Comparison between fractionation and bioavailability of trace elements in rhizosphere and bulk soils

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Abstract

Rhizosphere is a microbiosphere and has quite different chemical, physical and biological properties from bulk soils. A greenhouse experiment was performed to compare the difference of fractionation and bioavailability of trace elements Cr, Ni, Zn, Cu, Pb and Cd between rhizosphere soil and bulk soil. In the meantime, the influence of air-drying on the fractionation and bioavailability was also investigated by using wet soil sample as a control. Soils in a homemade rhizobox were divided into four zones: rhizosphere, near rhizosphere, near bulk soil and bulk soil zones, which was designated as S₁, S₂, S₃ and S₄. Elemental speciations were fractionated to water soluble, exchangeable and carbonate bound (B1), Fe–Mn oxide bound (B2), and organic and sulfide bound (B3) by a sequential extraction procedure. Speciation differences were observed for elements Cr, Ni, Zn, Cu, Pb and Cd between the rhizosphere and bulk soils, and between the air-dried and wet soils as well. The concentrations of all six heavy metals in fraction B1 followed the order of S₂ > S₃ > S₁ > S₄ and for B2, the order was S₂ > S₃ ≈ S₄ > S₁. For B3, the order was S₁ > S₃ ≈ S₄ > S₂, while for Cd the order was S₂ > S₃ ≈ S₄ > S₁. The air-drying increased elemental concentration in fractions B1 and B2 by 20–50% and decreased in fraction B3 by about 20–100%. Correlation analysis also indicated that the bioavailability correlation coefficient of fraction B1 in rhizosphere wet soil to plants was better than that between either air-dried or nonrhizosphere soils. Therefore, application of rhizosphere wet soils should be recommended in the future study on the speciation analysis of trace elements in soils and bioavailability. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Fractionation; Bioavailability; Rhizosphere; Air-drying

1. Introduction

Speciation is defined as a process leading to the identification and determination of the different chemical and physical forms of an element existing in a sample (Kot and Namiesnik, 2000). In the case of soils the speciation of heavy metals is generally related to their physico-chemical forms existed: simple and complex ions in interstitial solution; exchangeable ions; associated with soil organic fractions; occluded or coprecipitated

tated with metal oxides, carbonates or phosphates and other secondary minerals; ions in the crystal lattices of primary minerals (Zhu and Alva, 1993). Based on the consideration of elements being associated with the different geochemical phases of soils there are numerous experimentally defined single and multiple sequential extraction procedures for the elemental speciation analysis (Shuman, 1985; Levesque and Mathur, 1988; Novozamsky et al., 1993; Ure et al., 1993; Leleyter and Probst, 1999). Among them the most widely applied methods were those recommended by Tessier et al. (1979) and by the Community Bureau of Reference (BCR) (Ure et al., 1993). BCR is now called the Standard Measurement and Testing Program of the European Community (STM). The main drawbacks of the

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sequential extraction are no information of true speciation of elements in soils available in addition to the nonselectivity of extractants used and redistribution of trace elements among geochemical phases during extraction processes (Shan and Chen, 1993; Howard and Brink, 1999). Although these disadvantages of elemental speciation analysis are recognized, the sequential extraction methods are unfortunately still used before the more effective methods available, especially in the case of study on the relationship between the elemental speciations and their bioavailability (LeClaire et al., 1984; Chlopecka and Adriano, 1996; Davidson et al., 1999).

Principally, the methods to study on the bioavailability of trace elements in soils should be analogous to the uptake of metals by biota and need a detailed knowledge of biological uptake mechanisms, particularly the transport of metals through the biological membrane (Town, 2000). In order to achieve these goals, many efforts should be made in the future to understand what speciation of metals present in a sample (Kaneta et al., 1986; Kot and Namiesnik, 2000), where the different forms of metals are localized in plants (Vazquez et al., 1992; Kramer et al., 1996; Kelley et al., 1999), how the different compounds of metals enter the plants apoplastically or intercellularly (Kramer et al., 1997; Salt and Kramer, 2000). Due to the extremely complicated mechanisms involved between the speciation of metals and their bioavailability, at the present time, various empirical approaches are often used to study the speciations of trace elements in soils and the contents of those elements in plants, including single regression (Randall et al., 1976; LeClaire et al., 1984; Chlopecka and Adriano, 1996) and multiple regression analyses (Davies, 1992; Maiz et al., 2000).

