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Short-term response of soil enzyme activities in a chlorpyrifos-treated mesocosm: Use of enzyme-based indexes

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ABSTRACT

Soil enzyme activities have been long used as indicators of soil contamination, and their integration into numerical indexes of microbial functional diversity is a practical approach in the environmental risk assessment of soil pollutants. However, suitable numerical indexes need to be developed and standardized for monitoring deterioration of soil quality by agrochemicals. Herein, a mesocosm study was performed to examine short-term responses of selected soil enzyme activities to chlorpyrifos (Lorsban® 4E). Hydrolases (carboxylesterase, acid phosphatase, β-glucosidase, urease and protease) and oxidoreductases (dehydrogenase and catalase) were measured in Andisols 14 d after an application with two doses (4.8 and 24 kg a.i. ha^{-1}) of chlorpyrifos. Both application rates caused a strong inhibition of carboxylesterase (62–78% of controls), acid phosphatase (56–60%) and β-glucosidase (43–58%) activities. Soil microbial activity was also reduced in pesticide-sprayed soils as indicated by the decreased dehydrogenase (47%) and catalase (38%) activities compared with control soils. However, only carboxylesterase activity showed a dose-dependent response with the chlorpyrifos application rate. An *in vitro* trial was further performed to provide evidence of a direct interaction between the enzyme (carboxylesterase, acid phosphatase and β-glucosidase) and the pesticide (chlorpyrifos and its main metabolites chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol). Results of these *in vitro* assays showed that the activity of carboxylesterase was directly affected by chlorpyrifos-oxon and, at less extend, by chlorpyrifos, whereas variations of both acid phosphatase and β-glucosidase activities were likely dependent on changes in microbial activity. Urease and protease activities did not change in pesticide-treated soils compared with pesticide-free soils. Despite the absence of response in these two N-cycling enzyme activities, four enzymatic indexes (geometric mean, weighted mean, "treated-soil quality index" [T-SQI] and "integrated biological response" [IBRv2] index) were significantly lower in the chlorpyrifos-sprayed soils compared with controls. Moreover, there was a significant ($r^2 = 0.87$, $P < 0.0001$) correlation between T-SQI and IBRv2 scores, which suggested that the IBRv2 index (an index used for assessing animal's health inhabiting contaminated sites) may be a complementary index in soil quality assessment.

1. Introduction

Extracellular soil enzymes are key catalysts in the decomposition of soil organic matter (Burns et al., 2013; Arnosti et al., 2014). These proteins are mostly released by microorganisms, so their activity levels are used as indicators of microbial functional diversity (Nannipieri et al., 2002; Paz-Ferreiro and Fu, 2013). Accordingly, the measurement of soil extracellular enzyme activities, together with other physicochemical and biological variables, is a widely accepted approach to assess soil deterioration by environmental contaminants (Rao et al., 2014). In particular, pesticides induce quick changes in the activity of some soil enzymes such as phosphatases, β-glucosidase, cellulase or urease (Riah et al., 2014), but linking these changes to pesticide exposure is not always simple. The enzyme response is generally the result of complex direct and indirect effects occurring on enzyme activity (Floch et al.,

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2011; Gianfreda and Rao, 2008). Moreover, multiple environmental fluctuating factors (location of enzyme in soil, physicochemical properties of soil, land use, among others) interfere in soil enzyme responses to pesticides, adding complexity to the assessment of soil deterioration by pesticide treatments.

In an attempt to give a solution to this challenge, some authors recommend the use of enzyme-based numerical indexes to explain the response of enzyme activities in soils under environmental stressors such as contamination (Puglisi et al., 2006; Paz-Ferreiro and Fu, 2013). For instance, the geometric mean (GMean) index has been satisfactorily used in the assessment of metal-contaminated soils (Hinojosa et al., 2004; Lessard et al., 2014), oil-contaminated saline soils (Gao et al., 2013), contrasting agricultural managements (Paz-Ferreiro et al., 2013), as well as in the evaluation of the effectiveness of bioremediation actions in metal-contaminated soils (Lu et al., 2015). Similarly, Mijangos et al. (2010) developed a novel enzymatic index of soil quality named 'treated-soil quality index' (T-SQI), which has been used to examine the effects of pesticide and fertilizer inputs on several microbial endpoints of soil quality (Muñoz-Leoz et al., 2013). However, far too little attention has been paid to application of these indexes in soils contaminated by pesticides.

Pesticides are still a chemical strategy needed to control pests' damage on crops, accordingly their global consumption has progressively increased since 2007 (Peshin and Zhang, 2014). It is now recognized that pesticide residues in soil threaten other environmental compartments such as groundwater (Arias-Estévez et al., 2008), surficial water-bodies (Blann et al., 2009), and non-target organisms such as earthworms (Pelosi et al., 2013) and soil microorganisms (Gianfreda and Rao, 2008). In Chile, for instance, there has been a gradual increase of pesticide consumption in the last decade, being the organophosphorus (OP) the main class of agrochemicals (SAG, 2012). Consequently, OP residues have been systematically detected in both agricultural fresh foodstuff such as fruits and vegetables (Muñoz-Quezada et al., 2012) and transformed products as olive oil (Fuentes et al., 2010). Because Chile is among the largest fruit exporters of Latin America and pesticide consumption in this country is increasing (SAG, 2012), development of biological indicators for monitoring contaminated soils by agrochemicals may be of great concern to regulatory and environmental agencies.

