Effects of bifenthrin exposure in soil on whole-organism endpoints and biomarkers of earthworm *Eisenia fetida*

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**Highlights**

- The toxicity of bifenthrin to *E. fetida* in soil was evaluated synchronously by multi-endpoints in different levels.
- Bifenthrin was low toxic in soil but its reproduction toxicity was demonstrated.
- EROD activity in earthworm was measured by HPLC and used as a biomarker to assess the effects of bifenthrin.
- The toxicity response of worms to the reduced bifenthrin in content, demonstrated the formation of toxic metabolites.

**Abstract**

In this study, toxic effects of bifenthrin in soil on earthworms were evaluated by acute and chronic toxic endpoints combined with a set of biomarkers. Bifenthrin was moderately toxic in 72-h filter paper test and low toxic in 14-d soil test. The exposure of earthworms to bifenthrin-polluted soil for 8 weeks showed that cocoons were inhibited by high dose of bifenthrin, and larvae were stimulated by low dose but inhibited by high dose of bifenthrin. Furthermore, 28-d soil test on the responses of enzymes associated with antioxidation and detoxification in worms showed that peroxidase (POD) was stimulated by bifenthrin, superoxide dismutase (SOD) inhibited in the early period but stimulated in the later period, glutathione S-transferase (GST) inhibited in the later period, and ethoxyresorufin-O-deethylase (EROD) inhibited at day 3 but markedly stimulated at day 28 at high dose. The different responses of these indexes indicated that multi indexes should be jointly taken into account for comprehensive evaluation of the environmental risk of contaminants in soil.

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**1. Introduction**

Pyrethroids are used worldwide to control a wide spectrum of insect pests with the characteristics of high potency, low acute toxicity and easy degradation (Casida and Quistad, 1998; Soderlund et al., 2002). The frequent use of pesticides results in accumulation of pesticide residues in the environment. Pyrethroids are ubiquitous in sediments at levels high enough to cause toxicity to *Hyalella azteca* (Weston et al., 2004). A recent study on pyrethroids encompassing 25 states across the USA shows that bifenthrin is the most frequently detected pyrethroid (58% of samples), followed by permethrin (31%) and cyfluthrin (14%) (Hladik and Kuivila, 2012). Also, residual pyrethroids can have secondary toxic effects on the non-target organisms (Vyjayanthi and Subramanyam, 2002).

Bifenthrin is a third-generation synthetic pyrethroid insecticide with the reputation of strong environmental persistence and high insecticidal activity (Mokry and Hoagland, 1990) and is applied commonly in agricultural settings against many insects and mite pests (Wang et al., 2014). As an α-cyano-substituted pyrethroid, bifenthrin is generally more toxic than other pyrethroids to both terrestrial and aquatic insects, and its higher toxicity may be related to its stronger influence on detoxification enzymes (Siegfried, 1993).

Up to now, the toxicity of bifenthrin to aquatic organisms has been studied frequently (Weston et al., 2005; Velisek et al., 2009a, 2009b; Beggel et al., 2011). Effective concentrations of bifenthrin
2. Materials and methods

2.1. Soil and earthworms

Soil (0–20 cm) was collected in Shenyang Ecological station, Liaoning Province, China with characteristics of pH 6.2, K–N 0.091%, total P 0.04%, total K 0.18%, organic matter content 1.65%, cation exchange capacity 12.3 cmol kg\(^{-1}\), water holding capacity (WHC) 32%, sand (>50 μm) 22%, silt (1–50 μm) 64%, and clay (<1 μm) 14%. Soil was air-dried and sieved through a 2 mm sieve.

Earthworms (Eisenia fetida) were bought from a breeder in Shenyang, and adult worms with well-developed clitellum and weight of 300–400 mg were chosen to adapt to the soil for more than one week. Before experiments, the earthworms were carried out to rinse out soil and cow dung on moist filter paper for 24 h at 20 °C.

