Environmental Science and Pollution Research (2018) 25:18938–18946
https://doi.org/10.1007/s11356-018-2065-4

RESEARCH ARTICLE

Biochemical and life cycle effects of triclosan chronic toxicity to earthworm Eisenia fetida

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Received: 22 January 2018 / Accepted: 17 April 2018 / Published online: 2 May 2018

Abstract

The study aimed at determining the response of adult *Eisenia fetida* earthworms to chronic exposure to triclosan (TCS) (10–750 mg kg^{-1}) in soil. TCS life cycle toxicity was evaluated by the means of survival, growth rate, and reproduction assessment. Biochemical responses including changes in the activity of antioxidative enzymes (catalase, superoxide dismutase, and glutathione reductase) and concentration of malondialdehyde (MDA) were determined. Significant reduction in the earthworm survival was observed only if the exposure to TCS was longer than 4 weeks. TCS reduced the growth rate of *E. fetida*; the weight of the fastest growing control individuals exceeded that for the slowest growing by factor of 2.56. Reproduction was the most sensitive life cycle parameter and was affected at the very low levels of TCS in the soil. The results showed that chronic exposure to TCS levels in the soil induced a significant increase in the activity of antioxidative enzymes and MDA concentration. Present study revealed that an integrated approach combining biochemical and life cycle endpoints would provide a more comprehensive assessment of the ecological effects of chronic TCS exposure on earthworms.

Keywords Antioxidative enzymes · Earthworm · Growth · Reproduction · Triclosan

Introduction

Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy)phenol) is a broad spectrum antimicrobial and antifungal agent widely used in industrial, medical, household, and personal care products. In recent years, there is an increasing concern about the effects of TCS on aquatic and terrestrial organisms as TCS has already been detected not only in the environment, but in living organisms and humans as well (Calafat et al. 2008; Guo and Iwata 2017).

Effluent discharge and sewage sludge/biosolid land application are the main routes for TCS to enter the environment. TCS has been detected in effluents across the world and TCS is listed among top 10 most commonly detected organic wastewater compounds for frequency and concentration (Brausch and Rand 2011). The efficiency of TCS removal from the wastewater depends on the wastewater treatment system, and commonly, the removal efficiency is higher than 90% (Bester 2003; McAvoy et al. 2002). Low levels of TCS are discharged with effluents to water bodies and pose a risk to aquatic biota. TCS hazard assessment based on hazard quotient (HQ) value showed that TCS in aquatic environment have the potential to cause chronic effects (Brausch and Rand 2011; Dann and Hontela 2011). Data from aquatic organism studies reviewed by Bedoux et al. (2012) showed that TCS mainly exhibit chronic toxicity to aquatic organisms and determined EC_{50} values were near TCS environmental concentrations.

As up to 30–50% of TCS during wastewater treatment sorbs to sludge and biosolids (Bester 2003; Waria et al. 2011), elevated levels of TCS in amended soils are observed (Chen et al. 2014). The possible pathways of TCS removal within the soil are biodegradation, photodegradation, volatilization, and leaching (Lozano et al. 2010). The biodegradation by microorganisms is the dominant removal mechanism and it occurs primarily under aerobic conditions. Biodegradation of

Capsule
Integrated approach combining biochemical and life cycle endpoints provide a more comprehensive assessment of the ecological effects of chronic TCS exposure on earthworms.

Responsible editor: Philippe Garrigues

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TCS in soils depends on environmental conditions and soil properties (such as temperature, soil pH, texture, and organic matter content). Half-lives of TCS in different soil types were reported to be between 2 and 58 days (Reiss et al. 2009; Wu et al. 2009), while Waria et al. (2011) reported TCS half-life of 421 days for a fine sandy soil type. In general, TCS is more persistent in the soil in anaerobic conditions and under field conditions rather than in laboratory incubation experiments (Ying et al. 2007). Land application of TCS containing sewage sludge or biosolids may negatively affect plant growth, soil respiration, and soil organisms (Reiss et al. 2009; Wang et al. 2014).

Earthworms can accumulate various pollutants from the soil as a result of direct soil contact and soil consumption. Bioaccumulation of TCS in earthworms in sewage amended soils is reported in laboratory (Higgins et al. 2011; Kinney et al. 2008) and field experiments (Macherius et al. 2014; Pannu et al. 2012). TCS has been shown to reduce survival and reproduction of earthworms (Amorim et al. 2010; Reiss et al. 2009; Schnug et al. 2013), induce oxidative stress and DNA damage (Lin et al. 2010, 2012, 2014), evoke weight loss, and alter metabolite content (Gillis et al. 2017). However, the majority of studies focused on short-term TCS toxicity and its bioaccumulation in earthworm. The aim of the study was to determine the response of the earthworms *Eisenia fetida* to chronic exposure to triclosan in the soil; the response of different *E. fetida* life cycle parameters was complemented with biochemical response analysis.

