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Ecological detoxification of methamidophos by earthworms in phaiozem co-contaminated with acetochlor and copper

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ABSTRACT

In view of the ubiquitous co-existence of methamidophos, acetochlor and copper (Cu) in agricultural soils, ecological detoxification of methamidophos in phaiozem by earthworms was examined using the detoxic incubation experiments with illumination. It was validated that the earthworm *Eisenia fetida* is a useful soil animal in the process of methamidophos detoxification as the assistance of soil microbes and enzymes. Due to the action of earthworms, the half life of methamidophos with concentration of 15 mg/kg in phaiozem could decrease from 5.61 days to 5.08 days. Dynamics of methamidophos detoxification by earthworms could conform to the logistic model. Under the condition of multiple pollution combined with acetochlor (20 mg/kg) and Cu (300 mg/kg), ecological detoxification of methamidophos by earthworms became complicated. Acetochlor played a promoting role in the biodegradation of methamidophos to some extent, while it was basically inhibited by Cu.

1. Introduction

As a typical organophosphorus insecticide, methamidophos has been widely applied to agricultural production in China, USA, the Netherlands and other countries in the world in view of its high effectiveness in killing various pests (Lin et al., 2000; Wu and Miyata, 2005; de Castro and Chiorato, 2007). Even in 1990, the amount of methamidophos applied to agricultural fields was up to 3.5×10^4 t (Lin et al., 2000), ranking the first in the application of insecticides in China. The normal dosage of methamidophos per hectare ranges from 187.5 to 900.0 g as the purified compound. Due to its effectiveness in killing various pests, it may be applied frequently according to the occurrence of pests in an agricultural field and other areas. And spraying is the normal method of application in fields. Because of its relatively short half-life, readily degradable property by chemical, biological, biochemical and ecological processes and high mobility in soils, methamidophos residues in soils have been seldom investigated (Hung et al., 2002; Athanasopoulos et al., 2005; Yen et al., 2000). However, with the increasing dosage of methamidophos applied and continual input to agricultural soils (Sun and Zhang, 2002), the amount of methamidophos residues in agricultural soils has even exceeded the self-purifying capacity of soil ecosystems (Liao et al., 2003; Zhou et al., 2004; García-de la Parra et al., 2006; Zhou and Wang, 2006). As a result, methamidophos has been detected in high concentrations in plants such as vegetables and crops, and thus leading to some direct and potential adverse effects on ecosystem health and environmental safety (de Castro et al., 2000; Spassova et al., 2000; Karlen et al., 2003;

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Battershill et al., 2004; Li et al., 2005). In other words, more and more attention has been paid to the ''pseudo-persistence'' of methamidophos in ecosystems in despite of its relatively short half-life.

According to our previous investigation, methamidophos is also one of the most frequently applied pesticides in Chinese coastal areas with a high economy-increasing rate, particularly in the phaiozem area of northeast China (Yu and Zhou, 2003; Zhou et al., 2004). With frequent application of the pesticide in agricultural production, there is a continual accumulation of methamidophos in soil environment (Yu and Zhou, 2005; Yu et al., 2005); although various environmental factors such as soil components, solution pH, and cationic exchange (CEC) had some influences on methamidophos sorption in soils, with the increase of mineral contents, organic matter, pH and CEC in soils the sorption of methamidophos increasing accordingly (Koleli et al., 2007). At the same time, some herbicides such as acethochlor and fungicides containing metals such as copper (Cu) are also applied widely in order to achieve high cropping rates (Yu and Zhou, 2003; Zhang et al., 2003). In addition, many large thermal power plants in the same area have been built in order to produce sufficient electric and heat energy for winter, and as a result, the fly ash containing Cu from coal combustion in the power plants becomes a point source of Cu pollution when waste material is finally deposited in the soil (Zhou et al., 2004; Klose and Makeschin, 2005; Wang and Zhou, 2005).

