Triclosan-induced changes in earthworm *Eisenia fetida* life cycle under elevated temperature

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Abstract
Antimicrobials are important for preserving the quality of life and public health. Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether, TCS) is one of the most used antimicrobials in both pharmaceuticals and personal care products and it is a frequently detected emerging organic contaminant (EOCs). Earthworms *Eisenia fetida*, with their thin epithelium and their feeding, are directly exposed to soil pollutants, making them highly important for the assessment of the toxicity of inorganic and organic contaminants to soil biota. With potentially far-reaching consequences for life on Earth, climate change is an increasingly urgent issue. There is a growing awareness of the importance of anticipating the interactions between natural and chemical stressors, and the way they affect organisms and their performance. The purpose of the study is to assess the effect of elevated temperatures on changes in the life cycle of earthworm *E.fetida* induced by triclosan. Triclosan-contaminated (10 - 750 mg TCS kg^{-1}) soil studies with *E.fetida* were performed at different temperatures (20°C and 25°C). Earthworm lifecycle parameters (mortality rate, weight growth, reproduction) were determined. Results of this study showed that elevated temperature enhanced the negative effect of triclosan on *E.fetida* mortality at ≥100 mg TCS kg^{-1} concentrations. Reproduction of *E.Fetida* was the most sensitive to TCS exposure and the lowest tested TCS concentration severely affected *E.Fetida* reproduction.
Keywords: triclosan, climate change, chronic toxicity, earthworms, temperature

1. Introduction

Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy) phenol) is a broad-spectrum antimicrobial and antifungal agent and it is a frequently detected emerging organic contaminant (EOCs). EOCs is a new class of environmental pollutants defined as “chemical substances that have no regulation and are suspected to negatively affect the environment or whose effects are unknown” (Geissen et al., 2015). TCS has been used in numerous health and personal care products: hand soaps, surgical supplies, shower gels, hand creams and lotions, toothpastes and mouthwashes, deodorants, textiles and toys (Dann and Hontela, 2011). The European Commission disapproved the use of TCS for hygienic purposes in 2017 (Official Journal of the European Union, L107). However, the use of triclosan is not prohibited and is still unregulated in many countries and TCS still is used in many personal care products (Weatherly and Gosse, 2017).

TCS can be released into the environment in different ways. Up to 450 t/year of TCS are consumed in Europe, approximately 96% of which is disposed to wastewater plants (SCCS/1392/10). Even though the removal rate of TCS during wastewater treatments is >80% (Reiss et al., 2002), TCS has become one of the top contaminants in water (Brausch and Rand, 2011) and ranks among the contaminants of concern worldwide. Owing to the hydrophobic lipophilicity of TCS (log Kow of 4.76) triclosan tends to accumulate in the living organisms and has been detected in biotic and abiotic environments: surface water, drinking water, sewage sludge, soil, water, and soil organisms (Chen et al., 2018; Dhillon et al., 2015). It was reported that 30-64% of the TCS was transferred to sewage sludge (Lozano, 2013), with which it also enters terrestrial ecosystems. The toxicity of triclosan to aquatic organisms is well documented (Dhillon et al. 2015), however data about the effects on soil organisms are comparatively limited (Lin et al., 2010). Climate change is an increasingly urgent problem with potentially far-reaching consequences for biodiversity. There is growing awareness of the importance of anticipating the interactions between natural and chemical stressors, and the way they affect organisms and their performance. There is a growing body of evidence that climate change will have significant impacts on the fate, distribution, and toxicity of environmental contaminants (Confalonieri et al., 2007). Among the terrestrial organisms most susceptible to changing environmental conditions are soil invertebrates (e.g., earthworms).

