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RESEARCH ARTICLE

Physiological and molecular responses of the earthworm *Eisenia fetida* to polychlorinated biphenyl contamination in soil

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Abstract Polychlorinated biphenyls (PCBs) are a class of man-made organic compounds ubiquitously present in the biosphere. In this study, we evaluated the toxic effects of different concentrations of PCBs in two natural soils (i.e. red soil and fluvo-aquic soil) on the earthworm *Eisenia fetida*. The parameters investigated included anti-oxidative response, genotoxic potential, weight variation and biochemical responses of the earthworm exposed to two different types of soils spiked with PCBs after 7 or 14 days of exposure. Earthworms had significantly lower weights in both soils after PCB exposure. PCBs significantly increased catalase (CAT), superoxide dismutase (SOD), and guaiacol peroxidase (POD) activity in earthworms exposed to either soil type for 7 or 14 days and decreased the malondialdehyde (MDA) content in earthworms exposed to red soil for 14 days. Of the enzymes examined, SOD activity was the most sensitive to PCB stress. In addition, PCB exposure triggered dose-dependent coelomocyte DNA damage, even at the lowest concentration tested. This response was relatively stable between different soils. Three-way analysis of variance (ANOVA) showed that the weight variation, anti-oxidant enzyme activities, and MDA contents were significantly correlated with exposure concentration or exposure duration ($P < 0.01$). Furthermore, weight variation, CAT activity, and SOD activity were significantly affected by soil type ($P < 0.01$). Therefore, the soil type and exposure time influence the toxic effects of PCBs, and these factors should be considered when selecting responsive biomarkers.

Keywords Acute toxicity · Anti-oxidant enzymes · Comet assay · *Eisenia fetida* · Malondialdehyde · Polychlorinated biphenyls

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Introduction

Polychlorinated biphenyls (PCBs) are a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl, which is composed of two benzene rings (DeRosa et al. 1998). Due to their non-flammability, chemical stability, and high dielectric constants, PCBs have been used as pesticide extenders and plasticizers, and have consequently been released, directly or indirectly, into the environment (Kavlock et al. 1996). As PCBs pose potential hazards to human health and environmental safety, the use of PCBs has been severely restricted or banned in many countries (Han et al. 2013). However, it is estimated that approximately 750,000 t of PCBs remain in the biosphere (Sanchez et al. 2000).

Although the production and usage of PCBs have been banned in China since the 1970s, a large proportion of old, PCB-containing transformers and capacitors remain in use, which results in the continued environmental input of PCBs, from both deliberate and accidental sources. In addition, the pool of PCBs remaining in the soil/sediment from the time when PCBs were widely used continues to present considerable environmental and human health risks (Zhang et al. 2012). Therefore, there is an urgent need to assess the risks of PCB-contaminated soils.

Earthworms, which constitute approximately 60–80% of the soil biomass, have close contact with the soil, affect soil structure and fertility, and have been used extensively as bioindicators of soil contamination. *Eisenia fetida*, which is relatively easy to handle in the laboratory and is sensitive to contaminants, is the most commonly used species of earthworm used for toxicity testing (Belmeskine et al. 2012). The toxic effect of PCBs in aquatic ecosystems has been studied extensively for many years (Coteur et al. 2001). However, few studies have assessed the toxicity of PCBs in terrestrial ecosystems, especially using earthworms as test organisms (Vlčková and Hofman 2012).

In many countries, environmental agencies set the guidelines for the protection of soils, which are always based on the sum of the concentrations of selected priority PCB congeners according to their frequency of occurrence and toxicity. For example, the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) chose seven PCBs, i.e., PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180, as indicator PCBs because they were the main components of commercial PCB commercial products, e.g., Aroclors. Furthermore, these seven PCB congeners were commonly found in the highest concentrations in abiotic samples from the environment and were frequently studied in accumulation experiments within tissues from birds, fish, and earthworms (Wågman et al. 2001; Hecker et al. 2006). Moreover, the seven selected PCB congeners contain different degrees of chlorination but have similar chemical structures (Li et al. 2012). These seven PCBs are also commonly measured and are used in the Netherlands Soil Protection Guidelines to represent soil PCBs (Teng et al. 2015). Therefore, we chose to use a mixture of these seven PCB congeners to evaluate the toxicity responses of earthworm in this study.

