Chemosphere xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Carbendazim dissipation in the biomixture of on-farm biopurification systems and its effect on microbial communities

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HIGHLIGHTS

- Microbiological impact of carbendazim at high doses on biopurification system was evaluated.
- Efficient carbendazim degradation occurred after three successive applications.
- Enzyme activities were affected, but recovered quickly after each carbendazim application.
- Carbendazim did not significantly change the structure of microbial communities.
- We demonstrate the robustness of this biological system from a microbiological viewpoint.

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form 26 May 2013
Accepted 29 May 2013
Available online xxxx

Keywords:
Microbial community
Pesticides
On-farm biopurification system
Carbendazim dissipation

ABSTRACT

The impact of repeated carbendazim (CARB) applications on the extent of CARB dissipation, the microbial diversity, the community level physiological profile (CLPP), and the enzymatic activity within the biomixture of an on-farm biopurification system was evaluated. After three successive CARB applications, the CARB dissipation efficiency was high; the efficiency of dissipation was 87%, 94% and 96% after each application, respectively. Although microbial enzymatic activity was affected significantly by CARB application, it could recover after each CARB pulse. Likewise, the numbers of cultivable bacteria, fungi and actinomycetes (as measured in CFUs) were slightly affected by the addition of CARB, but the inhibitory effect of the pesticide application was temporary. Denaturing gradient gel electrophoresis (DGGE) and Biolog Ecoplate assays demonstrated that the microbial populations remained relatively stable over time when compared to the control. The results obtained herein therefore demonstrate the high dissipation capacity of this biomixture and highlight the microbiological robustness of this biological system.

1. Introduction

On-farm biopurification systems, termed "biobeds", are a biotechnological tool widely distributed in Europe and, more recently, in South America as well. Such systems were designed and implemented to mitigate point source contamination by agriculture pesticides (Torstensson and Castillo, 1997). This system is designed with several components, of which the principal component is the biomixture (Castillo et al., 2008). The biomixture is typically composed of straw, peat and topsoil in a volumetric proportion of 2:1:1. The traditional biomixture (Swedish biomixture, see Torstensson and Castillo, 1997) has been modified to alternative biomixtures in other countries for specialised purposes (Coppola et al., 2007; Karanasios et al., 2010). An efficient biomixture must have the capacity to retain and degrade contaminants by promoting the development of numerous and robust degrading microorganisms, especially white rot fungi. Such organisms can degrade pesticides with extracellular enzymes, such as phenoloxidases (Castillo et al., 2008). Several systems have been developed in recent years for pesticide degradation using traditional and modified biomixtures (Fogg et al., 2003; Coppola et al., 2007; Vischetti et al., 2008; Karanasios et al., 2010; Tortella et al., 2012), including full-scale model biobeds (Spliid et al., 2006; Omirou et al., 2012). However, the effects of the pesticides and their interactions with the microbial communities of the biomixture systems are not yet fully understood (Castillo et al., 2008; Karanasios et al., 2012). In soil, pesticides are known to have a significant effect on local microorganisms, causing changes in nutrient turnover rates, the microbial community structure and soil quality (Chowdhury et al., 2008; Kalia and Gosal, 2011; Muñoz-Leoz et al., 2011, 2013; Cycón et al., 2012; Imfeld and Vuilleumier, 2012). It is therefore reasonable to expect that pesticides might also affect the microbial populations in the biopurification system biomixtures. A better understanding of the microbiology of

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0045-6535/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.chemosphere.2013.05.084

such biomixtures will be crucial to understand the impact of pesticides on the microbial functional diversity and microbial communities within the biomixture. This information is needed to ensure the long-term sustainability of the biological systems for agricultural purposes. The current literature suggests that microbial communities in a pesticide-contaminated biomixture are adversely affected, though recovery is normally observed over time. Vischetti et al. (2008) reported a negative effect of chlorpyrifos and metalaxyl on the microbial communities of an alternative biomixture (composed of vine/branches, urban wastes/garden compost and soil), but as soon as the pesticide concentration decreased, the microbial activity was found to recover. Similar results were reported by Coppola et al. (2011) and by Marinozzi et al. (in press) regarding the effects of different fungicides on the microbial diversity of modified biomixture systems (composed of either compost and straw or pruning residues and straw, respectively). Interestingly, the effect of pesticides on the traditional biomixture of straw, peat and soil, which is the most widely used biomixture for on-farm biopurification systems, has not been extensively studied and is therefore the subject of this research. Here, carbendazim (CARB, methyl 2-benzimidazole carbamate) was used as a model contaminant. CARB is a broad-spectrum benzimidazole fungicide that is widely used to control foliar diseases on arable crops such as cereals, sugar and fodder beet, oil seed rape and fruits (EFSA, 2010). CARB is also a major degradation product of other common fungicides, including benomyl and thiphanate-methyl (Mazellier et al., 2002). CARB poses a high environmental concern due to its persistence in soil and its known adverse effect on soil microorganisms (Grogan and Jukes, 2003; Yan et al., 2011). The estimated single first order DT_{50} of CARB (time needed for disappearance of half the chemical) is 26–40 d (at 20 °C and 10 kPa soil moisture) (EFSA 2010). However, comprehensive information regarding the route of aerobic degradation of CARB in soil is lacking and detailed identification and quantification of degradation metabolites not available (EFSA 2010).

