Effect of ryegrass (*Lolium multiflorum* L.) growth on degradation of phenanthrene and enzyme activity in soil

S.L. Liu[1], Z.H. Cao[2], H.E. Liu[1]

[1]*College of Resources and Environment, Henan Agricultural University, Zhengzhou, P.R. China*
[2]*Key Laboratory of Soil Environment and Pollution Remediation, State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, P.R. China*

ABSTRACT

A 75-day pot experiment was carried out to study the effect of growth of ryegrass (*Lolium multiflorum* L.) on degradation rate of spiked phenanthrene (the concentration was 5, 50, 200 mg/kg) in soil. The results showed that ryegrass growth enhanced the degradation of phenanthrene spiked in the soil, thus making the content of extractable phenanthrene lower ($P < 0.05$) in the ryegrass planted pots than that of pots without ryegrass. In the treatments of 5, 50 and 200 mg/kg of phenanthrene, phenanthrene degradation rate reached 81.1, 90.4 and 85.0%, respectively, while in pots without ryegrass they were only 73.5, 86.2 and 67.6%, respectively, and ryegrass growth shorted phenanthrene half-life. Ryegrass growth enhanced activities of polyphenol oxidase, dehydrogenase and increased the content of microbiological biomass carbon, thus raised the degradation rate of phenanthrene in the soil. High concentration of phenanthrene inhibited soil biological activity, and in turn the effect of soil biology on phenanthrene degradation. Therefore, the findings disclose the biological and enzymological mechanisms of the plant enhancing phenanthrene degradation. It was also found that ryegrass is rather tolerant to polycyclic aromatic hydrocarbons, but high phenanthrene concentration affected ryegrass growth.

Keywords: polycyclic aromatic hydrocarbons; planted ryegrass; phytoremediation, degradation rate

Polycyclic aromatic hydrocarbons (PAHs) are by-products from the incomplete combustion or pyrolysis of organic materials (Gao and Zhu 2004). Since PAHs are potentially toxic, mutagenic and carcinogenic, sixteen of them have been considered as priority pollutants by the US Environmental Protection Agency.

The effect and fate of PAHs in nature are of great environmental and human health concern, and many studies focused on the remediation of PAHs polluted soils (Gao and Zhu 2004, Vácha et al. 2005, Gao et al. 2006). The use of phytoremediation for removal PAHs from the contaminated soils is well documented (Aprill and Sims 1990, Reilley et al. 1996, Bient et al. 2000, Dominiguez-Rosado and Pichtel 2004, Gao and Zhu 2005, Mueller and Shann 2006, Jensen et al. 2012). Liste and Alexander (2000) reported enhanced degradation of pyrene by nine plant species and noted that pyrene was reduced by 74% in planted soil compared to less than 40% in unplanted soil. Fu et al. (2012) found that short-term planting of alfalfa inhibited the dissipation of benzo[a]pyrene from the soil by 8.9%. Chen et al. (2003) found that 37.7% and 30.4% of

Supported by the Natural Science Foundation of China, Grants No. 40031010 and 40432005, and by the Key Technology Projects of the Henan Province, Project No. 1221 0211 0123.

^{14}C-pyrene was mineralized in the soil planted with tall fescue and switchgrass, respectively, while only 4.3% mineralization was observed for unplanted control. The presence of plants enhanced the removal of PAHs, which may result from the enhanced degradation of organic compounds in the rhizosphere soil, because of the higher densities and greater activities of microorganisms than in the surrounding soil (bulk soil) (Xu et al. 2005, 2009, Jensen et al. 2012). And a plant may secrete 10–20% of its photosynthate in root exudates. Some exudated compounds may serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms that are capable of degrading organic pollutants (Vácha et al. 2010). Yoshitomi and Shann (2001) found that the addition of root exudates stimulated the mineralization of ^{14}C-pyrene in an unplanted soil to the same degree as observed in an actual rhizosphere. This indicates that plant root exudates have the potential to increase the degradation of xenobiotics by promoting the growth of soil micro-organisms (Huang et al. 2004).