This study seeks to provide a simple enzyme-based approach to assess the impact of OP treatments on soil enzyme activities. Therefore, the aims were: 1) to determine whether a single application of chlorpyrifos causes short-term changes in soil enzyme activities covering the main biogeochemical cycles, 2) to explore chemical-biological interactions that account for chlorpyrifos-induced changes in soil enzyme activities, and 3) to assess the potential of simple enzymatic indexes of soil quality for discriminating adverse effects in chlorpyrifos-treated soils, with particular emphasis on the "Integrated Biological Response version 2" index (IBRv2). The IBRv2 index, proposed by Sanchez et al. (2012), integrates the response of multiple biomarkers (biological responses to environmental contaminants at sub-individual level of biological organization demonstrating departure from normal status, Walker, 2014) to provide a measurement of the animals' health status inhabiting contaminated environments. In recent years, there has been an increasing amount of literature showing the potential of the IBRv2 index in the ecological risk assessment of contaminated aquatic ecosystems (Raphael et al., 2014; Catherine et al., 2015; Marques et al., 2016; Vieira et al., 2016). Altogether, results in this study should be useful not only to standardize enzyme-based indexes for soil quality monitoring purposes, but also to increase the potential use of the IBRv2 index beyond the frontier of aquatic toxicology.

2. Materials and methods

2.1. Site description and experimental design

The study site is located at the Faculty of Agriculture of the University of Concepción (Chillán, Biobio Region, Chile; 36°35′53″S and 72°04′59″W, elevation 137 m a.s.l., Fig. 1). The climate is temperate Mediterranean; characterized by an average annual temperature of 14 °C (average coldest temperatures in July ranging from 3.5° to 5 °C, and an average highest temperature in January of 29 °C). The annual precipitation is 1006 mm and annual pan evaporation is 1257 mm. The mesocosm study was performed in an abandoned agricultural field of 1150 m^2, which was organically cultivated with blueberry (*Vaccinium corymbosum* L.). We selected this field because this kind of crop has experienced a huge growing in the last decades in Chile, accompanied with a high consumption of chlorpyrifos as indicated in Briceño et al. (2012). At the time of the mesocosm study, some blueberry plants were still present on the experimental field together with a dense weed cover. Grass was removed from each plot before pesticide application (Fig. 1). Soil is an Andisol (Humic haploxerands) with a loam texture (32.7% sand, 47.4% silt and 19.9% clay), a pH value of 6.53 ± 0.14 (1:5 w/v in water, mean ± SD, n = 6), an electrical conductivity of 61.53 ± 6.51 μS cm^{-1}, and a total organic carbon of 43.7 ± 0.7 mg C g^{-1} dry soil (dichromate redox colorimetric method by Skjemstad and Baldock, 2007).

Chlorpyrifos was applied as an emulsified formulation named Lorsban® 4E (48% w/v chlorpyrifos, Dow AgroScience Chile, S.A., Chile) in a split-plot design, which consisted of nine 1-m^2 plots. We chose the highest acceptable application rate recommended by the manufacturer (10 l ha^{-1}), and a dose 5-fold higher to examine their impact on soil enzyme activities. Therefore, three plots were treated with 4.8 kg a.i. ha^{-1} (recommended dose), and three plots received 24 kg a.i. ha^{-1} (5× dose), using a hand operated sprayer. The other three plots acted as controls. The initial predicted environmental concentrations ($PEC_{S,0}$) associated to 4.8 and 24 kg a.i. ha^{-1} were 9.5 and 45.7 mg chlorpyrifos kg^{-1} dry soil, which were estimated using the equation (FOCUS, 2006): $PEC_{S,0} = A \times (1 - fint)/(100 \times depth \times bd)$, where $PEC_{S,0}$ is the initial soil concentration (mg kg^{-1}) of chlorpyrifos immediately following a single application, A is the application rate (g ha^{-1}), f_{int} is the fraction of pesticide intercepted by the crop canopy, *depth* is the mixing depth (cm), and *bd* is the dry bulk soil density (g cm^{-3}). In $PEC_{S,0}$ calculations, we assumed a bulk soil density of 1.05 g cm^{-3} (Sandoval et al., 2007), a depth soil layer of 5 cm of pesticide penetration, and no crop interception as weed cover was removed from the plots.

A soil sample was collected from the upper layer from each plot after 14 d of pesticide application (each sample was a composite of five sub-samples per plot). This sampling time was chosen according to a previous laboratory study that showed a significant effect of chlorpyrifos on soil esterase activities at a short-time scale (Sanchez-Hernandez et al., 2015). Samples were stored in plastic bags at 4–5 °C, and transported to the laboratory of ecotoxicology (Univ. Castilla-La Mancha, Spain) where were kept at −30 °C until analysis (1 month).

2.2. Chemical analysis of chlorpyrifos residues

Chlorpyrifos concentrations were determined in an Agilent 1200 Series HPLC system, equipped with an automatic injector, a vacuum degasser, a quaternary pump and a diode-array detector. The pesticide extraction followed the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure described in the AOAC official method 2007.01 (Lehotay, 2007), with some modifications