 3 indicate that quite similar correlation coefficients (r) of Cu, Zn, Cd, and Mn were

Table 1

Ranges and means of total metal contents in the bulk soils (rhizosphere soils are given in parentheses), and wheat roots and shoots

Element	Content in soils (mg/kg dry soil)			Content in roots (µg/kg)			Content in shoots (µg/kg)		
	Range	Mean	CV (%)	Range	Mean	CV (%)	Range	Mean	CV (%)
Ni	1.1–72.5 (5.1–80.0)	37.2 (40.9)	57.7 (57.1)	1.3–5.7	2.7	48.2	0.6–3.6	1.5	59.9
Cu	5.3–53.0 (7.1–58.0)	23.3 (22.6)	48.1 (53.1)	5.3–18.4	9.7	37.8	5.5–14.7	8.2	30.1
Zn	31.2–286.1 (30.7–309.0)	169.7 (190.9)	44.3 (36.5)	23.8–162.3	57.9	64.2	30.8–106.2	53.4	37.3
Cd	0.08–0.8 (0.02–0.8)	0.3 (0.3)	74.4 (73.3)	0.3–1.3	0.7	45.0	0.1–0.7	0.3	59.0
Pb	2.2–47.0 (1.0–55.0)	16.9 (15.4)	73.9 (86.9)	1.3–8.5	4.1	52.6	1.2–5.2	3.2	38.9
Mn	104.3–629.6 (87.1–650.0)	407.5 (379.7)	59.9 (42.2)	28.2–159.3	85.9	52.2	26.4–167.0	103.2	52.5

All determinations were conducted in triplicate.

Fig. 1. Relationships between the selected soil properties of CEC, pH, SOM, CryFe, AmorFe, and CryMn contents of bulk and rhizosphere soils. The dashed is $y = x$ line.

obtained by the A-RHIZO and RHIZO methods, although r values of Ni and Pb were improved by the A-RHIZO and by RHIZO methods, respectively. When it comes to other extraction methods, r values from bulk soils were just observed on average as good as those obtained from rhizosphere soils or even much better for DTPA extraction versus metal concentration in roots. Based on a comparison among correlation coefficients obtained by various methods, it can be seen that both A-RHIZO and RHIZO methods had often relatively higher correlation coefficients than, at least equal to, that obtained by any of the other extraction methods. All the heavy metals extracted by the A-RHIZO method were significantly correlated with metal concentrations in wheat roots with r ranging from 0.45 to 0.89, while only RHIZO extractable Ni from rhizosphere soils showed no significant correlation to root Ni. In contrast,

only 2–3 heavy metals extracted by CaCl_2 , BCR1 and EDTA methods from both bulk and rhizosphere soils were significantly correlated to the corresponding metal concentrations in wheat roots. For DTPA method, five heavy metals (Cu, Zn, Cd, Pb and Mn) extracted from bulk soil were significantly correlated to root metals with r ranging from 0.45 to 0.70, while only Pb extracted from rhizosphere soil was correlated to root Pb contents.

In most cases, for wheat shoots the simple correlation coefficients were often lower than those obtained for wheat roots for both bulk and rhizosphere soils (Table 3). Chaignon et al. (2003) concluded that metals in shoots are not a good indicator of metal phytoavailability. Thus, the following discussion will be limited to the relationship between metals in roots and in bulk soil pools.

3.5. Multiple regression analysis between extractable metals and phytoavailability

Stepwise multiple regression models for metals in roots as a function of extracted metals in soil solution fractions and selected soil properties including pH, CEC, SOM, AmorFe, CryFe and CryMn contents and compound variables, which were obtained by multiplying metal concentrations in soils by various soil properties of bulk soils, are presented in Table 4. For simplicity, the coefficients of all equations were omitted,

Table 2

Significance levels of extractants (LMWOAs, EDTA, DTPA, CaCl_2 and BCR1) and soil fractions (bulk and rhizosphere soils), or their interaction effect on metal extractability

