Survival, growth, detoxifying and antioxidative responses of earthworms (*Eisenia fetida*) exposed to soils with industrial DDT contamination

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ABSTRACT

The survival, growth, activity of the biotransformation system phase II enzyme glutathione-S-transferase (GST) and the oxidative defense enzyme catalase (CAT) of earthworms exposed to the contaminated soils from a former DDT plant and reference soils were investigated, and compared with the corresponding indicators in simulated soil-earthworm system, unpolluted natural soils with spiked-in DDT series, to identify the toxic effects of DDT on earthworms and their cellular defense system in complex soil system. The results indicated that DDT level in the contaminated soils was significantly higher than that in the reference soils with similar level of other pollutants and soil characters. The mortality, growth inhibition rates, GST and GST activities of earthworms exposed to the contaminated soils were significantly higher than that in reference soils. The contribution of historical DDT in contaminated soils to earthworms was confirmed by the DDT spiked tests. DDT spiked in soils at rates of higher than 200 mg·kg⁻¹ was significantly toxic to both the survival and the growth of earthworms. DDT significantly stimulated GST and CAT activity in earthworms after 14 days. The CAT and GST activities were also stimulated by DDT exposure at rates of 100 mg·kg⁻¹ after chronic exposure (42 days). The results provide implications for validating the extrapolation from laboratory simulated soils criteria to contaminated soils and for making site risk assessments.

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1. Introduction

1,1,1-Trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) was the first widely used synthetic pesticide and was liberally applied around the world for decades due to its high efficiency and low cost. Their inadvertent use and strong tendency to bioaccumulate has caused considerable harm to ecosystems, and its use was prohibited beginning worldwide in 1972 [1]. However, because of their extreme persistence, exposure to DDT remains widespread [2–4]. Inputs to ecosystems continue, both by illegal use and the continuing production and application in some countries such as India and in Africa as a very effective antimalarial. DDT even predominate over all organic chemicals among all matrices, especially in soils [5]; current levels in some biotic tissues can be comparable to the high levels found 30 years ago [6]. So the hazard of DDT in the ecosystem is not negligible.

Assessing risk from DDT-contaminated soils is particularly challenging since a mixture of chemicals including pesticides, industrial products and heavy metals is generally present and the bioavailability

varies with the soil characteristics [7]. The risk of DDT in contaminated soils could be assessed more quantitatively by identifying the responses of biomarkers to DDT in both a simplified simulated ecosystem and the complex soils themselves.

Earthworms play a key role in biogeochemical processes in soil ecosystems. Biomarkers for assessing the toxicity of chemicals on earthworms such as the survival, growth and reproduction are reliable, however, a great portion of these have been tested in simplified paper contact or artificial soils [8–13] and may lack ecological significance; so that it is essential to conduct tests with contaminated natural soils to allow the laboratory data to be interpreted and extrapolated to the contaminated field.

Organisms have defense mechanisms to protect the cell from the toxin or to defend it against adverse effects. Biochemical response capacities of defense system enzymes such as biotransformation system phase II enzyme glutathione-S-transferase (GST), the antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) provide results that are sensitive, informative, reproducible and can indicate potential toxicity in organisms such as fish, bivalves, aquatic worms, African common toad, mussels exposed to toxicant [14–17]. Some evidences indicate that the induction of the CAT activity is considered valid biomarkers of ambient oxidative pollutants in earthworm toxicology [18–20]. GST activity has been used as a

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potential biomarker of earthworms exposed to heavy metals [21] and pesticides [22] since GST is a detoxification enzyme involved in both the conjugation of glutathione with xenobiotic and antioxidative defenses [23]. However, the response activity of GST and CAT of earthworms exposed to soils with historical DDT contamination are unclear.

