Evaluation of bioavailability of light rare earth elements to wheat (*Triticum aestivum* L.) under field conditions

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Abstract

Current bioavailability assessments of light rare earth elements (LREEs) are often carried out under greenhouse conditions. This work was to identify which soil properties are mostly related to LREE bioavailability and what extraction method is the most promising approach for assessing bioavailability of LREEs to wheat under field conditions. A comparison was made between four commonly used extractants (DTPA, EDTA, CaCl₂ and CH₃COOH), and the results suggested that the LREE concentrations in wheat roots significantly correlated with soil pH and amorphous Fe oxide contents in soils, but were independent of soil organic matter (SOM), crystalline Fe and Mn oxide contents, and cation exchange capacity (CEC). The contents of LREEs in shoots were independent of any soil properties. Both DTPA- and EDTA-extractable LREEs were significantly correlated to LREE concentrations in wheat roots and shoots. DTPA extractable LREEs were comparable to LREEs in wheat roots, however, the EDTA extractable LREEs overestimated the LREE accumulation in wheat roots. Neither root nor shoot LREEs showed significant correlation with CH₃COOH extractable LREEs, suggesting that the CH₃COOH extraction method was not suitable for predicting LREE bioavailability. CaCl₂ method was unable to estimate the LREE bioavailability due to poor data distribution in correlation analysis. Overall, DTPA extraction method was preferred to other extraction methods for estimating bioavailability of LREEs to wheat.

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Keywords: Light rare earth elements; Extraction method; Bioavailability; Wheat; Field study; Soil properties

1. Introduction

Fertilizers containing rare earth elements (REEs) with predominant proportion La and Ce are widely used in agriculture in China in order to increase crop yields of some plant species, particularly if moisture is limited (Xu et al., 2002; Shtangeeva, 2004). Since 1990s, millions of tons of such fertilizers have been applied. As a result, REEs in China have been found to be accumulated in soils, crops, and enter the food chain (Chua et al., 1998; Wen et al., 2001). Others sources (sewage sludge, incinerator bottom ashes and rainwater) can also contribute to environmental contamination by REEs (Zhang et al., 2001; Zhang and Liu, 2004). The adverse health effect and potential detrimental environmental issue caused by REEs are concerned by environmental scientists. Possible

health risks for humans could not be excluded and health status of children should be examined from health risk assessment perspective in rare earth mining areas (De Boer et al., 1996; Tong et al., 2004). High concentrations of REEs may cause significant damage to ecosystem (Barry and Meehan, 2000). Some studies also focused on the toxic effects of REEs to plants from occupational and environmental exposures and the long-term hazardous environment effects (Sax, 1984; Suzuki et al., 1992). To evaluate the ecotoxicological risks of REEs, the first step is to estimate the bioavailability of REEs.

REE bioavailability is measured by assessing the proportion of REEs that is accumulated by an organism or that can cause a biological response. It is the common concept nowadays that total concentration of metals in soils is not a good indicator of bioavailability, and a good tool for potential risk assessment neither (Qian et al., 1996; Chen et al., 1996). Extraction procedures using a selective chemical extractant, such as strong chelating agents, mild neutral salts, organic or inorganic acid

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Fig. 1. Soil and wheat sampling locations. (1) Beijing, (2) Hebei 1, (3) Hebei 2, (4) Hebei 3, (5) Hebei 4, (6) Tianjin, (7) Shandong, (8) Henan, (9) Shanxi, (10) Sha'anxi, (11) Chongqing, (12) Sichuan, (13) Hubei, (14) Anhui, (15) Jiangsu, (16) Zhejiang 1, (17) Zhejiang 2, (18) Zhejiang 3, (19) Fujian, (20) Yunnan.

(Lu et al., 2003; Wang et al., 2003; Wang et al., 2004) could provide information on the potential mobility and bioavailability of REEs. Bioavailability of REEs depends on the chemical speciation of REEs in soils and plant species (Zhang and Shan, 2001; Hu et al., 2002; Wang et al., 2004). Shan et al. (2003) found that REEs in the labile rhizosphere soil solution, including free metal ions and inorganic and organic complexes, were mostly bioavailable. REEs in water soluble, exchangeable and carbonate bound fractions were significantly correlated with REE uptake by wheat (Wang et al., 2001) and alfalfa (Cao et al., 2000). CaCl_2 and CH_3COOH extracted REEs were reported to be best correlated with REE contents in shoots of wheat (Lu et al., 2003). $0.1 \text{ mol l}^{-1} \text{ HCl}$ gave a good estimate of the wheat available REEs in soils (Li et al. 2001). A mixture of malic and citric acids could be also used as a predictor of available REEs (Zhang et al. 2000). The above studies were inconsistent and made comparisons difficult. However, most of the results were obtained under greenhouse conditions. Extrapolation of the results to the field might be problematic due to the complicated growth conditions. Therefore, the aims of this work are (1) to identify which soil properties are mostly related to LREE bioavailability; (2) to search for the most efficient extraction method for evaluation of LREE bioavailability to wheat under field conditions by comparing EDTA, DTPA, CaCl_2 and CH_3COOH extraction methods.

