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Responses of Microorganism in the Rhizosphere of Winter Wheat Seedlings to a Low Concentration of Lead

Xia Jia, Suiming Dong and Chunjuan Zhou
School of Environment Science and Engineering, Chang'an University,
Yanta Road, No. 126, Xi'an 710054, China

Abstract: Pot experiments were conducted to investigate the effect of a low concentration of lead (<300 mg/kg, soil pH = 8.45) on the rhizosphere microbial community of winter wheat seedlings. The ability of the microorganisms to utilize carbon sources was notably altered and a principal component analysis also showed that the microbial functional diversity was significantly affected by a low concentration of lead. The number of fungi decreased significantly ($p<0.01$) under the stress of a low concentration of lead. The fungal/bacterial population ratio decreased markedly with the increase of lead during three growth stages. Our results would be helpful for future proposals and concerning decisions of environmental quality standards for soils with regard to lead thresholds for agricultural land.

Keywords: Low concentration of lead, rhizosphere microorganism, winter wheat seedlings

INTRODUCTION

The main sources of soil pollution are improper waste dumping, abandoned industrial activities, incidental accumulation, atmospheric fallout and agricultural chemicals (Alloway, 1994; Joubert *et al*., 2007). Furthermore, soil contamination with heavy metal is a serious environmental hazard and is costly to remediate (Lin *et al*., 2010). Among heavy metals, lead is a nonessential element that can be taken up by plants and its subsequent accumulation along the food chain is a potential risk to human health (Lin *et al*., 2010). The mobility of lead in the soil depends on physical, chemical and biological processes, which mainly include plant rhizosphere microbial activities and plant root growth behavior. Rhizosphere microbes play a crucial role in energy flow, element cycling and the turnover of organic matter in ecosystems and a well-functioning microbial community is a prerequisite for soil fertility and the resilience to external factors (Niklińska *et al*., 2005). The rhizosphere is a zone with enhanced microbial activity and the rhizosphere microbial community is an important factor in determining the survival and sustainable growth of plants (Zhang *et al*., 2011). Chronic exposure to elevated concentrations of lead can change the composition and function of rhizosphere microorganisms through the direct effects of lead and the indirect influences of changes in the root exudates under the stress of lead (Martínez-Iñigo *et al*., 2009). However, a low concentration (<300 mg/kg dry weight soil, soil pH>7.5) of lead is widely recognized as being potentially nontoxic to living organisms (Malkowski *et al*., 2002; Pang *et al*., 2001). Although the effect of a low concentration of lead on plant growth and metabolism is insignificant, its influence on plant growth is evident when accompanied by other heavy metals, such as cadmium (Zhao *et al*., 2002; Du *et al*., 2010). The composition of trace elements in the soil will represent a permanent threat to the function of soil ecosystems, likely through the disturbance of the microbial community. However, due to a lack of relevant studies, it remains unclear how rhizosphere microbial communities respond to a low concentration (<300 mg/kg dry weight soil, soil pH>7.5) of lead. In addition, detailed research on the effect of a low concentration of lead on rhizosphere microorganisms is also needed for establishing future environmental quality standard for soils. Currently, the lead threshold for agricultural land in China was 350 mg/kg dry weight soil when the soil pH value is over 7.5 (Environmental quality standard for soils, GB 15618-1995). However, it is not certain whether the current threshold for lead is reasonable for all types of soil and the maintenance of a lasting soil micro ecology function for cultivation.

The aim of this study was to test the sensitivity of microbial communities in the rhizosphere of winter wheat seedlings to a low concentration of lead (<300 mg/kg dry weight soil, pH = 8.45). To understand the role of microbial communities, information on the functional diversity, which is represented by the catabolic potential of the community, is essential. The Biolog system, which quickly, economically and effectively determines diversity profiles, was used to examine the catabolic potential of the microorganisms

Corresponding Author: Xia Jia, School of Environment Science and Engineering, Chang'an University, Yanta Road, No. 126, Xi'an 710054, China, Tel.: 13772931503

and the evaluation of Colony-Forming Units (CFUs) was used to assess the microorganism population in the rhizosphere of winter wheat seedlings.

