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Changes in exposure temperature lead to changes in pesticide toxicity to earthworms: A preliminary study

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ABSTRACT

The occurring climate changes will have direct consequences to all ecosystems, including the soil ecosystems. The effects of climate change include, among other, the changes in temperature and greater frequency and intensity of extreme weather conditions. Temperature is an important factor in ecotoxicological investigations since it can act as a stressor and influence the physiological status of organisms, as well as affect the fate and transport of pollutants present in the environment. However, most of so far conducted (eco)toxicological investigations neglected the possible effects of temperature and focused solely on the effects of toxicants on organisms. Considering that temperature can contribute to the toxicity of pollutants, it is of immense importance to investigate whether the change in the exposure temperature will impact the strength of the toxic effects of pollutants present in soil ecosystems. Therefore, in the present study the toxicity of several commonly used pesticides to earthworms was assessed under different exposure temperatures (15, 20 and 25 °C). The results showed that changes in exposure temperature lead to changes in susceptibility of earthworms to particular pesticides. Namely, exposures to the same pesticide concentration at different temperatures lead to different toxicity responses. Increase in exposure temperature in most cases caused increase in toxicity, whereas decrease in temperature mostly caused decrease in toxicity. This preliminary study points to need for an in-depth investigation of mechanisms by which temperature affects the toxicity of pesticides and also provides important data for future research on the effects of temperature change on the soil ecosystems.

1. Introduction

Soil is a non-renewable resource and its preservation is crucial for sustainable future. However, soil is under increasing pressure of intensification of its use for different purposes, as well as under increasing exposure to different pollutants. The pollution of soil may arise from different sources and among wide variety of soil pollutants pesticides play an important role (Finizio and Villa, 2002). Pesticides are being used for control of different pests and weeds; however, after application they can move down the soil column and affect many non-target soil organisms and consequently have adverse effects on soil ecosystems. According to the specific organisms they are used to control, pesticides are generally classified into insecticides, fungicides and herbicides. Although each group specifically targets certain organisms, they can all exert toxic effects on different soil organisms (e.g. Cortet et al., 2002; Jänsch et al., 2005; Lo, 2010; Zhang et al., 2010; Yasmin and D'Souza, 2010; Wang et al., 2012a; Milanović et al., 2014).

Earthworms are important members of the soil food webs, can constitute a major fraction of the soil invertebrate biomass and act as ecosystem engineers (Lee, 1985; Edwards and Bohlen, 1996). Besides their role in soil quality and functioning, earthworms are also important for the investigation of effects of soil pollutants. Since earthworms are in constant interaction with soil, through dermal contact and ingestion they are exposed to pollutants present in the soil and are suitable species for ecotoxicological assessment of soil pollution (Schreck et al., 2008). Based on different morphological and behavioral characteristics, earthworms can be divided into three main ecological groups—epigeic, endogeic and anecic species (Bouché, 1977) and in the present study the compost epigeic species *Eisenia fetida* was chosen as model organism. Although this species is usually not significantly represented in the environment, it is commonly used for toxicity testing in the laboratory conditions and is recommended by the OECD (1984). The usage of this "laboratory" species is appropriate for preliminary assessment of toxicity whereas in further ecotoxicological investigations other environmentally more relevant species should be included.

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Earthworms are ectotherm organisms and temperature is an important factor which can influence different physiological and biochemical processes. In previous studies it was shown that both low and high temperatures can affect the behavior of earthworms, lead to changes in growth and reproduction, as well as the metabolic activity of organisms, and affect the intake rate of toxic substances (e.g. Presley et al., 1996; Fayolle et al., 1997; Wever et al., 2001; Perreault et al., 2006; Tripathi et al., 2011). Fluctuation of temperature in the environment is common occurrence and organisms have developed different adaptations which ensure the maintaining of homeostasis. However, the extreme weather conditions, in terms of extreme temperatures, can result in the emergence of temperature stress in organisms in the environment. Given that climate change projections indicate that the frequency, intensity and duration of extreme climate events will increase in the future, it is important to investigate whether the temperature at which are organisms exposed to pesticides affects the strength of pesticides' toxic effects. So far, many studies have investigated the effects of pesticides on earthworms, however, very little information is available on the impact of exposure temperature on the pesticide toxicity. Bindesbøl et al. (2009) investigated effects of freezing temperatures on toxicities of abamectin and carbendazim, De Silva et al. (2009) investigated influence of temperature and soil type on the toxicities of chlorpyrifos and carbofuran, Lima et al. (2015) investigated effects of carbaryl under low and high temperatures, and Garcia et al. (2008) assessed effects of three pesticides on the avoidance behavior under temperate and tropical conditions. These investigations showed that changes in temperature may result in changes in pesticide toxicity, but the results obtained in these studies are not unambiguous which is somewhat expected given that neither the measured endpoints nor the experimental conditions of the experiment were the same. In the present study the toxicity of pesticides to earthworms was assessed under low and high temperatures using standardized toxicity test. The usage of standardized test enabled the comparison of the toxicities of investigated pesticides as well as comparison of the obtained results with other studies which also used standardized test.

