ORIGINAL ARTICLE

Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose–response model and PCR-RAPD

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Abstract A laboratory study was conducted to evaluate the response of soil enzyme activities (namely dehydrogenase, phosphatase and urease) to different levels of trace element pollution in soil representative area. The improved ecological dose model and random-amplified polymorphic DNA (RAPD) were used to assess soil health. The 50% ecological dose (ED_{50}) values modified by toxicant coefficient were calculated from the best-fit model, and determination values from the regression analysis for the three enzyme activities were studied after the incubation periods. The results showed that the elevated heavy metal concentration negatively affects the total population size of bacteria and actinomycetes and enzymatic activity; dehydrogenase ($ED_{50} = 777$) was the most sensitive soil enzyme, whereas urease activity ($ED_{50} = 2,857$) showed the lowest inhibition; combined pollution or elevated toxicant level would increase disappearing RAPD bands, and the number of denoting polymorphic bands was greater in combined polluted soils. All three mathematical modified models satisfactorily described the inhibition of soil enzyme activities caused by Cd and Pb, by giving the best fit.

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Introduction

It has been demonstrated repeatedly that heavy metals adversely affect biological functions in soil, including the size, activity and diversity of the soil microbial community (Chander et al. 2001), and the activity of enzymes involved in C, N, P and S transformation (Moreno et al. 1999; Belyaeva et al. 2005). Enzymatic reactions are inhibited by heavy metals by (1) complexation of the substrate, (2) combining with the protein-active groups of the enzyme or (3) reacting with the enzyme–substrate complex (Nannipieri 1994; Dick 1997; Moreno et al. 2003).

Among the different enzymes in soils, dehydrogenase, urease and phosphatases are important in the transformation of different plant nutrients. Dehydrogenase activity reflects the total oxidative activity of the microbial biomass (Nannipieri et al. 1990) and being involved in central aspect of metabolism, does not function extracellularly. Urease catalyzes the hydrolysis of urea into ammonia or ammonium ion depending on soil pH, and carbon dioxide. Among the enzymes that are involved in soil N cycling, urease is the most prominent (Tabatabai and Bremner 1972; Cookson 1999). Phosphorus is one of the essential plant nutrients. A large part of soil phosphorus occurs in organic forms. Phosphatases play an important role in transforming organic phosphorus into inorganic forms, suitable for plants. The inhibition of heavy metal pollution on urease and dehydrogenase activity was reported by many scientists (Zheng et al. 1999). Heavy metal is toxic to almost all bacteria, by inhibiting basic cellular functions,

which are linked with energy metabolism (Walker et al. 2000; Lorenz et al. 2003). As a result, decreases in microbial populations have been reported in the soils polluted with heavy metal compounds (Hiroki 1993; Renella et al. 2005).

Random-amplified polymorphic DNA (RAPD) has been widely used in species classification and phylogenetic analysis, resistance gene identification, and genetic analysis of populations (Dweikat et al. 1993; Atienzar et al. 2002), as it is quick, simple and inexpensive. In fact, RAPD analysis has become one of the most popular DNA-based methods for assessing genetic diversity in plants (Liu et al. 2005) and has been used in DNA analysis of soil microbial community (Yang et al. 2000). RAPD fragments are detected after agarose gel electrophoresis and ethidium bromide (EB) staining by visualizing band shifts, missing bands or the appearance of new bands in a DNA gel electrophoresis. Detection of genotoxic effect using RAPD involves the comparison of profiles generated from control (unexposed) and treated (exposed) DNA. Using multiple primers also helps ensure that a sufficiently large region of the target DNA is scanned when an estimate of overall variance between samples is desired (Ogram and Feng 1997).

Several authors (Moreno et al. 1999, 2001) have quantified the effect of heavy metals on various soil enzyme activities by determining the ecological dose 50% (ED_{50}), the concentration of the heavy metal at which the enzyme, or other biological activities, is reduced to 50% of the uninhibited value. Traditionally, only single trace element additions to soils have been used in most studies for ED_{50} determination, and scarce information is available on the possible synergic effects of multiple trace elements (Yang et al. 2006). Renella et al. (2003) studied the effect of Cd on the ED_{50} on acid and alkaline phosphatase activities of three contrasting soils with or without Cu and/or Zn. They demonstrated the occurrence of additive effects of Cu and Zn on Cd toxicity to these enzyme activities. This indicates that the calculation of ED_{50} values for enzyme activities in the presence of several trace elements is required to interpret data from field studies in which complex trace element pollutants are involved.