Earthworms (*Eisenia fetida*) are the key component of terrestrial ecosystems since they play a role in organic matter decomposition, nutrient mobilization, soil bioturbation and soil structure improvement (Lavelle et al., 2006). Earthworms are directly exposed to soil pollutants with their thin epithelium and their feeding; thus, they are highly relevant for the assessment of the bioavailability, bioaccumulation and toxicity of inorganic and organic contaminants to soil biota and is widely used as model organism in ecotoxicology tests (Frund et al., 2010). While an increasing body of knowledge has demonstrated the physiological and biochemical effects induced by TCS, however, changes in the bioaccumulation and ecotoxicity of TCS due to climate change have not been investigated in terrestrial organisms. Aim of the study was to assess the effect of elevated temperature on changes in the life cycle of earthworm *Eisenia fetida* induced by triclosan.

Methodology

The experiment was carried out in the regulated environment chambers according to modified OECD Protocol No. 222- Earthworm Reproduction Test (Eisenia fetida) (OECD, 2004). 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 4.38 ± 0.15%, pH 6.52 ± 0.083, water content 60 % of the maximum water holding capacity (SWC). The constituents of artificial soil were air-dried, mixed thoroughly, and weighted (750 g) into plastic containers. The TCS (Alfa Aesar GmbH & Co KG) was dissolved in acetone and applied to the soil at 5 different concentrations (in mg kg−1 of soil): 10, 100, 250, 500, and 750. Each treatment was prepared in triplicates. The experiment was performed under different combinations of air temperature (20°C and 25°C). The duration of the experiment was 8 weeks. Fifteen earthworms were added to each container. Water content in each container was checked weekly, the earthworms were weekly supplied with oatmeal (approximately 0.5 g per earthworm). Earthworm life cycle indicators such as weight, mortality was measured after 2, 4, and 8 weeks of TCS exposure, the reproduction parameters (number of cocoons and juveniles) were calculated at the end of the experiment.

Factorial analysis of variance (ANOVA) was used to assess the concentration effect on estimated life cycle endpoints. Significant differences between treatments were determined by *t* test and $p < 0.05$ was considered to be significant. All the statistical analysis was carried out using Statistica software.

2. Results

4.1. Mortality

Mortality (%) of *E.Fetida* under different temperature regimes (21 °C and 25 °C) is presented in Fig.1. Time, TCS concentration, and the temperature had a significant effect on mortality (ANOVA, F = 5.95 - 18.4, p<0.05). No mortality was observed in control and low TCS concentrations (10 mg kg^{-1}) after short-term exposure to TCS (2 weeks) under both tested air temperatures. The highest tested TCS concentration (750 mg kg^{-1}) during short-term exposure evoked low (8.33 %) mortality, which increased after sub-chronic exposure (4 weeks) and reached 100 % after chronic exposure (8 weeks) under 21 °C air temperature. After short-term exposure to TCS under elevated temperature (25°C) the mortality of *E.fetida* reached 22 % and 47 % at 500 mg TCS kg^{-1} and 750 mg TCS kg^{-1} treatments respectively, continued to increase along the experiment time and reached ~80 % of mortality

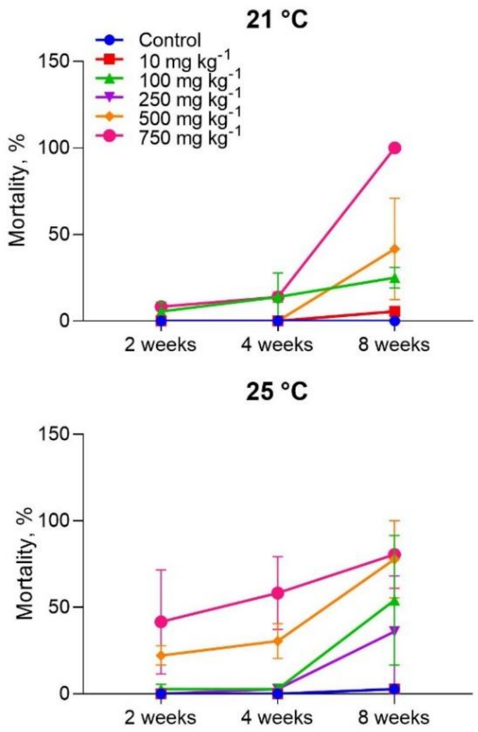

Figure 1. *Mortality (%) of earthworm E.fetida after 2, 4 and 8 weeks of exposure to different TCS concentrations under different air temperatures (21 °C and 25° C)*

after chronic exposure.

