Journal of Environmental Management 228 (2018) 451–457

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Glyphosate and aminomethylphosphonic acid degradation in biomixtures based on alfalfa straw, wheat stubble and river waste

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ARTICLE INFO

Keywords:
Biopurification systems
Biobeds
Herbicide
Wastewater
AMPA

ABSTRACT

The aim of the work was to evaluate novel biomixtures for their use on biopurification systems (BPS) in Argentina also called biobeds. Glyphosate and aminomethylphosphonic acid (AMPA) degradation was evaluated on biomixtures containing local materials: alfalfa straw (As), wheat stubble (Ws), river waste (Rw) and soil. Glyphosate, AMPA concentrations and biological activity were followed with time. Soil was used as control. Glyphosate initial concentration was 1000 mg kg^{-1}. Glyphosate disappeared almost completely after 63 days in all tested biomixtures. For Ws, WsRw and AsRw glyphosate degradation was around 99% and for As 85%. The biomixture Ws showed the highest glyphosate degradation rate. In all cases AMPA was formed and degraded to concentrations between 60 and 100 mg kg^{-1}. In the control with only soil, glyphosate was degraded 53% and AMPA concentration at the end of the test was 438 mg kg^{-1}. We conclude that alfalfa straw, wheat stubble and river waste are local materials that can be used in the preparation of biomixtures since they showed higher glyphosate degradation capacity and less AMPA accumulation compared to the soil alone. Also, the presence of river waste did enhance the water retention capacity.

1. Introduction

Environmental contamination by pesticides may be caused from point or diffuse sources. Diffuse contamination takes place during the application of pesticides in the field, mainly due to runoff or drift losses. Point source contamination occurs as a result of accidental spills at the place of pesticide manipulation, during the filling of the spraying equipment or due to the management of pesticides residues left outside and inside the tank. Even when precautions are taken, there is a risk of potential ground and surface water contamination (Castillo et al., 2008).

Biopurification systems (BPS), as biobeds and biofilters, are designed to collect and decontaminate accidental spills or waste liquids with a high concentration of pesticides and, therefore, avoid the contamination of surface- and ground waters. BPS are low cost systems that basically consist of waterproofed excavations or containers filled with a biologically active matrix, called biomixture, and covered by a vegetal layer (Castillo et al., 2008). The biomixture consists on soil, lignocellulosic materials and a humified organic substrate mixed at variable volumetric ratios. It has a high microbial activity and it is the main component of the BPS, allowing the retention and subsequent biological degradation of the pesticides. The original biomixture that was designed for the Swedish biobed (Torstensson and Castillo, 1997) was made of wheat straw (rich in lignocellulose), soil and peat. The straw allows the development of ligninolytic fungi that in turn promote the enzymatic degradation of pesticides. The soil provides adsorption capacity and acts as a source of pesticide-degrading bacteria. Peat also contributes to pesticide adsorption, moisture control and the decrease of pH that promotes the growth of fungi (Castillo and Torstensson, 2007).

In order to achieve a low cost and sustainable BPS, the composition of the biomixture must be adapted according to the availability of agricultural residues and local materials. For example, straw has been replaced by sunflower, olive and vine crop residues (Karanasios et al., 2010), bagasse (Roffignac et al., 2008) or oat husks, barley husks or sawdust (Urrutia et al., 2013). Furthermore, an important challenge is to find materials that can replace peat as it is a scarce and expensive resource (Gao et al., 2015; Karas et al., 2015). Peat has been replaced by different types of compost (Omirou et al., 2012; Karanasios et al., 2010) or spent mushroom substrate (Gao et al., 2015).

In order to use the BPS in Argentina it is necessary to find biomixtures based on locally available materials but that can still keep high degradation efficiencies. Once the crops are harvested, a significant amount of "crop residues" are left and they are commonly called stubbles. The most available lignocellulosic materials in Argentina are wheat stubble and

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https://doi.org/10.1016/j.jenvman.2018.09.009
Received 20 March 2018; Received in revised form 24 August 2018; Accepted 2 September 2018

alfalfa straw. In 2016, the total cereal production in Argentina was 67 million tons (of which about 80% corresponds to corn and wheat) and the alfalfa forage and silage production reached 39 million tons according to FAO 2016 (FAOSTAT, 2016). An alternative material to peat is river waste that consists on plant residues accumulation under anaerobic conditions, common in certain areas of the Paraná Delta River (Di Benedetto et al., 2004) and that is currently used in flower production for the formulation of substrates.

It has been demonstrated that several enzymes can act in pesticides degradation in soil, such as phosphatases, hydrolases and carboxylesterases (Tortella et al., 2012). In this sense the determination of biological activities such as hydrolytic activity based on the Fluorescein Diacetate activity (FDA) is one approach to monitor the potential effect of pesticides in biological activities. FDA (3′,6′-diacetylfluorescein) has been used to determine amounts of active fungi and bacteria since it is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases (Schnürer and Rosswall, 1982).

