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Role of different earthworms in a soil polluted with oxyfluorfen herbicide. Short-time response on soil biochemical properties

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ABSTRACT

In this paper we studied in the laboratory the bioremediation effects of an oxyfluorfen herbicide-polluted soil with and without three earthworms (*Eisenia fetida*, *Lumbricus terrestris* and *Allolobophora molleri*) and its influence on soil biochemical properties over 90 days. Glutathione-S-transferase activity (GST) and weight in earthworms were measured at four different incubation times (3, 15, 60 and 90 days). Cocoon numbers, average weight per cocoon and hatchlings per cocoon were measured 30 days after the oxyfluorfen exposure. Herbicide in soils and the earthworms was determined during the incubation period. To observe the effects of bioremediation of the contaminated soil, ATP, and urease, phosphatase and arylsulphatase activities were measured. Although the bioaccumulation factor of oxyfluorfen was 1.4- and 3.5-fold higher in *A. molleri* than for *E. fetida* and *L. terrestris*, respectively, compared to the unpolluted soils, the GST activity of *A. molleri* significantly decreased by 19% in soils polluted with oxyfluorfen, whereas for *E. fetida* and *L. terrestris* the GST activity significantly decreased by 23.3% and 34.2%, respectively. Furthermore, the earthworms' weight, cocoon numbers, average weight per cocoon and hatchlings per cocoon showed a pronounced decrease among *L. terrestris*, *E. fetida* and *A. molleri*, respectively, in polluted soils. When the concentration of herbicide decreased, so did enzyme activity. This decrease inhibition was more pronounced in soils with *A. molleri* than *E. fetida* and *L. terrestris*. Of the three species tested, *A. molleri* appears to the best suited for bioremediation of oxyfluorfen-contaminated soil.

1. Introduction

In recent years, the continued use of pesticides has been widespread in agricultural production. These organic compounds can help minimize economic losses caused by weeds, insects, and pathogens (Tejada et al., 2011; Jovana et al., 2014). Nevertheless, pesticides can also give rise to several important environmental problems because not only are many toxic to the target species, but also to non-target organisms, mainly due to physiological similarities between them (Santos et al., 2010), thus causing a high risk of soil contamination (Tejada et al., 2014; Xu et al., 2014). Pesticide-contaminated soils therefore require remediation in order to mitigate the hazardous effects of these agrochemicals.

Earthworms are non-target organisms in the use of pesticides. There is abundant literature showing that these organisms are used as biomarkers of the toxicity that organic pesticides may cause in soil (Jovana et al., 2014; Velki et al., 2014; Xu et al., 2014). Earthworms have extensive contact with the soil, and thus their tissues are subject to rapid exposure to the chemicals and their subsequent accumulation (Tejada and Masciandaro, 2011; Xu et al., 2014). Observing the response of earthworms after applying pesticides to soil can provide information on their tolerance to, and their capacity to remediate the toxic effects of herbicides in soil (Tejada et al., 2011).

Some tests based on enzyme activities such as cellulase, gluthatione-S-transferase, acetylcholinesterase, superoxide dismutase or catalase activities, as well as reproduction and morphological parameters have been used to monitor the contamination of various inorganic and organic compounds in soil (Tejada and Masciandaro, 2011; Lin et al., 2012; Tejada et al., 2013; Sanchez-Hernandez et al., 2014). In this respect, glutathione-S-transferase is an important detoxification enzyme and its activity has been used as a potential bioindicator and biomarker of earthworms' responses to heavy metals (Lukkari et al., 2004; Tejada et al., 2010) and pesticide exposure (Booth et al., 2001; Xiao et al., 2006).

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However, the response of earthworms to different xenobiotic compounds in soil differs greatly depending on the species. Physiological differences between earthworms, soil properties and pesticide concentration in soil are factors that affect differently the enzyme activities and morphological and reproductive parameters on worms (Tejada et al., 2011). In this respect, Tejada et al. (2011) found different chlorpyrifos insecticide sensitivity in two species of earthworms (*Eisenia fetida* < *Lumbricus terrestris*).

On the other hand, soil biochemical parameters can be used as potential soil quality indicators for sustainable management because they are sensitive to ecological stress and land management practices (Ceccanti et al., 2006). ATP (adenosine 5′-triphosphate) and soil enzymatic activities have been used as indicator parameters of the soil microbial biomass activity, and they can also be useful for interpreting the intensity of microbial metabolism in soil. Soil ATP reflect the actual microbial activity in the soil (Bastida et al., 2006). Respect to the hydrolases enzymes, urease is involved in the hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants (Nannipieri et al., 1990; Kizilkaya and Bayrakli, 2005). Phosphatase is the enzyme involved in the hydrolysis of organic phosphorus to different forms of inorganic phosphorus (García et al., 2002; Kizilkaya and Bayrakli, 2005; Bastida et al., 2006). Arylsulphatase is the enzyme involved in the mineralization of ester sulphate in soils (García et al., 2002; Kizilkaya and Bayrakli, 2005; Bastida et al., 2006). They are, therefore, of great use for understanding the bioremediation of contaminated soils (Tejada and Masciandaro, 2011).

