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Technical Note

Chlorpyrifos degradation in a biomixture of biobed at different maturity stages

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ABSTRACT

The biomixture is a principal element controlling the degradation efficacy of the biobed. The maturity of the biomixture used in the biobed affects its overall performance of the biobed, but this is not well studied yet. The aim of this research was to evaluate the effect of using a typical composition of Swedish biomixture at different maturity stages on the degradation of chlorpyrifos. Tests were made using biomixture at three maturity stages: 0 d (BC0), 15 d (BC15) and 30 d (BC30); chlorpyrifos was added to the biobeds at final concentration of 200, 320 and 480 mg kg^{-1}. Chlorpyrifos degradation in the biomixture was monitored over time. Formation of TCP (3,5,6-trichloro-2-pyrinidol) was also quantified, and hydrolytic and phenoloxidase activities measured. The biomixture efficiently degraded chlorpyrifos (degradation efficiency >50%) in all the evaluated maturity stages. However, chlorpyrifos degradation decreased with increasing concentrations of the pesticide. TCP formation occurred in all biomixtures, but a major accumulation was observed in BC30. Significant differences were found in both phenoloxidase and hydrolytic activities in the three maturity stages of biomixture evaluated. Also, these two biological activities were affected by the increase in pesticide concentration. In conclusion, our results demonstrated that chlorpyrifos can be degraded efficiently in all the evaluated maturity stages.

1. Introduction

Pesticides play an important role in the success of modern farming and food production. However, surface and groundwater can be contaminated with pesticides due to their inadequate management. Point sources such as accidental spillages during tank filling or cleaning the spraying equipment have been identified as the major contamination risks (Müller et al., 2002). Pesticide contamination is extremely harmful for the environment and constitutes an important public topic at world level. It is important to develop novel processes for the control and treatment of this type of pollution, namely by taking part of the catalytic potential of biological systems that can be used to reduce pesticide contamination.

Biobeds are biological systems developed in Sweden and had been successfully used to reduce point source contamination by pesticides (Torstensson and Castillo, 1997; Torstensson, 2000; Vischetti et al., 2004). The principal component of the biobed systems is the biomixture. The typical biomixture is composed by straw:peat:soil in the proportions 50:25:25 vol.% (Castillo et al., 2008). Biomixture efficiency is based on the ability to retain and degrade pesticides and, therefore, a good biomixture must have good adsorption capacity and high biological catalytic activity for pesticide degradation (Castillo et al., 2008). There are several factors that can affect biomixture efficiency, such as temperature, composition, moisture, and maturity (age) of the biomixture (Castillo et al., 2008). Most of these factors have been evaluated in the biomixture (Fogg et al., 2003a,b; Fogg and Boxall, 2004), but the influence of using biomixtures at different maturity stages has not been extensively studied thus far. Castillo et al. (2008) reported that biomixture age is important because the progressive biodegradation of the component allows a series of microbial reactions and enzymatic activities that enable an efficient degradation of pesticides and avoid metabolite accumulation. Castillo and Torstensson (2007) suggested that a good practice to be accomplished is a precompost the biomixture before pesticide contamination. In this same sense, Castillo et al. (2008) reported that more research is needed to evaluate changes in pesticide degradation during maturation of the biomixture. Fogg et al. (2003b) reported that isoproturon and chlorotalonil were degraded (>90%) in a biomix precomposted for 80–100 d. However, comparisons between different maturity times were never performed.

Chlorpyrifos [*O,O*-diethyl *O*(3,5,6-trichloro-2-pyridyl) phosphorothiate] is a broad-spectrum organophosphorus insecticide widely used throughout the world. It has low water solubility (1.4 mg L^{-1}) and a high affinity for soil organic carbon (K_{oc} = 8.5 L g^{-1}) (Racke et al., 1996). Reduction in the abundance of microbial communities in soil has been observed after contaminations with chlorpyrifos (Chu et al., 2008). Also, chlorpyrifos bounds to soil constituents that may be introduced into rivers by surface runoff from agricultural lands (Wu and Laird, 2004).

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Degradation of chlorpyrifos leads to the formation of a major metabolite that has been identified as 3,5,6-trichloro-2-pyrinidol (TCP). This metabolite presents antimicrobial properties due to the presence of the chlorine atoms in its structure, which, when removed from the structure, can be toxic to chlorpyrifos-degrading microflora (Singh et al., 2003). It has also been reported that TCP in soil can delay chlorpyrifos degradation (Racke et al., 1990).

The influence of biomixture maturity in pesticide degradation is not extensively studied. This is important for defining adequate conditions for the initial establishment and correct preparation of a biomixture. Therefore, the aim of this study has been to evaluate three stages of maturity of the biomixture and their effect on pesticide degradation and biological activities.

2. Materials and methods

2.1. Chemicals

Analytic-grade (99% purity) chlorpyrifos and TCP were purchased from Sigma–Aldrich. Formulated chlorpyrifos (Clorpirifos S480) of 48% w/v was purchased from ASP Chile. MBTH (3-methyl-2-benzothiazolinone hydrazone), DMAB (3-(dimethylamino) benzoic acid) were purchased from Aldrich. All others chemicals and solvents were of analytical reagent grade and were purchased from Equilab and Merck Chile.