Glyphosate [N-(phosphonomethyl) glycine], a synthetic phosphonate compound with stable carbon-phosphorus (C—P) bond is the active ingredient of broad spectrum post-emergent, and non-selective systemic herbicide (Li et al., 2016). Glyphosate-based formulations have become the dominant herbicides on a global scale (Cuhra et al., 2016). Recent studies revealed that glyphosate occurs in soil, surface water, and groundwater, and residues are found at all levels of the food chain, such as drinking water, plants, animals, and even in humans (Milan et al., 2018). Aminomethylphosphonic acid (AMPA) is a degradation product resulting from phosphonate degradation (Wang et al., 2016). It can be a metabolite of glyphosate microbial degradation in soils (Borggaard and Gimsing, 2008). In a spatially wide occurrence study, Battaglin et al. (2014) showed that glyphosate is detected without AMPA in only 2.3% of 3732 water and sediment samplesand that AMPA is detected without glyphosate in 17.9% of samples. AMPA is strongly adsorbed to soil particles and moves with the particles towards the stream in rainfall runoff. In urban areas, AMPA comes from phosphonates and glyphosate in wastewater. AMPA is reported to be persistent in soils and sediments (Grandcoin et al., 2017). Based on recent reports on potential chronic side effects of glyphosate (Battaglin et al., 2014), the World Health Organization reclassified the herbicide glyphosate as probably carcinogenic to humans in 2015 (WHO, 2015).

In Argentina the use of glyphosate pesticide increased from 1 million liters in 1991 to 200 million liters in 2013 (Casafe, 2013; Binimelis et al., 2009). The occurrence of glyphosate and AMPA have been reported in the water and sediments of streams from rural and suburban basins of our country (Argentina) within the provinces of Buenos Aires, Santa Fe and Córdoba (Castro Berman et al., 2018).

In this context, the aim of this work was to evaluate glyphosate and AMPA degradation employing different biomixtures prepared with local materials (soil, alfalfa straw, wheat stubble and river waste).

Glyphosate degradation and the presence of its main metabolite AMPA were followed with time. Biological activity, as fluorescein diacetate hydrolysis (FDA), was also followed. Soil alone was run as control.

2. Materials and methods

2.1. Biomixtures preparation

Biomixtures were prepared using an agricultural soil, two different lignocellulosic materials (alfalfa straw or wheat stubble) and river waste. Experiments were conducted using three independent replicates. The soil was obtained from a field in the north of Santa Fe province, Argentina (29° 42′ 59″ S and 60° 5′ 35″ W) with more than 20 years of continuous soybean cultivation where glyphosate was applied. The local soil acts as an inoculum of pesticide-adapted microbiological populations (De Wilde et al., 2007). The kind of soil according to its taxonomy is aquic Argiudoll. Alfalfa straw and wheat stubble were collected from the same field as the soil. River waste is a commercial product (Santa Isabel S.A. vivarium), used for cultivation of plants. Physicochemical properties of all components are shown in Tables 1–3.

Table 1
Soil physicochemical properties.

Parameter	Soil
Granulometry (%)	Sand 6.4; Silt 66.6; Clay 27.0
C (g kg^{-1})	19.7
Organic matter ((g kg^{-1}))	34.0
P (mg L^{-1})	0.023
Actual density (g cm^{-3})	2.67
Porosity (%)	70.7
pH[a]	5.96
Ashes ((mg kg^{-1}))	948.
K[b] (mg kg^{-1})	462.7
Ca[b] (mg kg^{-1})	184.9
Mg[b] (mg kg^{-1})	84.4
Na[b] (mg kg^{-1})	10.4
N (g kg^{-1})	1.53
C/N Ratio	12.9

[a] Determined in a mixture of air-dried soil and deionized water (1:2.5 w/v).
[b] Values corresponding to total content.

Both glyphosate and AMPA concentrations were below the Limit of Detection in all substrates (0.6 μg L^{-1} for both according the method reported by Sasal et al., 2015). The soil was sieved (3 mm), the stubble and straw were cut into pieces of approximately 2–3 cm and the river waste was used directly.

The biomixtures were prepared by mixing the components with a shovel in the proportions shown in Table 4 and 15 L of biomixture were placed in 30 L glass boxes (20 cm × 30 cm × 50 cm) (Fig. 1). Temperature, pH and moisture (expressed as water weight/dry material weight) were registered daily. Moisture was adjusted to 60–70% and kept constant during the whole experiment by adding distilled water. Moisture and pH measurements were performed with a garden meter (TFA). Soil as the only component was run in parallel as control. The moisture was adjusted at the same value range as recommended by Castillo et al. (2008).

After preparation the biomixtures were maturated for 50 days before the addition of glyphosate according to the recommendation of previous studies (Castillo et al., 2008; Roffignac et al., 2008; Karanasios et al., 2012; Góngora-Echeverría et al., 2017). The commercial glyphosate formulation Eskoba® was sprayed over the surface of the biomixtures and soil alone at a concentration of 1000 mg glyphosate kg^{-1} dry biomixture. This high concentration value selected is related to the residues produced in the area (mainly rinsing water of commercial containers and water belonging from spray tank washing (De Wilde et al., 2007).

The experience was performed for 63 days and samples were taken immediately after glyphosate application (day 0) and after 10, 16, 25, 43 and 63 days. The experience time up to 63 days was chosen taking into account the half-life of glyphosate in soil and according previous studies of glyphosate degradation in biomixtures (Roffignac et al., 2008; Góngora-Echeverría et al., 2017); a typical field half-life of 47 days has been suggested (Lawrence, 2002).

Each sample was a composite of several subsamples taken at different positions of the biobed employing a soil sampler and was

Table 2
Physicochemical properties of the lignocellulosic materials.

Parameter	Alfalfa straw	Wheat stubble
Organic matter (%)	79.5	82.2
Dry material (%)	89.6	91.3
Ashes (%)	10.1	9.1
Raw or crude fiber (%)	23.6	38.4
P (%)	0.4	Not detected
N (%)	2.3	0.46
Density (g cm^{-3})	0.08	0.06