Effects of heavy metal Cd pollution on microbial activities in soil

Weilin Shi[1], Xiying Ma[1]

[1] University of Science and Technology, Kerui Road 1, Gaoxin, Section, Suzhou 215011, Jiangsu, China

▌ Abstract
Heavy metal contamination of soil occurs when heavy metals are introduced to soil through human activities, leading to the gradual deterioration of the ecology and environment. Microorganism activity reflects the intensity of various biochemical reactions in soil, and changes in it reflect the level of heavy metal pollution affecting the soil. The effects were studied of heavy metal Cd on the microbial activity of soil at different concentrations by investigating the respiratory intensity, urease activity, and catalase activity in forest soil and garden soil. The results showed that the respiratory intensity, urease and catalase activities in the garden soil were all higher than in the forest soil. Cd has obvious inhibitory effects on microbial activities. The three parameters exhibited a downward trend with increasing concentrations of Cd. Catalase activity increased when the mass concentration of Cd reached 1.0 mg/kg, indicating that low concentrations of Cd can promote the activity of some microorganisms. Respiratory intensity and urease activity also increased when the concentration reached 10.0 mg/kg, showing that respiratory intensity and urease activity have strong response mechanisms to adverse conditions. The effective state of Cd in soil, as well as inhibition of microbial activity, decreased with incubation time.

▌ Key words
heavy metals, microbial activity, soil respiratory, soil enzyme activities

INTRODUCTION

Heavy metals, such as Fe, Mn, Cd, Hg, and Co, are introduced into soil on a large scale through anthropogenic sources, such as municipal solid waste, mining activities, use of fertilizers and pesticides in agriculture, and industrial emissions of "three wastes". Soil pollution occurs when the concentration of these heavy metals becomes significantly higher than that of the background, gradually deteriorating the ecology and environment [1]. Among heavy metals, Cd pollution is the most serious. It reduces the biological activity of soil microorganisms, and thus affects crop yield and quality. Furthermore, Cd is difficult to degrade and remains in the soil for a long period, eventually accumulating in soil or plant edible parts and further poisoning animals and humans through the food chain [2, 3]. Excessive intake of Cd can lead to prostate cancer, kidney cancer, and other diseases [4, 5].

Microorganisms are an important component of soil; their activity reflects the intensity and trend of various biochemical reactions in soil [6]. Changes in microbial community structure and diversity serve as an important biological indicator for evaluating the quality and status of soil [7].

Soil respiration is an exchange process between soil and CO_2 in the atmosphere. Its intensity is an important index for measuring total microbial activity [8, 9]. Soil enzyme serves as a biological index of soil quality and as an indicator for evaluating soil fertility [10]. It is usually used to monitor the pollution and fertility of soil, and to evaluate the quality of soil and the environment [11]. The reaction of catalase and urease in soil is sensitive to heavy metals, and they can reflect the toxic effects of heavy metals on soil microorganisms [12].

With the rapid development of industrialization in the People's Republic of China, large amounts of Cd are introduced to soil through various means, leading to the contamination of farmland and other soils, which seriously threatens human health and ecological security [12]. Many developed countries have lain down soil standards for ecological conservation based on the eco-toxicology theory. Such a standard has not been developed in China because of the lack of an ecotoxicological basis [13, 14]. In the present study, forest and garden soils were selected as research objects to investigate the respiratory intensity, catalase and urease activities of soil at various concentrations of Cd. As important biological indexes, they can assist in the assessment of Cd contaminated soil, and offer a scientific basis for early warning for different soils contaminated by Cd.

MATERIALS AND METHOD

Soil sample collection and processing. The soil samples included a type of yellow-brown soil sampled from Shangfang Mountain Forest Park (unpolluted forest soil) of Suzhou, and common garden soil (vegetable field garden soil). First, areas were designated for the selection and collection of samples, according to S type and five points sampling methods, the upper soil layer was stripped, and fresh underground soil obtained at a depth of 10–20 cm. The sampled fresh soil samples were spread to a thin layer of 2–3 cm thickness in a clean ceramic pot. Subsequently, objects such as small insects, plant debris, and gravel were removed, and the samples placed in a well-ventilated and cool room for drying in natural air. Finally, the soil samples were ground and sieved through a 2-mm nylon mesh, after which they were used for the experiments.

