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Influence of soil temperature and moisture on biochemical biomarkers in earthworm and microbial activity after exposure to propiconazole and chlorantraniliprole

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ABSTRACT

Predicted climate change could impact the effects that various chemicals have on organisms. Increased temperature or change in precipitation regime could either enhance or lower toxicity of pesticides. The aim of this study is to assess how change in temperature and soil moisture affect biochemical biomarkers in *Eisenia fetida* earthworm and microbial activity in their excrements after exposure to a fungicide - propiconazole (PCZ) and an insecticide - chlorantraniliprole (CAP). For seven days, earthworms were exposed to the pesticides under four environmental conditions comprising combinations of two different temperatures (20 °C and 25 °C) and two different soil water holding capacities (30% and 50%). After exposure, in the collected earthworm casts the microbial activity was measured through dehydrogenase activity (DHA) and biofilm forming ability (BFA), and in the postmitochondrial fraction of earthworms the activities of acetylcholinesterase (AChE), catalase (CAT) and glutathione-S-transferase (GST) respectively. The temperature and the soil moisture affected enzyme activities and organism's response to pesticides. It was determined that a three-way interaction (pesticide concentration, temperature and moisture) is statistically significant for the CAT and GST after the CAP exposure, and for the AChE and CAT after the PCZ exposure. Interestingly, the AChE activity was induced by both pesticides at a higher temperature tested. The most important two-way interaction that was determined occurred between the concentration and temperature applied. DHA and BFA, as markers of microbial activity, were unevenly affected by PCZ, CAP and environmental conditions. The results of this experiment demonstrate that experiments with at least two different environmental conditions can give a very good insight into some possible effects that the climate change could have on the toxicity of pesticides. The interaction of environmental factors should play a more important role in the risk assessments for pesticides.

1. Introduction

Over the past few decades, there has been extensive research into the toxicity of pesticides and other chemicals on earthworms. Earthworms were given such attention due to their widely known beneficial roles in a number of soil processes and their sensitivity to contaminants, environmental stress and impact on soil quality. Depending on the objective of research, different endpoints are investigated, such as survival, reproduction, avoidance, biochemical biomarkers, gene expression, etc. Most of these studies are conducted in laboratories under the standard exposure conditions. However, climate change could modify the influence that tested chemicals have on organisms. Negative impacts of various pollutants and contaminants may intensify as a consequence of the forecasted temperature increases (1.1–6.4 °C by the year 2100) under the current global warming perspectives (IPCC, 2007). The change in a precipitation regime is also predicted, particularly an increase during winter and a decrease during summer in central Europe (Alcamo et al., 2007). Climate change could have both indirect and direct effects on pesticides (Kattwinkel et al., 2011). The indirect effects include changes in exposure to pesticides due to the shifts in cultivation towards higher latitudes and extension of cultivation periods (Tubiello et al., 2002; Bloomfield et al., 2006). The potential enhancement of volatility and degradation of pesticides could affect their efficiency against pests and, therefore, pesticide application rates could increase (Noyes et al., 2009). This increase of pesticide application could be both in their quantity, but also in the extent of

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application area (Koleva and Schneider, 2009). In terms of direct effects, climate change might have an impact on pesticide decomposition and toxicity (Kattwinkel et al., 2011), particularly in the expected alternations in temperature and precipitation (Noyes et al., 2009). It has been shown that the pesticide degradation is dependent on soil moisture and temperature (Kookana et al., 2010). Besides being dependent on the temperature, the toxic effects of pesticides are also linked with other stressors.

Frequently, that results in increasing toxicity with increasing temperature (Kattwinkel et al., 2011). Changes in soil moisture are also associated with differently toxic and environmentally mobile metabolites (Van den Berg et al., 1999). Changes in temperature alter toxicokinetics of toxicants (Lydy et al., 1999), boost an organism's metabolic activity and thus uptake the rates of toxicants (Martikainen and Krogh, 1999; Lima et al., 2015). Soil temperature and soil moisture are key factors influencing growth, survival, fecundity and activity of earthworms (Edwards and Bohlen, 1996) and, indirectly, influencing the earthworm habitat and availability of food (Curry, 2004). Moreover, soil temperature and moisture affect most of the life cycle traits, such as weight, cocoon incubation time, onset of sexual maturity, reproduction and life span. Increase in temperature may accelerate the growth and reproduction rate of earthworms (Uvarov et al., 2011). It has also been shown that soil moisture and temperature have influence on biomarkers in *Aporrectodea caliginosa* earthworms (Booth et al., 2000). Several studies dealing with the influence of soil moisture on effects of different chemicals found a synergistic response (Bindesbøl et al., 2005; Friis et al., 2004; Long et al., 2009; Lima et al., 2011). Toxicity can be different depending on the tested pesticide, temperature and type of soil or a measured endpoint (De Silva et al., 2009). Papers focusing on the combination of toxicity of chemicals and change of both soil moisture and temperature are scarce. Gonzàlez-Alcaraz and van Gestel (2016a, 2016b) studied the bioaccumulation and toxicity of metals/metalloids in earthworms and enchytraeids under different scenarios of climate changes while Bandow et al. (2014) studied the interactive effects of environmental factors and pesticides on collembola. They pointed out that an interaction of environmental factors has not been examined within the chemical risk assessment, but might become more relevant if global climate change accelerates (Bandow et al., 2014).

