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Natural wastes rich in terpenes and their relevance in the matrix of an on-farm biopurification system for the biodegradation of atrazine

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ABSTRACT

A preliminary assay was performed to evaluate if natural wastes of low cost and rich in terpenes, such as pine needles, eucalyptus leaves and orange peels, added individually (5% w/w) into a microcosm simulating the matrix (peat based biomixture) of an on-farm biopurification system can be used to enhance the biodegradation of atrazine (ATZ) at 10 and 20 mg of active ingredient (a.i) of ATZ per kg of biomixture (mg a.i kg^{-1}) and their enzyme activities. Accumulation of the degradation products deisopropylatrazine (DIA), deethylatrazine (DEA) and hydroxyatrazine (HA) were evaluated, and the volatile compounds emitted from the biomixture were also monitored. Addition of natural wastes allowed for a higher rate of biotransformation at both the ATZ concentrations evaluated. However, significant differences were observed with the addition of the orange peels (98%) and the eucalyptus leaves (95%) when compared to the control. ATZ biotransformation was not enhanced at either concentration when pine needles were added, but instead, this addition resulted in a decrease of the degradation efficiency compared with the control. The DIA, DEA and HA metabolites were detected in all treatments without clear formation patterns. Enzyme activities were negatively affected, especially following the addition of pine needles or eucalyptus leaves. However, the entire enzyme activities evaluated recovered quickly. In conclusion, the results obtained from this study demonstrated the relevance of using natural wastes rich in terpenes in a peat-based biomixture of a biopurification system to potentially enhance the ATZ biodegradation.

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; ATZ) is a widely used herbicide and is applied in large quantities on agriculture worldwide (Clausen et al., 2004; Tao and Tang, 2004). According to reports from the United States Environmental Protection Agency (USEPA), 34,000 metric tons of this product is used annually in the USA (Wulfeck-Kleier et al., 2010). The intensive application rates of ATZ have resulted in several environmental problems generally by diffuse (nonpoint) or localised (point) sources (Cox, 2001). Several works have reported that point sources, such as accidental spillage, tank filling, cleaning of the spraying equipment and intentional disposal, are the major sources of contamination for the soil and water in agricultural systems (Gan and Koskinen, 1998; Schulz, 2004; Lima et al., 2009). Therefore, the contamination of the groundwater by pesticides is an important issue to consider when using these pesticides; an alternative strategy to reduce the risk of point source contamination by pesticides is an on-farm biopurification system, commonly referred to as a "biobed" (Torstensson and Castillo, 1997; Castillo et al., 2008). Biobeds are built using simple and cheap materials. The principal component is the biomixture (peat-based biomixture) composed of volumetric proportions of straw, peat and soil (2:1:1) (Torstensson and Castillo, 1997). However, for the purposes of adaption, the substrate composition of the peat-based biomixture have been modified in some countries (Karanasios et al., 2010a,b; Marinozzi et al., 2012). Several studies have demonstrated that biomixtures can retain and degrade a wide variety of pesticides (Castillo et al., 2008; Kravvariti et al., 2010; Karanasios et al., 2010a; Tortella et al., 2012). However, there is a lack of studies examining strategies to increase the capacity of

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pesticide dissipation in the peat-based biomixture, although it has been widely recommended biomixture for establishing biobeds (Castillo et al., 2008).

Strategies such as the addition of volatile organic compounds (VOCs) into the soil (i.e. terpenes) have been reported to enhance the biodegradation of environmental pollutants (Tyagi et al., 2011; Dudášova et al., 2012). In this sense, natural wastes rich in terpenes, such as orange and tangerine peels, pine needles and ivy leaves, have been evaluated for their ability to enhance the biodegradation of polychlorinated biphenyls (PCBs), trichloroethylene and 2,4-dichlorophenol in soil (Suttinun et al., 2004; Rhodes et al., 2007; Dudášova et al., 2012). However, the use of natural wastes rich in terpenes in a peat-based biomixture of biobed systems has not been evaluated in depth. Several reports indicate that adding citrus peels to a garden-compost based biomixture does not result in a significant difference in the degradation of the insecticide chlorpyrifos when compared with a biomixture containing only garden compost (Coppola et al., 2007). Other authors have reported that citrus peels caused a decrease in pesticide degradation in olive leaves compost based biomixtures; this result was attributed to the antimicrobial properties of the phenolic compounds found in the citrus peels (Karanasios et al., 2010a). However, preliminary results using a peat-based biomixture have shown that the addition of pure terpenes, such as eucalyptol and limonene, at low concentrations (50 μg kg^{-1}) enhanced the degradation of atrazine (Tortella et al., 2013). However, a short stimulatory effect was observed, and this was primarily attributed to the rapid rate of terpene volatilisation. Therefore, natural wastes with high terpene content could be used as terpene sources. The main objective of our investigation was to evaluate the feasibility of adding natural wastes rich in terpenes into a peat-based biomixtures in an effort to improve ATZ biotransformation when high concentrations of pesticide are added.

