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Toxicity of cadmium to soil microbial biomass and its activity: Effect of incubation time on Cd ecological dose in a paddy soil*

LIAO Min (廖 敏)†1, LUO Yun-kuo (罗运阔)2, ZHAO Xiao-min (赵小敏)2, Huang Chang-yong (黄昌勇)1

(1*School of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310029, China*)

(2*School of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, China*)

†E-mail: liaomin@zju.edu.cn

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Abstract: Cadmium (Cd) is ubiquitous in the human environment and has toxic effect on soil microbial biomass or its activity, including microbial biomass carbon (C_{mic}), dehydrogenase activity (DHA) and basal respiration (BR), etc., C_{mic}, DHA, BR were used as bioindicators of the toxic effect of Cd in soil. This study was conducted to determine the effects of Cd on soil microbial biomass and its activity in a paddy soil. The inhibition of microbial biomass and its activity by different Cd concentrations was described by the kinetic model (M1) and the sigmoid dose-response model (M2) in order to calculate three ecological doses of Cd: ED_{50}, ED_{10} and ED_5. Results showed that M2 was better fit than M1 for describing the ecological toxicity dose effect of cadmium on soil microbial biomass and its activity in a paddy soil. M2 for ED values (mg/kg soil) of C_{mic}, DHA, BR best fitted the measured paddy soil bioindicators. M2 showed that all ED values (mg/kg) increased in turn with increased incubation time. ED_{50}, ED_{10} and ED_5 of C_{mic} with M2 were increased in turn from 403.2, 141.1, 100.4 to 1000.7, 230.9, 144.8, respectively, after 10 d to 60 d of incubation. ED_{50}, ED_{10} and ED_5 of DHA with M2 increased in turn from 67.6, 6.2, 1.5 to 101.1, 50.9, 41.0, respectively, after 10 d to 60 d of incubation. ED_{50}, ED_{10} and ED_5 of BR with M2 increased in turn from 149.7, 6.5, 1.8 to 156.5, 50.8, 35.5, respectively, after 10 d to 60 d of incubation. So the ecological dose increased in turn with increased incubation time for M2 showed that toxicity of cadmium to soil microbial biomass and its activity was decreased with increased incubation time.

Key words: Cadmium, Soil microbial biomass, Basal respiration, Dehydrogenase activity, Ecological dose, Paddy soil
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INTRODUCTION

Cadmium (Cd) is ubiquitous in the human environment and has been recognized as one of the most deleterious heavy metal pollutants (Robards and Worsfold, 1991; Christine, 1997). It may easily move from soil to food plants through root absorption and accumulate in their tissues (Oliver, 1997). In this way, Cd may enter the food chain and affect human health (Adriano, 1986). Among many heavy metals polluting soil, Cd is of concern because of its potentially harmful effects on not only humans and animals, but also the most adverse effects on microbial biomass and its activity which play an important role in the biological cycles of almost all the major plant nutrients cycling, soil nutrient cycling and in maintaining soil fertility (Smith, 1996; Jose *et al.*, 2002; Yao *et al.*, 2003). Cd can also cause changes in the size, composition and activity of soil microbial community (Giller *et al.*, 1998). There were many studies on this topic (Brookes, 1995; Nannipieri *et al.*, 1997; Giller *et al.*, 1998). However, little is known about the toxicity effect of Cd on the microbial biomass and its activity of paddy soils. Paddy soil widely distributed in China and Asia is important resource for food production. In China, more than 30 million hectares located mainly in the area south of the Changjiang River are important rice production regions (Zhang

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and Gong, 2003) contaminated by Cd in widespread area.

In response to the apparent need to easily quantify the influence of pollutants on microbe-mediated ecological processes in various ecosystems, the concept of an ecological dose (ED_{50}) was developed, which is the toxicant concentration that inhibits a microbe-mediated ecological process by 50% (Babich *et al.*, 1983). However, a 50% reduction in a basic ecological process may be too extreme for the continued functioning of a paddy soil and so, lower percentage values of inhibition (5%, 10% or 25%) equivalent to ED_5, ED_{10} or ED_{25} must be established. These values may be more suitable criteria for protecting soil quality and for assessing the sensitivity of a paddy soil ecosystem subjected to heavy metals pollution stress (Doelman and Haanstra, 1989; Kostov and Van, 2001). Different researchers proposed several mathematical models for calculating the ecological dose value. Haanstra *et al.*(1985) used a sigmoid curve to describe the inhibitions of urease and phosphatase activity as a function of the natural logarithm of the concentration of heavy metals, while Speir *et al.*(1995) used two models based on enzyme inhibition kinetics.