Therefore, we suggest modifying the dose–response model to evaluate the ED_{50} values for enzymatic activities when several heavy metals coexist in soil ecological system. The aims of this paper were to (1) investigate the effects of Cd and Pb combined stress on soil microbe population and genetic diversity using RAPD method, (2) assess the effects of Cd and Pb combined pollution on soil enzyme activities and calculate the ED_{50} values for enzymatic activities using modified ecological dose–response model.

Materials and methods

Characterization, sampling, and treatment of soils

Unpolluted soil from farmland surface layer (0–15 cm) was collected in May 2008, Yangtse River Basin, Shanghai, China (31°02′N, 121°52′E). Fresh soil was sieved through a 4 mm sieve and mixed with Cd/Pb, and then placed into plastic pots with 40 cm in height and 20 cm in diameter. Cd was applied as $CdCl_2 \cdot 2.5H_2O$ and Pb was applied as $Pb(NO_3)_2$. The tested soil was paddy soil and the main soil parameters are: soil pH 8.18; organic matter 16.17 g kg^{-1}; total N and P 1.14 and 1.36 g kg^{-1}, respectively; CEC 15.60 cmol kg^{-1}; the concentrations of Cd, Cu, Zn, Pb and As 0.18, 22.9, 38.1, 15.1 and 7.4 mg kg^{-1}, respectively.

The soil was incubated under aerobic-controlled conditions in greenhouse which was located in Shanghai Jiaotong University, with 22–25°C average temperature. The experimental treatment for heavy metal addition level is shown in Table 1 and all treatments were replicated three times. Soil moisture was maintained constant by auto spray water device controlling house moisture at regular intervals throughout the incubation period. After 7, 14 and 35 days of incubation, soil subsamples were assayed for dehydrogenase, urease and phosphatases. Soil subsamples at 35 days were assayed for soil microbe population and genetic diversity. Five soil holes (1 cm diameter) were taken to a depth of 20 cm and were then mixed (about 100 g) as one sample.

Enumeration of major soil microbial population groups

The enumeration of the soil microflora was done by the dilution plate method (Nair and Subba-Rao 1977). The total colony forming units (cfu) of bacteria, fungi and actinomycetes (Allen 1959) were recorded on Ken Knight and Munaier's agar (Allen 1959), Martin's rose bengal agar (Martin 1950) and Jensen's agar (Jensen 1951) media, respectively. The plates were incubated at 28°C and microbial population was calculated and expressed as cfu $\times 10^n$ g^{-1} air dried soil, where 10^n was dilution factor.

Determination of enzymatic activity

Soil urease activity was determined by the method of Tabatabai and Bremner (1972), expressed as NH_4-N mg kg^{-1} h^{-1}. Dehydrogenase activity was tested by reduction of 2,3,5-triphenyltetrazolium chloride (TTC). After 24 h at 37°C, the triphenyl formazan (TPF) released was extracted with methanol and assayed at 485 nm in an UV spectrophotometer. The unit of dehydrogenase activity was TPF mg kg^{-1} h^{-1}. Acid phosphatase activity was determined by the method of

Table 1 Number of 13 treatments responding to heavy metal content (mg kg^{-1} soil)

	A	B	C	D	E	F	G
Metal content	Cd10	Cd20	Cd50	Cd100	Pb50	Pb100	Pb200
	H	I	J		K	L	CK
Metal content	Pb500	Cd10 + Pb50	Cd20 + Pb100		Cd50 + Pb200	Cd100 + Pb500	Control

Tabatabai and Bremner (1969). The *p*-nitrophenol (PNP) in the filtrate was determined colorimetrically at 410 nm after 1 h incubation with *p*-nitrophenyl phosphate, expressed as PNP mg kg^{-1} h^{-1}.

