A METHOD FOR PREDICTING BIOAVAILABILITY OF RARE EARTH ELEMENTS IN SOILS TO MAIZE

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(Received 5 December 2002; Accepted 13 August 2003)

Abstract—A single-extraction procedure using low-molecular-weight organic acids (LMWOAs) as extractant and the first and second steps of a three-step extraction procedure recommended by the European Community Bureau of Reference (BCR; now European Community Standards, Measurement and Testing Programme, Brussels, Belgium) were performed to extract the light rare earth elements (LREEs) La, Ce, Pr, and Nd from wet rhizosphere soil. The extracted soil solutions were successively filtered through membranes with a pore size of less than 0.45 μm and a molecular weight cutoff of less than 1 kDa, which were termed colloidal and truly dissolved fractions, respectively. Apoplastically and symplastically bound LREEs in maize roots were experimentally distinguished by ultrasound-assisted desorption with 1 mM CaCl2 solution at 0°C in ice-cooled water bath. When the LMWOA extraction method was used, a good correlation was obtained between LREEs in soil colloidal and truly dissolved fractions and LREEs bound to apoplasm and symplasm of maize root. Both apoplastically and symplastically bound LREEs are the result of bioavailability. However, a poor correlation was obtained between LREEs in fractions water soluble, exchangeable and carbonate bound (B1) and Fe-Mn oxide bound (B2) of the BCR method and LREEs in apoplasm and symplasm and in intact roots. Hence, the LMWOA extraction method is recommended for measuring the bioavailability of LREEs in soils.

Keywords—Bioavailability, Light rare earth elements, Maize, Low molecular weight, Organic acids extraction method

INTRODUCTION

During the past decades, rare earth elements (REEs) have been widely applied in both industry and agriculture. Particularly, millions of tons of fertilizers containing REEs are used worldwide because of the increase in agricultural productivity. Since the 1990s, such fertilizers have been applied in China, and they were estimated to cover approximately 3.7 × 10^7 ha in 1993 and 1.6 to 2.0 × 10^7 ha in 1995 [1,2]. Therefore, more and more REEs enter the environment anthropogenically. Several reports have concerned the toxic effects of REEs from occupational and environmental exposures [3,4]. So, it is important to study the bioavailability of REEs.

At present, the general methods for predicting bioavailability are established by correlating the organism accumulation or toxicity with amounts in various soil pools as determined by a variety of extraction methods [5–9]. However, most of data derived from such studies tend to be inconsistent, making interpretation difficult [10–12]. The reasons for this are that metal bioavailability depends on many factors, such as plant species and soil properties [12–15]. In addition, the present approaches of bioavailability do not take into account metal speciation in soils and uptake mechanisms by plants. The main reasons for this are the inherent difficulties in obtaining detailed information concerning the biologically active speciation of metals in soils and uptake by plants. Considering this situation, one can understand why no universally applicable methods are available for measuring the bioavailability of metals in soils [16]. To achieve this practical goal, appropriate computer programs and laboratory- or field-analytical techniques need to be developed.

The alternate way to predict the bioavailability of metals in soils is to simulate the real-world field conditions as closely as possible (i.e., considering all effects of soil properties and plant species on bioavailability as a whole). It is generally recognized that the rhizosphere is distinguished from bulk soil by the influence of plant roots [17]. It is a zone of increased microbial activity and low-molecular-weight organic acids (LMWOAs). According to present knowledge, LMWOAs play an important role not only in carbon metabolism but also in root–soil interactions [18,19]. In our previous study [13], wet rhizosphere soil and dry bulk soil were compared for bioavailability, and application of wet rhizosphere soil was strongly recommended for fractionation and bioavailability.

Uptake kinetics of metals in soil solution by plants is assumed to be the rate-limiting step of bioavailability [20]. Time course of metal accumulation and concentration-dependent uptake kinetics of metals by plants are characterized by an initial rapid component, which is followed by a slower linear phase of accumulation. The rapid component is interpreted to represent accumulation in apoplasm, whereas the slower linear phase is thought to result from transport across the plasma membrane [21–23]. Organic acids increased desorption of REEs from soil and enhanced their uptake in soil solution by roots. These uptake kinetics studies indicated that organic acids influence accumulation and uptake of metals in plants, thus influencing bioavailability.