2. Materials and methods

2.1. Chemicals

Analytical grade (98%) atrazine (2-chloro-4-(ethylamino)-6-isopropylamine-s-triazine; ATZ), deisopropylatrazine (DIA), deethylatrazine (DEA) and hydroxyatrazine (HA) were supplied by Chem Service. Formulated atrazine (50% p/v; Atrazina SC500) was supplied by Dow Agrosciences, and 3-methyl-2-benzothiazolinone hydrazone (MBTH), and 3-(dimethylamino) benzoic acid (DMAB) were purchased from Sigma Aldrich (Steinheim, Germany). The ATZ stock solution for the degradation experiments was prepared by dissolving aliquots of commercially formulated pesticides in sterilised distilled water.

2.2. Biomixture preparation

A traditional peat-based biomixture was prepared by mixing andisol top soil (0–20 cm depth, 30.7% sand, 41.8% silt, 27.4% clay, 18% organic matter, pH 6.1) with no history of ATZ application, commercial peat (29.67% organic carbon) and winter wheat straw (43% organic carbon) in a 1:1:2 proportion, respectively (Torstensson and Castillo, 1997). The straw was cut in small fragments (approximately 3 mm) using a food processor, and the soil and peat were sieved (3 mm). The constituents were vigorously mixed to obtain a homogeneous biomixture (Castillo et al., 2008). Except for the control, the biomixture was mixed with one of three natural wastes: eucalyptus leaves, pine needles or orange peels (5% w/w). The biomixture was put in polypropylene bags to mature, and the moisture content was adjusted by adding sterilised distilled water to 60% of the water-holding capacity. The biomixture was stored in the dark at 20 ± 2 °C for 30 days prior to being used in the experiments.

2.3. Degradation studies

After the maturation process, a bulk sample (2.0 kg) of the biomixture containing orange peels, eucalyptus leaves or pine needles was placed in a glass container (40 × 20 × 10 cm deep), and a predetermined volume of commercially formulated ATZ was sprayed separately to each biomixture; the ATZ was diluted with sterilised distilled water to obtain a certain concentration of ATZ (10 and 20 mg a. i. kg^{-1}), thereby simulating a spill of pesticide, and to obtain 60% biomixture moisture (WHC). All assays were performed in triplicate. The control treatment received the same volume of sterilised distilled water without the ATZ. Each container was covered with plastic film containing several aeration holes to avoid excessive evaporation, and the container was incubated in the dark at 25 ± 2 °C for 60 days. Biomixture moisture was maintained by the regular addition of sterilised water. Samples of the biomixture were collected at fixed intervals (0, 5, 10, 15, 30, 40 and 60 days) to determine the amounts of residual pesticide and to perform other biological assays. Immediately after mixing, treated and control samples were removed and stored at −20 °C until analysis. ATZ and its metabolites were extracted from the biomixture (10 g) by shaking at 350 rpm for 2 h, followed by ultrasonication for 30 min in 20 mL of a 4:1 methanol:water mixture; the samples were then centrifuged at 10,000 rpm. The supernatant (5 mL) was filtered through a 0.2 μm PTFE membrane (Millipore) and analysed by liquid chromatography (HPLC), according to Aguilera et al. (2009) with slight modifications as described below. The extraction technique was validated through the contamination of biomixture samples (with and without natural wastes) with ATZ at 1, 10 and 20 mg kg^{-1} dry weight. The average recoveries for these ATZ applications were 94 ± 1.7%, 93.3 ± 2.6% and 95.3 ± 0.7%, respectively. Only the biomixture samples containing orange peels had an average recovery of 90.2 ± 1.7%, 90.1 ± 1.4% and 91.3 ± 1.7%, respectively, demonstrating that a small fraction of ATZ (between 2 and 4%) was adsorbed by the orange peels.

2.4. Enzyme activities

Dehydrogenase activity (DHA) was calculated from a standard curve created using 2,3,5-triphenyltetrazoliumchloride (TTC) as a substrate, as described previously by Casida (1977). DHA activity was expressed as μg TPF g^{-1} h^{-1}.

β-glucosidase was evaluated using *p*-nitrophenyl-β-D-glucopyranoside as a substrate. Absorbance at 398 nm was read using a UV–Vis Spectronic Genesis™ 2 PC spectrophotometer and was compared with a standard curve created using *p*-nitrophenol (Sigma). β-glucosidase activity was expressed as μg *p*-nitro phenol g^{-1} h^{-1} (Masciandaro and Ceccanti, 1999).

Phenoloxidase activity was determined in all degradation assays and was performed using MBTH/DMAB (Castillo et al., 1994). Because no correction was made for the possible presence of lignin peroxidase (LiP) and laccase (Lac) activity, the measurement represents the sum of the manganese peroxidase, LiP and Lac activities and is expressed as total phenoloxidase activity (Castillo and Torstensson, 2007).

Hydrolytic activity was determined in all degradation assays and was measured by monitoring the hydrolysis of fluorescein diacetate (FDA) (Schnürer and Rosswall, 1982).

2.5. Atrazine analysis

The samples were injected using a Rheodyne 7725 injector with a 20 μL loop into a Merck Hitachi HPLC system equipped with a L-7100 pump and a L-7455 diode array detector. The detector was set at 290 nm, and a C18 column was used (Superspher RP-C18,