Materials and methods

Experimental design

The adult *E. fetida* earthworms with well-developed clitellum were taken from a breeding culture kept in the laboratory of Vytautas Magnus University. The selected earthworms (420 ± 116 mg) were acclimatized for 7 days. All procedures were carried out according to the modified OECD guidelines for the testing of chemicals (OECD 2004) with earthworm *E. fetida*.

The artificial soil composition was as follows (by dry weight): 70% quartz sand, 20% kaolin clay, and 10% *Sphagnum* peat. The soil organic matter content was 1.68 ± 0.04% and pH 6.40 ± 0.05. The constituents of artificial soil were air dried, mixed thoroughly, and weighted (500 g) into plastic containers. Soil was spiked with solutions of TCS (Alfa Aesar GmbH & Co KG) (TCS was solved in acetone) to obtain the final required water content (45–50% of the maximum water holding capacity) and TCS concentrations in soil. The concentrations of TCS in soil were (in mg kg^{-1} of soil): 10, 100, 250, 500, and 750. The highest tested concentrations were based on TCS effect concentrations reported in earlier studies (Amorim et al. 2010; Reiss et al. 2009; Žaltauskaitė and Miškelytė 2014); the lowest tested TCS concentration could be find in sewage sludge and soil (Chalew and Halden 2009). Each treatment was prepared in triplicate. Two controls were executed in parallel: only water added and a solvent (acetone) control. In further analysis, solvent control was used as no statistical significant differences were observed between solvent and water controls.

Ten earthworms were added to each covered container. Water content in each container was checked weekly. The earthworms were exposed to TCS for 8 weeks at 20 °C under constant light (600 lx). The earthworms were weekly supplied with oatmeal (approximately 0.5 g per earthworm). The unconsumed food was removed prior to resupplying a new portion. Survival and growth were measured on a weekly basis by counting and weighing the surviving earthworms in each container. The earthworms were returned to the same test soil. After 56 days of exposure, the soil was sorted twice to collect cocoons and juveniles. After the experiment, earthworms were removed from the soil, cleaned, and placed on moistened filter paper in Petri dishes for 48 h to void their gut content. After the depuration, the earthworms were frozen at − 80 °C until further analysis.

Biochemical assays

All procedures were carried out at 4 °C. Earthworm tissues were homogenized in prechilled mortar with pestle with potassium phosphate buffer (1:9 *w*/*v*, pH 7.8) containing 1 mM EDTA and 250 mM sucrose. The homogenates were centrifuged at 13000×*g* for 30 min at 4 °C and the supernatant was used for further analysis.

Protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as standard. The activity of catalase (CAT, expressed as nmol H_2O_2 mg $protein^{-1}$ min^{-1}) was measured by modified method described by Clairbone (1985). The enzyme extract, H_2O_2, and K phosphate buffer were mixed and the change in absorbance (240 nm) was recorded. Glutathione reductase (GR) activity was determined by measuring the decrease in the absorbance (340 nm) during NADPH oxidation and expressed as nmol NAPH oxidized mg $protein^{-1}$ min^{-1}. Superoxide dismutase (SOD) was measured using nitroblue tetrazolium according to Giannopolitis and Ries (1977) and was expressed as SOD units mg $protein^{-1}$. The content of MDA was estimated by formation of thiobarbituric acid reactive substances according to the method described by Mensah et al. (2012). The concentration of MDA was calculated using the extinction coefficient 1.56×10^5 M^{-1} cm^{-1}.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to assess the concentration effect on estimated life cycle and

biochemical endpoints. Significant differences between treatments were determined by Tukey's test and $p < 0.05$ was considered to be significant.

The probability of survival (S(t)) was estimated using a surviving model (Jager et al. 2011):

$$S(t) = \exp^{-H(t)}, \quad (1)$$

where H(t) denotes the individual's cumulative hazard at time t. It was presumed that the instantaneous hazard (probability to die) was constant with time and it was assumed to increase linearly with the concentration. H was estimated as follows:

$$H(t) = \lambda_0 t + \alpha c_w t, \quad (2)$$

where λ_0 is background hazard rate, c_w is external metal concentration in the soil, and α is slope parameter. The parameters of the model were estimated using a log-likelihood function.

Effective concentration (EC_{50}) values were calculated using the logistic dose-response model. Earthworm growth (measured as fresh weight) rate during the study period was determined by linear regression and the slope of the curve (b) was used as a prediction of growth rate (g week^{-1}). Significance of difference between the linear regression slopes of earthworm growth rate curves for different TCS concentrations was assessed using *t* test. All the statistical analysis was carried out using R version 2.15.2 (R Development Core Team 2004) and Statistica software.