DNA extraction and RAPD

Soil samples of 5 g were mixed with 13.5 ml of DNA extraction buffer [100 mM Tris–HCl (pH 8.0), 1.5 M NaCl, 1% CTAB] and 100 μl of proteinase K (10 mg ml^{-1}) in centrifuge tubes by horizontal shaking at 225 rpm for 30 min at 37°C. After the shaking treatment, 1.5 ml of 20% SDS was added, and the samples were incubated in a 65°C water bath for 2 h with gentle end-over-end inversions every 15–20 min. The supernatants were collected after centrifugation at 6,000×*g* for 10 min at room temperature and transferred into 50 ml centrifuge tubes. Supernatants from the three cycles of extractions were combined and mixed with an equal volume of chloroform-isoamyl alcohol (24:1, v/v). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h or overnight. The pellet of crude nucleic acids was obtained by centrifugation at 16,000×*g* for 20 min, resuspended in TE buffer [10 mM Tris–HCl (pH 8.0), 1 mM EDTA (pH 8.0)] to give a final volume of 500 μl. DNA was then purified by the low-melting-point agarose gel recovery method (Zhou et al. 1996).

PCR amplification reaction in a 20 μl total volume contains 2 μl 10× Taq buffer, 2 mM MgCl$_2$, 1 unit Taq DNA polymerase, 0.25 mM dNTP (supplied by Sangon, Shanghai), 25 pmol primer, and 10 ng soil DNA. DNA amplification was carried out in a MJ research PT-200 thermocycler with the following procedure: an initial denaturing step at 94 μl for 3 min; 40 cycles for 30 s at 94°C (denature), 45 s at 36°C (annealing), 90 s at 72°C (extension), and a final elongation step at 72°C for 5 min. PCR products were separated by electrophoresis on 1.8% agarose gel and after which the gels were stained with ethidium bromide (EB) solution (0.015%) in distilled water and photographed. The standard DNA samples (1 kb DNA ladder marker) were used as molecular size marker.

Table 2 Sequences of ten primers used in this experiment

No. of primers	Sequences of primers	Percentage of GC
S1	TAGGCGGCGG	80
S2	CTGCTGGGAC	70
S3	TCTCCCTCAG	60
S4	GAGGCCCGTT	70
S5	CACTTCCGCT	60
S6	GTCGCCGTCA	70
S7	CTCCCCAGAC	70
S8	TTGCAGGCAG	60
S9	GCGTCGAGGG	80
S10	GTAAGCCCCT	60

RAPD fingerprints profiles

The photographic plates were scanned into computer and analyzed using a computer image analysis system (ChampGel^{3200}, Beijing). We selected 10 random primers (Table 2) having good repetition from 50 random primers to amplify the microbial community DNA from the treated soils. Polymorphism observed in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to control RAPD profiles. The presence and absence of amplified fragments were scored (Wang et al. 2007).

Coefficient of DNA sequence similarity was defined following the formula of Nei and Li (1979) as follows: $S_{xy} = 2N_{xy}/(N_x + N_y)$, where S_{xy} is the coefficient of DNA sequence similarity between DNA samples x and y; N_{xy} represents the numbers of RAPD fragments shared between DNA samples x and y; N_x and N_y are the numbers of RAPD fragments from DNA samples x and y, respectively. Coefficient of DNA sequence similarity can reflect the difference in the DNA sequences between soil microbial communities.

Modified ecological model for soil enzyme activity under heavy metal stress

The two kinetic models proposed by Speir et al. (1995), and the sigmoidal dose–response model of Haanstra et al. (1985), were used to assess the inhibition of enzymatic

activity by heavy metal. The author tried to introduce toxicant coefficient to ecological dose–response model, which could be used to assess enzymatic activity under heavy metal synergic effects:

$$E_i = T_i \times C_i \tag{1}$$

$$R_i = \sum_{i=1}^{m} E_i \tag{2}$$

where C_i is concentration of heavy metal in soil, E_i is the potential ecological risk factor for a given substance, T_i is the "toxic-response" factor for the given substance, i.e., Cu = Pb = 5, Zn = 1, Cd = 30, Cr = Ni = 2 (He et al. 1998). For example, the E_i for the treatment with addition of Cd 10 mg kg^{-1} soil and Pb 20 mg kg^{-1} soil was 300 and 100, respectively, so the R_i of the treatment was 400.

