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Comprehensive analysis of degradation and accumulation of ametryn in soils and in wheat, maize, ryegrass and alfalfa plants

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ABSTRACT

Ametryn is a selective herbicide belonging to the triazine family and widely used for killing annual grasses or weeds in China and other parts of the world. However, reports on its environmental risk assessment with regard to soil and crop contamination are limited. In this study, accumulation of ametryn in wheat, maize, ryegrass and alfalfa crops along with ametryn residues in the soil planted with the plants were comparatively investigated. Soil enzyme activities and low molecular weight organic acids (LMWOAs), as well as antioxidant and degradation enzyme activities in plant tissues were measured. The maximum accumulation of ametryn was found in shoots and roots of wheat and alfalfa. Ryegrass had the maximum ametryn translocation factor (TF) from roots to shoots, with more than three times over the other crops. The ametryn residue in ryegrass-planted soil was much lower than that in soil planted with others. The residual content of ametryn in crop-planted soils was ordered as rhizosphere soil < bulk soil < non-rhizosphere soil < control (without plants). Activities of catalase (CAT), glutathione S-transferase (GST) and laccase (LAC) in ametryn-exposed ryegrass were significant higher than those in non-ametryn exposed ryegrass. The maximum activities of CAT in ryegrass shoot and root were increased by 6.16- and 28.84-fold over the control, respectively. Exudation of organic acids in the crop was induced by ametryn and contributed a lot to the degradation of the herbicide. Thus, ryegrass was shown to have a relatively strong ability to remove ametryn from ametryn-contaminated soil and its plant tissues as well.

1. Introduction

The current mechanism for toxicology of herbicide triazine is the disrupting of photosynthetic electron flow in photosystem II (PSII) through blocking the activity of D1 protein (Mimmo et al., 2015). Ametryn is a triazine herbicide and was designed for being phytotoxic to PSII in plants (Sandoval-Carrasco et al., 2013). As an effective killer of many broadleaf and grass weeds, ametryn is widely used in fields of many crops such as maize, pineapple and sugarcane for many decades (Navaratna et al., 2012). However, overuse of triazine herbicides (such as ametryn and atrazine) not only contaminates soils and waters but potentially affects crop production and food safety as well (McDonald et al., 1999; Jablonowski et al., 2011; Powell et al., 2011; Ibrahim et al., 2013). For example, the content of atrazine was detected at 102 μg L^{-1} in river basins of agricultural areas, 42 μg L^{-1} in surface waters and 21 μg L^{-1} in groundwater (Jablonowski et al., 2011; Powell et al., 2011). The widespread triazine residue has become a great environmental concern, which receives much attention for the researchers who investigate its ecotoxicology and seeks biodegradation of the herbicide in contaminated soils (Gerhardt et al., 2009; Zhang et al., 2014).

Soil is the major sink of the globally consumed herbicide residues, because they comprise more than 80% of the chemicals used in field soil (Fang et al., 2001). However, soils are also the major sites where the residues of herbicides are degraded. Degradation of soil herbicides relies on chemical, physical and biological factors, and each of them individually or coordinately influences the process of sediment, transformation or degradation of herbicides within soil-crop systems (Arias-Estévez et al., 2008). The organic compounds can be effectively removed by microorganisms in soil (Guo et al., 2009; Ma et al., 2010), where the rapid degradation of the contaminants occurs in rhizosphere by depending on the LMWOAs contributed by root exudates and specific enzymes such as CAT, PO (phenol oxidase) and DHA (dehydrogenase) (Li and Yang, 2013; Sui and Yang, 2013; Lu et al., 2015). While soils are contaminated by organic toxicants such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and petroleum, most of them are freely absorbed by crops (Johnson et al., 2005; Song