To our knowledge, no convincing data correlate metals in soil colloidal solutions and truly dissolved fractions with apoplastically and symplastically bound metal. Therefore, the purpose of the present study was to develop a method for evaluating bioavailability of the light REEs (LREEs) La, Ce, Pr, and Nd in soils to plants. This was done by correlating LREEs
in soil pools with LREEs bound to apoplasm and symplasm. A comparison was also made between the LMWOAs extraction method and the first and second steps of the European Community Bureau of Reference (BCR; now European Community Standards, Measurement and Testing Programme, Brussels, Belgium) sequential extraction method to test their suitability for measuring bioavailability.

MATERIALS AND METHODS

Hydroponic experiments

Maize seeds (Zea mays) were thoroughly rinsed with water and germinated on filter paper. After the maize seeds were germinated for 36 h at 20°C in the dark, uniformly germinated maize seeds were placed in quartz sand containing deinonized water for 7 d. Then, the seeds were transferred to a hydroponic culture dish containing a nutrient solution of 2 mM Ca(NO₃)₂, 2 mM KH₂PO₄, 2 mM KCl, 0.75 mM K₂SO₄, 0.65 mM MgSO₄, 0.1 mM ethylenediaminetetraacetic acid (EDTA) disodium salt, and the following micronutrients: 0.05 μM CuSO₄, 0.01 μM ZnSO₄, 0.01 μM MnSO₄, 0.01 μM H₂BO₃, 0.05 μM (NH₄)₂MnO₂₃. The nutrient solution was replaced every 3 d, and the pH was adjusted to 5.8.

Eighteen-day-old seedlings grown in the nutrient medium were separated into three culture dishes. To fix the plants vertically, each culture dish was covered by a thin plastic plate with many holes (diameter, ~0.5 cm). The plant roots were immersed in the culture solution so that the shoots could grow upward. To test the effects of acetic or malic acids on the uptake of La³⁺ by roots (La(NO₃)₃·6H₂O, analytical-reagent grade; Chemical Reagent of Beijing, Beijing, China), three experiments were preformed: 200 μM acetic acid plus 100 μM La³⁺, 200 μM malic acid plus 100 μM La³⁺, and 100 μM La³⁺. The plants were grown in the hydroponic uptake solution for different time intervals of 0 to 48 h and then harvested. The plant roots were ultrasound-extracted into 5 mM EDTA-Na₂ at 0°C with continuous shaking for 15 min to desorb apoplastically bound La. The roots were then washed with deinonized water and dried to constant weight in an oven at 65°C for 36 h for the determination of La. Each experiment was performed in triplicate.

Soil culture experiments

Soil samples were collected from 15 rural areas in China and 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 with 0.4 g/kg each of N and P as a solution of (NH₄)₂CO₃ and KH₂PO₄, respectively. 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 pH was measured using a soil:water ratio of 1:1 (w/v). Soil organic matter and cation-exchange capacity were determined using standard methods [24]. These properties are given in Table 1.

A homemade rhizobox [13] was used to plant maize. The dimensions of the rhizobox were 120 × 100 × 150 mm (length × width × height). The rhizobox was divided into three sections: a central or rhizosphere zone (width, 20 mm), which was surrounded by nylon cloth (300 mesh), and left and right nonrhizosphere zones (width, 50 mm each). A total of 1.5 kg of treated soils was added to each rhizobox. Two rhizoboxes were used for each soil. maize seedlings used for the optimization of CaCl₂ desorption were planted only in Beijing soil.

Uniformly germinated seeds with the radical emerged were sown in soil and left in the rhizobox to equilibrate overnight. The soil was moistened by adding deinonized water daily. The corn was harvested eight weeks after planting. The soil adhering to the maize root was defined as rhizosphere soil. This soil was immediately used for metal fractionation or frozen in a refrigerator (−18°C) for analysis. No significant difference was observed between metal fractionation from fresh or freezing soils [25].

Ultrasound-assisted washing and desorption of apoplastically bound La from intact maize roots

After harvesting, the living maize roots were thoroughly washed first with tap water and then with deinonized water. The roots were washed further with ultrasound-assisted washing (35 W, 25 kHz) for different time intervals of 0 to 60 min in a deinonized water bath. After that, the roots were desorbed with different concentrations of 0 to 10 μM CaCl₂ solution in 5 μM Mes-Tris solution (pH 6.0; Beijing Chemical Factory, Beijing, China) for different time intervals of 0 to 90 min at 0°C in an ice-cooled water bath with continuously shaking. The desorbed roots were washed three times with deinonized water to remove Ca²⁺, and the roots were cut from the shoots, blotted with paper tissues, and dried to constant weight at 65°C for 36 h for determination of LREEs. The maize leaf was thoroughly washed with tap water first and then with deinonized water. Finally, the leaf was dried to constant weight in an oven at 65°C for 36 h for determination of LREEs. All desorption procedures were performed in quadruplicate.

