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Interactive effect of dissolved organic matter and phenanthrene on soil enzymatic activities

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Abstract

The investigation of dissolved organic matter (DOM) on urease, catalase and polyphenol oxidase activity in a phenanthrene (Phe)-contaminated soil was conducted under laboratory incubation conditions. Values of soil enzymatic activity depended mainly on incubation time. In the initial 16 days, urease activity increased, and was followed by a decrease. In the initial 8 days, catalase activity decreased and then increased. Variation of polyphenol oxidase activity was just the reverse of catalase activity. After 30 days of incubation, no pronounced difference among treatments with Phe, Phe and DOM, and control were detected in urease, catalase and polyphenol oxidase activity. Phe might inhibit urease and catalase, and stimulate polyphenol oxidase. DOM could improve inhibition of Phe in soil urease and catalase activity during the initial period of applying DOM. Nevertheless, DOM had no significant effect on polyphenol oxidase activity in the Phe contaminated soil. There was a negative correlation between catalase and polyphenol oxidase (r = –0.761***), and catalase and urease (r = –0.554**). Additionally, a positive correlation between polyphenol oxidase and urease was also detected (r = 0.701***). It is implied that the formed DOM after application of organic wastes into soils may counteract the inhibition of polycyclic aromatic hydrocarbons in soil enzyme activities.

Key words: polycyclic aromatic hydrocarbons; dissolved organic matter; phenanthrene; soil enzymes
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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a unique class of persistent organic pollutants (POPs), which contain two or more fused aromatic rings made up of carbon and hydrogen atoms (Douben, 2003). They are ubiquitous organic pollutants and are potentially carcinogenic, mutagenic and toxic to both human and non-human organisms. Therefore, PAHs have been a hot topic in environmental studies during recent years.

More than 90% of PAHs in the environment reside in surface soil (Wild and Jones, 1995). There is an increasing concern about the ecotoxicity of PAHs in soil. Pollutants introduced into soil exert an influence on the microbiota, which manifests as changes in biological parameters such as enzyme activities, and microbial counts. There is mounting evidence that soil biological parameters may have potential as early and sensitive indicators for measuring the degree of soil degradation in both natural and agro-ecosystems. This is because they particularly suit to measure the impact of pollution on the quality of soil (Dick and Tabatabai, 1992; Gianfreda et al., 2005). An alteration of their activity and diversity may result, and in turn this will be reflected by reduced soil quality (Schloter et al., 2003). Soil enzyme activities are the driving force behind all biochemical processes occurred in soil, including the decomposition of organic contaminants and the detoxification of xenobiotics (Margesin et al., 2000b). Their evaluation may provide useful information about soil microbial activity and be helpful to determine the effects of soil specific environmental conditions (Dick et al., 1996).

In recent years, the majority of studies regarding the effect of PAHs on soil enzymatic activities have focused on the enzymes participating in the biological transformation processes of C, N, and P such as β-glucosidase, sucrase, protease, urease, and phosphatase, and the oxidation of aromatic compounds like dehydrogenase, catalase, phenoloxidase, and laccase (Verrhiest et al., 2002; Andreoni et al., 2004; Gianfreda et al., 2005). The results regarding a relationship between PAHs and soil enzymatic activities are in contradiction (Margesin et al., 2000a; Andreoni et al., 2004; Baran et al., 2004; Gianfreda et al., 2005). The occurrence of the contradiction depends, to a large extent, on categories of soil, enzyme, and PAHs, contents of PAHs in polluted soil, and experimental conditions (Kucharski et al., 2000; Baran et al., 2004; Gianfreda et al., 2005).

Application of organic residues as a source of organic matter is a common practice to improve physical, chemical

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and biochemical properties of soil (Martens et al., 1992; Giusquiani et al., 1995; Bellamy et al., 1995; Barker, 1997; Entry et al., 1997), and is also an effective way to dispose of organic solid wastes (Sumner, 2000; Sánchez-Monedero et al., 2004; Avery et al., 2004; Overcash et al., 2005). In China, the total annual amount of organic materials is more than 13.3 billion tons (Ju et al., 2005). Most of this waste is applied to soil in order to maintain cropland fertility. Historically, recycling organic material in agriculture has been a longstanding tradition in China (FAO, 1978).