Elevated temperature enhanced the negative TCS effect to *E.fetida* mortality at ≥100 mg TCS kg^{-1} treatments. Our results indicate that TCS does not evoke acute toxicity, however sub-chronic and chronic exposure might lead to lethal effects.

It is consistent with our previous studies (Žaltauskaitė and Miškelytė, 2018, Žaltauskaitė and Miškelytė, 2014) where no acute lethal TCS toxicity was found under ambient 21 °C air temperature treatments. It was also reported that 14 days of TCS exposure to *Eisenia fetida* at a range of 0-1026 mg TCS kg^{-1} did not result in lethal toxicity (Reiss et al., 2009). In our study elevated temperature enhanced the negative TCS effect to *E.fetida* mortality at the concentration higher than 100 mg TCS kg $^{-1}$ concentrations. Other studies with bivalves have demonstrated that warming conditions alter their sensitivity to organic and inorganic pollutants (Attig et al., 2014; Moreira et al., 2017; Nardi et al.,2018). Our data also suggest that elevated temperature altered *E.Fetida* sensitivity to higher TCS concentrations that had led to higher mortality.

4.2. Fresh weight

Temperature, time, TCS concentration, and their interaction had a significant effect on *E.Fetida* weight growth (ANOVA, F= 4.88-181.46, p<0.001). It was observed that with increasing TCS concentration, the fresh weight of *E.Fetida* decreased at all time points examined under both temperature regimes (Fig.2). During short-term exposure, *E.Fetida* exposed to ≥100 mg TCS kg^{-1} treatments at 21 °C did not gain in weight in comparison with initial weight. The 14% weight loss (p>0.05) was observed at 750 mg TCS kg^{-1} treatments. In contrast, weight loss at elevated air temperature (25 °C) was observed at ≥500 mg TCS kg^{-1} treatments, however, these changes were not statistically significant (p>0.05). Elevated temperature statistically significantly (p<0.05) enhanced *E.fetida* weight growth at ≤250 mg TCS kg^{-1} treatments during all examined time endpoints. Temperature is among the most important abiotic factors controlling the activity of earthworms (Lowe and Butt, 2005).

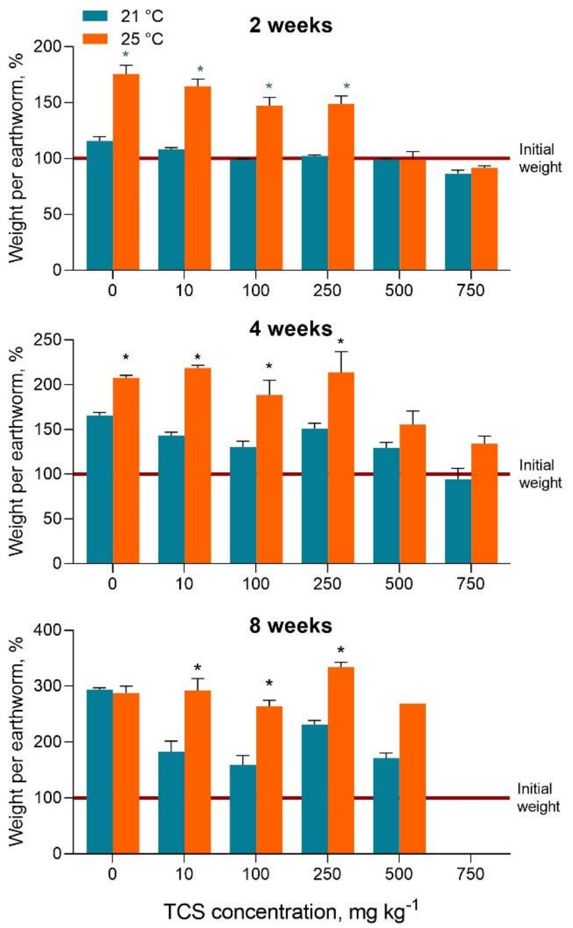

Figure 2. *Weight change (% of initial weight) of earthworm E. fetida after 2, 4, and 8 weeks exposure to different TCS concentrations under different air temperatures (21 °C and 25° C). The asterisk * indicates a significant difference between different air temperature regimes (p<0.05)*