Factors	Significance levels P					
	Ni	Cu	Zn	Cd	Pb	Mn
Extractant	0.000	0.000	0.000	0.000	0.000	0.000
Soil type	0.001	0.509	0.137	0.115	0.377	0.172
Extractant $\times$ soil fractions	0.000	0.969	0.001	0.197	0.206	0.675

Fig. 2. Means and standard deviations of extractable metals from 20 soils, where significant differences ($P < 0.05$) in extractable metal concentrations between soil fractions (bulk and rhizosphere soils) were indicated with an asterisk and the extractability differences among various methods were lettered (the same letters are not significantly different according to multiple comparisons with Bonferoni correction at the level $P < 0.05$).

while the negative sign of the variables was retained. Correlation coefficients (r values) allow a holistic evaluation of the equations for metal phytoavailability to wheat. When soil properties' variables were included the correlation coefficients were often increased. For example, soil CEC significantly enhanced the predictability of A-RHIZO for Pb and Ni. SOM improved the predictability of BCR1 for Cu, while pH and CEC greatly improved the predictability of CaCl_2 method for Zn, Pb and

Ni. The ability of the EDTA method to predict metal availability for Cd, Ni and Mn was significantly improved by including soil CryMn content, CEC and pH, respectively.

A comparison of the multiple regression equations between the A-RHIZO and other extraction methods showed that the multiple regression equations of the A-RHIZO method were the simplest with only one variable for each of the six metals studied and the correlation coefficients (r) for Cu, Zn and Pb

Table 3

Linear correlation coefficients (r) between metal concentrations in wheat roots and shoots and metals extracted from bulk and rhizosphere soils by different extraction methods ($n = 20$)

	Element	A-RHIZO	RHIZO	CaCl_2	BCR1	EDTA	DTPA
Root	Cu	0.83***	0.79***	0.31 (0.05)	0.25 (0.60**)	0.71*** (0.66**)	0.64** (0.41)
	Zn	0.89***	0.91***	0.56* (0.59**)	0.70*** (0.44)	0.71*** (0.63**)	0.62** (0.36)
	Cd	0.74***	0.75***	0.72*** (0.73***)	0.65** (0.60**)	0.56* (0.71***)	0.70*** (0.34)
	Pb	0.61**	0.71***	0.51 (0.16)	0.69*** (0.30)	0.44 (0.27)	0.45* (0.52*)
	Ni	0.53*	0.29	0.18 (0.32)	0.25 (0.06)	0.43 (0.26)	0.41 (0.33)
	Mn	0.45*	0.48*	0.40 (0.22)	0.24 (0.09)	0.25 (0.26)	0.49* (0.29)
Shoot	Cu	0.74***	0.78***	0.26 (0.15)	0.54** (0.49*)	0.44 (0.65**)	0.32 (0.46)
	Zn	0.79***	0.70***	0.78*** (0.68***)	0.72*** (0.55*)	0.53* (0.67**)	0.36 (0.02)
	Cd	0.43	0.76***	0.49* (0.46)	0.76*** (0.19)	0.41 (0.28)	0.40 (0.34)
	Pb	0.43	0.39	0.50* (0.42)	0.42 (0.36)	0.47* (0.48*)	0.60** (0.60**)
	Ni	0.15	−0.04	0.18 (0.40)	−0.01 (0.15)	0.31 (−0.15)	0.43 (0.06)
	Mn	0.31	0.33	0.06 (0.22)	0.26 (0.14)	−0.01 (0.16)	0.41 (0.31)

A-RHIZO, adjusted rhizosphere-based method; RHIZO, rhizosphere-based method; BCR1, the first step of European Community Bureau of Reference method.

***, ** and * indicate significant correlations at the probability level of $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

Values in parenthesis correspond to those of the rhizosphere soils.