A large amount of dissolved organic matter (DOM) is immediately and significantly presented after organic residues are added to soil (Zsolnay and Görlitz, 1994; Jensen et al., 1997; Chantigny et al., 2000). DOM is the most active component in both terrestrial and water ecosystems (Kalbitz et al., 2000; Neff and Asner, 2001). It can facilitate the mobility of contaminants (Magee et al., 1991; Kalbitz and Wennrich, 1998; Charles et al., 2002), and affect the bioavailability of contaminants such as heavy metals, PAHs in soil or water (Landrum et al., 1985; Kukkonen and Oikari, 1991; Kukkonen and Pellinen, 1994). Hence, it is presumed that DOM is able to elevate or alleviate the toxicity of PAHs to soil biological organisms. However, little information is available on the influences of DOM on enzymatic activities in PAH-contaminated soils. The investigation of the combined effects of DOM and PAHs on soil enzymatic activity is vital for evaluating variation in soil quality after the application of organic wastes in PAH-polluted soils.

Phenanthrene (Phe), one of the 16 PAHs selected as priority pollutants by the American Environmental Protection Agency, has been used as a model compound of PAHs because of its ubiquity and high detection rate in the environment (Huang et al., 1998; Xiao et al., 2004). In many cases, the activity of urease appears to be more sensitive to pollution than that of other soil enzymes (Bååth, 1989). Catalase is a biomarker of PAHs-mediated oxidative stress (Lionetto et al., 2003). Polyphenol oxidase may give indications on the oxidative potential of soil (Gianfreda et al., 2005). The purpose of this study was to determine the impact of DOM originated from pig manure and green manure on urease, catalase, and polyphenol oxidase activities in a Phe-polluted soil under laboratory conditions.

1 Materials and methods

1.1 Soil sampling and processing

Surface paddy soil samples (0–20 cm), Udic Luvisols, were collected from Moling (31°51′47″N, 118°49′85″E), a suburb of Nanjing, Jiangsu Province, China. After removing all stones, visible roots and fauna in the soil samples, the soil samples were air dried at room temperature and sieved to pass a 1-mm screen. Subsample was employed to determine soil physiochemical properties using standard methods recommended by the Chinese Society of Soil Science (Lu, 1999) and the remaining sample was used for the soil incubation experiment.

The main characteristics of the selected soil were: pH 6.70 (in water), 19.4 g/kg organic carbon, 1.90 g/kg total N, 0.57 g/kg total P, 20.2 cmol/kg cation exchange capacity (CEC), 51.4% clay (particle size < 0.002 mm), 39.1% of silt (particle size between 0.002 and 0.05 mm), 9.5% of sand (particle size > 0.05 mm).

1.2 Organic wastes preparation and DOM extraction

Pig manure and green manure (*Vicia faba* L.) were selected as sources of DOM, due to their application in cropland as common organic manure in China. They were collected from a farm of Nanjing Agricultural University, oven-dried at 40°C, ground and sieved (< 1 mm mesh) for subsequent use.

The DOM extraction was conducted by mixing dry solid sample with Milli-Q water at 1:10 (*W/V*) for pig manure, and 1:40 (*W/V*) for green manure. The samples were then shaken for 16 hr at 200 r/min on a rotating shaker. Extracts were centrifuged for 20 min at 12,000 r/min at 4°C and filtered through a 0.45-μm membrane. They were stored in amber glass bottles for a maximum of 24 hr at 4°C until utilization. The DOC of DOM was determined with a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). DOC contents were 12,904 mg C/kg for pig manure, and 380,560 mg C/kg for green manure.

1.3 Preparation of PAHs-contaminated soil and incubation

Soil incubation was carried out in 800 mL Erlenmeyer glass beakers containing 200 g air dried, sieved (1 mm) soil. Phe was added to the whole soil (i.e., 200 g) once as an acetone solution. The concentrations of Phe in the soil were designed as 100 and 200 mg/kg dry weight soil in the light of the reports of Eriksson et al. (2000) and Cai et al. (2008). In the control, the same amount of acetone was added. Soils spiked with acetone were stored in the dark for 2 days when the acetone was completely volatilized. After volatilization of acetone, DOM (Milli-Q water extractant) was applied into soils at a rate of 100 mg DOC/kg dry weight soil, which was within the range of DOM concentrations shown by Williams et al. (2002).