2. Materials and methods

2.1. Soil and wheat samples

Twenty cultivated surface soils and the corresponding wheat seedlings from the same field were collected from 15 different

Chinese provinces during February 2005 (Fig. 1). Within each sampling unit, both soil and wheat subsamples were taken from several different locations (5–10 locations, depending on the uniformity and size of the sampling unit), and were mixed into one composite sample. Moist soils and wheat samples were preserved separately in polyethylene bags during transport to the laboratory. All wheat seedlings were cut to the size of 3–7 cm for

Table 1
Extraction procedures used in this study

Extractant	Procedure	Fraction	Reference
CaCl_2	2.00 g soil was mixed with 20 ml $0.01 \text{ mol l}^{-1} \text{ CaCl}_2$ in a 50 ml plastic centrifuge tube, and shaken for 3 h	Easily exchangeable	Novozamsky et al. (1993)
CH_3COOH	1.00 g soil was mixed with 20 ml 0.11 mol l^{-1} acetic acid in a 50 ml plastic centrifuge tube, and shaken for 16 h	Exchangeable and carbonate bound	Ure et al. (1993)
EDTA	2.00 g soil was mixed with 20 ml 0.05 mol l^{-1} EDTA and its pH was adjusted with ammonia solution to 7.0 in a 50 ml plastic centrifuge tube, and the suspension was shaken for 1 h	Organically bound and metals occluded in oxides and secondary clay minerals in part	Wear and Evans (1968)
DTPA	10.0 g soil was mixed with 20 ml 0.005 mol l^{-1} DTPA+ $0.01 \text{ mol l}^{-1} \text{ CaCl}_2$ + $0.01 \text{ mol l}^{-1} \text{ TEA}$, pH of suspension was adjusted to pH 7.3 in a 50 ml plastic centrifuge tube, and shaken for 2 h	Iron and manganese oxides bound	Lindsay and Norvell (1978)

Table 2
Selected properties of soils and total LREE contents in soils

Site	pH	CEC	SOM	Fe (g/kg)		CryMn	Total LREEs (mg/kg)			
		me/100 g	%	CryFe	AmorFe	g/kg	La	Ce	Pr	Nd
Beijing	7.40	12.50	2.96	2.02	0.76	0.12	27.12	45.76	7.65	21.54
Hebei 1	7.48	12.81	1.56	3.38	1.10	0.25	9.89	23.86	2.69	9.57
Hebei 2	7.19	8.34	1.88	1.85	0.93	0.12	9.95	24.01	2.70	9.63
Hebei 3	5.92	14.89	2.11	4.10	2.07	0.19	4.65	14.15	1.84	4.68
Hebei 4	7.59	10.82	2.91	1.30	0.44	0.10	20.82	34.81	4.43	17.29
Tianjin	7.24	14.29	1.66	2.47	1.01	0.22	13.55	38.29	4.62	14.19
Shandong	7.31	9.52	1.97	1.62	0.85	0.11	25.67	43.82	6.20	20.56
Henan	7.38	18.56	1.28	2.24	0.95	0.17	26.35	45.52	6.75	21.42
Shanxi	7.34	8.12	1.12	1.22	0.31	0.11	25.52	43.84	6.21	21.05
Sha'anxi	6.18	16.91	1.14	5.63	0.94	0.65	33.53	56.48	11.54	25.35
Chongqing	6.98	27.30	1.1	5.13	0.97	0.19	35.68	63.24	11.15	28.03
Sichuan	6.74	18.95	4.46	4.42	2.39	0.12	33.86	59.54	11.09	26.69
Hubei	5.30	7.10	1.81	6.18	2.93	0.93	5.23	9.92	1.74	5.55
Anhui	5.98	14.25	2.51	8.42	6.18	0.16	34.38	60.09	12.76	26.76
Jiangsu	7.36	7.55	1.1	1.37	0.49	0.11	23.33	39.31	5.86	18.90
Zhejiang 1	5.30	6.99	2.18	2.17	1.42	0.09	30.77	60.5	11.61	22.27
Zhejiang 2	7.25	9.96	3.48	2.93	1.32	0.42	18.18	43.18	6.41	14.41
Zhejiang 3	5.24	18.16	3.88	3.43	2.89	0.05	27.39	56.01	10.70	22.80
Fujian	6.82	6.92	2.13	1.69	1.10	0.06	23.86	45.19	8.59	17.07
Yunnan	6.06	6.65	1.30	0.80	0.38	0.05	7.05	16.06	3.26	6.50
Medium value of world soil							40	50	7	35
Range of world soil							7–180	3–170	3–12	4–63