The aims of the present study were to evaluate the dissipation of CARB within the traditional biomixture and to evaluate the impact of repeated high-concentration CARB applications on the microbial composition, functional microbial diversity and enzymatic activities of the biomixture system.

2. Materials and methods

2.1. Chemicals

Analytical standard carbendazim (CARB) (99% purity) was purchased from Sigma Aldrich, Chile. Commercial formulation CARB (Itabarb 50%) was supplied by Solchem Ltda., Chile. MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-(dimethylamino) benzoic acid) were purchased from Sigma Aldrich, Chile. All other chemicals and solvents were of analytical reagent grade and were purchased from Equilab Ltda. and Merck S.A (Chile).

2.2. Biomixture preparation

The biomixture was prepared by mixing top soil, commercial peat (36.6% organic carbon) and winter wheat straw (34.0% organic carbon) in the volumetric proportion of 1:1:2. Topsoil (0–20 cm) was collected from the experimental station Maquehue (Andisol Freire series; 38°50′S, 72°41′W) at La Frontera University; this site had no history of CARB contamination. The soil was mainly composed of sand (30.7%), silt (41.8%), clay (27.4%), and organic matter (18%) and had a pH of 6.1. Wheat straw was cut in fragments of approximately 3 mm using a food processor, and the soil and peat were sieved through a 3 mm mesh. The constituents were then mixed vigorously and homogenised by hand mixing. The resulting biomixture was placed in a polypropylene bag and the moisture content corrected to approximately 60% of its water holding capacity (WHC) using sterile distilled water. The biomixture was left to mature for 150 d at 25 ± 2 °C; moisture content was kept stable by regular additions of sterile distilled water.

2.3. Experimental design and CARB treatments

Bulk samples (2.0 kg) of mature biomixture were placed in six glass containers (40 × 20 × 10 cm deep). Three of these containers were artificially contaminated with CARB (commercial formulation) taken from a stock solution (600 mg L^{-1}). CARB was sprayed to a final concentration of 40 mg a.i kg^{-1}, together with the appropriate amount of distilled water necessary to guarantee a WHC of 60%. The applied CARB dose was approximately forty-fold higher than the recommended field dose (approximately 1 mg kg^{-1} of soil), in order to mimic a pesticide spill on the biomixture. Two additional CARB doses (40 mg a.i kg^{-1}) were subsequently applied to the contaminated biomixtures, at 30 and 60 d. CARB was added in high dose because the biomixture of the biobeds was designed to degrade pesticide residues from accidental pesticide spills. Moreover, CARB was added to the biomixture as commercial formulations because co-adjuvants or surfactants present in commercial CARB may affect both degradation rates and their impact on soil microbial communities (Beigel et al., 1999). Biomixture control assays (triplicates) received the same amount of sterile distilled water as CARB-contaminated biomixtures, but no pesticide was sprayed in these assays. Each container was covered with a perforated plastic film to avoid excessive evaporation and incubated in the dark at 25 ± 2 °C for a period of 90 d. The biomixture moisture was kept stable by regularly adding sterile distilled water. At fixed intervals, biomixture samples were collected to determine the level of residual CARB and other biological parameters. Residual CARB was extracted from 5 g of the biomixture with 30 mL of acetonitrile of HPLC grade (2 h shaking at 350 rpm and 30 min of ultrasonication). The samples were then centrifuged (13 500*g*), and the resulting supernatant was filtered using a PTFE membrane (0.2 μm pore size; Millipore) and analysed by liquid chromatography (HPLC). The extraction technique was validated through the contamination of the biomixture samples with CARB at dry weights of 1, 10 and 40 mg kg^{-1}. The average recovery numbers after CARB addition were 91 ± 1.7, 93.4 ± 2.6 and 95.1 ± 0.89%, respectively. The CARB degradation in the biomixture followed first-order kinetics, and the CARB concentration at a given post-application time (t) could be described by the equation $C = C_0 \cdot e^{-kt}$. The CARB half-life was determined using the equation $t_{½} = \mathrm{Ln}(2)/k$.

2.4. Microbial analysis

2.4.1. Determination of enzyme activities

Dehydrogenase activity (DHA) of the biomixture was determined by 2,3,5-Triphenyl Tetrozolium Chloride (TTC) reduction technique (Casida 1977). The biomixture DHA is therefore expressed as μg TPF produced g^{-1} h^{-1}.

Acid (AP) and alkaline phosphatase (AKP) activities were determined according to the method of Tabatabai and Bremner (1969), using *p*-nitrophenyl phosphate (0.05 M) as substrate. The phosphatase activity is expressed as μg p-nitrophenol g^{-1} h^{-1} produced.

Hydrolitic activity was measured by monitoring fluorescein diacetate hydrolysis (FDA) according to Schnurer and Rosswall (1982) with slight modifications. 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}.

Please cite this article in press as: Tortella, G.R., et al. Carbendazim dissipation in the biomixture of on-farm biopurification systems and its effect on microbial communities. Chemosphere (2013), http://dx.doi.org/10.1016/j.chemosphere.2013.05.084