Results

Low earthworm mortality was observed in the control, though it did not exceed the criteria of test validity (OECD 2004). No significant effect (ANOVA, $F < 1.37$, $p > 0.05$) of TCS on the survival of earthworms was found during the first 3–4 weeks of exposure (Fig. 1). Sub-chronic and chronic exposure to TCS (from the 4–5th week) reduced the earthworm survival, however only in the 100–750 mg kg^{-1} treatments. The sharper increase in mortality of earthworms was observed only from the 6–7th week of exposure at all treatment concentrations. Exposure to 500 mg kg^{-1} evoked the death of more than 20% of earthworms after 6 weeks of exposure, and at the end of the experiment, the mortality of earthworms reached 44.44%. Calculation of the LC_{50} values was not possible as the mortality at the highest test concentration did not exceed 50%. Fitted survival model has also proved that TCS had not posed a significant risk of the death of the earthworms (model parameters λ_0, α, and $\chi 2$ are presented in Fig. 1).

None of the tested TCS concentrations evoked weight loss during the exposure period and TCS had a significant effect on the final earthworms' weight (ANOVA, $F = 32.71$, $p < 0.01$) (Fig. 2). The earthworms exposed to TCS have reached lower final weight than control earthworms except for treatments with 100 and 500 mg kg^{-1} (Tukey, $p < 0.01$). As some differences in the temporal pattern of earthworms' growth exposed to different TCS concentrations were observed, earthworm growth rate (g week^{-1}) was calculated. Earthworm growth rate decreased with TCS concentration in the soil (Table 1). Comparison of earthworm growth rates (*t* test) throughout the experiment indicated significant differences between the treatments with TCS and control except for the treatment with 100 mg kg^{-1}. The smallest growth rate was detected in the treatment with 750 mg kg^{-1}, and at the end of the experiment, the weight of the fastest growing control individuals exceeded than that for the slowest growing by factor of 2.56.

As the significant TCS impact on the earthworm survival was observed only from the 4–5th week of exposure, we calculated the earthworm growth rate for 0–4 (early phase) and 4–8 (second phase) weeks of experiment. Some differences in the growth rate patterns were found between these two periods. In the early phase of the experiment (0–4 weeks), significant differences between the treatments with all TCS exposure concentrations and control were found, except for the growth rates in the treatment of 100 mg kg^{-1} (*t* test, $p = 0.93$). The growth rate during the early phase of the experiment showed the tendency to decrease along with TCS concentration ($R^2 = 0.64$, $p = 0.056$). Whereas in the second phase of the experiment (4–8 weeks), the growth rate of TCS-exposed earthworms did not differ from the control (except the 10 mg kg^{-1} treatment (*t* test, $p = 0.002$)) and no relationship between TCS concentration and growth rate was found ($R^2 = 0.01$, $p = 0.87$). Moreover, earthworms exposed to 10 and 250 mg kg^{-1} of TCS started to lose their weight during this phase of the experiment. The earthworms exposed to 500–750 mg kg^{-1} of TCS started to grow faster at the second phase of the experiment than during the early phase (0–4 weeks). Although the growth rate of earthworms in the 750 mg kg^{-1} treatment increased in the second phase, it remained very low. Generally, control earthworms and earthworms exposed to low levels of TCS (10–250 mg kg^{-1}) grew faster in the early phase of exposure and the growth rate decreased in the second phase (*t* test, $p < 0.05$). Earthworms exposed to higher TCS concentrations (500–750 mg kg^{-1}) grew faster in the second stage of exposure (4–8 weeks) than in the beginning of their exposure to TCS (0–4 weeks).

TCS had a significant effect on the cocoon production (ANOVA, $F = 23.55$, $p < 0.001$); however, post hoc (Tukey) comparisons indicated no significant differences between treatments (Fig. 3a). The lowest TCS concentration had no significant adverse effect on earthworm reproduction. The exposure to 100–750 mg kg^{-1} dramatically reduced the cocoon production by 79.46–99.31% ($R^2 = 0.51$, $p = 0.003$). The cocoon production rate EC_{50} was 35.41 ± 12.83 mg kg^{-1}. Juveniles were found only in the control and in the treatments with 10 and 100 mg TCS kg^{-1} (Fig. 3b). TCS had a highly significant detrimental effect on juveniles' hatching (ANOVA,