To reduce the complexity and the influence of environmental factors, standard soils are commonly used in earthworm ecotoxicological tests, including artificial soil recommended by the Organization for Economic Cooperation and Development (OECD) and the natural standard soil LUFA 2.2 (OECD 1984; Marabini et al. 2011). Artificial soil with known doses of a single PCB congener is commonly used in earthworm ecotoxicological tests (OECD 1984; Lokke and Gestel 1998). However, PCBs usually appear as mixtures in the soil, and the use of artificial soil may reduce the relevance of the results of environmental risk assessments, as the soil characteristics (e.g. the organic carbon (OC) and clay contents and pH) can affect the bioavailability of the PCBs and influence the fate of contaminants and their toxicity to earthworms (Hofman et al. 2008; Delannoy et al. 2015), making PCB risk assessment technically challenging (Zhang et al. 2014; Sforzini et al. 2015). The bioavailability of PCB 153 was shown to differ significantly between natural and artificial soils (Vlčková and Hofman 2012), and the toxic effects of PCB153 on earthworms varied in different soil types (Šmídová and Hofman 2014). Therefore, we chose two natural soils (red soil and fluvo-aquic soil) with different characteristics, such as pH and organic matter content and collected soil from the regions that were reported to be contaminated with PCBs. For instance, the concentration of $\sum_7$ PCBs (PCB 28, 52, 101, 138, 153, 180, and 118) in the fluvo-aquic soil was reported to be in the range of 0–373 μg kg^{-1} (average 46.2 μg kg^{-1}) in Tianjin (Hou et al. 2013), and that of $\sum_7$ PCBs (PCB 28, 52, 101, 138, 153, 180, and 118) in the red soil was in the range of 0–129 μg kg^{-1} (average 14.9 μg kg^{-1}) in Jiangxi, which is relatively high in the global context, and might pose a threat to human health and the environment (Li et al. 2010; Teng et al. 2015). Thus, it is important to evaluate and compare the toxicity of PCBs in these two soils.

Biomarkers are sensitive parameters used to gauge the toxic effects of substances. Examining different biological responses provides useful information for deciphering the mechanisms underlying chemical toxicity as well as characterizing the potential impact on the fitness of an organism. To improve risk assessment in the environment at the early stages of contamination, multiple biomarkers should be combined (Sforzini et al. 2015). In addition to detecting weight variation at the organismal level, observing changes in antioxidant enzyme activity and DNA damage at the molecular and cellular levels might reveal sensitive, sub-lethal responses of organisms to toxicants after short periods of time (Bonnard et al. 2010; Brown et al. 2010; Whitfield Åslund et al. 2011). The induction of antioxidant enzyme activity is attributed to the saturation of antioxidant defenses of the cell against reactive oxygen species (ROS), indicating high oxidative stress (Koivula and Eeva 2010). The enzymes superoxide dismutase (SOD), guaiacol peroxidase (POD), and catalase (CAT) are involved in the detoxification of O_2^- (SOD) and H_2O_2 (CAT, POD), and prevent the formation of ROS (Liu et al. 2011). For the PCB contamination, impairments of immune and metabolic functions and genotoxic damage were often used for evaluating the stress, while few reports were focused on developing a suite of oxidative stress related biomarkers, especially in the earthworms (Sforzini et al. 2015).

Therefore, we aimed to examine the effect of different biological levels of the sum of seven ($\sum_7$) representative PCBs (28, 52, 101, 118, 138, 153, and 180) on multiple toxic biomarkers of *Eisenia fetida*, including weight variation, antioxidative responses such as the malondialdehyde (MDA) content, the antioxidants SOD, POD, and CAT, and also DNA damage. In addition to examining the effects of soil types (red soil and fluvo-aquic soil) on PCB toxicity using these biomarkers, we also considered the effects of exposure duration and PCB concentration.

Materials and methods

Chemicals and reagents

The standard PCB mixture, which included PCB 28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB 180, and which had a purity of >95%, was obtained from AccuStandard (New Haven, USA). All reagents used in this study were of analytical grade and purchased from Nanjing Chemical Reagent Company (China), unless otherwise indicated.

Test organisms and soil

The earthworm *Eisenia fetida* was purchased from a commercial earthworm cultivating farm (Nanjing, China). Adult worms with a clitellum (i.e. at least 2 months old) and an individual weight of 300 to 600 mg were selected.

Two widely distributed soil types (fluvo-aquic soil and red soil) in China were collected from the surface layer (0–20 cm) of uncultivated, unpolluted fields in Jiangxi and Tianjin (Duan et al. 2015). Soils were air-dried and passed through a 2 mm mesh. The physical and chemical properties of both soils were recorded (Table 1).

Experimental design

The acute toxicity test was performed according to the OECD guideline (OECD 1984) with slight modifications. A mixture containing seven different PCB congeners (28, 52, 101, 138, 153, 180, and 118) was added to acetone and sprayed evenly into 100 g aliquots of soil, followed by thorough mixing to produce the following final concentrations: 0 (control), 0.25, 0.5, 1, 5, and 10 mg kg^{-1}. The soils were then transferred to pre-cleaned 250-mL glass containers. To ensure that the acetone had completely evaporated, these containers were incubated in an exhaust hood for at least 1 day. Ten worms were kept in each jar, and each treatment had eight replicates. The test containers were incubated at 20 ± 2 °C under continuous light to ensure that the worms remained in the test soil. The humidity was adjusted by adding deionized water to obtain 60% of the maximum water holding capacity. The earthworms were manually collected at 7 and 14 days, incubated in darkness at 22 °C to void the gut for 24 h, washed with deionized water, gently dried with absorbent paper, frozen in liquid nitrogen, and stored at −70 °C before analysis (the mortality rate is shown in supplied material, Fig. S1).