The physical and chemical properties of the soil samples were determined using conventional methods. The results are shown in Table 1. For calculating pH, the soil samples and deionized water were fully mixed with a volume ratio of 25:10, and allowed to remain static for 30 min. Total nitrogen

Address for correspondence: Weilin Shi, University of Science and Technology, Kerui Road 1, Gaoxin, Section, Suzhou 215011, Jiangsu, China
E-mail: 291740901@qq.com

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Table 1. Characterization of the used soil samples

Elements	pH	Total phosphorus	Total nitrogen	Organic matter	Cd
Forest soil	5.87	1.85	2.34	43.80	3.3×10^{-5}
garden soil	5.58	0.69	1.70	13.08	5.6×10^{-5}

was measured using the semi-micro Kjeldahl method; total phosphoric was measured using the sulfuric acid-perchloric acid digestion method; and organic matter was determined using the $K_2Cr_2O_7$ volumetric method. The concentration of Cd was measured using the full decomposition method and flame atomic absorption spectrophotometry of dispelled soil samples [14]. The concentrations of Cd in the forest soil and garden soil samples were 3.3×10^{-2} mg/kg and 5.6×10^{-2} mg/kg, which did not exceed the secondary standard of the "Environmental Quality Standard for Soils" (GB15618–1995) (Cd£0.3 mg/kg), and the first grade standard of soil environmental quality in Jiangsu Province (Cd £0.2 mg/kg) [15]. Therefore, the two soil samples were identified as unpolluted soils.

Experimental design. Method of adding exogenous Cd into soil. A stock solution of Cd 2.0 g/L was prepared by dissolving 3.723g Cd sulfate in 1,000 ml water in a volumetric flask. Cd was added to 500 g soil samples at mass concentrations of 0 (control sample), 1.0, 5.0, 10.0, and 30.0 mg/kg by evenly spraying 0.25, 1.25, 2.50, and 7.50 mL of the Cd stock solution. The samples were put into 500 mL beakers that were placed in a biochemical incubator at a constant culturing temperature for 7–28 days, in which the water content was maintained at 30%. For each test sample, three parallel groups were designed.

Determination of soil respiration intensity. The respiration intensity of soil was measured using the direct breath titration method [16]. Twenty-five grams of the soil sample were uniformly laid in a closed container, and a 25 ml beaker containing 1.0 mol NaOH solution (10 ml) placed in the container. The container was then sealed and positioned in a constant temperature incubator (28 °C). CO_2 from soil was absorbed by the NaOH solution, the residual CO_2 was titrated by hydrochloric acid, and the amount of CO_2 was calculated according to the consumption of hydrochloric acid.

Volumetric method for measuring soil catalase [17]. Twenty grams of the soil sample was put into a 100 ml conical flask, and injected with 40 mL distilled water and 5 ml 0.6% (mass fraction) hydrogen peroxide solution. The flask was sealed with a cork and oscillated in on a reciprocating shaker for 20 min. The reaction was then stopped by adding 5 ml 1.5 mol/L sulfuric acid. Finally, the liquid in the bottle was filtered with a quantitative filter paper, and 25 ml of the 0.02 mol/L liquid was titrated from mineral chameleon to reddish color.

Colorimetric method determination of soil urease [18]. One gram of dry soil sample was placed in a 100 ml triangle flask, and 0.5 ml toluene was added. After 15 min, 10 ml urea solution 10% (mass fraction) and 20 ml citrate buffer (pH=6.7) were added with even mixing. Subsequently, the flask was placed in a constant temperature incubator (28 °C) for 24 h. After filtration, 1 ml of the solution was put into a 50 ml volumetric flask, and 20 ml of distilled water added. Then,

4 ml sodium phenate solution and 3 ml sodium hypochlorite were successively added by mixing. After 20 min, it was coloured, weighed, and subjected to colorimetric analysis at 578 nm, using a spectrophotometer within 1 h.

Data processing. The experimental data of each test group were collected and averaged to the measured values of the three parallel groups. The data were then processed and line error analysis performed using Microsoft Excel 2010 and Origin 7.5 software.

