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Removal of pesticides and ecotoxicological changes during the simultaneous treatment of triazines and chlorpyrifos in biomixtures

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HIGHLIGHTS

- Removal of triazines and chlorpyrifos in biomixture, bioaugmented biomixture and soil.
- Final removal (60 d) was similar in soil and biomixture, but faster in the latter.
- Fungal bioaugmentation of the biomixture delayed pesticide removal and detoxification.
- Fast detoxification in soil and biomixture according to tests on *Daphnia magna*.
- Unclear detoxification patterns (phytotoxicity) despite high herbicide removal.

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ABSTRACT

Biopurification systems constitute a biological approach for the treatment of pesticide-containing wastewaters produced in agricultural activities, and contain an active core called biomixture. This work evaluated the performance of a biomixture to remove and detoxify a combination of three triazine herbicides (atrazine/terbuthylazine/terbutryn) and one insecticide (chlorpyrifos), and this efficiency was compared with dissipation in soil alone. The potential enhancement of the process was also assayed by bioaugmentation with the ligninolytic fungi *Trametes versicolor*. Globally, the non-bioaugmented biomixture exhibited faster pesticide removal than soil, but only in the first stages of the treatment. After 20 d, the largest pesticide removal was achieved in the biomixture, while significant removal was detected only for chlorpyrifos in soil. However, after 60 d the removal values in soil matched those achieved in the biomixture for all the pesticides. The bioaugmentation failed to enhance, and even significantly decreased the biomixture removal capacity. Final removal values were 82.8% (non-bioaugmented biomixture), 43.8% (fungal bioaugmented biomixture), and 84.7% (soil). The ecotoxicological analysis revealed rapid detoxification (from 100 to 170 TU to <1 TU in 20 d) towards *Daphnia magna* in the biomixture and soil, and slower in the bioaugmented biomixture, coinciding with pesticide removal. On the contrary, despite important herbicide elimination, no clear detoxification patterns were observed in the phytotoxicity towards *Lactuca sativa*. Findings suggest that the proposed biomixture is useful for fast removal of the target pesticides; even though soil also removes the agrochemicals, longer periods would be required. On the other hand, the use of fungal bioaugmentation is discouraged in this matrix.

1. Introduction

Agriculture represents an important sector in worldwide economy; therefore the use of pesticides for pest control in agriculture is common to maximize production. The use of these chemicals results in negative impacts on ecosystems when they enter water bodies by diffuse or point source contamination (Karanasios et al., 2012). Diffuse contamination includes surface runoff, leaching and drainage, as the major important pathways (Vymazal and Březinová, 2015); in the meantime, point source contamination includes the pollution processes derived from leakages or improper handling of pesticide application equipment due to incorrect disposal of residues or washing waters (De Wilde et al., 2007), and can be controlled by good practices in the field.

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The adsorption by activated carbon and advanced oxidation processes represent potential, though costly physicochemical strategies to remove pesticides from wastewater. One biotechnological, low-cost approach to reduce point source contamination is the use of biopurification systems (BPS), which are intended to eliminate pesticide residues by microbial action. Degradation in this matrix is possible thanks to the presence of the biomixture, the biologically active component of BPS. The biomixture is composed of three materials: a lignocellulosic substrate, employed to enhance the colonization and activity of ligninolytic fungi, known for their capacity to transform organic pollutants such as pesticides (Mir-Tutusaus et al., 2014; Rodríguez-Rodríguez et al., 2013); soil, commonly pre-exposed to the target pesticide, which provides an adapted microbial community (Sniegowski et al., 2012); and finally, a humic-rich component to enhance the retention of the pesticides in the matrix (Karanasios et al., 2012).

Considering that microbial degradation is one of the main processes involved in the environmental decontamination of pesticides, bioaugmentation of the BPS using pesticide-primed materials (Grundmann et al., 2007; Sniegowski and Springael, 2015) or specialized pesticide-degrading bacterial isolates or consortia to enhance biodegradation, is a matter of appeal in environmental biotechnology (Karas et al., 2016; Verhagen et al., 2013). In particular, considering that ligninolytic fungi may play a leading role in the processes taking place within the biomixture due to the high content of lignocellulosic materials in this matrix (Rodríguez-Rodríguez et al., 2013), the bioaugmentation with such microorganisms is of high interest.

Determination of biomixture performance usually relies on demonstrating the elimination of the parent compounds by means of analytical techniques; however, the transformation of the pesticides may result in the production of toxic metabolites, even of higher toxicity than the original compound. Therefore, the application of ecotoxicological assays represents a global approach to determine the detoxification potential of these matrices. Toxicity tests on *Daphnia magna* permitted to determine acute detoxification of biomixtures used for carbamates degradation (Chin-Pampillo et al., 2015; Rodríguez-Rodríguez et al., 2017), as well as the inability to detoxify a complex mixture of pesticides (Huete-Soto et al., 2017b). Moreover, detoxification in seed germination tests supported the removal of several herbicides (Huete-Soto et al., 2017b). Similarly, chronic toxic effects in fish were demonstrated in biomixture leachates, in spite of achieving high degradation rates in this matrix (Ruiz-Hidalgo et al., 2016).