Beside earthworms, pesticides affect the response of soil microbial communities and possible ecological implications of such exposure raised some serious concerns (Imfeld and Vuilleumier, 2012). The effects of pesticides on soil microbial community became even more complicated with the incorporation of microbial interactions with other non-target organisms such as earthworms. It is known that earthworms could stimulate abundance and activity of pesticide degraders (Liu et al., 2011; Sanchez-Hernandez et al., 2014), while pesticides could have direct effects on both earthworms (Stepić et al., 2013; García-Pérez et al., 2016) and microbes (Imfeld and Vuilleumier, 2012; Petric et al., 2016). The effects of pesticides on soil microbes can be either stimulating or inhibitory.

For the purposes of this research, we chose two pesticides that are classified as nontoxic to earthworms, and their possible adverse effects on earthworms are scarcely studied. One is propiconazole (PCZ), a fungicide from a triazole family, widely used as a systemic foliar fungicide (Konwick et al., 2006). They can be widely distributed into soil after treatment (Wang et al., 2008; Gao et al., 2013). Concerning soil microorganisms, propiconazole stimulated cellulase and invertase activities (Ramudu et al., 2011), while certain negative effects were noted in relation to microbial abundance and soil microbial community structure (Yen et al., 2009). It also decreased the substrate induced respiration, radioactively labelled leucine incorporation (Fernández-Calviño et al., 2017) and phosphatase activity (Kalam and Mukherjee, 2002). The second pesticide used in this research is a novel insecticide chlorantraniliprole (CAP), an anthranilic diamide which has a specific mode of action: it activates ryanodine receptors that can release stored calcium and, hence, cause an impaired muscle contraction regulation (Cordova et al., 2007; Larson et al., 2012). Due to the differential receptor selectivity of CAP between ryanodine receptors in various animal groups, CAP is relatively safe for mammals, birds and fish, but very highly toxic for several aquatic invertebrates (Lavtižar et al., 2016). These features make CAP rapidly replacing other groups of insecticides (Rodrigues et al., 2015).

The aim of this paper is to determine whether changes in temperature and soil moisture have an impact on the toxicity of PCZ and CAP to *Eisenia fetida* earthworm at environmentally relevant concentrations. Therefore, we have made a combination of two temperatures (20 °C and 25 °C) and two soil moisture (30% and 50% WHC) treatments according to Gonzalez-Alcaraz et al. (2015). The impact was examined through the measurement of biochemical biomarkers. Additionally, as the earthworm casts are an important source of substrate for microorganisms in soil (e.g. increased active surface, earthworm cast — microbial hotspot), we have tested the impact of the two pesticides on the microbial activity in the casts through the dehydrogenase activity and the biofilm forming ability. Moreover, we have also tested whether the impact, if any, persists 10 days after the exposure to pesticides in several time steps.

2. Materials and methods

2.1. Organisms

Adult earthworms (*Eisenia fetida*) were obtained from a culture maintained in our laboratory. All earthworms were adults, four months old, with well-developed clitellae and weighted between 250 mg and 450 mg. Prior to each exposure, earthworms were removed from the culture and placed on a damp filter paper overnight to void their gut content.

2.2. Chemicals

All reagents used were of analytical grade. 5,5'dithiobis-2 nitrobenzoic acid (DTNB), acetylthiocholine iodide (AcSChI), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), bovine serum albumin (BSA), 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(phenyl) tetrazolium chloride (INT) and Crystal Violet.

The following commercial preparations of pesticides were used — Bumper 25 EC, as a propiconazole formulation (250 g/L propiconazole), and Coragen 20 SC, as a chlorantraniliprole formulation (200 g/L chlorantraniliprole).

2.3. Experimental set-up

Preliminary experiments were conducted in order to determine concentration range that is sublethal and environmentally relevant. In all preliminary experiments biomarkers that are measured in subsequent experiments were also measured. Preliminary experiments comprised a 72-h filter paper test (according to OECD, 1984), and an artificial soil test for 7 and 14 days under the standard conditions (20 °C, 50% WHC).

In the final experiments, four concentrations were applied for each pesticide. Firstly, the highest recommended concentration relevant for field application was calculated according to the label of the commercial formulations. To enable a comparison, an application rates were transformed into $\mathrm{mg_{a.i.}\ kg_{dw\ soil}}^{-1}$. An average soil depth of 0.1 m and an average soil bulk density of 1.5 g $\mathrm{cm^{-3}}$ was taken into account to facilitate calculations. This environmentally relevant concentrations were labelled as a C3 concentrations. Apart from this concentration, 2-fold and 4-fold lower and 2-fold higher concentrations were also applied. The applied concentrations were: 20.825 μg $\mathrm{kg^{-1}}$ (C1), 41.65 μg $\mathrm{kg^{-1}}$(C2), 83.3 μg $\mathrm{kg^{-1}}$ (C3) and 166.6 μg $\mathrm{kg^{-1}}$ (C4) for propiconazole (PCZ); and 5 μg $\mathrm{kg^{-1}}$ (C1), 10 μg $\mathrm{kg^{-1}}$(C2), 20 μg $\mathrm{kg^{-1}}$