CEC = cation exchange capacity.

SOM = soil organic matter.

CryFe = crystalline Fe oxide contents.

AmorFe = amorphous Fe oxide contents.

CryMn = crystalline Mn oxide contents.

roots and 11–21 cm for shoots. Roots and shoots were firstly rinsed thoroughly with tap water and subsequently with de-ionized water, and finally with ultrasonic-assisted washing for extra 20 min to remove the fine adhering soil particles. After washing, wheat shoots and roots were dried at 60 °C for 48 h and separately finely ground before analysis. Subsamples of soils were air-dried and small pieces of broken roots and any other extraneous materials were carefully removed, prior to sieving <1 mm using a nylon fiber sieve and stored for subsequent analysis of soil characteristics. The remaining portions of soils were refrigerated at –18 °C prior for measuring soil humidity and extraction. Soil organic matter (SOM) content was determined using the Walkley–Black procedure (Nelson and Sommers, 1982). Soil pH was measured at a soil to 0.01 mol l^{–1} CaCl₂ solution ratio of 1:5(w/v). CEC was determined by standard methods (Rhoades, 1982). Cation exchange capacity (CEC) of the soils with pH <7.0 was determined by the BaCl₂ method, while the CEC of soils with pH >7.0 was determined by

the CH₃COONa method. Amorphous Fe oxide was extracted by shaking 1.00 g of soil in 30 ml of acidified ammonium oxalate buffer (0.175 mol l^{–1} (NH₄)₂C₂H₄+0.1 mol l^{–1} H₂C₂O₄, pH 3.0) for 2 h (Loeppert and Inskeep, 1996). Crystalline Fe and Mn oxides were determined by the oxalate–ascorbic acid extraction method of Shuman (Shuman, 1982).

2.2. Extraction procedure

Field moist soils were subjected to all of the extraction procedures in order to avoid metal speciation change during drying process. The detailed operations and nominal target fractions in soils were described briefly in Table 1. For all extraction procedures, after shaking, the suspensions were centrifuged at 7000 g for 20 min and the supernatant was filtered through a disposable 0.45 µm cellulose acetate membrane filter prior to LREE determination. All extractions including blanks were carried out in triplicate.

Table 3
Total contents of LREEs in wheat (mg kg^{–1} DM) and the correlation between LREE in wheat roots and shoots

Element	In roots		In shoots		Equations	R
	Range	Mean	Range	Mean		
La	636–10,642	3171	114–7209	1320	Shoot–La=0.38×Root–La	0.642**
Ce	1310–23,462	7170	228–15,695	2766	Shoot–Ce=0.42×Root–La	0.715***
Pr	152–2510	801	25–1730	306	Shoot–Pr=0.40×Root–La	0.642**
Nd	578–10,234	3126	103–6909	1205	Shoot–Nd=0.41×Root–La	0.668***

R=the correlation coefficient ** and ***=the probability level of $p<0.01$ and $p<0.001$, respectively.

Table 4
Linear correlation coefficients (*R*) between LREE concentrations in wheat shoots and roots and soil properties (*n*=20)

	Element	pH	CEC	SOM	CryFe	AmorFe	CryMn
Root	La	−0.73***	0.17	0.18	0.44	0.57**	0.03
	Ce	−0.70***	0.16	0.21	0.34	0.50*	0.02
	Pr	−0.73***	0.19	0.19	0.39	0.50*	0.03
	Nd	−0.73***	0.19	0.21	0.39	0.51*	0.02
Shoot	La	−0.38	0.10	0.40	0.09	0.41	−0.20
	Ce	−0.40	0.09	0.42	0.09	0.39	−0.16
	Pr	−0.38	0.12	0.41	0.09	0.41	−0.19
	Nd	−0.41	0.12	0.41	0.11	0.43	−0.19

*, ** and ***=the probability level of $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

CEC = cation exchange capacity.

