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Atrazine dissipation and its impact on the microbial communities and community level physiological profiles in a microcosm simulating the biomixture of on-farm biopurification system

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HIGHLIGHTS

- Effect of atrazine on microcosm simulating a peat based biomixture of on-biopurification system was evaluated.
- Atrazine dissipation, enzyme activities and microbial communities were assessed.
- Efficient atrazine degradation and short-term inhibitory effect on microbial activity were observed.
- Atrazine did not significantly change the structure of microbial communities.
- We demonstrate the microbiological robustness of peat based biomixture when high atrazine dose is treated.

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ABSTRACT

The effects of repeated atrazine application (40 mg a.i. kg^{-1}) on its degradation, microbial communities and enzyme activities were studied in a peat based biomixture composed by straw, soil and peat in the volumetric proportions of 2:1:1 that can be used in on-farm biopurification system. Atrazine removal efficiency was high (96%, 78% and 96%) after each atrazine application and did not show a lag phase. Microbial enzyme activities were reduced significantly with atrazine application but rapidly recovered. Microbial diversity obtained by BiologEcoplate™ was similar after the first and second atrazine application. However, an inhibitory effect was observed after the third application. After each atrazine application, culturable fungi were reduced, but rapidly recovered without significant changes in culturable bacteria and actinomycetes compared to the control. Denaturing gradient gel electrophoresis (DGGE) patterns demonstrated that microbial community structure remained relatively stable in time when compared to the controls. In conclusion, our results demonstrated that after successive ATZ applications, the peat based biomixture had a good degradation capacity. Moreover, microbiological assays demonstrated the robustness of the peat based biomixture from a microbiological point of view to support pesticide degradation.

1. Introduction

On farm biopurification systems, commonly known as "biobeds", are a biotechnological tool used to reduce pesticide point source pollution [1]. The principal component is the biomixture [2], which is composed traditionally of straw, peat and soil in a volumetric proportion of 2:1:1. However, some lignocellulosic substrates of the biomixture have been replaced in some countries, for adaptation purposes [3–5]. This composition promotes the development of numerous microorganisms, especially white rot fungi, which can degrade pesticides through extracellular enzymes, e.g., phenoloxidases [2]. Several studies have been reported to examine pesticide biodegradation in the biomixture of a biopurification system [6–12] and in a full-scale biobed model [13,14].

Pesticides are synthesised to inhibit the growth of target microorganisms, but their effects can often be extended to non-target soil microorganisms as has been well reported in the literature [15,16], causing changes in microbial community structure and soil quality [16–18]. Thus, it is expected that pesticides will also affect the microbial communities in the biomixture of biopurification systems. However, few studies up to now have been presented detailed information on pesticide effect on the microbial

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community structure in a traditional biomixture of biobed system [2,19]. Therefore, a better understanding on the dynamics of the microbial communities and enzyme activities in the biomixture after pesticide exposure in this biopurification system is crucial to know if possible changes induced in microbial communities and their activities can influence the pesticide biodegradation and their microbiological sustainability. Research to date suggests that the composition of microbial communities in compost based biomixtures contaminated with pesticides is reduced, but recovery from the effects of pesticides is normally observed over time. For example, Vischetti et al. [9] reported that the microbial biomass (MBC) content in a compost based biomixture composed of vine-branches, urban wastes-garden compost following the addition of chlorpyrifos and metalaxyl negatively affected the MBC, but this was recovered as the pesticide concentration decreased. Similar results regarding the effect different fungicides had on microbial diversity in a modified biomixture consisting of compost and straw were reported by Coppola et al. [20]. Denaturing gradient gel electrophoresis (DGGE) showed a transient change of microbial diversity that was correlated to fungicides application. Recently, Marinozzi et al. [5] reported that the application of three fungicides showed adverse effects in the structure of biomixture microorganisms. However, negative effects were transitory, and recoveries of microbial parameters were observed 60 d after the pesticide application. However, these effects could be more severe when pesticides are more persistent, applied repeatedly at high concentrations or occur in combination with other toxic compounds [2,7,9,21]. Although Sniegowski et al. [10] reported that in biobed system linuron mineralisation not was affected when the biomixture was exposed to cold period and to pesticide mixture. Interestingly, Sniegowski et al. [10] reported that variations in overall microbial communities in biobed were associated with environmental changes as cold or drought period and not related with pesticide application. Moreover, changes in microbial communities related with pesticide application were observed when specific target bacterial genera were evaluated. In another work, Sniegowski et al. [11] reported that bioaugmentation of on-farm biopurification system with pesticide primed-soils, showed the proliferation of specific microorganisms responsible of the linuron mineralisation. However, it was not clear in the biomixture composed by non-primed soil. In this sense, Bers et al. [12] reported that the presence of linuron in the biomixture could activate important unknown biological mechanism involved in linuron degradation. According to the mentioned above, variation and effects caused by pesticides in the biomixture are just beginnings to be explored.

Atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine] (ATZ) it is a herbicide widely used in agricultural activities in the world to control broadleaf weeds in corn (*Zea mays* L.) and wheat (*Triticum aestivum*) production. ATZ has been identified as a moderately persistent pesticide and their movements through environment are key factors influencing its potential to contaminate soil and water [22]. Moreover, ATZ has been reported as one of the major pesticide requiring an important attention and therefore the development of effective methods for decontamination of contaminated matrices [23,24] and little information has been reported in the literature regarding its degradation and their microbiological effects in the biobed system.

Thus, the aim of the present study was to evaluate the impact of ATZ applied repeatedly at high concentrations on microbial functional diversity, microbial communities, enzyme activities and its degradation in a biopurification system background composed by straw:peat:soil in the volumetric proportions of 2:1:1. This biomixture was selected because is widely used in biobed system and little information is reported from microbiological point of view, compared to compost based biomixtures.

2. Materials and methods

2.1. Chemicals

Analytical standards of ATZ (99% purity) were purchased from Chem Service (West Chester, USA). The commercial formulation of ATZ (Atranex 50 SC) was obtained from Agan Chemicals Manufacturers Ltd. MBTH (3-methyl-2-benzothiazolinone hydrazone), DMAB (3-(dimethylamino) benzoic acid) were purchased from Aldrich.

2.2. Biomixture preparation

The biomixture was prepared by mixing top soil, commercial peat (organic carbon 39.6%) and winter wheat straw (organic carbon 43%) in the volumetric proportions of 1:1:2, respectively. The soil (30.7% sand, 41.8% silt, 27.4% clay, organic matter 18%, pH 6.1) was collected (0–20 cm) from the experimental station Maquehue (Andisol Freire series; 38°50′ S, 72°41′ W) of La Frontera University (Temuco-Chile), which was without an ATZ application history. The straw was cut into small fragments (3 mm) using a food processor, while the soil and the peat were sieved (3 mm). The constituents were mixed vigorously and homogenised by hand. The biomixture was placed inside of a polypropylene bag for the maturation process during 150 d at 25 ± 2 °C and the moisture content was adjusted with sterilised distilled water (SDW) to approximately 60% of its water holding capacity (WHC) and stored. Chemical characterisation is available as supplementary material (Table S1).

2.3. Biomixture treatment

After the maturation process, bulk samples (2.0 kg) of the biomixture were placed into glass containers (40 cm × 20 cm × 10 cm deep) in triplicate and artificially contaminated. ATZ was applied with a predetermined volume according to a commercial formulation, which allowed for a proper dilution with SDW to give a certain level of ATZ (40 mg a.i. kg^{-1}) and to obtain biomixture moisture of 60% (WHC). The ATZ dose applied was approximately forty-fold above the recommended field dose, to simulate a pesticide spill on the biomixture. The treatments consisted of three successive doses of ATZ at the same concentration on 0, 30 and 60 d. A biomixture control received the same amount of SDW without ATZ. Control and treatment were incubated at 25 ± 2 °C for 90 d. The biomixture moisture was maintained by regular addition of SDW. At fixed intervals and between each ATZ application, samples of the biomixture were collected for the determination of residual ATZ and biological parameters. Pesticide recovery is available in supplementary material.

2.4. Enzyme activities

Dehydrogenase activity (DHA) was calculated using a standard curve using 2,3,5-triphenyltetrazoliumchloride (TTC) as a substrate according to Casida et al. [25]. DHA was expressed as μg TPF produced g^{-1} h^{-1}.

Acid and alkaline phosphatase activities were determined according to Tabatabai and Bremmer [26] using *p*-nitrophenyl phosphate (0.05 M) as a substrate. Phosphatase activities were expressed as μg p-nitrophenol g^{-1} h^{-1} produced.

Phenoloxidase activity was determined using the MBTH/DMAB method proposed by Castillo et al. [27] and Castillo and Torstensson [28].

2.5. Community level physiological profiles (CLPP)

CLPPs were assessed by the BiologEcoplate™ system (Biolog Inc., CA, USA). Microplates containing 96-wells filled with 31 sole