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Transformation of Copper Fractions in Rhizosphere Soil of Two Dominant Plants in a Deserted Land of Copper Tailings

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Abstract This paper studied the transformation of copper fractions in rhizosphere and non-rhizosphere soils of *Festuca arundinacea* and *Trifolium repens* in deserted land of copper tailings. The results showed that the proportion of organic-bound or exchangeable contents to the total contents of copper in rhizosphere increased by 9.81 and 10.42%, while the contents of carbonate-bound or Fe–Mn oxides-bound decreased by 2.96 and 1.82%, respectively. The growth and absorption of *F. arundinacea* and *T. repens* accelerated the transformation of copper fractions in rhizosphere soils. The effect of root system of *T. repens* was prominently greater than that of *F. arundinacea*.

Keywords Copper tailings · Copper · Fractions · Rhizosphere · *Festuca arundinacea* · *Trifolium repens*

Because of the effects of plant absorption, root secretion and microorganism movement in soil, rhizosphere soil differs from the nature one on many aspects including physical, chemical, biological characteristics. These differences directly affected the chemical processes of heavy metals in the rhizosphere soil, and their availability and toxicity to plants. Therefore, studies on rhizosphere environment is especially important to understand the environmental implications of heavy metals soil-plant interfaces, and their noxious effects in soil as well as the resistance and endurance of plants (Nye 1981; Chaignon et al. 2002; Chaudhuri et al. 2003). In recent years, a large number of scientific researches have been reported about the effects of rhizosphere environment on the change of heavy metal fractions. Considerable advances have been achieved in the areas of fraction transformation and availability of heavy metals in rhizosphere in soil solution, solid soil and their hyperaccumulative uptake process (Bernal et al. 1994; Chaignon et al. 2002; Lin et al. 1998; Chen et al. 2005; Wang et al. 2003). However, as a result of interactions between plants and heavy metals, the changes in heavy metal fractions in rhizosphere environment is particularly affected by discrepancy of plant classes or in response to the changes in rhizosphere environment. Presently, most studies on rhizosphere environment either are focused on the content changes of heavy metals (or their available fractions) or employ only solid soil of rhizosphere, or artificial rhizosphere environment such as rhizosphere box, rhizosphere bag etc. (Lin et al. 2000). As a matter of fact, these simulated rhizosphere environments differ greatly from the natural growth environment. Therefore, it is crucial to employ natural growth environment to investigate how environmental action can influence heavy metals in soil-plant system, how heavy metal fractions are transformed in solid soil of rhizosphere in response to the environment action, and how these changes affect on plant's resistance to these heavy metals.

As an important base for copper mining and smelting in China, Tongling City is situated in the south of Anhui Province, on the south bank of the low middle reaches of Yangtze River. A number of studies on activity of enzyme in soil and patterns of vegetation succession on the deserted land of copper tailings in Tongling City had been reported (Wang et al. 2003, 2004). However, so far, no research has been reported about transformation of heavy metal fractions in rhizosphere soils of wild plants that naturally grow in these large deserted lands of copper tailings. *Festuca*

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arundinacea and *Trifolium repens* were dominant plants in the deserted land of copper tailings in Tongling City, which appeared to have ability to restore the deserted land of copper tailings. Meanwhile, they had a superior quality to be used as lawn grass. For this reason, investigations had been conducted on the deserted copper tailings from July 2003 to May 2006. *T. repens* and *F. arundinacea* that naturally grew on the copper tailings were chosen for the experiments. To provide reliable data for vegetation reconstruction and ecosystem protection of land with copper tailings, this experiment investigated the transformation of Cu fractions in rhizosphere environment in a large-scale deserted land of copper tailings by comparing the samples from rhizosphere to non-rhizosphere soils.

