Relationship between the extractable metals from soils and metals taken up by maize roots and shoots

Wei-Sheng Wang, Xiao-Quan Shan ^{*}, Bei Wen, Shu-Zhen Zhang

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

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Abstract

Two extraction procedures, i.e. a single extraction procedure using low-molecular-weight-organic-acids (LMWOAs) as extractant and a sequential extraction procedure recommended by the European Community Bureau of Reference (BCR), were performed to extract metal fractions from wet rhizosphere soil. And the extracted soil solutions were further fractionated as colloidal and truly dissolved fractions. Heavy metals in maize roots were experimentally defined as metals adsorbed on cell wall and metals taken up by cross-membrane by washing with CaCl_2 . The correlation coefficients between extractable metals from soil and taken up by maize roots and shoots were compared between two extraction methods, and a good correlation was obtained if LMWOAs were used. In contrast, the correlation coefficients were poor when the BCR method was used.

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1. Introduction

It is common conception nowadays that the total concentrations of metals in soils are not a good indicator of bioavailability, or a good tool for potential risk assessment either, due to the different and complex distribution patterns of metals among various chemical species or solid phases (Chen et al., 1996). In recent years, the experimentally defined chemical fractionation procedures have been applied to identify plant-available forms of trace metals in soils. There were a variety of reports on the single and/or sequential extraction procedures for available metals in the literatures (Novozamsky et al., 1993; Houbat et al., 1996; McGrath, 1996; Kennedy et al., 1997; Maiz et al., 1997; Krishnamurti and Naidu, 2002). Among these methods, the most widely used procedures were those recommended by

Tessier et al. (1979) and European Community Bureau of Reference (BCR), now the Standard Measurements and Testing Programme of the European Community (Ure et al., 1993). These extraction procedures have been used to partition metals into fractions defined as water soluble, exchangeable and carbonate bound (fraction B1), Fe–Mn oxide bound (fraction B2), organic materials and sulfide bound (fraction B3). However, sequential extraction methods were suffered from nonselectivity of extractants and trace element redistribution among phases during the extraction processes (Shan and Chen, 1993; Tu et al., 1994; Howard and VandenBrink, 1999). To date, no methods have been considered as universally applicable for the assessment of metal fractions in soils and plant bioavailability. Most of data derived from bioavailability studies tend to be inconsistent, making interpretations difficult (Davies, 1992; Qian et al., 1996; Sauvé et al., 1996). The reasons for this fact are that metal bioavailability depends on many factors such as plant species and soil properties. Different fractions of soil metals vary considerably in their chemical reactivity and bioavailability (Qian et al., 1996; Li et al., 1998;

^{*} Corresponding author. Tel.: +86-10-6292-3560; fax: +86-10-6292-3563.

E-mail address: xiaoquan@mail.rcees.ac.cn (X.-Q. Shan).

Zhang et al., 2000; Wang et al., 2001). In addition, the present approach for bioavailability prediction does not take into account the real chemical speciation of metals in soils and uptake mechanisms of metals by plants either. The main reasons for this are the inherent difficulties in obtaining the detailed information on the biological active speciation of metals in soils and uptake through biological membrane of plants. Considering this situation one can understand why there are no universally experimental methods available for prediction of bioavailability of metals in soils in the literature (Sauvé et al., 2000). To obtain practical goal, appropriate computational written programs and laboratory or field analytical techniques need to be developed along this direction of establishing the relationship between the chemical metals speciation in soils and uptake routes by plants in the future.

The alternative way to predict the bioavailability of metals in soils is to simulate the real field conditions, under which the plants grow on soils. It seems that the most promising empirical approach nowadays was the effective soil solution concentration (Zhang et al., 2001). It took into account not only the diffusional and convective transport of solutes to plant roots, but also the resupply of metal solutes from solid phase under the deficiency conditions. However, this method could only be used for soils of its moist content at or above 80%. More serious drawback of the method is that the soil solutes were taken from bulk soil surface rather than from rhizosphere soils, indicating the empirical conditions are still far away from the real field conditions.