MATERIALS AND METHODS

Methods:

Pot experiment and soil sampling: Four levels of lead (50, 120, 180 and 230 mg/kg dry weight soil, respectively) were selected by the environmental quality standard for soils (GB 15168-1995) in China. The pot (H 46 cm×D 36 cm) experiment was conducted using winter wheat seedlings. The soil for the pot experiment was collected from cornfield in Central Shaanxi, China (108° 54' E, 34° 16' N), using the top 20 cm layer at 8 locations. Some properties of the soil are as following: pH, 8.45; organic m content, 17.17; total nitrogen, 1.12; available P, 71.17 g/kg; exchangeable K, 575.00; soluble salt 0.74; total lead, 7.35 mg/kg, respectively and soil type is brown soil.

The soil was air-dried, sieved to a diameter of <2 mm, mixed and homogenized for treatments with $(CH_3COO)_2$ Pb. The plastic pots were filled with 15 kg of the contaminated soil after it was consolidated for 30 days; three replicates for each treatment were prepared. The control treatments (7.35 mg/kg dry weight soil) were left uncontaminated. The soil moisture was brought to 60% of field capacity and the pots were placed in the open air. Winter wheat (*Triticum aestivum* L.) seeds were planted in each pots on October 1st, 2010, with 100 seedlings in each pot after emergence. The pots were maintained at 60% field capacity (using constant weight) by irrigation during the seedling growth stage. The soil strongly adhering to the roots and within the space explored by the roots was considered the rhizosphere soil (Sinha *et al*., 2009). Rhizosphere soil samples were collected when the winter wheat seedlings had grown for three weeks, seven weeks and twelve weeks. The winter wheat roots were collected from five areas in each pot and the rhizosphere soil was carefully collected, mixed and homogenized to obtain 0.3-0.5 kg. Each sample was then divided into three subsamples and analyzed for the microbial functional diversity, the number of microorganisms and soil water content.

Soil microbial analysis: The Colony-Forming Units (CFUs) of the bacteria, actinomyces and fungi were determined using a modified plate dilution technique on meat-peptone agar, Gause's starch agar and Martin agar, respectively (Yang *et al*., 2009). The number of each group of microbes was determined using the average of three replicate soil samples.

The Biolog EcoPlate system (Biolog Inc., USA) was used to discriminate the metabolic diversity of the microorganisms in the rhizosphere of the wheat seedlings. The rhizosphere soil samples were homogenized with sterile saline (0.85% NaCl) into slurries, with a final soil concentration of 1.000 g dry weight/L. The carbon source utilization patterns of the microbial communities were assessed using Biolog 96-well Eco-Micro-plates, which contained three replicate wells of 31 carbon sources and a water blank (Douterelo *et al*., 2010). A 150-μL aliquot of the soil suspension was added to each well of the microplate, which was then incubated in the dark at 28°C for 168 h until no further color development occurred. The color development in the wells was measured at 590 nm every 12 h.

Analysis methods: The intensive metabolism of carbon sources was evaluated by determining the area under the absorbance versus time curve for each well using an excel-based trapezoidal approximation. The area of the trapezoidal was as follows:

$$S = \sum_{i=1}^{n} \frac{v_i + v_{i-1}}{2} \times (t_i - t_{i-1})$$

where, v_i was the value of the AWCD when the Eco-Micro-plates were cultured for i time (Guckert *et al*., 1996). The Average Well Color Development (AWCD) was calculated for each microplate as AWCD = ∑ (C-R) /N, where C is the raw absorbance in each well, R is the absorbance in the control well (A1 well) and N is the number of substrates in the plate. The functional diversity index of the rhizosphere microbial community was calculated based on the data recorded over 168 h.

Shannon and Weaver (1963) diversity index (H') was calculated to evaluate the microbial functional diversity, as follows: H' = -∑pi ln (pi), where pi is the proportional color development of the well in relation to the total color development of all of the wells of a plate. AWCD for six categories of carbon source was calculated as AWCD = ∑ (Ai - Ai1) /n, where Ai is the relative absorbance in the i well, Ai1 is the relative absorbance in the Ai1 well and n is the number of substrates selected.

A Principal Component Analysis (PCA) was used to normalize the Biolog absorbance values based on the AWCD values. All of the statistical analyses were all conducted using SPSS 15.0 software. All of the values are expressed as the mean±S.E. (n = 3).