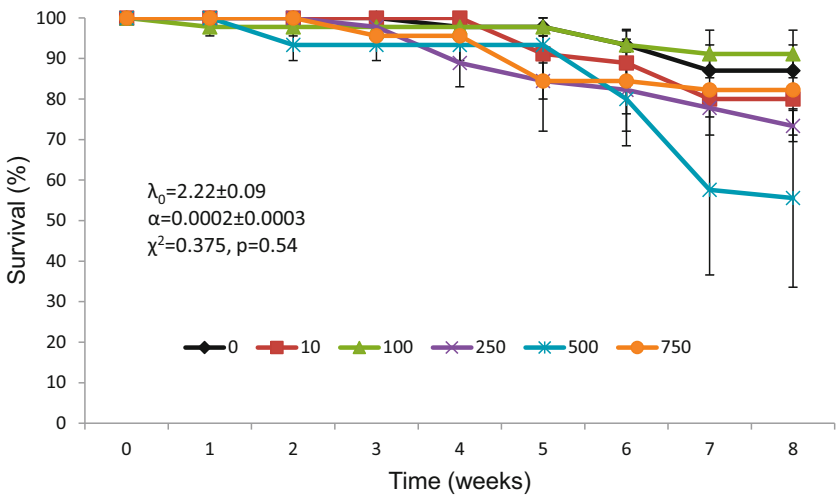

Fig. 1 Survival of earthworms *Eisenia fetida* exposed to triclosan in artificial OECD soil for 8 weeks

$F = 112.45$, $p < 0.001$), as juvenile number in the 100 mg TCS kg^{-1} treatment was by 8.14 times lower than in the control and no juveniles were found in other treatments with higher TCS concentrations.

The biochemical effects of TCS expressed as the changes in the activity of antioxidant enzymes (SOD, CAT, and GR) were analyzed (Fig. 4a–c). TCS had a significant effect on SOD activity (ANOVA, $F = 15.12$, $p < 0.01$) and SOD significantly increased after exposure to TCS at 10–250 mg/kg. The peak of SOD was observed in the 100–250 mg/kg treatment groups, reaching 3.92–4.22-fold level of that in the control earthworms. The level of SOD in the earthworms exposed to the highest TCS concentrations (500–750 mg kg^{-1}) was higher than in control earthworms though lower than in the earthworms exposed to lower TCS concentrations (Tukey, $p < 0.05$). The changes in CAT activity were very similar to that of SOD (ANOVA, $F = 7.09$, $p < 0.01$). The highest CAT activity in *E. fetida* was observed in the treatments of 100 and 250 mg kg^{-1}; the CAT activity was by 95.53 and 54.30% higher than that of controls. Further increase in TCS concentrations in soil led to a slight decrease in CAT activity in comparison with that in the 100–25 mg kg^{-1} treatments, though the level of CAT was still above the level of controls. No regular changes in the activity of GR were observed. An increase in GR was observed at all TCS exposure levels; however, the significant increase was observed only in the treatment with 750 mg kg^{-1} (Tukey test, $p < 0.05$). The GR activity was 249% of that of the control after exposure to the highest TCS concentration (750 mg kg^{-1}). TCS had a significant effect on the malondialdehyde (MDA) concentrations (ANOVA, $F = 8.97$, $p < 0.01$) (Fig. 4d). Content of MDA in the tissue of earthworms exposed to TCS was significantly

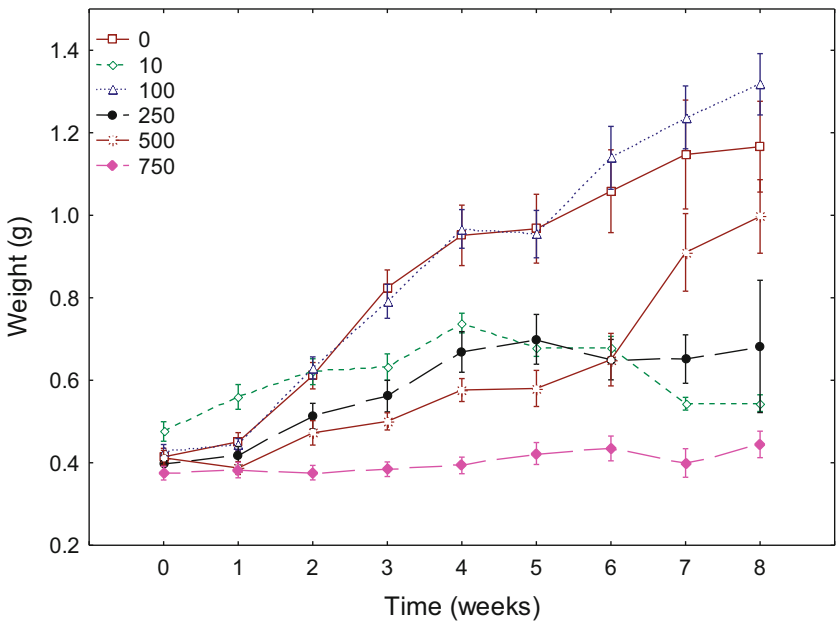

Fig. 2 Fresh weight of earthworms *Eisenia fetida* exposed to triclosan in artificial OECD soil for 8 weeks