It must be pointed out that plants exude many low molecular organic acids, which can influence the speciation of elements in soil solutions and the uptake of elements by plants. Rhizosphere is a small but important subsystem of pedosphere, and the properties of rhizosphere are different largely from those of bulk soils (Krafczyk et al., 1984; Curl and Truelove, 1986). It also seems that there are different speciations present in dried and wet soils (Kennedy et al., 1997). The aim of this study is to compare the elemental speciation and bioavailability between the rhizosphere soils and bulk soils. A comparison is also made between the dry and wet soils.

2. Materials and methods

2.1. Soils

Soil samples were collected from 15 rural area around China, representing typical Chinese soil with different physical and chemical properties. All soils were taken from the surface layer (0–20 cm) of cultivated

Table 1
Selected properties of soils

Sample	Geographical origin	pH	CEC (cmol/kg)	OM (%)
1	Jiamusi	5.97	27.03	4.30
2	Lanzhou	7.43	10.68	2.53
3	Shaanxi	8.25	25.88	1.75
4	Shanxi	7.92	21.86	4.12
5	Yuxi	4.45	25.78	1.37
6	Kunming	5.55	26.74	4.22
7	Shandong	5.94	30.2	3.62
8	Jiangxi	5.49	14.24	1.53
9	Shanghai	5.53	36.65	3.02
10	Hangzhou	7.05	19.76	3.28
11	Henan	7.73	18.12	2.03
12	Wuhan	7.27	53.98	2.37
13	Changsha	5.50	43.11	2.28
14	Hefei	6.71	24.95	2.09
15	Dongguan	7.45	15.92	1.37

soils. The soils were air-dried, ground and sieved to pass through 1 mm of 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 by using standard methods (Lao, 1988). These properties are given in Table 1.

2.2. Greenhouse experiment

A homemade rhizobox (Fig. 1) (Youssef and Chino, 1989) was used to plant wheat. The dimension of the

Fig. 1. Sketch diagram of rhizobox.

rhizobox was $120 \times 100 \times 150$ (length $\times$ width $\times$ height, mm). The rhizobox was divided into three sections: a central zone, or rhizosphere zone (20 mm in length), which was surrounded by nylon cloth (300 mesh), and left and right nonrhizosphere zones (50 mm in length). Each nonrhizosphere soils was further evenly separated into three subzones by nylon cloth, which referred to as near rhizosphere zone, near bulk soil zone and bulk soil zone according to distance from the rhizosphere zone to the boundary of the rhizobox. The previously air-dried soils were treated with 0.4 g/kg N and P, as 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, and ground to pass through 1 mm of mesh. To each rhizobox was added 1.5 kg of the treated soil. Soils were not sterilized.

Wheat seeds (*Triticum aestivum* L.) were thoroughly rinsed with water, and germinated on the filter paper. After wheat seeds were germinated for 24 h at 20 °C in the dark, uniformly germinated seeds with radical emerged were sown in soil, which had been left in pots to equilibrate overnight. 30 seeds were sown per rhizosphere zone and subsequently thinned to 20 plants. Plants were grown under the greenhouse conditions of temperature ranged from 20 to 25 °C during the day and 8 to 15 °C during the night. The relative humidity was kept around 15% by addition of deionized water daily.

Plants were harvested 8 weeks after germination. The aboveground parts of the plants were first harvested as shoots. Rhizobox was then dismantled. The roots were remained completely in the central zone, while the mycelium was found to perforate the nylon cloth. Roots were washed out with tap water to remove soil, both the roots and shoots were then rinsed with deionized water and dried at 60 °C for 48 h. Then the dried sample was finely ground for speciation analysis.