Earthworm weight variations

The weight of living earthworms was measured at 0, 7, and 14 days of exposure to the soil. This value was divided by the number of the living earthworms to calculate the percentage of weight variation, using the following formula:

$$WR_c\% = (W_0/10 - W_t/n)/(W_0/10) \times 100\%$$

where

$WR_c\%$ is the percentage of weight variation for concentration c,
W_0 is the total weight of the living earthworms on 0 day,
W_t is the total weight of the living earthworms after t days of exposure, and n is the number of earthworms alive after t days of exposure.

Comet assay

Earthworm coelomocytes were extracted according to a non-invasive manner, as described by Eyambe et al. (1991). Three earthworms were incubated in extrusion medium (composed of 5% ethanol, 2.5 mg kg^{-1} EDTA, 95% saline, and 10 mg kg^{-1} guaiacol glyceryl ether (pH 7.3)) for 3 min. The coelomocyte extractions were centrifuged (4 °C, 3000 rpm, 10 min) to remove the supernatant and recover the cell pellet, washed in phosphate-buffered saline (PBS, pH 7.4), and centrifuged again. These steps were repeated three times. Finally, the coelomocytes were placed on ice prior to the comet assay. The comet assay was determined by the method reported by Duan et al. (2015). Images of the single-cell gel electrophoresis were analyzed using the Comet Assay Software Project (CASP) (Końca et al. 2003), and 100 cell cores were counted per slide. The parameter used to quantify the extent of DNA damage was olive tail moment (OTM), which is defined as the distance between the center of gravity of the head and the center of gravity of the tail and the fraction of total DNA in the tail (Khan et al. 2012).

Enzyme activity and MDA content assays

The earthworms were ground with a prechilled mortar and pestle in 50 mM Tris-sucrose buffer (1:9, *w*/*v*, pH 7.5) under ice-cold conditions. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C to recover the supernatant, which was stored at −70 °C until use for enzyme activity assessment and protein determination.

The supernatant was thawed just prior to the assay to avoid multigelation. The protein content was determined by the Coomassie brilliant blue method developed by Bradford (1976) using bovine serum albumin (BSA) as the standard. The total superoxide dismutase (SOD) activity was

Table 1 Physical and chemical properties of the two test soils

Soil	pH (1:2.5 *w/w*)	Organic matter (g/kg)	Clay content (%)	C/N	Texture	CEC (cmol kg^{-1})
Red soil	4.89	9.95	52	5.85	Clay loam	9.76
Fluvo–aquic soil	7.71	34.53	43	10.02	Clay loam	20.94

CEC cation exchange capacity

determined by measuring the inhibition of nitroblue tetrazolium chloride (NBT) reduction, as described by Song et al. (2009). Catalase (CAT) activity was determined as described by Song et al. (2009) using H_2O_2 solution as the substrate. The activity was calculated based on the decrease in ultraviolet absorption at 250 nm per unit time due to decomposition of H_2O_2 by CAT in the sample. Guaiacol peroxidase (POD) activity was determined according to the method of Song et al. (2009). The supernatant was added to 3 mL of reaction mixture containing 19 mL of 30% H_2O_2, 50 mL potassium phosphate buffer (100 mM, pH 6.0), and 28 mL guaiacol. Changes in absorbance were recorded at 470 nm. One activity unit of POD (U mg^{-1} Pr) was defined as the amount of enzyme that caused an increase of 0.01 absorbance unit min^{-1}. The malondialdehyde (MDA) content was used as an indicator of lipid peroxidation. The MDA content was calculated by measuring the absorbance at 532 nm using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Gonzalez Flecha et al. 1991). The final results are expressed as nmol MDA mg^{-1} protein.

Statistical analysis

Statistical analyses were performed using the SPSS program (SPSS 20.0). All values are presented as mean ± standard deviation (SD). A three-way ANOVA test was applied to detect whether PCB concentration, duration of exposure, soil type, and their interaction affected indicators (the weight variation, SOD activity, CAT activity, POD activity, and MDA content). One-way ANOVA and the least significance difference (LSD) test ($P < 0.05$) were used to determine the comparisons and differences among the six treatments. A Mann-Whitney *U* (two-tailed) test was used to determine significant differences ($P < 0.01$) between the treatment and control groups, because the data from the comet assay failed to meet the normality test.

Results

Earthworm weight variations

Earthworm weight was reduced during the 14 days exposure to red soil, whereas it was increased in fluvo-aquic soil (Table 2). The reduction in earthworm growth increased with increasing PCB concentration and was shown to be dose-dependent in both soils. ANOVA revealed that the weight variation of earthworms was significantly affected by PCB concentration, exposure duration, and soil type, and also by the interactions between these parameters (Table 3).

Comet assay

The OTM values of earthworms in all PCB treatment groups were significantly different from those of the control treatment ($P < 0.001$) in both soils after exposure for 7 and 14 days (Fig. 1), even at the lowest concentration tested, implying that PCBs caused serious DNA damage to the coelomocytes of earthworms. The OTM values increased with increasing doses of PCB and increasing exposure times but did not significantly differ between the two soil types (Table 3).