In the present study the changes of different microbial indicators in a paddy soil contaminated by a wide range of Cd levels were measured to evaluate the effects of Cd toxicity on the sensitivity of soil microbial indicators. The microbial indicators used were microbial biomass carbon (C_{mic}), dehydrogenase activity (DHA) and basal respiration (BR). Statistical test of non-linear correlation was used to evaluate which of the previously mentioned models best fit the experimental data. We calculated the ED values for Cd using two models and compared changes in ED values in a paddy soil under different incubation periods

MATERIALS AND METHODS

Soil

The laboratory incubation experiment at constant temperature of 25 °C was carried out with a paddy soil sampled from near Jinhua City, Zhejiang Province, China. The soil samples used were collected from the surface layer (0–15 cm) of an agricultural field used for paddy rice. After removal of surface water, the fresh soil was brought to laboratory immediately after the collection, hand picked to remove discrete plant residues and large soil animals (earth warms, etc.). Soil samples were homogenized and air-dried at room temperature to facilitate the sieving, passed through a 2-mm sieve and stored at 4 °C prior to analyze. A sub-sample of the soil was taken, air-dried, ground, and analyzed for various physicochemical characteristics listed in Table 1.

Table 1 Some physicochemical characteristics of the soil used in the experiment

Parameters	Value	Parameters	Value
pH (H_2O)	4.74	CEC (cmol/kg)	7.328
WHC (g/kg)	510	Sand (g/kg)	278
O. C. (g/kg)	15.25	Silt (g/kg)	562
Av. N (mg/kg)	106.40	Clay (g/kg)	160
Av. P (mg/kg)	13.34	Soil texture	Silt loam
Total content of Cd (mg/kg)	BDL[#]		

[#]Below detection limit

Soil treatments and incubation conditions

The pretreated soils passed though 2 mm sieve in portions of 500 g were oven-dried, placed into 1000 ml plastic beakers. Eighteen plastic beakers were prepared, containing six Cd levels of cadmium nitrate. All treatments were carried out in triplicates (6 treatments). The soil samples were first adjusted to their required level of moisture contents (40% of its water-holding capacity, WHC, which was calculated according to Table 1) by adding distilled water and pre-incubated for 14 d at 25 °C to stabilize microbial activity. After 14 d pre-incubation, placed into a set of beakers with designated amounts of Cd nitrate applied in solution form to achieve several final Cd concentrations in the soil: 0 (background), 10, 20, 40, 80, 150 mg/kg soil. The moisture contents in the treated soils were adjusted to 60% WHC and then incubated at 25 °C for 0 d, 10 d, 20 d, 40 d, 60 d, respectively. The soil moisture was kept at the same level (60% WHC) by adding distilled water at regular intervals throughout the incubation period. At the end of the incubation for 0 d, 10 d, 20 d, 40 d, 60 d, respectively, the soil samples were taken out and analyzed for C_{mic}, DHA, BR.

Soil microbial parameters assay

All of the following parameters results reported were averages of triplication assays and analysis was used to calculate ED values. Statistical analyses were performed using SPSS 11.0 software.

Soil samples for the determination of C_{mic} were taken by fumigation-extraction (FE) method based on the difference between C extracted with 0.5 mol/L K_2SO_4 from chloroform ($CHCl_3$)-fumigated and unfumigated soil samples using a K_c factor of 0.45 (Vance *et al.*, 1987; Ghamry *et al.*, 2000; Milne and Haynes, 2004). The contents of K_2SO_4-extracted C in the $CHCl_3$-treated and untreated soils were determined using a Shimadzu TOC-500 carbon analyzer (Zhang and Zhang, 2003). DHA was assayed by the method described by Zhu (1996a; 1996b). This method involved colorimetric determination (at 492 nm wavelength on a 722 type spectrophotometer) of 2,3,5-triphenyl formazan (TF) produced by the reduction of 2,3,5-Triphenyltetrazolium Chloride (TTC) by soil microorganisms. The absorbance value obtained photometrically was converted to TF using its standard curve. DHA was expressed by TF generated contents. BR was assayed by the method of seal incubation in erlenmeyer flask, described by Jin *et al.*(1998). Soil samples for BR were placed in a sealed flask with a small bottle of 0.1 mol/L NaOH in it, incubated for 2 d at 25 °C. The CO_2 produced was measured after 48 h by using 0.1 mol/L HCl dripping into the 0.1 mol/L NaOH. BR was expressed as mg CO_2-C/kg soil per hour by CO_2 content released. The CO_2 content was calculated by the formula described by Lu (2000).

