Ecotoxicology (2020) 29:551–558
https://doi.org/10.1007/s10646-020-02205-4

Effects of Cd and Pb on diversity of microbial community and enzyme activity in soil

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Accepted: 23 March 2020 / Published online: 11 May 2020

Abstract

Pollution due to heavy metals is a serious global environmental problem, particularly in China. It is thus important to study the effects of heavy metal pollution, especially in mining areas. Cadmium(Cd) and lead(Pb) severely damage the microbial life in soil. The concentration of heavy metals and their toxic effects on microbes and enzymes in soil were examined in this study using contaminated soil samples. The Biolog method was used to analyze the characteristics of the microbial community. The results showed that the addition of Cd^{2+} and Pb^{2+} in different concentrations has a significant impact on microbial and enzyme activity in soil. With an increase in their concentrations, activities of the microbial community and enzymes decreased gradually. Each index related to the structure of the microbial community in soil decreased, indicating that pollution due to Cd and Pb reduced its size and functional activity. This study provides a reference for future research on the functional diversity of the microbial community in soil and plays its role in their environmental management.

Keywords Heavy metal pollution · Enzyme activity · Microbial community · BIOLOG

Introduction

China has a vast territory containing a large area of cultivated land and mineral resources. However, with rapid industrialization and excessive mining for minerals, problems such as soil pollution due to heavy metals have arisen (Xu et al. 2020). According to Wei and Yang (2010), heavy metals, especially lead(Pb) and cadmium(Cd), have accumulated in China's urban and agricultural soil owing to anthropogenic activities.

Heavy metal pollution in soil occurs mainly in mining areas (Fakayode and Onianwa 2002). Heavy metals accumulate in soil from weathering, industrial effluents, agricultural chemicals, and toxic gases in the air that eventually mix with the soil due to rain (Wang et al. 2004). Moreover, heavy metals are diffused through the atmosphere and water bodies in the surrounding areas. Acosta et al. (2011) analyzed two tailings ponds of an abandoned Pb–zinc(Zn) mine and found that the concentration of heavy metals was higher than in distant areas. High concentrations of soluble Zn^{2+} and Cd^{2+} have seeped into streams and groundwater Meshalkina et al. (1996) observed high levels of S, V, and As in soil 1–2 km from a Russian sulfuric acid production plant.

According to Huang et al. (2018), more than 70% of land in suburbs contaminated by mines has been used for agriculture. Heavy metals in soil affect the local ecology and agricultural products. Papaioannou et al. (2019) treated soil samples with wastewater containing heavy metals to reduce the beet dry matter yield. Antoniadis et al. (2017) found that heavy metals tend to bioaccumulate in the food chain and gradually affect the safety of residents in the surrounding areas. Heavy metals tend to settle in polluted areas for a long time owing to their long half-lives, which poses daunting challenges to the ecological restoration of mining areas (Chen and Guo 2014). Chao et al. (2016) found that the structure of the microbial community in mining areas was significantly different from that in unmined areas. To protect the ecology of mining areas and the health of local residents, it is imperative to study pollution due to heavy metals in soil.

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The microbial communities in soil are very sensitive to heavy metal stress. The volume of microbes and the structure of the community change when the soil is stressed by heavy metals. Therefore, the microbial diversity in soil is an important characteristic of soil quality and a sensitive indicator of soil ecology (Vinhal-Freitas et al. 2017). Zhao et al. (2019) analyzed the microbial community in the soil of a mining area and found that *Proteobacteria* and *Firmicutes* were highly resistant to heavy metals. The absorption and adsorption of microorganisms can help reduce the mobility and biological toxicity of heavy metals. The microorganisms in soil thus have a certain restorative effect on soil contaminated by heavy metals. This has practical significance for research on changes to the microbial population, plant population, and the structure and metabolic function of the microbial community when polluted by heavy metals in soil. By researching these issues, the resistance of microorganisms to heavy metals in soil can be better understood.

In the present work, we prepared the contaminated soil samples with lead chloride ($PbCl_2$) and cadmium chloride ($CdCl_2$) solution, and examined the effect of Pb^{2+} and Cd^{2+} on the microbial communities and enzyme activities in soil.