2.2. Chemicals

Bifenthrin (97% purity) was purchased from Shanghai Forever chemical firm. Glucose-6-phosphate, Glucose-6-phosphate dehydrogenase, β-Nicotinamide adenine dinucleotide 2-phosphate reduced tetrasodium salt (NADPH), 7-Ethoxyresorufin, Resorufin, Ethylene Diamine Tetraacetic Acid (EDTA), DL-Dithiothreitol (DTT), methanol, acetonitrile, glutathione, Tris, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Bovine serum albumin (BSA), Coomassie brilliant blue G-250 and 1-Chloro-2,4-dinitrobenzene were purchased from Sigma-Adrich. P-Nitro-Blue tetrazolium chloride (NBT), methionine, riboflavin and guaiacol were purchased from Sinopharm Chemical Reagent, China. Other chemicals were bought from a chemical firm in Shenyang.

2.3. Acute toxicity tests

2.3.1. Filter paper contact test

Filter paper contact test was conducted in accordance with OECD guideline (OECD, 1984). Filter paper was cut to fit the inside of test glass vials (5.0 × 6.5 cm). For each treatment and control there were ten replicates. Each replicate had one worm. Concentrations spanned a wide range from 62.5 to 2500 μg cm\(^{-2}\). Worms were considered dead when they had no response under mechanical stimulus at the front end. Mortality was calculated at 48 h and 72 h.

2.3.2. Soil contact test

Sampled soil test was carried out based on the OECD guidelines (OECD, 1984) with some modifications. Bifenthrin dissolved in 50 mL of acetone was added to sampled soil to get the concentration of 40, 80, 120, 160, 200, 240 and 280 mg kg\(^{-1}\). Each concentration has 3 containers. After balancing for 48 h, ten worms per container were placed in 500 g soil wetted with distilled water with moisture content of 60% WHC and incubated at 20 ± 1 °C (12:12 light:dark cycle). Mortality was assessed at day 7 and day 14. Controls were treated in the same way as the bifenthrin treatments in all tests.

2.4. Reproduction toxicity test

Earthworms were exposed to bifenthrin at sub-lethal concentrations of 0, 10, 20, 40, 60, 80 mg kg\(^{-1}\) for 8 weeks based on the LC\(_{50}\) of soil acute toxicity tests. For each concentration, there were three containers, each containing 10 adult earthworms. Earthworms were fed with 0.5 g of cow dung per worm once a week. The weights of worms, the number of cocoons and larvae in each dose group were counted every two weeks. The growth-inhibition rate of earthworms was calculated as follows:

\[
\text{GIRn} = \frac{W_0 - W_i}{W_0} \times 100\%
\]

GIRn is the growth-inhibition rate for dose group n; W\(_0\) is the
weight on week 0; Wj is the weight on week j (Shi et al., 2007).

2.5. Determination of biomarkers activity

2.5.1. Sample preparation

Earthworms were exposed to soil with concentrations of 0, 10, 20, 40, 60, 80 mg kg⁻¹ of bifenthrin over 28 days, with three replicates for each concentration, and 20 earthworms in 1000 g soil per replicate. At day 3, 7, 14, 21 and 28, earthworms exposed to each concentration were collected in triplicate for analysis, with three earthworms pooled together as one replicate. Worms were kept on wet filter paper for 24 h to defecate, cut into pieces, washed with 0.15 M KCl, and then homogenized for 1 min in 5 mL homogenization buffer (250 mM sucrose, 50 mM pH 7.5 tris, 1 mM DTT and 1 mM EDTA) by Ultra-Turrax IKA T18 at 12,000 rpm. All procedures were carried out at 4 °C. The homogenate was centrifuged at 15,000g at 4 °C for 30 min. 1.0 mL of the supernatant was taken out for analysis of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione S-transferase (GST). Then the remaining supernatant was centrifuged at 150,000g for 1.5 h at 4 °C to obtain microsomes. The microsomes were then suspended in 1 mL of store buffer (250 mM sucrose, 50 mM pH 7.5 tris, 1 mM DTT and 20% glycerol) and stored at −80 °C before used to determine ethoxyresorufin-O-deethylase (EROD) activity.

