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研究简报

土壤微生物ATP、脱氢酶和尿素酶活性的ED$_{50}$值评价外源镧的生物毒性

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The ED$_{50}$ Values for Assessing Lanthanum Toxicity on Microbial ATP Content and Dehydrogenase and Urease Activities of Paddy Soil

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Rare earths are applied widely in Chinese agriculture to improve crops nutrition and incidentally in fertilizers, as yet little is known of their effect on the biological function of the soil. An incubation experiment was performed to assess lanthanum toxicity on microbial ATP content and dehydrogenase and urease activities in paddy soil, which was assayed after adding La for 4h, 1d, 1, 4, 8, and 16 weeks. The relative ED$_{50}$ values were calculated by two kinetic models (model 1 and model 2) and one sigmoidal dose-response model (model 3). Model 1 was successful in calculating the ED$_{50}$ values for the ATP content, dehydrogenase and urease activities. The ED$_{50}$ values relating to ATP content and dehydrogenase and urease activities were 1086.5 and 897.8 and 1112.5 mg La kg^{-1} soil, respectively. The ED$_{50}$ values of ATP content and dehydrogenase activity increase with incubation time, but the ED$_{50}$ value of urease activity decreases. After 16 weeks, La amendment has no inhibition of urease activity. The present dosage of mixed rare earths, about 0.15mg kg^{-1} soil year^{-1}, applied in agriculture can hardly affect ATP content and dehydrogenase and urease activities in the paddy soil even over a long period.

Keywords: **exogenous lanthanum** **soil microbial ATP** **dehydrogenase activity** **urease activity** **ED$_{50}$**

0 Introduction

Rare earths are now applied widely in China, which can improve crops yields and the their qualities[1]. The beneficial effects may be due to the stimu-

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latory effects of these elements on the nutrient uptake by plants or on the increasing of chlorophyll synthesis in the plants[2] . While a lot of researches have been done on the improved nutrition of crops after application of rare earths, much less attention has been paid to the deterioration of soil quality due to the application of rare earths for years[3]. Scientists have discovered that accumulation of rare earths is significant after 3 months of agricultural application[4], and rare earth elements (REEs) hardly migrate downward in soil profiles[5]. REEs have a lasting effect on soil microbial community structure, biodiversity, N mineralization, and enzyme activity[6] . So, we consider that the added rare earths might be the pollutants. It is important to ascertain the maximum amounts of REEs that can be supported by a soil without any effect on its quality. In order to quantify easily the influence of pollutants on microbe-mediated processes in soil, an ecological dose 50% (ED_{50}) concept was developed, which is the concentration of a toxicant that inhibits a microbe-mediated ecological process by 50%. The ED_{50} can also be useful in determining which factors affect REEs toxicity, such as time, soil abiotic properties, and sensitivity of exogenous chemical properties[7].

During the last few decades, lots of attention has been paid to pollution action of heavy metal, such as Cu, Pb, Cd, Zn[8]. Different mathematical models have been used to calculate the ED_{50} values[9,10]. Recently, more and more information has been obtained on bioavailability of REEs in soil[11~14]. ATP and enzyme are the sensitive indicator of microbial metabolism in soil. As far as we know, no significant research has yet been done on the influence of lanthanum on ATP content and dehydrogenase and urease activities in soils. The aim of this study was to assess the inhibitions of the ATP content and dehydrogenase and urease activities in paddy soil by adding La. These soil parameters were assayed at different periods in order to assess the effect of duration of exposure to exogenous lanthanum pollution.

1 Material and Methods

1.1 Soil Samples and Handling

Soils were surface sampled (0 ~ 20cm) from a farmland in West Tiaoxi catchments (40km southwest to Taihu Lake) . After culling plants, roots, stones, and animals in soil, the 0 ~ 20cm topsoil was sieved (2mm), homogenized and stored in polyethylene bags at 4℃. Before the experiment, soil samples were pre-incubated for 7 days at 25℃ to stabilize microbial activity. The basic properties of the soil are shown in Table 1.

1.2 Experimental Design

The soils (1000g) were treated with 50mL of $LaCl_3$ solutions to give the La concentration ranging from 5 to 4000mg · kg^{-1} soil (20 different La concentrations in soils) . Untreated soils served as controls. The soil moisture was adjusted to 60% of water holding capacity with distilled water and then the soils were incubated under controlled conditions (25℃ and darkness) for 4h, 1day, 1, 4, 8 and 16 weeks.

1.3 Soil Analyses

Microbial ATP was extracted and determined from soil subsamples of 2g by the procedure of reference 15. Soil dehydrogenase activity was determined using p-iodonitrotetrazolium chloride (INT), as reported in reference 16, and expressed as μg iodonitrotetrazolium formazan (INTF)/(h g soil). Soil urease activity was determined by the buffered method[17], and expressed as μg NH_4^+-N/(h g soil).

