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Behavior of two pesticides in a soil subjected to severe drought. Effects on soil biology

Luis Franco-Andreu[a], Isidoro Gómez[a], Juan Parrado[b], Carlos García[c], Teresa Hernández[c], Manuel Tejada[a,*]

[a] Grupo de investigación "Edafología ambiental", Departamento de Cristalografía, Mineralogía y Química Agrícola, E.T.S.I.A. Universidad de Sevilla, Crta de Utrera km. 1, 41013 Sevilla, Spain
[b] Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, C/Prof. García González 2, 41012 Sevilla, Spain
[c] Departamento de Conservación de Suelos y Agua y Manejo de Residuos Orgánicos, Centro de Edafología y Biología Aplicada del Segura, CEBAS-CSIC, PO Box 4195, 30080 Murcia, Spain

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ABSTRACT

The behavior of oxyfluorfen herbicide and chlorpyrifos insecticide applied at different concentrations in a soil submitted to a severe and continued 120-day drought and their influence on soil enzymatic activities (dehydrogenase, urease, β-glucosidase and phosphatase activities) and soil biodiversity (analyzed by phospholipid fatty acids) were studied under controlled laboratory conditions. Two levels of irrigation were employed: (1) watered soils, where the soils were maintained at 60% of their water holding capacity, and (2) non-watered soils, without irrigation. In watered soils, the oxyfluorfen herbicide and the chlorpyrifos insecticide both caused a toxic effect on both soil enzymatic activity and soil diversity. This decrease was greatest when the highest dose of herbicide was applied to the soil. The application of pesticides to the non-watered soil caused a greater inhibition of soil enzymatic activities and microbial population possibly due to the combined effect of both pesticide toxicity and soil drought conditions. For this reason, the degradation of pesticides in non-watered soil was lower than in the case of watered soils, which probably made the persistence in soil of both pesticides longer. This suggests that, in drought conditions, soil pollution by these pesticides increases over time.

1. Introduction

Recent global circulation models predict an increase in temperatures and a decrease in rainfall in the Mediterranean area in a not-too-distant future, increasing drought conditions in soil (IPCC, 2007).

In agricultural soils developed in semi-arid Mediterranean ecosystems, water availability is often the most limiting factor for crop growth, due to the combination of high temperatures and low rainfall (Sardans et al., 2006; Hueso et al., 2011, 2012; Curiel Yuste et al., 2014). Therefore, these harsher drought conditions and the consequent sharp decrease in available water can cause severe alterations in such ecosystems, affecting both the growth and yield of agricultural crops.

Furthermore, in Southern European agricultural soils oxyfluorfen and chlorpyrifos are in common use in order to minimize economic losses caused by weeds and insects, as well as to maintain current production, quality and yield levels (Gamón et al., 2003; Coppola et al., 2007; Fenoll Serrano et al., 2010; Hermosin et al., 2013). Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene] is a diphenyl ether selective contact herbicide with residual activity. It is more readily absorbed by leaves, and especially buds, than by roots with little translocation. Chlorpyrifos [($C_9H_{11}Cl_3NO_3$ PS) or (O,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridil phosphorothioate)] is a broad-spectrum organophosphate insecticide that is widely used for insect pest control in agriculture and for soil and foliar treatments in different crops (Korade and Fulekar, 2009; Zhang et al., 2012).

Under non-drought conditions, some authors have shown the toxic effect of both pesticides upon soil microorganisms, affecting both soil microbial population growth and activity (Gómez et al., 2014; Tejada et al., 2011, 2014, 2016). However, in the climatic predictions discussed above, the behaviour of these pesticides under dry Mediterranean soil conditions is unknown.

The current literature indicates that soil enzymatic activities react faster than physical variables and/or after any chemical

* Corresponding author.
E-mail address: mtmoral@us.es (M. Tejada).

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change in the soil. They may, therefore, be useful as early indicators of the various biological changes that may occur in soil (García et al., 2000; Masciandaro et al., 2004; Tejada et al., 2014, 2016). Furthermore, the phospholipid fatty acids (PLFA are also useful when measuring microbial community structure in soil (Frostegård et al., 1993, 2011; Bååth, 2003). Since soil enzymatic activities and microbial biodiversity depend on soil moisture (Bérard et al., 2011; Hueso et al., 2011, 2012; Curiel Yueste et al., 2014), both biological parameters could be used to understand the interaction of oxyfluorfen and chlorpyrifos with soil moisture.

The aim of this study was to examine, under controlled laboratory conditions, the behavior of oxyfluorfen herbicide and chlorpyrifos insecticide in a soil submitted to a severe and continued 120-day drought and its influence on soil enzymatic activities and soil biodiversity.

2. Material and methods

2.1. Soil and pesticide 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 were reported elsewhere (Rodríguez-Morgado et al., 2014; Tejada et al., 2014) and are summarized in Table 1.

Two pesticides were used. Firstly, the oxyfluorfen herbicide was used. The commercial formulation Fenfen (24% p v^{-1}, 240 g l^{-1}) was purchased from Lainco, S.A. (Spain). The second pesticide used was chlorpyrifos insecticide. The commercial formulation Senator® 48 (48% chlorpyrifos) was purchased from Bayer CropScience (Madrid, Spain).