2.5.2. Enzyme assay

CAT activity was determined based on the method of Xu et al. (Xu et al., 1997). 100 µL of supernatant was added into a tube containing 3 mL of 50 mM (pH 7.8) phosphate buffer which contained 50 mM H₂O₂ for CAT activity measurement at 240 nm. One unit of CAT activity was defined as the enzyme quantity required to consume half of H₂O₂ in 100 s at 25 °C. POD activity was determined according to the method of Kochba (Kochba et al., 1977). The reaction was carried out by adding 100 µL of supernatant into 3 mL of reaction buffer which was prepared by adding 19 µL of 30% H₂O₂ and 28 µL of guaiacol into 50 mL of potassium phosphate buffer (50 mM, pH 7.8). The absorbance at 470 nm was recorded at the beginning and after incubation for 3 min, respectively. One unit of POD activity was defined as the amount of enzyme that caused an increase of 0.01 absorbance units per minute. SOD activity was determined as described by Giannopolitis (Giannopolitis and Ries, 1977). The reaction system contained 50 µL of supernatant, 2.9 mL of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, 130 mM methionine, 750 µM NBT, and 100 µL of 20 µM riboflavin. The tubes were shaken and illuminated with 4000 Lx fluorescent tubes for 20 min, following which the tubes were covered with a black cloth. Absorbance of the mixture was read at 560 nm. One unit was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate (U mg⁻¹ Protein).

GST activity was determined according to the method of Habig et al. (Habig et al., 1974) with the extinction coefficient of 9.6 mM⁻¹ cm⁻¹. Reaction system contained 150 µL of 100 mM (pH 6.5) potassium phosphate containing 0.1% Triton X-100, 20 µL of 10 mM glutathione, 20 µL of supernatant and 10 µL of 20 mM CDNB. The change rate of absorbance at 340 nm during the initial 4 min was recorded. The GST activity was expressed as nmol min⁻¹ mg⁻¹ Protein.

EROD activity was assessed by the HPLC method with some modifications based on Belaz and Hanioka (Hanioka et al., 2000; Belaz and Oliveira, 2013). The incubation system was composed of 800 µL of incubation buffer (100 mM pH 7.8 HEPS, 5 mM glucose-6-phosphate, 1 mg ml⁻¹ BSA, 5 mM MgCl₂, 1 unit mL⁻¹ glucose-6-phosphate dehydrogenase), 50 µL of 7-Ethoxyresorufin (100 µM), and 300 µL of microsomes, and 50 µL of NADPH (5 mM) was added to start the reaction. After 20 min, 800 µL of ice-cold methanol was added to stop the reaction. Then, the mixtures were put on ice for 30 min and centrifuged at 10,000g for 15 min at 4 °C to obtain the supernatant to measure the content of resorufin by HPLC (Thermo Fisher Ultimate 3000).

Samples were injected into a reverse-phase C18 column (Thermo Hypersil Gold 150 × 4.6 mm, 5 µ) at 35 °C following the eluent consisting of 20 mM phosphate buffer (pH 6.8), methanol and acetonitrile (52: 45: 3, v/v/v) at a flow rate of 0.8 mL min⁻¹, and detected using fluorescence detector at 530 nm (excitation) and 582 nm (emission). The EROD activity was expressed as the production of resorufin as pmol min⁻¹ mg⁻¹ Protein. The protein concentration was assayed according to the method of Bradford (Bradford, 1976).

2.6. Bifenthrin degradation in soil

The determination of bifenthrin in soil at day 3, 7, 14, 21 and 28 was carried out according to the method of Zhang et al. (2009) with some modifications. 5 g soil was soaked with 25 mL petroleum ether; acetone (v/v = 2:1) after 1 min of vortex oscillation for 24 h. The soaked soil was extracted for 30 min by a KQ-250DE ultrasonic cleaner. After 10 min, the microsomes were centrifuged again. 2 g NaCl and 2 g Na₂SO₄ were added to the supernatant after centrifuging at 4000 rpm for 10 min, and the organic layer was dried under N₂ blow with 50 °C water bath and dissolved with 2 mL hexane for purification by Florisil SPE column (1 g, 6 mL, Thermo). The eluent was dried under N₂ and dissolved to 2 mL with hexane for the analysis by GC-ECD with a DB-1 column (Thermo Finnigan TRACE GC).