The two kinetic models were:

$$v = \frac{c}{1 + bR_i} \quad \text{(Model 1)}$$

$$v = \frac{c(1 + aR_i)}{1 + bR_i} \quad \text{(Model 2)}$$

where v is the response variable, and a, b and c are fitting parameters with positive values and $b > a$. Model 1 describes the full inhibition of v by R_i. Model 2 describes the partial inhibition. By fitting the equation of Models 1 and 2 to the experimental data, it is possible to calculate the 50% ecological dose (ED$_{50}$) values from the relationship:

$$\text{ED}_{50} = \frac{1}{b} \tag{3}$$

The mathematical equation for the sigmoidal dose–response model (Model 3) is

$$v = \frac{a}{1 + e^{b(x-c)}} \quad \text{(Model 3)}$$

where v is again the response variable, x is the natural logarithm of R_i, a is the uninhibited value of v, b is a slope factor, and c is the natural logarithm of ED$_{50}$. The ED$_{50}$ values are calculated using the following expression:

$$\text{ED}_{50} = e^c \tag{4}$$

This model describes a logistic curve, which represents the relationship between the measured activity and the natural logarithm of toxicant coefficient.

Data analysis

The values of the constants a, b and c of these models were estimated using the Levenberg–Marquardt method to solve non-linear curve fit by origin 7.5. Two-way ANOVA and least significant difference (LSD) at 5% confidence level were performed on each dependent variable by SPSS 12.0

Results

Soil microbe population

The results of quantitative analysis of soil microbial populations were shown in Fig. 1. The soil microbe populations were far lower under heavy metal pollution than under control treatment, and soil microbe populations under different toxicant level showed significant difference. Bacteria showed a marked decrease in population size with increasing toxicant level for the contaminated soil samples and it was more sensitive to heavy metal for bacteria than for other soil microbe; actinomycetes in population size responded similarly with bacteria, but the variance was smaller in actinomycetes population size than in bacteria; fungi seemed not to be sensitive to heavy metal, and there was no big discrepancy on population size, although all treatments showed significant difference on population size compared to control.

Effects on enzymatic activities

Figure 2 shows the evolution of the three enzyme activities measured during the experimental period. The results from our experiment showed that elevated heavy metal concentration and toxicant level differently impacted on soil enzyme activities. An inhibition of phosphatase, urease and dehydrogenase activity under heavy metal stress occurred with increasing incubated time and toxicant level. The decrease was more significant on urease activity than on phosphatase and dehydrogenase compared to control.

RAPD profiles and genetic similarity

The size of DNA obtained from the soil samples was about 23.1 kb (Fig. 3) and the DNA yields were about
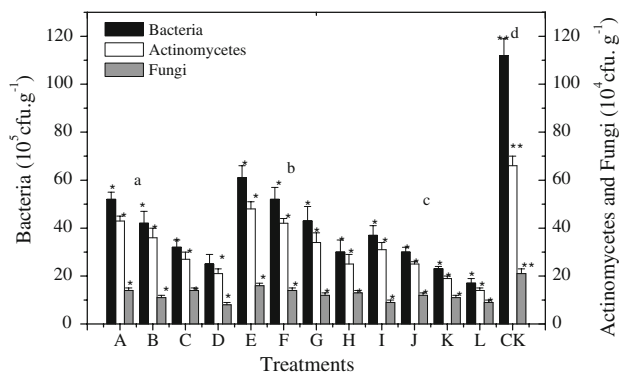