Table 1 Estimated growth rate of *Eisenia fetida* exposed to triclosan (TCS) in artificial OECD soil for 8 weeks

TCS concentration, mg kg^{-1}	Growth rate, g week^{-1} (0–8-week period)	Growth rate, g week^{-1} (0–4-week period)	Growth rate, g week^{-1} (4–8-week period)
0	0.111	0.143	0.060
10	0.010*	0.059*	− 0.052*
100	0.120	0.141	0.096
250	0.046*	0.068*	− 0.003
500	0.056*	0.044*	0.097
750	0.008*	0.004*	0.009

*Growth rate significantly different from the control (determined by *t* test)

higher ($p < 0.05$) than in control earthworms by 4.24–7.46 times; however, no significant differences between TCS treatments were observed.

Discussion

Our results indicate that TCS do not evoke acute toxicity, but longer exposure to this compound may lead to lethal effects. It is consistent with the results of our previous study where no acute lethal TCS toxicity was observed (Žaltauskaitė and Miškelytė 2014). Reiss et al. (2009) reported that 14 days of exposure of *Eisenia fetida* to TCS at the range of 0–1026 mg kg^{-1} induced no lethal toxicity and did not disturb the growth of earthworms. The 14-day LC_{50} for *E. andrei* was estimated to be approximately 866 mg kg^{-1}, though the survival was significantly reduced only at the highest tested concentration (range 0–1080 mg kg^{-1}) and the NOEC was reported to be approximately 320 mg kg^{-1} (Amorim et al. 2010). A similar 14-day LC_{50} (541.89 mg kg^{-1}) for *E. fetida* was reported by Wang et al. (2015). Whereas, Schnug et al. (2013) determined the 28-day LC_{50} for *E. fetida* of 552 mg kg^{-1} in sandy loam soil. Unfortunately, we cannot compare the obtained chronic toxicity results as data of chronic TCS toxicity to earthworms are lacking and we have not determined LC_{50}. As more pronounced mortality of earthworms was observed only after 4–5 weeks of exposure, it may be presumed that only chronic exposure to TCS may result in reduced survival of *E. fetida*.

Single endpoints' studies, depending on endpoints' sensitivity and ecological relevance, might underestimate or overestimate the possible chemical effects on organisms. Therefore, the complex analysis combining low level (sub-organismal) measurements with life cycle parameters may give a better understanding of chemical impact on biota and enable better prediction of possible consequences at population level (Forbes et al. 2008; Spurgeon et al. 2005). Unlike the survival, TCS severely affected the other *E. fetida* life cycle parameters, such as growth and reproduction. TCS possible impact to earthworm growth rate was not studied previously and we cannot compare our results. Dramatic earthworm weight loss was observed after 24- and 48-h earthworm exposure to 0.0001–1 mg cm^{-2} TCS using filter paper contact test (Gillis et al. 2017). Decrease in *E. fetida* weight was also observed after their exposure to TCS for 7 days (Ma et al. 2017). In our study, the earthworms exposed to TCS grew slower and it resulted in lower final weight (Table 1, Fig. 2). Control earthworms and earthworms exposed to low levels of TCS (10–250 mg kg^{-1}) grew faster in the early phase of exposure (1–4 weeks) and their growth rate decreased in the second phase (4–8 weeks). It may indicate that sub-chronic exposure is insufficient to reduce significantly the growth rate and only more prolonged exposure may result in reduced growth rate. Other possible explanation of such phenomenon