The purpose of this study was to examine PAH removal in the presence of plants to contribute to the technology for field phytoremediation in practice. Furthermore, the phenanthrene is the representative of 3-ring PAHs, studies also found that the *n*-octanol/water partition coefficient of phenanthrene was 4.47; it accumulated in soil very rapidly (McCarthy and Jimenez 1985). And removal mechanism of phenanthrene by phytoremediation, especially rhizodegradation was not clear. So, phenanthrene was selected as the target compound, and the ryegrass was selected to represent a wide range of grass plant. It aims to obtain basic information about plant contributions to the promoted removal of PAHs in soils on a quantitative scale. The results from this work may advance our understanding of the phytoremediation mechanisms of PAHs.

MATERIAL AND METHODS

Tested soil collecting and phenanthrene spiking. The tested paddy soil was sampled in the upper horizon (0–20 cm) from Changshu Agricultural Experiment Station, Academia Sinica, soil pH (in H_2O) was 6.96, its total carbon (TC) content was 5.93%, and its total N, P, K were 2.25, 0.75, 17.4 g/kg, respectively. The free Fe_2O_3 content was 16.3 g/kg; and CEC 21.6 $cmol_+$/kg. The air dried soil was passed through a 2-mm steel sieve. Five gram of soil were soaked in acetone containing phenanthrene (purity > 97%, Sigma Chemical Co., Ltd., Germany). The acetone was evaporated and the treated soil was diluted by mixing with uncontaminated soil to obtain a final concentration of 5, 50 and 200 mg phenanthrene/kg soil (oven dry weight base). After adjusting the soil moisture content to 60% soil water holding capacity, the soil was stored in a growth chamber for 12 days before use.

Pot experiment design and sample collection. A fully randomized design experiment was conducted in a greenhouse at the Institute of Soil Science, Chinese Academy of Sciences. There were eight treatments: (1) no phenanthrene spiked, planted ryegrass; (2) no phenanthrene spiked, without ryegrass; (3) phenanthrene concentration of 5 mg/kg and planted ryegrass; (4) phenanthrene concentration of 5 mg/kg and without ryegrass; (5) phenanthrene concentration of 50 mg/kg and planted ryegrass; (6) phenanthrene concentration of 50 mg/kg and without ryegrass; (7) phenanthrene concentration of 200 mg/kg and planted ryegrass; (8) phenanthrene concentration of 200 mg/kg and without ryegrass. There were 15 replicates for each treatment. Twenty ryegrass (*Lolium multiflorum* L.) seedlings (germinated in distilled water and grown 20 days) were transplanted in pots containing 250 g of the soil either with phenanthrene or not, and the ryegrass seedlings transplanted date was considered as 0 days. During plant growth, the soil keeping field moisture capacity to 60% as needed by watered distilled water, and on the 15th, 30th, 45th, 60th, 75th day, three replicates were harvested, soil and plant samples were collected for phenanthrene content determination.

The soils were carefully collected, homogenized, air dried and passed through a 2 mm sieve. The soil without ryegrass treatment was considered as non-rhizosphere soil and the soil planted with ryegrass was considered as rhizosphere soil. All samples (soils, ryegrass shoots and roots) were freeze-dried, bagged and stored at 4°C before analytical treatment. Both shoots and roots were weighed for the determination of fresh weight in the same time.

Phenanthrene extraction and determination

Extraction. The following procedure, adapted from Song et al. (1995), was used to extract and

determine the phenanthrene concentrations in soil samples.

Five grams of soil was mixed with 20 mL dichloromethane, and then extracted for 30 min by ultrasonic agitation. Samples were centrifuged at 2000 rpm for 5 min. The supernatant was the soil phenanthrene extractant.

The methanol was removed from 10 mL of soil phenanthrene extractant in a rotary evaporator until dry. Cyclohexane (2 mL) was added and the mixture was vigorously shaken with a vortex mixer for 1 min. A 0.5 mL aliquot of the slurry was passed through a silica gel column. The column was washed with dichloromethane: hexane (1:1 v/v) and the first 1 mL of liquid were discarded. The following 2 mL of liquid was collected and dried with N_2 gas. 2 mL acetonitrile was added and shaken with a vortex mixer and then injected directly into the HPLC.

Chromatographic conditions. Solvents that constituted the mobile phase were A (cyclohexane) and B (water). The flow rate and the column temperature were set at 0.5 mL/min and 30°C, respectively. Other parameters adopted were as follows: injection volume, 40 µL; detection wavelength, 251 nm.