SOM = soil organic matter.

CryFe = crystalline Fe oxide contents.

AmorFe = amorphous Fe oxide contents.

CryMn = crystalline Mn oxide contents.

2.3. Determination of LREEs in soils and plants

Total LREE concentrations in soils, and in wheat shoots and roots were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Electron Company, USA) with Rh as an internal standard after digestion with 3 ml of $\text{HNO}_3\text{--HClO}_4\text{--HF}$ (1:1:1, v/v) in PTFE vessel under high-pressure conditions (Zhang and Shan, 1997). For quality control of low levels of LREEs in wheat 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 LREE concentrations determined in this work were reliable. LREE concentrations in soil solution obtained from extraction methods were also determined by ICP-MS.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., USA). Linear correlation analysis was performed for soils to visualize the relationship between wheat LREE concentrations in shoots and roots, and soil extractable LREEs or soil properties.

3. Results and discussion

3.1. LREEs in soils and wheat, and extractable LREEs from soils

The total LREE contents in soils varied considerably, ranging from 4.65–35.68 (La), 9.92–63.24 (Ce), 1.74–12.76 (Pr), and 4.68–28.03 mg kg^{-1} (Nd) (Table 2). The contents of each LREEs followed the order $\text{Ce}>\text{La}\geq\text{Nd}>\text{Pr}$. No significant contamination of LREEs in soils was found because most of the total LREE contents in the studied soils were in the background range of REEs in world soils. Among them Ce and Pr contents in six and eight soils were higher than the medium values of world soils, respectively. Both La and Nd contents of all the studied soils were below the medium values of world soils.

Total concentrations of LREEs in wheat roots were higher than those in shoots (Table 3). Significant correlation was observed between LREE contents in roots and shoots in this work. The coefficients between REE contents in shoots and roots ranged from 0.38 to 0.42, indicating that LREE accumulation ratio of shoots to roots were about 38–42% by mean.

The extractable LREEs by the four extractants followed the order $\text{EDTA}>\text{DTPA}>\text{CH}_3\text{COOH}>\text{CaCl}_2$, which were in the range of 21.2–32.7% (EDTA), 6.9–11.6% (DTPA), 0.53–0.87% (CH_3COOH), and less than 0.1% (CaCl_2) of total LREEs in soils. The reasons for this order were mainly ascribed to their different extractability. EDTA is a strong chelating reagent, which has been reported to extract labile and non-labile metals, including exchangeable and weakly organically bound fractions (Bermond et al., 1998). Iron and manganese oxides are potentially extracted from acidic soils by DTPA (O'Connor, 1988). The high concentration of acetic acid can extract weakly organically bound metals (Payà-Pérez et al., 1993) and most of calcium and mineral bound metals (Whalley and Grant, 1994). CaCl_2 is mainly able to extract easily exchangeable metals.

3.2. Relationship between soil properties and bioavailability of LREEs

Linear correlation analysis was performed in order to define the relationship between LREE concentrations in wheat and soil properties (Table 4). LREE concentrations in shoots were not correlated ($p>0.05$) to any soil variables, suggesting that shoot accumulation of LREEs was not influenced by soil properties. Negative correlations were found between soil pH and root LREE concentrations, whereas the amorphous Fe oxide

Table 5
Linear correlation coefficients (*R*) between extractable LREEs and soil properties

Extractant	Element	pH	CEC	SOM	CryFe	AmorFe	CryMn
DTPA	La	−0.86***	0.08	0.32	0.54*	0.74***	0.10
	Ce	−0.84***	0.04	0.33	0.42	0.64**	0.12
	Pr	−0.82***	0.15	0.37	0.48*	0.68***	0.07
	Nd	−0.82***	0.16	0.36	0.48*	0.67***	0.06
EDTA	La	−0.39	0.03	0.44	0.32	0.84***	0.11
	Ce	−0.64**	0.07	0.38	0.43	0.84***	0.12
	Pr	−0.45*	0.08	0.51*	0.36	0.79***	0.10
	Nd	−0.47*	0.13	0.52*	0.39	0.78***	0.12
CaCl_2	La	−0.70***	0.07	0.37	0.16	0.40	0.03
	Ce	−0.68***	0.07	0.37	0.13	0.35	0.00
	Pr	−0.69***	0.06	0.35	0.12	0.35	0.02
	Nd	−0.71***	0.04	0.33	0.12	0.34	0.00
CH_3COOH	La	0.15	0.29	0.12	0.26	0.08	0.25
	Ce	0.02	0.28	0.06	0.17	0.02	0.21
	Pr	0.13	0.26	0.11	0.23	0.04	0.27
	Nd	0.16	0.26	0.15	0.25	0.06	0.28

*, ** and ***=the probability level of $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

CEC = cation exchange capacity.