In this paper, the survival, growth, activities of GST and CAT of earthworms exposed to the contaminated soils surrounding a former DDT plant were investigated, and compared with the corresponding indicators in simulated soil-earthworms system, unpolluted natural soils with spiked-in DDT series, to identify the toxicity effects of DDT on earthworms and their cellular defense system in real soil system. The results provide implications for validating the extrapolation from simulated soils criteria to natural soils and site risk assessment.

2. Materials and methods

2.1. Tested earthworms, chemicals, and soils

Mature earthworms (*Eisenia fetida*) at 3 months old with a well-developed clitellum were obtained from a farm in Beijing in which they were fed by animal manure. They were acclimatised for 48 h with the natural soils before test.

The DDT (95% purity) used in this study was produced at the Tianjin Chemical Plant, China.

Natural soils for the earthworms' exposure test with spiked-in DDT were collected from Yanghe catchment in Hebei province, China. The physico-chemical characters are as follows: pH = 8.05, TC = 2.96%, TN = 0.05%, TP = 0.70 g·kg⁻¹. The soils were regarded as *unpolluted* with undetectable DDT, HCH and heavy metals.

DDT contaminated soils were collected from barren sites in a former DDT plant in Hebei province, China. Soils from eight sites inside the plant were collected as the *contaminated soils*, and eight sites surrounding the plant as the *reference soils*. Ten subsamples from each of the sampling sites were collected at the soil depth of 0–20 cm. All subsamples were thereafter pooled to obtain one representative sample of about 1.5 kg for each site.

Samples of 50 g were homogenized by sifting them through steel mesh (2-mm grid size), freeze dried at –20 °C, and then stored at –4 °C until being analyzed by chemical analysis. All the other soils left were air-dried, homogenized by sifting through steel mesh (2-mm grid size) and kept in PVC bags for the earthworms' exposure test.

2.2. Earthworms exposure protocol

Earthworms were exposed in unpolluted soils spiked with series concentrations of DDT. The soils moisture contents were adjusted to 35% of the basic substrate's dry weight by adding deionized water. DDT was dissolved with 2 ml acetone and thoroughly mixed into the soils at concentrations of 50, 100, 200, and 400 mg·kg⁻¹ dry soil. The glass containers had been allowed to evaporate for a minimum of 1 h in an exhaust hood to ensure that the acetone had completely evaporated. Three replicates were used for each dose. Ten adult earthworms were introduced into a glass container with 520 g of wet soils. The containers, which were covered with perforated transparent membrane to access to light, limit the evaporation of moisture and prevent the worms from escaping, were kept in an incubation chamber (20 ± 1 °C, 70–90% relative humidity with continuous illumination at 400–800 lx) throughout the test period. From days 15 to 42, additional food (finely ground and dried cow dung, 0.5 g cow dung per worm) was added once a week to every container. The same procedure, but with acetone and deionized water, was used as solvent control and control. During the test period earthworms were separated from the test substrate, counted, cleaned with deionized water, and weighed on days 7, 14, 28 and 42. All the replicates were examined on each sampling date and the worms were then put back to the containers.

Earthworms were also exposed to DDT contaminated soils and reference soils. 10 earthworms were introduced into a glass container with 520 g wet soils from each site. Four replicates were used for each of the sites. The exposure protocol was the same as that in those of spiked test except that no DDT were added. Earthworms were separated from the test substrate, counted, cleaned with deionized water, weighed, and then returned to the containers on days 7, 14, 28 and 42.

2.3. Mortality and growth inhibition of earthworms

The mortality was determined by counting the number of dead earthworms. Earthworms were considered dead if they lacked movement and did not respond to a definite tactile stimulus to the anterior end. Because earthworms tend to disintegrate quickly after death, they were considered to have died if they were missing.

The weight of earthworms in each dose group reported from the various exposure periods was then used to compute the growth inhibition as follows:

$$GI_n = \frac{(W_0 - W_t)}{W_0} \times 100\%, \quad (1)$$

where GI_n is the growth inhibition for dose group n , W_0 is the weight on day 0 and W_t is the weight after t days of exposure.