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et al., 2007; Tang et al., 2010; Yu et al., 2011). Plants have developed sophisticated strategies to deal with the adverse impact of the toxicants. Several mechanisms for detoxification and degradation of xenobiotics have been proposed (Kawahigashi, 2009). Glutathione *S*-transferases (GSTs) and cytochrome P450 monooxygenases (P450s)-mediated catabolic processes have been implicated in the mechanism for modification and degradation of toxicants (Lu et al., 2016; Jensen and Møller, 2010; Tan et al., 2015). Several other novel pathways or components such as salicylic acid and laccases are also found to be involved in the degradation and detoxification of organic toxicants (Jönsson et al., 1998; Lu et al., 2015; Huang et al., 2016). The removal of toxic pesticides using plants that has potential to accumulate and degrade the organic xenobiotics has been a promising perspective approach, because it is eco-friendly, economically feasible and environmental safety (Kumar et al., 2013; Mimmo et al., 2015). Recent studies have shown that some monocot or dicot grasses have high tolerance to toxic pesticides (Cui and Yang, 2011; Yu et al., 2011; Li and Yang, 2013; Lu et al., 2015), and some genotypes and varieties (or cultivars) can efficiently remove pesticides from pesticide-polluted soil (Tang et al., 2010; Ibrahim et al., 2013; Ghosh et al., 2015; Xiao et al., 2015). Nevertheless, some other plants may clean up hazardous organic compounds through interaction with microorganisms (Merini et al., 2009; Jin et al., 2012; Li and Yang, 2013). To facilitate the catabolism of pesticides already absorbed in plants, special desirable plants for highly effective extraction of the toxicants are needed and selected for genetic engineering (Kawahigashi, 2009). Up to now, no literature is available for degradation of ametryn-contaminated soils and in plants. Understanding bioaccumulation and developing a degradation system that mitigates ametryn contamination in soils is critical and applicable. Ryegrass (*Lolium multiflorum*) and alfalfa (*Medicago sativa*) are the species tolerant to the adverse environmental stresses, and can effectively degrade herbicides from toxicant-polluted soils (Li and Yang, 2013; Sui and Yang, 2013). Maize (*Zea mays*) and wheat (*Triticum aestivum*) are food crops and their ability to tolerate adverse environments largely depends on genetic background. In this regard, we assumed that these plants should have different mechanisms responsible for the tolerance and detoxification to the toxic herbicide ametryn. Hence, the present study selected the four different crops to comparatively analyze ametryn accumulation and degradation in plants and ametryn-exposed soils. The aim of the study was to figure out the potential of the plant species in accumulation of ametryn and evaluate their capacity for scavenger of ametryn in the contaminated soil.

2. Materials and methods

2.1. Materials

Ametryn (with a purity of 97%) was provided by Dalian Huiyuan Fine Chemicals Co., Ltd., China. Ametryn-free soil (pH 7.25; organic matter, 1.40%; total N, 1.26 g kg^{-1}; available P, 34.3 mg kg^{-1}; and available K, 91.5 mg kg^{-1}) was collected from the Experimental Station of Nanjing Agricultural University, Nanjing, China (Eutric gleysols, N 32.03°; E 118.84°) (Cui and Yang, 2011). The soil was air-dried, manually crumbled, crushed and passed through a 3 mm sieve for plant cultivation and a 1 mm sieve for assay of soil enzymes.

2.2. Growth and plant treatment

Seeds of wheat (cv. Zhengmai 7), ryegrass (cv. Changjiang II), alfalfa (cv. Longdong) and maize (cv. Nan 8) were surface-sterilized with 3% solution of H_2O_2 for 15 min, rinsed and germinated for 24 h. The germinating seedlings were sowed in soil which was mixed thoroughly with a certain amount of ametryn (solved in acetone), with the final concentration of ametryn being 1 mg kg^{-1}. Twenty uniform seedlings grew on the soil surface of a plastic container (1 L) with 1120 g soils mixed with ametryn. The soil was watered to maintain 60% of relative water content of the soil. Treatments were repeated in triplicate. Plants were grown in a climate chamber under the controlled conditions (light intensity, 300 μmol m^{-2} s^{-1}; photoperiod, 14/10 h light/dark cycle at 25/20 °C at day/night; soil moisture 60%) for 10 d. Shoots and roots of plants were separately harvested and immediately analyzed. Soils adhered tightly to the root system were collected as rhizosphere soil (RS). Soils unattached tightly to the root system were thought of the non-rhizosphere soil (NRS). All collected rhizosphere and non-rhizosphere soils were mixed as bulk soils (BS) (Wenzel, 2009; Sui and Yang, 2013). Soils were analyzed immediately after collected.