Materials and methods

Medium

The Martin medium was used to culture fungi in 1 L of distilled water, 10 g glucose, 5 g peptone, 1 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 18 g agar, 3.3 mL of 10 g/L Bengal red solution, and 3 mL of 10 g/L streptomycin solution prepared just before use. The improved Gause medium was used to culture the actinomycete in 1 L of distilled water, 20 g starch, 0.5 g NaCl, 1 g KNO_3, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.5 g K_2HPO_4, 0.5 g MgSO4·$7H_2O$, and 18 g agar. The beef extract-peptone agar medium was used to culture the bacteria in 1 L of distilled water, 5 g peptone, 3 g beef paste, and 18 g agar.

Soil samples

The soil samples were collected from Buxian Mountain in uncontaminated form with abundant vegetation. The samples were collected from four corners and the center of a piece of land 20 m × 20 m at a depth of 10–15 cm to avoid biases. They were mixed thoroughly, air dried, crushed in a ceramic mortar, and sieved through a <2 mm mesh. Then, they were divided into two parts and put in aseptic polythene bags. The first sample was used to prepare soils contaminated by heavy metals, and determine the structural characteristics of the microbial community and enzyme activity in soil. The second sample was used as control check (CK). The two samples were stored in refrigerator at 4 °C for further use.

Simulating heavy metal pollution

The concentration gradients of the added Cd^{2+} were 11.2, 16.8, 22.4, and 28.0 mg/kg. The concentration gradients of the added Pb^{2+} were 10.35, 20.70, 31.05, and 41.40 mg/kg.

Soil samples added with Cd^{2+} at 280 mg/kg and Pb^{2+} at 414 mg/kg were prepared by using 100 g of air-dried soil soaked in 0.5 mol/L of a cadmium chloride ($CdCl_2$) solution and a 0.5 mol/L lead chloride ($PbCl_2$) solution, respectively. The samples were then mixed with the air-dried soil in proportion to the above-mentioned amount of contaminated soil samples as shown in Table 1.

The eight soil samples were examined for the microbial analysis of soil, enzyme activity, and the structure of the microbial community. All experiments were carried out in triplicate.

Microbial analysis of soil

For microbial analysis, 10 g of soil was put into 100 mL of distilled water in a 250 mL flask. It was stirred with a magnetic stirrer for 30 min to thoroughly suspend the soil particles. The suspension was then diluted to obtain dilutions of 10^{-5}, 10^{-6}, and 10^{-7}. These suspensions were then added to the previously prepared medium according to the

Table 1 Preparation of contaminated soil samples with Cd^{2+} and Pb^{2+}

Concentration of the added Cd^{2+} (mg/kg)	Untreated soil (g)	Soil with 280 mg/kg of Cd^{2+} (g)	Concentration of the added Pb^{2+} (mg/kg)	Untreated soil (g)	Soil with 414 mg/kg of Pb^{2+} (g)
11.2	48.0	2.0	10.35	48.75	1.25
16.8	47.0	3.0	20.70	47.50	2.50
22.4	46.0	4.0	31.05	46.25	3.75
26.8	45.0	5.0	41.40	45.00	5.00

required concentration, respectively. The 10^{-5} soil suspension was inoculated into the Martin medium, the 10^{-6} suspension was inoculated into the improved Gause medium, and the 10^{-7} suspension was inoculated into the beef extract-peptone agar medium. These media were then inoculated with 200 µL of inoculum in a shaker incubator for 4 days at 30 °C.

Assays of enzyme activity in soil

Different assays were followed to detect enzyme activity in soil, including urease, sucrase, and acid phosphatase. Urease activity in soil was assayed by the indophenol blue colorimetric method (Huang et al. 2015). For sucrase, the 3,5-dinitrosalicylic acid colorimetric method was used (Li and Zheng et al. 2016), whereas acid phosphatase was determined by the phenyl phosphate disodium colorimetric method (Li and Geng et al. 2016).