Extraction LREEs and size fractionation of extracted rhizosphere soil solution

Two extraction procedures (i.e., the LMWOAs extraction method and the first and second steps of the three-step BCR method [26]) were adopted in the present study. The conditions are given briefly in Table 2. Among LMWOAs, acetic, formic, citric, and malic acids were relatively abundant [18]. Ten-millimolar acetic, formic, citric, and malic acids in a molar ratio of 2:2:1:1 were used as an extractant. The pH of the

### Table 1. Selected properties of soils

<table>
<thead>
<tr>
<th>Sample</th>
<th>Site</th>
<th>pH</th>
<th>CEC</th>
<th>OM</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Jiamusi</td>
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<td>27.03</td>
<td>4.30</td>
</tr>
<tr>
<td>2</td>
<td>Yuxi</td>
<td>6.05</td>
<td>25.78</td>
<td>1.37</td>
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<td>26.74</td>
<td>4.22</td>
</tr>
<tr>
<td>4</td>
<td>Shandong</td>
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<td>30.20</td>
<td>3.62</td>
</tr>
<tr>
<td>5</td>
<td>Jiangxi</td>
<td>5.49</td>
<td>14.24</td>
<td>1.53</td>
</tr>
<tr>
<td>6</td>
<td>Shanghai</td>
<td>7.62</td>
<td>36.65</td>
<td>3.02</td>
</tr>
<tr>
<td>7</td>
<td>Hangzhou</td>
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<td>19.76</td>
<td>3.28</td>
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<td>8</td>
<td>Hefei</td>
<td>5.58</td>
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<td>2.09</td>
</tr>
<tr>
<td>9</td>
<td>Wuhan</td>
<td>6.65</td>
<td>53.98</td>
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</tr>
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<td>Changsha</td>
<td>5.20</td>
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<tr>
<td>12</td>
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<td>7.83</td>
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<tr>
<td>14</td>
<td>Chaozhou</td>
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<td>15</td>
<td>Xiangli</td>
<td>4.57</td>
<td>12.9</td>
<td>2.46</td>
</tr>
</tbody>
</table>

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*All study site locations are found in China.

1 Cation-exchange capacity.

2 Organic matter.
LMWOAs extractant was adjusted to different soil pH with 0.1 M HNO₃ or NaOH before extraction.

After extraction, soil solutions were centrifuged at 4,000 g for 30 min. For the BCR method, soil solutions were filtered only through a cellulose acetate membrane (pore size, 0.45 μm). For the LMWOAs extraction method, soil solutions were successively filtered through a cellulose acetate membrane (pore size, 0.45 μm; Chemical Reagent of Beijing) and a regenerated cellulose membrane (molecular weight cutoff, 1 kDa; Millipore®, Bedford, MA, USA). The filtrates passing through the 0.45-μm and 1-kDa membranes are referred to as the colloidal and truly dissolved fractions, respectively. All extraction experiments were performed in duplicate.

Reagents and glassware

The La(NO₃)₃·6H₂O, Ca(NO₃)₂·4H₂O, KH₂PO₄, KCl, K₂SO₄, MgSO₄·7H₂O, EDTA-Fe, EDTA-Na₂, CuSO₄·5H₂O, ZnSO₄·7H₂O, MnSO₄·H₂O, H₂BO₃ and (NH₄)₆Mo₇O₂₄·4H₂O, acetic acid, formic acid, citric acid, and malic acid were of analytical-reagent grade. The HClO₄ was of guaranteed grade. All chemical reagents were purchased from Chemical Reagent and from Millipore, respectively.

Statistical analyses

Statistical and correlation analyses were performed with the statistical software Microcal Origin (Microcal Software, Northampton, MA, USA).