2.3. Fractionation analysis

The rhizosphere soil was sieved gently to remove the roots by keeping the root mass intact as much as possible. Rootlets that passed through the sieve were subsequently removed with a pair of tweezers. The soils

taken from two near rhizosphere soil zone, near bulk soil zone, bulk soil zone of the left and right were mixed separately for further speciation analysis. The four soil samples collected from the rhizosphere, near rhizosphere, near bulk soil and bulk soil zones were designated as S_1 , S_2 , S_3 and S_4 respectively, in the remainder of the study.

A portion of 1.000 g of the wet samples of S_1 , S_2 , S_3 and S_4 were weighed immediately after sampling and homogenizing and put into 50 ml polypropylene centrifuge tubes for speciation analysis with a three-stage sequential extraction procedure proposed by BCR (Ure et al., 1993) (Table 2). They were specified as fraction B1: water soluble, exchangeable and carbonate bound; fraction B2: Fe–Mn oxide bound; and fraction B3: OM and sulfide bound. At the same time, subsamples were taken to determine the soil moisture content. A portion of soils S_1 and S_4 was air-dried, ground and passed through 1 mm mesh. The air-dried samples were extracted with the same fractionation analysis procedure as wet sample except for only 0.5000 g of dried soil was weighed. All the speciation analysis was performed duplicated and the average values were used for further analysis.

2.4. Determination of elements

Total contents of Cr, Ni, Zn, Cu, Pb, and Cd in plants and soils were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (VG Plasma-Quard 3, Fisons Instruments, UK) after samples were decomposed with 3 ml of HNO_3 – HF – HClO_4 (1:1:1) under high pressure conditions (Zhang and Shan, 1997). The ranges and mean values are presented in Table 3. The trace elements in each fraction were also determined by ICP-MS. The optimal operation conditions for the determination were reported previously (Li et al., 1998).

2.5. Reagent

All reagents used were of analytical reagent grade or better.

Table 2
Extractant and procedure used for fractionation of BCR

Step	Phase	Procedure
1	B1: water soluble, exchangeable and carbonate bound	0.1 M CH_3COOH , 20 ml, room temperature, shake for 16 h
2	B2: Fe–Mn oxide bound	0.1 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ (pH 2 with HNO_3), 20 ml, room temperature, shake for 16 h
3	B3: OM and sulfide bound	30% H_2O_2 , 5 ml, room temperature, 1 h, followed 85 °C, 1 h, occasionally shake; add 5 ml 30% H_2O_2 , 85 °C, 1 h; add 1 M NH_4 OAC (pH 2 with HNO_3) 25 ml, room temperature, shake 16 h

Table 3

Total concentrations of trace elements in soils, shoots and roots ($\mu\text{g/g}$)

Element	Concentration in soil		Concentration in root		Concentration in shoot	
	Range	Mean	Range	Mean	Range	Mean
Cr	15.19–57.65	44.13	5.51–11.52	8.93	0.60–0.89	0.71
Ni	6.31–30.05	19.50	2.71–7.02	4.29	0.27–1.28	0.47
Zn	28.18–71.95	50.13	22.2–54.00	34.90	9.47–39.34	16.03
Cu	5.79–66.05	25.20	6.21–29.67	15.22	4.01–15.71	6.56
Pb	19.22–53.61	29.91	4.29–10.60	7.98	0.36–20.00	2.38
Cd	0.26–0.76	0.54	0.09–0.53	0.24	0.04–0.33	0.09

2.6. Statistics

Statistic analysis was performed using the software of Microcal_Origin_5.0 (Northampton, USA).

3. Results and discussion

3.1. Speciation difference between air-dried and wet samples

Routinely, air-dried soil samples were used in soil analysis because it is easy to operate. However, when the soils were air-dried some of their chemical and physical properties may be altered. Such changes may be unimportant to total concentration but can significantly affect the speciation of trace elements in soils (Kennedy et al., 1997). Therefore, the veracity of using dried soil samples for the speciation analysis and bioavailability should be reconsidered.