Determination of soil microbial biomass C and enzyme activity. Soil microbial biomass C was determined using the chloroform fumigation extraction method as described by Vance et al. (1987). Soil polyphenol oxidase activity was measured using standard colorimetric methods (Dick et al. 1988), soil dehydrogenase activity was determined using the TTC method described by Casida et al. (1964).

Statistical analysis. Treatment effects were compared using the analysis of variance (ANOVA), and comparisons of means were carried out using the Duncan's test ($P < 0.05$). All statistical analyses were performed using the software Statistical Package for Social Sciences (SPSS 11.5 for Windows, USA).

RESULTS AND DISCUSSION

The biomass of ryegrass. The biomass of ryegrass in the soil contaminated with different concentrations of phenanthrene was shown in Figure 1. There was no significant difference in the dry weight biomass of ryegrass with addition of phenanthrene before the 60 days of the experimentation ($P < 0.01$). But at the 75th day, the ryegrass dry weight of 200 mg/kg phenanthrene treatment was significantly lower than those of the other 3 treatments. This indicated that phenanthrene had smaller effect on the total biomass of ryegrass during early growth, and ryegrass could grow normally in high concentration phenanthrene-contaminated soil, but during later growth, the plants exhibited apparent signs of toxicity stress in higher concentration phenanthrene-contaminated soil, and restrained plant growth.

Extractable phenanthrene concentration in soil. Figure 2 showed that the extractable concentration of phenanthrene in soils gradually decreased with incubation. In three treatments, extractable phenanthrene concentration reduced rapidly during the first 15 days, and then the degradation rate slowed down. When the ryegrass was presented, the soil microorganisms may take part in the degradation of phenanthrene, so the extractable phenanthrene concentration decreased quickly. The results indicated that ryegrass can accelerate the degradation of phenanthrene in the soil, because in the three application levels, the phenanthrene degradation rates in the treatments planted with ryegrass were significantly higher than that in the treatments without ryegrass ($P < 0.05$). Within the 75-day growth period, the degradation rates of phenanthrene added at the dosages of 5, 50 and 200 mg/kg treatments with ryegrass were 81.1, 90.4, 84.9%, respectively; whereas the phenanthrene degradation rates in the treatments without ryegrass were 75.3, 86.6, and 67.6%, respectively.

Figure 2 also reveals that the phenanthrene degradation kinetics complied with exponential model.

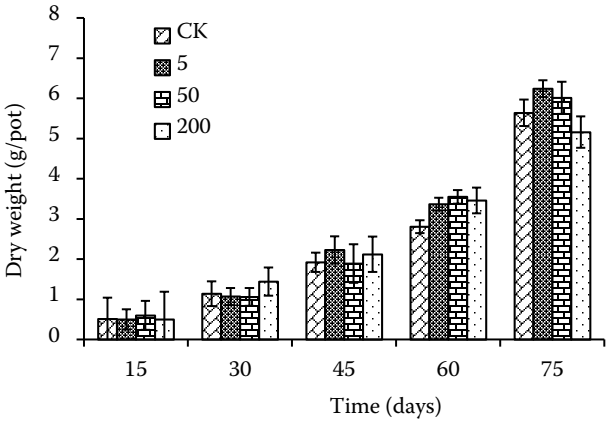

Figure 1. Dry weight of ryegrass in soils different in degree of phenanthrene contamination. CK – 0 mg/kg phenanthrene; 5 – 5 mg/kg phenanthrene; 50 – 50 mg/kg phenanthrene; 200 – 200 mg/kg phenanthrene