Materials and Methods

The deserted land of Shizishan copper tailings was selected as the research site. This deserted land was roughly 20 hm^2, piled up by copper tailings. It lies in Village Chaoshan in southeastern Tongling City, with mountainous areas on three sides and a dam that is about 100 m on the other side. About 10 years ago, the discharge of copper tailings was stopped. With few ecesis plants, the surface of the deserted land was commonly unstable and unstructured with poor water and fertilizer retention ability. Interestingly, the areas with improved soil quality were often accompanied with the existence of ecesis plants, particularly, *F. arundinacea* and *T. repens*. Similar size of plants (*F. arundinacea* and *T. repens*, 10 cm in height) were dug out from soil. The tiny soil layer ($\sim$1 mm in thickness from the surface of root system) was picked as rhizosphere soil samples. For each species, 8 spots were chosen, and total 16 rhizosphere soil samples were collected. The non-rhizosphere soil samples were taken from the corresponding spots about 8–10 cm surrounding the above plant root systems. Eight spots were chosen for each species, and total 16 non-rhizosphere soil samples were gathered. Based on the depth of *F. arundinacea* and *T. repens* plants, the soil samples were collected within the depth between 0 and 20 cm. To investigate plant absorbability of exchangeable fraction of Cu in rhizosphere soil, we collected plant samples with different size, divided them into 4 groups based on their heights (<5, 5–10, 10–20 and >20 cm), and took the average biomass of all plants in one group as the biomass of that group. Each group had 10 plants. The corresponding rhizosphere soil samples or non-rhizosphere soil samples were also collected and divided into 4 groups. All plants and soil samples were then brought into laboratory for analysis.To evaluate how growth time of *F. arundinacea* or *T. repens* plants affected Cu fraction distribution in the rhizosphere soil, the growth of *F. arundinacea* and *T. repens* plants was periodically recoded from January 2004, and the plant samples and rhizosphere soil samples were collected since the plants were 2–3 cm above the ground (10 plants and 10 soil samples for each species were collected). Those samples were labeled as in 30, 60, 90 and 120 days, respectively. According to the sequential fractionation method and error adjustment method for remaining liquid (Lin et al. 1998), a proportion of liquid to soil of 10:1 was made for Cu fraction extraction. The extraction of Cu fractions was based on the following definition: exchangeable, carbonate-bound, Fe–Mn oxides-bound, organic-bound and residual. The content of residual fraction was estimated by the difference between the total Cu content and the summation of contents of exchangeable, carbonate-bound, Fe–Mn oxides-bound, and organic-bound fractions. Soil pH and the contents of water-soluble salts, organic matter and N, P, and K were measured by the methods of Environmental Monitoring of China (1992). The content of Cu was determined by atomic adsorption spectrophotometer (AAS) [AA6800, Shimadzu, Japan]. To avoid cross-contamination of Cu or other metals, all receptacles had been soaked in 2% HNO_3 for more than 24 h before used.

Data were analyzed using SPSS (Version 11.0), and regression, correlation analysis and *t*-test were used for determining the significance of difference among various groups of plant and soil samples. At the same time, we used relative change percent (%) defined as following formula:

$$R = \left[\frac{\sum_{i=1}^{n}\left(C_{ij}/C_{iT}\right)}{\sum_{i=1}^{n}\left(C_{0ij}/C_{0iT}\right)} - 1\right] \times 100\%$$

where R is the relative change percent, C_{ij} is the Cu content of fraction j in rhizosphere, C_{iT} is the total content of Cu in rhizosphere soil, C_{0ij} is the Cu content of fraction j in non-rhizosphere, C_{0iT} is the total content of Cu in non-rhizosphere soil, n is number of samples in one group, and it equals 10 in this study.

Results and Discussion

Because of the influences of plant root absorption, secretion, the activities of microbes in the soil, etc., the rhizosphere soil of the *F. arundinacea* and *T. repens* plants had prominent differences in chemical composition compared to the non-rhizosphere soil (Table 1).