Mathematical models

The kinetic model proposed by Speir *et al*.(1995) and the sigmoid dose-response model proposed by Haanstra *et al*.(1985) were used to calculate the ED values and evaluate the suitability of these models for describing Cd inhibition of soil microbiological and biochemical properties. The algebraic expression of kinetic model was:

$$v = \frac{c}{(1+bi)} \qquad \text{(Model 1)}$$

The constants b and c were always positive. The constant c represents the uninhibited value of the tested parameter, and the constant b depends on the curve slope. Model 1 describes the full inhibition of v (tested parameter) by i, the concentration of inhibitor (Cd concentration). For data fitting Model 1, it was possible to calculate the ecological dose values from the relationships:

$$ED_{50} = \frac{1}{b};\ ED_{10} = \frac{1}{9b};\ ED_5 = \frac{5}{95b}$$

Model 1 describes the concave rectangular hyperbolic relationships between v and i; the mathematical equation for the sigmoid dose-response model was:

$$y = \frac{a}{\{1+\exp[b(x-c)]\}} \qquad \text{(Model 2)}$$

where y is the tested parameter, x the natural logarithm of Cd concentration, a the uninhibited value of y, b a slope parameter indicating the inhibition rate, and c the natural logarithm of ED_{50}. The ED values were calculated using the following expressions:

$$ED_{50} = \exp(c);\ ED_{10} = \exp[c - (\frac{2.2}{b})];$$

$$ED_5 = \exp[c - (\frac{2.9}{b})]$$

Model 2 describes a logistic curve which is the relationship between the measured activity and the natural logarithm of the inhibitor concentration. The value of the coefficient of determination (r^2) of the non-linear regression was only determined at $P<0.05$.

RESULTS

The ED_{50}, ED_{10} and ED_5 values calculated with the above two models for C_{mic}, DHA and BR are shown in Tables 2–4. These ED values were calculated for cadmium pollution soil after the different incubation periods mentioned (10 d, 20 d, 40 d and 60 d).

Two mathematical models described the inhibition situation of C_{mic} (Table 2). The values of the coefficient of determination (r^2) were high (0.8562–0.9957) after the addition of Cd during the

Table 2 Values of r^2 (P<0.05) obtained from the regression analysis of Model 1 (M1) and Model 2 (M2) which best describe the inhibition of soil microbial biomass carbon (C_{mic}) by Cd, and ED values (mg Cd/kg soil) predicted from these models

Treatments	Model	r^2	ED_{50}	ED_{10}	ED_5
10 d of incubation	M1	0.8562	1000.0	111.1	52.6
	M2	0.9957	403.2	141.1	100.4
20 d of incubation	M1	0.9545	1250.0	138.9	65.8
	M2	0.9639	612.6	164.7	108.4
40 d of incubation	M1	0.9077	1000.0	111.1	52.6
	M2	0.9774	649.9	168.7	109.8
60 d of incubation	M1	0.8859	1111.1	123.5	58.5
	M2	0.8682	1000.7	230.9	144.8

Table 3 Values of r^2 (P<0.05) obtained from the regression analysis of Model 1 (M1) and Model 2 (M2) which best describe the inhibition of soil dehydrogenase activity (DHA) by Cd, and ED values (mg Cd/kg soil) predicted from these models

Treatments	Model	r^2	ED_{50}	ED_{10}	ED_5
10 d of incubation	M1	0.8567	178.6	19.8	9.4
	M2	0.9812	67.6	6.2	1.5
20 d of incubation	M1	0.8513	66.2	7.4	3.5
	M2	0.9128	69.1	13.0	7.7
40 d of incubation	M1	0.8038	103.1	11.5	5.4
	M2	0.8147	95.5	48.3	38.9
60 d of incubation	M1	0.7954	119.0	13.2	6.3
	M2	0.7477	101.1	50.9	41.0

Table 4 Values of r^2 (P<0.05) obtained from the regression analysis of Model 1 (M1) and Model 2 (M2) which best describe the inhibition of soil microbial basal respiration (BR) by Cd concentration, and ED values (mg Cd/kg soil) predicted from these models

Treatments	Model	r^2	ED_{50}	ED_{10}	ED_5
10 d of incubation	M1	*n.f.	–	–	–
	M2	0.9967	149.7	6.5	1.8
20 d of incubation	M1	0.9256	163.9	18.2	8.6
	M2	0.9813	150.4	7.1	2.7
40 d of incubation	M1	0.8265	163.9	18.2	8.6
	M2	0.9752	154.8	43.8	29.3
60 d of incubation	M1	0.9205	208.3	23.1	11.0
	M2	0.9373	156.5	50.8	35.5