2.2. Preparation of the biomixture and maturity conditions

The biomixture was prepared by mixing an Andisol top soil without chlorpyrifos application history (37.1% sand, 34.2% silt, 28.7% clay, organic matter 12%, pH 5.9), commercial peat (organic carbon 39.67%) and winter wheat straw (organic carbon 43%) in the volumetric proportions of 1:1:2 respectively, in the same form of the traditional Swedish biomixture. The straw was cut using a food processor to obtain small fragment (3 mm approximately) to obtain homogeneous biomixture according to Castillo et al. (2008) and mixed with top soil and peat. Soil and peat were also sieved to homogenize (3 mm). The biomixture was put in polypropylene bags for the maturation process, moisture content was adjusted with distilled water to 60% of their water holding capacity and stored in dark at 20 °C ± 2. Polypropylene bags were maintained under these condition during the following time of maturation: 0 d (fresh biomixture), 15 and 30 d, namely BC0, BC15 and BC30 respectively, prior to being used in the chlorpyrifos experiments. The major chemical properties of the biomixture in the three different times compost are shown in Table 1.

2.3. Degradation studies

Formulated chlorpyrifos containing the active ingredient (Clorpirifos S480) was used in the degradation studies. One bulk sample of biomixture (2500 g dw) of BC0, BC15 and BC30 was separated into 84 sub-samples (25 g) and placed in glass flask (250 mL). 63 sub-samples were individually treated with formulated chlorpyrifos at an application rate of 200, 320 and 480 mg kg^{-1} of active ingredient chlorpyrifos approximately. 21 sub-samples per each concentration were established and 21 sub-samples were used for control purposes in biological assays to BC0, BC15 and BC30. The three concentrations used were to simulate an accidental spill of pesticide. After mixing, the flasks were incubated in the dark for 40 d at 20 ± 2 °C. The biomix moisture level was maintained through periodic application of distilled water. Immediately after mixing, treated samples and control samples were removed and stored at −20 °C until their analysis. Chlorpyrifos residues and TCP were extracted from biomixture (5 g) by shaking (350 rpm, 2 h) and ultrasonication (30 min) with 6 mL of acidified acetone (acetone + water + concentrated phosphoric acid, 98:1:1 by volume) per gram of substrate (Coppola et al., 2007). Later, the samples were centrifuged at 10000 rpm and the supernatant (5 mL) was filtered with PTFE membrane (0.2 μm pore size; Millipore) and analyzed by liquid chromatography (HPLC) as is described below.

Table 1
Chemical characterization of the biomixture in the three different stages (d) of maturity evaluated.

Parameter	0 d	15 d	30 d
N (mg kg^{-1})	27	25	26
P (mg kg^{-1})	19	13	12
K (mg kg^{-1})	997	1095	1153
pH (water)	6.1	6.6	6.9
Organic matter (%)	21.1	20	22
Na (cmol + kg^{-1})	1.8	1.8	1.8
Ca (cmol + kg^{-1})	17.9	19.8	20.2
Mg (cmol + kg^{-1})	5.6	5.4	5.6
Al (cmol + kg^{-1})	0.02	0.01	0.02
Cation exchange capacity (CEC) (cmol + kg^{-1})	27.8	29.8	30.6
Sum of bases (cmol + kg^{-1})	27.8	29.8	30.6

2.4. Determination of biological activities in the biomixture

Phenoloxidase activity was determined in all degradation assays and was performed using MBTH/DMAB (Castillo et al., 1994). Briefly, samples (10 g) of the biomixture were agitated (150 rpm, 2 h) with 25 mL of a 100 mM succinate–lactate buffer (pH 4.5). Samples were centrifuged (4000 rpm, 20 min). The supernatant of each sample was collected, filtered through 0.45 μm membrane (National Scientifics filter unit) and measured immediately. The reaction mixture contained 300 μL of 6.6 mM DMAB, 100 μL of 1.4 mM MBTH, 30 μL of 20 mM $MnSO_4$, 1560 μL of the filtered sample and, the reaction is initiated with the addition of 10 μL of 10 mM H_2O_2. The reaction is followed in a spectrophotometer Spectronic Genesis 2PC at 590 nm ($\varepsilon = 0.053\ \mu M^{-1}\ cm^{-1}$). Because no correction was made for the possible presence of lignin peroxidase (LiP) and laccase (Lac) activity, this measurement may represent the sum of manganese peroxidase, LiP and Lac (Castillo and Torstensson, 2007) and is expressed as phenoloxidase activity.

Hydrolytic activity was determined in all degradation assays and measured by monitoring fluorescein diacetate hydrolysis (FDA) according to Schnürer and Rosswall (1982) with slight modifications. Briefly, 1 g of biomixture was incubated in a 30 mL conical flask with 9.9 mL of sterile 60 mM sodium phosphate buffer, pH 7.8. The reaction was started by adding 0.1 mL of a FDA solution (2.0 mg mL^{-1}). After 1 h of incubation at 25 ± 1 °C, 10 mL of acetone was added to stop the reaction. A_{490} was measured in a spectrophotometrically after removal of the biomixture by centrifugation and filtration. The concentration of the released fluorescein was calculated by a calibration curve with standard quantities of FDA and the results were expressed as μg FDA $g^{-1}\ h^{-1}$.

2.5. Pesticide analysis

For pesticide analysis the samples were injected with a Rheodyne 7725 injector with a 20 μL loop in a Merck Hitachi HPLC system equipped with L-7100 pump, and an L-7455 diode array detector. The detector was set at 290 nm and the column was a C18 column (Superspher RP-C18, 5 μm 4.6 × 150 mm). The mobile phase consisted of 60% CH_3CN and 40% water/acetic acid (95.3/4.3 v/v) with a flow rate of 1 mL min^{-1} at 25 °C. The chlorpyrifos and TCP recovery was >85% in the biomixture. The retention times