In general, the nutrient contents of the rhisosphere soil are increased in some extent, and the overall soil quality was improved. The secretion of organic acid in the rhizosphere lowered the pH from an alkaline soil of the deserted land of copper tailings to be more neutral one. The content of organic matter was increased by an average of 27.8%

Table 1 Physical and chemical properties of soils

Category	pH	Organic matter (g/kg)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Water-soluble salt (g/kg)	Total Cu (mg/kg)
Non-rhizosphere soil of *F. arundinacea*	8.01 ± 0.23[a]	4.33 ± 0.25	0.28 ± 0.05	2.02 ± 0.08	41.42 ± 3.87	1.61 ± 0.14	978.76 ± 67.26
Rhizosphere soil of *F. arundinacea*	7.46 ± 0.17	5.96 ± 0.37	0.31 ± 0.04	2.23 ± 0.11	42.35 ± 4.11	1.77 ± 0.18	920.45 ± 50.43
P[b]	0.00	0.01	0.03	0.03	0.02	0.00	0.00
Non-rhizosphere soil of *T. repens*	7.75 ± 0.24	6.76 ± 0.54	0.61 ± 0.05	2.98 ± 0.33	48.75 ± 4.64	1.83 ± 0.19	812.65 ± 65.47
Rhizosphere soil of *T. repens*	7.13 ± 0.11	8.64 ± 0.65	0.74 ± 0.05	4.08 ± 0.49	51.67 ± 5.89	2.02 ± 0.15	736.69 ± 50.69
P	0.01	0.00	0.01	0.04	0.04	0.01	0.01

[a] Stdevp, n = 8

[b] Concomitant probability of *t*-test

although its value was still relatively low compared with the non-rhizosphere soil. The contents of water-soluble salts in rhizosphere soils were enhanced in different extent, while the content of total Cu decreased remarkably.

Similar distribution of Cu among various fractions was observed in rhizosphere and non-rhizosphere soils; the residue fraction accounted for the majority of the total contents of Cu, whereas exchange fraction was relatively low (Table 2). The rhizosphere soils demonstrated similar trend of changes of Cu contents in fractions. The rhizosphere soils exhibited high relative change percentages for organic-bound and exchangeable fractions compared to the non-rhizosphere soils. The change of relative change percentage of exchangeable fraction was noticeably 10.42%, while that of organic-bound Cu was 9.81%. Consequently, the contents of carbonate-bound and Fe–Mn oxide-bound decreased by 2.96 and 1.82%, respectively.

Because plant growth and root system metabolism are incessant processes, the rhizosphere environments are under the influence of ceaseless variations, resulting in a dynamic movement of Cu fractions in rhizosphere soils. The contents of bio-available heavy metals are commonly correlated to soil physicochemical properties. Therefore, they directly affect the growth of plants, which can further influence the distribution of heavy metal fractions in rhizosphere soils. The results in this investigation showed that the biomass of *F. arundinacea* or *T. repens* increased as the plant absorption of Cu increased (Table 3). It could be presumed that Cu in plants mostly came from direct absorption of available fractions in the soil. Interestingly, although plant absorption increased prominently with growth, compared to the change of Cu availability in non-rhizosphere soil, the contents of available Cu in rhizosphere soil of *F. arundinacea* and *T. repens* were elevated rather than reduced. Despite a small decrease subsequently, the content of available Cu in the rhizosphere soil was still generally greater than that of non-rhizosphere soil. This demonstrated that the absorption of Cu by *F. arundinacea* and *T. repens* induced Cu transformation from non-available fractions into available fractions.

There was an obvious increase in organic matter content in the rhizosphere soil of *F. arundinacea* and *T. repens* (Table 1), and this remarkably enhanced Cu content in organic-bound fraction (Table 2). The secretion of organic acids of root systems of *F. arundinacea* and *T. repens* decreased the pH of soil that were in the vicinity of the roots, resulting in the increase of Cu content in the exchangeable fraction. As a result of this, Cu contents in carbonate-bound fraction, Fe–Mn oxides-bound fraction, and even organic-bound fraction were transformed into

Table 2 Distribution of Cu fractions and results (*P*) of *t*-tests in *F. arundinacea* and *T. repens* rhizosphere soils