Both the wet and dried rhizosphere soils (S_1) and bulk soils (S_4) were subjected to the fractionation analysis with BCR sequential extraction procedure and the distribution of trace elements in fractions B1, B2 and B3 was compared. Since a similar tendency can be found in S_1 and S_4 for all elements Cr, Ni, Zn, Cu, Pb and Cd. Results of Cr in S_1 are displayed as an example (Fig. 2). As it can be seen clearly that Cr in fractions B1 and B2 of air-dry samples is higher than that of wet samples. While in fraction B3, a rather reverse phenomenon appeared. For different elements in soils changes in the fractionation between wet and dried samples are different. Numerical comparisons for these changes are summarized in Table 4, which revealed the significant influence of air-drying on the fractionation of trace elements. The mean values of ratio of elemental speciation from wet to dried samples for all elements in fractions B1 and B2 are around 70–80% or even up to 50%. While in fraction B3, the ratio is almost 116–230% for all these elements. Rapin et al. (1986) studied the influence of air-drying on heavy metals' speciation in sediment samples, and Shuman (1980) and Barlett and James (1980) pointed out that air-drying the soils caused increased extractable Mn and Fe. The reasons for the

Fig. 2. Comparison of chromium concentrations in fractions B1, B2 and B3 between air-dried and wet rhizosphere soil (soil zone in S_1).

fractionation change are mainly focused on the chemical change of Fe, Mn, S and OM due to the oxidation of soil material occurred during the air-drying process. The oxidation of S and OM would release those elements initially organically and sulfide bound (fraction B3) into more labile and exchange phase (fractions B1 and B2). However, the newly formed Fe–Mn oxides would be highly redox reactive and they maybe immediately reduced through the oxidation of OM. Although the extractants and procedures they adopted were slightly different from that used in this paper, their elucidation can be borrowed to explain to some extent the results in this paper. Since the chemical oxidation of

Table 4
Ratio of fractionations extracted from wet samples to dried samples (w/d)

Element	Fraction	S ₁		S ₄	
		Range (%)	Mean (%)	Range (%)	Mean (%)
Cr	B1	57.51–88.33	72.52	52.50–93.90	73.10
	B2	28.33–84.25	67.73	47.70–96.70	74.20
	B3	101.20–133.00	116.69	106.21–275.50	166.60
Ni	B1	56.53–91.24	74.31	45.60–90.40	77.20
	B2	48.47–94.54	81.73	32.50–85.20	75.40
	B3	101.41–146.16	114.72	131.68–434.30	229.73
Zn	B1	52.37–87.14	75.11	31.30–84.50	57.80
	B2	29.61–96.54	77.20	20.90–83.90	44.20
	B3	103.57–208.80	128.60	119.60–419.07	231.21
Cu	B1	51.61–84.26	65.93	18.50–90.10	63.50
	B2	30.64–93.88	74.73	22.50–58.70	59.10
	B3	104.31–162.33	113.60	105.11–42.05	155.26
Pb	B1	49.71–94.39	70.98	31.70–88.50	72.50
	B2	31.39–91.74	77.86	25.40–92.80	83.71
	B3	100.50–149.25	130.46	109.90–171.4	113.10
Cd	B1	49.71–90.18	88.97	51.50–93.10	56.10
	B2	59.00–98.28	88.46	59.80–97.40	61.25
	B3	109.60–131.58	120.10	109.40–251.74	135.90

the soil OM and S occurs during the air-drying process, together with the reduction of Fe–Mn oxides, the amounts of trace elements in fraction B1 and fraction B2 of the wet soils were less than that of dry soils. While, in contrast, the organically bound and sulfide bound metals (B3) of wet soils were greater than that of air-dried soils. The experimental results came up to our expectation.

3.2. Comparison of relationship between speciation and bioavailability of trace elements in dried and wet samples

Single correlation analysis was performed to investigate the relationship between different chemical fractionations of Cr, Ni, Zn, Cu, Pb and Cd in soils and their plant concentrations. Both the shoot and root were treated in correlation analysis separately. The correlation coefficients are displayed in Tables 5 and 6, respectively. Except Cd the concentrations of trace elements in each soil fractions correlated poorly with that in the shoots. For Cd, the correlation coefficients of S₁ and S₄ to fraction B1 was found to be good ($r_{1,w} = 0.8666^{**}$, $r_{1,d} = 0.7948^{**}$; $r_{4,w} = 0.6849^{**}$, $r_{4,d} = 0.5996^{**}$), which clearly demonstrated that the correlation coefficients obtained by wet soil samples were better than that by air-dried samples. It also can be seen from Table 6 that the concentrations in fraction B1 have significant correlation with the root uptake. This is in good accordance with our previous findings (Li et al., 1998;