All soils were then moistened up to 80% of maximum field water holding capacity. After thorough mixing, the treated soils were incubated at 28°C under aerobic conditions for 30 days. The moisture content was maintained throughout the experiment weighing.

Soil without the addition of Phe and DOM was utilized as a control. A randomized complete plot design with three replicates per treatment was used. The soil incubation experiment was performed with the following 7 treatments: the control; soil + 100 mg/kg Phe (Phe100); soil + 100 mg/kg Phe + 100 mg/kg DOM derived from pig manure (Phe100 + PM100); soil + 100 mg/kg Phe + 100 mg/kg DOM derived from green manure (Phe100 + GM100); soil + 200 mg/kg Phe (Phe200); soil + 200 mg/kg Phe + 100 mg/kg DOM derived from pig manure (Phe200 + PM100); soil + 200 mg/kg Phe + 100 mg/kg DOM derived from green manure (Phe200 + GM100).

Three beakers per treatment were withdrawn at each

sampling point. Soil was homogenized prior to sampling. Soil enzymatic activity measurements were conducted on day 0, 4, 8, 16 and 30.

1.4 Analysis of soil enzyme activities

Urease is involved in the hydrolysis of C–N bonds of some amides and urea (Madejón et al., 2001a), and more sensitive to pollution (Bååth, 1989). Catalase normally acts on the H_2O_2 produced in the reduction of $O_2^{-\bullet}$. Since reactive oxygen species may be produced in PAH metabolism, an induction of this enzyme after PAH exposure is expected (Barreira et al., 2007). Polyphenol oxidases catalyze the hydroxylation of monophenols to *o*-diphenols using molecular oxygen and the oxidation of *o*-diphenols to *o*-quinones (Girelli et al., 2004). Therefore, activities of the three enzymes are affected by PAHs in soil.

Urease activity was measured as described by McGarity and Myers (1967). Five gram soil was placed in a 50-mL Erlenmeyer flask, 1 mL toluene was added to the soil in the flask, and the contents were allowed to stand for approximately 15 min until the toluene had completely penetrated the soil. Then, a 20-mL potassium citrate-citric acid buffer (pH 6.7) and a 10-mL of 10% urea solution were added. The flasks were stoppered, shaken and then incubated at 37°C for 24 hr. A control, in which 10 mL distilled water was substituted for the urea, was conducted simultaneously. After incubation, the contents of the flasks were filtered. The ammonia released by hydrolysis of urea was determined on the filtrate by the colorimetric indophenol blue method. The unit of urease activity was reported as mg NH_4^+-N released/(kg dry soil·24 hr).

Polyphenol oxidase was analyzed with pyrogallic acid as substrate. The mixture of 1 g soil and 10 mL of 1% pyrogallic acid was incubated at 30°C. A 4-mL disodium hydrogen phosphate-citric acid buffer (pH 4.5) was added after 2 hr incubation and purpurigallin was extracted by ether. It was then measured by a spectrophotometer (Shimadzu, UV-2550, Kyoto, Japan) at 430 nm. Polyphenol oxidase activity was expressed as mg purpurigauin/(g dry soil·2 hr) (Zhou, 1987).

Catalase activity was determined by $KMnO_4$ titration method with H_2O_2 as substrate. The mixture of 2 g soil, 40 mL distilled water and 5 mL of 0.3% H_2O_2 was shaken for 20 min at 25°C. After filtration, 5 mL of 1.5 mol/L H_2SO_4 was added to 10 mL extract and the remaining H_2O_2 was measured by 0.1 mol/L $KMnO_4$ solution. Catalase activity was expressed in mL 0.1 mol/L $KMnO_4$ solution titrated/(g dry soil·20 min) (Zhou, 1987).

All determinations of enzymatic activity were performed in triplicates, and all values reported are averages of the three trials performed on oven-dried soil (105°C).

1.5 Statistical analysis

Means and standard deviation of triplicates were determined and all the figures presented included standard errors of the data. Analysis of variance (One-way ANOVA) was carried out. The means were compared using the least significant difference (LSD) test, with a significance level of $p < 0.01$. All statistical calculations were performed using Excel and SPSS 12.0 computer programs. Pearson correlation coefficients were calculated, and significance was set at $^{**}p < 0.01$ and $^{***}p < 0.001$.