Fig. 1 Microbial diversity of different treatments. (*Note*: different letters show significantly different at $p < 0.05$ within groups of different metal treatment and * shows significantly different at $p < 0.05$ compared to control.)
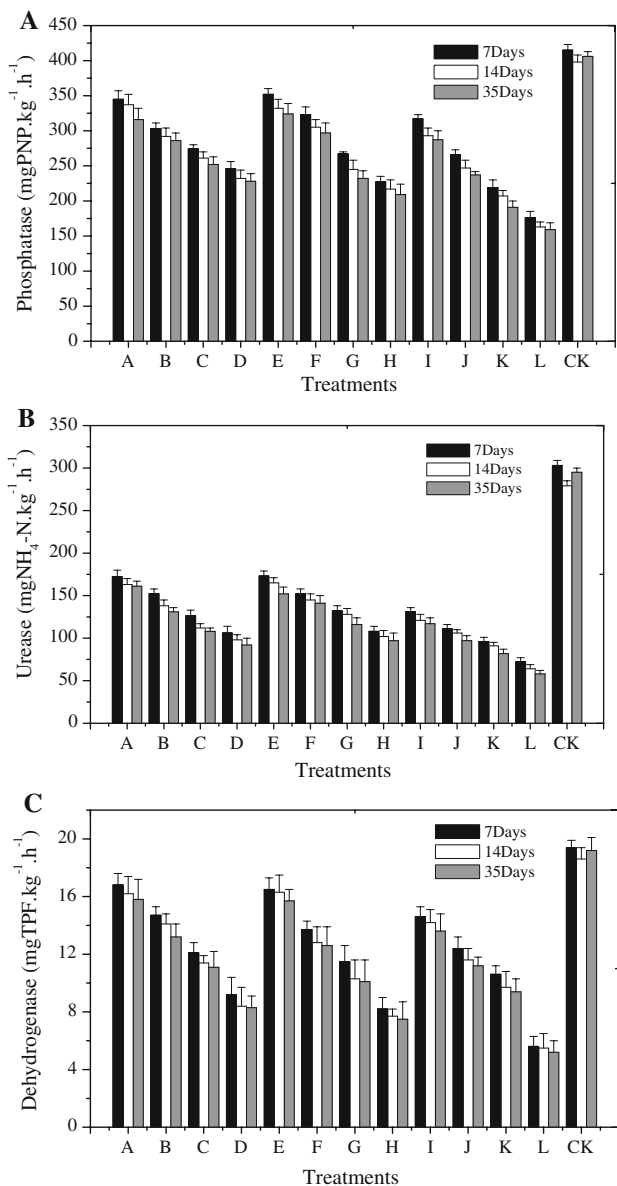
Fig. 2 Changes of **a** phosphatase, **b** urease and **c** dehydrogenase

15.3 ± 1.5 µg g^{-1} of soil. The concentration and purity of DNA extracted were usually measured at OD$_{260}$ and by 260/280 nm absorbance ratio. The purity grade of DNA extracted from the control and the treated soils was in the range of 1.66–1.75, and the concentration obtained was approximately 180 ng µl^{-1}.

The RAPD fingerprints showed substantial differences between different treatments, with apparent changes in the number and size of amplified DNA fragments, e.g., using primer S4 (Fig. 4). The number of disappearing RAPD bands was greater than that of the appearing bands (Table 3). The greater number of disappearing bands was 22 (I, J and K) and the greater number of new bands was 20 (E). Combined pollution or elevated toxicant level would increase disappearing RAPD bands compared to control. The number of denoting polymorphic bands, in which the greatest value was P (%) = 49.4%, was greater in combined polluted soils. In all cases, polymorphisms were due to the loss and gain of amplified bands in the treated samples compared with the control. In our present study, control treatment had smaller coefficient of DNA sequence similarity to I, J, K and L than to the other samples, and combined pollution caused smaller coefficient of DNA sequence similarity to other samples (Table 4).

Discussion

Effects of heavy metal on microbe population

The effects of heavy metal on the number of culturable bacteria remain unclarified (Bååth 1989), but in this investigation, the total number of bacteria and actinomycetes was reduced with elevated heavy metal concentration. Various studies have found that fungi are more resistant than bacteria to long-term heavy metal contamination (Bååth 1989; Fliessbach et al. 1994; Frostegård et al. 1996). The results were also observed in our study. Soil microbial community structure was an important component in regulation of the soil microbial activity in our experiments. Metal toxicity experiments often study effects on microbial activity parameters solely (e.g., Welp 1999; Kristensen et al. 2003; Smolders et al. 2004). Our results can be used to estimate the potential of heavy metals in contaminated field soils to negatively affect soil microbial community responses, and it is similar with microbe population results.

Effects of heavy metal on soil microbial community DNA sequence and similarity

Recently, RAPD technique has been successfully utilized to detect various types of DNA damage and mutation in animals, bacteria and plants induced by pollutants