To quantitatively reflect the degree of inhibition of enzyme activity in soil by different concentrations of heavy metals, the enzyme activity inhibition rate was used, which was estimated as follows (Fang et al. 2017):

$$\textit{Enzyme activity inhibition rate} = \left[1 - \frac{\textit{Enzyme activity of the treated sample}}{\textit{Enzyme activity of control samples}}\right] \times 100\% \quad (1)$$

Analysis of structural characteristics of microbial community

The structural characteristics of the microbial community in soil were analyzed by using Biolog-ECO microplate analysis (Garland 1996). The moisture content of soil was calculated by the drying method. Soil samples were first activated by incubation at 25 °C for 24 h, and 10 g (dry weight) of the activated soil was added to 90 mL of a 0.145 mol/L sodium chloride (NaCl) solution in a 250 mL flask. The flask was shaken for 30 min. The supernatant was diluted to 10^{-3}, inoculated at 150 µL per well to the ECO plate, and incubated in the dark at 25 °C for 7 days. The aseptic water was added into the control well instead of the supernatant.

The metabolic activity of microorganisms is shown by the 590 nm value minus the 750 nm value. Well optical density values under 0.06 were set to zero (Classen et al. 2003). The average well color development (AWCD) was calculated using the following equation (Wang et al. 2019):

$$\mathrm{AWCD} = \sum(C - R)/31, \quad (2)$$

where C is the absorbance of each well, and R is the absorbance of the control well in the plate (i.e., blank). The AWCD value reflected the overall utilization of 31 carbon sources (Choi and Dobbs 1999; Kumar et al. 2017; Shrestha et al. 2019).

The data for the 72 h culture were selected for analysis, and the Simpson index (D), Shannon diversity index (H), and McIntosh index (U) were calculated to characterize the functional diversity of the microbial community in soil. The parameter was estimated as follows (Zak et al. 1994; Yang et al. 2013):

$$D = \sum \frac{n_i(n_i - 1)}{N(N - 1)} \quad (3)$$

$$H = -\sum_{i=1}^{s} p_i \ln p_i \quad (4)$$

$$U = \sqrt{\sum n_i^2}, \quad (5)$$

Where $n_i = C_i - R$, C_i is the absorbance of each well and R is the absorbance of the control well; S is the number of wells in which color changes occurred, i.e., 31; P_i is the ratio of the relative absorbance of each well ($C_i - R$) to the sum of the relative absorbance of the entire plate. When calculating the Simpson index (D), the data were expanded 1000 times to prevent from having a negative value (Wang et al. 2010).

Results and discussion

Microbial culture in soil contaminated with Cd^{2+} and Pb^{2+}

The results in Table 2 show that the highest microbial growth occurred in CK, and the addition of heavy metals significantly affected the growth of the microorganisms. After the addition of the heavy metals, the growths of the three colonies were affected. With increasing concentration of heavy metals, the impact became more significant.

The number of fungal communities in Cd-contaminated soil decreased by more than 75% and that of actinomycetes decreased by more than 88%. It is clear that Cd pollution had a greater impact on the growth of actinomycetes in soil. The number of fungi communities in Pb-contaminated soil decreased by more than 31%, that of actinomycetes decreased by more than 88%, and that of bacterial communities decreased by more than 27%. It is clear that Pb pollution had the greatest influence on the growth of actinomycetes in soil and the smallest impact on the growth of bacteria.

Cd pollution had a greater impact on the growth of microorganisms than Pb pollution. The two heavy metal had the greatest influence on the growth of the actinomycetes and the smallest impact on that of the bacteria.

Table 2 Results of culturing the fungi, actinomycete, and bacteria in soil contaminated with Cd^{2+} or Pb^{2+}

Sample (mg/kg)		Fungi ($\times 10^5$ cfu/mL)	Actinomycete ($\times 10^6$ cfu/mL)	Bacterial ($\times 10^7$ cfu/mL)
	CK	16 ± 1.2	25 ± 1.7	38 ± 2.5
Cd^{2+}	11.2	4 ± 0.6	3 ± 0.3	16 ± 1.0
	16.8	3 ± 0.3	3 ± 0.2	14 ± 1.5
	22.4	3 ± 0.2	2 ± 0.08	25 ± 2.1
	28.0	1 ± 0.05	1 ± 0.02	0
Pb^{2+}	10.35	11 ± 0.9	3 ± 0.5	30 ± 2.5
	20.70	7 ± 0.2	3 ± 0.3	26 ± 3.1
	31.05	4 ± 0.15	1 ± 0.02	18 ± 1.7
	41.40	1 ± 0.03	0	10 ± 0.8