Among numerous pesticides being used in the environment for different purposes, 12 pesticides commonly used in agriculture and orcharding were selected for this study. Since adverse effects of pesticides on earthworms can be very diverse depending on the mode of action, for the toxicity assessment 5 insecticides, 2 fungicides and 5 herbicides were chosen. Regarding the insecticides, neonicotinoid imidacloprid, oxadiazine indoxacarb, pyrethroids alpha-cypermethrin and lambda-cyhalothrin, and combination of organophosphate chlorpyrifos and pyrethroid cypermethrin were investigated. All these insecticides primarily affect nervous system—neonicotinoids interfere with the transmission of stimuli in the nervous system causing irreversible blockage of acetylcholine receptors; oxadiazines act as voltage-gated sodium channel blockers; pyrethroids cause excitation of the sodium and potassium channels of neurons and the delay of closing of the channels during the phase of depolarization and organophosphatesinhibit the action of enzyme acetylcholinesterase (AChE) leading to accumulation of acetylcholine, excessive stimulation of the cholinergic receptors and disruption of neural activity (Stenersen, 2004; Casida, 2009). In case of fungicides, the toxicity of combined preparation of difenoconazole and propiconazole, and combined preparation of azoxystrobin and cyproconazole was assessed. Difenoconazole, propiconazole and cyproconazole belong to class of triazole fungicides which act as inhibitors of ergosterol biosynthesis. Since biosynthesis of these ergosterols is critical to the formation of cell walls of fungi, the lack of normal sterol production slows or stops the growth of the fungus (Uesugi, 1998). Azoxystrobin belongs to strobilurin fungicides which act as respiration inhibitors. Azoxystrobin binds to the ubiquinone (Qo) site of cytochrome b which forms part of the cytochrome bc1 complex in the fungal mitochondrial membrane. This binding disrupts the transfer of electrons and prevents the ATP formation (Bartlett et al., 2002). From the group of herbicides following were used: tembotrione (inhibitor of pigment synthesis—inhibits 4-hydroxyphenylpyruvate dioxygenase (HPPD), which leads to chlorophyll destruction by photooxidation and causes bleaching of emerging foliar tissue); imazamox (inhibits acetolactate synthase (ALS), an enzyme required for the synthesis of essential branched chain amino acids, valine, leucine, and isoleucine); diquat (photosystem I inhibitor causing formation of hydroxyl radicals that destroy unsaturated lipids including membrane fatty acids and chlorophyll); fluazifop-*p*-butyl (inhibits acetyl CoA carboxylase, an enzyme that catalyzes an early step in fatty acid synthesis, leading to failure of cell membrane integrity); and glyphosate (inhibitor of the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP), which is necessary for the formation of the aromatic amino acids tyrosine, tryptophan, and phenylalanine) (Duke, 1990; Stenersen, 2004; Casida, 2009).

In the present study effects of insecticides, fungicides and herbicides commonly used in agriculture and orcharding were assessed under different exposure temperatures. The main objective of this study was to investigate the strength of the toxicity of each pesticide to earthworms and to determine whether the changes in exposure temperatures will lead to significant changes in the toxicity of the investigated pesticides.

2. Materials and methods

2.1. Earthworms

The adult *E. fetida* were purchased from local supplier and acclimatized in the laboratory. Prior to use, earthworms were separated, rinsed with water and kept in Petri dishes on damp filter paper in darkness for 3 h to void the gut contents (OECD, 1984). The earthworms used in this assay were all adults with well-developed clitellae.

2.2. Pesticides

Commercial preparations of following pesticides were used: (1) insecticides: Rapid (Herbos d.d.) as imidacloprid preparation (200 g L^{-1} a.i.), Direkt (Genera d.d.) as alpha-cypermethrin preparation (100 g L^{-1} a.i.), Avaunt (AgroChemMaks d.o.o.) as indoxacarb preparation (150 g L^{-1} a.i.), Pinurel D (Pinus Agro d.o.o.) as combined preparation of chlorpyrifos and cypermethrin (500 g L^{-1} of chlorpyrifos and 50 g L^{-1} of cypermethrin), Karate Zeon (Syngenta Agro d.o.o.) as lambda-cyhalothrin preparation (50 g L^{-1} a.i.); (2) fungicides: Rias 300 (Syngenta Agro d.o.o.) as combined preparation of difenoconazole and propiconazole (150 g L^{-1} of difenoconazole and 150 g L^{-1} of propiconazole), Amistar Extra (Syngenta Agro d.o.o.) as combined preparation of azoxystrobin and cyproconazole (200 g L^{-1} of azoxystrobin and 80 g L^{-1} of cyproconazole); (3) herbicides: Laudis (Bayer d.o.o., Bayer CropScience) as tembotrione preparation (44 g L^{-1} a.i.), Pulsar 40 (BASF Croatia d.o.o.) as imazamox preparation (40 g L^{-1} a.i.), Reglone Forte (Syngenta Agro d.o.o.) as diquat preparation (150 g L^{-1} a.i.), Fusilade Forte (Syngenta Agro d.o.o.) as fluazifop-*p*-butyl preparation (150 g L^{-1} a.i.), Ouragan System 4 (Syngenta Agro d.o.o.) as glyphosate preparation (360 g L^{-1}).

2.3. Toxicity tests

Earthworms were exposed to pesticides using the filter paper contact toxicity method (OECD, 1984). Filter paper contact test is simple method which excludes the impact and contribution of