RESULTS AND DISCUSSION

Effects of low concentrations of lead on rhizosphere microflora: Table 1 shows the effects of the low concentration of lead on the rhizosphere soil microflora. The number of bacteria at low concentrations of lead

Table 1: The number of microbes (counts/g dry soil) in the rhizosphere of the winter wheat seedlings

		Treatments				
Growth stage	Items	The control	T1[a)]	T2	T3	T4
Three weeks	Bacteria ($\times10^7$/g)	3.79±0.01$^{Ab)}$	3.52±0.91^{B}	3.42±0.91^{C}	3.030±1.00^{D}	2.760±0.22^{E}
	Fungi ($\times10^5$/g)	1.79±0.10^{A}	1.59±0.11^{B}	1.00±0.01^{C}	1.250±0.09^{D}	0.880±0.03^{E}
	Actinomycete ($\times10^7$/g)	0.73±0.02^{A}	3.16±1.00^{B}	2.70±0.83^{C}	2.630±0.19^{D}	2.170±0.95^{E}
	Total ($\times10^7$/g)	4.54±0.15^{A}	6.70±2.02^{B}	6.12±1.75^{C}	5.670±1.29^{D}	4.940±1.21^{E}
Seven weeks	Bacteria ($\times10^7$/g)	2.57±0.17^{A}	2.24±0.57^{B}	2.46±0.52^{C}	2.140±0.19^{D}	2.517±0.22^{E}
	Fungi ($\times10^5$/g)	4.55±0.53^{A}	2.90±0.22^{B}	2.95±0.86^{C}	2.620±0.22^{D}	2.470±0.82^{E}
	Actinomycete ($\times10^7$/g)	2.94±0.43^{A}	2.01±0.35^{B}	2.57±0.42^{C}	1.980±0.31^{D}	1.950±0.43^{E}
	Total ($\times10^7$/g)	5.51±1.13^{A}	4.26±1.14^{B}	5.03±1.80^{C}	4.130±0.73^{D}	4.460±1.27^{E}
Twelve weeks	Bacteria ($\times10^7$/g)	0.74±0.02^{A}	0.75±0.02^{A}	0.95±0.09^{a}	1.030±0.02^{a}	1.920±0.87^{Bb}
	Fungi ($\times10^5$/g)	1.55±0.09^{A}	1.44±0.05^{AB}	1.32±0.09^{B}	1.350±0.09^{B}	1.010±0.08^{C}
	Actinomycete ($\times10^7$/g)	1.30±0.21^{A}	1.32±0.11^{B}	0.62±1.22^{C}	1.020±0.12^{D}	1.200±0.42^{E}
	Total ($\times10^7$/g)	8.74±0.41^{A}	8.79±0.24^{B}	10.14±1.40^{C}	11.375±0.22	20.450±1.83^{D}

[a)]: The T1, T2, T3 and T4 treatments were 50, 120, 180 and 230 mg/kg dry weight soil, respectively, the same below; [b]: Different capital letters in the same row at the same growth stage indicate significant differences at p<0.01; LSD test; Each value represents the mean±S.E.

Table 2: The intensive metabolism of carbon sources by rhizosphere microorganisms

	Treatments				
Growth stage	The control	T1	T2	T3	T4
Three weeks	273.63±0.32^{A}	285.80±0.99^{B}	256.27±0.57^{C}	243.79±0.87^{D}	255.51±0.16^{C}
Seven weeks	265.25±0.54^{A}	258.97±0.55^{E}	250.22±0.68^{C}	246.66±0.92^{D}	200.73±0.52^{B}
Twelve weeks	273.01±0.73^{A}	222.85±0.72^{B}	270.96±0.42^{C}	220.08±0.87^{D}	237.62±0.65^{E}

Different capital letters in the same row at the same growth stage indicate significant differences at p<0.01; LSD test; Each value represents the mean±S.E.

decrease significantly (p<0.01) by 7.27-27.28% at the third week and by 1.99-16.19% at the seventh week; conversely, the abundance increased notably by 0.54-159.30% at the twelfth week. The number of fungi significantly (p<0.01) decreased by an average of 7.47-50.92% at the 3 time points. The amount of actinomyces with the addition of lead increased significantly (p<0.01) by an average of 198.21-334.34% at the third week and decreased significantly (p<0.01) by averages of 12.84-33.64% and 7.63-52.54% at the 7th and 12th weeks, respectively. The total number of microorganisms significantly (p<0.01) increased under the lead treatments, with the exception of the seventh week. Bacteria were predominant in the rhizosphere of the winter wheat seedlings, accounting for 46.46-94.09% of the total number of microorganisms at the low concentration of lead, followed by actinomycetes at 5.86-53.46% and fungi, accounting for 0.05-0.40%.