1.4 Mathematical Analysis of Data

The two kinetic models proposed in reference 10 and one sigmoidal dose-response model proposed in reference 9 were used to assess the inhibitions of soil biological and biochemical properties by La. The algebraic expressions of kinetic models were $v = c(1 +$

Table 1 Basic Properties of the Soils used in Incubation Experiments

classification	pH($CaCl_2$)	organic matter /(g · kg^{-1})	cation exchange capacity /(cmol · kg^{-1})	water holding capacity/%	composition/%		
					sand	silt	clay
paddy soil	4.80	3.80	17.90	54.20	51.4	35.7	12.9

b_i) (model 1) and $v = c(1 + a_i)/(1 + b_i)$ (model 2). The contents a, b and c were always positive, with $b > a$. Model 1 describes the full inhibition of v (tested parameter) by i, the concentration of inhibitor, and model 2 describes the partial inhibition. For data fitting model 1 was possible to calculate the ecological dose by the equation of $ED_{50} = 1/b$. In the case of model 2 the equation was $ED_{50} = (1 - a/b)(b - a)$. Model 2 describes a concave rectangular hyperbolic relationship between v and i, with asymptote ca/b parallel to but above the x-axis. The equation for the sigmoidal dose-response model was $y = a/(1 + e^{b(x-c)})$, where y is the tested parameter, x is the natural logarithm of La concentration, a is the uninhibited value of y, b is a slope factor value and c is the natural logarithm of ED_{50}.

2 Results

The ED_{50} values calculated with the three models are shown in Table 2. Some typical plots showing the fitting of the measured parameters to the models used are presented in Figs. 1 ~ 3. With the exception of the urease activity at 16 weeks of contamination, the inhibitions of the tested parameters by La concentrations in soil were described by at least one of the tested models. Model 3, which describes a sigmoidal dose-response curve, was less successful than models 1 and 2 in fitting the experimental data. Only in three cases (dehydrogenase activity at 1 day and urease activity at 1 day or 1 week) the r^2 values of model 3 were higher than 0.900. The ED_{50} values of ATP content were always found to be higher in model 1 than in model 2 under same concentration. This is not the case for dehydrogenase and urease activity.

Generally the ED_{50} values of dehydrogenase and urease activities predicted by model 1 and model 3 are similar when their correlation coefficients (r^2) were higher than 0.900. The ED_{50} values of dehydrogenase activity, predicted by model 1 and model 2, are similar because the asymptote value of model 2 is close to zero. The same phenomenon was observed for the urease activity after adding La for 4 weeks (Fig. 3). The ED_{50}

Table 2 ED_{50} values of ATP, and Urease and Dehydrogenase Activities in Soils after 4 hours, 1 day, 1, 4, 8, and 16 weeks of Exogenous La Contamination

			incubation time					
			4 h	1 day	1 week	4 weeks	8 weeks	16 weeks
ATP	model 1	ED_{50}	1179.4	1678.5	3214.7	2587.4	5347.8	19109.0
		r^2	0.936	0.902	0.924	0.956	0.917	0.968
	model 2	ED_{50}	N. F.	1545.1	2728.4	2417.5	4177.9	N. F.
		r^2	—	0.879	0.901	0.912	0.905	—
		asymptote	—	11.5	24.1	17.9	56.4	—
	model 3	ED_{50}	1086.5	1612.8	2751.8	2416.7	8103.6	16155.0
		r^2	0.813	0.848	0.820	0.784	0.490	0.385
dehydrogenase	model 1	ED_{50}	918.5	1514.6	2458.4	2144.7	2816.3	3172.7
		r^2	0.956	0.966	0.925	0.908	0.923	0.955
	model 2	ED_{50}	1128.9	1446.2	N. F.	2075.8	N. F.	N. F.
		r^2	0.897	0.911	—	0.892	—	—
		asymptote	14.9	7.1	—	4.3	—	—
	model 3	ED_{50}	897.8	1401.4	2145.6	1948.7	2458.9	2751.8
		r^2	0.854	0.918	0.784	0.812	0.771	0.676
urease	model 1	ED_{50}	3451.2	2784.5	1538.5	1645.4	1112.5	N. I.
		r^2	0.941	0.925	0.914	0.971	0.958	—
	model 2	ED_{50}	N. F.	N. F.	N. F.	1351.2	1209.1	N. I.
		r^2	—	—	—	0.884	0.912	—
		asymptote	—	—	—	9.74	6.54	—
	model 3	ED_{50}	3066.7	2688.3	1500.2	1603.6	1054.3	N. I.
		r^2	0.784	0.912	0.905	0.782	0.633	—

* N. F. indicates no fit of the data to the model; r^2 and asymptote values, although calculable, are not valid. N. I. indicates no inhibition of urease activity by La amendment. ED_{50} and asymptote values are expressed as $mg \cdot kg^{-1}$.