Data are mean ± SD of three replicates

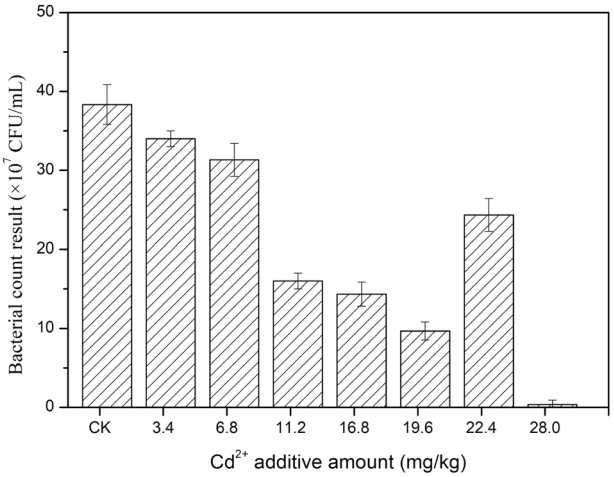

Fig. 1 Results of culturing bacteria in Cd-contaminated soil

The most deleterious effect was observed on the bacteria as no growth was determined in the highest Cd-contaminated soils. The experiment was repeated to eliminate operational errors. The results were also confirmed by using the following Cd concentrations: 3.4, 6.8 and 19.6 mg/kg.

Figure 1 shows that at low Cd concentrations, the number of bacteria generally decreased with an increase in the added amount. When the added Cd^{2+} was 22.4 mg/kg, the number of bacteria increased but was still lower than that in untreated soil samples.

Heavy metal-resistant microorganisms are often found in soil, and low concentrations of Cd may promote their growth. The number of bacteria increased at 22.4 mg/kg of Cd, and a Cd-resistant bacterial group became the dominant species at this concentration. Research has shown that the number of bacteria in the soil treated by low concentrations of Cd^{2+} does not exhibit any regularity, but high concentrations of Cd have a significant inhibitory effect on microorganisms in soil.

Effects of heavy metals on enzyme activities in soil

The absorbance of each sample was measured by the colorimetric method and the activity of various enzymes was calculated by a standard curve. The results are shown in Table 3.

The above results indicate that different concentrations of Cd^{2+} and Pb^{2+} had posed different degrees of inhibition on the activities of the tested enzymes, and this inhibition increased with the concentration of heavy metal ions.

Urease was more strongly inhibited by Cd than by Pb as 94.3% of its activity was inhibited at 28.0 mg/kg of Cd. With an increase in the concentration of heavy metal ions, the activity of urease in soil decreased significantly. Urease activity decreased by nearly 50% as Cd^{2+} increased and from 0.205 to 0.167 mg as Pb^{2+} increased. Therefore, urease in soil is highly sensitive to Cd^{2+}. Gao et al. (2008)

Table 3 Results of assay and inhibitory rates of urease, sucrase, and acid phosphatase in soil

Sample (mg/kg)		Urease activity/mg	Inhibitory rate (%)	Sucrase activity/mg	Inhibitory rate (%)	Acid phosphatase activity/mg	Inhibitory rate (%)
	CK	0.438 ± 0.055	0	11.64 ± 0.85	0	0.94 ± 0.06	0
Cd^{2+}	11.2	0.049 ± 0.002	88.8	3.74 ± 0.27	67.9	0.23 ± 0.03	75.7
	16.8	0.034 ± 0.005	92.2	2.72 ± 0.31	76.7	0.20 ± 0.04	78.6
	22.4	0.031 ± 0.003	93.2	2.58 ± 0.46	77.9	0.19 ± 0.02	79.9
	28.0	0.025 ± 0.002	94.3	2.46 ± 0.19	78.9	0.15 ± 0.03	83.9
Pb^{2+}	10.35	0.205 ± 0.042	53.2	4.68 ± 0.71	59.8	0.16 ± 0.04	83.5
	20.70	0.187 ± 0.048	57.3	4.37 ± 0.42	62.5	0.15 ± 0.01	84.6
	31.05	0.168 ± 0.020	61.6	4.32 ± 0.18	62.9	0.14 ± 0.03	84.9
	41.40	0.167 ± 0.031	61.9	3.48 ± 0.22	70.1	0.14 ± 0.02	85.2