It is generally recognized that rhizosphere is distinguished from bulk soil by the root influences (Anderson et al., 1993). It is a zone of increased microbial activity and low-molecular-weight-organic-acids (LMWOAs). According to the current knowledge LMWOAs play an important role not only in carbon metabolism but also in root–soil interactions (Jones, 1998; López-Bucio et al., 2000). Unfortunately, no one considers the importance of rhizosphere zone in establishing correlation of metal fractions in soils and plant availability. It must be pointed out that many factors can influence metal fraction and bioavailability such as interactions with physicochemical properties of soils, and genotype differences of plants, etc. Therefore, the use of bulk soils in the soil testing purposes such as bioavailability prediction is problematic. The importance of using rhizosphere wet soil in bioavailability is addressed in our previous reports (Wang et al., 2001).

Another important reason related with the uncertainty of predicting bioavailability may be the sampling strategies. Sampling of plants may introduce the highest errors in the final results (Markert, 1993; Kovacheva et al., 2000). Unfortunately, a difficult matter is the quantitative removal of fine soil grain from root surface, which may introduce 100–300% error in the final results

(Markert, 1996; Kovacheva et al., 2000). The effect of washing process on bioavailability needs to be examined.

In time-dependent kinetic studies of root metal absorption in plants, an initial rapid accumulation was observed, followed by a slower linear phase of accumulation (Maria and Cogliatti, 1988; Zhang and Taylor, 1989; Lasat et al., 1996). It was suggested that the initial phase represented rapid binding to the cell walls and that the subsequent slower phase was due to transport across the plasma membrane into the cytoplasm. So separation of the root metals into these two compartments for the test of bioavailability may be worthwhile.

Of course, the prerequisite for promising bioavailability approach is to simulate the real-world field conditions, under which plants grow on soils. In our previous studies the use of rhizosphere wet soils and LMWOAs as extractant for available labile soil solution fractions were strongly recommended (Zhang et al., 2000; Shan et al., 2003). The aims of the present study are to examine: (1) Does the washing of plant roots affect the bioavailability? (2) Does the cell wall adsorption or cross-membrane uptake of metals in plant roots determine the bioavailability? (3) Is the experimentally defined total colloidal soil fraction or truly dissolved fraction more available by plants?

2. Materials and methods

2.1. Soils

Soil samples were collected from 15 rural areas around China, represented typical Chinese soils with different physical and chemical properties. All soils were taken from the surface layer (0–20 cm) of cultivated soils. The air-dried soils were fertilized to 0.4 g N and P/kg soil with solution of KH_2PO_4 and $(\text{NH}_4)_2\text{CO}_3$. After the soil and solution were mixed thoroughly, the soils were air-dried again, ground and sieved to pass through a 1 mm plastic mesh for further use. Precautions were taken to avoid contamination during sampling, drying, grinding and storage. Soil properties of pH, organic matter (OM) and cation exchange capacity (CEC) were determined using standard methods (Lao, 1988). These soil properties are given in Table 1.

2.2. Greenhouse experiment

A homemade rhizobox (Wang et al., 2001) was used to plant maize. The dimension of the rhizobox was $120 \times 100 \times 150$ (mm, length $\times$ width $\times$ height). The rhizobox was divided into three sections: a central zone or rhizosphere zone (20 mm in width), which was surrounded by nylon cloth (300 mesh), and left and right nonrhizosphere zones (50 mm in width). To each rhizobox was

Table 1
Selected properties of soils

Sample	Site	pH	CEC (cmol kg ⁻¹)	OM (%)
1	Jiamusi	5.32	27.03	4.30
2	Yuxi	6.05	25.78	1.37
3	Kunming	4.71	26.74	4.22
4	Shandjong	5.76	30.20	3.62
5	Jiangxi	5.49	14.24	1.53
6	Shanghai	7.62	36.65	3.02
7	Hangzhu	6.90	19.76	3.28
8	Hefei	5.58	24.95	2.09
9	Wuhan	6.65	53.98	2.37
10	Changsha	5.20	43.11	2.28
11	Dongguan	7.13	15.92	1.37
12	Beijing	7.83	14.1	2.99
13	Ankang	7.30	14.4	2.10
14	Chaozhou	5.69	14.1	3.14
15	Xiangli	4.57	12.9	2.46

added 1.5 kg of the treated soil. The maize used for washing was planted in plastic pots (20 × 18 cm, inner diameter × height). The soil was collected from Beijing.

Maize seeds (*Zea Mays*) were thoroughly rinsed with water, and germinated on a filter paper. After the maize seeds were germinated for 36 h at 20 °C in the dark, uniformly germinated seeds with radical emerged were sown in the soil. The relative humidity of soils was kept around 15% by adding deionized water daily. The maize was harvested 8 weeks after planting. The rhizosphere and nonrhizosphere soils were collected and kept in refrigerator (−18 °C) for further use in the remainder of the study.