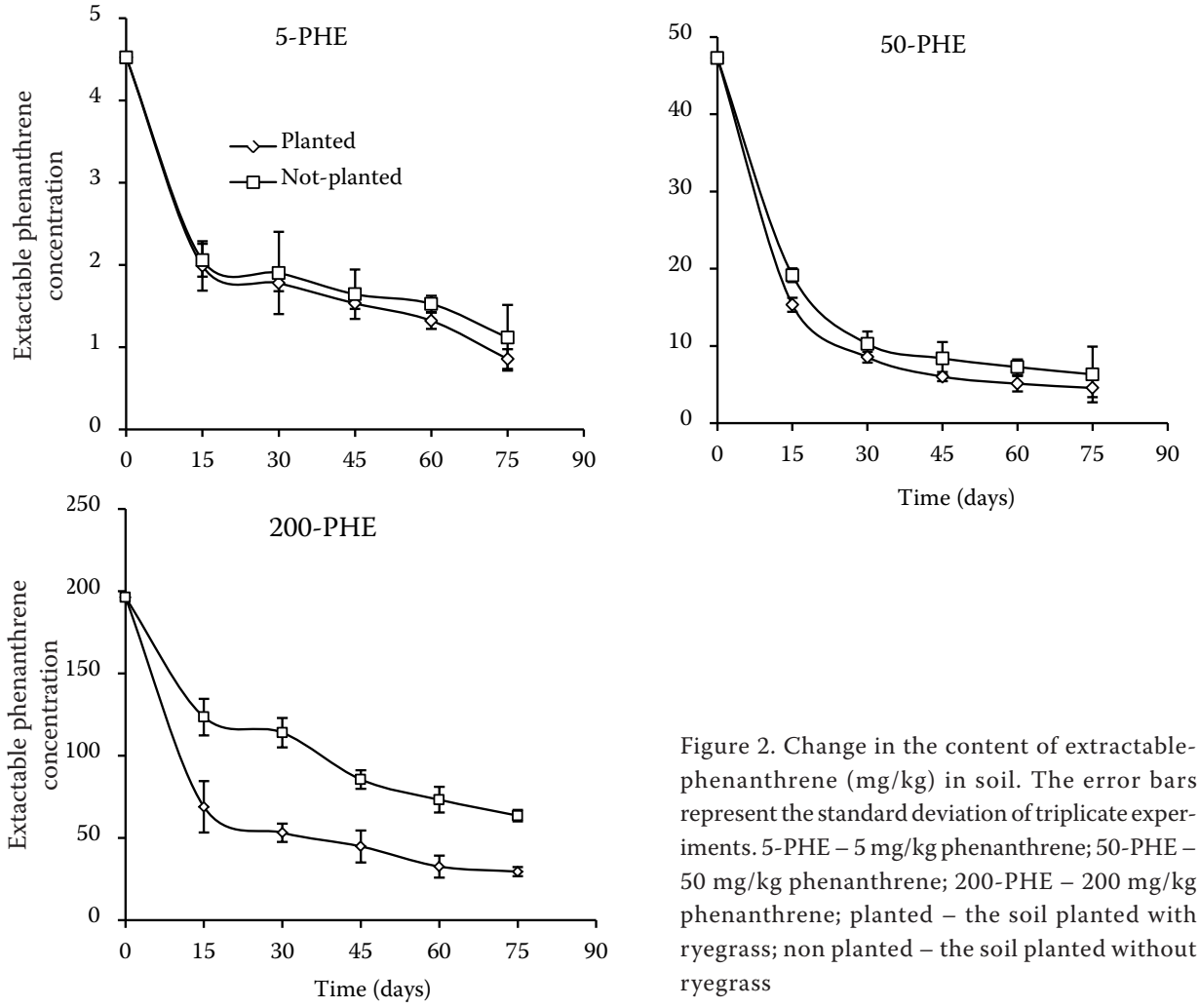

Figure 2. Change in the content of extractable-phenanthrene (mg/kg) in soil. The error bars represent the standard deviation of triplicate experiments. 5-PHE – 5 mg/kg phenanthrene; 50-PHE – 50 mg/kg phenanthrene; 200-PHE – 200 mg/kg phenanthrene; planted – the soil planted with ryegrass; non planted – the soil planted without ryegrass

The degradation dynamics could express with the following equations (Table 1). Table 1 shows that phenanthrene half-life in planted ryegrass treatment was significantly shorter than that of unplanted ryegrass treatments; it indicated that planted ryegrass enhanced phenanthrene degradation in the soil.

Table 2 showed the difference of phenanthrene concentrations between unplanted soil and planted with ryegrass soil, and it reflected the enhanced degradation of phenanthrene by ryegrass. There was no significant difference in soil phenanthrene concentration between low and intermediate levels concentration phenanthrene treatment, but the effect of plant in high concentration phenanthrene was significant, and this indicated that high concentration of phenanthrene perhaps resulted in toxicity to plant growth, but phytoremediation of PAHs contaminated soil was more suitable for high level phenanthrene contamination.