Fig. 3 Genomic DNA extracted by CTAB in the treated samples (A–CK). *M* the DNA marker; the bands at bottom 23,130 bp
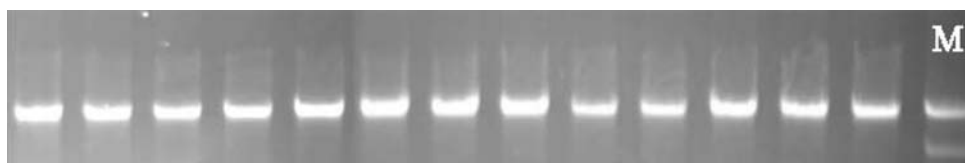
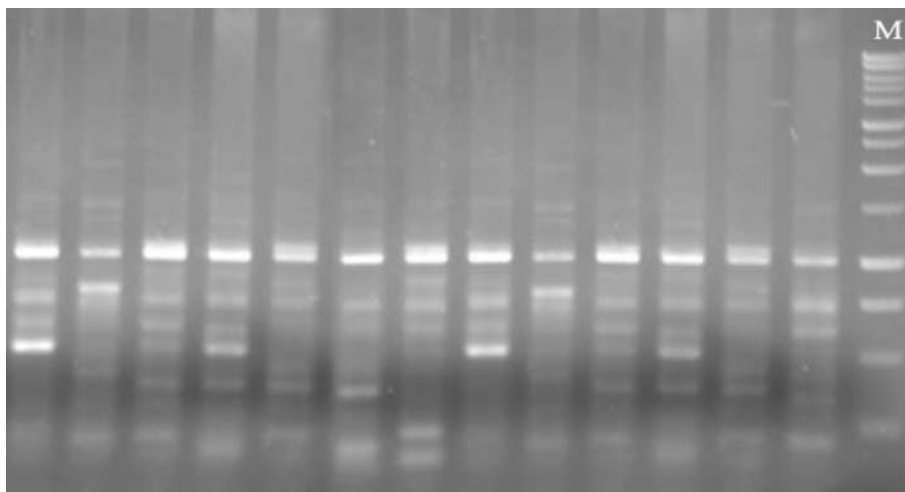**Fig. 4** The RAPD fingerprints of primer S4 from the treated samples (A–CK)

Table 3 Changes of total bands, and of polymorphic bands and varied bands in treatments

No.	A		B		C		D		E		F		G		H		I		J		K		L		CK
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
S1	0	2	2	1	1	0	0	2	1	1	2	1	2	3	1	1	0	1	1	1	2	1	1	1	9
S2	1	1	2	3	3	2	2	2	2	1	2	2	2	3	2	1	2	2	2	1	0	2	2	2	6
S3	1	2	0	3	3	2	0	1	1	3	1	2	1	1	1	1	2	2	1	4	2	3	2	1	8
S4	1	2	2	1	1	1	1	1	2	2	2	1	0	0	1	1	2	3	1	2	0	3	1	3	9
S5	2	2	0	1	1	2	0	2	1	2	1	3	2	1	1	2	2	1	2	3	2	1	1	2	8
S6	0	3	1	2	2	0	1	1	2	3	1	1	1	2	2	2	2	4	1	3	2	3	1	2	8
S7	1	2	1	1	1	2	2	2	2	2	1	3	2	2	1	1	3	2	2	3	1	2	1	1	7
S8	2	1	1	1	1	2	1	1	2	1	0	0	0	1	0	2	1	3	3	1	2	2	2	3	9
S9	2	2	2	2	2	0	1	1	2	2	2	1	1	1	1	3	1	2	2	1	2	3	1	4	6
S10	1	2	1	2	0	1	2	3	1	1	2	3	2	1	2	2	1	2	0	2	2	2	2	2	7
Total	11	19	12	17	12	15	10	16	17	18	14	17	13	17	12	16	16	22	15	22	15	22	14	21	77
a + b	30		29		27		26		35		31		30		28		38		37		37		35		
P%	38.9		37.7		35.1		33.8		45.5		40.4		39.1		36.4		49.4		48.1		48.1		45.5		

a appearance of new bands, *b* disappearance of normal bands, *a + b* polymorphic bands, *P* value of polymorphism compared to the control

Table 4 Coefficients of microbial community DNA sequence similarity of different soil samples

No.	A	B	C	D	E	F	G	H	I	J	K	L	CK
A	1												
B	0.64	1											
C	0.68	0.64	1										
D	0.66	0.69	0.62	1									
E	0.55	0.53	0.54	0.68	1								
F	0.56	0.52	0.51	0.68	0.74	1							
G	0.52	0.57	0.55	0.51	0.64	0.68	1						
H	0.59	0.63	0.56	0.62	0.68	0.66	0.70	1					
I	0.47	0.42	0.46	0.40	0.46	0.51	0.48	0.50	1				
J	0.43	0.51	0.47	0.49	0.42	0.43	0.46	0.48	0.71	1			
K	0.45	0.49	0.44	0.42	0.49	0.45	0.39	0.40	0.69	0.74	1		
L	0.47	0.51	0.48	0.52	0.54	0.56	0.58	0.54	0.66	0.73	0.66	1	
CK	0.52	0.51	0.54	0.47	0.53	0.49	0.49	0.52	0.43	0.39	0.39	0.41	1