CAT activity

CAT activity was significantly increased ($P < 0.05$) in earthworms after a 7 days exposure to contaminated red soil compared to the control, and a significantly higher level of CAT activity was detected at PCB concentrations of greater than 0.5 mg kg^{-1} after 14 days (Fig. 2a). For fluvo-aquic soil, CAT activity was significantly higher than that of the control at all doses, except for after a 7 days exposure to 0.25 mg kg^{-1} PCBs (Fig. 2b). When the duration of exposure increased, the CAT activity decreased in red soil at the same PCB concentration, but in fluvo-aquic soil, the CAT activity did not significantly change, except at 10 mg kg^{-1} PCB treatment, where it significantly decreased. At the same PCB concentrations, the CAT activity was higher in red soil than in fluvo-aquic soil. ANOVA analysis showed that the CAT activity of earthworms was significantly affected by PCB concentration, exposure duration, and soil type (Table 3), as well as by interactions between these parameters, except for the interaction between soil type and PCB concentration.

SOD activity

SOD activity was significantly higher in earthworms subjected to PCB treatment for 7 and 14 days than in the control for both soils, even at the lowest concentration tested (Fig. 3). SOD activity increased with increasing exposure duration at all concentrations tested and in both soils. Under the same PCB treatment, SOD activity was higher in red soil than in

Table 2 The percentage of weight variation (mean + SD) in *Eisenia fetida* exposed to two types of soil containing different doses of polychlorinated biphenyls for 7 and 14 days

	Red soil		Fluvo–aquic soil	
PCB concentration (mg/kg)	The percentage of weight variation after 7 days (weight lost %)	The percentage of weight variation after 14 days (weight lost %)	The percentage of weight variation after 7 days (weight gained %)	The percentage of weight variation after 14 days (weight gained %)
0	5.1 ± 0.024 f	12.3 ± 0.124 E	28.3 ± 0.069 f	33.4 ± 0.075 F
0.25	9.0 ± 0.019 e	15.4 ± 0.086 D	24.0 ± 0.116 e	27.4 ± 0.095 E
0.5	13.2 ± 0.083 d	14.3 ± 0.069 D	20.2 ± 0.100 d	22.4 ± 0.088 D
1	15.9 ± 0.077 c	20.5 ± 0.089 C	13.5 ± 0.087 c	16.7 ± 0.055 C
5	24.0 ± 0.142 b	25.6 ± 0.055 B	11.4 ± 0.084 b	13.8 ± 0.073 B
10	28.5 ± 0.104 a	36.2 ± 0.071 A	6.2 ± 0.114 a	10.2 ± 0.087 A

The results of one-way ANOVA are presented in the table. Different lowercase and uppercase letters indicate statistical difference between treatments at 7 and 14 days, respectively (LSD test, $P < 0.05$)

fluvo-aquic soil. In addition, SOD activity of the earthworms was significantly affected by PCB concentration, exposure duration, and soil type (Table 3).

POD activity

For red soil, POD activity significantly increased in the earthworms after exposure to 5 and 10 mg kg^{-1} PCBs at 7 days, but at 14 days, a significant increase was only detected for the 10 mg kg^{-1} PCB treatment. For fluvo-aquic soil, POD activity also significantly increased with increasing PCB concentration at 5 and 10 mg kg^{-1} (Fig. 4). With increasing time of PCB exposure, POD activity decreased in earthworms maintained in both red soil and fluvo-aquic soil. POD activity did not significantly differ based on soil type, while PCB concentration and exposure duration had significant effects on POD activity (Table 3).

MDA content

For red soil, the MDA content in the PCB treatment groups did not significantly differ from that of the control group after 7 days of exposure (Fig. 5). After 14 days of treatment, a significant decrease in MDA content was observed at PCB concentrations of 0.25, 5, and 10 mg kg^{-1}. For fluvo-aquic soil, the MDA content did not change with increasing PCB concentration or exposure duration. The soil type did not influence the MDA content. As revealed by ANOVA analysis, the MDA content of the earthworms was significantly affected by both PCB concentration and exposure duration (Table 3).

Discussion

Traditional risk assessment has routinely focused on exposure to a single chemical in the soil. However, in natural

Table 3 Summary of the effect of polychlorinated biphenyl treatment on various *Eisenia fetida* biomarkers

	d.f.	The percentage of weight variation	CAT	SOD	POD	MDA
Concentration	5	1526.9***	315.5***	323.0***	15.0***	4.1**
Duration	1	29.4***	99.3***	142.3***	35.5***	21.3***
Soil	1	43,686.5***	87.0***	69.7**	2.7	0.2
Duration × concentration	5	6.5***	56.6***	1.6	1.5	2.0
Duration × soil	1	463.2***	14.9***	3.0	0. 7	0.9
Soil × concentration	5	35.9***	2.3	0. 2	0.3	0.3
Duration × soil × concentration	5	20.8***	6.8***	0.9	0.3	0.1

F-values for the three-way ANOVA on the effects of concentrations (0, 0.25, 0.5, 1, 5, or 10 mg kg^{-1}) and soils (red soil or fluvo-aquic soil) on the growth inhibition and catalase activity (CAT), superoxide dismutase activity (SOD), guaiacol peroxidase activity (POD), and malondialdehyde (MDA) content of *Eisenia fetida* after 7 and 14 days of polychlorinated biphenyl exposure

df degrees of freedom

Cases where the concentration, soil type, or the duration of exposure had a significant effect (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