Besides the non-point pollution damage caused by the increasing use of methamidophos due to its mobile characteristics in soil environment, methamidophos also may affect the soil microbe directly. Su et al. (2007) examined toxic effects of methamidophos in agricultural phaiozem on *nifH* gene, namely the nitrogenase iron protein gene, which is one of the oldest functional genes in the history of gene evolution served in nitrogen fixation of bacteria using the denaturing gradient gel electrophoresis (DGGE) and the sequencing approaches in a microcosm experiment. It was showed that the medium tested concentration (150 mg/kg) of methamidophos caused the most apparent changes in *nifH* gene diversity at the first week, while the high concentration (250 mg/kg) of methamidophos produced prominent effects on *nifH* gene diversity in the following weeks, and joint toxic effects of methamidophos and acetochlor on *nifH* gene were also apparent.

On the other hand, the self-purification and detoxification function of soil ecosystems is increasingly degraded with unceasing application of chemical pesticides and the multiple pollution by more than one pollutant (Simonich and Hites, 1995; Zhou, 1995; Zhou, 2003; Wang and Zhou, 2005; Zhou, 2006), especially with the application of methamidophos, because the biological toxicity of the pesticide is very strong (Zhou et al., 2004) and the desorption of the pesticide from soils is very rapid and its bioavailability is also high (Yu, 2004; Worek et al., 2007). Although people know in theory that the self-purification ability of soil ecosystems are mainly related to the activity of microorganisms as the main force decomposing organic pollutants (Awasthi et al., 2000; Zhang et al., 2003; Zhou et al., 2004), whether earthworms themselves and enzymes from the activity of earthworms in soils can degrade methamidophos, contribute to the degradation of methamidophos and promote the decomposing function of microorganisms or not is still vague, especially under the condition of multiple pollution combined with other chemicals (Zhou, 1995; Zhou and Wang, 2006). Thus, the research on earthworm detoxification of methamidophos in phaiozem co-contaminated with other chemicals including acetochlor and Cu is of practical and scientific significance, and will provide a useful basis for strengthening methamidophos detoxification in complicated environments where the multiple pollution takes place (Zhou, 1995; Zhou and Wang, 2005).

2. Materials and methods

2.1. Soil sampling

Samples of the tested soil, phaiozem (black soil), as a typical zonal soil in northeast China, were collected from a fallow field (47°26′N, 126°38′E) in the Hailun Station of Agro-ecological Experimentation, Chinese Academy of Sciences, which is situated at Hailun County, Heilongjiang Province, China. No agricultural chemicals have been used in the past decades at the sampling site, which is located in the continental temperate monsoon zone, with a dry and cold winter and a warm and wet summer. Annual mean temperature is about 1.5 °C. Annual precipitation averages range between 500 and 600 mm, of which 70% occurs between May and September. The length of the annual frostless period is only around 30 days (Yu and Zhou, 2003; Zhang et al., 2003). Basic physical and chemical properties of the tested soil are listed in Table 1.

2.2. Chemicals and reagents used

Commercial formulations of methamidophos (O, S-dimethyl phosphoroamido thiolate, $CH_3SCH_3OPONH_2$), 40% of miscible oil reagent (pH 6.37), and acetochlor ($C_{14}H_{20}ClNO_2$), 50% of miscible oil reagent, were used as the tested agrochemicals. And $CuSO_4{\cdot}5H_2O$ of analytic grade was used as the added heavy metal. Besides, all other reagents used in this work were at analytic grade.

2.3. Detoxic experiments

The tested concentrations (Table 2) of methamidophos, acetochlor and Cu in phaiozem were set according to our

Table 1 – Basic physical and chemical properties of the tested soil

Sampling depth (cm)	0–20
Soil pH[a]	6.58
OM (g kg^{-1})	37.83
Total N (g kg^{-1})	2.56
Total P (g kg^{-1})	0.61
Total K (g kg^{-1})	26.00
Particle size (%)	
Sand	33.8
Silt	39.6
Clay	26.6

[a] Soil pH was determined on the basis of pH-H_2O.