2.3. Ametryn analysis

Ametryn in soil was quantified according to the method described previously (Cui and Yang, 2011), with minor modification. Soil (10g) was extracted ultrasonically with 25 mL acetone–water (3:1, v:v) for 30 min. The extracting solution was centrifuged at 4000*g* for 5 min and filtered. The process was repeated in triplicate. The filtrate was concentrated to remove acetone at 40 °C by a rotary vacuum evaporator. The residual water was extracted with petroleum ether for three times, each time with 15 mL. The organic phase was pooled and dried to dryness. The residue was dissolved in 2 mL methanol and analyzed by high performance liquid chromatography (HPLC) with Waters 515 pump and 2487 ultraviolet (UV) detector (Waters Technologies Co. Ltd.). The operating condition was set as following: UV detection length, 254 nm; hypersil reversed phase C_8 column (Inertsil, 250 mm × 4.6 mm i.d.); room temperature; mobile phase, methanol: water (70:30, v/v); flow rate, 0.6 mL min^{-1}; injection volume, 20 μL.

Ametryn in plant tissues was analyzed based on the method described previously (Lu et al., 2016). Fresh tissues of shoot (2g) were ground with 25 mL petroleum ether and extracted ultrasonically for 30 min. The mixture was centrifuged at 4000*g* for 5 min and filtered. The process was repeated in triplicate. The filtrate was evaporated to dryness. The residue was re-dissolved in 3 mL acetone. The dissolved solution was added to a glass column containing 5g of activated silica gel. The column was washed with 30 mL diethyl ether/petroleum ether (2:98, v/v), and elutes were collected and evaporated to dryness. The residue was dissolved in 2 mL methanol and analyzed by HPLC. For analysis of ametryn in root (2g), the extraction, purification and analysis were carried out according to the method of soil samples.

The accuracy of the method was evaluated by the recovery test conducted with spiked samples. Free-ametryn soil, shoots and roots of wheat, maize, ryegrass and alfalfa were spiked with standard ametryn at three concentration levels of 0.2, 1.0 and 2.0 mg kg^{-1}, respectively. Spiked recoveries of ametryn in soil and shoot/root of plants were showed in Table 1. Under conditions, the limit of detection (LOD) of the analytical method was 0.005 mg kg^{-1} for soil and 0.01 mg kg^{-1} for plants.

2.4. Assays of enzyme activities in plant tissues

Fresh tissues (0.3g shoot or root) were ground and extracted with 1.5 mL 50 mM Na-phosphate buffer (pH 7.8) consisted of 1.0% (w/v) poly-vinylpyrrolidone and 0.1 mM EDTA under ice-cold condition. The homogenate was centrifuged at 12,000*g* at 4 °C for 20 min. The supernatant was taken as the enzyme source for measurement of catalase (CAT, EC1.11.1.6), glutathione S-transferase (GST, EC2.5.1.18) and laccase (LAC, EC 1.10.3.2) activities and the protein concentration in the extracts was determined (Jiang and Yang, 2009).

CAT activity was assayed by the method of Jiang and Yang (2009). The consumption of H_2O_2 was measured for 2 min as the decrease in absorbance at 240 nm in 3 mL reaction medium containing 100 mM potassium phosphate buffer, 15 mM H_2O_2 and 50 μL enzyme extract. GST activity was assayed in 3 mL reaction mixture containing 100 mM potassium phosphate buffer (pH6.5), 1 mM reduced glutathione (GSH), 1 mM 1-chloro-2,4-dinitro-benzene (CDNB) and 0.1 mL enzyme extract