These results (Figure 1, Table 2) indicated that ryegrass significantly enhanced phenanthrene degradation in the soil, and the degradation rates in medium and low concentration phenanthrene treatments were significantly higher than at the high phenanthrene concentration, it can be said that the endurance of ryegrass was greatest under the highest phenanthrene application rate.

The result was similar to the previous results. Previous experiments with PAHs such as anthracene and pyrene (Günther et al. 1996, Reilley et al. 1996, Ling and Gao 2004, Kirk et al. 2005) showed a very rapid dissipation of these compounds in the rhizosphere of several plants in the early stages (40 days) followed by slow rates. These authors also reported that degradation of pyrene was much faster in rhizosphere soil than in bulk soil. Binet et al. (2000) studied the fate of eight PAHs (3–6 rings) in the rhizosphere, and showed that ryegrass was able to accelerate the

Table 1. Phenanthrene degradation kinetics parameters in different treatments

Amount of phenanthrene (mg/kg)		Degradation kinetics equations	Correlation coefficient (r)	Half-life/(days)
5	planted	$C = 3.475\ e^{-0.0185t}$	0.938 3	17.8
5	non planted	$C = 3.402\ e^{-0.0153t}$	0.911 6	20.9
50	planted	$C = 29.215\ e^{-0.0292t}$	0.923 4	5.3
50	non planted	$C = 31.689\ e^{-0.0251t}$	0.923 1	9.5
200	planted	$C = 131.06\ e^{-0.0226t}$	0.922 8	12.0
200	non planted	$C = 173.56\ e^{-0.0145t}$	0.976 2	38.0

Planted – the soil planted with ryegrass; non planted – the soil planted without ryegrass

dissipation of a range of PAHs, including 5 and 6 ring PAHs such as dibenzo (a, h) anthracene and benzo (g, h, i) perylene which have a low solubility and bioavailability.

Soil microbial biomass C. Figure 3A indicated that there was no significant difference in microbial biomass carbon among the treatments within the first 45 days of the experiment. However, at later stages, the microbial biomass carbon in 50 mg/kg phenanthrene treatment soil was increased faster than other treatments, and followed by 5 mg/kg, CK, and 200 mg/kg. In general, the microbial biomass carbon of 50 mg/kg phenanthrene treatment soil was significantly higher than that of 5 mg/kg treatment, and both treatments were significantly higher than that of CK and 200 mg/kg treatments on the 60th day. There was no significant difference between 5 mg/kg and 50 mg/kg treatment, but both treatments were significantly higher than that of CK and 200 mg/kg at day 70th. This indicated that low and intermediate levels of phenanthrene addition stimulated the soil microorganism activity rapidly to degrade phenanthrene, while high level phenanthrene added soil could produce toxicity on microorganism, and restrained the soil microorganism growth, so the degradation rate was lower.

Polyphenol oxidase activity in soil. Plant roots can enhance the dissipation of organic pollutants in soils, and this is mainly attributed to supply of root exudates for co-metabolic processes (Yoshitomi and Shann 2001). When plant roots are stressed, e.g. in PAH-contaminated soil, the plants can exude certain enzymes to degrade or transform the pollutants (Gramss 1997). Studies revealed a range of phenol oxidizing enzyme activities, including tyrosinase (EC 1.14.18.1), catechol oxidase (EC 1.10.3.1), ascorbate oxidase (EC 1.10.3.3) and laccase (EC 1.10.3.2) associated with plant root system in soils with organic pollutants (Colpaert and Van Laere 1996, Liste and Prutz 2006).