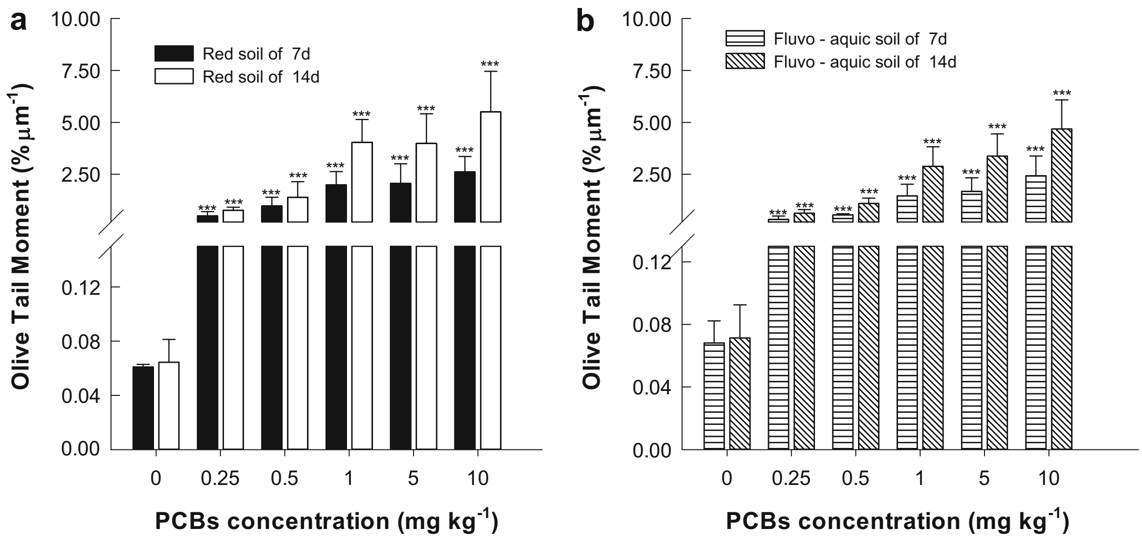

Fig. 1 Effects of polychlorinated biphenyls on DNA damage, as evaluated using the comet assay. *Eisenia fetida* coelomocytes were exposed for 7 and 14 days to **a** red soil and **b** fluvo-aquic soil. The data are expressed as olive tail moment (OTM). The results of a Mann-Whitney *U* (two-tailed) test are presented in the graphs. *Bars with stars* (***) indicate statistical significance versus the control group ($P < 0.001$)

environmental matrices, PCBs usually occur as complex mixtures, and their combined effects may be toxic to organisms. It is both time-consuming and expensive to evaluate the risk of each single PCB congener separately in the soil. In addition, risk assessments based on individual components tend to underestimate the effects associated with the toxic actions of mixtures (Yang et al. 2015). Hence, toxicity studies of PCB mixtures with soil organisms are urgently needed.

Bioassays that directly assess the ecological toxicity of polluted soil are considered to be more accurate than chemical assays. Biomass, a highly sensitive parameter, was used to determine the effects of various concentrations of contaminants on *E. fetida* (Wu et al. 2011). In the present study, the weight of earthworms significantly decreased in red soil but increased in fluvo-aquic soil (Table 2). This result might be due to the higher organic matter content of fluvo-aquic soil, which could provide more nutrients for the earthworms. However, the weight of the earthworms in both soils decreased with increasing PCB concentration, indicating that chemical stress eventually inhibited growth. This finding