SOM = soil organic matter.

CryFe = crystalline Fe oxide contents.

AmorFe = amorphous Fe oxide contents.

CryMn = crystalline Mn oxide contents.

Table 6

Linear correlation coefficients (R) between LREE concentrations in wheat shoots and roots and LREEs extracted by different extraction methods ($n=20$)

	Element	CaCl ₂	CH ₃ COOH	EDTA	DTPA
Root	La	0.76***	0.09	0.64**	0.91***
	Ce	0.77***	0.25	0.73***	0.83***
	Pr	0.80***	0.11	0.69***	0.90***
	Nd	0.82***	0.05	0.71***	0.91***
Shoot	La	0.80***	0.33	0.58**	0.67**
	Ce	0.81***	0.38	0.66**	0.77***
	Pr	0.79***	0.36	0.68***	0.75***
	Nd	0.79***	0.31	0.70***	0.78***

** and ***=the probability level of $p<0.01$ and $p<0.001$, respectively.

contents were positively correlated with root LREEs. However, in the present work, CEC, SOM and crystalline Fe and Mn oxide contents had little effect on the bioavailability of LREEs. The adverse effect of pH on root LREE contents was consistent with the general expectation of greater bioavailability of LREEs at lower soil pH, since LREEs would be precipitated and less released from soils as the soil pH increased (Cao et al., 2001; Zhang and Shan, 2001). Amorphous Fe was the main factor controlling the adsorption capacities of LREEs in soils (Liu and Ran, 1993). Cao et al. (2001) reported that LREE release might originate from dissolution of Fe and Mn oxyhydroxides under reduction and lower pH conditions. Thus, the more amorphous

Fe contents, the more LREEs in soils and the bigger bioavailable pool of LREEs.

3.3. Relationship between soil properties and extractable LREEs by various methods

Linear correlation analysis between the extractable LREEs and soil properties are shown in Table 5. This work verified that soil pH, Fe oxide and SOM contents could influence LREE extractability of different extractants. Both DTPA- and EDTA-extractable LREEs were significantly negatively correlated with soil pH and positively correlated with amorphous Fe oxide contents, suggesting that DTPA and EDTA were able to extract more LREEs in the soils with low pH and high amorphous Fe oxide contents. DTPA-extractable LREEs also correlated with soil crystalline Fe oxide contents with the exception of Ce, and the correlation coefficients were about 0.50, which were much lower than those between DTPA- and EDTA-extractable LREEs and amorphous Fe oxide contents. In addition, EDTA-extractable Pr and Nd were significantly related to SOM contents. Negative correlation was also found between soil pH and the CaCl₂-extractable LREEs. By contrast, CH₃COOH-extractable LREEs showed no correlation with any soil properties. The inverse effect of soil pH on metal extractability was always obtained, which may partly attribute to the increasing displacement

Fig. 2. Linear correlation between accumulated LREEs in wheat roots and extracted LREEs by various extraction methods.

of adsorbed metal ions into extractant solution (or soil solution in real situations) by H^+ ions as acidity increases (Rieuwerts et al., 2006).

3.4. Evaluation of LREE bioavailability using various extraction methods

Linear correlation analysis demonstrated that the concentrations of LREEs in both wheat roots and shoots were significantly correlated to DTPA-, EDTA- and $CaCl_2$ -extractable LREEs from soils (Table 6). The correlation coefficients (r) between accumulated LREEs in roots and extractable LREEs by DTPA method (ranging of 0.83–0.91) were the highest among these extraction methods, followed by $CaCl_2$ (ranging of 0.77–0.82) and EDTA methods (ranging of 0.64–0.73). As far as the relationship between the extractable REEs from soils and REEs in shoots is concerned that three extraction methods had quite similar correlation coefficients although the $CaCl_2$ method seems to be slightly better. In contrast, no significant relationship was found between CH_3COOH extractable LREEs and LREE contents in wheat roots or shoots, suggesting that CH_3COOH method was not suitable to predict LREE bioavailability to wheat under field conditions.