Table 2 – Tested concentrations of pollutants in various treatments

Treatment	Concentration (mg kg^{-1})			Activity of earthworms
	Methamidophos	Cu	Acetochlor	
Natural	15			No
Earthworm	15			Yes
Copper	15	300		No
Earthworm + Cu	15	300		Yes
Acetochlor	15		20	No
Earthworm + Ace	15		20	Yes

previous study (Yu and Zhou, 2003) and different treatments were devised on the basis of statistical principles. Before the detoxic experiment, healthy and mature earthworms (*Eisenia fetida*) with a similar size and the weight ranging from 250 to 400 mg after a 3-month growth were pre-incubated in the control soil for 1 week.

According to Table 2, one or two pollutant(s) with the designed concentration(s) were mixed in a pot with 500 g of air-dried soil which had been passed through a 1-mm sieve. After having regulated the soil water potential at −33 kPa, 20 earthworms (*E. fetida*) were put to the soil samples. All the treatments were incubated for 18 days in a biochemical incubator (LRH-250-A type, made in Guangzhou, China) in dark with a constant temperature of 20 ± 1 °C. Every day, a little deionized water was sprinkled on the soil surface in order to maintain the soil moisture. Every 3 days, about 30.0 g incubated soil was sampled and analyzed for the presence of methamidophos. Each treatment was replicated three times.

2.4. Extraction and determination

The method of extracting and determining methamidophos after the modification by Yu (2004) on the basis of the suggestion by Shi et al. (2001) was adopted. After the modification, the extraction rate of methamidophos was effectively increased and higher than the method by Shi et al. (2001), in particular, the recovery rate of methamidophos was up to 87.3–97.1%. The main procedures of the modified method were described as follows.

At first, 20.0 g of air-dried soil samples and 4.0 g of NaCl were added to a triangle flask with 50 ml of water. Having been shaken for 1.0 h and centrifuged for 5.0 min at 5000 r/min, the extract solution was removed. Then another 30 ml of water were added to the centrifuged soil samples. After shaken and centrifuged as the first time, the extracted solutions at the separated times were collected together into a funnels. The aqueous extract was saturated with ammonium sulfate, and further extracted for 15 min using 50 ml of ethyl acetate, 30 ml of a mixture of ethyl acetate and methanol (5:1, v/v), and 20 ml of ethyl acetate. The organic extracts were passed through sodium sulfate without water and brought to a volume of 100 ml for the determination of methamidophos using the gas chromatogram method.

Determination conditions of the gas chromatography (GC): (1) chromatogram pole: capillary pole (30 cm of length and 0.32 mm of inner radius) with HP-210, 19091C-613 (50% trifluoropropyl ME siloxame; (2) temperature: 220 °C at the pole, 130 °C at the sample-injecting hole, 240 °C at the nitrogen phosphorus detector (NPD); (3) velocity of gas flow: 30 ml (nitrogen)/min, 3 ml (hydrogen)/min, and 60 ml (air)/min; (4) volume of injected sample solutions (tested solution and standard solution), 1.0 μl; (5) reservation time, 2.506 min. Samples were quantified using the surface method. The peak area of samples determined was converted into mass corresponding to a standard curve. Thus, the concentration of methamidophos in soils can be calculated according to following formula:

$$\mathrm{Ys} = \frac{M' \times V'}{\text{Sample mass (kg)} \times \text{volume (ml) of sample injected}} \quad (1)$$

where Ys is the concentration (mg/kg) of methamidophos in soils, M' the mass (mg) corresponding to the peak area, and V' is the fixed volume (ml) of the final extracted material.

2.5. Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA) for factors of pollutant concentrations and sampling intervals. Multiple comparisons of significant differences in degradation rate of methamidophos among different treatments as well as sampling intervals were made using the least significant difference (LSD) test ($p < 0.05$). The proposal system software (DPS, v3.01 professional) was used to perform the ANOVA and multiple comparisons. Due to the fact that the degradation of methamidophos in soils is usually related to the activity of microbes and can be described with first order kinetics, the degradation kinetics of methamidophos can be fitted to different models (linear, exponential and logistic) using the DPS software. Then the half life values of methamidophos were calculated using the optimal model.