2.3. Washing procedure of maize roots

The living maize roots were first washed thoroughly with tap water to remove soil adhered to roots. Then, the roots were washed with ultrasound-assisted bath at 35 W, 25 kHz for various periods. After ultrasound-assisted washing the roots were washed with different concentrations of CaCl₂ solution. Among the reagents used for root washing, CaCl₂ was often used (Lasat et al., 1996), different concentrations of CaCl₂ solution in 5.0 mmol l⁻¹ Mes–Tris solution, pH 6.0, for different washing periods at 0 °C in ice bath were examined. Afterwards, the roots were washed three times with deionized water to remove Ca²⁺. Finally, the roots were then cut down from the shoots and dried to constant weight at 65 °C for 36 h for the determination of heavy metals. The maize shoots were thoroughly washed with tap water and then washed with deionized water. Then, the shoots were dried to constant weight in oven at 65 °C for 36 h for further use.

2.4. Fractionation of metals in soil

Two extraction procedures were compared. The first two steps of BCR method and a single extraction procedure were adopted in this study. Among the reported LMWOAs, acetic acid, formic acid, citric acid and malic acids were frequently found to be the abundant organic acids (Jones, 1998; Li et al., 2003). The detailed extraction conditions are listed in Table 2. After extraction, the extractant was centrifuged at 4000 g for 30 min. Then, the supernatant was filtrated through 0.45 μm cellulose acetate membrane, which was referred to total colloidal fraction. For the BCR procedure, heavy metals in the filtrate were directly determined. For the LMWOAs extraction procedure, the filtrate was successively ultrafiltered through molecular weight cutoff of 1 kD membrane. The fraction obtained by ultrafiltrating through 1 kD membrane was referred to the truly dissolved fraction. The concentrations of heavy metals in each fraction were determined. All samples were performed duplicated except four repeated experiments were performed for washing procedure.

2.5. Digestion of plant tissues

The maize root and leaf samples were digested with 2 ml HNO₃–HClO₄ (1:1, v/v) under high pressure condition, and the detailed experimental conditions were given previous (Zhang and Shan, 1997).

2.6. Determination of heavy metals

All reagents used were of analytical grade or better. Stock solutions of each metal for sample analysis, 1000 mg l⁻¹, were obtained from Research Center for Chinese

Table 2
The extraction procedures adopted in this experiment

Procedure	Extractant	Volume (ml)	Extraction condition
First step of BCR (B1 fraction)	0.11 mol l ⁻¹ CH ₃ COOH	20	0.60 g wet soil, shaking 16 h, at room temperature
Second step of BCR (B2 fraction)	0.1 mol l ⁻¹ NH ₂ OH · HCl ^a	20	0.60 g wet soil, shaking 16 h, at room temperature
Single extraction	10 mmol l ⁻¹ mixed organic acid ^b	30	6.60 g wet soil, shaking 12 h, at room temperature

^a pH was adjusted to 2.0 by 0.1 mol l⁻¹ HNO₃.

^b Acetic acid:formic acid:citric acid:malic acid = 2:2:1:1 (molar ratio), its pH values were adjusted with dilute HNO₃ or NaOH to the soil pH.

Standard Materials. High purity of water (18 MΩcm) was prepared by double distillation of deionized water in a fused quartz still (Westdeutsche Quarzschmelze, Gesthacht Germany). Nitric acid was purified by sub-boiling distillation in quartz still using ultrapure grade HNO₃ as feed. Prior to use, all beakers, calibrated flasks and other glassware cleaned sequentially with tap water, neutral detergent, tap water, then in nitric acid (1 + 1) for 48 h and cleaned with double distilled deionized water before use.

Total concentrations of heavy metals Cr, Co, Mo, Ni, Zn, Cu, Cd and Pb in various soil colloid-size fractions and maize samples were determined by inductively coupled plasma mass spectrometry (VG PlasmaQuard 3, Fisons Instruments, UK). The operating conditions for the determinations were optimized. ¹¹⁵In at 10 ng ml⁻¹ in 2% nitric acid was used as an internal standard to compensate for matrix suppression and signal drifting during analysis.