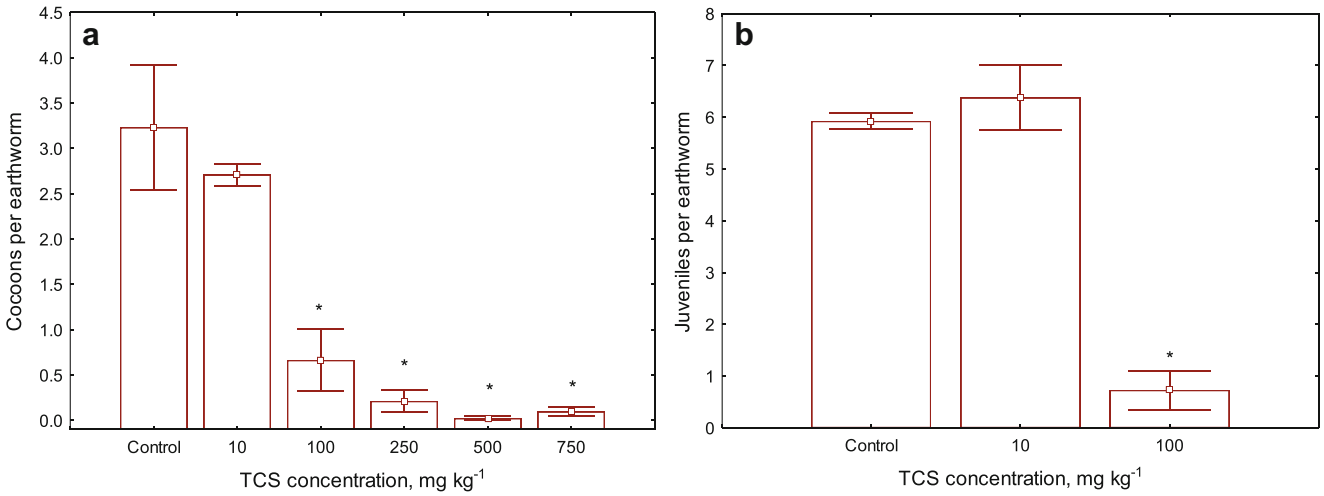

Fig. 3 Cocoon production by earthworms *Eisenia fetida* (**a**) and juvenile number per earthworm (**b**) exposed to triclosan in artificial OECD soil for 8 weeks. Asterisk * indicates significant differences from the control ($p < 0.05$)