3. Results

3.1. Analysis of variance (ANOVA) and selection of degradation kinetics models

ANOVA revealed that there were markedly significant ($p = 0.004$) differences in methamidophos degradation rate among treatments with added Cu or acetochlor in the presence or absence of earthworms. And the differences among the six sampling intervals were also markedly significant ($p = 0.0001$). It was revealed by fitting degradation kinetics of methamidophos to linear, exponential and logistic models that the logistic model was the most optimal in fitting the degradation kinetics of methamidophos in phaiozem,

Table 3 – Multiple comparisons of significant differences in the degradation rate of methamidophos among different treatments added copper or acetochlor in the presence or absence of earthworms using the LSD test ($p < 0.05$)

Time (days)	Natural (%)	Earthworm (%)	Cu (%)	Earthworm + Cu (%)	Acetochlor (%)	Earthworm + Acetochlor (%)
3	39.1 ± 0.85 a	35.7 ± 5.1 ab	35.9 ± 5.6 ab	26.8 ± 0.8 b	31.5 ± 2.1 ab	32.5 ± 1.7 ab
6	45.6 ± 9.1 c	52.5 ± 8.3 c	65.6 ± 0.6 b	68.4 ± 0.8 ab	75.7 ± 0.6 ab	77.2 ± 1.2 a
9	70.7 ± 1.1 bc	83.1 ± 0.7 a	63.3 ± 2.7 c	71.5 ± 1.5 bc	74.2 ± 0.1 abc	77.1 ± 0.2 ab
12	86.1 ± 0.6 a	96.3 ± 0.0 a	85.9 ± 1.3 a	87.3 ± 0.7 a	91.5 ± 2.6 a	94.9 ± 0.2 a
15	98.0 ± 0.1 a	98.5 ± 0.1 a	93.3 ± 0.0 a	91.2 ± 0.4 a	97.7 ± 0.1 a	98.1 ± 0.0 a
18	91.3 ± 0.3 ab	95.9 ± 0.1 a	83.6 ± 2.2 b	86.1 ± 0.6 ab	90.6 ± 0.0 ab	87.9 ± 0.1 ab

Data presented in mean ± S.D.

although the previous study showed that the degradation of methamidophos in phaiozem under natural conditions could conform to a dynamical process expressed using the quadratic equations with one unknown quantity.

3.2. *Detoxification of methamidophos by earthworms*

The natural degradation rate of methamidophos in phaiozem was 39.1% in the first 3 days (Table 3). The degradation of methamidophos in phaiozem under natural conditions could conform to a dynamical process (Fig. 1) expressed using the following logistic model:

$$DR' = \frac{101.32}{1 + e^{1.43-0.25t}} \quad (r = 0.977, \ p = 0.01) \tag{2}$$

where DR′ is the natural degradation rate (%) and t is the time (day). It can be calculated on the basis of Eq. (2) that the half life of methamidophos in phaiozem was 5.61 days.

As shown in Fig. 1 and Table 3, earthworms (*E. fetida*) can play a promoting role in the degradation processes of methamidophos in phaiozem. The degradation rate in soils incubated with earthworms was generally higher than that in natural soils after incubated for 6–18 days (Table 3). The detoxification of methamidophos in phaiozem incubated with earthworms (*E. fetida*) can be expressed by the following regression equation:

$$DR = \frac{100.55}{1 + e^{1.84-0.36t}} \quad (r = 0.989, \ p = 0.003) \tag{3}$$

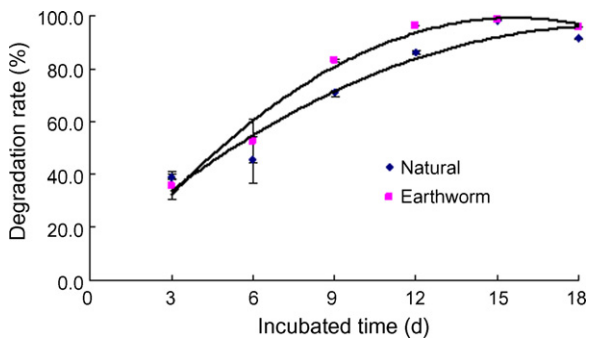


Fig. 1 – Dynamics of methamidophos degradation in phaiozem by earthworms and under natural conditions. p (earthworm × incubated time) = 0.01.

where DR is the detoxification rate (%) of methamidophos by earthworms. According to Eq. (3), the half life of methamidophos in phaiozem was cut down to 5.08 days. This should be basically responsible for the detoxification by earthworms *E. fetida*.