2.4. Enzyme activities

Three individuals from each replicate were picked up randomly, gut cleaned and ground in liquid nitrogen. Samples were then thawed on ice and homogenized in TRIS buffer (100 mM, pH 7.5) in a 0.25 w/v ratio at 4 °C for 1 min at 12,000 rpm. The homogenate was centrifuged at 9000 g for 30 min.

CAT activity was measured as described in Claiborne [24] and Saint-Denis et al. [25,26]. The variation of absorbance at 240 nm due to the dismutation of H₂O₂ was measured. Assay conditions were as follows: 67 mM Tris buffer, pH 7.4, 15 mM H₂O₂ and 100 µg sample proteins in 3 ml incubate. Incubation was carried out at 25 °C and the reaction was initiated by the addition of H₂O₂.

GST activity was measured using the method described in Saint-Denis et al. [25,26]. The assay was conducted by monitoring the appearance of the conjugated complex of CDNB and GSH at 340 nm over time. Assay conditions were as follows: 100 mM Tris buffer, pH 7, 2 mM GSH, 1 mM CDNB, 100 µg sample proteins in 3 ml incubate. The reaction was conducted at 25 °C and initiated by the addition of GSH.

Protein content was assayed according to Bradford [27] with BSA as the reference substance.

2.5. Chemical analysis

The soil samples were analyzed for DDTs and HCHs, the details were given in Shi [28]. In brief, samples of 10 g of freeze-dried soil were mixed with 25 ml of hexane/acetone (1:1), and then were shaken in an ultrasonic bath for 20 min. The extracts obtained were concentrated for subsequent cleaning, transferred to a Florisil column, and eluted with an 80 ml eluting mixture (15% acetone/hexane). The eluates were then concentrated and made into a final volume of 1 ml with hexane for gas-chromatographic analysis. The levels of DDTs (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD) and HCHs (α -HCH, β -HCH, γ -HCH and δ -HCH) were measured by a HP 6890 GC-ECD. Recovery from the soils for chlorinated organics ranged from 95.4–99.2% for DDTs to 92.6%–97.6% for HCHs, and the detection limits were between 0.06–0.37 ng g⁻¹ d.w. for DDTs and 0.06–0.15 ng g⁻¹ d.w. for HCHs.

The samples were analyzed for metals and the details were given in Xu [29]. Briefly, total concentrations of Cd, Cu, Ni, Pb, Cr and Zn in the digestion solution were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The digestion for arsenic (As)

determination was done using aqua regia (HCl: HNO₃, V/V, 3:1). The analytical precision was within 10% variability.

Contents of soil Total Carbon (TC) and Nitrogen (TN) were detected by the Universal CHNOS Elemental Analyzer (Elementar vario EL III, Germany). Total Phosphorus (TP) concentrations were determined colorimetrically using the molybdate method following H₂SO₄–HClO₄ digestion. Soil moisture content was determined from 20 g of fresh soil (n = 3) at 105 °C for 24 h. Soil pH was measured in deionised water (1:2.5, soil:water) using a pH electrode.

2.6. Statistical analysis

LC₅₀ values were computed using Probit Regression modeling for the mortality data. The data for growth inhibition and enzyme activity were subjected to a one-way analysis of variance (ANOVA) using the LSD post hoc pairwise multiple comparison procedure for contrast of the differences among treatment means. The Shapiro-Wilk test and Levene's test were used to ensure the normality assumption and the homogeneity of variances, respectively. Logarithmic transformation was applied to the dependent variables where necessary. The Kruskal-Wallis H test was used to compare the differences of pollutants and soil physico-chemical characteristics between the contaminated and the reference sites since the variables could not represent normality and homoscedasticity even after transformation. A Pearson correlation analysis was done to determine the relationship between DDTs and mortality and growth inhibition rates. Results with P less than 0.05 were considered significant. All statistics were performed using the SPSS® software (SPSS® Inc., 2010).