Zhang and Shan, 2001). The reason for different correlation between the shoots and roots and soil fractions may be due to the short culture period of plant. Since the wheat has been planted only for 8 weeks, the trace elements maybe mainly accumulated in roots and only little amount was transported to the shoots. Therefore, the relationship between the root uptake and elemental concentration in soil fractions will be used in the following discussion. Generally, all elements accumulated in root correlated well to the concentration of trace elements in fraction B1, while no significant correlation was found between the root accumulation and extracted concentration of elements in fraction B2 and B3. This phenomenon was also reported by others (Chlopecka and Adriano, 1996; Li et al., 1998). It can be clearly seen from Table 6 that the correlation coefficients obtained with wet samples were also better than that obtained with dried samples. For instance, the correlation coefficients of element Cr ($r_{1,w} = -0.7609^{**}$, $r_{1,d} = -0.5803^{*}$) showed this trend. This means that bioavailability of trace elements can be expressed well by using wet samples instead of dried samples.

3.3. Speciation difference among different soil zones by using wet samples

A comparison was also made between the extracted elements of soils obtained from rhizosphere, near rhizosphere, near bulk soil and bulk soil by using wet soil

Table 5

Correlation coefficients for trace elements in shoots and soil fractions ($n = 15$)

Shoot	Fraction	S ₁		S ₂	S ₃	S ₄	
		d	w	w	w	w	d
Cr	B1	0.2339	0.2832	−0.2290	−0.1041	−0.1806	−0.1468
	B2	0.2184	0.5311*	−0.1263	0.1965	0.2211	0.2827
	B3	0.1601	−0.1754	0.0698	0.0245	−0.3451	−0.0034
Ni	B1	−0.3377	−0.3525	−0.3433	−0.2488	−0.5130	−0.4184
	B2	0.0307	−0.0819	−0.2204	−0.2371	−0.1639	−0.0595
	B3	−0.4209	−0.4871*	−0.1414	−0.3498	−0.2624	−0.0300
Zn	B1	−0.2169	0.43216*	0.0505	0.0582	0.1203	−0.0735
	B2	0.3647	0.5895	0.0825	0.0385	−0.0482	−0.2429
	B3	−0.0762	0.1600	0.3425	−0.0024	−0.1379	0.1570
Cu	B1	−0.0187	−0.3134	−0.1016	0.1247	−0.1578	−0.1488
	B2	−0.1284	−0.0671	0.2545	0.2903	0.1425	0.0960
	B3	0.2150	0.2284	0.2340	0.0771	0.3650	0.0850
Pb	B1	−0.1386	−0.2102	−0.2326	−0.1034	−0.1692	−0.1071
	B2	0.0221	−0.0241	−0.1594	−0.0509	−0.0261	0.0131
	B3	−0.1170	0.0098	−0.0923	−0.0402	−0.2838	0.3356
Cd	B1	0.7949**	0.8666**	0.8277**	0.5775**	0.6849**	0.5995**
	B2	0.2647	0.1081	0.4849**	0.6832**	0.8287**	0.6257**
	B3	0.2486	0.2363	−0.3022	0.1609	0.6833**	0.0891

d = air-dried soil sample; w = wet soil sample.

* Significant at 0.10 probability level.

** Significant at 0.050 probability level.

Table 6

Correlation coefficients for trace elements in root and soil fractions ($n = 15$)

