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Mechanism of the effect of pH and biochar on the phytotoxicity of the weak acid herbicides imazethapyr and 2,4-D in soil to rice (*Oryza sativa*) and estimation by chemical methods

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ABSTRACT

The existing form of an ionizable organic compound can simultaneously affect its soil adsorption and plant bioactivity. In this experiment, the adsorption and bioactivity of two weak acid herbicides (WAHs), imazethapyr and 2,4-D, were studied to explore the predominant mechanism by which the soil pH and the addition of biochar can influence the phytotoxicity of WAHs in soil. Then, the WAH concentration extracted by hollow fiber-based liquid-phase microextraction ($C_{HF\text{-}LPME}$), the in situ pore water concentration (C_{IPW}) and the added concentration (C_{AC}) were employed to estimate the phytotoxicity. The results showed that with increased pH from 5.5 to 8.5, the phytotoxicity of the WAHs to rice increased about 1-fold in the soil, but decreased in aqueous solutions, the IC_{50} values for imazethapyr and 2,4-D at pH 5.0 were 3- and 2-fold higher than that at pH 8.0. In addition, the soil adsorption decreased, indicating that the adsorption process was the dominant factor for the variation of the phytotoxicity of the WAHs in the tested soil instead of the decreasing bioactivity. The concentration that inhibits plant growth by 50% (IC_{50}) calculated by the C_{AC} in different pH and biochar soils ranged from 0.619 to 3.826 mg/kg for imazethapyr and 1.871–72.83 mg/kg for 2,4-D. The coefficient of variation (CV) of the IC_{50} values reached 65.61% for imazethapyr and 130.0% for 2,4-D. However, when IC_{50} was calculated by C_{IPW} and $C_{HF\text{-}LPME}$, the CVs of the IC_{50} values decreased to 23.51% and 36.23% for imazethapyr and 40.21% and 50.93% for 2,4-D, respectively. These results suggested that C_{IPW} and $C_{HF\text{-}LPME}$ may be more appropriate than C_{AC} for estimating the phytotoxicity of WAHs.

1. Introduction

Currently, ionizable organic chemicals (IOCs) represent a large percentage of the agricultural market (Kah and Brown, 2006). An estimated fifty percent of 150,000 pre-registered compounds at the European Chemicals Agency are ionizable, including acids, bases and zwitterions (Franco et al., 2010). The bioavailability of IOCs has raised worldwide concern regarding the impact of speciation (i.e., the fractional amount of neutral and ionized forms), which is influenced by the variable pH values ranging from 6 to 9 in natural water (Rendal et al., 2011b). The pH and pKa of IOCs have been considered key elements that dominated the existing form of IOCs (Anskjær et al., 2013; Boström and Berglund, 2015). The toxicity and bioaccumulation of IOCs in biological testing often increased with an increased neutral form of the IOCs (Nichols et al., 2015; Xing et al., 2012).

Moreover, different existing forms of IOCs have been regarded as one factor influencing the adsorption of IOCs, which alters their bioavailability (Bresnahan et al., 2000; Ellis et al., 2007). Researchers have proven that the neutral form of IOCs is often much more strongly adsorptive in soils than the anion in a nonlinear process, but a common rule has not been formulated stating that the adsorption behavior of a weak base would predictably change with pH changes, depending on the physicochemical properties of IOCs and the soil properties (Kah and Brown, 2006). Meanwhile, amended biochar could affect the bioavailability of IOCs by increasing the soil adsorption capacity (Khorram et al., 2015). We found that the adsorption capacity increased and the phytotoxicity of an IOC (sulfentrazone) to rice decreased under these conditions, although the addition of biochar increased the soil carbon content and pH (Liu et al., 2016b).

The adsorption of a weak acid herbicide (WAH) often increases with

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increasing organic carbon content and decreasing pH, and this change may lead to reduced phytotoxicity of the WAH. However, a decreasing pH results in an increasing percentage of neutral molecules in the soil environment, leading to increased phytotoxicity of the WAH. These two aspects are contradictory, and there are no reports on this topic available in the literature. Therefore, it is urgent to determine which factor is the governing factor affecting the phytotoxicity of WAHs.

In general, an organism takes up organic chemicals from soil pore water (Sijm et al., 2000). Pore water concentrations, regarded as effective concentrations in soil, have been used to estimate the bioavailability of hydrophobic organic contaminants (Van der Wal et al., 2004) and IOCs (Liu et al., 2012b), but the estimation has been useless when the existing forms of the organic contaminant are significantly different (Liu et al., 2012b) because the different forms have distinct bioactivities (Rendal et al., 2011a). Therefore, a new biomimetic method that can act as a surrogate for plant uptake is required to estimate the bioavailability of IOCs.

A three-phase hollow fiber-based liquid-phase microextraction (HF-LPME) method was used to detect microscale pollutants in an environment (Payan et al., 2010). Because the extraction process of HF-LPME is similar to the toxicokinetic ion-trapping model that could be used to explain the pH-dependent toxicity of IOCs (Neuwoehner and Escher, 2011), we adopted the concentration extracted by HF-LPME to estimate the toxicity of sulfadiazine to *Daphnia magna* in a solution with different pH values (Liu et al., 2016a). It was unknown whether the HF-LPME could estimate the phytotoxicity of IOCs in the complex matrix soil.