Many studies have reported the bioaccumulation of various herbicides in earthworms (Booth et al., 2001; Tejada et al., 2011; Lin et al., 2012; Sanchez-Hernandez et al., 2014), yet regardless of all these data, the bioaccumulation of oxyfluorfen in earthworms has not been reported.

Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] is a diphenyl ether herbicide with residual activity and contact, selective. It is more readily absorbed by the leaves, buds especially, than by the roots and, has little translocation.

Furthermore, no studies using different earthworms species to remediate soils polluted with herbicides have been performed. For this reason, the objective of this study was to investigate the effect of three earthworm species (*E. fetida* Savigny, 1826; *L. terrestris* Linnaeus, 1758; and *A. molleri* Rosa, 1889) on restoring an oxyfluorfen herbicide-polluted soil and their influence on soil biochemical properties.

2. Materials and methods

2.1. Soil, organic wastes and herbicide characteristics

The soil used in this experiment is a Calcaric Regosol (FAO, 1989). Soil samples were collected from the 0–25 cm surface layer. The main soil characteristics are shown in Table 1 and the methodology used to determine the soils physical and chemical parameters is described in Gómez et al. (2014).

Table 1
Characteristics of the experimental soil (mean ± standard error). Data are the means of three samples.

pH (H_2O)	7.9 ± 0.2
Coarse sand (g kg^{-1})	486 ± 49
Fine sand (g kg^{-1})	130 ± 25
Silt (g kg^{-1})	123 ± 29
Clay (g kg^{-1})	260 ± 35
Total N (g kg^{-1})	0.93 ± 0.08
Organic matter (g kg^{-1})	17 ± 1

The herbicide used in this experiment was oxyfluorfen. The commercial formulation Fenfen (24% p v^{-1}, 240 g l^{-1}) was purchased from Lainco, S.A. (Spain). The rate applied to the soil was 4 l ha^{-1} (recommended application rate).

2.2. Experimental earthworms

Three earthworms species were selected for the bioaccumulation trials: (1) *E. fetida* were bred in laboratory cultures on organic waste materials, mainly vermicomposts; (2) *L. terrestris* were collected in the field in soils rich in organic matter and, in an area that had not been treated with pesticides for 20 years; and (3) *A. molleri*. This latter is an earthworm that lives in soils that are rich in organic matter with substantial vegetative remains and sufficient moisture (Tejada et al., 2013). The earthworms were collected from behind the ETSIA greenhouse (University of Sevilla, Spain) from soil untreated with pesticides.

2.3. Incubation procedure

Two kg of soil were pre-incubated in 5-l containers at 25 °C for 7 days at 30–40% of their water-holding capacity, according to the procedures defined by Tejada (2009). After this pre-incubation period, soil samples were treated with oxyfluorfen and with and without the different experimental earthworms. An unamended polluted soil without earthworms was used as control. The incubation treatments are detailed as follows:

1. C, control soil, non-polluted soil without earthworms.
2. CEF, non-polluted soil with *E. fetida*.
3. CLT, non-polluted soil with *L. terrestris*.
4. CAM, non-polluted soil with *A. molleri*.
5. C + O, oxyfluorfen-polluted soil without earthworms.
6. CEF + O, oxyfluorfen-polluted soil with *E. fetida*.
7. CLT + O, oxyfluorfen-polluted soil with *L. terrestris*.
8. CAM + O, oxyfluorfen-polluted soil with *A. molleri*.

The herbicide was applied at a pressure of 3 kg cm^{-2} and 300 l ha^{-1} of application using a laboratory treatments machine equipped with Teejet 80.02 E.VS flat fan fuzes.

Three replicates of each treatment were kept in mesocosms at 25 °C for 3, 15, 45 and 90 days, respectively. Twenty five earthworms of each experimental species (*E. fetida* ~450 mg fresh weight; *L. terrestris* ~304 mg fresh weight; *A. molleri* ~970 mg fresh weight) were included in each mesocosm. Before being used in our experiment individuals of each species were selected after morphological examination. Earthworms were placed on damp filter paper for 48 h to empty their guts and then weighed. Each mesocosm was covered with fine nylon mesh to prevent the earthworms from escaping as well as to prevent soil loss.

2.4. Soil analysis

At days 3, 15, 45 and 90 of the incubation period and for each treatment, adenosine triphosphate (ATP) was extracted from soil using the Webster et al. (1984) procedure and measured as recommended by Ciardi and Nannipieri (1990). Twenty ml of a phosphoric acid extractant was added to 1 g of soil, and the closed flasks were shaken in a cool bath. The mixture was then filtered through Whatman paper and an aliquot was used to measure the ATP content by means of luciferin-luciferase assay in a luminometer (Optocomp 1, MGM Instruments, Inc.). Soil urease activity was determined by the Kandeler and Gerber method (1988), using urea as substrate. Phosphatase activity was measured using p-nitrophenyl phosphate as substrate (Tabatabai