2.7. Statistical analysis

SPSS 21.0 was used for data analysis. Kolmogorov-Smirnov test and Levene’s test were used for normality assumption and homogeneity of variances. One-way analysis of variance (ANOVA) with post hoc comparisons (LSD tests) was used to determine significant differences among treatments (p < 0.05). Logarithmic transformation was applied when data could not meet homogeneity. EROD activity at day 14 followed log-normal distribution. The non-parametric Kruskal-Wallis test was used when the variable could not meet the normality and homogeneity after transformation. Cocoons during 6–8 weeks were tested by non-parametric Kruskal-Wallis test, and there were no significant differences among treatments (P = 0.072). LC₅₀ and associated 95% confidence intervals were estimated using a probit equation.

3. Results

3.1. The acute toxicity

Positive correlations were found between the concentration of bifenthrin and the mortality of earthworms both in filter paper and in soil test. No earthworms survived at 72 h when exposed to 2500 µg cm⁻² of bifenthrin (Fig. 1A). The 48 h and 72 h LC₅₀ of bifenthrin were 440.5 µg cm⁻² and 163.5 µg cm⁻², respectively (Table S1). In soil test, the mortality reached 93.3% as earthworms exposed to 280 mg kg⁻¹ group for 14 d (Fig. 1B). The LC₅₀ of bifenthrin in soil for E. fetida were 226.0 mg kg⁻¹ at day 7 and 166.1 mg kg⁻¹ at day 14 (Table S1). The change of mortality in filter paper test was drastic during 0–1000 µg cm⁻² and then steadied. The mortality in soil test increased continuously with the increase of concentration.
3.2. Reproduction toxicity

3.2.1. Growth inhibition

Fig. 2 showed the growth inhibition of bifenthrin on earthworms after exposure of 2, 4, 6 and 8 weeks. The concentration-related growth inhibition on earthworm was significant during the whole exposure period (Pearson correlation $r = 0.931$, $P < 0.01$; $r = 0.908$, $P < 0.01$; $r = 0.812$, $P < 0.01$ and $r = 0.822$, $P < 0.01$). At 10 mg kg$^{-1}$, no significant effect on growth was determined at whole period. The inhibition effect on worm growth increased with prolonging the exposure time. For example, in the 80 mg kg$^{-1}$ groups, the growth inhibition rates were 13.96% at 2 weeks, 16.95% at 4 weeks, 18.62% at 6 weeks and 25.33% at 8 weeks, respectively.

3.2.2. Cocoon reproduction and larva incubation

Table 1 illustrated the influence of bifenthrin on the cocoon reproduction of every two weeks. In the 10–20 mg kg$^{-1}$ groups, the cocoons were more or less similar to that of the control during the first six weeks, and slightly more during 6–8 weeks. In the 40 mg kg$^{-1}$ group, the cocoons were stimulated by bifenthrin during 4–6 weeks significantly. As the concentration increased to 60 and 80 mg kg$^{-1}$, significant inhibition effects occurred during the first 4 weeks. There were only 2.3 and 2.7 cocoons at first 2 weeks. The inhibition effects of 60–80 mg kg$^{-1}$ groups weakened with the prolongation of exposure.

The effect of bifenthrin on larva incubation was shown in Table 1. Compared to the control, the 10 mg kg$^{-1}$ groups stimulated the larva incubation significantly after 2 weeks. The larvae from 40 mg kg$^{-1}$ group were similar to those of the controls before 6 weeks exposure, but significant higher during 6–8 weeks. As the concentration increased to 60 and 80 mg kg$^{-1}$, the larva incubation was inhibited significantly during the whole exposure and only 1.3 and 2 larvae were observed at the first 4 weeks.

3.3. Biomarkers

3.3.1. Antioxidant enzyme activity

In this study, antioxidant enzyme activities (CAT, POD and SOD) were determined to reflect the bifenthrin stress on earthworms in soil. Fig. 3A indicated at day 3, CAT activity was increased by lower dose of bifenthrin and reduced by high dose (40–80 mg kg$^{-1}$). During the exposure time of 7–21 d, the CAT activities of all bifenthrin groups were similar to those of the controls with no significant differences. As the exposure time prolonged to 28 d, 20 and 40 mg kg$^{-1}$ bifenthrin induced the CAT activity significantly, and the CAT activity of 20 mg kg$^{-1}$ bifenthrin group was 2.26 times as big as that of the control.