2 Results

2.1 Urease activity

The changes in soil urease activity during the incubation are presented in Fig. 1. Urease activity was within the range of values usually found in soil (Nannipieri et al., 2002). During the incubation period from day 0 to day 16, soil urease activity exhibited a pronounced increase with the exception of the control. In the control, on the first 4 days of incubation, a decrease was observed, followed by an increase in soil urease activity. At day 16, soil urease activities reached the highest values for all treatments. After that, a decrease in soil urease activities occurred. At day 30, there was no significant difference in urease activity among the seven treatments. During the whole incubation time, soil urease activities were significantly lower than those of the control samples (without the addition of Phe and DOM) except on day 30. This indicated that the addition of Phe in the presence and absence of DOM did not stimulate soil urease activity, on the contrary, inhibited soil urease activity. The results were in good agreement with the investigation conducted by Gianfreda et al. (2005). After 30 days, the inhibition effect of Phe on soil urease activity disappeared.

It was noted that soil urease activity increased dramatically during the first 4 days. Hence, the first 4 days could be considered the period in which soil was most sensitive to urease activity measurement. This means that it is better to determine soil urease activity within the first 4 days of

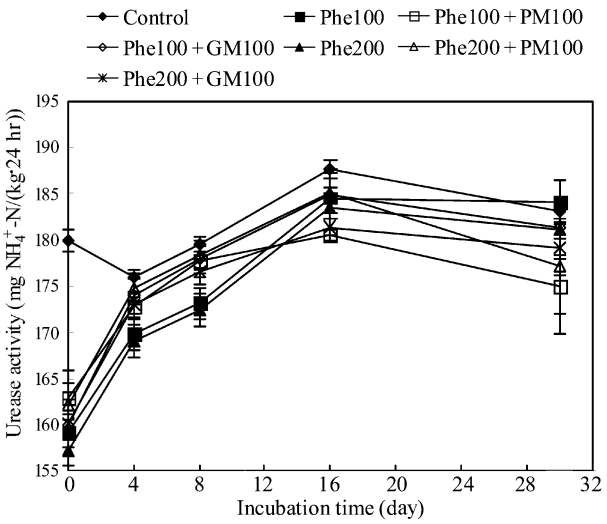

Fig. 1 Dynamic changes of urease activity in PAHs-contanminated soils in the presence and absence of DOM during incubation for 30 days. Phe100: the treatment with 100 mg/kg Phe; Phe100 + PM100: the treatment with 100 mg/kg Phe and 100 mg DOC/ kg DOM derived from pig manure; Phe100 + GM100: the treatment with 100 mg/kg Phe and 100 mg DOC/kg DOM derived from green manure, Phe200 the treatment with 200 mg/kg Phe; Phe200 + PM100: the treatment with 200 mg/kg Phe and 100 mg DOC/kg DOM derived from pig manure; Phe200 + GM100: the treatment with 200 mg/kg Phe and 100 mg DOC/kg DOM derived from green manure.

incubation for the evaluation of toxic effect by Phe.

2.2 Catalase activity

Variations in soil catalase activity during the whole incubation period were different from those observed in soil urease activity. In the control, there was no significant fluctuation in soil catalase activity over 30 days of incubation. The average soil catalase activity in the control was (2.93 ± 0.19) mL/(g dry soil·20 min), with a minimum of (2.77 ± 0.10) mL/(g dry soil·20 min) and a maximum of (3.23 ± 0.03) mL/(g dry soil·20 min) (Fig. 2).

For soil samples spiked with Phe in the presence and absence of DOM, catalase activity noticeably declined during the first 8 days of incubation (Fig. 2). However, after the eighth day, a progressive increase in soil catalase activity was observed until the termination of incubation (i.e., day 30). At day 30, no significant difference in soil catalase activities was detected among any of the seven treatments. In the light of catalase activity, the first 8 day constituted the period most sensitive to measuring the toxic effect of Phe.

2.3 Polyphenol oxidase activity

The activity of polyphenol oxidase showed a reverse trend in comparison with that of catalase in soil samples treated with Phe or Phe + DOM: a rapid increase in the first 8 days of incubation followed by a pronounced decrease in activity (Fig. 3).