Changes of enzyme activity in soil reflect the degradation activity of soil micro-organisms and plant roots (Wyszkowski and Wyszkowska 2005). Polyphenol oxidase is an important oxidoreductase in soils and can catalyse the degradation and transformation processes of aromatic compounds (Andrew and John 2000, Miya and Friestone 2001). The difference of polyphenol oxidase between planted with ryegrass soil and unplanted ryegrass soil indicated that ryegrass root system affects intensity of soil polyphenol oxidase activities. Figure 3B shows that there was no significant difference in enzyme activity among the treatments within the first 30 days of the experiment, it indicated that there was no significant effect on soil polyphenol oxidase activities when added phenthrene into soil

Table 2. Difference of soil phenanthrene concentration between treatments with or without ryegrass growth

Amount of phenanthrene added (mg/kg)	Days after addition					
	0	15	30	45	60	75
5	0	0.073	0.118	0.114	0.206	0.257
50	0	3.82	1.72	2.37	2.15	1.76
200	0	54.6	60.9	40.7	40.6	34.0

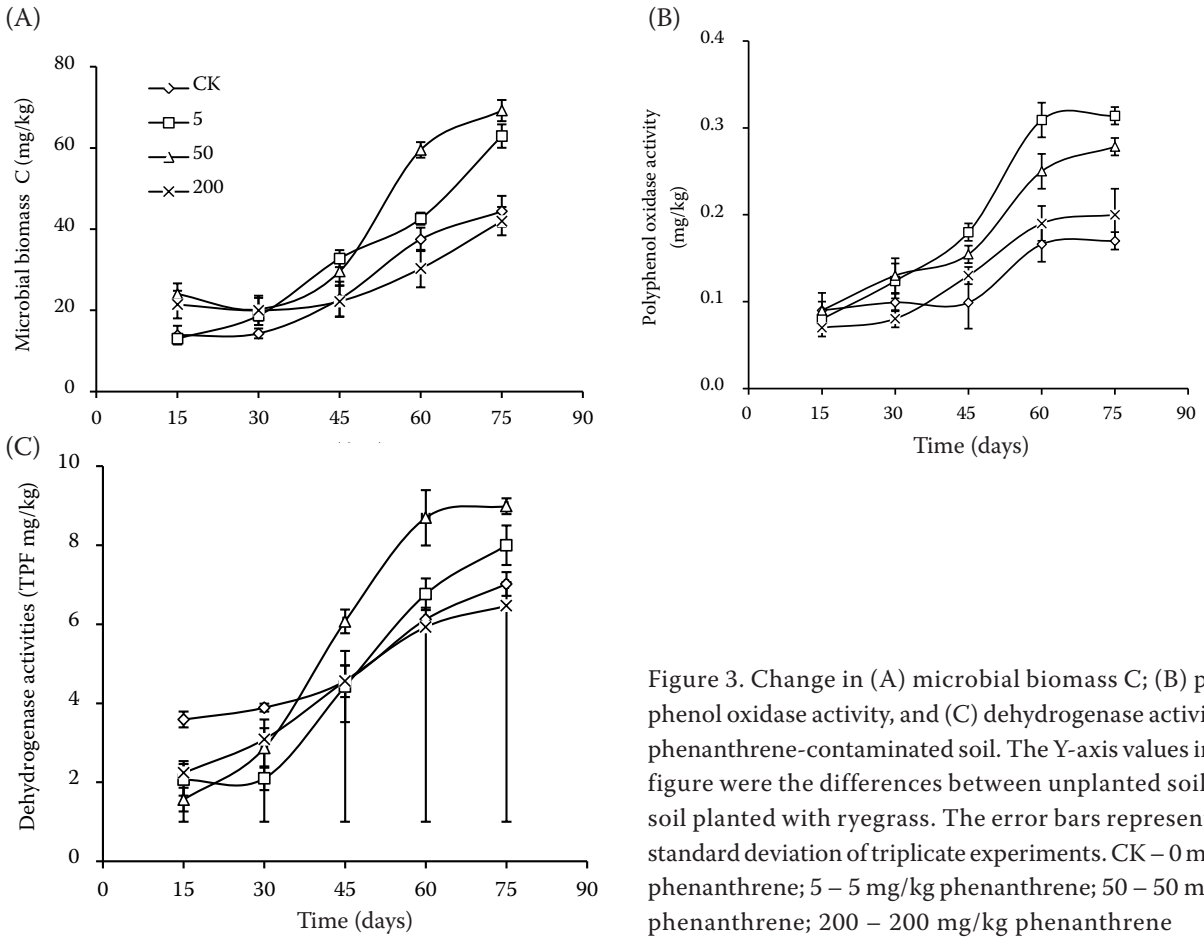

Figure 3. Change in (A) microbial biomass C; (B) polyphenol oxidase activity, and (C) dehydrogenase activity in phenanthrene-contaminated soil. The Y-axis values in the figure were the differences between unplanted soil and soil planted with ryegrass. The error bars represent the standard deviation of triplicate experiments. CK – 0 mg/kg phenanthrene; 5 – 5 mg/kg phenanthrene; 50 – 50 mg/kg phenanthrene; 200 – 200 mg/kg phenanthrene