RESULTS AND DISCUSSION

Effect of organic acids on uptake of La by roots under hydroponic culture conditions

To test whether LMWOAs could stimulate uptake of La by maize roots, a series of hydroponic experiments were performed according to the conditions given in Materials and Methods. The time course of La uptake in the presence or absence of acetic and malic acids is shown in Figure 1. No obvious differences were found between the uptake of La with or without acetic and malic acids at the first uptake stage from 0 to 1 h. However, when the uptake time was prolonged further, from 2 to 48 h, the uptake of La by maize roots was enhanced by acetic and malic acids. A t test indicated significant differences between the uptake of La with and without malic acid (α = 0.05), except for the time point of 34 h. For acetic acid, significant differences were also observed when the uptake time was longer than 2 h (α = 0.05), except for the time points of 22 and 34 h because of the large standard deviation of the control.

![Fig. 1. Time course of lanthanum uptake by maize roots in the presence or absence of acetic and malic acids: 100 μM La⁺³ + 200 μM acetic acid (●), 100 μM La⁺³ + 200 μM malic acid (▲), and 100 μM La⁺³ only (○). Results are expressed as the mean ± standard deviation (n = 3). DW = dry weight.](image-url)
The results clearly demonstrated that malic acid and acetic acid stimulated the uptake of La, but this enhancement was masked by apoplastic binding at early treatment times. The reason for the enhanced uptake by acetic and malic acids likely reflects higher membrane influx, because the background of apoplastically bound La was low. On the other hand, soluble organic acids may increase the carrying capacity of the nutrient solution for La by the formation of soluble organo-La complexes. High concentration of organic acids promoted trace metal adsorption onto the root surface [28], and plant roots were able to liberate trace metals from dissolved organometallic complexes once they were associated in the roots [29].

**Optimization of root-washing conditions:**

**Ultrasound-assisted washing of maize roots**

After the roots were thoroughly washed with tap water and then with deionized water, bundles of 10 intact maize seedlings were immersed with their roots in 500-ml beakers with deionized water for ultrasound-assisted washing. After washing for different time intervals of 0 to 60 min, the concentrations of LREEs in roots were determined. The results are schematically shown in Figure 2. Concentrations of LREEs in roots decreased slightly with increasing washing time from 0 to 20 min and then remained unchanged when the washing time was further prolonged to 60 min. If the concentrations of LREEs in the intact roots before ultrasound-assisted washing were normalized as 100%, the concentrations of LREEs in maize roots after ultrasound-assisted washing were approximately 86, 91, 91, and 87% for La, Ce, Pr, and Nd, respectively. The results demonstrated that ultrasound-assisted washing was necessary. A washing time of 20 min was adopted for the remainder of the present study.

**Desorption of apoplastically bound LREEs**

To distinguish between apoplastically bound and symplastically bound LREEs, a desorption procedure was tested using CaCl₂ as the desorption reagent. This was done at 0°C after the ultrasound-assisted washing. The effect of various CaCl₂ concentrations on removal of apoplastically bound LREEs from roots was studied. The results are shown in Figure 3. The concentrations of LREEs in intact maize roots decreased rapidly with increasing CaCl₂ concentration from 0 to 0.5 mM. A plateau was observed when the CaCl₂ concentration was increased from 0.5 to 10 mM. Quite similar phenomena have been reported in the literature [21–23]. These data suggest that the initial rapid decrease represented the absorption of LREEs by root apoplasm and that the subsequent slower phase was caused by uptake of LREEs by root symplasm. Hereafter, LREEs desorbed with 0.5 mM CaCl₂ are referred to as apoplastically bound, and LREEs that remained after CaCl₂ desorption are designated as symplastically bound. In these experiments, 1 mM CaCl₂ was chosen to distinguish between apoplastically and symplastically bound LREEs.

**Effect of desorption time on removal of apoplastically bound LREEs**

The maize roots were desorbed with 1 mM CaCl₂ at 0°C for different periods of time, and then the concentrations of LREEs in the desorbed roots were determined. The results indicated that the concentrations of LREEs in maize roots decreased very rapidly over a desorption time from 0 to 5 min and then remained almost unchanged with further increasing desorption time up to 60 min (Fig. 4). For La and Ce, a slight decrease was observed when desorption time was further prolonged to 90 min. This experimental evidence verified that apoplastically bound LREEs were easily removable and that the symplastically bound LREEs were quite stable and unlikely to be liberated from the cytosol via efflux across the plasma.
membrane back into the external solution. According to these results, a desorption time of 30 min was adopted for the remainder of the present study.