In the control, soil polyphenol oxidase activities varied from (0.40 ± 0.02) to (0.44 ± 0.02) mg purpurigauin/(g dry soil·2 hr), with a mean of (0.42 ± 0.01) mg purpurigauin/(g dry soil·2 hr). Because polyphenol oxidase is activated and induced by aromatic compounds (Zhou, 1987), and the control is not spiked with Phe or DOM, these lead to no pronounced variation in soil polyphenol oxidase activity in the control during 30 days of incubation. In addition, during the initial 4 days of incubation, no noticeable difference among the seven treatments was found. Compared to the control, soil polyphenol oxidase activities in the other six treatments were much higher from day 4 to day 6. On day 30, there was no significant difference in polyphenol oxidase activities among any of the seven treatments.

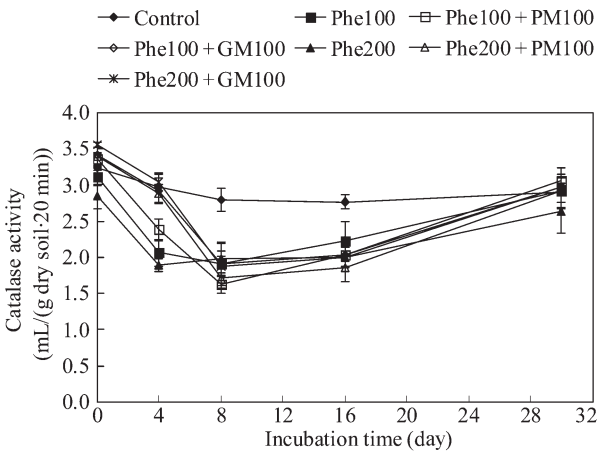

Fig. 2 Dynamic changes of catalase activity in PAHs-contanminated soils in the presence and absence of DOM during incubation for 30 days. For abbreviations, see Fig. 1.

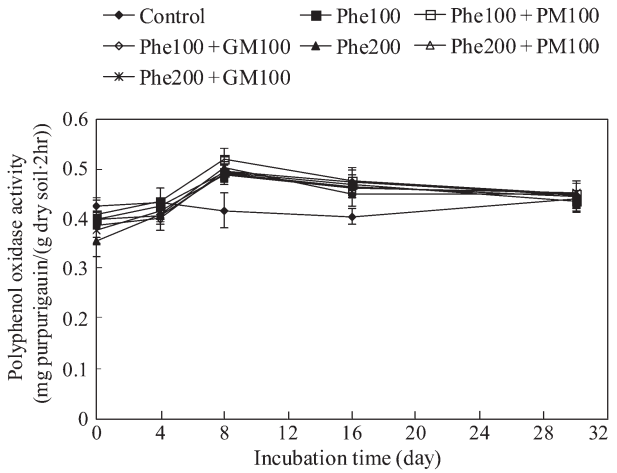

Fig. 3 Dynamic changes of polyphenol oxidase in PAHs-contanminated soils in the presence and absence of DOM during incubation for 30 days. For abbreviations, see Fig. 1.

3 Discussion

3.1 Incubation time and soil enzyme activity

Values of soil enzymatic activities (Figs. 1, 2, and 3) depend, to a large extent, on incubation time. Similar conclusions have been drawn by Shen et al. (2005) in their study on the combined effect of PAHs and heavy metal on soil urease activity. In the present study, the evolution of enzyme activity during the incubation period can be separated into two different stages: a first stage lasting from the beginning of the incubation to day 8 or day 16, during which a great activation of polyphenol oxidase and urease or an inhibition of catalase was recorded as a result of the addition of Phe or Phe and DOM; and a second stage lasting from day 8 or day 16 to the end of the incubation (i.e., day 30), during which a major decrease or increase in the enzymatic activities in soils treated with Phe or Phe and DOM was recorded. This was in accordance with the results obtained by Kizilkaya and Bayrakli (2005). The increase period occurs due to microbial growth, enzymatic induction and available substrate for enzymes stimulated by adding Phe or Phe and DOM to the soil. But the drop in enzyme activity is caused by the stress effect of Phe or Phe and DOM, the reduction of available substrate and the decrease in bioavailability of Phe, a source of carbon and energy for microorganisms. Enzyme activities tended to return to control values after 30 days of Phe or Phe and DOM addition. Madejón et al. (2001a) have also observed similar results. The presented results indicated that variations of soil enzyme activities occurred in the initial period of spiking with Phe or Phe and DOM. After that period, no difference in enzymatic activities was detected as compared to the control. However, during the initial period, soil enzyme activity varied drastically, and the soil enzyme activity determined in this stage is the most suitable for evaluating the degree of Phe pollution in soils and the impact of DOM.