RESULTS AND DISCUSSION

Soil respiration intensity. The respiration intensity of soil, which is characterized by the amount of CO_2 released in 24 h from 25.0 g of the soil sample, represents the effect of Cd stress on microbial respiration in soil for 7 days (Fig. 1). The respiration intensity of the garden and forest soil soils showed a downward trend with the increasing concentration of Cd. However, the intensity was slightly higher in a 10.0 mg/kg sample than in a 5.0 mg/kg sample, indicating that Cd exhibits a hermetic effect on microbial activity. This may lead to some abnormal reaction, that is, microbial activity was stimulated at a certain concentration of Cd. However, this effect will decrease when the concentration exceeds this level. Comparing the two soil samples, the respiration intensity in garden soil was clearly higher than that of forest soil. Furthermore, the respiration intensities of the two samples also showed a decrease with the increasing concentrations of Cd within the same incubation time (Figs. 2 and 3).
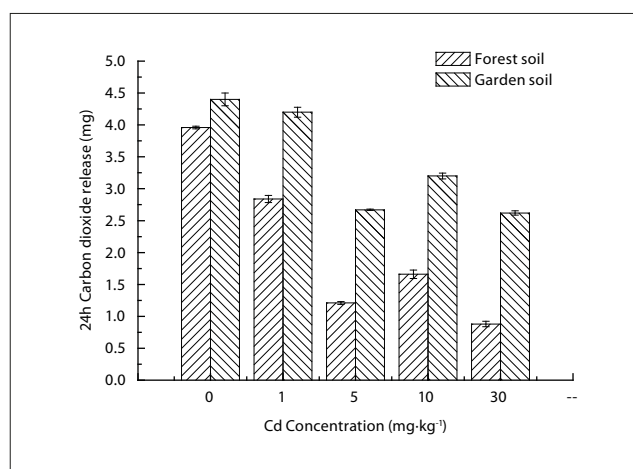
Figure 1. Soil respiration in different soils

After 21 days, the change was not significant with time for the same Cd concentration. This may reflect the toxicity of Cd diminishing with time. The results show that the increase of the respiration intensity at higher Cd concentration is a reaction mechanism for adverse conditions [19]. Liu et. al. [20] showed that the inhibitory effect of Cd on respiration increases with increasing Cd concentration.

Soil enzyme activities. Soil catalase activity was determined using the titration of mineral chameleon-the volume difference between undecomposed hydrogen peroxide and a blank sample. A larger volume of mineral chameleon indicates stronger soil catalase activity. As shown in Fig. 4,
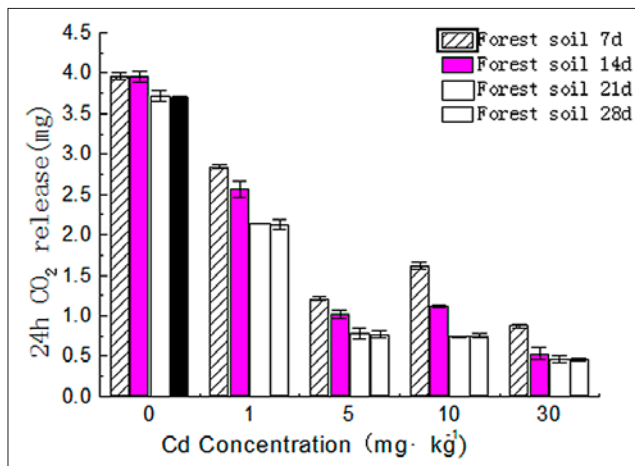
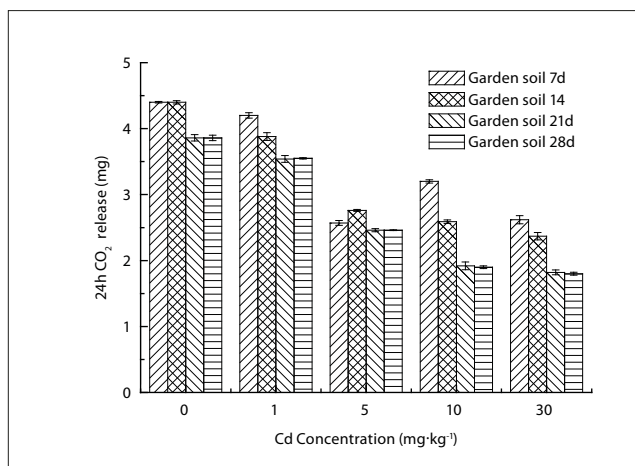**Figure 2.** Effect of Cd on soil respiration in forest soil