3. Results

3.1. Earthworms exposed to contaminated soils

3.1.1. The physico-chemical characteristics and residue levels of pollutants in contaminated and reference soils

The sites description and soil characteristics for both the contaminated and the reference soils are shown in Table 1. The soil physico-chemical characteristics between the contaminated and the reference sites were similar, no significant differences existed for the soils (pH, Chi-square = 0.750, P = 0.627; TC, Chi-square = 0.750, P = 0.627; TN, Chi-square = 0.500, P = 0.964; TP, Chi-square = 0.750, P = 0.627).

In the contaminated natural soils, DDTs co-existed with HCHs and metals. The residue levels of DDTs, HCHs and metals in soils in the contaminated sites and the reference sites are shown in Fig. 1A and B respectively. By comparing with the guide value of Environmental Quality standards for soils (GB 15618-1995), the main pollutants in

Fig. 1. The Cumulative distribution frequency of pollutants residual level in soils of DDTs plant area. A) contaminated sites; B) reference sites. Note: The dot lines mean the guide values (Environmental Quality standards for soils) of the corresponding pollutants with the same color.

the soils were screened out as Cd and DDT, with the rates of residue level exceeding the guide value at 100% and 87.5%, respectively.

There was no significant difference between the residue levels of Cd in the two groups ($P > 0.05$), so do all the other pollutants.

DDTs level in the contaminated sites was significantly higher than that in reference sites ($P < 0.05$). *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT were the main residue of DDT family in the contaminated soils, with the average percentage of 43.9%, 23.4% and 23.7% respectively, while

Table 1

The sites description and selected soil characters in soils from the former DDTs plant area.

Sample groups	Site no.	Historical landuse	Present landuse	pH	TP(g·kg ⁻¹)	TN(%)	TC(%)
Contaminated soils	H1	DDT storage unit	Industrial barren land	7.67	0.61	0.08	3.12
	H2	DDT production unit	Industrial barren land	8.11	0.63	0.14	3.90
	H3	Dicofol production unit	Industrial barren land	8.50	0.74	0.09	1.95
	H4	Waste storage area	Industrial barren land	8.43	0.61	0.14	4.24
	H5	Dicofol storage plant	Industrial barren land	8.83	0.76	0.17	6.40
	H6	Waste storage area	Industrial barren land	9.55	0.51	0.07	4.83
	H7	Waste storage area	Industrial barren land	7.64	0.75	0.10	4.40
	H8	Waste storage area	Industrial barren land	8.51	0.69	0.16	4.01
Reference soils	H9	Office and residential area	Farmland	8.48	0.64	0.12	2.74
	H10	Office and residential area	Farmland	8.56	0.68	0.10	2.09
	H11	Office and residential area	Farmland	8.49	0.78	0.11	3.52
	H12	Farmland	Farmland	8.50	1.02	0.13	5.43
	H13	Farmland	Farmland	8.46	0.85	0.10	2.43
	H14	Farmland	Farmland	8.43	0.68	0.09	2.52
	H15	Farmland	Farmland	8.52	0.55	0.08	2.26
	H16	Farmland	Farmland	8.53	0.80	0.08	2.43

less *p,p'*-DDT (8.57%) and more *p,p'*-DDD, *p,p'*-DDE were detected in the reference sites.

3.1.2. Mortality of earthworms

The mortality of earthworms exposed to the soils collected from the areas of pesticide plant after 7, 14, 28 and 42 days are shown in Fig. 2. During the acute exposure period (7 days and 14 days), almost all the earthworms survived in the soils in reference sites, in which the DDT remnant level ranged between 0.09–6.70 mg·kg⁻¹. In 50% of the soils in the contaminated sites, with DDT ranging from between 3.11–23.80 mg·kg⁻¹, the mortality of earthworms was zero, while in the other 4 sites (DDT ranging from between 3.11–287.43 mg·kg⁻¹) the mortality level was between 3.33–53.3%.