POD activities in worms exposed to the control and bifenthrin treatments were shown in Fig. 3B. The POD activity was significantly stimulated by bifenthrin with a positive dose–response correlation from day 3 to day 14. The POD activities of the 80 mg kg$^{-1}$ group at day 3, 7 and 14 were 6.8, 3.45 and 3.25 times as big as that of the control. POD activities in bifenthrin groups were stimulated significantly at day 14, and the CAT activity of 20 mg kg$^{-1}$ bifenthrin group was 2.27 times as big as that of the control.

Changes of SOD activity of earthworms exposed to bifenthrin were showed in Fig. 3C. At day 3 and day 7, bifenthrin inhibited SOD activity in earthworms significantly. The SOD activity at control was 5.1 times as big as that of 80 mg kg$^{-1}$ group at day 3, SOD activities in bifenthrin groups were stimulated significantly at day 14, and the SOD activity at 40 mg kg$^{-1}$ bifenthrin was 2.27 times as big as that of the control. Thereafter, SOD activity was induced by low-
Table 1
Cocoon reproduction and larva incubation of earthworm influenced by bifenthrin in soil.

<table>
<thead>
<tr>
<th>Treatment mg kg⁻¹</th>
<th>Cocoons</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–2 weeks</td>
<td>2–4 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>6 ± 2.0ab</td>
<td>1.7 ± 0.6a</td>
</tr>
<tr>
<td>10</td>
<td>6.7 ± 1.2ab</td>
<td>1.3 ± 0.6ab</td>
</tr>
<tr>
<td>20</td>
<td>4 ± 2.7bc</td>
<td>1.3 ± 0.6ab</td>
</tr>
<tr>
<td>40</td>
<td>7.0 ± 1.7a</td>
<td>2±1a</td>
</tr>
<tr>
<td>60</td>
<td>2.3 ± 0.6c</td>
<td>0.3 ± 0.6b</td>
</tr>
<tr>
<td>80</td>
<td>2.7 ± 0.6c</td>
<td>0.3 ± 0.6b</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 3). Figures followed by different letters in the same column are significantly different at p < 0.05.

Fig. 3. Activities of (A) CAT, (B) POD, (C) SOD, (D) EROD and (E) GST in earthworms E. fetida after exposure to bifenthrin in soil for 3, 7, 14, 21 and 28 days. Error bars indicated standard errors (n = 3). Data with different letters means significant difference at P < 0.05.
concentration bifenthrin but was close to that of the control at high-dose bifenthrin.

3.3.2. Metabolic detoxification enzymes

Fig. 3D showed the responses of EROD activity of earthworms exposed to bifenthrin in soil. At day 3, there was no big difference between the CYP1A1 activity at low dose of bifenthrin (10–20 mg kg\(^{-1}\)) and that of the control; however, high dose (40–80 mg kg\(^{-1}\)) inhibited the EROD activity significantly. At the 7th day, the EROD activities were induced by bifenthrin treatments. And at the 14th and 21th day, no prominent differences of EROD activities between the bifenthrin groups and the control were observed. However, when exposed for 28 d, the stimulation effects of bifenthrin were increased, and the EROD activity increased with the increase of concentration. The EROD activity at 80 mg kg\(^{-1}\) bifenthrin was 4.08 times as big as that of the control.

As shown in Fig. 3E, at day 3, the GST activity in earthworm was increased by low dose of bifenthrin and reduced by high dose. The GST activities at high dose of bifenthrin (40–80 mg kg\(^{-1}\)) were significantly lower than those at low dose. At the 14th day, 10 mg kg\(^{-1}\) bifenthrin significantly induced the GST activity, which was 1.48 times as big as that of the control. With the extension of exposure time, the significant inhibition effect of bifenthrin on GST activity was observed at day 21 and 28.