Of course, the desorption procedures using CaCl₂ and other complexing ligands (e.g., EDTA) cannot allow a clear distinction between apoplastically and symplastically bound La. Such desorption methods also cannot differentiate between La bound to apoplasm during the uptake culture and background, undesorbed La of apoplasm. Nevertheless, quite similar desorption methods were used [23].

Concentrations of LREEs in B1 and B2 fractions

Fraction B1 was referred to as water soluble, exchangeable, and carbonate bound, which was considered as a mobile form and easily available to plants. Fraction B2 was designated as bound to iron and magnesium oxides and was normally recognized as less mobile. The LREEs in fractions B1 and B2 were only approximately 1% and 10 to 15% of total LREEs in soils, respectively, although the percentage varies with the given soil. This suggested that only a small portion of the total soil LREEs was available to plants.

Distribution of LREEs between soil colloidal and truly dissolved fractions

Different definitions exist of rhizosphere soil. Soil loosely adhering to the roots was gently shaken off, and then the soil adhering to the root was washed off with distilled water. This latter soil fraction represented the rhizosphere soil [30]. In the present study, the space of the rhizosphere zone was small (20 × 100 × 150 mm), and 10 maize seedlings were planted in each zone. After eight weeks of plant growth, the rhizobox was dismantled. Because very dense roots were observed in the rhizosphere zone and the mycelium perforated the nylon cloth, the soil adhering to maize roots was defined as rhizosphere soil. The wet rhizosphere soil was subjected to metal fractionation immediately or frozen in a refrigerator at −18°C before fractionation.

The wet rhizosphere soil was subjected to LMWOAs and BCR extractions. Then, a soil solution fractionation procedure was followed. The LREEs in rhizosphere soil solutions obtained by LMWOAs extraction were successively filtered with 0.45-μm and 1-kDa membranes. Filtrate obtained using the 0.45-μm membrane usually includes both colloidal and truly dissolved fractions. In the present experiment, if the concentrations of LREEs in the <0.45-μm fractions were normalized as 100%, the LREEs in the <1-kDa fraction were 46.6, 49.3, 46.7, and 48.8% for La, Ce, Pr, and Nd, respectively. Comparative analysis of extractable LREEs by LMWOAs and BCR extraction procedures revealed that the extractable amounts of LREEs in the B1 fraction were much higher than those in the <0.45-μm fraction when LMWOAs extraction was used. This difference may be attributable to the higher concentration of acetic acid used in the first step of BCR than that of organic acids in the LMWOAs extraction procedure.

The initial motive for fractionating the soil solutions into colloidal and truly dissolved fractions was to test whether metals in truly dissolved fractions are more available to plants. The hydroponic results (Fig. 1) support this assumption (i.e., in the presence of acetic and malic acids, La uptake was enhanced). If one compares the correlation coefficients between La in the <0.45-μm and <1-kDa fractions and symplastically bound La, this was also true (Table 3).

Distribution of root LREEs among apoplasm and symplasm

If the concentrations of LREEs in intact roots were normalized as 100%, symplastically bound LREEs were approximately 72.6, 68.0, 70.8, and 68.3% for La, Ce, Pr, and Nd, respectively. Apoplastically bound La, Ce, Pr, and Nd were approximately 27.4, 32.0, 29.2, and 31.7%, respectively. These results may suggest that LREEs in roots were mainly present in root symplasm.

Correlation between apoplastically bound, symplastically bound, and total LREEs in intact roots and LREEs in colloidal and truly dissolved fractions of extracted rhizosphere soil solutions

A simple regression analysis was used for bioavailability of LREEs by correlating apoplastically bound, symplastically bound, and total LREEs in intact maize roots with LREEs in various soil solution fractions obtained by the LMWOAs and BCR methods. The correlation coefficients obtained by using the LMWOAs extraction method are given in Table 3.

As can be seen from Table 3, the correlation coefficients between the concentrations of LREEs bound to apoplasm, symplasm, and intact roots and those in soil colloidal and truly dissolved fractions (i.e., \( r_{0.45-μm,Apop} \), \( r_{0.45-μm,Tc} \), \( r_{1-kDa,Symp} \), \( r_{1-kDa,Apop} \), and \( r_{1-kDa,Tc} \)) ranged from 0.811 to 0.931 at the 0.05 probability level, and not much difference was observed between them statistically. The correlation coefficients of \( r_{0.45-μm,Symp} \) were slightly lower but still statistically significant. Considering the slight difference between the correlation coefficients of apoplastically and symplastically bound LREEs, one might conclude that both the apoplastically bound and symplastically bound LREEs were the result of bioavailability. If the correlation coefficients between the LREEs in soil colloidal fraction and truly dissolved fraction were not compared, it may support the expectation that better correlation coefficients were not obtained using the truly dissolved fraction. This may indicate that LREEs in both colloidal and truly dissolved fractions have similar bioavailabilities.