Table 4
Stepwise multiple regression models relating metal concentrations in wheat roots to extractable metals by various methods, soil properties and compound variables which were obtained by multiplying extractable metal concentrations in soils by various soil properties of bulk soils

Methods	Regres. steps	Cu variables	Zn variables	Cd variables	Pb variables	Ni variables	Mn variables	r
A-RHIZO	1	Cu × SOM	Zn	Cd	Pb × CEC	Ni × CEC	Mn × pH	0.469*
	2							
BCR1	1	Cu × SOM	Zn	Cd	Pb	CEC	ns	0.688***
	2	Cu × CEC				CryFe		0.780***
CaCl ₂	1	SOM	Zn × pH	Cd × CEC	Pb × pH	CEC	ns	0.688***
	2		Zn × CEC	CEC		Ni × CryFe		0.846***
EDTA	1	Cu	Zn	Cd × CryMn	ns	Ni × CEC	Mn × pH	0.688***
	2		Zn × SOM			CryFe		0.780***
DTPA	1	SOM	Zn	Cd	Pb	CEC	Mn × CEC	0.521*
	2					Ni × CryMn		0.802***

r, The correlation coefficient; A-RHIZO, adjusted rhizosphere-based method; BCR1, the first step of European Community Bureau of Reference method; codes, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; ns, not significant.

were the highest. For BCR1, CaCl₂, EDTA, and DTPA methods soil properties' variables always need to be entered to their multiple regression equations. Although CaCl₂ and DTPA methods had the highest r for Cd and Ni, and for Mn, respectively, more than one variable was needed to be entered to their equations, and no significant improvements were obtained compared to A-RHIZO method.

3.6. Soil properties versus metal extractability

Multiple regression analysis was also used to correlate extractable metals with soil properties (including total soil metal) to determine the influence of soil properties on metal extractability (Table 5). For simplicity, only the variables (inclusive of sign) and the correlation coefficients, r values, are given. Soil properties significantly influenced the extractability of metals from soils. The most important soil properties influencing metal extractability varied with both the extraction method and the metal of interest. The most important soil properties affecting the extractability of metals by the A-RHIZO method were SOM (Cu), pH (Cu, Cd, Pb and Mn), CryFe (Ni) and CryMn (Mn). SOM (Cu, Zn, Cd and Pb), CryMn (Mn) and pH (Mn) had the most prominent effects on the extractability of metals by the EDTA method. SOM (Cu, Zn, Cd and Pb), pH (Cd, Ni and Mn), CEC (Zn) and CryMn (Mn) were the most relevant factors for the DTPA method. Soil pH (Zn, Cd, Ni, Pb and Mn), CryFe (Cd and Ni) and CryMn (Pb) contents influenced the extractability of metals when using the CaCl₂ method. For the BCR1 method, SOM (Cu and Ni), CryFe (Cd) and CryMn (Pb), AmorFe (Pb) and pH (Pb and Mn) were the key parameters controlling the extractability of metals.

4. Discussion

4.1. Bulk soils versus rhizosphere soils

The rhizosphere is commonly defined as the zone where root activity significantly influences soil properties. Rhizosphere soil differs from bulk soil in a range of biochemical, chemical and physical processes that occur as a consequence of root growth, water and nutrient uptake, respiration and rhizodeposition (Hinsinger et al., 2005). Tao et al. (2003) found increases in pH, DOC and microbial activity in the rhizosphere of maize during cultivation. In contrast, Chen et al. (2006) observed that the pH in rhizosphere soils of tea plants was lower than that in bulk soils, while SOM and CEC were higher than those of bulk soils under field conditions. In the present work, no significant differences were observed in pH, CEC and SOM on average between rhizosphere soils and bulk soils, although pH values in some cases were considerably higher or lower in rhizosphere soils compared to bulk soils (Fig. 1). Hinsinger et al. (2003) reported that root induced pH changes in the rhizosphere by release of charges carried by H⁺ or OH⁻ to compensate for an unbalanced cation–anion uptake at the soil–root interface. The effect of rhizosphere on soil pH also seemed to be dependent on the initial bulk soils. Chaignon et al. (2002) reported that alkalisation in an acidic soil and acidification in a calcareous soil really

Table 5
Stepwise multiple regressions between the extractable metals and the properties of bulk soils