During the chronic exposure period (28 days and 42 days), the mortality of earthworms remained the same as that in the acute period, except that in site H2, the mortality of earthworms increased from 53.3% (14 days) to 93.3 (28 days) and 100% (42 days), respectively. A significant positive correlation was shown between the DDT residue level in the soils from the contaminated sites and mortality of earthworms exposed to such soils at 14, 28 and 42 days (14 days, $R = 0.977$; 28 days, $R = 0.985$; 42 days $R = 0.985$).

3.1.3. Growth inhibition of earthworms

The growth inhibition rates of earthworms exposed to soils in the pesticide plant area at 7, 14, 28, and 42 days are shown in Fig. 3.

During both the acute and chronic exposure period, the growth inhibition rates of earthworms exposed to soils in reference sites were almost all negative, except in H15 and H16 in chronic period, the growth inhibition rates were around 3% and 7% in 28 and 42 days respectively, which proved that the earthworms grew well in the reference soils.

The growth inhibition rates in sites H1, H2, H3 and H4 were almost all positive, ranging between 2.44%–48.96%, and increasing with the increase of exposure time. The growth of earthworms were inhibited in some of the contaminated sites which had higher DDT residue levels. The growth inhibition rates of earthworms in site H7 increased from 18% at day 7 to 25% at day 14, but decreased to 14.2% at day 28 and 11.5% at day 42. The growth of earthworms were inhibited in site H7 but the inhibition in chronic period was less than that in acute period. In the other 3 contaminated sites, the growth inhibition rates were negative but higher than those in reference sites.

The inhibition rates in soils in the contaminated sites were significantly higher than those in the reference sites (ANOVA, 7 days, $F = 7.328$, $P = 0.018$; 14 days, $F = 5.646$, $P = 0.034$; 28 days, $F = 7.614$, $P = 0.016$; 42 days, $F = 9.209$, $P = 0.010$); The growth inhibition rates of earthworms at 7, 14 and 28 days were significantly correlated

Fig. 2. Mortality of earthworms exposed to soils in the contaminated and reference sites.

Fig. 3. Rates of growth inhibition in earthworms exposed to soils in the contaminated and reference sites.

with the residue level of DDT in soils (7 days, $R = 0.518$, $P = 0.040$; 14 days, $R = 0.509$, $P = 0.044$; 28 days, $R = 0.535$, $P = 0.033$; 42 days, $R = 0.229$, $P = 0.411$).

3.1.4. Enzyme activities in earthworms

The character description of CAT and GST activities in earthworms exposed to soils in the contaminated and reference sites at 14, 28, and 42 days are shown in Fig. 4 A and B.

The CAT activities of earthworms during the chronic exposure (42 days) in the contaminated sites were significantly higher than those in reference sites (ANOVA, $F = 13.133$, $P = 0.003$), however no significant difference between the two groups during the short exposure time (14 days, $F = 1.399$, $P = 0.257$; 28 days, $F = 0.565$, $P = 0.466$) were observed. The stimulation of CAT activity in earthworms by DDT contamination may occur after long time exposure.

During both the acute and chronic exposure period, the GST activities in earthworms in the contaminated sites were significantly higher than those in reference sites (ANOVA, 14 days, $F = 6.951$, $P = 0.020$; 28 days, $F = 8.803$, $P = 0.011$; 42 days, $F = 28.183$, $P = 0.000$), and the corresponding DDT residue level in the contaminated sites was also significantly higher than that in reference sites, an induction of GST activity in earthworms by DDT contamination in soils may occur.

3.2. Earthworms exposed in soils spiked with DDT

3.2.1. Mortality of earthworms

Mortality of the earthworms exposed to soils spiked with DDT during acute and chronic periods is presented in Fig. 5. All worms survived in the (solvent) controls and at treatments with DDT concentrations 50 mg·kg⁻¹, while few survivors were found at the treatments at DDT concentrations of 200 and 400 mg·kg⁻¹. Mortality increased with the increase of DDT concentration and exposure time, the mortality levels for 28 days being 90% and 100% after exposure to DDT at 200 and 400 mg·kg⁻¹, respectively.