Shoot	Fraction	S ₁		S ₂	S ₃	S ₄	
		d	w	w	w	w	d
Cr	B1	−0.5032**	−0.6576**	0.4131	−0.1776	0.2759	−0.0942
	B2	0.1438	−0.0591	−0.0759	−0.1714	0.4305*	0.1783
	B3	0.3736	0.4583*	0.1752	0.1673	−0.4256	−0.3121
Ni	B1	0.4700*	0.5012*	0.3215	0.2883	0.3475	0.3109
	B2	0.0280	0.1349	0.3103	0.2558	0.2964	0.2483
	B3	−0.0245	−0.1743	0.3536	0.3444	0.0126	0.4579*
Zn	B1	−0.3400	−0.6345**	−0.3539	0.0014	−0.2628	−0.0609
	B2	0.1306	0.2366	0.4362	0.2362	0.3673	0.5581**
	B3	0.3900	0.1075	0.1420	0.2864	0.3680	0.4593*
Cu	B1	0.6911**	0.7408**	0.5172*	0.6295**	0.5258*	0.3253
	B2	0.5218*	0.5218*	0.3495	0.2594	0.5450**	0.5807**
	B3	0.4898*	0.4898*	0.5442**	0.5503**	0.1749	0.2840
Pb	B1	−0.7029**	−0.7116**	0.5481**	−0.2105	−0.2010	−0.1823
	B2	0.0610	0.0827	0.0034	−0.0175	0.0044	0.0245
	B3	−0.1148	−0.2553	−0.3907	−0.1791	−0.1315	−0.1331
Cd	B1	0.5803**	0.7906**	0.6552**	0.7544**	0.7222**	0.6965**
	B2	0.4202	0.1914	−0.0451	0.4520	0.7456**	0.6756**
	B3	0.0378	0.1918	−0.2238	0.1575	0.8501**	0.1196

d = air-dried soil sample; w = wet soil sample.

* Significant at 0.10 probability level.

** Significant at 0.050 probability level.

Table 7
Fractionation difference of elements in different soil zones

Fraction	Element	Range (%)				Mean (%)			
		S ₁	S ₂	S ₃	S ₄	S ₁	S ₂	S ₃	S ₄
B1	Cr	0.27–2.32	0.61–2.38	0.51–2.03	0.34–1.71	0.89	1.05	0.91	0.71
	Ni	1.30–13.63	1.68–15.79	1.39–14.02	0.5–9.71	5.39	6.09	5.71	2.95
	Zn	1.51–10.1	2.43–9.56	3.09–8.42	1.31–7.50	4.33	5.69	5.12	3.69
	Cu	1.33–5.71	1.82–12.17	1.01–5.57	0.37–4.32	2.97	4.36	3.27	1.68
	Pb	0.36–1.81	0.69–8.06	0.69–3.51	0.28–1.12	1.11	2.83	2.73	0.60
	Cd	8.11–31.67	11.6–43.7	8.15–31.52	2.52–16.26	15.7	22.9	20.5	7.60
B2	Cr	0.99–3.50	1.11–3.99	0.54–4.43	0.52–3.83	1.55	1.73	1.59	1.61
	Ni	2.47–20.49	4.07–30.03	1.50–23.79	1.87–23.23	8.19	11.09	8.43	8.08
	Zn	2.29–16.01	2.21–16.79	1.11–12.35	1.35–17.69	8.31	9.11	5.71	6.39
	Cu	1.23–8.35	1.71–10.68	0.99–27.9	1.26–20.9	4.13	5.62	5.22	6.03
	Pb	1.74–25.56	8.28–40.61	1.78–45.71	1.68–28.82	11.01	15.55	14.01	13.46
	Cd	11.22–46.44	19.11–42.19	11.97–32.22	12.18–35.84	27.05	33.93	24.81	25.93
B3	Cr	14.01–88.87	11.07–75.04	6.29–63.63	7.34–60.95	28.91	22.51	24.53	22.73
	Ni	33.74–79.33	13.02–52.49	10.74–86.15	11.86–68.13	69.02	21.73	34.27	33.61
	Zn	9.46–44.36	4.31–12.05	2.32–30.12	2.56–19.64	17.27	5.93	12.93	13.04
	Cu	32.41–85.39	6.84–55.80	11.41–81.01	7.44–86.02	56.49	24.53	44.73	44.82
	Pb	25.04–70.49	17.92–58.25	14.98–64.93	13.41–0.67	53.78	28.01	39.53	39.13
	Cd	11.69–35.74	47.22–89.52	10.70–69.4	12.27–58.57	16.64	52.41	26.46	27.87

The relative values were expressed as the concentrations in fraction B1, B2 and B3 to the total concentrations of elements of interest in soil.

