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RESEARCH ARTICLE

Influence of exogenous lead pollution on enzyme activities and organic matter degradation in the surface of river sediment

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Abstract As lead is one of the most hazardous heavy metals in river ecosystem, the influence of exogenous lead pollution on enzyme activities and organic matter degradation in the surface of river sediment with high moisture content were studied at laboratory scale. The dynamic changes of urease, catalase, protease activities, organic matter content, and exchangeable or ethylenediaminetetraacetic acid (EDTA)-extractable Pb concentration in sediment were monitored during different levels of exogenous lead infiltrating into sediment. At the early stage of incubation, the activities of catalase and protease were inhibited, whereas the urease activities were enhanced with different levels of exogenous lead. Organic matter content in polluted sediment with exogenous lead was lower than control and correlated with enzyme activities. In addition, the effects of lead on the three enzyme activities were strongly time-dependent and catalase activities showed lower significant difference ($P<0.05$) than urease and protease. Correlations between catalase activities and EDTA-extractable Pb in the experiment were significantly negative. The present findings will improve the understandings about the ecotoxicological mechanisms in sediment.

Keywords Exogenous lead pollution · Sediment · Urease · Catalase · Protease · Organic matter

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Introduction

With the development of the industrial production, large amount of heavy metal pollutants have been discharged into rivers. That represents a long-term health hazard to animals, plants, and humans (Zeng et al. 2006; Zhu et al. 2013). Lead is a widespread heavy metal existing in environment. Mankind is faced with the risk of lead contamination on a global scale (Finster et al. 2004; Feng et al. 2004). Major anthropogenic sources of lead include base metal mining, ore processing, and smelting; battery manufacturing; uncontrolled disposal of Pb-containing products, such as spent batteries and computer parts; Pb-based paints; and the continuous use of lead in ammunition and fishing tackle (Sun et al. 2011). All of the above anthropogenic activities could increase environmental lead concentration to a level that is potentially toxic to various organisms (Johnson et al. 1995; Munoz et al. 2012).

Ecological system of river sediments are frequently subjected to stress factors involving large inputs of organic matter (OM), heavy metals, and biological activity in the benthic compartment. Sediments are generally considered as sinks for heavy metals and metalloids in river ecosystem (Barceló 2007; Yao et al. 2007; Mishra et al. 2008; Mao et al. 2014). Lead can be absorbed onto sediments and released again under certain conditions. With respect to the potential release of lead from sediments, changes of pH, salinity, redox potential conditions, and resuspension of polluted sediments are primary factors (Ho et al. 2012; Zhang et al. 2014). The free lead ion (Pb^{2+}) is considered to be the most toxic form in sediments and the total amount of Pb in sediments cannot predict the bioavailability and toxicity of lead (Pagnanelli et al. 2004; Clozel et al. 2006). There are numerous complexities that affect Pb accumulation and transportation in river sediments. These include varying pH, changes of redox potential over time and space, organic carbon bonding sites, effect of benthic

organisms, and other potential ligands presenting in the sediments. The toxicity and concentration of Pb^{2+} in sediments vary with dissolved organic compounds, hydroxide, carbonate, chloride, and hardness (calcium and magnesium) (Luoma and Davies 1983; Rinklebe and Shaheen 2014; Li et al. 2014). Furthermore, the biological magnification of Pb is unobvious in organisms (Mikac et al. 2001). Therefore, it is essential to control lead pollution and understand the mobility and potential ecological risks of lead.

Microorganisms in sediment play a key role in the processing of organic carbon, and they are regarded as the major producers of ectoenzymes on the surface of sediment. Variations in microbial diversity and community structure can affect organic matter. In turn, organic matter affects the ecosystem function of a river ecosystem. Sinking and deposited organic matter serves as a high-quality food source, being a potentially important structuring mechanism in sediment. Organic matter quality has been described to influence sediment bacterial community and biomass, density and productivity of microorganisms (Kirchman et al. 2005; Ikenaga et al. 2010). Microorganisms are the first to suffer the negative impacts of lead pollutants when exogenous lead polluted river sediment and have to cope with toxic Pb during their growth (Zhu et al. 2013; Yang et al. 2014a; Pan and Yu 2011). The exposure of microorganisms to metals always inhibits microbial growth and enzyme activities (Yang et al. 2014b). Enzyme activities in the system of river sediment are reliable indicators for the rate of biotic transformation processes (Zhou et al. 2005). Urease, catalase, and protease are ubiquitous enzymes in sediment and participate in the recycling of nutrients. Urease could catalyze hydrolysis of urea to form carbon dioxide and ammonia as a nickel-dependent enzyme (Li et al. 2013; Wightwick et al. 2013). Catalase is an antioxidant enzyme present in all aerobic organisms. Catalase works as a detoxification system inside living cells against reactive oxygen species formed as a by-product of different metabolic reactions (Mullineaux et al. 2006). It also plays a role in maintaining redox homeostasis of the cell as a part of the antioxidant response system. Most sediment microorganisms can produce proteases to express proteolytic activities, by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein (Valerie et al. 2013). Proteolysis is an important process with regard to N-cycling in many ecosystems, as proteolysis is considered to be a rate-limiting step during N mineralization in sediment (Weintraub and Schimel 2005). Under unfavorable conditions, protease plays a role in the interactions of sediment organisms via cleavage of the cell wall proteins and function in the survival of microorganisms (Simona et al. 2004; Valerie et al. 2013). Microbial enzymes might be affected by lead due to the potential inhibition to both enzymatic reactions and complex metabolic processes (Zhang et al. 2008). In addition, enzyme activities have been recommended as standard biochemical indicators for assessing the general condition of sediment microbial populations (Liang et al. 2003; Munoz et al. 2012) and enzyme assays can reflect the biological functioning of sediment organisms.

It is well known that moisture content is a very important factor in sediment system. Both microbial activity and community size decrease as the moisture content decreases below the optimum level (Cho et al. 2001). The rate of the biotransformation processes in river sediment is directly related to the parameter "moisture." In addition, there are some connections between moisture content and enzyme activities. Previous researches reported significant correlations of phosphatase activity with changes in moisture over time under field conditions (Speir and Cowling 1991). Hinojosa et al. (2004) observed different enzymes have different responses to moisture content. Alkaline phosphatase and urease activities significantly increased with high level of moisture content, whereas β-glucosidase did not show any significant changes (Hinojosa et al. 2004). However, there are only a few studies showing the importance of pretreatment to standardize moisture level in enzyme assays in sediment.

The objective of this study was to gain new insights on the effect of exogenous lead pollution on enzyme activities and organic matter degradation in the surface of river sediment. The sediments we used in our experiment were kept at high moisture content. The dynamic changes of urease, catalase, protease activities, organic matter content, and exchangeable or ethylenediaminetetraacetic acid (EDTA)-extractable Pb concentration in sediment were systematically studied during different levels of exogenous lead infiltrating into sediment. An attempt was made to evaluate the relationships between enzyme activities with cultivated time, different treatments, and organic matter content in sediment. Furthermore, correlation analyses between exchangeable or EDTA-extractable Pb concentration and enzyme activities were discussed in detail. The relationship between organic matter degradation and enzyme activities in sediment was studied to enhance our understanding of the mechanisms of biogeochemical processes.

Materials and methods

Sediment collection and preparation

Sediment was collected from the surface layer (0–20 cm) of Xiangjiang River in Changsha of Hunan Province (28° 50′ 10″ N, 112° 52′ 56″ E) by using a gravity corer consisting of an acrylic pipe with 8-cm inner diameter and 100-cm length, a sediment catcher, and a clear vent. The sediment was brought to the laboratory and kept at 4 °C (to reduce biological metabolic activity) until acclimation (1 week maximum). In order to standardize substratum structure, the natural sediments

Table 1 Physicochemical characteristics analysis of the used sediment

Temperature (°C)	Moisture content (%)	pH	Organic matter (g/g)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Soluble–exchangeable Pb (mg/kg)	EDTA-extractable Pb (mg/kg)
20	71.6	7.64	0.058	2.14	0.173	11.840	58.03

were sieved (pore size 10 mm) and homogenized in glass jars using a clean spatula. A sample of fresh sediment was analyzed after sediment collection to determine the moisture content and the organic matter content. Moisture content was measured by weighing each replicate sample before and after drying at 105 °C until weight stabilization. The content of organic matter (OM) was determined after drying at 90 °C for 48 h and incineration at 550 °C for 4 h. Degradation rates of organic matter were calculated by the following formula:

$$D = m_i - m_{i+1} \quad (1)$$

where m_i represent the content of organic matter at each sampling day. And some basic physicochemical properties of the fresh sediments were analyzed subsequently and the results were listed in Table 1.