during ryegrass growth prophase. However, there was a significant effect on soil polyphenol oxidase activities at later stages (45 days ~ 75 days). In general, the polyphenol oxidase activity of 50 mg/kg phenanthrene treatment soil was significantly higher than that of 5 mg/kg treatment, and in both the treatments it was significantly higher than that of 200 mg/kg treatment. This indicated that when low and intermediate levels of phenanthrene added soil, stimulated the plant and soil microorganism rapidly growth and excreted lots of polyphenol oxidase, while higher level phenanthrene (200 mg/kg) added soil could produce toxicity on microorganism, and restrained the soil polyphenol oxidase activities.

Dehydrogenase activity in soil. Figure 3C showed that there was no significant difference in dehydrogenase activity among the treatments during the first 30 days of plant growth. After 45 days, the dehydrogenase activity of 50 mg/kg phenanthrene treatment soil was significantly higher than other 3 treatments ($P < 0.05$). After 75 days the enzyme activity at the 50 mg/kg phenanthrene application rate was significantly higher than the 5 mg/kg phenanthrene application rate of phenanthrene, and the dehydrogenase activity at the 5 mg/kg phenanthrene application rate of phenanthrene was also significantly higher than the highest rate of phenanthrene and CK ($P < 0.05$). The reason was the same as the polyphenol oxidase activities in soil and also indicated why the degradation rate in the highest phenanthrene concentration was lowest.

These results (Figure 3) indicated that ryegrass plantation enhanced activities of polyphenol oxidase, dehydrogenase, and increased content of microbiological biomass C, that is, the soil biological activity, thus raising the degradation rate of phenanthrene in the soil. And soil biological activity varied sharply from treatment to treatment. Higher concentration of phenanthrene inhibited soil biological activity, and in turn the effect of soil biology on phenanthrene degradation. Therefore, the findings disclosed the biological and enzymological mechanisms of the plant enhancing phenanthrene degradation.

REFERENCES

Andrew A.M., John W.G. (2000): Ectomycorrhizas – extending the capabilities of rhizosphere remediation. Soil Biology and Biochemistry, *32*: 1475–1484.

Aprill W., Sims R.C. (1990): Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere, *20*: 253–265.

Binet P., Portal J.M., Leyal C. (2000): Dissipation of 3-6-ring polycyclic aromatic hydrocarbon in the rhizosphere of ryegrass. Soil Biology and Biochemistry, *32*: 2011–2017.

Casida L.C., Klein D.A.Jr., Santoro T. (1964): Soil dehydrogenase activity. Soil Science, *98*: 371–376.

Chen Y., Banks M.K., Schwab A.P. (2003): Pyrene degradation in the rhizosphere of tall fescue (*Festuca arundinacea*) and switchgrass (*Panicum virgatum* L.). Environmental Science and Technology, *37*: 5778–5782.

Colpaert J.V., Van Laere A. (1996): A comparison of the extracellular enzyme activities of two ectomycorrhizal and leaf-saprotrophic basidiomycete colonizing beech leaf litter. New Phytologist, *134*: 133–141.

Dick R.P., Myrold D.D., Kerte E.A. (1988): Soil polyphenol oxidase activity and its standard measure methods. Soil Science Society of America Journal, *52*: 512–516.

Dominiguez-Rosado E., Pichtel J. (2004): Phytoremediation of soil contaminated with used motor oil: II. Greenhouse studies. Environmental Engineering Science, *21*: 169–180.

Fu D., Teng Y., Luo Y., Tu C., Li S., Li Z., Christie P. (2012): Effects of alfalfa and organic fertilizer on benzo[a]pyrene dissipation in an aged contaminated soil. Environmental Science Pollution Research International, *19*: 1605–1611.

Gao Y.Z., Zhu L.Z. (2004): Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere, *55*: 1169–1178.