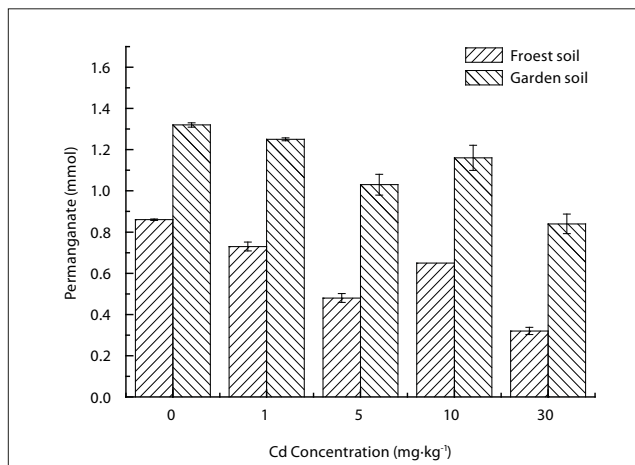**Figure 3.** Effect of Cd on soil respiration in rural soil

Figure 4. Soil catalase activity in different soils

7 d catalase activity in the garden and forest soil samples generally showed a downward trend with increasing Cd concentration. However, catalase activity was higher at a Cd concentration of 10.0 mg/kg than at 5.0 mg/kg. In addition, catalase activity in the garden soil was clearly higher than that in the forest soil. Catalase activity in the garden and forest soils both decreased with increasing Cd concentration for the same culturing time (Figs. 5 and 6). For 21 d culturing, catalase activity presented a small change trend for the same
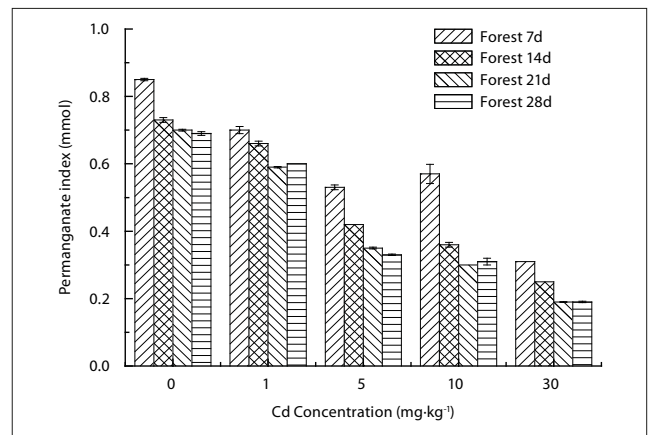

Figure 5. Effect of Cd on catalase activity of catalase in forest soil
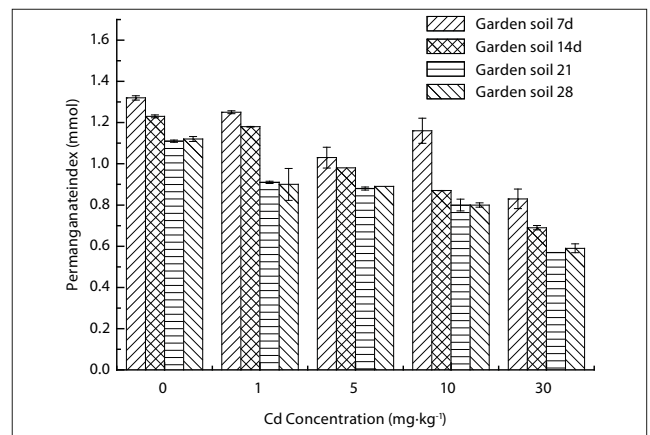

Figure 6. Effect of Cd on catalese activity in garden soil

Cd concentration. Gao et.al. [21] found that soil catalase activity decreases with the increasing availability of heavy metals, which is consistent with the results of this study.