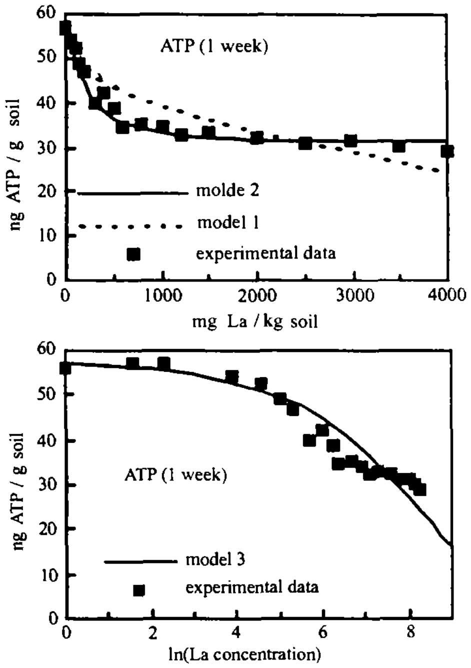

Fig. 1 Experimental data and calculated plots of the relationship between ATP content and total La concentration according to model 1 (describing full inhibition), model 2 (describing partial inhibition), and model 3 (the sigmoidal dose-response model)

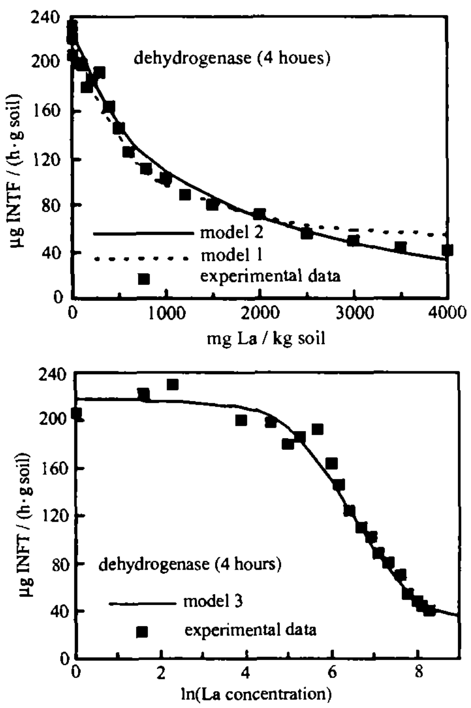

Fig. 2 Experimental data and calculated plots of the relationship between dehydrogenase activity and total La concentration according to model 1 (describing full inhibition), model 2 (describing partial inhibition), and model 3 (the sigmoidal dose-response model)

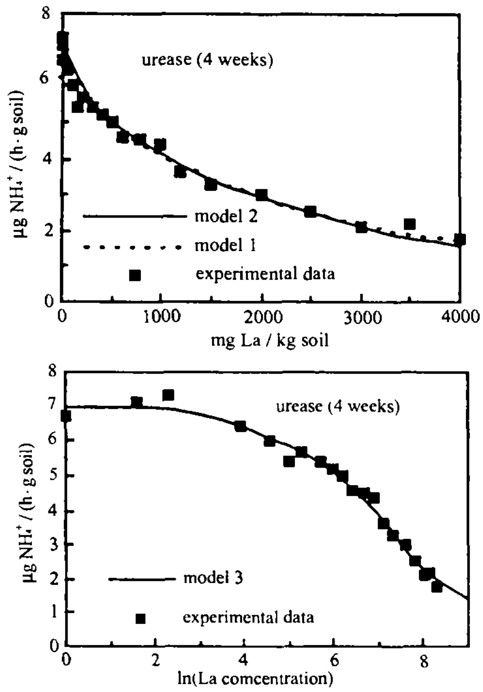

Fig. 3 Experimental data and calculated plots of the relationship between urease activity and total La concentration according to model 1 (describing full inhibition), model 2 (describing partial inhibition), and model 3 (the sigmoidal dose-response model)

values for ATP content and dehydrogenase activity peaked after 16 weeks whereas the highest value of urease activity was reached after 4 hours when the values were calculated by model 1 (Table 2). At 16 weeks, we can discover no inhibition of urease activity by La amendment.