3.3. *Effects of copper pollution on methamidophos detoxification*

It was shown in Fig. 2a and Table 3 that except for the degradation rate at the sixth day of incubation, the degradation rate of methamidophos in phaiozem treated with 300 mg/kg of Cu was lower than that under natural conditions although the differences between them were not very much significant. Obviously, the addition of Cu to soil affected the natural detoxification of methamidophos in phaiozem. The degradation dynamics of methamidophos in phaiozem co-contaminated with Cu (Fig. 2a) could be described using the logistic model:

$$DR = \frac{89.96}{1 + e^{1.19-0.30t}} \quad (r = 0.942, \ p = 0.038) \tag{4}$$

However, because the degradation rate (65.6%) in soil co-contaminated with Cu after incubated for 6 days was significantly higher than that in nature soil (45.6%) (Table 3), the half life of methamidophos calculated from Eq. (4) was 4.74 days, which is lower than that in natural soil.

The detoxification rate of methamidophos by earthworms *E. fetida* in phaiozem co-contaminated with Cu was lower than that without Cu except for that with the 6-day incubation (Table 3). As a whole, there were inhibitory effects by Cu on the detoxification of methamidophos in phaiozem incubated with earthworms *E. fetida*. This trend is clearly depicted in Table 3. The degradation kinetics of methamidophos in phaiozem co-existed with Cu were shown in Fig. 2b and its fitted logistic model was described as follows:

$$DR = \frac{86.79}{1 + e^{2.34-0.55t}} \quad (r = 0.974, \ p = 0.012) \tag{5}$$

However, the half life of methamidophos calculated from (5) was 4.79 days, which was lower than that in natural soil with or without earthworms. It could be concluded from Table 3 and the half life values of methamidophos calculated from (4) and (5) that the degradation of methamidophos was almost the same between those in Cu contaminated soil with and without earthworms.

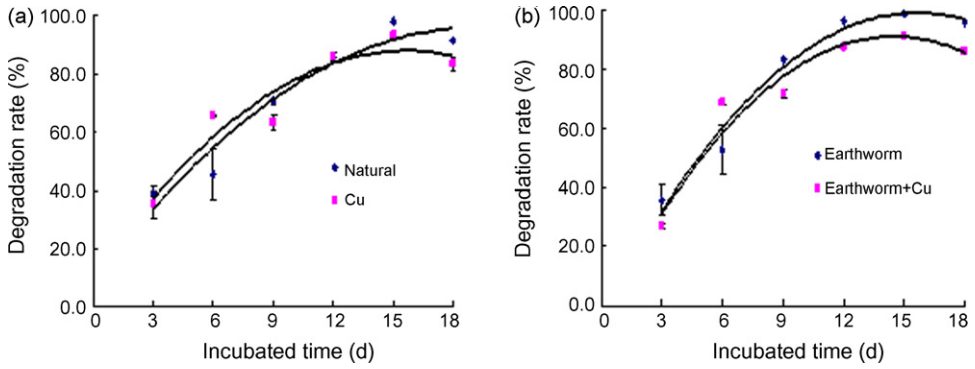


Fig. 2 – Influences of Cu pollution on the dynamics of methamidophos degradation. p (earthworm × incubated time) = 0.01.

3.4. *Effects of acetochlor pollution on methamidophos detoxification*

In the incubation experiment, the degradation rate of methamidophos in phaiozem co-contaminated with acetochlor (20 mg/kg) was basically higher than that under natural conditions at the most time (Table 3) although the differences between them were insignificant. In other words, the addition of acetochlor to soil could promote the natural processes of methamidophos detoxification in phaiozem to a certain extent. Degradation dynamics of methamidophos in phaiozem co-contaminated with acetochlor were shown in Fig. 3a and could also be described using the following logistic model:

$$\mathrm{DR} = \frac{91.24}{1 + e^{2.19 - 0.56t}} \quad (r = 0.958, \ p = 0.024) \tag{6}$$

According to Eq. (6), it can be calculated that the half life of methamidophos in phaiozem was further cut down to 4.29 days due to the promoting action of acetochlor.