3. Results and discussion

3.1. Ultrasound-assisted washing of maize roots

After the maize roots were thoroughly washed with tap water followed by deionized water for several times, the maize roots were put into beakers containing deionized water for ultrasound-assisted washing under the conditions described in the experimental section. The effect of washing time on the removal of fine soil grains from the roots is schematically shown in Fig. 1, where Zn, Cu and Pb were selected as typical metals. The results showed that the concentrations of heavy metals in roots remained almost unchanged over the washing periods of 0–60 min. Based on this finding one may conclude that the washing by tap water followed by deionized water was effective to remove the soil grains adhered to maize roots, and the ultrasound-assisted washing could hardly improve washing further. So in the

Fig. 1. Relationship between the heavy metals in maize roots and ultrasound-assisted washing. Results are expressed as the mean ± S.D. (*n* = 4).

remainder of the experiments, no ultrasound-assisted washing was adopted.

3.2. Optimization of CaCl₂ washing to distinguish cell wall adsorption from cross-membrane uptake

The effect of CaCl₂ washing with different concentrations of CaCl₂ in 5.0 mmol l⁻¹ Mes–Tris buffer, pH 6.0 on the concentrations of heavy metals in roots is shown in Fig. 2. In order to see it more clearly only Zn, Cu and Pb were chosen as the representative of heavy metals. As it can be seen quite clearly that the concentration of heavy metals in maize roots decreased rapidly when the concentration of CaCl₂ increased from 0 to 0.5 mmol l⁻¹ and then almost remained unchanged over the concentration range of CaCl₂ from 0.5 to 5.0 mmol l⁻¹. However, Zn, Cu and Ni in maize root gradually decreased again when the concentration of CaCl₂ was further increased from 5.0 to 10 mmol l⁻¹. Based on the above study, the CaCl₂ concentration of 1.0 mmol l⁻¹ was used in the remainder of the present study. Hereafter, the metals washed away with the concentration of 0.5 mmol l⁻¹ CaCl₂ were referred as cell wall adsorption,

Fig. 2. Effect of CaCl_2 concentration on metal desorption from roots. Results are expressed as the mean $\pm$ S.D. ($n = 4$).

and the metals remained after CaCl_2 washing were designated as cross-membrane uptake. Quite similar phenomenon was reported in the literature (Maria and Cogliatti, 1988; Zhang and Taylor, 1989; Lasat et al., 1996). It was suggested that the initial phase represented rapid entry into root-free space and binding to the cell walls and the subsequent slower phase was due to transport across the plasma membrane into the cytoplasm (Maria and Cogliatti, 1988). The metals by cell wall adsorption and cross-membrane uptake were separately correlated with metal fractions in soil in the remainder of the study.

3.3. The optimization of washing time

The maize root was washed with 1.0 mmol l^{-1} CaCl_2 solution in 5.0 mmol l^{-1} Mes-Tris buffer, pH 6.0, for different periods, and the washing time was optimized and the results are shown in Fig. 3, where only Zn, Cu and Pb were shown there. A rapid decrease of metals in roots was observed in the first 6 min followed by an

Fig. 3. Effect of CaCl_2 washing time on metal desorption from roots. Results are expressed as the mean $\pm$ S.D. ($n = 4$).

unchanged profile up to washing time of 60 min. And the rapid decrease profile may correspond to heavy metals adsorbed by cell wall of plant roots, the unchanged profile may mean that heavy metals entered cell membrane and difficult to be washed down. Considering little change in heavy metals in the maize roots over the washing time range of 15–60 min, the washing time of 30 min was adopted.

3.4. Heavy metal concentrations in B1 and B2 fractions

For a comparison, the sequential extraction procedure was also adopted and fraction B1 (water soluble, exchangeable and carbonate bound) and fraction B2 (Fe–Mn oxides bound) were also determined. The concentrations of different heavy metals in fraction B2 were similar or higher than those in fraction B1 except for the concentration of Cr (data not shown).