Soil urease activity. Urease activity was characterized using the amount of ammonia produced by decomposition urea in 24 h. Larger amounts of ammonia reflected higher urease activity. 7 d soil urease activity in the garden and forest soils both exhibited a declining trend with increasing Cd concentration (Fig. 7). Urease activity was clearly higher in the garden soil than in the forest soil at the Cd concentration of 1.0 mg/kg. At higher concentrations, Cd had a strong
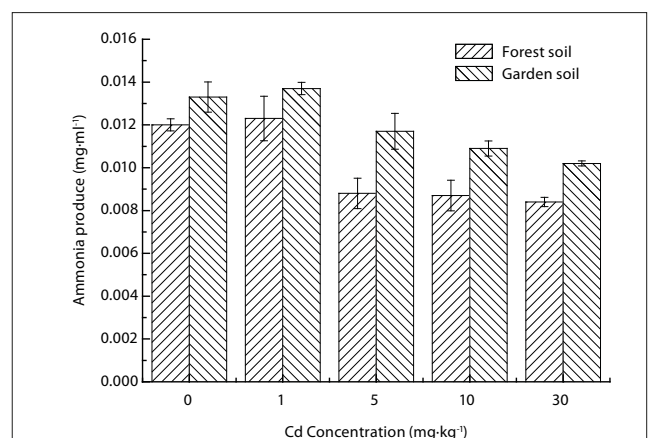

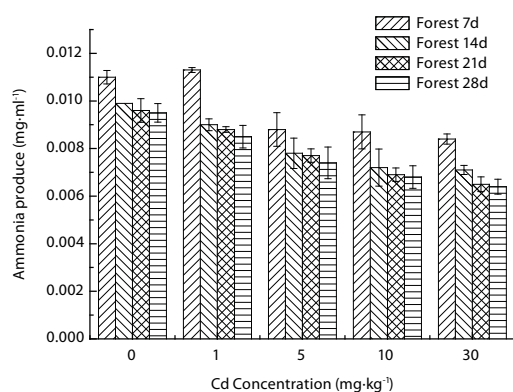**Figure 7.** Soil urease in different soils

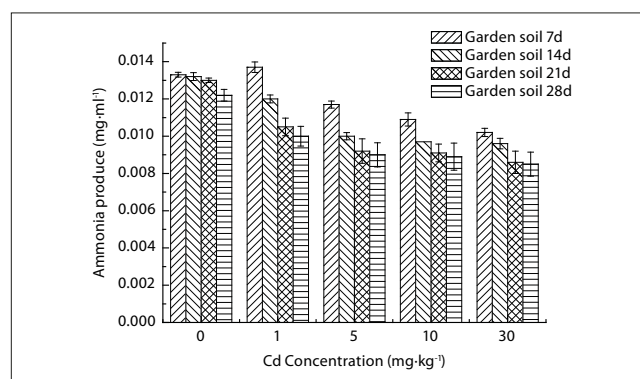**Figure 8.** Effect of Cd on urease activity in forest soil

Figure 9. Effect of Cd on urease activity of urease in garden soil

inhibitory effect on urease activity. Under Cd stress, urease activity in the garden soil was clearly higher than in the forest soil. show that urease activity does not change significantly with Cd concentration for 21 d culturing. The results are consistent with those obtained by Meng et al. [22], who reported that a single heavy metal can promoteed soil enzyme activity when the mass fraction of heavy metals was low; in contrast, it inhibits soil enzyme activity when the mass fraction of heavy metals is high.

From the results of the presented study, it was found that the activity of microorganisms in the garden soil sample was stronger than that in the forest soil sample under the same concentration of Cd. This is because the physical and chemical properties of the two types of soils are different. The concentration of total phosphorus, nitrogen and organic matter in the garden soil was lower than that in the forest soil, whereas the intrinsic concentration of Cd was higher than that in the forest soil. Therefore, the adaptability of microorganisms was stronger in the garden soil.

CONCLUSIONS

Differences in the physical and chemical properties of soil strongly influence the ecotoxicological effects of Cd and effective state of Cd. Because the physical and chemical properties of garden soil and forest soil differ, microbial activity was found to be stronger in garden soil than in forest soil under the same concentration of Cd. The results showed that Cd promotes soil respiration and catalase activity at a concentration of 10.0 mg/kg, and stimulates soil urease activity at a concentration of 1.0 mg/kg. The effects of Cd

vary with different biochemical processes; it has a strong restraining effect on soil catalase and urease, and the effect is stronger for urease than for catalase. According to the related research, the effect of Cd in soil will diminish with time. Changes in microbial activity in soil exhibited a declining trend in the presented study, which could be attributed to the weakening of the effects of Cd with time.

Acknowledgements

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