Experimental design

A stock solution containing 1.0 g/l Pb (II) was prepared by dissolving $Pb(NO_3)_2$ in ultra-pure water. The exact concentration of the stock solution was determined by flame atomic absorption spectrometry (Perkin-Elmer AA700, USA). All of the other inorganic chemicals were of analytical grade and were purchased from Shanghai First Reagent Co., China. Test solutions were prepared by diluting the stock solution to the desired Pb (II) concentrations of 0, 10, 50, 100, 200, 500, and 1,000 mg/l, respectively. The experiment was carried out in 500-ml flasks containing 300-g portions of homogenized sediment. Flasks were labeled as S0 (control), S1 (10 mg/l), S2 (50 mg/l), S3 (100 mg/l), S4 (200 mg/l), S5 (500 mg/l), and S6 (1,000 mg/l). The moisture content of homogenized sediment in each flask was adjusted to 70 %. After 7 days of incubation, the sediments were supplemented and mixed with 100 ml of 0, 10, 50, 100, 200, 500, and 1,000 mg/l Pb (II) solutions, respectively. They were used to simulate different degrees of exogenous lead pollution. To avoid the effects of sampling on sediment, three similar flasks for each concentration were prepared in the same way. Each replicate sediment sample was mixed thoroughly. Perforated plastic covers were used to restrict evaporation and permit gas exchange. Each flask was performed in water-bathing constant temperature vibrator under 50 rpm at 20 °C for 45 days. The moisture content was maintained at the initial level by using deionized water in the entire incubation period. Subsamples were collected from the flasks periodically after the addition of exogenous lead (1, 3, 7, 10, 15, 20, 30, and 45 days). Subsamples from flasks of the same treatment were combined and then analyzed in three replicates. After each sampling, the weight of sediment in the flask reduced and the moisture content remained unchanged. The pH was in the range 7.5–7.9 in the whole experiment.

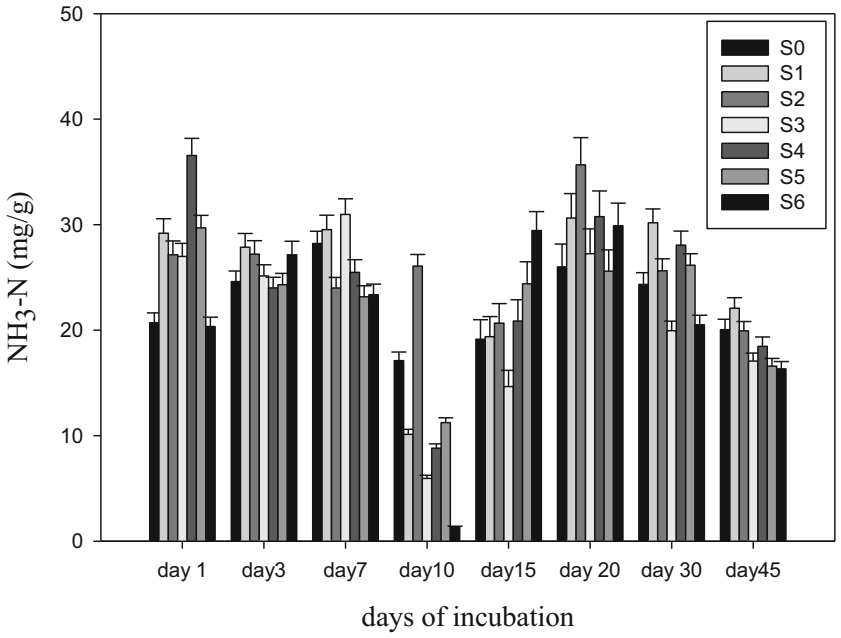

Fig. 1 The effect of lead on urease activity (milligrams of NH_4–N per gram of sediment per hour): during the different incubation periods with Pb addition. The bars represent the standard deviations of the means (n=3)

Sediment enzymes activity assays

Enzyme assays were analyzed to reflect microbial activity and represent a range of processes involved in nutrient cycling and decomposition. Sediment enzyme activities were assayed in triplicate air-dried pooled samples as described by Li et al. (2008). The urease activity in the sediment was measured by colorimetric method using toluene and 10 % urea. In this procedure, a solution of urea (10 %) and citrate buffer (pH 7) were added to sample in hermetically sealed flasks and then incubated for 24 h at 37 °C. The ammonium content of the centrifuged extracts was determined by a modified indophenol blue reaction. Catalase activity was measured by the titrimetric method using potassium permanganate ($KMnO_4$) titration. In the procedure, a solution of peroxide (0.3 % H_2O_2) was added to sediment as substrate and vibrated for 20 min, then filtered for titration. Protease activity was determined by the content of amino acid. Two grams of sediment sample was incubated for 24 h at 30 °C with 0.5 ml of toluene and 10 ml of phosphate buffer (pH 7.4) containing 1 % (w/v) of gelafusal. After incubation, the mixture was filtered and 5 mm of the supernatant was treated with 0.5 ml of sulfuric acid solution (0.1 N) and 3 ml of sodium sulfate (20 %), followed by addition of 1.0 ml of ninhydrin (2 %, w/v). After boiling water bath for 10 min, mixture was measured at 560 nm in a spectrophotometer.

Lead extraction and analysis

The soluble–exchangeable and EDTA-extractable Pb concentration were determined as follows: (i) Soluble exchangeable: The 1 g air-dried sediment which passed through 100 meshes sieve was extracted for 2 h with 8 ml of 1 M $MgCl_2$ (pH 7.0) with continuous agitation. (ii) EDTA extractable: The 5 g air-dried sediment which passed through 100 meshes sieve was extracted for 1 h with 25 ml of 0.05 M EDTA ammonium solution (pH 7.0) with occasional agitation. Separation was effected by centrifuging and Pb concentrations in the supernatant were determined by an atomic absorption spectrometer (Huang et al. 2006). All the measurements were carried out in triplicate.

Statistical analysis

Data were the means of three replicates, the standard deviations were used to analyze experimental data. The standard errors of means were below 1.2 % (n=3). Statistical analyses were performed using the software package SPSS 19.0 for Windows (SPSS, Germany). One-way analysis of variance (ANOVA) and test of homogeneity of variances were used to determine differences of urease activities, catalase activities, protease activities and the degradation rates of organic matter among the treatment groups. We used Tukey's b test

Table 2 Significant difference analysis of urease activity (milligrams of NH_4–N per gram of sediment per hour) in sediment under different levels of exogenous lead

Incubation days	Different treatments						Test of homogeneity of variances	One-way ANOVA	Two-way ANOVA		
	S0	S1	S2	S3	S4	S5	S6	P value		P_1	P_2	P_3
1	20.75(1.15)^{a}	29.45(1.47)^{b}	27.05(1.33)^{c}	26.29(1.61)^{c}	36.12(1.40)^{d}	29.49(1.61)^{b}	19.85(1.57)^{a}	0.34	0.00*	0.00*	0.00*	0.00*
3	24.61(1.65)^{a}	27.86(1.86)^{b}	27.21(0.94)^{b}	25.14(1.69)^{a,b}	24.01(1.95)^{a}	24.31(1.77)^{a}	27.15(2.49)^{b}	0.59	0.00*			
7	28.21(1.63)^{a}	29.53(1.31)^{b}	23.99(2.22)^{c}	30.97(1.32)^{d}	25.48(1.16)^{e}	23.16(2.09)^{c}	23.36(1.30)^{c}	0.09	0.01*			
10	17.10(1.07)^{a}	10.12(1.23)^{a,b}	26.07(1.27)^{c}	5.95(2.02)^{b,d}	8.82(1.12)^{b,e}	11.23(1.00)^{b,f}	1.36(2.04)^{g}	0.00	0.00*			
15	19.14(1.17)^{a}	19.38(1.02)^{a}	20.67(1.26)^{a,b}	14.66(2.46)^{c}	20.87(1.05)^{b}	24.40(2.01)^{d}	19.44(2.07)^{a}	0.00	0.00*			
20	26.00(1.05)^{a}	30.63(1.71)^{b}	35.66(2.03)^{c}	27.24(1.27)^{a,d}	30.76(2.00)^{b}	25.59(1.28)^{a}	29.89(1.46)^{b}	0.02	0.00*			
30	20.33(1.22)^{a}	30.18(1.15)^{b}	25.62(2.06)^{b,c}	19.95(1.23)^{a}	28.07(1.03)^{b}	28.16(1.08)^{b}	20.51(2.00)^{a}	0.00	0.00*			
45	20.05(1.57)^{a}	18.08(1.36)^{b}	18.93(1.41)^{c}	17.97(2.14)^{b}	17.46(1.83)^{d}	16.59(1.37)^{e}	16.33(0.94)^{e}	0.07	0.00*			