*n.f.: the model did not fit the experimental data, r^2 values are calculable but the level of significance for the regression was not P<0.05

whole incubation period. The ED_{50} values calculated with Model 1 (M1) were much higher than that of Model 2 (M2) in different incubation times, but in 60 d of incubation, they were near. While ED_{10} and ED_5 values with M2 were higher than those with M1 for Cd stress paddy soil. The ED_{50}, ED_{10} and ED_5 values with M2 were all increased with increase of incubation time. In all levels of incubation time, the ED_{50}, ED_{10} and ED_5 values with M1 were near to each other, respectively, but all of them were unstable with incubation time going on. The ED_{50}, ED_{10} and ED_5 values in 20 d of incubation all were highest using M1 during the whole incubation period, respectively, while these highest values of M2 were observed in 60 d of incubation. After 10 d and 40 d of Cd exposure, respectively, the lowest values of ED_{10} and ED_5 cal-

culated with M1 were observed. ED_{10} and ED_5 values of 10 d were equal to those of 40 d, respectively. So the change trend of M2 with incubation time was obvious and regular.

For DHA, the coefficients of determination for the two models were from 0.7477 to 0.9812 during the whole incubation period. M1 and M2 described well the inhibition of DHA, except for 60 d after the addition of Cd, whose coefficients of determination were 0.7954, 0.7747 for M1 and M2, respectively (Table 3). In general, the ED_{50} values predicted from the two models were very near correspondingly, except for 10 d of incubation, when there was greater discrepancy in the predicted ED_{10} and ED_5 values. From 20 d to 60 d of incubation, The ED_{50}, ED_{10} and ED_5 for M1 were increased in turn with incubation time. While ED_{50}, ED_{10} and ED_5 values with M2 were all increased in turn with incubation time. The ED_{10} and ED_5 values with M2 were higher than those of M1 at all spans of incubation time, respectively, except for 10 d of incubation. The ED_{50} values of M1 were lower than those of M2 in 20 d of incubation, higher in other incubation days. From 20 d to 60 d of incubation, the predicted ED_{10} and ED_5 values were higher (about two to seven times), respectively, with M2 than with M1. While in 10 d incubation, the predicted ED_{10} and ED_5 values with M1 were about three or six times that with M2, respectively.

For soil microbial basal respiration (BR), the r^2 values for M1 and M2 also were very high, except for at the 10 d of incubation with M1 (Table 4). In this case the low Cd inhibition of soil microbial basal respiration and dispersion of data produced a poor fit to the mathematical models used. At 10 d of incubation, great discrepancy was observed between the ED values predicted by the two models. M2 gave better fitted the measured data. With this model, the ED values for the Cd stress of paddy soil were lower in the values of r^2 with M1 than with M2 in all stretches of incubation time. This finding may reflect reality because the r^2 values for this model are very high in all cases. r^2 of M2 were from 0.9373 to 0.9967. ED_{50}, ED_{10} and ED_5 values of M2 were all increased with incubation time, but these values of M1 were very near in all cases, respectively, except for 10 d of incubation. For M2 in 10 d of incubation, ED_{50}, ED_{10} and ED_5 values were the lowest, while they were the highest in 60 d of incubation, respectively. For M1 in 10 d of incubation, the ED_{50}, ED_{10} and ED_5 values did not exist, while these values were completely equal in 20 d and 40 d of incubation, respectively. Then, in 60 d of incubation, they were increased, respectively. So M1 could not describe well the inhibition of soil microbial basal respiration under Cd stress in the paddy soil, though their r^2 values were very high (from 0.8265 to 0.9256) except for 10 d of incubation.

DISCUSSION

Tables 2–4 show that ED_{50}, ED_{10} and ED_5 values calculated with M1 were near with incubation time, respectively, except for DHA and BR in 10 d of incubation, while M2 showed that all ED values were increased in turn with prolongation of incubation time. This presumably was the reason that M1 was a full inhibition model. For M2, heavy metal cadmium could have an aging characteristic after entering soil, resulting in lower availability and lower toxicity for Cd with prolongation of incubation time (Martinez *et al.*, 2003). Results showed that compared to M1, M2 describes better the ecological toxicity dose effect of cadmium on soil microbial biomass and its activity in the paddy soil. M2 for ED values of C_{mic}, DHA, BR best fitted the measured paddy soil bioindicators.