Hence, the objective of this study was to elucidate the mechanism of the effect of pH and biochar on the phytotoxicity of 2 WAHs, imazethapyr and 2,4-D (chemical structures shown in Fig. 1) to rice, and to determine which factor (pH or biochar) governed the phytotoxicity of WAHs between the adsorption by soil and the bioactivity to rice when the existing form of the compound changed from a neutral molecule to an ion as the pH and biochar increased. Then, we explored whether determining the concentration extracted by HF-LPME was an available method to evaluate the phytotoxicity of imazethapyr (pKa = 3.9) and 2,4-D (pKa = 2.6) to rice in the soil. If the concentrations were bioavailable, and if they could be used to calculate the phytotoxicity of WAHs to rice, then the dose-response curve and IC_{50} should be the same regardless of the soil (Xu et al., 2007).

2. Materials and methods

2.1. Chemicals and soils

The herbicide imazethapyr (97.0%) was purchased from Shandong Qiaochang Chemical Co., Ltd. (QCC) in China, and 2,4-D (98%) was purchased from Shandong Keyuan Pharmaceutical Co., Ltd. in China. HPLC-grade methanol was used, and the other chemicals and solvents were analytical grade. A Q3/2 Accurel® PP polypropylene microporous hollow fiber membrane (200 μm wall thickness, 600 μm inner diameter, 0.2 μm pore size, and 75% porosity) was obtained from Membrana GmbH (Wuppertal, Germany).

Surface (0 ~ 10 cm) soils were collected from Changsha in Hunan Province. The physical and chemical properties of the soil samples were as follows: organic matter content (OMC), 3.01%; total N, 0.29%; cation exchange capacity (CEC), 21.3 cmol/kg; sand, 26.7%; silt, 65.2%; clay, 8.09%; and pH, 5.54. The soil was air-dried and passed through a 2-mm sieve. Biochar was collected directly from a field in Changle Yueyang City in Hunan Province (E113.273788, N 28.863936). The biochar contained 72.86% crude ash, 12.11% elemental carbon, and 0.55% nitrogen, and the pH was 11.04.

2.2. Bioassay

A mass of 100 g of dry weight equivalents of the sieved soils was placed in a plastic pot. Based on the preliminary bioassay tests (data not shown), the soil pH was adjusted with calcium hydroxide and hydrochloric acid to pH values of 5.5, 7.0, and 8.5 for imazethapyr and to pH values of 5.5 and 8.5 for 2,4-D. The treated soil was mixed thoroughly with the desired aqueous solutions of herbicide in water to give initial concentrations of 0.25, 0.5, 1, 2, 4, and 8 mg/kg for imazethapyr and 1.25, 2.5, 5, 10, and 20 mg/kg for 2,4-D. Soil samples that were mixed with the same amount of water without chemicals were used as the controls. The soil moisture content was maintained at the soil water holding capacity (30%). The treated soils were covered with aluminum foil and equilibrated for 24 h at 25 °C, and then, 10 germinated rice seeds were planted on the treated soil surface.

To eliminate the effect of soil adsorption on phytotoxicity, the phytotoxicity of two herbicides was observed in the solutions with different pH values. Pure water that was used for the solutions was produced in-house (Millipore, Bedford, MA, USA). The herbicide solutions used for the phytotoxicity tests were prepared with buffers. 2-(N-morpholino) ethanesulfonic acid (MES) hydrate (3 mM) and HEPES (2 mM) were added to achieve stable pH levels of 5.0 and 8.0, respectively. Hydrochloric acid and sodium hydroxide were used to adjust the pH of the buffer solutions. Ten germinated rice seeds were randomly placed in a plastic cup containing 5 mL of the herbicide solution; the cup was sealed with plastic wrap that was reinforced by a rubber band.

The planted pots were randomly placed in an artificial climate incubator (Ningbo Saifu Laboratory Instrument Factory, Zhejiang, China) at 28 °C and 80% humidity under a 16-h/8-h light/dark cycle (light 350 $\mu E/m^2/s$). Three replicates of each treatment were performed, and the plants were harvested on day 7 to measure their growth (plant height).

2.3. Batch sorption experiment

Adsorption experiments were conducted with the batch equilibration method recommended by the Organization for Economic Co-

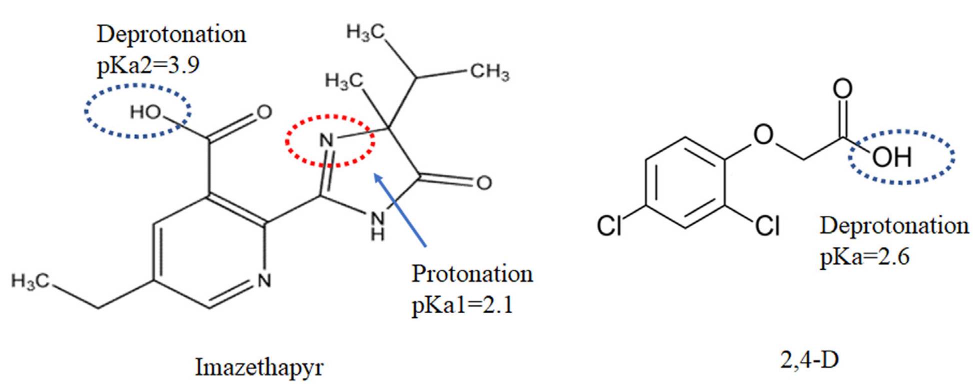

Fig. 1. Imazethapyr and 2,4-D chemical structures.