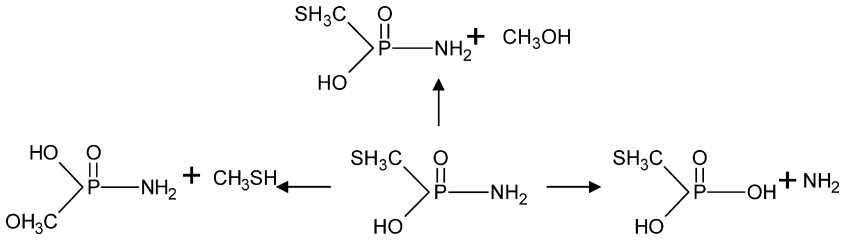
The dynamics of methamidophos detoxification by earthworms *E. fetida* in phaiozem co-contaminated with acetochlor were shown in Fig. 3b and can be described using the following logistic model:

$$\mathrm{DR} = \frac{91.24}{1 + e^{2.45 - 0.64t}} \quad (r = 0.961, \ p = 0.021) \tag{7}$$

According to Eq. (7), it can be calculated that the half life of methamidophos by earthworms *E. fetida* in phaiozem co-contaminated with acetochlor was 4.12 days, which was lower than that (5.08 days) without external acetochlor, but almost same as that (4.29 days) in acetochlor added soil without earthworms. However, it was suggested in Table 3 that just as the case in the Cu contamination test, except for that in the 6-day, the degradation rate in acetochlor contaminated soil with earthworms was lower than that in natural soil with earthworms.

4. Discussion

General speaking, the degradation of methamidophos in phaiozem and other soils under natural conditions should derive from the activity of microorganisms, plants, animals and enzymes released from plant roots, animals and microorganisms to a great extent (Cao and Wang, 1996), similar to biodegradation of other organic pollutants in contaminated soils (Kumar et al., 2006). This proposition has been testified by some reports (Yen et al., 2000; Yuan et al., 2000) about the microbiological and biochemical degradation of methamidophos; this process can be described using the following chemical reaction formula:



(8)

It was reported by Yen et al. (2000) that the half life of methamidophos in soils was 1.11–13.2 days, dependent on soil pH. Compared with the overall range, the half life of methamidophos in phaiozem is in the middle of the range. In other words, methamidophos in phaiozem is normally degradable.

In ecotoxicology, exposure time or degradation time of a pollutant is an important index to denote the magnitude of relative toxicity (Connell et al., 1999; Clements and Newman,

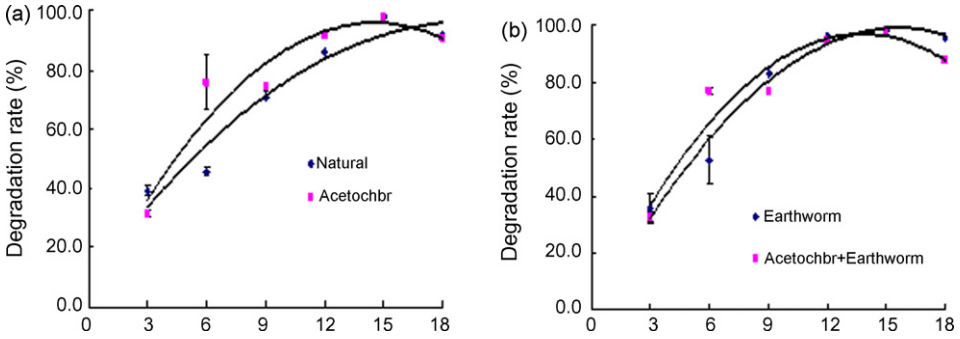


Fig. 3 – Influences of acetochlor pollution on the dynamics of methamidophos degradation. *p* (earthworm × incubated time) = 0.01.