* Significant differences at the 0.05 level (P<0.05)

Values are means (n=3) with standard deviation in parentheses; P_1=significance level between treatments; P_2=significance level between times; P_3=significance level of interaction effect between treatments and times. Means followed by different letters within the same row are significantly different at P<0.05 according to Tukey's b test and Games–Howell post hoc test at the 5 % probability level; Tukey's b test was used when test of homogeneity of variance is passed (P>0.05) and Games–Howell test was used for the case of equal variance not assumed (P<0.05)

for multiple comparisons between different treatments when test of homogeneity of variance was not significant and Games–Howell post hoc test for the case of equal variance not assumed when appropriate. Two-way ANOVA was used to test the individual effect of time and treatment, and the interaction effect between them. Differences were considered statistically significant at $P<0.05$. Linear correlation analyses used to determine the relationships between enzyme activities and the concentration of soluble–exchangeable Pb or EDTA-extractable Pb and content of organic matter, respectively.

Results and discussion

Effect of exogenous lead in sediment on urease activities

Figure 1 demonstrates dynamic changes of urease activities during incubation time with different initial Pb concentrations in sediments. The urease activities with exogenous lead pollution were higher than control on day 1 and day 3 and lower than control on day 7 except S1 and S3. Urease activities for both low and high concentrations of exogenous lead were significantly lower than control on day 10 except S2. The lowest urease activity was found in S6 on day 10. Stimulation of urease activities were observed in sediments with medium and high concentrations of exogenous lead on day 15, day 20, and day 30. The urease activities at five concentrations studied were lower than control except S1 on day 45. Statistical analysis of data by one-way ANOVA and two-way ANOVA showed urease that indicated significant differences ($P<0.05$) in the exposure time and different treatments (Table 2). Significant interaction effects of both time and treatment on urease activities were observed statistically (Table 2).

Several studies have indicated that urease activities decreased with the increasing Pb concentrations (Pan et al. 2011; Hinojosa et al. 2008; Liang et al. 2003). However, in this study, it was found that exogenous lead did not cause inhibition of urease activities in high-dose experiment (Fig. 1). Rather, the high concentrations of exogenous lead caused a significant increase in urease activity 1 day after application, although this effect was not apparent in the day 3 and day 7 samples (Fig. 1). This might be an indication of a direct effect on sediment microorganisms resulting in a short-term increase in enzyme concentration due to their release from dying cells (Yan et al. 2013; Modolo et al. 2015). In this study, we found that exogenous lead had a stimulating effect on urease even at the highest concentration and that urease activity in S1 was higher than control on day 45. This was consistent with previous researches that sediment containing low levels of metals has a stimulating effect on microbial activity (Fliessch et al. 1994; Pan et al. 2011). Urease activities showed significant changes with exposure time. It was noticed that the urease activities in sediment with medium and high concentrations of exogenous lead increased after day 10. A proper mechanism to explain this situation may be the ageing phenomenon of heavy metal, the tolerance and adaptation of microorganism to the sorption of Pb, which change the bioavailability of Pb (Huang et al. 2010; Munoz et al. 2012). The small percentage of available Pb existing in sediment can influence the metabolic activities of microorganisms. Due to long-time processes (ageing), the bioavailability of Pb decreased with time and exogenous lead associated with natural organic matter and the penetration of exogenous lead into

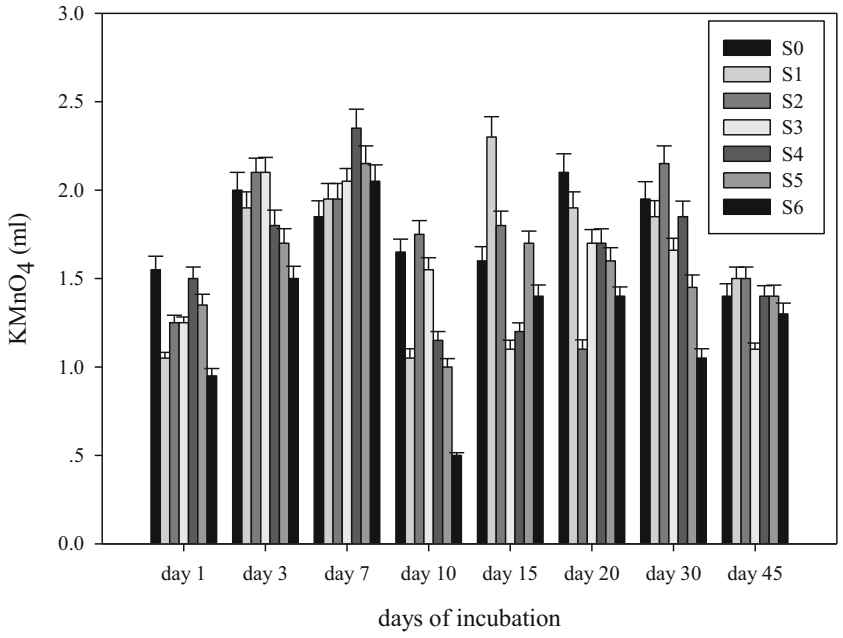

Fig. 2 Changes of catalase activity in the control without Pb addition and the treatments with Pb in concentration of 10, 50, 100, 200, 500, and 1,000 mg/l at different incubation times. Vertical bars denote SE ($n=3$)

small pores in sediment (Trivedi and Axe 2000; Lock and Janssen 2003; Huang et al. 2008). In conclusion, the results indicate that sediment microorganisms are not destroyed by lead exposure in spite of the observed inhibitory effects.

Changes of catalase activities under Pb stress

The sensitivities of catalase to different levels of exogenous lead in sediments are shown in Fig. 2. The catalase activities were lower than control (S0) with increased Pb concentration during day 1 and day 3 except S2 and S3 (Fig. 2). Whereas catalase activities in sediments with exogenous lead evidently increased in contrast to control on day 7, but decreased with Pb concentration on day 10 except S1. The catalase activity was inhibited by higher Pb concentrations in the exogenous Pb treatment when the added Pb concentration reached 100 mg/l, whereas the low Pb concentration from 10 to 50 mg/l activated the enzyme activity on day 10 and day 15. On day 20 and day 30, the trends were significantly downward; on day 45, the catalase activity showed little difference with increased Pb concentration, and the lowest point occurred in S3. The results of one-way ANOVA and two-way ANOVA showed strong effect of time and treatment on catalase activities and interaction effect was significant (Table 3).

The exogenous lead can induce oxidative stress on sediment microorganisms. The catalase is a significant component of the cell defense mechanism against oxidative stress, as it scavenges hydrogen peroxide (H_2O_2) to oxygen and water. All aerobic microorganisms have evolved complex inducible repair mechanisms, in the form of this enzyme, to alleviate the damaging effects of active oxygen. The exogenous lead might inhibit the catalase activities by inducing oxidative stress at the early stage. Furthermore, catalase is typically localized in peroxisomes. Peroxisomes are essential subcellular organelles present in almost all eukaryotic cells and are lacking in DNA and translational machinery (Wolf et al. 2010). The peroxisome is considered to be a protective compartment within eukaryotic cells that shields the surrounding cytoplasm from many toxic or harmful compounds which are produced and detoxicated by enzymes within this organelle (Wolf et al. 2010; Pan et al. 2011). The protective effect of peroxisome in catalase might be the reason why catalase activities increased after inhibition under exogenous lead pollution. At present, very little information is available on the enzyme activation mechanism in the presence of Pb. An increase in the catalase enzyme activity in the presence of Pb was reported and the stimulating effect for the enzyme stemmed from Pb associated with Fe–Mn oxide (Liu et al. 2003). The observed activation of calalase in sediment in the presence of Pb probably was a result of the reaction between the Pb ion in sediment and the functional groups of catalase. According to Moreno et al. (1999), the decrease in the microbial indicators measured with increasing incubation time was presumably