When measuring the sucrase activity in Pb-contaminated soil, all soil samples were diluted 10 times because absorbance was large and the reading was unstable. Data are mean ± SD of three replicates.

have shown that the activity of urease can be used as an indicator for pollution due to mercury (Hg) and Cd.

Sucrase in soil acts as a hydrolase and its activity can affect the carbon cycle. It is also an important indicator of nutrient supply capacity, and soil fertility and quality. The data showed that Cd^{2+} and Pb^{2+} had an inhibitory effect on sucrase activity in soil, but Cd^{2+} were more toxic. 11.2 mg/kg of Cd^{2+} led to a reduction in sucrose from 11.64 to 3.74 mg, which inhibited its activity by 67.9%. Therefore, Cd^{2+} had a significant influence on sucrase activity in soil. Similarly, when Pb^{2+} was at 10.35 mg/kg, the sucrase in soil decreased by 59.8% and enzyme activity recorded a significant drop.

The inhibitory effects of Cd^{2+} and Pb^{2+} on acid phosphatase activity in soil were similar, but the inhibition of Pb^{2+} was greater than that of Cd^{2+}. When the additive amounts of Pb^{2+} were from 20.70 to 41.40 mg/kg, acid phosphatase activity in soil was nearly constant. It may be that 31.05 mg/kg of Pb^{2+} was the maximum inhibitory concentrations of acid phosphatase in soil. In addition, Pb^{2+} inhibited acid phosphatase in soil, whereas the inhibition range of Cd^{2+} was wider.

In summary, Cd^{2+} inhibited urease and sucrase in soil more strongly than Pb^{2+}, and Pb^{2+} inhibited acid phosphatase activity more strongly than Cd^{2+}. The order of inhibition of Cd^{2+} on the three enzymes was urease > acid phosphatase > sucrose. Because urease is the most sensitive to contamination by Cd^{2+}, it can be used as an indicator of Cd pollution. The order of inhibition of Pb^{2+} on the three enzymes was acid phosphatase > sucrose > urease. Because acid phosphatase in soil is the most sensitive to pollution from Pb^{2+}, it can be used as an indicator of Pb pollution.

Correlation between enzyme activities in soil and heavy metal pollution

Soil microbes are an important part of the soil ecosystem, and there is a correlation between the degree of heavy metal pollution and enzyme activities in soil. The correlation and regression analyses of the microbial community and enzyme activity in soil with Cd and Pb are shown in Table 4 and Table 5.

The results show that there is significant negative correlation between the number of fungal communities in soil and the degree of pollution due to the two heavy metals, and the fitting effect was good. This shows that fungi in soil were sensitive to pollution due to two heavy metals. The number of bacterial communities in soil was significantly negatively correlated only with the degree of Pb pollution, whereas the

Table 4 Correlation between microorganisms and enzyme activities in soil and the amount of heavy metal additive

		Fungi	Actinomycete	Bacteria	Urease activity	Sucrase activity	Acid phosphatase activity
Cd^{2+}	Pearson	−0.897[a]	−0.856	−0.627	−0.840	−0.872	−0.858
	Sig.	0.039	0.064	0.258	0.075	0.054	0.063
Pb^{2+}	Pearson	−0.993[b]	−0.785	−0.995[b]	−0.792	−0.786	−0.718
	Sig.	0.001	0.116	0.000	0.111	0.115	0.172

[a]Correlation is significant at the 0.05 level (2-tailed)

[b]Correlation is significant at the 0.01 level (2-tailed)