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 method is based on the assumption that there is a relationship between the extractable fraction of metals and the bioavailability of metals to plants, i.e., a good correlation indicated that a certain fraction of total metal in the soils was available to plants (Wang et al., 2004).

Generally, a comparison of the r -values of linear correlation analysis for extraction methods does not suggest one or the other to be a better predictor of crop accumulation (McBride et al., 2003). The reliability of a linear correlation analysis is dependent on good distribution of samples across the interval of interest. Thus single linear correlation between root LREE concentrations (y axis) and extractable LREEs from soils (x -axis) were plotted in Fig. 2. Inspection of the data, however, revealed that the correlation of $CaCl_2$ extraction method was biased by the dominating effect of Zhejiang3 soil on the correlation analysis. Most of the outlier data points were near the y -axis in Fig. 2- $CaCl_2$ ($CaCl_2$ extractable $La < 0.01 \text{ mg kg}^{-1}$). A similar pattern of data distribution was observed for Ce, Pr and Nd for $CaCl_2$ extraction method as well. These were mainly due to the extremely low extractable LREEs by $CaCl_2$, resulting in small variance of extractability of LREEs among most soils. Thus $CaCl_2$ method seemed not to be a good approach for assessing LREE bioavailability to wheat. By contrast, the relationships obtained by both DTPA and EDTA methods revealed a monotonic trend that was dependent on good distribution of data. The slope values of regression equations that indicated the average ratio of extracted LREE quantity (mg kg^{-1}) to root accumulated LREEs (mg kg^{-1}) obtained by DTPA method were close to 1 (ranged from 1.04 to 1.30) (Fig. 2), demonstrating that the accumulated LREEs in wheat roots is comparable to the DTPA-extractable pool from soils. While the correlation slopes of the EDTA method were from

0.65 to 0.71, indicating that EDTA-extractable LREEs would overestimate the accumulation of LREEs in wheat roots.

Ideally, a good method for predicting LREE bioavailability should mimic the root–soil interactions as closely as possible. For the DTPA method, the strongest soil factors controlling the extractability of LREEs were soil pH and amorphous Fe oxide contents, which were the most important factors influencing the LREE concentrations in roots (Tables 4 and 5). It might indicate that DTPA extraction could mostly reflect the soil property-related factors that modulate wheat uptake of LREEs. This deduction was further supported by the results that DTPA-extractable LREEs were best correlated to wheat root LREEs. On the contrary, CH_3COOH extractability could hardly be affected by soil properties (Table 5), and no significant relations were obtained between extractable LREEs by CH_3COOH and root LREE contents.

In previous greenhouse works, Zhang et al. (2000) found that CH_3COOH extraction was a poor indicator of REE availability to wheat. While the LREE fraction extracted by CH_3COOH was also reported to correlate significantly with LREE contents in both roots and shoots of wheat, and this fraction represented the bioavailable pool after rare earth fertilizer application (Zhang and Shan, 2001). These contradictions really reflected the complication and uncertainty of REE bioavailability. The reason for this is probably due to the so called residence time effect or ageing effect, which significantly influenced the fractionation of exogenous metals in soils (Lu et al., 2005). Metals were possibly transformed from easily extractable fractions to more stable fractions during the ageing processes, thus became less bioavailable with increasing residence time in soils. Hence any acceptable extraction method for assessing bioavailability of LREEs should be tested under field conditions. In addition, soils studied in this work were not significantly contaminated by LREEs, more detailed field study is needed to test whether DTPA and EDTA methods still remain acceptable for predicting LREE bioavailability in LREE-contaminated soils.

4. Conclusion

LREE accumulation in wheat roots were influenced by soil pH and amorphous Fe oxide contents in soils, while SOM, CEC, crystalline Fe and Mn oxide contents had little effects. Shoot LREEs were independent of soil properties. Based on the relationship between the extractable REEs and REEs in wheat or soil properties one may conclude that the DTPA method best reflected soil-wheat root interactions as soil properties influenced the uptake of LREEs, thus the DTPA method was the most efficient approach for evaluation of LREE bioavailability. EDTA method was also a good approach for assessing bioavailability of LREEs to wheat, although the extractable LREEs by EDTA always overestimated the accumulation of LREEs in wheat roots. CH_3COOH and $CaCl_2$ methods seemed not a good choice of estimating bioavailability of LREEs to wheat.

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