This study aimed to evaluate the removal of a mixture of three herbicides (atrazine, terbuthylazine and terbutryn) and the insecticide chlorpyrifos in a previously optimized biomixture made of coconut fiber, compost and soil (Chin-Pampillo et al., 2015); in order to determine a potential enhancement in the process, the same biomixture was also used after bioaugmentation with the ligninolytic fungus *Trametes versicolor*. To determine the relative efficiency of the biomixtures, their performance was compared to the removal in soil, in which a reduced removal was expected. To estimate the potential detoxification due to take place in parallel to pesticide elimination in the biomixtures and soil, ecotoxicological assays (acute toxicity on *D. magna* and seed germination tests in *Lactuca sativa*) were conducted.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards atrazine (6-chloro-*N*2-ethyl-*N*4-isopropyl-1,3,5-triazine-2,4-diamine, purity 99.0%) and terbuthylazine (*N*2-*tert*-butyl-6-chloro-*N*4-ethyl-1,3,5-triazine-2,4-diamine, 98.5%), were acquired from Dr. Ehrenstorfer (Augsburg, Germany); terbutryn (*N*2-*tert*-butyl-*N*4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, 98.1%) and chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, 99.5%) were obtained from Chem Service Inc. (West Chester, Pennsylvania, USA). Commercial formulations of atrazine (Atranex 90 WG®, 90% w/w), terbutryn (Terbutrex 50 SC®, 50% w/v), terbuthylazine (Terbusol 50 SC®, 50% w/v) and chlorpyrifos (Solver 48 EC®, 48% w/v) were acquired from a local store. Carbofuran-d_3 (surrogate standard, 98.0%) and linuron-d_6 (internal standard, 98.5%) were purchased from Dr. Ehrenstorfer. Solvents and extraction chemicals are listed in Ruiz-Hidalgo et al. (2014).

2.2. Experimental setup

A biomixture containing coconut fiber, compost and soil at a volumetric composition of 45:13:42 (pH 6.4; C 4.83%; N 0.32%; C/N 15.2; P 0.22%; Ca 0.48%; Mg 0.71%; K 0.19%; S 0.07%; Fe 31 192 mg kg^{-1}; Cu 94 mg kg^{-1}; Zn 91 mg kg^{-1}; Mn 521 mg kg^{-1}; B 66 mg kg^{-1}; EC 0.6 mS cm^{-1}) was employed for the removal of triazine herbicides and the organophosphate insecticide chlorpyrifos. To obtain the bioaugmented biomixture, this matrix was inoculated with the fungus *T. versicolor* (ATCC42530) in the form of a mycelial suspension (Rodríguez-Rodríguez et al., 2017). Removal assays were performed in trays (20 × 15 × 9.5 cm) containing approximately 200 g of the biomixture. Six trays containing the biomixture were prepared; three of them were bioaugmented with *T. versicolor* mycelial suspension, which was added at a ratio of 3 mL/g biomixture. Three additional trays were prepared containing soil with no record of preexposure to the target pesticides. All the trays were spiked with a mixture of the commercial formulations of herbicides and chlorpyrifos, to give a final concentration of 40 mg kg^{-1} of each pesticide, and then were incubated in static conditions at 25 °C until the end of the assay. Water content losses were frequently adjusted according to weight determinations in each system. Samples were periodically withdrawn over a 60 d-period to determine the concentration of pesticides and to perform ecotoxicological assays.

2.3. Analytical procedures

2.3.1. Extraction and quantification of pesticides

Extraction of pesticides was carried out following a method described by Ruiz-Hidalgo et al. (2014), which employs a mixture of water and acidified acetonitrile (formic acid 1% v/v) as extractant. Carbofuran-d_3 and linuron-d_6 were added as surrogate and internal standard, respectively. Analyses were performed by LC-MS/MS using ultra high performance liquid chromatography (UPLC-1290 Infinity LC, Agilent Technologies, CA) coupled to a triple quadrupole mass spectrometer (Agilent Technologies model 6460). Chromatographic separation was done at 40 °C by injecting 6 μL samples in a Poroshell 120 EC-C18 column (100 mm × 2.1 mm i.d., particle size 2.7 μm), and using acidified water (formic acid 0.1% v/v, A) and acidified methanol (formic acid 0.1% v/v, B) as mobile phases. The mobile phase flow was 0.3 mL min^{-1} at the following conditions: 30% B for 3 min, followed by a 15 min linear gradient to 100% B, 4 min at 100% B and 0.1 min gradient back to 30% B, followed by 4 min at initial conditions. Selected transitions, LOD and LOQ for the analytes are shown in Table 1. Conditions of the mass spectrometry detector are described in Chin-Pampillo et al. (2015).

Removal values for each pesticide were determined from triplicate systems per matrix, as percentages with respect to the initial concentration quantified in the samples. Differences in the removal of the treatments (biomixture, bioaugmented biomixture and soil) at each sampling point were determined by one-way ANOVA and