The LC₅₀ values were calculated in this study to depict the acute and chronic lethal effects of DDT on earthworms. The acute toxicity of DDT to earthworms after 14 days exposure was higher than the chronic toxicity after 42 days exposure, with median lethal concentrations (LC₅₀) of 273.8 and 146.4 mg·kg⁻¹, respectively.

3.2.2. Growth inhibition of earthworms

The growth inhibition rates of earthworms at 7, 14, 28, and 42 days for the DDT are shown in Fig. 6. The growth inhibition rates for DDT appeared to be time-dependent, with longer exposure duration resulting

Fig. 4. CAT and GST activities in earthworms exposed to soils in the contaminated and reference sites.

in higher inhibition. Growth inhibition rates of earthworms exposed to DDT-spiked soils were also significantly higher than those of the controls. The decrease in weight was shown to be dose-dependent over both the acute (7, 14-day) and chronic (28, 42-day) exposure period. This was confirmed by ANOVA test ($P = 0.009$ for the 7-day, $P = 0.20$ for the 14-day exposure, $P = 0.006$ for the 28-day exposure and $P = 0.007$ for the 42-day exposure). The growth inhibition of earthworms treated with the higher doses (200 and 400 mg·kg⁻¹) were significantly higher than those for treatments at the controls and

Fig. 5. The mortality of earthworms (*Eisenia fetida*) exposed to the soil spiked with DDT.

Fig. 6. The growth inhibition rates of earthworms (*Eisenia fetida*) exposed to the soils spiked with DDT.

lower concentrations (50, 100 mg·kg⁻¹) in both the acute and chronic exposure periods (7 days, 28 days and 42 days) (LSD test, $P < 0.05$).

3.2.3. Enzyme activities in earthworms

The CAT and GST activities in earthworms after 14, 28, and 42 days exposures to DDT are presented in Fig. 7 A and B. DDT significantly induced CAT activity in earthworms after 14 days exposure (ANOVA, $F = 5.553$, $P = 0.007$). Post-hoc comparison indicated that CAT activity was significantly increased at the concentration of 100 and 200 mg·kg⁻¹ DDT (LSD, $P < 0.05$). However, after a 28-d exposure, there were no significant (ANOVA, $F = 1.417$, $P = 0.307$) changes observed in the activity of CAT between the controls and DDT treatments. At the chronic exposure time (42 days), the CAT activities were also stimulated by DDT (Chi-square = 7.615, $P = 0.022$), significant increase was shown at treatment of 100 mg·kg⁻¹ ($P < 0.05$) compared with controls and lower dose DDT (50 mg·kg⁻¹) treatment.

Fig. 7. The CAT and GST activities in earthworms (*Eisenia fetida*) exposed to the soils spiked with DDT.

DDT significantly stimulated GST activity in earthworms after 14 days (ANOVA, $F = 3.411$, $P = 0.038$). Post-hoc comparison indicated that GST activity was significantly increased at 50, 100 and 200 $\text{mg} \cdot \text{kg}^{-1}$ DDT (14 days) (LSD, $P < 0.05$). After chronic exposure (42 days), GST activity of earthworms treated by 100 $\text{mg} \cdot \text{kg}^{-1}$ DDT was significantly induced (LSD, $P < 0.05$).

4. Discussions

4.1. Identification of DDT contribution to earthworms in contaminated soils

In the former DDT plant, DDT had been the main product during the 1968–1982 periods until production was banned in 1983. Dicofol, product made from DDT, became the alternative product until 2007 when the plant was closed. The soils in the plant were contaminated by the production and storage of DDT and dicofol. The DDT residue level in the contaminated soils was significantly higher than that in the reference sites, and both DDT and its metabolites DDD and DDE co-existed. These results are in agreement with other reports regarding industrial sites as the most contaminated lands [30].