3 Discussion

The ED_{50} values of ATP content predicted by model 2 were much smaller than those calculated from model 1, probably due to high asymptote values. By fitting the data according to model 2, microbial biomass ATP content did not fall to zero but to an asymptote parallel to but above the x-axis (Fig. 1). It may be possible that some of the enzyme activities involved in the ATP synthesis were not inhibited by the increase in La concentration. In this case it can be erroneous to calculate the ED_{50} value because the decrease in ATP content by La pollution was not higher than 50% of the initial value. When the asymptote values tend to zero, model 1 and model 2 tend to equality and the ED_{50} values were similar, such as dehydrogenase activity at 4 weeks and urease activity at 8 weeks (Table 2). The ED_{50} values predicted by model 1, which describe a total inhibition, were also higher than those calculated by model 3.

The ED_{50} values of ATP content and dehydrogenase activity predicted by model 1, increase with incubation times, which indicate more and more weakening inhibition by La amendment (Fig. 4). Possibly microbial and/or chemical process made La less available to soil microorganisms, and/or resistant microbial species were selected on prolonging the La contamination[18]. REEs in soil may dissolve in soil soluble liquor, absorb in the surface of gummed body, lie in soil mineral, and react with other chemical materials[19]. Toxicity is closed related with chemical form of La in soil[4,6]. Soluble, organic, exchangeable, absorption, and oxide are mutable and unstable, but relict is stable. Self-purification of soil depends on binding capacity (BC), which is controlled by pH, organic matter, composition, and Fe-Mn oxide[20]. After entering into soil, La is fixed and exchanged easily by organic matter and Fe-Mn oxide, which leads La to decrease significantly. Microorganisms can solubilize cations in soil by producing water-soluble organic compounds or by becoming soluble metal ligands or by releasing microbial metabolic products and these result in the physico-chemical conditions of the soil environment are changed. Microorganisms can also render metals insoluble in soil by producing water-insoluble organic compounds or ligands that form insoluble metal complexes[21]. Fixture role of soil was strengthened with incubation times. A lower pH value and higher organic matter of soil in this study change more easily microbial and chemical process. Therefore, effects of La on microbial biomass ATP content and on dehydrogenase activity take place in the early stage after adding La. The inhibitions of ATP and dehydrogenase activity may be lessened with incubation times, but never are eliminated in short term.

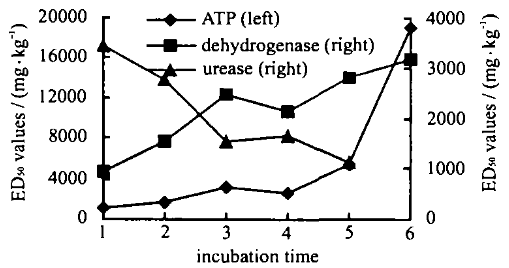

Fig. 4　ED_{50} values predicted by model 1 Change with incubation times

Incubation time: 1: 4 hours; 2: 1 day; 3: 1 week; 4: 4 weeks; 5: 8 weeks: 6: 16 weeks.

The ED_{50} values of urease activity decrease with incubation time, which indicate more and more strengthening inhibition by La amendment (Fig. 4). However, no inhibition of urease activity was observed at 16 weeks while the ED_{50} values of dehydrogenase activities generally peaked at 16 weeks. Urease activity of a soil can reflect the contribution of both intracellular and extracellular enzymes, which can be adsorbed on inorganic colloids or enclosed in humic complexes. Longer incubation periods can eliminate the inhibition of urease activity by La contamination[22]. The decrease in ATP content or the inhibition of dehydrogenase activity by La may be due to the negative effects of the REEs on the activity of microbial species sensitive to La pollution[23]. This can also be true for the inhibition of urease activity by La; however, in this study the possibility that La inhibits the activity of extracellular urease stabilized by soil colloids can not be excluded. Some studies show that the soil tested contains about 150mg rare earths kg^{-1}[3,6,11], and the concentrations of the rare earths in Chinese soils vary with soil type, ranging from 108 to 480mg · kg^{-1}[24]. The ED_{50} values relating to ATP content and dehydrogenase and urease activities were 1086.5 and 897.8 and 1112.5mg La/kg soil, respectively. The present dosage of mixed rare earths, about 0.15mg · kg^{-1} soil $year^{-1}$[1,3], applied in China can hardly affect ATP content and dehydrogenase and urease activities in the paddy soil even over a long period.

4　Conclusions

Model 1 was the most one in calculating the ED_{50}

values for ATP content and dehydrogenase and urease activity by La contamination. The ED_{50} values relating to ATP content and dehydrogenase and urease activity were 1086.5 and 897.8 and 1112.5 mg La/kg soil, respectively. However, the sensitivity of ATP content and dehydrogenase and urease activity by La amendment is significantly different. With increasing of incubation times, the ED_{50} values of ATP content and dehydrogenase activity increase in comparison with the decrease of urease activity. The ED_{50} values generally increased with the duration of La exposure, probably due to the selection of more resistant microbial populations, lower pH value, higher organic matter content, cation exchange capacity and soil colloids.

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