The responses of the number of the rhizosphere microorganisms to the low concentrations of lead in the three growth stages were most likely due to the shift of the root exudation patterns. Several studies have demonstrated that root exudation is a major factor controlling microbial activity and community structure in the rhizosphere (Kozdroj and van Elsas, 2000; Baudoin *et al*., 2003; Kumpiene *et al*., 2009). Therefore, the release of carbohydrates, organic acids, amino acids and vitamins from the winter wheat roots had a direct effect on the microbial populations in the rhizosphere (Martínez-Iñigo *et al*., 2009; Zhang and Wang, 2002). In addition, lead can stimulate the elongation of the root, increase the root biomass and promote the formation of root hairs in *Syringa vulgaris* populations (Wierzbicka and Panufnik, 1998) and lead may have had a similar effect on the winter wheat seedlings in the present study. Hence, there would be some differences in the quality and the quantity of the root exudates, which would influence the number of microorganisms in the rhizosphere. Moreover, the direct effect of the low concentration of lead on the number of microorganisms was not negligible. This direct effect would become weak because the absorption and accumulation ability of the roots to lead would intensify with the duration of the growth of the seedlings (Lin *et al*., 2003). The difference of the proportion of each microorganism contributing to the total suggested that some changes occurred in the composition and structure of the microbial communities for adapting to this special environment of low concentrations of lead. As there is a positive and significant correlation between the microbial biomass and the number of microbes and a higher fungal/bacterial population ratios may also generally reflect a more sustainable agroecosystem (Tobor-Kaplon *et al*., 2005; De Vries *et al*., 2006; Song *et al*., 2008), the decrease of the fungal/bacterial population ratios at the low concentration of lead (Table 1) predicts that the function of the soil microecology in the rhizosphere of the winter wheat seedlings would become weak in the future.

Temporal changes in the intensive metabolism of carbon sources: The intensive metabolism of carbon sources by the rhizosphere microorganisms at the low concentration of lead decreased significantly (p<0.01) in the three growth stages, with the exception of T1 at the third week (Table 2). The intensive metabolism of

Table 3: The utilization ratio of six carbon sources by rhizosphere microorganisms (absorbance)

Growth stage	Treatments	Carbohydrate	Carboxylic acids	Amino acid	Polymer	Phenolic acid	Propylamine
Three weeks	Control	1.12±0.05^{A}	1.22±0.01^{A}	0.69±0.02	0.79±0.02^{A}	0.55±0.03^{Aa}	1.13±0.03^{Aa}
	T1	0.98±0.01^{B}	1.10±0.02^{Aa}	0.65±0.04	0.57±0.04^{Ba}	0.67±0.05^{Bb}	1.09±0.04^{Aa}
	T2	1.22±0.02^{C}	1.28±0.03^{Ab}	0.67±0.05	0.85±0.04^{A}	0.70±0.03^{Ba}	0.86±0.04^{B}
	T3	0.94±0.03^{B}	1.16±0.02^{A}	0.68±0.03	0.55±0.05^{Ba}	0.60±0.02	1.26±0.02^{Ab}
	T4	0.95±0.01^{B}	1.95±0.06^{B}	0.61±0.03	0.71±0.02^{b}	0.60±0.02	1.02±0.01^{Aa}
Seven weeks	Control	1.06±0.12^{a}	1.01±0.03^{A}	0.58±0.02^{A}	0.56±0.00^{a}	0.59±0.03^{a}	1.15±0.10^{A}
	T1	1.06±0.11^{a}	1.14±0.02^{A}	0.54±0.01^{A}	0.71±0.02^{b}	0.73±0.01^{Ab}	0.80±0.02^{B}
	T2	0.84±0.10^{b}	0.92±0.12^{B}	0.38±0.01^{B}	0.55±0.01^{a}	0.50±0.00^{B}	0.53±0.01^{C}
	T3	1.00±0.10^{a}	1.82±0.12^{C}	0.56±0.01^{A}	0.66±0.02	0.57±0.01^{a}	0.78±0.02^{C}
	T4	0.98±0.01^{a}	1.09±0.11^{A}	0.45±0.01^{B}	0.78±0.02^{b}	0.56±0.10^{Ba}	1.01±0.06^{A}
Twelve weeks	Control	0.95±0.02^{a}	1.22±0.01^{A}	0.72±0.01^{A}	0.90±0.02^{A}	0.63±0.02	1.08±0.02^{A}
	T1	0.98±0.12^{a}	0.92±0.02^{B}	0.56±0.01^{B}	0.80±0.01^{A}	0.54±0.01^{A}	0.70±0.03^{B}
	T2	0.89±0.01^{b}	0.83±0.02^{B}	0.58±0.01^{B}	0.59±0.02^{B}	0.62±0.01	0.81±0.02^{B}
	T3	1.02±0.02^{a}	1.24±0.02^{A}	0.50±0.00^{B}	1.01±0.01^{A}	0.68±0.02^{B}	0.95±0.06^{AB}
	T4	0.98±0.04^{a}	0.80±0.02^{B}	0.36±0.01^{C}	0.67±0.01^{B}	0.61±0.02	0.69±0.04^{C}