Table 2 shows that M1 ED_{50} values were very high (more than 1000 mg Cd/kg soil) and nearly the same with incubation time, which could be because higher Cd dose imposed full inhibition on the microbial metabolic processes and microbial growth. ED_{50} values with M2 increased in turn with incubation time. This was possibly due to the slow increase in cadmium resistance in soil microorganisms (Bewley and Stotzky, 1983) under relatively lower cadmium concentration. M1 ED_{10} and ED_5 values were also very near, respectively, regardless of incubation time. But M2 ED_{10} and ED_5 values were increased in turn from 20 d to 60 d of incubation time.

In most cases, the soil microbiological indicators measured decreased with increasing Cd concentrations, which indicated that Cd had toxic effect on both the microbial metabolic processes and microbial growth. The microbial indicators with M2 decreased with the incubation time for ED_{10} and ED_5 of DHA, while BR and C_{mic} were same as DHA, except for 10 d of incubation. This was presumably due to the de-

crease with time of the substrates easily available to microorganisms (Moreno *et al.*, 1999). The ED values obtained for Cd were much greater than the maximum concentration of Cd permitted in soils by EU (European Union) legislation (3 mg/kg soil). However, for safety, a value one tenth or one thousandth that of ED_{50} (depending on the statistical reliability of this value) was proposed as a maximum limit for heavy metal in soils (Doelman and Haanstra, 1989). Other authors used the ED_{10} and ED_5 values to propose regulatory criteria for heavy metals in soils (Scott and Pedersen, 1995). These ED_{10} and ED_5 values may be more suitable indicators of the sensitivity of an ecosystem to a given stress, because a 50% reduction in a basic ecological process may be too extreme for its continued functioning (Babich *et al.*, 1983). Discrepancy observed in ED_{50} values calculated from the two models can be due to the great elongation of the initial part of the curve of M1 and thus small variation in the rate of decrease of the studied bioindicator can produce a large variation in ED_{50} values calculated from this model. M1 is a full inhibition model and the minimum possible value of the studied bioindicator is always higher than zero, except for soil microbial basal respiration.

In general, for this experiment, the addition of Cd to the soil increased the inhibitory effects on biological parameters. The addition of lower Cd could stimulate soil microbial biomass and its metabolic activity. The first time addition of Cd to soil can obviously inhibit soil microbial biomass and its metabolic activity, then favoring a selection of Cd resistant microorganisms with prolongation of addition time (Kandeler *et al.*, 2000). Furthermore, soil organic matter contributes to adsorption of Cd and thus restricts the movement of this heavy metal through the soil profile (Saviozzi *et al.*, 1983). In general, the ED values increased noticeably over time after soil Cd treatment. Doelman and Haanstra (1984) and Speir *et al.*(1995) also reported an increase in ED_{50} over time for different heavy metals, when the inhibition of soil microbial parameters by soil heavy metal pollution was studied. It is possible that this is due to an increase in Cd resistance in microorganisms as observed by other authors (Bewley and Stotzky, 1983). C_{mic} had been suggested as possible indicators of soil environmental quality in toxicity assays (Yao *et al.*, 2000). DHA is related to a group of intracellular enzymes present in active soil microorganisms. Enzymatic activity had been used as an index of overall microbiological activity in toxicity assays (Nannipieri *et al.*, 1997). DHA was largely affected by increasing Cd concentration in the paddy soil after 10 d, perhaps because metals cannot be taken up by soil and accumulated inside microorganisms at once. Differences in C availability in the media had been shown to have profound effect on the toxicity of Cd (Brynhildsen *et al.*, 1988). In 60 d, however, the soil had higher ED_{50}. Perhaps this is due to either a complexation of Cd with the humic substances or a different evolution of the soil microbial community. In general, the soil microbial basal respiration measurements fit M2 better (Table 4). This model assumes that a portion of the measured activity is not affected by high soil Cd concentrations. This means that the full inhibition of the measured activity is never 100% of the control value (Dixon and Webb, 1979).

In conclusion this study suggests that the addition of Cd to the paddy soil increased the inhibitory toxic effect on microbial function, a finding which may be of great use for the paddy soils with severe Cd contamination. Examples of such soils would be non-agricultural soils near smelters or mines. However, Cd bioavailability in paddy soils can change with time. The values of the ecological dose that produce a specific level of inhibition of soil microbial parameters, are suitable for setting a maximum limit of Cd concentration in order to avoid irreversible effects on soil functionality. However, for other soils a different indicator may produce a better fit to these models. So to safeguard the "health" of the soil, it is necessary to evaluate multiple indicators when using ED_x values as a criterion to set a maximum permissible concentration of a contaminant in soil. Since ED values changed with duration of incubation, it seems that a long-term incubation is better for estimating ED values for a soil, in order that the microbial community has enough time to adapt to the stress conditions.

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