The correlation coefficients between the concentrations of LREEs in fractions B1 and B2 of BCR and LREEs bound to apoplasm, symplasm, and intact roots ranged from 0.185 to 0.599, and none was significantly correlated (\( p > 0.05 \)). The poor correlation coefficients may indicate that the BCR extraction method was not suitable for the bioavailability of LREEs in maize roots. However, as can be seen from Table 4, the correlation coefficients of LREEs in maize leaves and those in the <0.45-μm, <1-kDa, and B1 fractions were sig-

<table>
<thead>
<tr>
<th>0.45-μm filtrate</th>
<th>1-kDa ultrafiltrate</th>
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</thead>
<tbody>
<tr>
<td><strong>La</strong></td>
<td><strong>Ce</strong></td>
</tr>
<tr>
<td>0.721</td>
<td>0.887</td>
</tr>
</tbody>
</table>

\( ^a \) All values are significant at the 0.05 probability level.
\( ^b \) Symplastically bound.
\( ^c \) Apoplastically bound.
\( ^d \) Total concentration in intact roots.
Table 4. Correlation coefficients of light rare earth elements (LREEs) in soil solution fractions (n = 15) using low-molecular-weight organic acids (LMWOAs) and European Community Bureau of Reference (BCR; Brussels, Belgium) methods with LREEs in maize leaves

<table>
<thead>
<tr>
<th>LMWOAs</th>
<th>0.45-μm filtrate</th>
<th>1-kDa ultrafiltrate</th>
<th>BCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>La</td>
<td>0.704 c</td>
<td>0.760 c</td>
<td>0.674 b</td>
</tr>
<tr>
<td>Ce</td>
<td>0.624 c</td>
<td>0.644 c</td>
<td>0.668 c</td>
</tr>
<tr>
<td>Pr</td>
<td>0.626 c</td>
<td>0.716 c</td>
<td>0.599 c</td>
</tr>
<tr>
<td>Nd</td>
<td>0.637 c</td>
<td>0.645 c</td>
<td>0.709 c</td>
</tr>
</tbody>
</table>

a The first step (B1 fraction) of BCR.
b The second step (B2 fraction) of BCR.
c Significant at the 0.05 probability level.

significantly correlated (p < 0.05). These phenomena were reported previously [13].

CONCLUSION

Based on the present study, several conclusions can be drawn.

First, acetic and malic acids have a positive effect on the uptake of La by maize roots, indicating that LMWOAs play a role in bioavailability.

Second, wet rhizosphere soil and LMWOAs should be used in combination when testing for bioavailability. Only in this case can good correlation coefficients between the metal concentrations in soil solution pools and in plant roots (or, sometimes, in shoots) be obtained. The BCR method was not suitable for measuring bioavailability.

Third, the LREEs in rhizosphere soil solutions can be fractionated into colloidal and truly dissolved fractions. Metal ions and metal–organic ligand complexes in the truly dissolved soil solution fraction were easily available to plants. For practical purposes, total metal concentrations in soil solutions are used because of insignificant difference between the correlation coefficients obtained with different colloidal fractions.

Fourth, both apoplastically and symplastically bound LREEs are the result of bioavailability. Currently, no techniques allow a clear distinction to be made between La in these two phases. Practically speaking, total root concentrations would be an indicator of bioavailability. A study of adsorption and uptake kinetics is needed to clarify further which of the two processes will be rate-limiting and most indicative of bioavailability.

Fifth, the errors caused by fine soil grains adhering to plant roots should be corrected, and an ultrasound-assisted washing procedure should be performed.

In summary, the LMWOAs extraction method mimics the real-world field conditions under which plants grow on soils. The LMWOAs extraction method probably can be widely used for measuring the bioavailability of metals in soils to plants.

Acknowledgement—This work was funded by the Chinese Academy of Science (Grant KZCX2-410) and National Natural Science Foundation of China (Grants 20177030, 40171086, and 20237010).

REFERENCES

27. Zhang SZ, Shan XQ. 1997. The determination of rare earth el-

W.-S. Wang et al.