Table 3 Significant difference analysis of catalase activity (milliliters of $KMnO_4$ per gram of sediment per hour) in sediment under different levels of exogenous lead

Incubation days	Different treatments						Test of homogeneity of variances	One-way ANOVA	Two-way ANOVA		
	S0	S1	S2	S3	S4	S5	S6	*P* value		P_1	P_2	P_3
1	1.55(0.12)^{a}	1.05(0.15)^{b}	1.25(0.13)^{c}	1.25(0.07)^{c}	1.50(0.11)^{d}	1.35(0.11)^{e}	0.95(0.14)^{f}	0.23	0.00*	0.00*	0.00*	0.00*
3	2.00(0.11)^{a}	1.90(0.12)^{b}	2.10(0.11)^{c}	2.10(0.13)^{c}	1.80(0.11)^{d}	1.70(0.14)^{e}	1.50(0.12)^{f}	0.42	0.00*			
7	1.85(0.11)^{a}	1.95(0.12)^{b}	1.95(0.21)^{b}	2.05(0.15)^{c}	2.35(0.13)^{d}	2.15(0.11)^{e}	2.05(0.15)^{c}	0.82	0.01*			
10	1.65(0.12)^{a}	1.05(0.21)^{b}	1.75(0.23)^{c}	1.55(0.10)^{d}	1.15(0.12)^{e}	1.00(0.15)^{f}	0.50(0.21)^{g}	1.00	0.00*			
15	1.60(0.11)^{a}	2.30(0.12)^{b}	1.80(0.13)^{c}	1.10(0.22)^{d}	1.20(0.01)^{e}	1.70(0.31)^{f}	1.40(0.12)^{g}	0.15	0.00*			
20	2.10(0.10)^{a}	1.90(0.12)^{b}	1.10(0.10)^{c}	1.70(0.10)^{d}	1.70(0.22)^{d}	1.60(0.13)^{e}	1.40(0.24)^{f}	1.00	0.00*			
30	1.95(0.11)^{a}	1.85(0.21)^{b}	2.15(0.15)^{c}	1.66(0.20)^{d}	1.85(0.22)^{b}	1.45(0.25)^{e}	1.05(0.15)^{f}	0.47	0.00*			
45	1.40(0.12)^{a}	1.50(0.23)^{b}	1.50(0.10)^{b}	1.10(0.14)^{c}	1.40(0.20)^{a}	1.40(0.10)^{a}	1.30(0.15)^{d}	0.01	0.00*			

* Significant differences at the 0.05 level ($P<0.05$)

Values are means ($n=3$) with standard deviation in parentheses; P_1=significance level between treatments; P_2=significance level between times; P_3=significance level of interaction effect between treatments and times. Means followed by different letters within the same row are significantly different at $P<0.05$ according to Tukey's *b* test and Games–Howell post hoc test at the 5 % probability level; Tukey's *b* test was used when test of homogeneity of variance is passed ($P>0.05$) and Games–Howell test was used for the case of equal variance not assumed ($P<0.05$)

due to the depletion of the substrates easily available to microorganisms.

The responses of sediment protease to different levels of Pb

The responses of protease to different levels of exogenous Pb in sediments are shown in Fig. 3. Generally, enzyme activities showed some irregular changes during 45 days following lead additions. Contrary to urease and catalase activities, we observed in sediment that a significant reduction of protease activity was detected after exogenous lead treatments on day 1. The protease activities in sediments polluted with low concentrations of exogenous lead were higher than control on day 3 and day 7. On day 10, day 15, and day 20, the protease activities in sediments with application of exogenous lead were lower than control. The exogenous Pb had no remarkable enhancement on protease activities before day 20. However, protease activities of all samples with exogenous lead application were higher than control. Protease activities showed significant difference ($P<0.05$) under different times and treatment by one-way ANOVA and two-way ANOVA (Table 4). There was significant interaction effect of both time and treatment on protease activities statistically.

Protease is a digestive enzyme that hydrolyzes peptide bonds and distributed in subclasses, depending on the catalytic mechanisms and substrate, as well as pH optimum. Protein breakdown and recycling depend on the levels of proteolytic enzymes and are an essential part of the response of microorganisms to environmental stress. Under unfavorable conditions, protease plays a role in the interactions of sediment organisms via cleavage of the cell wall proteins and function in the survival of microorganisms (Valerie et al. 2013). Our results demonstrated that the exogenous lead elicited considerable negative effects on protease activities. The inhibition of protease may be due to different molecular and cellular toxic effects of lead, inhibition of DNA repair, oxidative damage, and cellular apoptosis (Waisberg et al. 2003). Pb can inhibit protease reactions, which may react with the enzyme–substrate complex, complex the substrate, or combine with the protein-active groups of the protease (Simona et al. 2004; Pereira and Castro 2014). Sulfhydryl groups of enzymes serve as groups or as catalytic sites involved in maintaining the correct conformation of the protein. The exogenous Pb may cause destruction of functional S–S bridges and bind to sulfhydryl groups. In addition, Pb is able to form complexes with amino acid, which might lead to inhibition of protease (Munoz et al. 2012; Yang et al. 2006). In natural sediment, under conditions of bioavailability, Pb exhibit toxic activity toward sediment biota which may lead to the decrease of the number and the activity of sediment microorganisms, thus the decrease on enzyme activities primarily by direct suppression of microbial growth in contaminated sediment. In this study, protease activities with exogenous lead pollution were higher than control at the end of the incubation (Fig. 3). This phenomenon is probably due to the addition of NO_3^{-1} and the mechanism of microorganism resistance to Pb. The mechanism of resistance of protease to metal takes two forms: one is the accumulation in the form of particular protein–metal association, and the other is blockage at the level of the cell wall and the systems of membrane transportation (Hassen et al. 1998). Protease

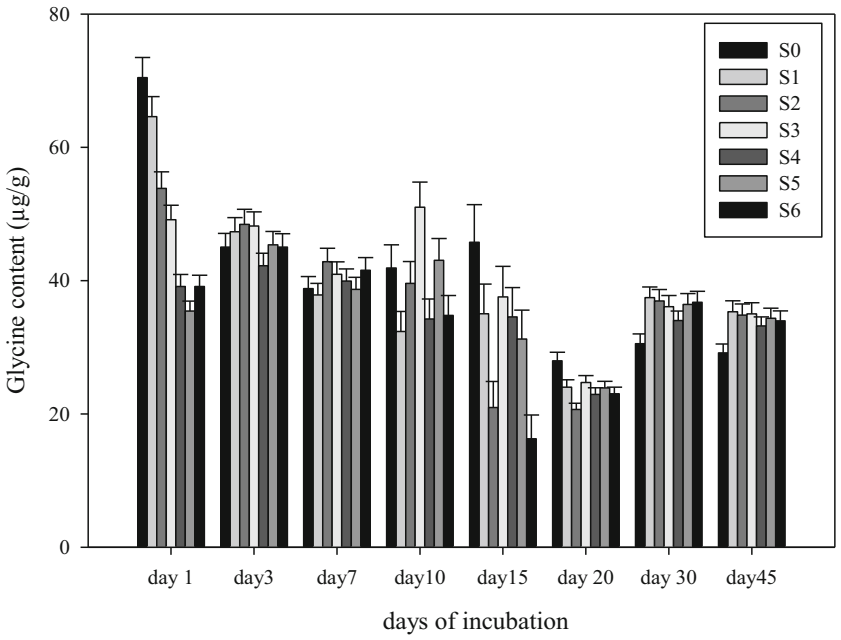

Fig. 3 Changes of protease activity in sediment under different levels of Pb stresses. Means of three replicated are shown at each exposure time. Vertical bars denote SE ($n=3$)

Table 4 Significant difference analysis of protease activity (micrograms of glycine per gram of sediment per hour) in sediment under different levels of exogenous lead