(Atienzar et al. 2002; Rong and Yin 2004). In our study, the results indicated that pollutants might decrease the richness of soil microbial community DNA sequence but the polymorphic at DNA sequence could still stay at high level. This may be that changes in the balance of microbial populations and genetic recombination contributed to the increased diversity (Bej et al. 1992).

The genomic template stability in soil was significantly affected by the addition of Cd and Pb in this study. Changes in oligonucleotide priming sites mainly due to genomic rearrangements and less likely to point mutations and DNA damage in the primer binding sites could act to block or reduce polymerization of DNA in the PCR reaction (Nelson et al. 1996). Pollutants could induce DNA damage such as single and double strand breaks, modified bases, abasic sites, etc., in organisms (Waisberg et al. 2003; Atesiet et al. 2004), which may also induce important structural changes that can significantly affect the kinetics of PCR events (Bowditch et al. 1993). Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations and large deletions or homologous recombination (Atienzar et al. 1999).

The result indicated that high concentration of Pb and Cd significantly affected the microbial communities on the diversity and genetic similarity. Moreover, this result was similar to the enzymatic activity in the control samples and the treated samples. Similar evidence was reported by Liu et al. (2005), which also demonstrated that changes in RAPD profiles induced by toxic pollution exposure could also be regarded as modifications in genomic DNA template stability.

Effects of heavy metal on enzymatic activities

Heavy metals were toxic to living organisms primarily due to their protein-binding capacity and hence ability to inhibit enzymes (Dick 1997). Pollutants can reduce enzymatic activity by interacting with the enzyme–substrate complex, denaturing the enzyme protein or interacting with the protein-active groups. And an indirect effect is also possible because changes in the community structure can modify the enzymatic activity (Nannipieri 1994). Results from our experiment indicated that heavy metal pollution caused an inhibition of microbe population and soil enzyme activities, but different dose heavy metal and incubation time showed effects discrepancy on enzymatic activity. Nannipieri (1994) and Lobo et al. (2000) found that the measurement of biochemical variables could show the toxic effects of heavy metals on the soil. According to

Moreno et al. (1999, 2003), the decrease in the microbial indicators measured with increasing incubation time was presumably due to the depletion of the substrates easily available to microorganisms.

Bioremediation can affect the soil biota by influencing the quantity and quality of organic substrates that reach the soil. It is well known that different plant species can associate with microbial communities with unique characteristics (Chen et al. 2002; Viketoft et al. 2005) probably due to differences in amount and quality of root exudates (Nguyen 2003). In natural soils, heavy metals exhibited toxic activity toward soil biota which may lead to the decrease of the number and the activity of soil microorganisms and reduce the rate of PAH microbial transformations playing an important role in dissipation of these compounds in the soil environment (Wild and Jones 1995). In this study, the results may indicate that enzyme inhibition would also affect bioremediation of organic pollutants mixed with heavy metals as well, because of reducing soil microbes' diversity and positive interaction of heavy metals and organic pollutants.

Figure 5 shows the ED_{50} values modified by toxicant coefficient calculated from the best-fit model, and determination values from the regression analysis for the three enzymatic activities studied after the incubation periods. All three modified models described well enzymatic activities, with a coefficient of determination (R^2) higher than 0.90, suggesting a high degree of compliance with these models. Although all three models were adequately fitted to experimental data, Model 2 was slightly best fitted in most of the cases. The highest ED_{50} values for phosphatase, urease and dehydrogenase activity, which responded to toxicant coefficient were 1,438, 2,857 and 777, respectively, were predicted using Model 2 (Fig. 5a, f and i). Dehydrogenase was the most sensitive soil enzyme, whereas urease activity showed the lowest inhibition, which was also observed by Hinojosa et al. (2008).