3.2 Phe, DOM and soil enzyme activities

Polycyclic aromatic hydrocarbons exhibit toxic properties that clearly inhibit soil enzymatic activity in most cases (Verrhiest et al., 2002; Maliszewska-Kordybach and Smreczak, 2003; Andreoni et al., 2004; Gianfreda et al., 2005). However, in some cases, a stimulating effect of PAHs on soil enzyme activity can be observed (Crowe and Olsson, 2001; Verrhiest et al., 2002; Chen et al., 2004). The influence of PAHs on enzyme activities depends mainly on the amount and composition of these compounds, and type of soil enzyme. As shown in Figs. 1 and 2, soil urease and catalase activities were inhibited in the treatments with 100 and 200 mg/kg Phe only during 30 days of incubation. Although the activities of the two enzymes were restricted in the Phe-contaminated soils, the urease activity exhibited a trend dissimilar to that of catalase: increased during the first stage of incubation, and decreased thereafter. The tendency was reversed in the case of catalase activity. The stress effect and adaptation of microorganisms to substrates contributed to the variation in catalase activity. Microorganism growth and a reduction in the bioavailability of Phe were responsible for the change in urease activity (Cerniglia, 1992). Enhanced polyphenol oxidase activites were also found (Fig. 3). Polyphenol oxidase is an enzyme activated and induced by aromatic compounds like PAHs (Zhou, 1987). Therefore, the presence of Phe can result in an increase in polyphenol oxidase activity. Gianfreda et al. (2005) observed a significant negative correlation between Phe/PAHs concentrations and urease activity. Chen et al. (2004) found that the activity of polyphenol oxidase was positively correlated with PAHs concentration. In the present work, although values of soil urease and catalase activity treated with 200 mg/kg Phe were a little lower than those treated with 100 mg/kg Phe, no pronounced difference in soil urease or catalase activity was found between the two treatments with paired *t*-test at 95% confidence level. As regards polyphenol oxidase activity, no pronounced difference between the Phe100 treatment and the Phe200 treatment was detected with paired *t*-test at 95% confidence level, either. The reason that no marked difference was observed in soil urease, catalase and polyphenol oxidase activity between the Phe100 treatment and the Phe200 treatment is the high amount of organic matter with 19.4 g/kg in the soil studied. Soil organic matter can mitigate the toxic effect of PAHs (Gensemer et al., 1998, 1999). The influence of PAHs on the enzymatic activity depends, to a significant level, on soil properties like total organic carbon content (Baran et al., 2004). Maliszewska-Kordybach et al. (2000) and Kucharski et al. (2000) have noted that the soil rich in organic matter considerably decreases the negative influence of PAHs on enzymatic activity. Hence, it can be concluded that the negative or positive correlation between concentration of PAHs and soil enzymatic activity is much more significant in the organic matter-poor soils.

DOM can stimulate microbial growth because it is an effective source of energy and carbon. Meanwhile, DOM can form complexes with PAHs by interactions of NH–π and π–π, hydrophobic effect, and partitioning (Zhan et al., 2007). Consequently, DOM may affect bioavailability and toxicity of PAHs. Our findings have demonstrated that DOM can alleviate the toxicity of PAHs to wheat seedling (Zhan et al., 2004). Nevertheless, limited information on the combined effect of DOM and PAHs on soil enzymatic activity was recorded.