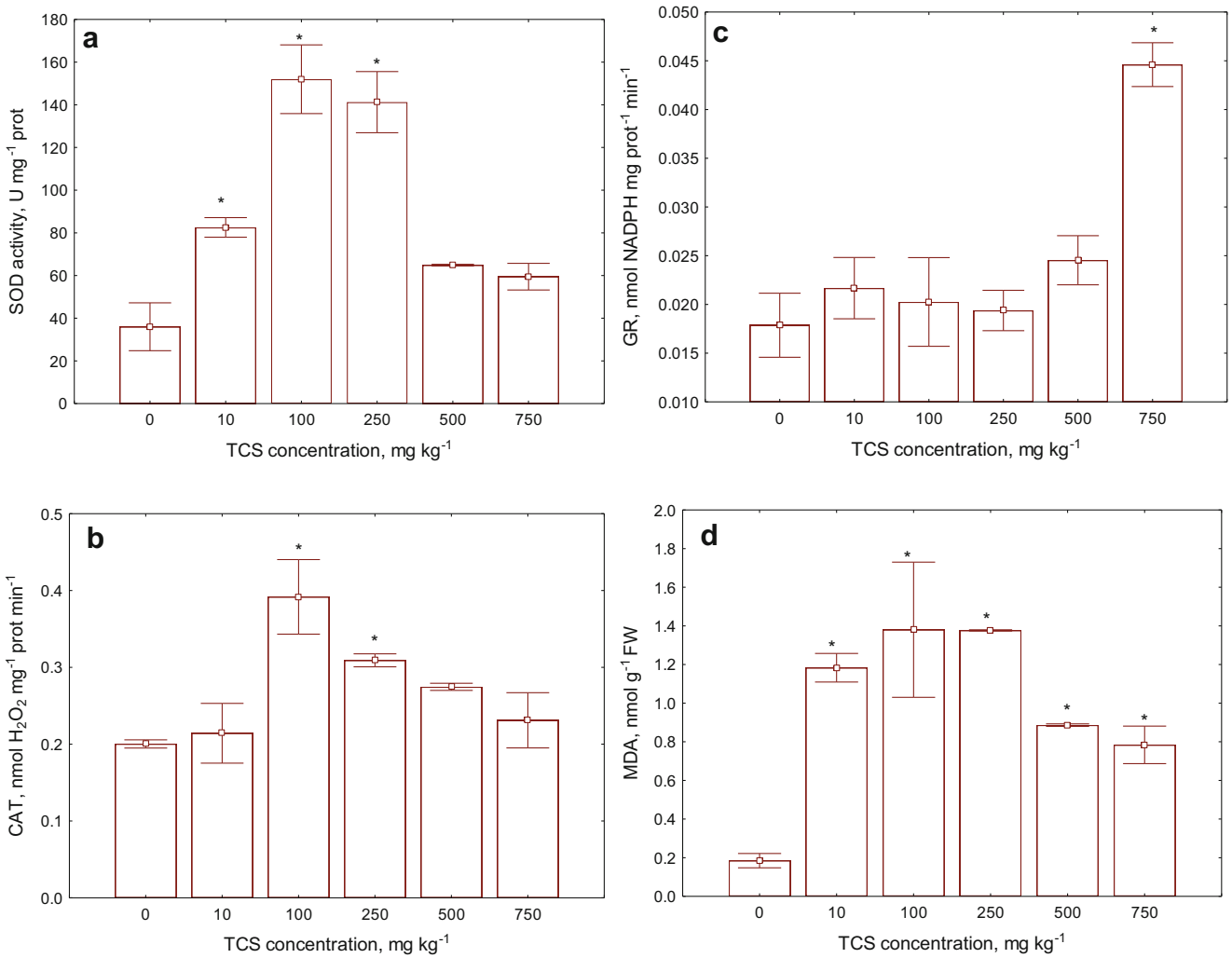

Fig. 4 The activity of SOD (**a**), CAT (**b**), GR (**c**), and MDA (**d**) concentrations in earthworms *Eisenia fetida* after 8-week incubation in OECD artificial soil contaminated with TCS. Asterisk * indicates significant differences from the control ($p < 0.05$)

could be the initiation of reproduction (Spurgeon and Hopkin, 1996) and it coincide with the observations that in the 10–100 mg TCS kg^{-1} treatment, cocoon production and juvenile hatchling were recorded (Fig. 3a, b).

The increase in earthworm growth rate exposed to 500–750 mg kg^{-1} of TCS within the second phase of the experiment might be related to a decreased density. Increased mortality from the week 6 in the treatment with 500 mg kg^{-1} resulted in a reduction in the mean number of earthworms in the sample, though no increase in mortality was observed in the treatment with 750 mg kg^{-1}. Thus, it is likely that a reduction in earthworm density in the 500 mg kg^{-1} treatment might be responsible for the increased growth rate at the second phase of experiment. Spurgeon and Hopkin (1999) investigated the life history patterns of *Lumbricus rubellus* bred from reference and metal-exposed (smelter) populations and concluded that lower density, due to higher mortality, was responsible for the faster growth of smelter earthworms than reference earthworms. Domínguez and Edwards (1997) have found that higher body weight and growth rate of *E. andrei* exposed to pig manure were at the lowest population density. Though Kamenga et al. (2003) pointed out that *E. fetida* population growth rate is less density dependent compared to *Lumbricus terrestris*.

This study shows that reproduction was the most sensitive endpoint and it suggests that even TCS residues in the soil may adversely affect earthworm reproduction. TCS application in the field has resulted in reduced juvenile proportions of *A. caliginosa* (Schnug et al. 2015). TCS deleterious effects on the earthworm reproduction were shown in several studies, though different EC_{50} values were determined. The study by Amorim et al. (2010) on the acute and chronic TCS toxicity to *E. andrei* showed that juvenile hatching was dramatically reduced and the EC_{50} value was 3.8 mg kg^{-1}. Similar *E. fetida* reproduction response was recorded by Wang et al. (2015); the 28-day EC_{50} was 6.17 mg kg^{-1}. High sensitivity of *E. fetida* reproduction was also observed by Schnug et al. (2013); the 28-day EC_{50} was of 0.87 mg kg^{-1}. Whereas, Lin et al. (2014)

determined 28-day EC_{50} for cocoon production was 142.11 mg kg^{-1} and no mortality was observed in the range of tested concentrations (up to 300 mg kg^{-1}). Summing up the findings of these studies, it could be seen that earthworm reproduction was altered at concentrations one to three orders of magnitude lower than those causing lethal toxicity and this is consistent with our results. Unfortunately, the possible mechanism of TCS toxicity to earthworms' reproduction has not been well defined, though impaired reproduction could be linked to TCS endocrine-disrupting properties (TCS is listed as a potential endocrine disruptor (WHO 2013). Estrogenic properties of TCS were discovered and the estrogenic effects were possibly mediated though an estrogen receptor (ER)-involved pathway (Jung et al. 2012) and existence of ER was shown in annelids (Keay and Thornton 2009). Endocrine disruption potential of TCS was also proved by increased level of vitellogenin in amphibians and fish (Ishibashi et al. 2004; Martins et al. 2017).

Biochemical responses against various environmental stressors are regarded as early warning indices of further possible adverse effects in sub-organism and organism levels. The analysis of biochemical response would be helpful to get more insight into TCS toxicity mechanism. Biochemical parameters could help to get more information analyzing the impact of chemicals to earthworm life cycle parameters and may be used in combination (Spurgeon et al. 2005; Žaltauskaitė and Sodienė 2014). The activities of enzymes (CAT, SOD, and GR) playing an important role in eliminating reactive oxygen species (ROS) are often used as biomarkers, indicating ROS production and they were used to study organism biochemical response to various pollutants (Han et al. 2016; Shi et al. 2013; Song et al. 2009).