Furthermore, toxicity of multi-metallic pollutants might be the result of complex interactions among metallic species, transformations dependent on soil properties, and the concomitant changes of pH that they entail (McBride 1989). This may be why ED_{50} values would be higher under combined pollution than under single pollution. In addition, enzymes in soils can be physically and chemically protected by soil constituents (organic and inorganic ligands), which interact with trace elements (Renella et al. 2003). However, it is not possible to determine if the decrease in soil enzymatic activities may be due to a direct metal inhibition of enzymes, to a lower synthesis and/or release of enzymes, or to a combination of both (Renella et al. 2005; Mench et al. 2006).
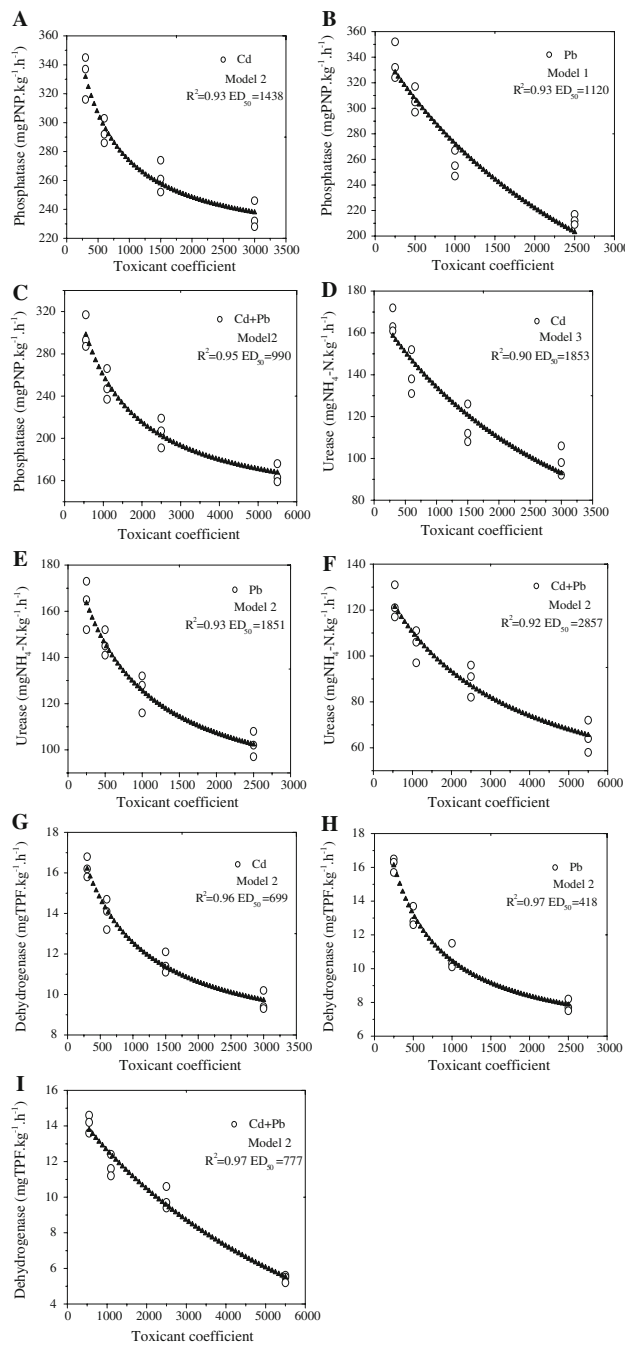
Fig. 5 Dose–response curves of enzyme activities shown correspond to the best-fitted model

These ED$_{50}$ values may be more suitable indicators of the sensitivity of an ecosystem to stress, because a 50% reduction of a basic ecological process may be too extreme for its continued functioning (Babich et al. 1983). Our results emphasize the need for the use of soil functioning indicators and toxicant coefficient calculated by ecological risk factor method, in addition to the current analytical chemical measurements, for the risk assessments and evaluations of contaminated sites.

Conclusion

All three mathematical modified models satisfactorily described the inhibition of soil enzyme activities caused by Cd and Pb, by giving the best fit. In this sense, a long-term monitoring of trace element bioavailability is necessary under field conditions in addition to more studies about the factors that regulate metal availability along time. The measurement of enzymatic activity can be considered a direct bioassay to evaluate recovery of functionality of soils as they are not always correlated with changes in labile metal pools. The research suggests that RAPD analysis in conjunction with modifying dose–response model would prove a powerful ecotoxicological tool and evaluating soil health method.

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