3.4. The degradation of bifenthrin in soil

Results (Table S2) showed a gradual decrease of bifenthrin in soil during the 28 d exposure. The degradation of bifenthrin corresponded to first-order kinetics with a coefficient of correlation of 0.82–0.99. For the 10 mg kg\(^{-1}\) group, the degradation rate of bifenthrin was 11.4% and 54.6% at day 3 and day 28, respectively; for the 80 mg kg\(^{-1}\) group, the bifenthrin was reduced by 63.9% and 42.40% after 3 and 28 d, respectively. The half-time of bifenthrin (10–80 mg kg\(^{-1}\)) in our soil was 30.01–59.24 d.

4. Discussion

Despite the widespread use of pyrethroid insecticides in the environment, few studies have been conducted to assess the toxic effects of pyrethroids including bifenthrin in soil. In this study, we focused on the toxic effects of bifenthrin on *E. fetida* in laboratory-spiked soil using the whole-organism endpoints and molecular biomarkers. The acute toxic effect of bifenthrin was time- and concentration-dependent. According to the classification of chemicals, bifenthrin was moderately toxic (LC\(_{50}\) = 100–1000 μg cm\(^{-2}\) in filter paper test and low toxic in soil test (LC\(_{50}\) > 10 mg kg\(^{-1}\)) (Roberts and Wyman Dorough, 1984; Institute for the Control of Agrochemicals (2004)). The low toxicity of bifenthrin in soil might because the bioavailability of chemicals could be modified by soil phy-chemical properties (Lanno et al., 2004). Although pyrethroids are weak toxins according their LC\(_{50}\)s in soil, their obvious sub-lethal toxicity to soil organisms was observed. When *E. fetida* was exposed to 25 mg kg\(^{-1}\) fenvalerate (14d-LC\(_{50}\) 40.77 mg kg\(^{-1}\)), dominant damage of cuticular membrane, and disintegration of circular and longitudinal muscles were observed (Saxena et al., 2014). When *E. fetida* was exposed to 5–50 mg kg\(^{-1}\) deltamethrin (14d-LC\(_{50}\) 423.9 mg kg\(^{-1}\)), dose-dependent toxic effects on growth and cellulose activity were showed (Shi et al., 2007). Thus, sub-lethal toxicity was important to assess the risk of bifenthrin in this study.

The change of biomass is a good indicator of chemical stress (Shi et al., 2007). In this study, the weights of earthworms were significantly inhibited by bifenthrin in soil with dose and time-dependent relationship (Fig. 2). In the environment, a relative biomass loss may be correlated with the strategy for survival (i.e., reducing food intake to avoid the toxins), and would have a substantial influence on population structure by reducing fecundity, recruitment and resistance to environmental stress because homeostatic energy demands are increased to dispose environmental contaminants (Burrows and Edwards, 2002; Amweg et al., 2005). The reproductive strategies were related to the ecological categories in earthworms (Barois et al., 1999). In the study, the reproductive strategies of *E. fetida* were influenced by bifenthrin. The changes of cocoon reproduction and larva incubation were sensitive to the toxicity of bifenthrin which agreed with previous study (Song et al., 2015).

Pollutants can induce excessive free radicals, which may result in oxidative damage to biological macromolecules (Liu et al., 2015). So CAT, POD and SOD activities have been applied to determine the oxidative stress in organisms (Holovská et al., 2005; Fatima et al., 2007; Song et al., 2009; Zhang et al., 2014). Pyrethroids (cypermethrin, fenvalerate and bifenthrin) have potential to cause severe oxidative stress to rats and mice (Kale et al., 1999; Dar et al., 2013; Jin et al., 2014). *Eisenia fetida* was demonstrated to be well-equipped to deal with electrophilic species and pro-oxidants (Saint-Denis et al., 1998). However, there are hardly any studies showing the oxidative stress on soil organisms. In this study, CAT, POD and SOD activity in *E. fetida* exposed to bifenthrin at sub-lethal concentrations were measured. The changes of CAT activities were slight and not significant in most of bifenthrin groups comparing with the controls. CAT activities varied in the controls of different exposure times because of variation in both biological factors and non-biological factors. Similar to our observation, no significant response was observed in CAT activity in *Eisenia fetida* affected by carbaryl and bromadiolone (Ribera et al., 2001; Liu et al., 2015). POD and SOD activity in earthworm were induced more significantly by bifenthrin. POD activity was stimulated at all concentrations during 3–14 d bifenthrin exposure, which might result from the H\(_2\)O\(_2\) accumulation. SOD activity was inhibited in worms in all bifenthrin treatments during the early exposure, which might be due to the saturated anti-oxidant defenses and the highly reactive O\(_2^\cdot\) (Wu et al., 2011). A decrease in SOD activity has also been reported in rats treated with bifenthrin and deltamethrin (Dar et al., 2013; Yousef et al., 2006). SOD activity in earthworm increased with the prolongation of exposure, possibly because of the production of O\(_2^\cdot\), which stimulated the SOD activity (Song et al., 2009). This study demonstrated exposure to bifenthrin resulted in oxidative damage to *E. fetida* in soil.