Incubation days	Different treatments						Test of homogeneity of variances	One-way ANOVA	Two-way ANOVA		
	S0	S1	S2	S3	S4	S5	S6	*P* value		P_1	P_2	P_3
1	76.45(1.27)^{a}	64.61(1.30)^{b}	53.83(1.98)^{c}	49.14(1.54)^{d}	39.12(1.55)^{e}	35.44(1.65)^{f}	39.12(1.50)^{e}	0.11	0.00*	0.00*	0.00*	0.00*
3	45.03(2.06)^{a}	47.33(1.39)^{b}	48.45(1.53)^{b}	48.20(1.68)^{b,c}	42.23(1.05)^{a}	45.38(1.12)^{b,d}	45.03(0.71)^{d}	0.06	0.00*			
7	38.79(1.24)^{a}	37.86(1.86)^{a}	42.84(1.59)^{b}	40.94(1.38)^{b,c}	39.93(1.67)^{c}	38.70(2.36)^{c}	41.56(1.25)^{b}	0.13	0.01*			
10	41.89(1.82)^{a}	32.36(1.87)^{b}	39.59(1.45)^{c}	41.03(1.57)^{a}	34.25(2.58)^{b}	43.05(1.51)^{a}	34.79(1.05)^{b}	0.31	0.00*			
15	45.75(1.52)^{a}	35.04(1.29)^{b}	20.97(1.36)^{c}	37.56(2.04)^{b}	34.56(2.51)^{b}	31.24(1.08)^{b}	16.27(1.82)^{d}	0.04	0.00*			
20	27.96(1.69)^{a}	24.01(1.87)^{b}	20.68(1.47)^{c}	24.71(2.08)^{c}	22.93(1.76)^{b,c}	23.90(1.91)^{c}	23.02(2.02)^{c}	0.79	0.00*			
30	30.54(1.72)^{a}	37.45(1.86)^{b}	36.96(1.02)^{b}	36.09(1.70)^{b}	34.02(1.35)^{c}	36.43(2.27)^{b}	36.75(1.38)^{b}	0.84	0.00*			
45	29.16(1.91)^{a}	35.33(2.69)^{b}	34.83(1.38)^{b}	35.11(2.01)^{b}	33.20(1.31)^{b}	34.35(1.82)^{b}	33.97(2.24)^{b}	0.75	0.00*			

* Significant differences at the 0.05 level ($P<0.05$)

Values are means ($n=3$) with standard deviation in parentheses; P_1=significance level between treatments; P_2=significance level between times; P_3=significance level of interaction effect between treatments and times. Means followed by different letters within the same row are significantly different at $P<0.05$ according to Tukey's *b* test and Games–Howell post hoc test at the 5 % probability level; Tukey's *b* test was used when test of homogeneity of variance is passed ($P>0.05$) and Games–Howell test was used for the case of equal variance not assumed ($P<0.05$)

showed enhanced plasticity with regard to the fitness trait (mass) during environmental stress and the higher lead load, when it changed.

Correlation analysis between exchangeable or EDTA-extractable Pb with enzyme activities

The changes of soluble–exchangeable Pb and EDTA-extractable Pb concentrations are shown in Fig. 4. It was found that the concentrations of soluble–exchangeable Pb in sediment with exogenous lead pollution rise from day 1 to day 15 and then declined till the end of the experiment except S6 declined from day 7. The concentrations of EDTA-extractable Pb increased along with exposure time among all treatments and achieved the highest in S6 in day 45.

The Pearson coefficients of correlation between lead and sediment enzyme activities were shown in Table 5. The Pearson's correlation analysis revealed that there were negative correlations between urease activities and EDTA-extractable Pb in the experiment. The relationships between catalase activities and EDTA-extractable Pb were significantly negative in the polluted sediments ($P<0.05$). The average correlation coefficients among urease and the soluble–exchangeable Pb or EDTA-extractable Pb were less than 0.50, which suggested that urease activities did not have close relationships with Pb concentration in the sediment. The number of catalase activities that correlated with EDTA-extractable Pb was larger than urease and protease.

With the pollution of exogenous lead in river sediment, the concentrations of soluble–exchangeable Pb rise at the early stage. The pollution of exogenous Pb increased Pb loading in sediments. Therefore, the water-soluble Pb complexes (which are usually thermodynamically less stable) increased in the studied sediments (Ayrault et al. 2014). The concentration of soluble–exchangeable Pb decreased on account of combination of Pb to clay minerals and Fe/Mn oxides or carbonate complexes (Usman et al. 2005; Reddy et al. 2010). Pb binding to clay minerals in sediment could lead to a significant decrease in the water-soluble and exchangeable varieties of Pb (Usman et al. 2005; Song and Sun 2014). Moreover, Pb had specific binding sites in the different solid phases within the sediments. Pb at low concentration preferred to undergo complexation reaction with strong binding sites (probably present in organic matter) in sediments (Chakraborty et al. 2012). Furthermore, after saturating relatively stronger binding sites, the toxic Pb started to occupy weaker binding sites with its increasing concentration in the sediments. EDTA as chelating agent has high metal removal efficiency, especially from bioavailable and labile fractions. Sediment extracting with EDTA first forms complexes with cationic metals, which are thermodynamically favorable and thus depletes the sediment of loosely bound

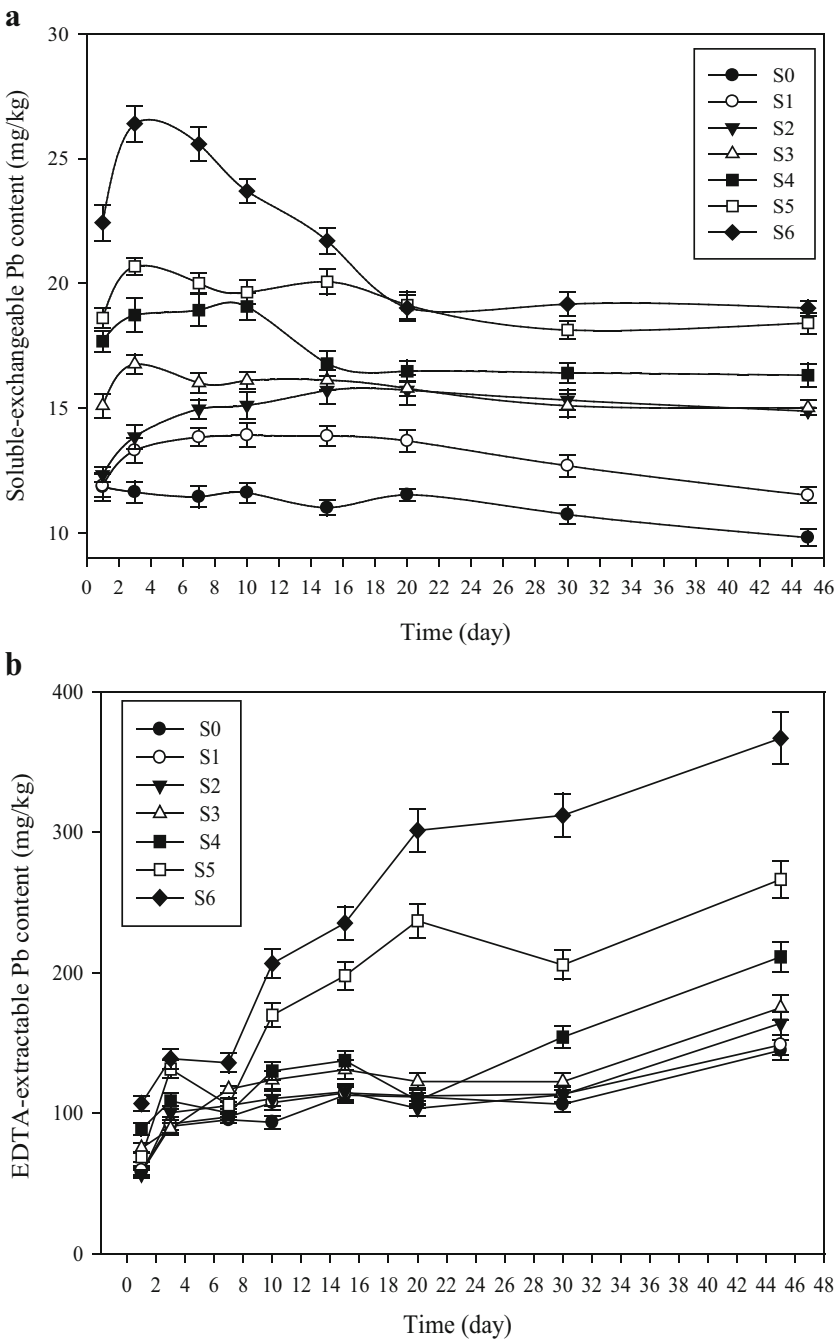

Fig. 4 Concentration of soluble–exchangeable Pb (a) and EDTA-extractable Pb (b) at different exposure time under different levels of exogenous lead in sediment. The bars represent the standard deviations of the means (n=3)

metals. In addition, EDTA can promote dissolution mechanism and partially breaks down the sediment structure, dissolving organic matter and sediment minerals (Jelusic and Lestan 2014), and thus indirectly releases more metals into the sediment solution. One possible explanation for the increasing concentrations of EDTA-extractable Pb along with exposure time is the kinetic model proposed by Olson and Shuman (1985), which was adapted (Lu et al. 1994; Chakraborty and Chakrabarti 2008; Chakraborty et al. 2012) to investigate the kinetic speciation of Pb in the coastal and estuarine sediments. The extraction of metals from the sediment using EDTA is represented by the following reaction (charges are omitted for simplicity):

$$M - \text{Sediment}_i + \text{EDTA} \xrightarrow{k_i} M - \text{EDTA} + \text{Sediment}_i \quad (2)$$

where i represents different binding sites on the naturally occurring heterogeneous complexant; M-Sediment$_i$ and M-EDTA represent a metal ion, M, bound to a sediment binding site, Sediment$_i$, and EDTA, respectively. As shown in Eq. (3), the concentration of metal extracted,