The lethal, growth inhibition, stimulation of CAT and GST activity in earthworms might be the integrated responses to all the pollutants in both the contaminated and reference sites, however, the two soil groups presented similar soil characteristics, HCH and metal pollution, but distinguished by different levels of DDT, the different responses of earthworms in the two groups could be subjected to the difference of DDT. The contribution of aged DDT contamination in soils to the survival, growth, antioxidative and detoxifying mechanism in the earthworms is also confirmed by the exposure test in the soils with spiked DDT.

The DDT residue level was also the baseline for setting the dose in the spiked test, so that the tests could be of ecological significance by simulating the real exposure scenario and distinguishing the combined effects of other co-existing pollutants in the real ecosystem.

4.2. Mortality and growth inhibition rates of earthworms exposed to contaminated soils

The mortality of earthworms exposed to DDT contaminated soils was comparable to those of the exposure test in natural soils with spiked DDT. In both the tests, DDT in concentrations lower than 100 $\text{mg} \cdot \text{kg}^{-1}$ had minimal effects on the survival of earthworms. In site H2 with DDT residue level of 287.43 $\text{mg} \cdot \text{kg}^{-1}$, which is close to the LC_{50} (14 days, 273.8 $\text{mg} \cdot \text{kg}^{-1}$) in the spiked test, the mortality (14 days) was 53.3%. This result confirms the lethal effects of DDT in complex soil system.

When the earthworms were exposed to contaminated soils, the worms' growth was inhibited by DDT not only at concentrations higher than 200 $\text{mg} \cdot \text{kg}^{-1}$, which was proven by the DDT spiked test, but also at sites with DDT residue of 2.83–12.54 $\text{mg} \cdot \text{kg}^{-1}$. No inhibition was found in the reference sites with DDT residue at 0–2 $\text{mg} \cdot \text{kg}^{-1}$, which is contrary to that in the spiked test in which all the growth inhibition rates were positive. This indicates that the inhibition of DDT in the low levels may be offset by other parameters beneficial to the worm's growth such as higher organic carbon, or antagonism/neutralizing effects of DDT with Cd, Zn, Pb or other chemicals.

4.3. Mortality and growth inhibition rates of earthworms exposed to soils spiked with DDT

Earthworms live inside the soil for all their life cycle and are inevitably exposed to the pollutants in soils, since they directly ingest it. Mortality and growth rates are the two individual level indicators which are easy to detect and can be applied frequently for assessing the toxicity of pollutants. There is ample evidence showing that earthworms died and their growth was inhibited by exposure to pesticides such as lindane, deltamethrin and chlorpyrifos etc. [13,31,32].

In this research, the mortality of earthworms exposed to soils spiked with DDT increased with the increase of concentration and exposure time, which indicates that such high levels of DDT are dangerous to the survival of earthworms. The LC_{50} (14 days) at 273.8 $\text{mg} \cdot \text{kg}^{-1}$ was much lower than the corresponding value (484.76 $\text{mg} \cdot \text{kg}^{-1}$) in artificial soils which have comparable total organic carbon content (TOC) [11], which means that the lethal effects of DDT in natural soils is greater than that in artificial soils. The cause may be lower bioavailability of organic pollutants in artificial soils [33].

DDT spiked in natural soils at concentration of 200 and 400 $\text{mg} \cdot \text{kg}^{-1}$ significantly inhibited the growth of earthworms. The inhibition of the growth of earthworms was similar to the results of DDT in artificial soils [11] and was in agreement with those reported on other organic pollutants in artificial soils. For example, a significant growth inhibition of earthworm *Lumbricus rubellus* was seen when exposed to PAH pyrene [34]. The mechanisms for earthworms growth inhibition by exposure to high concentration of DDT may be result of reduced food intake to avoid the toxins and consuming more energy to combat deleterious effects of contaminants.