The different lowercase and capital letters in the same column at the same growth stage indicate significant differences at $p<0.05$ and $p<0.01$, respectively; LSD test; Each value represents the mean±S.E.

carbon sources by rhizosphere microorganisms at the low concentration of lead was the highest for the T1 and the lowest for the T3 treatments at the third week. The order of the intensive metabolism of the carbon sources was control >T1>T2>T3>T4 at the 7th week and control >T2>T>T1>T3 at the 12th week.

The intensive metabolism of the carbon sources by the Excel-based trapezoidal approximation showed obvious differences of the rhizosphere microbial activity between the low concentration of lead and the control.

The utilization rate of six carbon sources by the rhizosphere microorganisms: Table 3 shows the utilization rate of six carbon sources by rhizosphere soil microorganisms under different treatments. At the low concentrations of lead, the rate of the metabolism of carboxylic acids was the fastest at the third and seventh weeks and the rate of the metabolism of carbohydrates was the fastest at the 12th week. Moreover, the difference in the metabolism of carbohydrates and carboxylic acids between the different treatments was significant ($p<0.01$). The sequence of the six carbon sources utilized exhibited notable changes at the low concentration of lead compared to the control at the three growth stages. However, the utilization of carbohydrates, carboxylic acids, amino acids and propylamines by the rhizosphere microorganisms decreased at the low concentrations of lead. The addition of a low concentration of lead also resulted in a decrease in the polymer utilization at the third and seventh weeks. The utilization of phenolic acid increased at the third week and primarily decreased at the seventh and 12th weeks.

Amino acids are known to be extruded from wheat roots and the addition of lead resulted in decreases in the amino acid and propylamine utilization. The paradox that the amino acid utilization by the rhizosphere microbes decreased with the addition of lead when the root exudation of amino acids most likely increased may be explained by the nature of nitrogen cycling in the soil (Nelson and Mele, 2007). Under normal circumstances, microbes will take up organic N sources and retain them, but when organic N concentrations increase, the mineralization of simple N compounds, such as NH_4^+ and NO_3^-, is stimulated (Nelson and Mele, 2007; Schimel *et al*., 2005). In the rhizosphere, this could result in a lessened reliance on amino acids and propylamine as N sources, as heterotrophs are more likely to utilize simper N compounds. However, the low concentration of lead also affected the utilization of carbohydrates, carboxylic acids and several other carbon sources that are associated with plant root exudation.

Diversity index in the utilization of media by the rhizosphere microorganisms: Table 4 shows the microbial diversity indices at the different concentrations of lead. According to the richness index, the number of carbon substrates utilized at the low concentration of lead was less than in the control. The microbial communities under the control treatment were able to use an average of 20-30 carbon substrates in the three growth stages. In contrast, only 16-29 carbon sources were metabolized by the microbial community at the low concentrations of lead. These values represented the ability of the soil microorganisms to metabolize different carbon sources in the three growth stages. The results suggested that significant changes occurred in the microbial metabolic diversity at the low concentrations of lead.