Table 5 Regression analysis of amount of Cd or Pb and enzyme activities in soil

		R-square
Cd^{2+} additive amount—fungi	$f(x) = -143.5x^{0.06595} + 172$	0.9229
Cd^{2+} additive amount—actinomycete	$f(x) = 0.0724x^3 - 1.834x^2 - 0.6036x + 30.37$	0.9662
Cd^{2+} additive amount—bacterial	$f(x) = -0.004952x^3 + 0.2632x^2 - 3.51x + 24.69$	0.8892
Cd^{2+} additive amount—urease activity	$f(x) = 1557x^2 - 783.8x + 44.66$	0.9690
Cd^{2+} additive amount—sucrase activity	$f(x) = -3.24x^3 + 59.1x^2 - 284.9x + 419.5$	0.9537
Cd^{2+} additive amount—acid phosphatase activity	$f(x) = 255.9x^2 - 316.9x + 71.07$	0.9859
Pb^{2+} additive amount—fungi	$f(x) = 0.07174x^2 - 3.989x + 45.46$	0.9997
Pb^{2+} additive amount—actinomycete	$f(x) = 0.3125x^2 - 9.45x + 40.93$	0.9492
Pb^{2+} additive amount—bacterial	$f(x) = -1.506x + 57.46$	0.9897
Pb^{2+} additive amount—urease activity	$f(x) = 2446x^2 - 1615x + 237.9$	0.9564
Pb^{2+} additive amount—sucrase activity	$f(x) = 2.594x^2 - 44.54x + 167$	0.9102
Pb^{2+} additive amount—acid phosphatase activity	$f(x) = 6.659e{-}x^{-16.13} + 1.004$	0.9880

x is the additive amount of Pb^{2+} and Cd^{2+}

correlation with Cd content was not significant and the fitting effect was poor. This indicates that the mechanism of Cd affecting the bacterial community is complex, and resistant bacteria might have multiplied under specific concentrations of Cd^{2+} (Griffiths and Philippot 2013).

The correlation between the heavy metal ions and enzymes in soil did not reach a significant level, but the enzymes were more significantly affected by Cd^{2+} than by Pb^{2+}. The effect of heavy metals on the enzymes is complex. Heavy metals can inhibit the growth of microorganisms, which in turn affects the synthesis of the enzymes. In addition, enzyme activity in soil increases due to "resistant-enzyme activity" caused by the proliferation of partial resistant- microorganisms (Griffiths and Philippot 2013). Enzyme activity in soil is also affected by other physical and chemical properties of the soil. Therefore, the results of different soil samples affected by heavy metals are different as reported in the literature. For example, Angelovičová et al. (2014) found the urease was significantly negatively affected by Pb and Zn, but the negative correlation between Pb and acid phosphatase was not significant. Zhao et al. (2015) found that heavy metals had no significant effect on urease activity, and Zhang et al. (2015) found no significant correlation between Pb content and enzymes in soil.

Effect of heavy metals on metabolic activity of microbial community in soil

Figure 2 shows that the AWCD value increased with culturing time, but hardly changed within 24 h after the inoculation because microbes require a certain response time to utilize different carbon sources. In the next 24 to 96 h, the AWCD value increased most rapidly. Thus, the microbial biomass in soil was abundant during this period. The growth rate from 96 to 192 h declined, possibly because of the depletion of carbon sources.

Within the first 72 h, only bacteria was visible, which is evident from the rapid rise in the AWCD value with time. After 72 h, the growth of fungi was evident and the curve was still on the rise. However, the growth rate was slow over time because the carbon source in the plate had been consumed.

Compared with uncontaminated soil, the AWCD values of microbes in soil with Cd and Pb decreased. Except for those of 10.35 mg/kg of Pb and 28.0 mg/kg of Cd, the curves showed that the AWCD value gradually decreased with an increase in the concentration of heavy metals.