Table 5 Correlation between sediment enzyme activities and the concentration of soluble–exchangeable Pb or EDTA-extractable Pb and content of organic matter

Sediment enzyme	Exposure time							
	Day 1	Day 3	Day 7	Day 10	Day 15	Day 20	Day 30	Day 45
Soluble–exchangeable Pb								
Urease	−0.020	−0.026	−0.600	−0.627	0.348	0.131	0.007	−0.863*
Catalase	−0.305	−0.857*	0.285	0.801*	−0.274	−0.725	−0.732	−0.369
Protease	−0.835*	−0.381	−0.330	−0.218	−0.831*	−0.662	0.485	0.413
EDTA-extractable Pb								
Urease	−0.053	−0.021	−0.729	−0.631	0.338	−0.277	−0.196	−0.831*
Catalase	−0.320	−0.903**	0.598	−0.838*	−0.253	−0.300	−0.920**	−0.236
Protease	−0.694	−0.418	0.129	−0.144	−0.575	−0.120	0.298	0.171
Organic matter								
Urease	0.229	−0.800	0.579	−0.271	−0.209	0.181	0.382	0.796*
Catalase	0.737	−0.346	−0.611	−0.037	0.349	0.269	0.955**	0.529
Protease	0.455	−0.561	−0.196	−0.023	−0.053	0.039	−0115	−0.021

* Correlation was significant at the 0.05 level; ** Correlation was significant at the 0.01 level

$c_{\text{M-EDTA}}$, rises exponentially over time to a limiting value, as shown in Eq. (3):

$$c_{M-\text{EDTA}}(t) = \sum_{i=1}^{n} c_{M-\text{Sediment}_i}\left(1 - e^{-k_i \cdot t}\right) \quad (3)$$

The model assumes that (1) the reactions are first-order or pseudo-first-order; (2) the reaction between M and EDTA is much faster than the dissociation reaction of M-Sediment$_i$ complexes, so that the dissociation reaction is the rate-determining step; and (3) the ratio between the concentrations of complexed metal and free metal is much larger than unity (Chakraborty et al. 2012).

The activities of sediment enzymes were affected by many factors, such as moisture, nutrition, temperature, pH, oxygen content, and the type of pollutants. Free metal ions are considered to be the most toxic, whereas metals that are complexed with organic compounds may be less available to sediment microbes (Zhang et al. 2001; Fangueiro et al. 2002). In addition, the tolerance to toxic effects of exogenous lead is strongly correlated to the concentrations of Pb (Ogilvie and Grant 2008). On the other hand, sediment microorganisms can

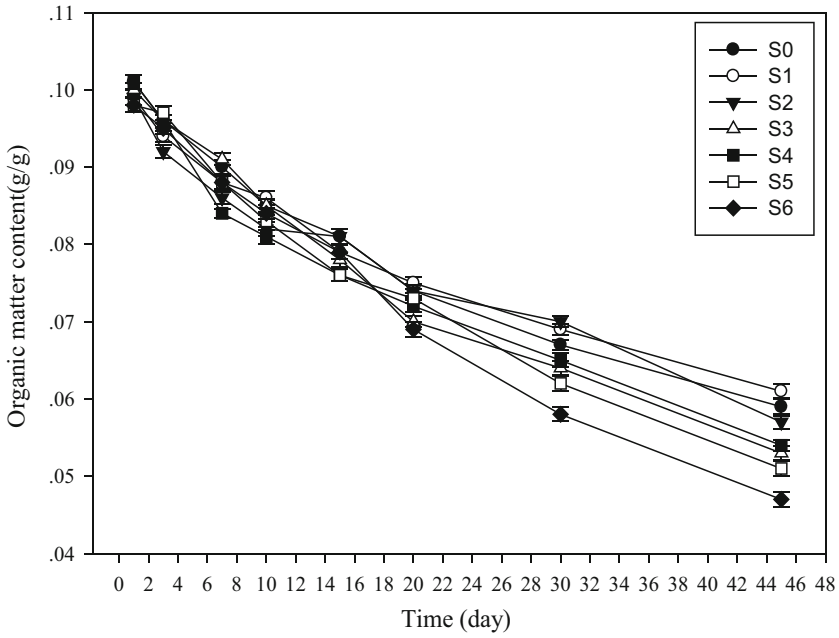

Fig. 5 The content of organic matter at different exposure time under different levels of exogenous lead in sediment. The bars represent the standard deviations of the means (n=3)

become adapted to the effects of exogenous heavy metals if these toxic elements have been present in the sediment for long periods of time (Chander and Joergensen 2008; Gómez-Sagasti et al. 2012).

Impact of exogenous Pb on degradation of organic matter

As shown in Fig. 5, the contents of organic matter in sediment decreased along with incubation time. The contents of organic matter in polluted sediments with exogenous lead were lower than control. Statistical analysis of data by one-way ANOVA and two-way ANOVA showed significant differences ($P<0.05$) in degradation rates of organic matter among treatments (in the presence of different levels of exogenous lead) till the end of the experiment (Table 6). Time also had a clear effect on the degradation rates of organic matter, and the interaction effect of both time and treatment was significant ($P<0.05$). Under the condition of exogenous lead pollution, the microbial community in sediments survived at the cost of increased degradation of organic matter during incubation and then entered a regime of reduced activities and halting of bacterial production (Almeida et al. 2007). During the degradation of organic matter, the sequential utilization of electron acceptors drove the biogeochemical dynamics in sediments. The biodegradation of organic matter occurred in the order of decreasing energy yield via aerobic oxidation, nitrate reduction, manganese reduction, iron reduction, sulfate reduction, and methanogenesis (ElBishlawi et al. 2013). On the other hand, the accumulation of organic matter in sediments with high moisture content stimulates the development of anoxia. The effects of anoxic conditions on the rate of organic matter decomposition are still controversial. Some researchers found little difference between oxic and anoxic rates of enzymatic activity (King 1986), while other authors (Danovaro et al. 2001) detected higher levels of activity in anoxic sediment layers.

Enzymes in sediment are strongly connected with important sediment properties, such as organic matter, and are physically and chemically protected by sediment constituents (organic and inorganic ligands), which interact with trace elements (Renella et al. 2003). Organic matter also can affect metal bioavailability and ecotoxicity. In this study, we found negative correlation between organic matter and protease (Table 5) and significant correlation between organic matter and urease on day 45. Significant correlation between organic matter and catalase was observed on day 30. This phenomenon may be related with the high capacity of the sediment to degrade organic matter and/or the ability of the microbial community to degrade specific compounds (ElBishlawi et al. 2013). This result indicated that the relationship between organic matter degradation and enzyme activities in sediment was more closely. Furthermore, enzyme activity related with the organic matter degradation may enhance our understanding about the mechanisms of biogeochemical processes.