4.4. CAT and GST activity of earthworms exposed to contaminated soils

Reports have shown that many pesticides (DDE, endosulfan, atrazine etc.) would generate reactive oxygen species (ROS), including superoxide anion radical ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($\text{OH}^{\bullet}$) in living organisms including earthworms [35–38]. The excess of ROS led to the oxidative stress and the deleterious effects to the organism, which were alleviated in the presence of antioxidant defense enzymes [39,40].

CAT is an anti-oxidant enzyme and catalyzes the degradation of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) [41]. During the chronic exposure (42 days), the higher CAT activity in earthworms exposed to the contaminated soils than that in reference sites is probably due to enhanced production of ROS caused by higher DDT and its metabolites (DDD and DDE) level in soils, and the induction of CAT may reflect an adaptation of earthworms for the removal of an excess of ROS (e.g. H_2O_2).

GST play important roles in the detoxification of xenobiotic compounds by conjugating reduced glutathione (GSH) to electrophilic xenobiotics and their metabolites as well as endogenously producing electrophiles, it also participate in antioxidative defenses by conjugating breakdown products of lipid peroxides to GSH [14,42,43]. In the present study, we observed the higher GST activity in earthworms exposed to the contaminated soils than that in the reference sites during the whole exposure period, which may suggest the magnitude of detoxify responses of earthworms to the residue DDT and its metabolites in soils.

4.5. CAT and GST activity of earthworms exposed to soils spiked with DDT

In the present spiked test, the significant increase in CAT activity of earthworms exposed to DDT for 14 days indicated that DDT led to oxidative stress in the earthworms, and CAT activity was induced for the removal of incurred ROS in the first stage. There was no significant difference in CAT activity observed between DDT-treatment groups and the control in the 28 days of exposure, probably because the longer exposure time led to an adaptive mechanism induced by DDT. However, CAT activity was induced again after 42 days of exposure, it may be due to the ROS generated by the increasing concentration of DDE and DDD, the metabolites of DDT. The results were in agreement with previous reports of the up-regulating of CAT activity in macrophytes and mussels exposed to lindane and endosulfan [36,44].

In the laboratory simulated test the enhancing of GST activity in earthworms at specific concentrations was observed. It demonstrates that the exposure of earthworms to DDT activated its phase II enzymatic protection system. DDT might be used as a substrate for phase II enzymes in earthworms. GST catalyzes the conjugation of glutathione

with DDT. The enhanced GST activity could also suggest an effective protection against DDT by increasing its capacity to scavenge ROS. This was in accordance with previous studies which linked increased GST activity in *Eisenia fetida*, *Bufo regularis*, *laevis* tadpoles, aquatic worm *Tubifex tubifex*, zebra mussel with organochlorine pesticides, acetochlor, triclosan and medicine [15,17,45–47].

The results prove the contribution of aged DDT in soils to the activation of both detoxification and antioxidant enzyme in earthworms, and also suggest that the induction of CAT and GST activity in *Eisenia fetida* could be considered effective biomarkers of DDT in soils.

5. Conclusions

Soils in a former DDT manufacturing plant remained severely contaminated with historical burden of residues. The growth inhibition rates of earthworms exposed to the contaminated soils were positively correlated with the DDT residue level, so was their mortality. An induction of GST and CAT activity in earthworms by aged DDTs (DDT, DDD and DDE co-existed) in soils may occurred.

DDT spiked in soils were significantly toxic to the growth and survival of earthworms. DDT significant induced CAT and GST activity in earthworms after 14 days exposure. Significantly increase of CAT and GST activity was shown after 42 days exposure.

The contribution of historical DDT in contaminated soils to lethal effects, growth inhibition, the antioxidative and detoxifying mechanism in earthworms were proven by both the DDT spiked tests and the comparison with the reference soils. The survival, growth and cellular defense response of earthworms can be potential biomarkers for assessing the risk of historical DDT contamination in soils.

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