3.5. Partitioning of heavy metals among soil colloid-size fractions

Traditionally, filtration is performed with a pore size of $0.45 \mu\text{m}$ membrane. However, this filtrate always includes colloidal fraction and truly dissolved metals. In order to determine the concentrations of truly dissolved metals, a home-made ultrafiltration device using a pore size of molecular weight cutoff of 1 kD membrane was used to separate truly dissolved fraction from soil colloidal fraction through $0.45 \mu\text{m}$ membrane. Hereafter, the fractions through $0.45 \mu\text{m}$ and 1 kD membrane filtration were referred as soil colloidal fraction and truly dissolved fraction. It was demonstrated that the concentrations of heavy metals in soil colloid fraction were higher than that in truly dissolved fraction when LMWOAs were used (data not shown). If the concentrations of metals in soil colloid fraction were normalized as 100%, the metal concentrations in truly dissolved fraction were about 72.4%, 68.4%, 78.1%, 66.3%, 40.6%, 79.5%, 83.4% and 52.0% for Cr, Co, Ni, Zn, Cu, Mo, Cd and Pb, respectively. Seven metals (Cr, Co, Ni, Zn, Mo, Cd and Pb) were mainly present in the truly dissolved fraction, it may mean that: (1) LMWOAs excreted by plants could transport metals from other forms into truly dissolved form by complexing process, (2) metals in the truly dissolved fraction may be easily available by plants. And for metals in B1 fraction, they were not fractionated into colloidal and truly dissolved fraction. The reasons are that: (1) our early studies showed that heavy metals were mostly existed in the truly dissolved fraction when the first step of BCR procedure was used, (2) the pH of the extractant of the first step of BCR procedure were about 2.0, which is nearly to the lower pH limit of the membrane, and it may introduce large error. An across analysis indicated that the extractable heavy metals in B1 fraction were much higher than that

obtained by LMWOAs, and these may be attributed to higher concentration of acetic acid used in the first step of BCR method.

3.6. Heavy metal concentrations in cell wall and cell membrane of maize roots

If the concentrations of heavy metals in intact roots were normalized as 100%, the concentrations of metals of cross-membrane uptake were about 70.6%, 66.7%, 76.1%, 77.4%, 83.2%, 80.1%, 77.0% and 62.7% for Cr, Co, Ni, Zn, Cu, Mo, Cd and Pb, respectively. And the concentrations of metals adsorption on cell wall were about 29.4%, 33.3%, 23.9%, 22.6%, 16.8%, 19.9%, 33% and 37.3% for Cr, Co, Ni, Zn, Cu, Mo, Cd and Pb, respectively. The results may suggest that heavy metals in roots mainly enter root cell membrane. The similar distribution of heavy metals among cell wall and cross-

membrane indicates that plant genotype dominantly determines the uptake of heavy metals.

3.7. Comparison of bioavailability between heavy metals in cell wall adsorption, cross-membrane uptake and total concentration in intact maize roots

A simple regression was used for the bioavailability correlation analysis of heavy metals in root cell wall, membrane uptake and total concentration in intact maize roots with soil fractions obtained by single extraction with LMWOAs and BCR's method. The correlation coefficients are given in Tables 3 and 4.

As it can be seen clearly from Table 3 that the correlation coefficients between the concentration of heavy metals in cell wall, cross-membrane and intact roots and that in soil colloid fraction and truly dissolved fraction were quite good, and there were insignificant differences

Table 3

Correlation coefficients (R) of heavy metals in soil fractions ($n = 15$) with that in plant roots using 10 mmol l^{-1} LMWOAs as extractant

	Soil colloidal fraction			Truly dissolved fraction		
	CMU	CWA	TC	CMU	CWA	TC
Cr	0.762 ^a	0.841 ^a	0.823 ^a	0.801 ^a	0.804 ^a	0.830 ^a
Co	0.768 ^a	0.857 ^a	0.835 ^a	0.864 ^a	0.829 ^a	0.851 ^a
Ni	0.800 ^a	0.807 ^a	0.867 ^a	0.752 ^a	0.845 ^a	0.825 ^a
Zn	0.782 ^a	0.833 ^a	0.854 ^a	0.784 ^a	0.793 ^a	0.802 ^a
Cu	0.843 ^a	0.811 ^a	0.860 ^a	0.759 ^a	0.846 ^a	0.831 ^a
Mo	0.813 ^a	0.812 ^a	0.850 ^a	0.787 ^a	0.844 ^a	0.919 ^a
Cd	0.904 ^a	0.842 ^a	0.858 ^a	0.812 ^a	0.846 ^a	0.875 ^a
Pb	0.735 ^a	0.767 ^a	0.827 ^a	0.734 ^a	0.772 ^a	0.746 ^a

CMU = cross-membrane uptake.

CWA = cell wall adsorption.

TC = total concentration in intact roots.

^a Significant at 0.05 probability level.

