[1]Effect of Phenanthrene（PHE）on Soil Enzyme Activity

Xuan Gong, Xi Chen

School of Resources and Civil Engineering, Northeastern University, Shenyang, China，110004

gongxuan@mail.neu.edu.cn

Abstract: The experiment is conducted to study the relationship between PHE and enzyme activity in the soil by studying the changes of soil enzyme activity when it is exposed to the contaminant, in which PAHs is selected as contaminant and sensitive soil enzyme is selected as eco-toxicological index for soil diagnoses of PAHs contamination. The research shows that when the additive PHE concentration >100μgKg-1, the urease activity is inhibited in the following 3 days, the dehydrogenase activity is inhibited while the phosphatase activity is activated in the following 7 days. When the concentration is in the range from 100 to 2400μgKg-1, no obvious changes of catalase activity are observed. It can be concluded that soil urease activity, dehydrogenase activity, and phosphatase activity maybe considered as diagnoses index for soil diagnoses of PHE contamination. And the observation from 1st to 7th days is the most important after adding PHE.

Keywords: PHE; soil enzyme activity; eco-toxicological index; inhibit; activate

There are many enzymes in the soil; they mainly come from microorganisms in the soil. The soil enzymes play an important role in carbon, nitrogen and phosphorus cycle of the soil, and they participate in many important metabolic processes, namely soil fertility and development, the formation and transformation of nutrients, organic decomposition, degradation etc. The soil enzyme activity reflects strengths and directions of different kinds of biochemical process existing in the soil. The soil enzyme activity is vulnerable to the physical, chemical and biological environment; the activity varies greatly under the condition of the pollution, so the soil enzyme activity is considered as a eco-toxicology index to judge the soil pollution degree and the ecological environment by many scholars caused by foreign substances. The research from YangZhixin shows: the soil enzymes activity inhibition effect caused by heavy metal is Cd>Zn>Pb, and urease is sensitive to heavy metal, and the inhibition is temporary，After a period of time the enzyme activity can be restore to a certain degree, the recovery time correlates to the soil enzyme inhibition intensity. Cd, Pb, Zn coexistence performs a negative effect of cooperative inhibition on urease, while shows a certain antagonism on catalase, but the effect on invertase and ALP decreases when the Cd concentration increases[1], LiuShuqing(1996) think the correlations between urease and catalase activity and Pb and Cd are the best conclusion[2]. So it is feasible to use urease and catalase activity as the main biochemical index for the pollution degree of the soil polluted by Pb and Cd. But the effects of PAHs on soil enzymes activity doesn't have many reports, adding to Carcinogenic, teratogenic and agenicity are paid more and more attentions, so here selects PHE –the representative of PAHs as the pollutant, by observing the changes of the soil enzyme activity in the pollutants, invests the relationship between PAHs and the soil enzyme activity and provides a theoretical basis for selecting soil enzyme activity as eco-toxicology index.

1. Materials and methods

1.1. Materials

1.1.1. Tested soils

The soils are black, which are from Helen county of heilongjiang province. After drying and grinding, the soils are set aside after sieving by 2mm griddle. The essential chemical property of the soils are in the table 1.

Table 1 the essential chemical property of the soils

Soil type	organic matter /g.kg^{-1}	Total N g.kg^{-1}	Total P g.kg^{-1}	Total K g.kg^{-1}	Available N mg.kg^{-1}	Available P mg.kg^{-1}	Available K mg.kg^{-1}	PH
black soil	40.70	2.11	3.99	18.97	277.72	17.55	123.09	6.52

1.1.2. Lab Sulphonic Acid

PHE, the product of the Fluka Company, the purity is more than 90%.

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1.2. Methods

1.2.1. Pre-culture

Take 1 Kg dry soil sample into porcelain basin (9.5 cm in diameter, high 10.5 cm), to which add distilled

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water till soil field is to its capacity, and then number, weigh the total quality of each porcelain basin and soil sample, add moisture loss according to the records of daily weighing, pre-cultivate it for a week.

1.2.2. Experiment design

The experiments have 5 treatments, each treatment has 3 replications. And the treatments having added the pollutants are took samples on 1st, 3rd, 7th, 14th and 28th day, then measure the activity of the urease, dehydrogenase, catalase and phosphatase in the soil. The experiment design is in the table2.