Methods	Regres. steps	Cu	Zn	Cd	Pb	Ni	Mn	r
A-RHIZO	1	SOM	ns	-pH	-pH	CryFe	CryMn	0.733**
	2	-pH	0.704**	0.714***	0.575**	0.699***	-pH	0.823***
BCR1	1	SOM	ns	CryFe	AmorFe	SOM	pH	0.779***
	2				-CryMn			
CaCl ₂	1	ns	0.556*	-pH	-pH	-pH	-pH	0.524*
	2			CryFe	-pH	0.739***		
	3			SOM	-CryMn	0.808***		
EDTA	1	SOM	0.666***	0.646**	0.658**	ns	CryMn	0.804***
	2		0.491*	0.689***	SOM	-pH	-pH	0.880***
DTPA	1	SOM	0.642**	0.734***	0.702***	0.511*	CryMn	0.846***
	2		CEC	-pH	0.768***		-pH	0.898***

r, The correlation coefficient; A-RHIZO, adjusted rhizosphere-based method; BCR1, the first step of European Community Bureau of Reference method. ***, ** and * indicate significant correlations at the probability level of $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively. ns, Not significant.

occurred in the rhizosphere of oilseed rape and tomato. Furthermore, fertilization regime (and especially the source of nitrogen used) and liming are well known to play an important role in determining the rhizosphere pH as documented in the early work by Riley and Barber (1971). In addition, CryFe, CryMn and AmorFe of rhizosphere soils were higher than those of bulk soils in most cases, which might suggest that some reactions in the rhizosphere were involved in the weathering of soil minerals. Roots secrete organic acids that are strong enough to dissolve calcium carbonate (Hinsinger et al., 2005). In this field investigation, management practices in the different fields, for example different weed management protocols, may also cause differences between the bulk and rhizosphere soils.

Although some differences in soil properties between rhizosphere and bulk soils exist, they seem to have little effect on the extractable amount of metals (except for Ni and Zn) by various methods from the two soil fractions (rhizosphere and bulk soils) (Fig. 2), which directly results in the approximate correlation coefficients obtained from both soil fractions (Table 3) and indicates that both bulk and rhizosphere soils are equally suitable for assessing metal phytoavailability, especially for A-RHIZO and RHIZO methods. This is in contrast with previous results obtained by Feng et al. (2005b). This may be due to difference in plant species (wheat versus barley) and growth conditions (greenhouse versus field). Another potential reason for the difference between the two studies was the likely planting of different wheat species in the different fields whereas one barley species was cropped in the pot experiments.

4.2. Evaluation of the A-RHIZO method based on a comparison with other extraction techniques

Many studies have shown that chemical extraction procedures can measure the metals in soil pools potentially available for plant uptake and/or accumulation (Kennedy et al., 1997). Single extraction methods were based on the assumption that there is a relationship between the extractable fraction of metals and the phytoavailability of metals to plants, i.e., a good correlation indicated that a certain fraction of the total metal in the soils was available to plants. Simple linear correlation analysis demonstrated that the A-RHIZO method was as good as the RHIZO method, and overall a better way than the other extraction methods for the evaluation of phytoavailability of all the six metals (Ni, Cu, Zn, Cd, Pb and Mn) in soils to wheat roots (Table 3). It should also recognize that depending on the metal other extraction methods were suited for assessing phytoavailability. For example, EDTA and DTPA methods seemed to be good for assessing phytoavailability of Cu, Zn and Cd, and of Cu, Zn, Cd, Pb and Mn, respectively, though the correlation coefficients were consistently lower than those of the A-RHIZO method (Table 3). The EDTA and DTPA extraction methods were originally developed for acidic soils (Hammer and Keller, 2002) and calcareous soils (Lindsay and Norvell, 1978), respectively. This may explain the limitations of the EDTA and DTPA techniques when applied to both acidic and calcareous soils.