Table 6 Significant difference analysis of degradation rates of organic matter (milligrams per gram of sediment) in sediment under different levels of exogenous lead

Incubation days	Different treatments						Test of homogeneity of variances	One-way ANOVA	Two-way ANOVA		
	S0	S1	S2	S3	S4	S5	S6	*P* value		P_1	P_2	P_3
1	1.07(0.12)^{a}	3.10(0.30)^{b}	3.33(0.57)^{b}	2.14(0.16)^{c}	3.00(0.55)^{b}	4.03(0.15)^{d}	3.97(0.15)^{d}	0.02	0.00*	0.00*	0.00*	0.00*
3	4.90(0.16)^{a}	5.03(1.39)^{a}	7.33(0.58)^{b}	4.00(1.00)^{a}	4.67(0.58)^{a}	3.30(0.52)^{a}	3.00(1.00)^{a}	0.52	0.00*			
7	5.67(0.58)^{a}	5.67(0.86)^{a}	5.00(1.00)^{a,b}	4.67(0.58)^{a}	11.00(1.20)^{c}	8.67(0.58)^{d}	6.67(0.58)^{b}	0.93	0.01*			
10	5.00(1.00)^{a}	1.97(0.87)^{b}	4.33(0.58)^{a,c}	6.17(0.28)^{d}	3.03(0.06)^{b}	5.33(0.57)^{d,e}	3.67(0.58)^{a,c}	0.07	0.00*			
15	4.33(0.58)^{a}	7.33(0.58)^{b}	5.00(1.00)^{a}	7.33(0.58)^{b}	4.67(0.58)^{a}	7.00(1.08)^{b}	4.67(0.33)^{a}	0.93	0.00*			
20	6.67(0.58)^{a}	3.67(0.55)^{b}	6.67(0.58)^{a}	8.00(1.00)^{a}	4.33(0.58)^{b}	3.33(0.58)^{b}	10.33(0.58)^{c}	0.94	0.00*			
30	6.67(0.58)^{a}	7.00(1.00)^{a}	4.33(0.58)^{b}	5.67(0.58)^{a,b}	6.67(0.58)^{a}	11.33(0.58)^{c}	10.67(0.58)^{c}	0.94	0.00*			
45	8.33(153)^{a}	8.00(1.00)^{a}	12.33(1.15)^{b}	10.67(0.58)^{b,c}	11.00(1.00)^{b}	10.67(0.58)^{b,c}	10.67(0.58)^{b,c}	0.47	0.01*			

* Significant differences at the 0.05 level ($P<0.05$)

Values are means ($n=3$) with standard deviation in parentheses; P_1=significance level between treatments; P_2=significance level between times; P_3=significance level of interaction effect between treatments and times. Means followed by different letters within the same row are significantly different at $P<0.05$ according to Tukey's *b* test and Games–Howell post hoc test at the 5 % probability level; Tukey's *b* test was used when test of homogeneity of variance is passed ($P>0.05$) and Games–Howell test was used for the case of equal variance not assumed ($P<0.05$)

Conclusions

The exogenous lead pollution changed the enzyme activities in sediments, and the enzyme activities correlated to lead concentration and exposure time. At the beginning of experiment, the activities of catalase and protease were inhibited in the Pb-contaminated sediment, whereas the urease activities were enhanced. Low levels of exogenous lead had a stimulating effect on microbial activity. There were significant negative correlations between catalase activities and EDTA-extractable Pb in the experiment, and urease activity may be a sensitive tool for assessing additive toxic lead to sediment biochemical parameters. The content of organic matter in sediments with exogenous lead pollution was lower than control at the end of experiment. Enzyme activities in sediment can affect the degradation of organic matter, and microorganisms showed strong tolerance and adaption to exogenous lead pollution. Further studies on the enzyme kinetics and mechanisms in heavy metal-contaminated sediment are needed, which would provide useful information for the development of bioremediation technologies of contaminated sediment and then to set up more in-depth analysis to increase progressive understanding of the involved ecotoxicological mechanisms.

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References

Almeida A, Cunha Â, Fernandes S et al (2007) Copper effects on bacterial activity of estuarine silty sediments. Estuar Coast Shelf Sci 73(3):743–752

Ayrault S, Le Pape P, Evrard O et al (2014) Remanence of lead pollution in an urban river system: a multi-scale temporal and spatial study in the Seine River basin, France. Environ Sci Pollut Res 21(6):4134–4148

Barceló D (2007) Effect-directed analysis of key toxicants in European river basins. A review (9 pp). Environ Sci Pollut Res 14(1):30–38

Chakraborty P, Chakrabarti CL (2008) Competition from Cu (II), Zn (II) and Cd (II) in Pb (II) binding to Suwannee River fulvic acid. Water Air Soil Pollut 195:63–71

Chakraborty P, Babu PV, Sarma VV (2012) A study of lead and cadmium speciation in some estuarine and coastal sediments. Chem Geol 294: 217–225

Chander K, Joergensen RG (2008) Decomposition of Zn-rich *Arabidopsis halleri* litter in low and high metal soil in the presence and absence of EDTA. Water Air Soil Pollut 188:195–204

Cho YC, Kwon O, Sokol RC et al (2001) Microbial PCB dechlorination in dredged sediments and the effect of moisture. Chemosphere 43: 1119–1126

Clozel B, Ruban V, Durand C, Conil P (2006) Origin and mobility of heavy metals in contaminated sediments from retention and infiltration ponds. Appl Geochem 21:1781–1798

Danovaro R, Manini E, Fabiano M (2001) Exoenzymatic activity and organic matter composition in sediments of the Northern Adriatic Sea: response to a river plume. Microb Ecol 44:235–251

ElBishlawi H, Shin JY, Jaffe PR (2013) Trace metal dynamics in the sediments of a constructed and natural urban tidal marsh: the role of iron, sulfide, and organic complexation. Ecol Eng 58:133–141

Fangueiro D, Bermond A, Santos E, Carapuca H, Duarte A (2002) Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extraction: search for optimal experimental conditions. Anal Chimi Acta 459:245–256

Feng H, Han XF, Zhang WG, Yu LZ (2004) A preliminary study of heavy metal contamination in Yangtze River intertidal zone due to urbanization. Mar Pollut Bull 49:910–915

Finster ME, Gray KA, Binns HJ (2004) Lead levels of edibles grown in contaminated residential soils: a field survey. Sci Total Environ 320: 245–257

Fliessch RA, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol Biochem 26:1201–1205

Gómez-Sagasti MT, Alkorta I, Becerril JM et al (2012) Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. Water Air Soil Pollut 223(6):3249–3262

Hassen A, Saidi N, Cherif M, Boudabous A (1998) Resistance of environmental bacteria to heavy metals. Bioresource Technol 64(1):7–15

Hinojosa MB, Carreira JA, García-Ruíz R et al (2004) Soil moisture pretreatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biol Biochem 36(10):1559–1568

Hinojosa MB, Carreira JA, Rodríguez-Maroto JM et al (2008) Effects of pyrite sludge pollution on soil enzyme activities: ecological dose–response model. Sci Total Environ 396(2):89–99

Ho HH, Swennen R, Cappuyns V et al (2012) Potential release of selected trace elements (As, Cd, Cu, Mn, Pb and Zn) from sediments in Cam River-mouth (Vietnam) under influence of pH and oxidation. Sci Total Environ 435:487–498

Huang DL, Zeng GM, Jiang XY, Feng CL et al (2006) Bioremediation of Pb-contaminated soil by incubating with *Phanerochaete chrysosporium* and straw. J Hazard Mater 134:268–276

Huang DL, Zeng GM, Feng CL, Hu S, Jiang XY et al (2008) Degradation of lead-contaminated lignocellulosic waste by *Phanerochaete chrysosporium* and the reduction of lead toxicity. Environ Sci Technol 42:4946–4951

Huang DL, Zeng GM, Feng CL, Hu S, Zhao MH et al (2010) Mycelial growth and solid-state fermentation of lignocellulosic waste by White-rot fungus *Phanerochaete chrysosporium* under lead stress. Chemosphere 8:1091–1097

Ikenaga M, Guevara R, Dean AL et al (2010) Changes in community structure of sediment bacteria along the Florida coastal everglades marsh–mangrove–seagrass salinity gradient. Microb Ecol 59(2): 284–295

Jelusic M, Lestan D (2014) Effect of EDTA washing of metal polluted garden soils: Part I. Toxicity hazards and impact on soil properties. Sci Total Environ 475:132–141

Johnson CE, Siccama TG, Driscoll CT, Likens GE, Moeller RE (1995) Changes in lead biogeochemistry in response to decreasing atmospheric inputs. Ecoll Appl 5:813–822

King GM (1986) Characterisation of β-glucosidase activity in intertidal marine sediments. Appl Environ Microbiol 51:373–380

Kirchman DL, Malmstrom RR, Cottrell MT (2005) Control of bacterial growth by temperature and organic matter in the Western Arctic. Deep-Sea Res Pt II 52:3386–3395

Li J, Zhao BQ, Li XY et al (2008) Effects of long-term combined application of organic and mineral fertilizers on soil microbiological properties and soil fertility. Scientia Agricultura Sinica 41(1):144–152

Li M, Cheng XH, Guo HX (2013) Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int Biodeterior Biodegrad 76:81–85