Effect of heavy metals on functional diversity of microbial community in soil

Table 6 shows the effect of Cd and Pb on functional diversity of microbial community.The Simpson index (*D*) is used to reflect the most common and dominant species in the treated

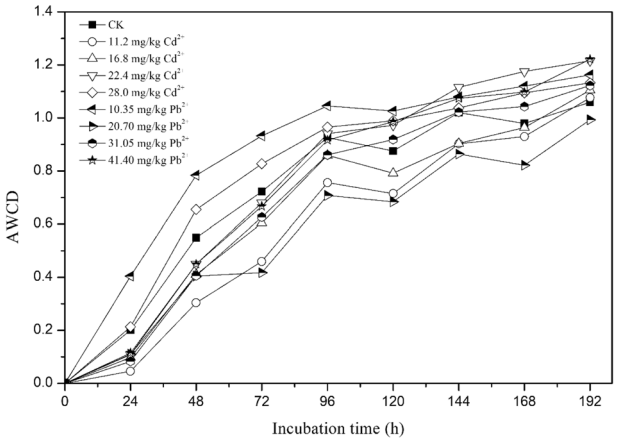

Fig. 2 Changes in the AWCD value of microbes in soil under different heavy metal treatments

Table 6 The Simpson index, Shannon index, and McIntosh index of microbial communities under heavy metal treatment

Sample (mg/kg)		Simpson index (*D*)	Shannon index (*H*)	McIntosh index (*U*)
	CK	0.01438	3.426	7.683
$Cd2^{+}$	11.2	0.00935	3.157	5.575
	16.8	0.01286	3.248	6.751
	22.4	0.01544	3.246	7.338
	28.0	0.01987	3.248	8.991
$Pb2^{+}$	10.35	0.01992	3.296	9.816
	20.70	0.02314	2.947	5.679
	31.05	0.01358	3.251	7.003
	41.40	0.01450	3.247	7.266

samples (Xu et al. 2008). It was found to increase with the concentration of Cd^{2+}. Therefore, there was no significant effect on community dominance at 22.4 and 28.0 mg/kg of Cd^{2+}. Only the Simpson index at 31.05 mg/kg of Pb^{2+} decreased by 5.5%, whereas other amounts of Pb^{2+} had no significant effect on community dominance.

The Shannon diversity index (*H*) is used to characterize the richness of microbial communities (Garland 1996; Dan et al. 2019). The data show that the addition of heavy metal influenced the richness of microbial communities, but there was no obvious effect of concentration gradient. The greatest impact on microbial richness was found at 11.2 mg/kg of Cd^{2+} and 20.70 mg/kg of Pb^{2+}, with a decrease of 7.8 and 14%, respectively.

The McIntosh index (*U*) is a diversity index based on the multidimensional space of the microbial community, and is used to reflect the uniformity of microbial communities in soil (Xu et al. 2008). Except for 10.35 mg/kg of Pb^{2+} and 28.0 mg/kg of Cd^{2+}, the amount of heavy metal added influenced the spatial diversity of microbes in soil compared

to untreated soil, but there was no prominent effect of concentration gradient. The McIntosh index was minimum at 11.2 mg/kg of Cd^{2+} and 20.70 mg/kg of Pb^{2+}, with a decrease of 27.4 and 26.1%, respectively.

Conclusion

The results of this study show that the microbial communities in soil were increasingly inhibited over time and the number of such colonies decreased with an increase in the concentration of Cd^{2+} and Pb^{2+}. The two heavy metals had the greatest influence on actinomycetes in soil, followed by fungi, and had the smallest influence on bacterial growth. However, Cd contamination had odd effects on the microbial communities, and this requires further investigation.

Cd^{2+} and Pb^{2+} also affected the activities of urease, invertase, and acid phosphatase in soil. Urease showed the highest sensitivity to Cd^{2+} and can be used as an indicator of Cd pollution. Acidic phosphatase had the highest sensitivity to Pb and can be used as an indicator of Pb pollution. The environmental quality of the contaminated soil needs further examination.

The AWCD value gradually increased with incubation time, and Cd and Pb inhibited the metabolic activities of the microorganisms in soil. The addition of heavy metals affected the microbial diversity index, but there was no prominent effect of gradient concentration. The Biolog method is effective and easy to perform, but only culturable microorganisms that can utilize carbon sources on the board can be reflected through the method. In the future, a variety of analytical methods should be used to assess the functional diversity of microbial communities in soil.

Funding This study was supported by the Fundamental Research Funds for the Central Universities (2019XKQYMS60).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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