Availability of metals to plants is complex and affected by several soil properties including soil pH, SOM, Fe–Mn oxide contents, and CEC (Haq et al., 1980). Thus multiple regression models considering soil characteristics could provide insight into the mechanisms of phytoavailability and improve the predictability of chemical extraction methods (Miner et al., 1997; Brun et al., 2001; Wang et al., 2004). Multiple regression analysis confirmed that the A-RHIZO method was a good choice for predicting metal availability to wheat. The regression equations of the A-RHIZO method were simplest and were dependent primarily on extractable metal concentrations. For the other extraction methods, more heavy metals' phytoavailability could be estimated based on multiple regression analysis than simple linear correlation analysis. For example, the BCR1 method was suitable for predicting phytoavailability of Cu, Zn, Cd and Pb. The CaCl_2 extraction is generally believed to extract only exchangeable metals. As calcium is the dominant cation in soils it is believed that the CaCl_2 extractant would reflect differences in binding strength or solubility between different soils (Novozamsky et al., 1993). The CaCl_2 method seemed good for predicting the phytoavailability of Zn, Cd, Pb and Ni to wheat with the inclusion of soil properties in its multiple regression equations.

4.3. The influence of soil properties on metal extractability

In addition to the chemical properties of the extractant used, soil characteristics can also govern metal extractability. Soil pH, CEC, SOM, iron and manganese oxide contents could, to some extent, influence metal extractability and hence influence metal phytoavailability (Table 5). In general, the most important variables were soil pH for A-RHIZO extractable Cu, Cd, Pb and Mn, SOM for extractable Cu, CryFe and CryMn contents for Ni and Mn, respectively. For strong chelating agents such as EDTA and DTPA, the most important soil property that influenced metal extractability was SOM. This suggested that metals loosely bound to soil organic matter could be extracted and was therefore potentially phytoavailable. This possibility may also be indicated directly by the good correlation between root Cu and SOM (Table 4). Under certain climatic conditions portions of SOM may become oxidized and consequently the associated metals may be released to the soil solution and thus made available to plants (Salomons, 1995). For the CaCl_2 method, soil pH seemed to play an important role in governing metal extractability. This is in agreement with many previous studies (Tipping et al., 2003; Rieuwerts et al., 2006). The observed influence of pH is partly attributed to the increasing displacement of adsorbed metal ions into the extractant solution with decreasing pH. Other processes could also explain the influence of pH, such as metal hydrolysis at higher pH followed by strong, preferential adsorption of the resulting metal hydroxo complexes and by precipitation. In addition, since the Ni extractability of both methods were positively related to CryFe contents in soils, the relatively higher Fe–Mn oxide contents in the rhizosphere soil may partly contribute to the more extracted Ni by RHIZO and CaCl_2 methods

from the rhizosphere than from the bulk soils in the present work.

However, some soil properties were intercorrelated, such relationships observed with metal extractability were artifacts of other associations. Similarly, the relationships between extractability and CEC may be related to CryFe levels. Positive relationships were also observed between metal extractability and the Fe and Mn oxide contents, suggesting that extractable metals might be associated with Fe and Mn oxides.

5. Conclusions

No significant differences were observed between extractable metals (except for Ni and Zn) from moist bulk and rhizosphere soils, which were evidence that moist bulk soil was suitable for assessing heavy metal phytoavailability. The present study demonstrated that the A-RHIZO method is, overall, a better way to estimate phytoavailability of all six metals (Ni, Cu, Zn, Cd, Pb and Mn) in soils to wheat roots under field conditions. From simple linear correlation analysis, A-RHIZO method often has highest correlation coefficients among the various extraction methods. From multiple regression analysis, no soil property parameters were required in most cases to be included into the equations to improve predictability. When including soil properties, other extraction methods seemed also able to predict phytoavailability reasonably well for some metals: DTPA for Cu, Zn, Cd, Pb, Ni and Mn, EDTA for Cu, Zn, Cd, Ni and Mn, BCR1 for Cu, Zn, Cd and Pb, and CaCl_2 for Zn, Cd, Pb and Ni. In addition, among soil properties SOM, pH, Fe–Mn oxide contents and CEC all significantly affected metal extractability, although the significance of the relevant soil properties varied with extraction method and with the metal of interest.

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