Category	Exchangeable (mg/kg)	Carbonate-bound (mg/kg)	Fe–Mn oxides-bound (mg/kg)	Organic-bound (mg/kg)
Non-rhizosphere soil of *F. arundinacea*	49.77 ± 2.22[a]	78.95 ± 4.11	61.24 ± 2.97	64.67 ± 7.46
Rhizosphere soil of *F. arundinacea*	51.05 ± 3.08	72.75 ± 4.35	56.79 ± 4.36	65.46 ± 6.31
P[b]	0.01	0.02	0.03	0.01
Non-rhizosphere soil of *T. repens*	41.15 ± 3.01	65.86 ± 5.51	48.49 ± 4.33	56.45 ± 5.39
Rhizosphere soil of *T. repens*	41.69 ± 4.15	57.48 ± 5.28	43.03 ± 2.47	57.52 ± 8.25
P	0.00	0.03	0.03	0.01

[a] Stdevp, n = 8

[b] Concomitant probability of *t*-test

Table 3 Contents of available Cu in different biomass of *F. arundinacea* and *T. repens* and their rhizosphere or non-rhizosphere soil

Plant	Dry weigh (g)	Contents of Cu in plant (mg/kg)	Contents of available Cu in non-rhizosphere soil (mg/kg)	Contents of available Cu in rhizosphere soil (mg/kg)
F. arundinacea	0.31	58.76 ± 4.25[a]	104.75 ± 12.33	115.24 ± 10.08
	0.53	81.22 ± 6.32	104.25 ± 10.64	123.75 ± 13.66
	0.65	89.76 ± 5.59	105.14 ± 14.78	125.15 ± 11.41
	0.88	88.04 ± 6.45	108.76 ± 12.53	126.32 ± 15.79
T. repens	0.21	61.35 ± 5.47	112.18 ± 12.25	119.64 ± 13.65
	0.45	89.49 ± 7.48	114.18 ± 13.06	138.49 ± 11.25
	0.63	92.77 ± 10.02	112.79 ± 12.45	132.75 ± 14.69
	0.85	91.84 ± 8.15	112.30 ± 10.08	128.69 ± 13.46

[a] Stdevp, n = 8

exchangeable fraction. Compared to the residual fraction, carbonate-bound fraction, Fe–Mn oxides-bound fraction, organic-bound fraction had a high potential to become bioavailable fraction, and therefore they served as a potential nutrition pool to plants. If the proportions of these fractions changed, the capacity and mobility of heavy metals in soil would change (Zhou and Sun 2002; Zhou et al. 2004). Thus, it could be inferred that the absorption of Cu by *F. arundinacea* or *T. repens* might be not necessary to only reason for the decrease of Cu content in the rhizosphere soil. The growth of plants and the secretion of root systems might be far more influential on the distribution of Cu fractions in soils than the plant absorption itself. In fact, the available fractions of Cu in soil are highly mobile, and subjected to lose by exotic factors, such as simple rainwater. As the weakly bound fractions, such as carbonate-bound fraction and Fe–Mn oxides-bound fraction, tend to resolve into available fractions. Therefore, the importance of these fractions should not be neglected.

Table 4 showed the change of Cu fractions in the rhizosphere soil influenced by growth time. Between 30th and 60th day of the growth period, the relative change percentage of the exchangeable fraction first rose rapidly, and then increased slightly. This level was kept until the end of the period. The change of the organic-bound fraction was similar to that of exchangeable fraction with somewhat smaller increase range. Compared to the non-rhizosphere soil, no matter how many times the plants grown, the Cu contents in carbonate-bound fraction and Fe–Mn oxide-bound fraction in the rhizosphere soil decreased evidently. In the first 60 days of plant growth, the proportions of those fractions to the total contents of Cu decreased rapidly, and then the speed of decrease slowed.