samples. The percentage range and mean values of these elements in fractions B1, B2 and B3 compared to the total concentrations in soils are given in Table 7. Although the changes for the trace elements are different, yet there are still some rules to be found. For all elements Cr, Ni, Zn, Cu, Pb and Cd the concentrations in fraction B1 followed the order of $S_2 > S_3 > S_1 > S_4$. For fraction B2 the order was $S_2 > S_3 \approx S_4 > S_1$. However, for fraction B3, the order was $S_1 > S_3 \approx S_4 > S_2$, while for Cd the order was $S_2 > S_3 \approx S_4 > S_1$. The reason for different elemental fractionations in different soil zones maybe ascribed to the plant root growth.

Based on the above researches one can draw a conclusion that the rhizosphere zone (S_1) has the rather low fractions B1 and B2 and the highest fraction B3 among the four soil zones. The decrease in water soluble, exchangeable and carbonate bound form was also reported previously (Youssef and Chino, 1989). The reasons for that were attributed to the increase of pH in rhizosphere (Romkens et al., 1999). The absorbed rate surpasses the available rate, since the trace elements were transported to rhizosphere by diffusion (Youssef and Chino, 1989). The exchangeable and water-soluble forms may be recomplexed by organic material secreted by roots (Marschner et al., 1989). Decrease in fraction B2, the Fe–Mn oxides bound form, can also be interpreted by reduction of oxides by organic acid secreted from the plant roots (Godo and Reisenauer, 1980). While the increase in fraction B3, organically bound forms, may be

mainly owing to the abundance of organic materials in rhizosphere.

For soil zone S_2 , the fact that it has the relative higher concentrations in fractions B1 and B2, and relative lower concentrations in fraction B3 among four zones may due to microorganism activity. Since the mycelium was found to perforate the nylon cloth between the soil zones S_1 and S_2 . Microorganism can oxidize the organic materials (Jones et al., 1996). This mechanism can partly account for the changes of elements in fraction B3 to fraction B1 and fraction B2. Unfortunately, no detailed information can be provided here to explain it. In the case of soil zone S_4 , which in most case was the lowest or second lowest part in fractionations. As it was the further away from plant, in other words, it was the least affected by plant growth. Therefore, the fractionation of heavy metals in this soil zones may mainly exist in fractions that cannot be extracted by the BCR into mobile forms.

Although research on rhizosphere has been a hotspot because of its important role in the agriculture and environments. The processes involved in the rhizosphere are rather intricacy, and many factors can influence the environmental behaviors of elements in the rhizosphere. The results here cannot be simply interpreted by one single reason, the above-mentioned simple explanation only provide a clue. Many efforts should be made in the future that more information can be obtained and the complicated mechanisms can be explained more reasonably.

3.4. Relationship between speciation and bioavailability of Cr, Ni, Zn, Cu, Pb and Cd in four soil zones using the wet soil samples

The simple correlation coefficients of trace elements in different soil zones and root concentrations are listed in Table 6. It can be easily seen that the correlation coefficients of fraction B1 in rhizosphere soil has a better values ($r_{\text{Ni}} = 0.50126^*$; $r_{\text{Cu}} = 0.7408^{**}$; $r_{\text{Cd}} = 0.79063^{**}$), while the other three soil zones have relatively poorer correlation coefficients. These results suggest that the rhizosphere soils could be used instead of bulk soils in predicting the availability of trace elements in soils.

4. Conclusion

Based on the above comparison of speciation of trace elements in soils and their bioavailability between the air-dried soils and wet soils, between the nonrhizosphere soils and rhizosphere soils the following conclusions can be drawn:

1. Chemical reaction taking place during the air-drying of the wet soil samples result in the increase in water-soluble, exchangeable and carbonate bound form of elements, which form is considered as the most easily uptake speciation by plants.
2. Rhizosphere soil is a small but important environmental zone in soils with quite different physicochemical properties mainly caused by low-molecular-weight organic acids.
3. In order to simulate the conditions close to that in the real environment it should be strongly recommended to use wet soil samples taken from the rhizosphere for the study in the speciation analysis and bioavailability.

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