Gao Y.Z., Zhu L.Z. (2005): Phytoremediation for phenanthrene and pyrene contaminated soils. Journal of Environmental Science, *16*: 14–18.

Gao Y.Z., Ling W.T., Wong M.H. (2006): Plant-accelerated dissipation of phenanthrene and pyrene from water in the presence of a nonionic-surfactant. Chemosphere, *63*: 1560–1567.

Gramss G. (1997): Activity of oxidative enzymes in fungal mycelia from grassland and forest soils. Journal Basical Microbiology, *37*: 407–423.

Günther T., Dornberger U., Fritsche W. (1996): Effect of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere, *33*: 203–215.

Huang X.D., Alawi E.Y., Penrose D.M., Glick B.R., Greenberg B.M. (2004): A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution, *130*: 465–476.

Jensen L.K., Honkanen J.O., Jæger I., Carroll J. (2012): Bioaccumulation of phenanthrene and benzo[a]pyrene in *Calanus finmarchicus*. Ecotoxicology and Environmental Safety, *78*: 225–231.

Kirk J.L., Klironomos J.N., Lee H., Trevors J.T. (2005): The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environmental Pollution, *133*: 455–465.

Ling W.T., Gao Y.Z. (2004): Promoted dissipation of phenanthrene and pyrene in soils by amaranth (*Amaranthus tricolor* L.). Environmental Geochemistry and Health, *46*: 553–560.

Liste H., Alexander M. (2000): Plant-promoted pyrene degradation in soil. Chemosphere, *40*: 7–15.

Liste H.H., Prutz I. (2006): Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere, *62*: 1411–1420.

McCarthy J.F., Jimenez B.D. (1985): Interactions between polycyclic aromatic hydrocarbons and dissolved humic materials: Binding and dissociation. Environmental Science and Technology, *19*: 1072–1076.

Miya R.K., Friestone M.K. (2001): Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environmental Quality, *30*: 1911–1918.

Mueller K.E., Shann J.R. (2006): PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees. Chemosphere, *64*: 1006–1014.

Reilley K.A., Banks M.K., Schwab A.P. (1996): Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbon in the rhizosphere. Journal of Environmental Quality, *25*: 212–219.

Song Y.F., Ou Z.Q., Sun T.H., Yediler A., Lorinci G., Kettrup A. (1995): Analytical method of polycyclic aromatic hydrocarbons in soil and plant samples. Chinese Journal of Applied Ecology, *6*: 92–96. (In Chinese)

Vance E.D., Brookes P.C., Jenkinson D.S. (1987): An extraction method for measuring soil microbial biomass-C. Soil Biology and Biochemistry, *19*: 703–707.

Vácha R., Čechmánková J., Skála J. (2010): Polycyclic aromatic hydrocarbons in soil and selected plants. Plant, Soil and Environment, *56*: 434–443.

Vácha R., Horváthová V., Vysloužilová M. (2005): The application of sludge on agriculturally used soils and the problem of persistent organic pollutants. Plant, Soil and Environment, *51*: 12–18.

Wyszkowski M., Wyszkowska J. (2005): Effect of enzymatic activity of diesel oil contaminated soil on the chemical composition of oat (*Avena sativa* L.) and maize (*Zea mays* L.). Plant, Soil and Environment, *51*: 360–367.

Xu S.Y., Chen Y.X., Lin Q., Wang W.X., Xie S.G., Shen C.F. (2005): Uptake and accumulation of phenanthrene and pyrene in spiked soils by ryegrass (*Lolium perenne* L). Journal of Environmental Science, *17*: 817–822.

Xu S.Y., Chen Y.X., Lin K.F. (2009): Removal of pyrene from contaminated soils by white clover. Pedosphere, *19:* 265–272.

Yoshitomi K.J., Shann J.R. (2001): Corn (*Zea mays* L) root exudates and their impact on ^{14}C-pyrene mineralization. Soil Biology and Biochemistry, *33*: 1769–1776.

Received on January 27, 2013
Accepted on April 19, 2013

Corresponding author:

Dr. Shi-Liang Liu, Henan Agricultural University, College of Resources and Environment, Zhengzhou, P.R. China
phone: + 86 371 6355 5504, fax: + 86 371 6355 5504, e-mail: shlliu70@163.com