Parallel changes in SOD and CAT activities were observed after the earthworm treatment with TCS in soil. The activities of these enzymes were stimulated by TCS with the highest activities in the treatments with 100–250 mg kg^{-1}. An increase in SOD activity may reflect the presence of superoxide radicals $O_2^{\bullet -}$ and their conversion to hydrogen peroxide (H_2O_2). Production of H_2O_2 stimulated the activity of CAT. At high TCS concentrations, the levels of antioxidant enzymes decreased and it indicates that ROS production exceeds the synthesis of antioxidant enzymes leading to damage to the antioxidant defense system (Wang et al. 2014). Lin et al. (2010) exposed *E. fetida* to 1–300 mg kg^{-1} TCS in the soil for 14 days and measured concentrations of antioxidant enzymes after 2, 7, and 14 days. It was observed that antioxidant enzyme response to TCS exposure differed depending on the exposure duration: CAT activity increased after a 2-day exposure and decreased after a 14-day exposure. After a 2-day exposure, an increase in SOD activity was recorded, but there were no regular changes in SOD activity after more prolonged (7 and 14 days) exposure. In the other study, Lin et al. (2012) found that SOD and CAT activity significantly increased in the tissue of earthworms after 28 days of TCS exposure only at TCS concentration ranges 50–100 mg kg^{-1} and 10–50 mg kg^{-1}, respectively. They found that MDA significantly increased only at 100 mg kg^{-1} and the increase was only 1.65-fold. Enhanced SOD and CAT activity was also observed after the 7-day earthworm exposure to TCS (10–1000 mg kg^{-1}) (Ma et al. 2017). Zhang et al. (2009) observed an increase in CAT and SOD activity after 4 h *E. fetida* exposure to low Cd concentrations and decrease after exposure to high Cd concentrations. GR plays an important role in cell protection by reducing oxidized glutathione (GSSG) to the functionally active reduced glutathione (GSG). In the present study, TCS impact on the activity of GR was less pronounced and significant increase in the GR activity was only at the highest TCS concentration. Our results indicate that TCS triggered relatively strong oxidative stress, reflected by high MDA concentrations, while antioxidative protection was stimulated to a lesser extent. It implies that the stimulation of antioxidant enzymes was insufficient to protect from the oxidative damage and the eliminating ability of SOD and CAT enzyme was exceeded. Insufficient stimulation of antioxidant enzymes leading to oxidative stress was shown in earthworms *E. fetida* exposed to TCS in paper contact test (Lin et al. 2012) and in barley after exposure to drought and copper (Kacienė et al. 2015).

Further, we tried to elucidate whether biochemical endpoints correspond well with studied life cycle parameters. The correlation analysis revealed that there was no significant relationship between studied life cycle parameters (mortality, growth rate, and reproduction) and biochemical endpoints (concentrations of antioxidant enzymes and MDA) with exception to the relationship between SOD and earthworm weight ($r = 0.54$, $p < 0.05$). Close relationship between SOD concentration and MDA ($r = 0.82$, $p < 0.01$) proves that SOD stimulation leads to high level of H_2O_2 and further lipid peroxidation. Insignificant relationship between life cycle and biochemical parameters could be due to very high biochemical parameter variation in time. Antioxidant enzyme response to various contaminant exposures differs depending on the exposure duration and general activity of antioxidant enzymes increases after short-term exposure (up to several days) and decreases after more prolonged exposure (Hayshi et al. 2013; Lin et al. 2010; Xiong et al. 2013). Decrease in antioxidant enzymes with time of exposure might be related with individuals' adaptation to chemicals or might indicate that chemicals could already have inhibited cell processes (Hartley-Whitaker et al. 2001). Further research in relationship between biochemical and life cycle parameters in different time periods is needed. Combining the different endpoints demonstrated that oxidative stress has led to the impairment of life cycle parameters though it could not fully explain chronic changes in life cycle parameters.

Conclusions

Results indicate that 10–750 mg kg^{-1} of TCS does not pose acute toxicity to the survival of soil key species earthworm *E. fetida*, though the survival was reduced after chronic exposure to TCS. TCS had a profound effect on the other life cycle parameters. Earthworms exposed to TCS grew slower, produced fewer cocoons, and juveniles were recorded only in the treatments with the lowest TCS concentration. These negative effects may have significant impact on the size and growth rate of the population as these life cycle parameters are very important for the population dynamics. Reproduction was the most sensitive endpoint and was affected at the very low levels of TCS. TCS triggered oxidative stress and it could partially explain the changes in life cycle parameters. We demonstrated that an integrated approach combining different biochemical and life cycle endpoints would provide a more comprehensive assessment of the ecological effects of chronic TCS exposure on earthworms.

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