Soil urease activities in the Phe100 + PM100 and Phe100 + GM100 treatments were significantly higher than those in the Phe100 treatment in the initial 16 days of incubation with paired *t*-test at 95% confidence level, but no pronounced difference between Phe100 + PM100 and Phe100 + GM100 was determined (Fig. 1). After 16 days, the differences in urease activity were not significant among the three treatments. A similar trend was shown among Phe200, Phe200 + PM100, and Phe200 + GM100. The pronounced difference in soil catalase activity between the treatment containing Phe and DOM, and the treatment containing Phe only be could found in the initial 8 days (Fig. 2). After 8 days, no significant difference among Phe100, Phe00 + PM100, Phe100 + GM100, Phe200, Phe200 + PM100, and Phe200 + GM100 were observed. Also, the marked difference in catalase activity between the treatments with PM100 and GM100 was not found. The above results indicated that DOM could alleviate inhibition of soil urease and catalase caused by PAHs during the initial period of applying DOM, and it seemed that the DOM derived from different sources of organic waste had little effect on alleviating inhibition of soil enzyme activity caused by PAHs. Some studies have shown that soil enzyme activity is stimulated by application of organic residues (Madejón et al., 2001a, 2001b; Maliszewska-Kordybach and Smreczak, 2003; Kizilkaya and Bayrakli, 2005). Our results regarding the impact of DOM on soil urease and catalase activity are consistent with these findings. DOM is a more easily utilized source of energy and carbon for microbial growth. When it is applied to soil, microbial growth will be enhanced. Thus, soil enzyme synthesis is accelerated (Martens et al., 1992). Besides improving enzyme synthesis, DOM can also form very stable complexion with organic pollutants (Baran et al., 2004; Zhan et al., 2007). These combined xenobiotics are unavailable to microorganisms, lowering their toxic effect on the microorganisms. Both of them are responsible for ameliorating inhibition of urease and catalase. Amelioration of inhibition by DOM during the initial period is due to the rapid decomposition of DOM by microorganisms. Zhou and Wong (2000) observed that the amount of DOM decomposed was up to 78% within 24 hr in the soils spiked with DOM. Compared to the source of DOM, the concentration of DOM plays a more important role in alleviating inhibition of soil enzymes. As for polyphenol oxidase activity, DOM did not exhibit a stimulating or suppressing effect. This is probably due to fewer benzene and phenol function groups in DOM (Zhan, 2005). Therefore, DOM has no marked effect on activation and inducement of polyphenol oxidase.

3.3 Relationship among urease, catalase and polyphenol oxidase

The relationship among enzyme activity, concentration of PAHs and soil properties has been widely reported (Margesin et al., 2000a; Baran et al., 2004; Gianfreda et al., 2005). However, the information regarding a relationship between enzymes in PAH-polluted soils has not been properly addressed. Urease significantly correlated with dehydrogenase and a correlation was found between dehydrogenase and phenoloxidase (Gianfreda et al., 2005). Dehydrogenase, polyphenol oxidase and catalase may reflect the oxidative potential of a soil. It can be inferred that there may be a good correlation between urease and catalase, and urease and polyphenol oxidase. This inference is supported by our results. In the present study, there was a good negative correlation between catalase and polyphenol oxidase (r = –0.761***), and catalase and urease (r = –0.554**) (Table 1). A good positive correlation between polyphenol oxidase and urease was also detected (r = 0.701***) (Table 1). Catalase and polyphenol oxidase can give indications on the oxidative-reductive potential of a soil (Lionetto et al., 2003; Gianfreda et al., 2005). Chen et al. (2004) also detected the negative correlation between catalase and polyphenol oxidase.

Table 1 Correlation between enzyme activities in Phe-contaminated soil

X	Y	Equation	r	n
CAT	PPO	$Y = -0.0517X + 0.573$	–0.761***	30
CAT	UR	$Y = -7.72X + 194$	–0.554**	30
PPO	UR	$Y = 144X + 111$	0.701***	30

CAT: catalase; PPO: polyphenol oxidase; UR: urease.
** $p < 0.01$; *** $p < 0.001$.

4 Conclusions

Values of enzyme activity in the PAH-contaminated soil depend, to a large extent, on incubation time. The evolution of enzyme activity can be divided into two stages during the incubation period: the first stage occurred in the initial 8–16 days of incubation and the second stage appeared after 8–16 days of incubation. Soil enzyme activity determined in the initial stage is the most suitable for evaluating the degree of Phe pollution in soils and the impact of DOM. Variations of soil enzyme activities occurred during 30 days of incubation in spiking with Phe or Phe and DOM, but no difference in enzymatic activity was detected as compared to the control at the end of the incubation (i.e., day 30).

DOM can alleviate inhibition of soil urease and catalase caused by PAHs during the initial period of application. But DOM does not exhibit a stimulating or suppressing effect on polyphenol oxidase activity. There is a good correlation among catalase, polyphenol oxidase and urease.

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