Table 2 experiment design

Code	PHE1	PHE2	PHE3	PHE4	PHE5
additive PHE concentration（$\mu g \cdot Kg^{-1}$）	100	300	600	1200	2400

1.3. Measuring methods

The measuring methods of the urease, dehydrogenase, catalase and phosphatase are Urea residual method [3], TTC method [4], titration [5], and colorimetric method.

1.4. Data processing

The Data processing, multiple comparisons are carried by SPSS11.0 software [6]。

2. Results and discussions

2.1. Multiple comparisons

The multiple comparisons of the experiment are carried by One-way ANOVA process of the SPSS11.0 software; the multiple methods take the control as a comparison, comparing the treatment above with the control, and observe the differences of the soil enzyme activity between the processed and the compared treatments. The results are in the table 3 in detail.

Table 3 the differences between the processed and the control

code	urease					dehydrogenase					catalase					phosphatase				
	1	3	7	14	28	1	3	7	14	28	1	3	7	14	28	1	3	7	14	28
PHE1	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-
PHE2	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-
PHE3	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-
PHE4	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-
PHE5	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-

Note: +says the differences of the soil enzyme activity between the processed treatment and the blank one are obvious;- says the differences is not significant.

2.2. Experiment results

2.2.1. The effect of PHE on the soil urease activity

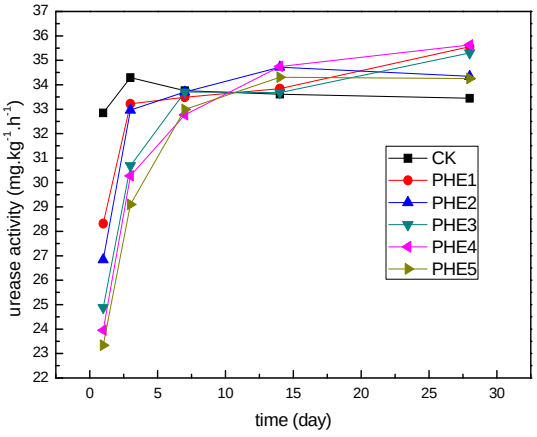

Fig.1 Effect of PHE on urease activity

The figure1 shows that urease activity of the 5 processed ones is lower than the control on the 1st day after adding PHE，when the concentration of the PHE is 100μg.Kg^{-1}, urease activity decreases by 14%,also the inhibiting effect increases with the increase of the quantity of additive PHE concentration, namely the inhibiting effect is PHE5>PHE4>PHE3>PHE2>PHE1. The urease activity of the PHE1 and PHE2 doesn't differ much from the control, the activity of PHE3 decreases by 11% than the control on the 3rd day, which is the same to what on the 1st day that the inhibiting effect increases with the increase of the additive quantity of PHE. The effect of PHE on soil enzyme activity is short because of the low quantity of PHE in PHE1 and PHE2. The difference between each treatment and the control is not obvious on the 7th day after adding PHE, which says the urease activity is recovered. According to the table 3 and figure 1 it says that each processed urease activity has significant changes on the 1st day after adding PHE, which says it is

the most important to observe the changes of soil enzyme activity when it is regarded as eco-toxicology index of the soil contaminated by PHE.

2.2.2. Effect of PHE on dehydrogenase activity

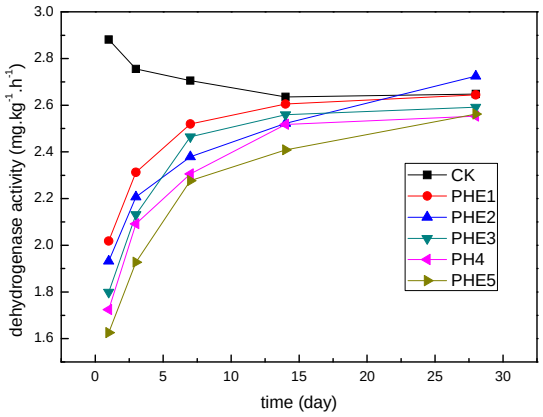

Fig.2 Effect of PHE on dehydrogenase activity

The result of the figure 2 indicates, the dehydrogenase activity decreases by 30% on the 1st day after adding dehydrogenase by comparing PHE1 to the control, the decreased degree of the dehydrogenase activity is PHE5>PHE4>PHE3>PHE2>PHE1. The activity of each treatment is lower than the control except the PHE2 on the 28th day, which says the dehydrogenase activity is inhibited after adding PHE. Because dehydrogenase activity is linked to the kinds and the quantity of microorganisms [7], while the kinds and quantity change after adding PHE, the dehydrogenase activity in inhibited because of the addition of PHE.