The increasing temperature might lead to greater earthworm growth and reproduction (Presley et al., 1996) as well as higher metabolic activity (Lee, 1985). In our study results were consistent with these findings.

4.3. Reproduction

Temperature, TCS concentration, and their interaction had a significant effect on *E.fetida* cocoon production (ANOVA, F= 6.21-113.27, p<0.05) where only TCS concentration had a significant effect on juveniles number (ANOVA, F=32.39, p<0.01). The results of this study showed that elevated temperature statistically significantly enhanced cocoons production at 10 mg TCS kg^{-1} treatment by 2.4 times (p<0.05) as well as the juvenile number by 7.3 times (p<0.05) (Fig.3). In addition, elevated temperature slightly enhanced (23.7%, p>0.05) juvenile number at control (0 mg TCS kg^{-1}) treatment, however, no positive effect of elevated temperature on cocoon's production was observed.

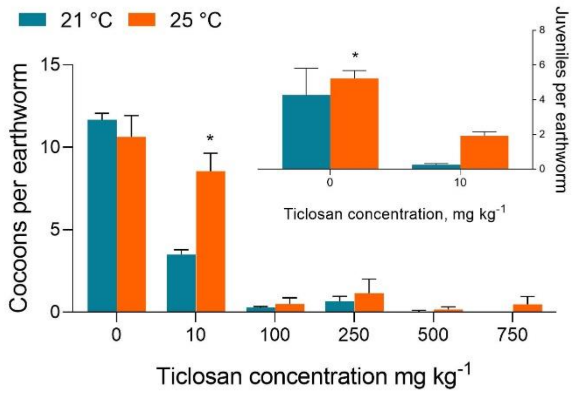

Figure 3. *The number of cocoons and juveniles of earthworm E. fetida 8 weeks exposure to different TCS concentrations under different air temperatures (21 °C and 25° C). The asterisk * indicates a significant difference between different air temperature regimes (p<0.05)*

Despite some positive effects of elevated temperature to *E.Fetida* reproduction, the overall results of this study showed that TCS even at the lowest tested treatment (10 mg TCS kg^{-1}) severely affected *E.Fetida* reproduction where the sharp decrease of the number of cocoons and juveniles under both air temperature regimes was observed. No juveniles were found at >10 mg TCS kg^{-1} treatments under both tested air temperature regimes and a dramatic decrease in cocoons production (more than 17.4 times at 21 °C and more than 9 times at 25°C) was observed. Even though it is reported (Presley et al., 1996) that elevated temperature enhances the reproduction of *E.Fetida* and the results of our study are consistent at some point where the positive effect of temperature was observed at ≤ 10 mg TCS kg^{-1} treatments, however, this study shows that reproduction is the most sensitive endpoint and suggests that even TCS residues in the soil may adversely affect earthworm reproduction. This is consistent with our previous studies (Žaltauskaitė and Miškelytė, 2018) as well as with Schnug et al. (2015) where he has reported that TCS application in the soil has resulted in juvenile numbers of *A. caligino*.

3. Conclusion

Results of this study indicate that elevated temperature enhanced the negative effect of triclosan to *E.fetida* mortality at ≥100 mg TCS kg^{-1} concentrations as well as statistically significantly ($p<0.05$) enhanced *E.fetida* weight growth at ≤250 mg TCS kg^{-1} concentrations during all examined time (8 weeks). Reproduction of *E.Fetida* was the most sensitive to TCS exposure and the lowest tested TCS concentration severely affected *E.Fetida* reproduction. These effects may lead to a decrease in earthworm growth population size.

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