The change of different fractions of heavy metals in crop rhizosphere soil under simulated environment had been studied by Chen et al. (2002, 2003). The results showed that the factions of heavy metals in rhizosphere soil had changed regularly in different time during plant growth period. Metal contents in exchangeable fraction, carbonate-bound fraction, Fe–Mn oxides-bound fraction were increased first, and then decreased. At the end, the contents of exchangeable fraction and carbonate-bound fraction were lower than those of non-rhizosphere soil. However, organic-bound fraction decreased gradually to the minimum, and then increased gradually during the rest of growth period. Our results, however, showed that the proportion of exchangeable fraction increased rapidly, and kept in the level until the end of the period, regardless of the growth time. Although the percentage of the change of the organic-bound fraction was less than that of exchangeable fraction, the increase was evident, and greater than that of the non-rhizosphere soil. Conversely,

Table 4 Relative change percent and results (*P*) of *t*-tests of Cu fractions in *F. arundinacea* and *T. repens* rhizosphere soils under different growth time

Plant	*F. arundinacea* (days)					*T. repens* (days)					P[a]
	0	30	60	90	120	0	30	60	90	120	
Exchangeable	3.65	5.83	9.09	8.44	8.56	5.01	8.78	12.24	12.16	11.80	0.00
Carbonate-bound	−0.94	−1.75	−2.12	−1.96	−1.94	−0.93	−2.06	−3.83	−3.64	−2.88	0.03
Fe–Mn oxides-bound	−0.43	−1.02	−1.32	−1.05	−0.98	−0.83	−1.93	−2.27	−1.98	−2.03	0.04
Organic-bound	3.02	6.45	8.23	8.15	8.18	4.01	8.12	10.64	9.67	9.20	0.01

[a] Concomitant probability of *t*-test

Cu contents of carbonate-bound fraction and Fe–Mn oxides-bound fraction decreased in the rhizosphere soil, and they tended to keep in a final level after a long time of growth. The possible explanation can be: compared with the simulated cultivation, the growth environment of natural plants is open and infinitely contaminative. The absorption of single plant has little impact on the total content of the contaminants in the environment. Some factors such as rainwater, animal and microbe actions, and the multiplication of other plants may prompt the change of the fractions of Cu in soil, and they transfer Cu fractions in physical spaces. In the condition of artificial growth, however, many factors are well controlled, and the impact of plant absorption on the assimilated fractions of Cu should not be ignored.

Zhang (1993) claimed that the species of leguminosae and gramineae were different genotypes, and they had a great difference in absorption of heavy metals and alimentary elements in terms of different abilities of biologic feedback and adjustability. Although both *F. arundinacea* and *T. repens* grew well and were preponderant plants in the deserted land of copper tailings in Tongling City, the distribution of Cu fractions in the rhizosphere soil were obviously different. The relative change percent of the contents of Cu fractions in *T. pratens* rhizosphere soil was prominently greater than that in *F. arundinacea* rhizosphere soil (Table 4). These differences might result from the obvious distinctions in genetic, ecological and physiological characters between *T. repens*, which belongs to leguminosae, and *F. arundinacea*, which belongs to Gramineae. This hypothesis was in consistent with the results by Chen et al. (2003).

The present study indicated that *F. arundinacea* and *T. repens* were dominant plants and grew well in the deserted land of copper tailings in Tongling City. The rhizosphere environment of *T. repens* possessed a prominently higher relative change percentage for each fraction of Cu than that of *F. arundinacea* (Table 4). In fact, the varieties of fractions of heavy metals in rhizosphere soil were result from the collective function of plants and heavy metals. The different rhizosphere environmental conditions of diversities of species led to the difference in the changes of fractions of heavy metals in rhizosphere. These differences inevitably influenced the bioavailability of heavy metals (Zhou et al. 2004). Therefore, when we evaluate the bioavailability of heavy metals, both physicochemical characteristics of the soil and plant species and their qualities on the soil must be considered (Bernal et al. 1994; Sterckeman et al. 2005). Not until a full understanding on the chemistry and biology of soil-plant system will we realize the most appropriate plant species with characteristics in endurance and resistance to be used for the restoration of the soil contaminated with heavy metals.

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