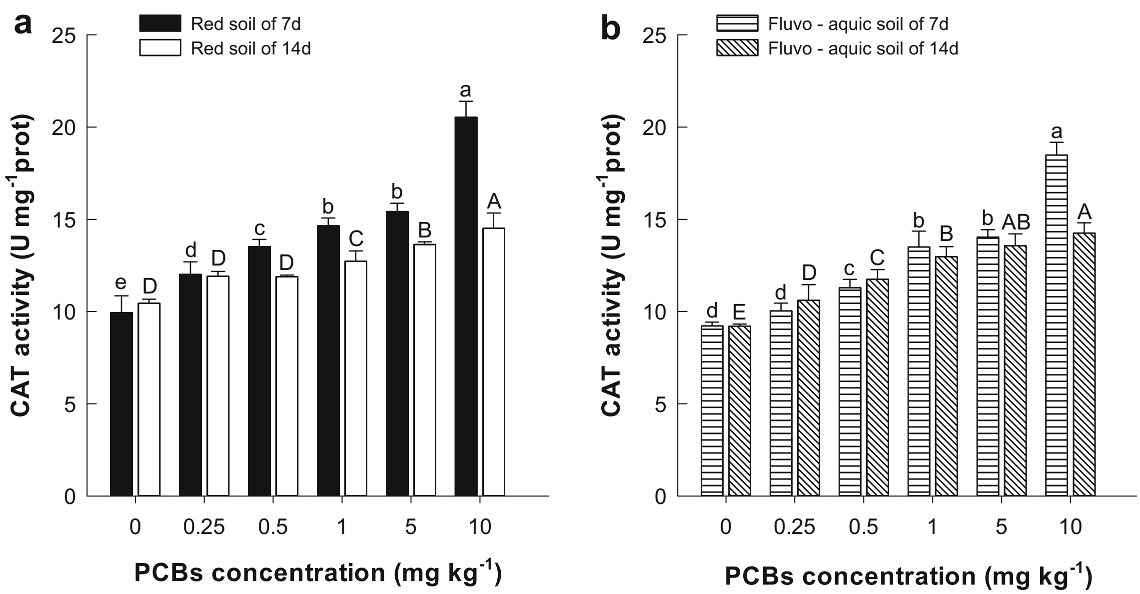

Fig. 2 Effects of polychlorinated biphenyls on catalase (CAT) activity in *Eisenia fetida*. **a** Red soil. **b** Fluvo-aquic soil. The results of one-way ANOVA are presented in the graphs. *Lowercase* and *uppercase letters* indicate statistically significant differences between treatments at 7 and 14 days of treatment, respectively (LSD test, $P < 0.05$)

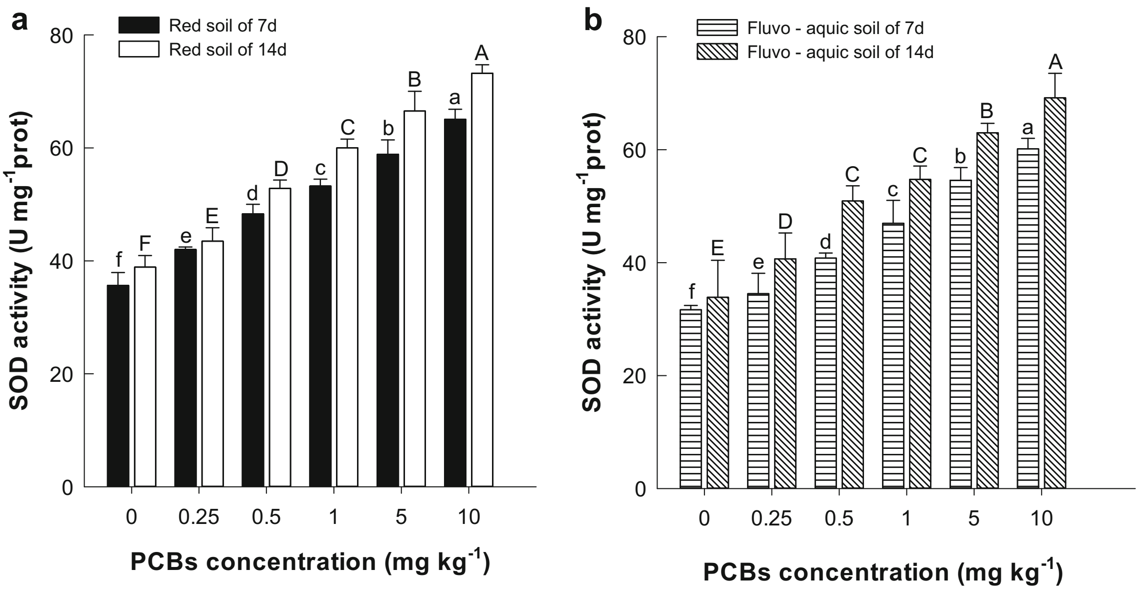

Fig. 3 Effects of polychlorinated biphenyls on superoxide dismutase (SOD) activity in *Eisenia fetida*. **a** Red soil. **b** Fluvo-aquic soil. The results of one-way ANOVA are presented in the *graphs*. *Bars with different lowercase* and *uppercase letters* indicate statistically significant differences between treatments at 7 and 14 days of treatment, respectively (LSD test, $P < 0.05$)

may be attributed to the natural survival strategy of earthworms; earthworms avoid toxins by reducing food intake, which subsequently results in weight loss (Mosleh et al. 2003). Similar adjustments were observed in fish, with reductions in daily intake levels and growth detected upon exposure to high doses of PCBs (Mohebbi-Nozar et al. 2013).

Our results showed that SOD and CAT activities increased after exposure to all doses of PCBs, including the lowest one tested (Figs. 2 and 3), whereas POD activity only significantly increased at PCB concentrations of 5 mg kg^{-1} and higher (Fig. 4). These results are in agreement with the findings of Parolini et al. (2013), who detected significant correlations between the induction of SOD and CAT in the freshwater bivalve zebra mussel (*Dreissena polymorpha*) exposed to PCBs. The differences in CAT and POD activity might be due to the fact that CAT plays a more dominant role than