2.2.3. Effect of PHE on catalase activity

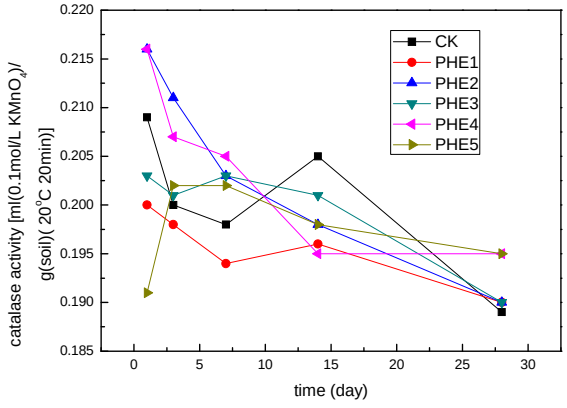

Fig.3 Effect of PHE on catalase activity

The fig.3 and the table 3 say there are not obvious differences between each treatment and the control. It says catalase activity doesn't affect after adding PHE in the experiment condition. Catalase is regarded as the aerobic microorganisms instructions (this enzyme doesn't exist in anaerobic microbe), also catalase activity links to the quantity of aerobic microorganisms and the soil fertility [8]. Therefore the effect of PHE on aerobic microorganisms can be thought subtle and so catalase activity doesn't change much.

2.2.4. Effect of PHE on phosphatase activity

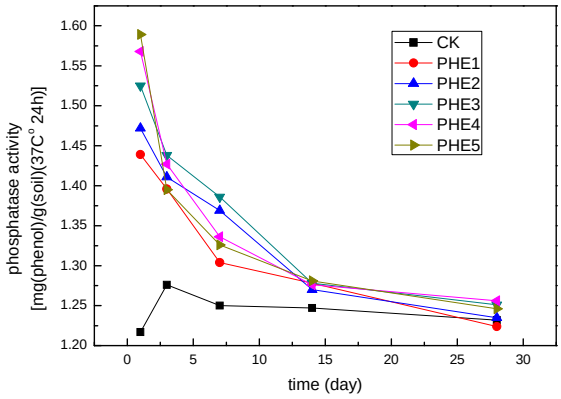

Fig.4 Effect of PHE on phosphatase activity

The fig.4 above says phosphatase activity increases by 18% by comparing PHE1 with the control and PHE5 increases by 31% on the 1st day after adding PHE, other treatments increase too, which shows PHE has a activation on phosphatase activity in the soil, the range of the activation is PHE5>PHE4> PHE3>PHE2>PHE. With the passage of time, each processed phosphatase activity gradually lowers, and activation gradually disappears. The catalase activity of the 5 processed doesn't differ much with the control. According to table 3 and Figure 4, it can be made the conclusion that the addition of phosphatase has certain activation on phosphatase activity and this activation increases with the increase of concentration added, which says soil phosphatase activity is sensitive to PHE, and phosphatase activity can also be regarded as eco-toxicological index of the soil contaminated by PHE.

3. Conclusions

(1)The effect of PHE on the soil enzymes activity is different: the activity of urease and dehydrogenase is inhibited by the addition of PHE, while phosphatase activity is activated; the effect of PHE on catalase activity is little.

(2) Some scholars show the extension of the higher plants is inhibited when the additive PHE concentration is ppm orders of magnitude in carrying the contaminated soil ecological toxicity experiments [9]. The result shows that when the concentration is 100μg.Kg^{-1}(100ppm orders of magnitude), soil urease, dehydrogenase and phosphatase activity changes much, which says the soil urease, dehydrogenase and phosphatase activity has a sensitive reaction to PHE, namely soil urease, dehydrogenase and phosphatase activity may be considered as

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diagnoses index of soil contaminated by PHE.

(3) The effects of PHE on soil urease, dehydrogenase and phosphatase activity differ from time to time: the inhibiting effect of PHE on soil urease activity is within 7 days, the inhibiting effect on oil dehydrogenase activity is within 14 days, and the activating effect of PHE on soil phosphatase activity is within 14 days.

(4) The activity of the soil urease, dehydrogenase and phosphatase has the most remarkable difference between the processed and the control on the 1st day after adding PHE, which says it is the most important to observe the changes within the 1st day after adding PHE by using soil urease, dehydrogenase and phosphatase activity as diagnoses index of soil contaminated by PHE.

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