2.2. Experimental design

In order to keep a soil in severe drought conditions over an extended set period of time (120 days) without risk of rain interrupting the experiment, different incubations were performed under laboratory conditions.

Two kg of dried and sieved (<2 mm) soil was placed in 5-L containers. Soil samples were mixed with oxyfluorfen herbicide at three concentrations [4 l ha^{-1} (recommended application rate), 2 l ha^{-1} (half recommended application rate), and 8 l ha^{-1} (twice recommended application rate)]. Soil was also mixed with chlorpyrifos insecticide at three concentrations [5 l ha^{-1} (recommended application rate), 2.5 l ha^{-1} (half recommended application rate), and 10 l ha^{-1} (twice recommended application rate)]. An unamended soil was used as control.

Two batches were moistened to 60% of the soil water-holding capacity (WHC) and incubated for 120 days. One of the batches was watered periodically to maintain the WHC at 40–60% (watered soils). The other batch was left to dry without watering (non-watered soils). The incubation treatments are detailed as follows:

1. S, untreated and watered soil throughout the experiment
2. S + O1, soil treated with oxyfluorfen at 4 l ha^{-1} and watered throughout the experiment
3. S + O2, soil treated with oxyfluorfen at 2 l ha^{-1} and watered throughout the experiment
4. S + O3, soil treated with oxyfluorfen at 8 l ha^{-1} and watered throughout the experiment
5. S + CL1, soil treated with chlorpyrifos at 5 l ha^{-1} and watered throughout the experiment
6. S + CL2, soil treated with chlorpyrifos at 2.5 l ha^{-1} and watered throughout the experiment
7. S + CL3, soil treated with chlorpyrifos at 10 l ha^{-1} and watered throughout the experiment
8. SD, untreated and non-watered soil throughout the experiment
9. SD + O1, soil treated with oxyfluorfen at 4 l ha^{-1} and non-watered throughout the experiment
10. SD + O2, soil treated with oxyfluorfen at 2 l ha^{-1} and non-watered throughout the experiment
11. SD + O3, soil treated with oxyfluorfen at 8 l ha^{-1} and non-watered throughout the experiment
12. SD + CL1, soil treated with chlorpyrifos at 5 l ha^{-1} and non-watered throughout the experiment
13. SD + CL2, soil treated with chlorpyrifos at 2.5 l ha^{-1} and non-watered throughout the experiment
14. S + CL3, soil treated with chlorpyrifos at 10 l ha^{-1} and non-watered throughout the experiment

Triplicate containers were randomly placed in an incubation chamber with controlled temperature (25 ± 1 °C). In watered soils, moisture loss was replaced by spraying containers weekly with distilled water to maintain their original weights.

Table 1
Characteristics of the experimental soil (mean ± standard error, n = 3).

	Soil
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})	17.2 ± 1.8
Humic-acid C (mg kg^{-1})	3250 ± 64
Fulvic-acid C (mg kg^{-1})	2933 ± 22
P (g kg^{-1})	14.5 ± 2.2
K (g kg^{-1})	20.3 ± 2.4
Fe (mg kg^{-1})	13.9 ± 2.8
Cu (mg kg^{-1})	10.4 ± 2.2
Mn (mg kg^{-1})	9.7 ± 1.5
Zn (mg kg^{-1})	6.6 ± 1.9
Cd (mg kg^{-1})	4.7 ± 1.3
Pb (mg kg^{-1})	2.4 ± 0.8
Ni (mg kg^{-1})	1.8 ± 0.3

2.3. Soil analysis

Gravimetric moisture content was measured by weighing soil samples before and after oven-drying at 105 °C for 48 h at days 5, 12, 26, 50, 79, 100 and 120 during the incubation period.

The activity levels of four soil enzymes for each treatment were measured at days 5, 12, 26, 59, 79, 100 and 120 during the incubation period. Dehydrogenase activity was measured as the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl) 5-phenyl tetrazoliumchloride to iodonitrophenylformazan (García et al., 1993). Urease activity was determined by the buffered method of Kandeler and Gerber (1988), using urea as the substrate. β-glucosidase activity was determined using *p*-nitrophenyl-β-D-glucopyranoside as the substrate (Masciandaro et al., 1994). Phosphatase activity was measured using *p*-nitrophenyl phosphate as the substrate (Tabatabai and Bremner, 1969).

For each treatment, phospholipids were extracted at days 10, 50 and 120 during the incubation period, (three replicates per treatment) using a chloroform–methanol extraction method based on Bligh and Dyer (1959). Phospholipids were transformed by alkaline methanolysis into fatty acid methyl esters (FAMEs), which were quantified by a gas chromatograph (TRACE GC Ultra, Thermo Scientific) fitted with a 60-m capillary column (BPX70 60 m X 0.25 mm ID X 0.25 μm film), using helium as carrier gas. The initial temperature was 150 °C for 0.5 min and it was increased to 180 °C