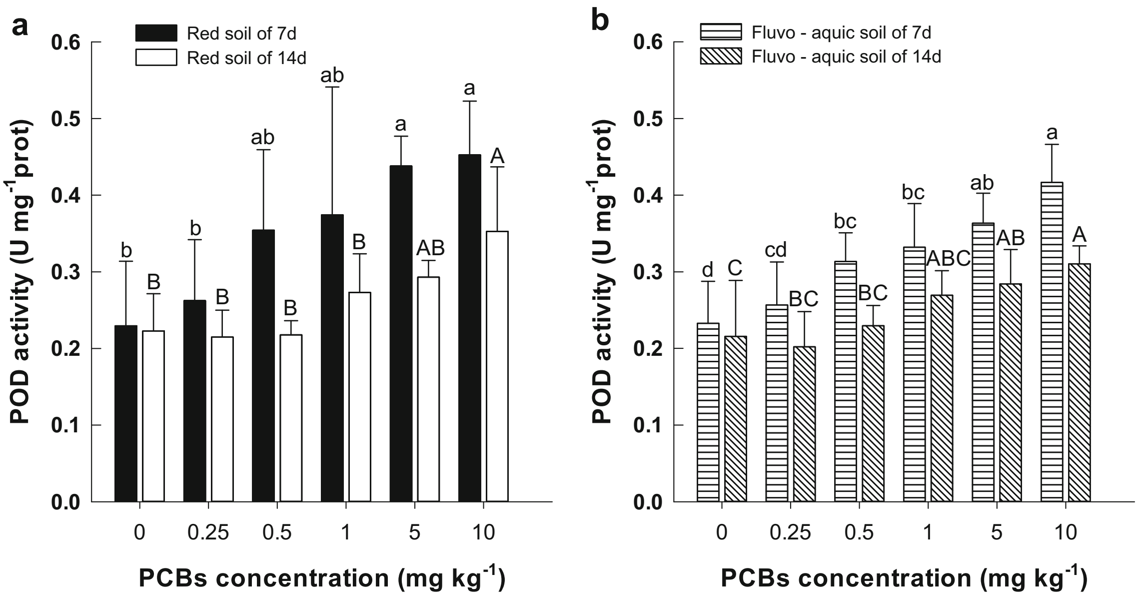

Fig. 4 Effects of polychlorinated biphenyls on guaiacol peroxidase (POD) activity in *Eisenia fetida*. **a** Red soil. **b** Fluvo-aquic soil. The results of one-way ANOVA are presented in the *graphs*. *Bars with different lowercase* and *uppercase letters* indicate statistically significant differences between treatments at 7 and 14 days of treatment, respectively (LSD test, $P < 0.05$)

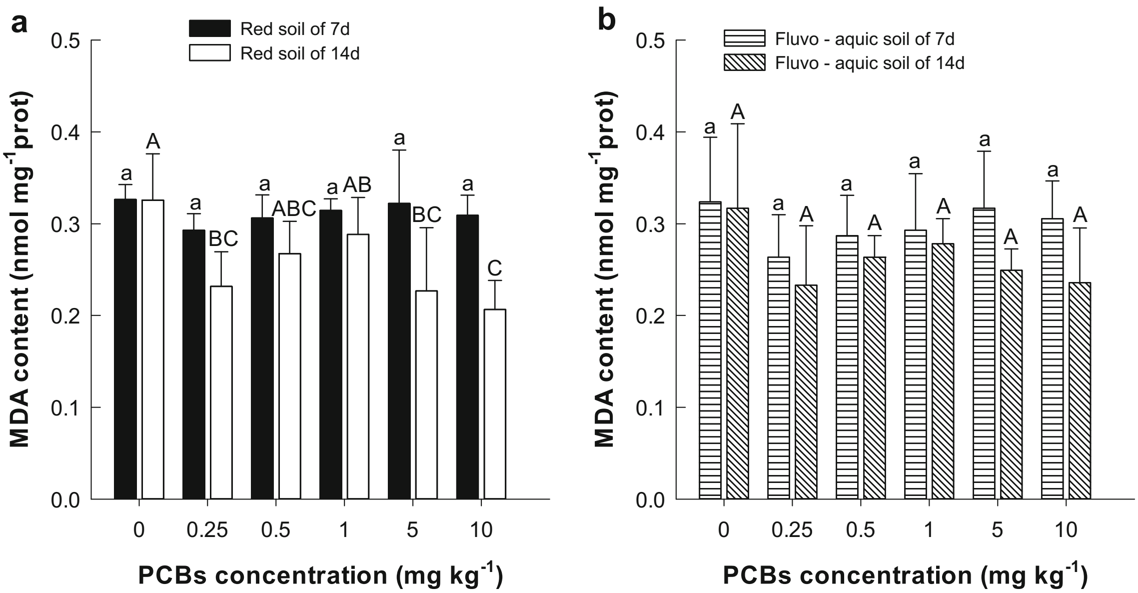

Fig. 5 Effects of polychlorinated biphenyls on malondialdehyde (MDA) content in *Eisenia fetida*. **a** Red soil. **b** Fluvo-aquic soil. The results of one-way ANOVA are presented in the *graphs*. *Bars with different lowercase* and *uppercase letters* indicate statistically significant differences between treatments at 7 and 14 days of treatment, respectively (LSD test, $P < 0.05$)

POD in eliminating H_2O_2; thus, POD would be more highly stimulated by H_2O_2 accumulation than CAT at higher PCB concentrations (Song et al. 2009; Duan et al. 2015).