CYP1A1, a dominant subform of CYP450 which involved in xenobiotic metabolism in living organisms, is widely used as a biomarker in ecotoxicological studies and specifically assessed by EROD (7-ethoxyresorufin O-deethylase) activity (Bonacci et al., 2003; Bozcaarmutlu et al., 2015). Up to present, only few studies reported the induction of EROD in terrestrial invertebrates (e.g. earthworm) exposed to pollutants successfully, because of low activity and interfering substances such as respiratory pigments (Lukkari et al., 2004; Cao et al., 2012; Sanchez-Hernandez et al., 2014). In this study, microsomal fraction of earthworm was used to measure EROD activity successfully based on the method we established for earthworm. Results showed EROD activity exhibited a biphasic response defined by an increase at low dose and decrease at high dose during short-time exposure. This inverted U-shaped relationship seems to be hormetic dose-response model in earthworms. Sanchez-Hernandez et al. (2014) observed the same type response in *Aporrectodea caliginosa* exposed to chlorpyrifos for 3 days. With the prolongation of exposure, the stimulated effect on EROD activity by bifenthrin was strengthened, showing the presence of bifenthrin induced CYP1A1 in *E. fetida*.

GST, a phase-II biotransformation enzyme, facilitates
conjugation of electrophilic substances with glutathione and the elimination of toxic compounds (Habig et al., 1974; Bernard et al., 2015). GST activity in earthworm was affected by pollutants such as PAHs, heavy metals and pyrethroids (Velki and Hackenberger, 2013a; Feng et al., 2013; Zhang et al., 2013). In the study, there were no significant differences between most bifenthin groups and controls before 14 d. The data is in line with previous study, in 2013a; Feng et al., 2015; Zhang et al., 2015). In the study, there are as PAHs, heavy metals and pyrethroids (Velki and Hackenberger, 2013a; Xu et al., 2015). Our results indicated that exposure to bifenthin in soil resulted in oxidative stress and biotransformation damage, followed by the decrease of growth and fecundity. All of these responses suggested that multi-endpoints are essential and important to diagnose the toxicity of bifenthin in soil. The degradation reaction of bifenthin in soil followed the first-order kinetic, and the half-life was between 30.1 and 59.2 d. However, with the degradation of bifenthin, the toxicity on worms was not decreased. In previous study, the inconsistence between the changes of toxic effect and the degradation of pyrethroids in soil has also been found (Song et al., 2015). This phenomenon might be due to the formation of toxic metabolites from bifenthin degradation, such as cyclopropanecarboxylic acid and 2-methyl-3-biphenyl methanol, because the toxicity of metabolite might be higher than the parental compound (Romero et al., 2012). Further studies are needed because of the inconsistence between the degradation of bifenthin and the enhanced toxicity response.

5. Conclusion

Sub-lethal exposure of Eisenia fetida to bifenthin produced evident toxicity responses including the reproduction toxicity, oxidative stress and metabolic detoxification toxicity. By integrating the acute and chronic toxic endpoints at the whole-organism level with a group of biochemical biomarkers, the information obtained in this study is useful for developing a scientific theoretical database for future risk assessment of pyrethroids in soil. The inconsistence found between the change of toxic effect and the degradation of bifenthin in soil indicated existence of possible toxic metabolic products, which need further work on the toxicity and transformation of bifenthin in soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.10.060.

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