The composition of microbial communities determines the biological quality of the soil and the resilience of soil microbial communities to contamination events is related to the high degree of functional redundancies of the microbes (Martínez-Iñigo *et al*., 2009). However, the decrease in the Shannon diversity index (H') and richness (S) showed that the low concentration of lead exerted a direct stress on the microbial community diversity; a decrease in the richness of culturable bacteria in the rhizosphere of *Syringa vulgaris* was also found under low concentrations of contaminants (Pb, Cr, Cd and Cu)

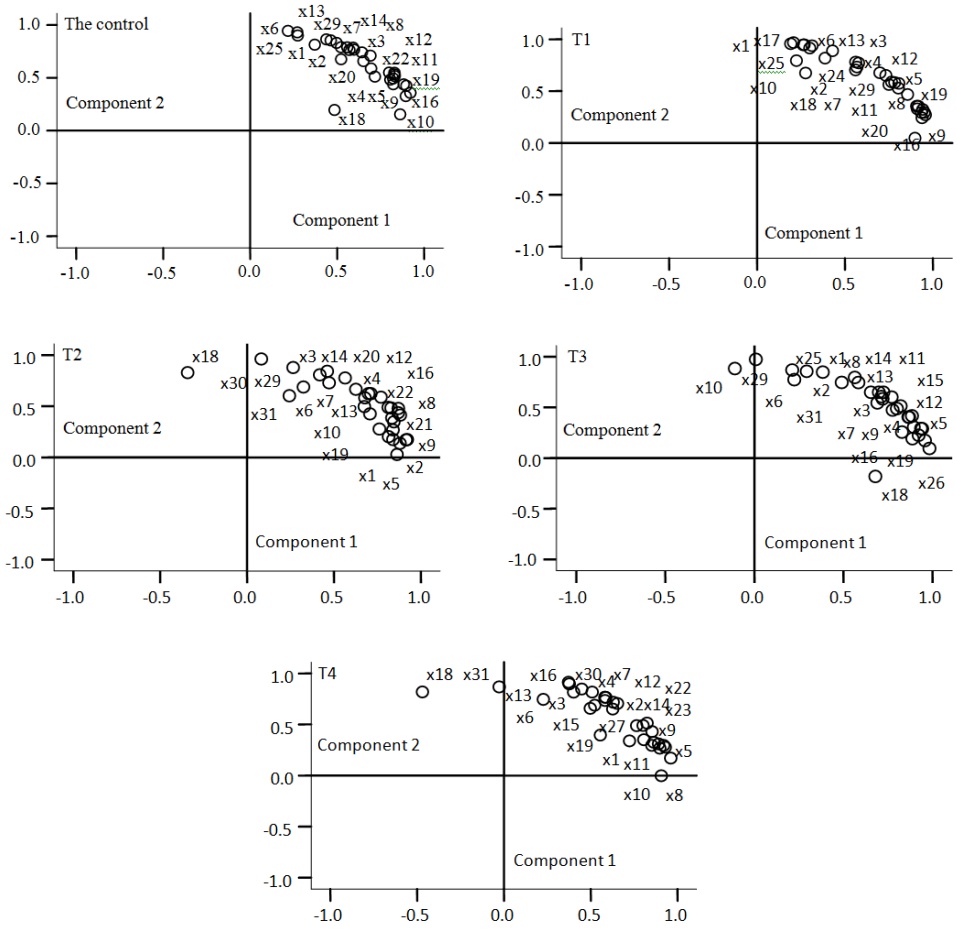

Fig. 1: Component plot in rotated space

X_1-X_{31}: D-Cellobiose, α-D-Lactose, β-Methyl-D-glucoside, D-Xylose, I-Erythritol, D-Mannitol, N-Acetyl-D-glucosamine, Glucose-1-phosphate, D-Galactonic acid-γ-lactone, D, L-α- Glycerophosphat, D-Glucosaminic acid, Pyruvic acid methyl ester, D-Galacturonic acid, γ-Hydroxybutyric acid, Itaconic, α-Ketobutyric acid, D-Malic acid, L-Arginine, L-Asparagine, L-Phenylalanine, L-Serine, L-Threonine, Glycyl-L-glutamic acid, Tween 40, Tween 80, α-Cyclodextrin, Glycogen, 2-Hydroxy benzoic acid, 4-Hydroxy benzoic acid, Phenylethylamine and Putrecine, respectively

Table 4: The diversity index of rhizosphere microorganisms under different treatments