MDA is formed when free radicals interact with unsaturated fatty acids in cellular membranes and MDA content can be used as a sensitive index for cellular oxidative injury (Martin-Diaz et al. 2009; Xue et al. 2009). However, few reports have examined the effects of PCBs on MDA levels in *E. fetida*. In our study, we found that the MDA content in earthworms remained stable, with the exception of those incubated in PCB-contaminated red soil for 14 days (Fig. 5). These results are likely due to the significant activation of antioxidant enzymes (e.g. SOD, CAT, and POD) observed in the same worms. These enzymes alleviate oxidative stress, and thereby were likely to have limited the accumulation of MDA in the earthworm tissues (Schmitt et al. 2007). After increased periods of PCB exposure in red soil, the earthworms were able to tolerate PCBs, as the increased antioxidant enzyme levels effectively reduced the levels of free radicals, which would in turn reduce MDA contents (Hao et al. 2014).

The comet assay is widely used to assess genotoxicity (González-Mille et al. 2010). We found that exposure to PCBs resulted in significantly elevated genotoxicity in *E. fetida*, even at the lowest concentration tested (0.25 mg kg^{-1}), in both soil types examined, with no significant difference between soil types (Fig. 1). Furthermore, the genotoxic response significantly increased with higher PCB concentrations and longer exposure times. Therefore, this response was relatively stable and sensitive, and can be regarded as a robust biomarker.

According to PCB surveys performed in the regions where soil was collected, the PCB concentrations used in this research ranged from concentrations (0.25 mg kg^{-1}) that were similar to those typically found in the environment (0.13 or 0.37 mg kg^{-1}), to concentrations that would be expected to result in the acute toxicity (Li et al. 2010; Hou et al. 2013; Teng et al. 2015). Thus, the laboratory tests were relevant to environmental concentrations and provide a representative precise assessment of the potential risks imposed by PCBs in the soil environment. Xenobiotics can induce oxidative stress in numerous species, which induces the production of reactive oxygen species (ROS), the activation of the antioxidant defense system (i.e. the biosynthesis of SOD, CAT, or POD), and oxidative damage, including the accumulation of the lipid peroxidation product malondialdehyde (MDA) due to ROS accumulation (Chen et al. 2017). Excessive ROS can permanently alter or damage DNA as well as lipids and proteins (Song et al. 2009). Our analyses showed that the biomarkers had varied sensitivities to PCB concentrations; the sensitivity ranking was as follows (in increasing order): MDA content < POD activity < percentage of weight variation < CAT activity < SOD activity < DNA damage. Significant increases in the DNA damage were induced in the earthworms, even at the lowest concentration of chemical tested (Fig. 1), while the MDA content was not changed much (Fig. 5). Therefore, DNA damage was the most sensitive parameter, which was in accordance with a previous research that DNA damage in earthworms could provide clear evidence of environmental impacts (Espinosa-Reyes et al. 2010). And the MDA content was the least sensitive biomarker. Similar results were found in earthworms exposed to benzo[a]pyrene (BaP)-contaminated soil (Duan et al. 2015).

When the earthworms were placed in soil not contaminated with PCBs, their biomarkers, including MDA contents and DNA damage, did not differ significantly between soil types, while the weight variation of the earthworms was significantly different. Furthermore, the difference in weight variation between earthworms maintained in the two soil types was similar for all PCB concentration levels. Three-way ANOVA analysis showed that soil type had a significant effect on the percentage of weight variation ($P < 0.001$), CAT activity ($P < 0.001$), and SOD activity ($P < 0.05$) (Table 3). These differences might be due to the different characteristics of the soil, such as pH and organic matter content. Soil pH might influence the conformation of natural soil organic matter, and soil organic matter might alter the affinity of the pollutant, which may in turn influence the bioavailability of PCBs in the soil and alter the toxicity of PCBs to earthworms (Hofman et al. 2008). However, the bioavailability of PCBs in these two natural soils has not been analyzed, and more research is needed to elucidate the underlying toxicity mechanisms.

Prolonging the duration of PCB exposure had significant effects on the percentage of weight variation ($P < 0.001$), POD activity ($P < 0.001$), CAT activity ($P < 0.001$), SOD activity ($P < 0.001$), and MDA content ($P < 0.001$) (Table 3). In agreement with these findings, Shi et al. (2013) showed that prolonged exposure to phenanthrene altered the activity of antioxidant enzymes in the earthworm's body. As ROS are eliminated after prolonged exposure to a toxin, the antioxidant enzyme activities also decrease.

Conclusions

PCBs induce physiological responses and genotoxic changes in *E. fetida* in natural soils. SOD activity and DNA damage were the most sensitive parameters of toxicity, and thus, can be used as early response biomarkers for toxicity assessment. The soil properties had significant effects on the percentage of weight variation of earthworms as well as on CAT and SOD activities. The exposure duration and PCB concentrations also significantly influence the toxic responses. In conclusion, when exploring the toxic effects of PCBs on earthworms (*E. fetida*) in natural soil, the duration of exposure and the characteristics of the soil should be considered.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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