2003; Zhou et al., 2004). When exposure time or degradation time of a pollutant is prolonged, the relative toxicity of the pollutant can be increased. Similarly, the relative toxicity of a pollutant decreases when its exposure time or degradation time is shortened. Undoubtedly, a decrease in the half life of methamidophos in phaiozem by earthworms can denote the promotion of methamidophos detoxification by earthworms. Moreover, the promotion of methamidophos detoxification in phaiozem by earthworms should be mainly responsible for the biological adsorption and assimilation of earthworms themselves, in particular, biological degradation of enzymes released from the activity of earthworms in soil. In fact the secretion from earthworms can perhaps play a key role in promoting and catalyzing methamidophos detoxification or biodegradation in phaiozem (Zhou et al., 2004).

It was reported by Liang and Zhou (2003) in studies of single and binary-combined toxicity of methamidophos, acetochlor and Cu acting on earthworms *Esisenia Foelide* that the single toxic sequence of the three chemicals was acetochlor > methamidophos > Cu. The values of their median lethal dose (LD_{50}) were 0.307 for acetochlor, 0.708 for methamidophos and 118.70 mg/l for Cu. Acetochlor and Cu could be absorbed by the earthworms through penetrating through the earthworm skins. The result also showed that Cu could swell the toxicity of methamidophos. And Cu in low concentration could decrease the toxicity of acetochlor; while in high concentration, Cu could increase the toxicity of acetochlor. There are also some adverse influences of Cu on the growth and development of microorganisms (Carbonell et al., 2000; Zhang et al., 2003; Zhou and Wang, 2006; Pamukoglu and Kargi, 2007), thus resulting in inhibitory effects on the degradation of methamidophos in phaiozem co-contaminated with Cu. However, the activity of some soil microorganisms can be stimulated at the beginning stage of heavy metal stresses (Zhou et al., 2004). This can explain why the detoxification rate of methamidophos in phaiozem co-contaminated with Cu could exceed that without Cu at the 6-day incubation and caused the lower half life of methamidophos compared with those in natural soil with or without earthworms. On the contrary, except for that with the 6-day incubation, the degradation rate of methamidophos in phaiozem co-contaminated with Cu by earthworms *E. fetida* was lower than that without Cu. And it was suggested that there was almost the same in the degradation of methamidophos between the Cu contaminated soils with and without earthworms. This phenomenon is perhaps dependent on two factors. Firstly, the degradation of methamidophos in soil was mainly performed by microorganisms and enzymes, while the co-incubation of earthworms had positive effects on the biodegradation of the chemical. Secondly, although there were adverse effects of Cu on the biodegradation of methamidophos by earthworms, effects on the degradation by degradative microorganisms and enzymes was dominant.

Acetochlor added to soil can inhibit the adsorption of methamidophos to soils (Liang and Zhou, 2003; Yu and Zhou, 2003). Thus, there will be more methamidophos bioavailable to degradative microorganisms. In this sense, the addition of acetochlor to soil can promote the detoxification of methamidophos in phaiozem. The compensatory action of acetochlor can elucidate a part of the fact that the degradation of methamidophos in phaiozem co-contaminated with acetochlor was higher than that in natural soil. However, in spite of the compensatory effect through chemical interactions among acetochlor, methamidophos and soil components, acetochlor had some adverse effects on the biodegradation of methamidophos in soils. With the increase in the incubating time, joint toxic effects of methamidophos and acetochlor can perhaps become the dominant factor influencing the detoxification of methamidophos in soils (Singh, 1999; Gallego et al., 2003; Zhou et al., 2004). This mechanism should be responsible for the lower degradation rate in acetochlor-contaminated soil with earthworms than that in natural soil with earthworms at most time during the incubation experiment.

So, it could be concluded that the biodegradation of methamidophos by microbes and enzymes in phaiozem was still the key pathway, which also could be illustrated by the degradation kinetics model because the logistic model is usually related to the activity of microorganisms. However, earthworms obviously had positive effects on the biodegradation process through possible pathways of biological absorption and assimilation, action of enzymes released from earthworms and other living organisms, or stimulation of the microbial community.

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