Growth stage	Treatments	Shannon's diversity index (H')	Richness (S)
Three weeks	Control	$3.27\pm0.02^{Aa)}$	20.67 ± 0.33^{Aa}
	T1	3.22 ± 0.01^{B}	20.33 ± 0.67^{a}
	T2	3.19 ± 0.02^{B}	19.67 ± 0.33^{b}
	T3	3.18 ± 0.04^{B}	18.33 ± 0.67^{B}
	T4	3.18 ± 0.02^{B}	16.67 ± 0.33^{C}
Seven weeks	Control	3.28 ± 0.02^{A}	30.67 ± 0.33^{Aa}
	T1	3.21 ± 0.01^{Ba}	25.33 ± 0.67^{B}
	T2	3.24 ± 0.03^{A}	29.33 ± 0.67^{Cb}
	T3	3.22 ± 0.02^{b}	27.67 ± 0.33^{C}
	T4	3.27 ± 0.01^{A}	28.67 ± 0.33^{C}
Twelve weeks	Control	3.27 ± 0.02^{A}	29.67 ± 0.33^{A}
	T1	3.17 ± 0.03^{B}	25.67 ± 0.33^{B}
	T2	3.26 ± 0.00^{A}	28.33 ± 0.67^{A}
	T3	3.25 ± 0.01^{A}	25.67 ± 0.33^{B}
	T4	3.21 ± 0.00^{B}	28.00 ± 0.00^{A}

[a)]: Different small and capital letters in the same column at the same growth stage indicate significant difference at $p<0.05$ and $p<0.01$, respectively; LSD test; Each value represents the mean±S.E.

through denaturing gradient gel electrophoresis (Martínez-Iñigo *et al*., 2009). In addition, the wheat seedling rhizosphere also exerted some influence on the microbial community diversity by means of the allelopathy of the root exudates at the low concentration of lead, although it is not well established that the rhizosphere is always selectively enriched with certain populations of microorganisms (Duineveld *et al*., 1998). It is generally accepted that the root exudates from sand plant species strongly determine the bacterial composition in the rhizosphere, producing plant genotype-specific community structures in the same soil (Jorquera *et al*., 2010). It is highly possible that the phenomenon of plant genotype-specific community structures also occurred for the winter wheat seedlings. Of course, the microbial community in the rhizosphere could be influenced by a wide variety of factors. From the quick response of microbial communities to

fluctuations at low concentrations of lead, it could be deduced that the soil health had decreased in the rhizosphere of the winter wheat seedlings and that the root health was poor due to the decline of the rhizosphere microbial functional diversity.

Analysis of the major components on the characteristics of the carbon source utilization: The first two principal factors accounted for 88.94, 91.50, 81.58, 86.66 and 84.48%, respectively and the First Principal Component axis (PC1) explained 81.46, 78.42, 69.74, 73.55 and 71.47% of the variation for the control, T1, T2, T3 and T4, respectively. The component plot in rotated space (Fig. 1) showed that the rhizosphere microbial functional diversity at the low concentration of lead increased overall. Moreover, the number of substrates utilized preferentially in the T1 treatment, which had load values exceeding 0.90, were more than in the control and consisted of D-galactonic acid-γ-lactone, D-cellobiose, D-mannitol, α-ketobutyric acid, D-malic acid, L-asparagine, L-phenylalanine, Tween 40 and Tween 80. The rhizosphere microbes in the control were more likely to use D-cellobiose, D-mannitol and Tween 80, which had a load value greater than 0.900, suggesting that 50 mg/kg lead stimulated the utilization of carbon by the rhizosphere microorganisms. However, the type of substrate utilized preferentially (load values >0.90) at the other low concentrations of lead was different from the control. These results indicated that there were significant changes in the responses of the rhizosphere microbial communities to the carbon sources among the control and low concentrations of lead. The principal component analysis showed that the ability of the rhizosphere microbes to utilize different carbon sources was affected by the low concentration of lead; moreover, the characteristic C source utilization patterns were different from the control.

CONCLUSION

Our results indicated that low concentrations of lead (<300 mg/kg dry weight soil, pH = 8.45) significantly affected the microbial population and functional diversity in the rhizosphere of winter wheat seedlings. This situation would further induce fluctuations in the rhizosphere soil micro ecology function and a deterioration of the soil environment for future cultivation. Thus, the effects of the low concentration of lead on the rhizosphere microbial characteristics should also be considered in decisions regarding environmental quality standards for agricultural land, in addition to plant growth, plant accumulation of lead and human health risks. Our results may be beneficial for proposals for decisions of lead thresholds for agricultural land for cultivation when the soil pH is over 7.5.

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