Table 4

Correlation coefficients (R) of heavy metals in soil fractions ($n = 15$) with that in plant roots using BCR extraction procedure

	B1 fraction			B2 fraction		
	CMU	CWA	TC	CMU	CWA	TC
Cr	0.653 ^a	-0.11	0.489 ^b	0.27	0.188	0.612 ^a
Co	0.748 ^a	0.103	0.707 ^a	0.022	-0.317	0.072
Ni	0.477 ^b	-0.344	0.552 ^a	0.547 ^a	-0.381	0.348
Zn	0.635 ^a	0.25	0.553 ^a	0.094	0.136	0.127
Cu	0.683 ^a	0.43	0.694 ^a	0.470 ^b	0.344	0.525 ^b
Mo	0.363	0.037	0.479 ^b	0.014	0.004	0.096
Cd	0.678 ^a	0.495 ^b	0.760 ^a	0.472 ^b	0.393	0.677 ^a
Pb	0.323	0.503 ^b	0.493 ^b	0.371	0.725 ^a	0.695 ^a

CMU = cross-membrane uptake.

CWA = cell wall adsorption.

TC = total concentration in intact roots.

^a Significant at 0.05 probability level.

^b Significant at 0.10 probability level.

Table 5

Correlation coefficients for heavy metals in soil fractions ($n = 15$) and maize leaves

	Soil colloidal fraction ^a	Truly dissolved fraction ^a	B1 fraction	B2 fraction
Cr	0.654 ^b	0.686 ^b	0.677 ^b	0.262
Co	0.597 ^b	0.663 ^b	0.618 ^b	0.040
Ni	0.555 ^b	0.643 ^b	0.469 ^c	0.184
Zn	0.519 ^c	0.595 ^b	0.498 ^c	−0.004
Cu	0.576 ^b	0.394	0.731 ^b	0.466 ^c
Mo	0.657 ^b	0.851 ^b	0.485	0.544 ^b
Cd	0.830 ^b	0.848 ^b	0.737 ^b	0.695 ^b
Pb	0.324	0.570 ^b	0.462	0.309

^a LMWOAs used as extractant.^b Significant at 0.05 probability level.^c Significant at 0.10 probability level.

between them from the point of view of statistics. Considering the small difference between the correlation coefficients of cell wall adsorption and cross-membrane uptake of heavy metals one might conclude that the cell wall adsorption and cross-membrane uptake were simultaneously accounted for the bioavailability. A good relationship between the concentrations of heavy metals in intact roots and that in soil supported this conclusion further. If compare the correlation coefficients between the heavy metals in soil colloid fraction and truly dissolved fraction it may beyond the expectation that no better correlation coefficients were obtained by using truly dissolved fraction. The reasons for this were obscure, however, the differences between the concentrations of heavy metals in soil colloidal fraction and in truly dissolved fraction could not reflected in the corresponding correlation coefficients.

If compare the correlation coefficients between the fractions B1 and B2 with cell wall, cross-membrane uptake and total concentration in intact roots it was easily found fraction B1 is bioavailable, however, the correlation coefficients were poor if compared with that obtained by LMWOAs. In addition, fraction B2 was not bioavailable by plants, which was reported previously (Li et al., 1998; Wang et al., 2001).

Correlation coefficients for heavy metals in soil fractions and in maize leaves are shown in Table 5. The results demonstrated that there was a poor relationship between the metal in fraction B2 and that in plants, and this phenomenon was often reported in the literature.

4. Conclusion

Based on the above study, the following conclusions can be drawn:

1. Wet rhizosphere soil and LMWOAs should be used for bioavailability test purpose, and in that case good

correlation coefficients can be obtained. The extractable metal fractions by using BCR method are a poor indicator of bioavailability.

2. Heavy metals in soil solution can be partitioned between different colloidal fractions, and metals in truly dissolved fraction are easily available by plants. However, for practical purpose total metal concentrations in soil solution can be still used due to only insignificant difference between the correlation coefficients obtained by different colloidal fractions.
3. Heavy metals adsorbed by plant root cell wall and taken up by cross-membrane were rationally responsible for plant availability. Hereafter, total content in plants can be used for bioavailability because the differences between the correlation coefficients were negligible for cell wall adsorption and cross-membrane uptake.
4. The errors caused by fine soil grains adhered to plant roots should be considered and a suitable washing procedure should be taken in the case of necessary.

Generally, a mimic simulation of real-world field conditions, under which plants grow on soils, and the combined effects of soil–root interactions as a whole should be the direction of future study of bioavailability.

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