Li Y, Yang RJ, Zhang AB, Wang SR (2014) The distribution of dissolved lead in the coastal waters of the East China Sea. Mar Pollut Bull 85: 700–709

Liang W, Wu Z, Cheng S, Zhou Q, Hu H (2003) Roles of substrate microorganisms and urease activities in wastewater purification in a constructed wetland system. Ecol Eng 21:191–195

Liu WX, Li XD, Shen ZG et al (2003) Multivariate statistical study of heavy metal enrichment in sediments of the Pearl River Estuary. Environ Pollut 121(3):377–388

Lock K, Janssen CR (2003) Influence of ageing on zinc bioavailability in soils. Environ Pollut 126:371–374

Lu Y, Chakrabarti CL, Back MH, Gregoire DC, Schroeder WH (1994) Kinetic studies of aluminum and zinc speciation in river water and snow. Anal Chim Acta 293:95–108

Luoma SN, Davies JA (1983) Requirements for modelling trace metal partitioning in oxidized estuarine sediments. Mar Chem 12:159–181

Mao LJ, Mo DW, Yang JH, Guo YY, Lv HY (2014) Rare earth elements geochemistry in surface floodplain sediments from the Xiangjiang River, middle reach of Changjiang River, China. Quat Int 336:80–88

Mikac N, Branica M, Harrison RM (2001) Total and organic lead distribution in water, sediment and organisms from the Eastern Adriatic Coast. Chem Speciation and Bioavailab 13:121–128

Mishra VK, Upadhyaya AR, Pandey SK, Tripathi BD (2008) Heavy metal pollution induced due to coal mining effluent on surrounding aquatic ecosystem and its management through naturally occurring aquatic macrophytes. Bioresour Technol 99:930–936

Modolo LV, de Souza AX, Horta LP et al (2015) An overview on the potential of natural products as ureases inhibitors: a review. J Adv Res 6(1):35–44

Moreno JL, Hernández T, García C (1999) Effects of a cadmium contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. Biol Fertil Soils 28:230–237

Mullineaux PM, Karpinski S, Baker NB (2006) Spatial dependence for hydrogen peroxidedirected signalling in light-stressed plants. Plant Physiol 141(2):346–350

Munoz AJ, Ruiz E, Abriouel H et al (2012) Heavy metal tolerance of microorganisms isolated from wastewaters: identification and evaluation of its potential for biosorption. Chem Eng J 210:325–332

Ogilvie LA, Grant A (2008) Linking pollution induced community tolerance (PICT) and microbial community structure in chronically metal polluted estuarine sediments. Mar Environ Res 65:187–198

Olson DL, Shuman MS (1985) Copper dissociation from estuarine humic materials. Geochim Cosmochim Acta 49:1371–1375

Pagnanelli F, Moscardini E, Giuliano V, Toro L (2004) Sequential extraction of heavy metals in river sediments of an abandoned pyrite mining area: pollution detection and affinity series. Environ Pollut 132:189–201

Pan J, Yu L (2011) Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. Ecol Eng 37:1889–1894

Pan XL, Zhang DY, Chen X, Bao AM, Li LH (2011) Antimony accumulation, growth performance, antioxidant defense system and photosynthesis of *Zea mays* in response to antimony pollution in soil. Water Air Soil Pollut 215:517–523

Pereira SIA, Castro PML (2014) Diversity and characterization of culturable bacterial endophytes from *Zea mays* and their potential as plant growth-promoting agents in metal-degraded soils. Environ Sci Pollut R 21(24):14110–14123

Reddy KR, Danda S, Yukselen-Aksoy Y, Al-Hamdan AZ (2010) Sequestration of heavy metals in soils from two polluted industrial sites: implications for remediation. Land Contamination Reclamation 18:13–23

Renella G, Ortigoza ALR, Landi L, Nannipieri P (2003) Additive effects of copper and zinc on cadmium toxicity on phosphatase activities and ATP content of soil as estimated by the ecological dose (ED50). Soil Biol Biochem 35:1203–1210

Rinklebe J, Shaheen SM (2014) Assessing the mobilization of cadmium, lead, and nickel using a seven-step sequential extraction technique in contaminated floodplain soil profiles along the central Elbe River, Germany. Water Air Soil Pollut 225(8):1–20

Simona C, Angela RF, de Santo AV (2004) Suitability of soil microbial parameters as indicators of heavy metal pollution. Water Air Soil Pollut 158(1):21–35

Song H, Sun Z (2014) Temporal variations and bioaccumulation of heavy metals in different *Suaeda salsa* marshes of the Yellow River Estuary, China. Environ Sci Pollut R 21(24):14174–14187

Speir TW, Cowling JC (1991) Phosphatase activity of pasture plants and soils: relationship with plant productivity and soil P fertility indices. Biol Fertil Soils 12:189–194

Sun GX, Wang XJ, Hu QH (2011) Using stable lead isotopes to trace heavy metal contamination sources in sediments of Xiangjiang and Lishui Rivers in China. Environ Pollut 159:3406–3410

Trivedi P, Axe L (2000) Modeling Cd and Zn sorption to hydrous metal oxides. Environ Sci Technol 34:2215–2223

Usman A, Kuzyakov Y, Stahr K (2005) Effect of clay minerals on immobilization of heavy metals and microbial activity in a sewage sludge-contaminated soil. J Soils Sediments 5:245–252

Valerie V, Klement R, Pavel F (2013) Proteolytic activity in soil: a review. Appl Soil Ecol 70:23–32

Waisberg M, Joseph P, Hale B et al (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192(2):95–117

Weintraub MN, Schimel JP (2005) Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biol Biochem 37:469–1475

Wightwick AM, Reichman SM, Menzies NW et al (2013) The effects of copper hydroxide, captan and trifloxystrobin fungicides on soil phosphomonoesterase and urease activity. Water Air Soil Pollut 224(12):1–9

Wolf J, Schliebs W, Erdmann R (2010) Peroxisomes as dynamic organelles: peroxisomal matrix protein import. FEBS J 277(16):3268–3278

Yan J, Quan G, Ding C (2013) Effects of the combined pollution of lead and cadmium on soil urease activity and nitrification. Procedia Environ Sci 18:78–83

Yang ZX, Liu SQ, Zheng DW, Feng SD (2006) Effects of cadium, zinc and lead on soil enzyme activities. J Environ Sci 18:1135–1141

Yang JY, Huang JH, Lazzaro A, Tang Y, Zeyer J (2014a) Response of soil enzyme activity and microbial community in vanadium-loaded soil. Water Air Soil Pollut 225(7):1–10

Yang Q, Lei AP, Li FL, Liu LN, Zan QJ, Shin PKS, Cheung SG, Tan NFY (2014b) Structure and function of soil microbial community in artificially planted *Sonneratia apetala* and *S.caseolaris* forests at different stand ages in Shenzhen Bay, China. Mar Pollut Bull 85: 754–763

Yao QZ, Zhang J, Wu Y, Xiong H (2007) Hydrochemical processes controlling arsenic and selenium in the Changjiang River (Yangtze River) system. Sci Total Environ 377:93–104

Zeng GM, Zhang C, Huang GH, Yu J, Wang Q, Li JB, Xi BD, Liu HL (2006) Adsorption behavior of bisphenol A on sediments in Xiangjiang River, Central-south China. Chemosphere 65:1490–1499

Zhang H, Zhao FJ, Sun B, Davison W, McGrath SP (2001) A new method to measure effective soil solution concentration predicts copper. Environ Sci Technol 35:2602–2607

Zhang J, Cheng SP, He F, Liang W, Wu ZB (2008) Effects of Cd^{2+} and Pb^{2+} on the substrate biofilms in the integrated vertical-flow constructed wetland. J Environ Sci 20(8):900–906

Zhang ZW, Xu XR, Sun YX et al (2014) Heavy metal and organic contaminants in mangrove ecosystems of China: a review. Environ Sci Pollut R 21(20):11938–11950

Zhou QH, Wu ZB, Cheng SP, He F, Fu GP (2005) Enzymatic activities in constructed wetlands and di-n-butyl phthalate (DBP) biodegradation. Soil Biol Biochem 37:1454–1459

Zhu JY, Zhang JX, Li Q, Han T, Xie JP, Hu YH, Chai LY (2013) Phylogenetic analysis of bacterial community composition in sediment contaminated with multiple heavy metals from the Xiangjiang River in China. Mar Poll Bull 70:134–139