Potential effects of rainwater-borne H_2O_2 on competitive degradation of herbicides and in the presence of humic acid

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ABSTRACT

In a previous piece of work, we reported some preliminary experimental results showing that hydrogen peroxide at a concentration range frequently encountered in rainwater could lead to degradation of three common herbicides (diuron, butachlor and glyphosate). However, the work was limited to the observation on the effects of Fenton process on the individual herbicides. In field conditions, different types of herbicides along with other organic molecules may occur concurrently. It is unclear how different herbicides and various organic molecules compete for the available hydroxyl radical. In this study, further laboratory experiments were conducted to observe the changes in the herbicides in the scenarios where multiple herbicides or humic acid are present. The results show that humic acid impeded hydroxyl radical-driven degradation of the diuron and butachlor. However, humic acid had no significant effects on reducing glyphosate removal rate. Glyphosate could compete strongly with the humic acid for the available hydroxyl radical in the reaction systems. The reactivity of glyphosate with hydroxyl radical was much higher than those of diuron and butachlor due possibly to its relatively simpler chemical structure, as compared to either diuron or butachlor, which are aromatic compounds that have higher chemical stability. Butachlor degradation was much weaker in the combined diuron and butachlor system than in the combined glyphosate and butachlor system. In the glyphosate-butachlor system, the opposite was observed. The findings have moved another step forward to understanding the potential role of rainwater-borne H_2O_2 in degrading herbicides in open water environments.

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1. Introduction

Herbicides are present in open water environments that receive agricultural runoff (Murray et al., 2010; Davis et al., 2013; Hijosa-Valsero et al., 2016). Understanding the chemical behaviour of herbicides in receiving water environments is essential for developing management strategies to minimize the ecological impacts of herbicides in aquatic ecosystems. Herbicides undergo decomposition via microbial degradation, photodegradation and other chemical degradation (Cullington and Walker, 1999; Salvestrini et al., 2002; Fenoll et al., 2013). Diuron, butachlor and glyphosate are among the most common herbicides in open water environments (Solomon and Thompson, 2003; Yu et al., 2003; Giacomazzi and Cochet, 2004). Diuron (N-(3,4-dichlorophenyl)-N,N-dimethyl-
urea) has moderate water solubility (Cabrera et al., 2010). Diuron can be decomposed by microbes both aerobically and anaerobically (Giacomazzi and Cochet, 2004). However, in surface water environments, anaerobic biodegradation is most likely to be limited to sediment-water interface (Attaway et al., 1982). The rate of microbial degradation of diuron may be affected due to the toxicity of diuron to the microbes involved (Guérin et al., 2008). The hydrolysis and photodegradation rates of diuron in natural water under circumneutral pH conditions are relatively low (Salvestrini et al., 2002, 2004). Like diuron, the hydrolysis rate of butachlor (N-butoxymethyl-2-chloro-2, 6-diethyl acetanilide) at circumneutral pH conditions is low. However, photodegradation of butachlor is very rapid (Zheng and Ye, 2001). Glyphosate (N-(phosphonomethyl)glycine) is highly soluble (Schuette, 1998) but it tends to be immobilized by adsorption to organic matter (Piccolo et al., 1996). Hydrolysis and photodegradation are unlikely to be major degradation pathways for glyphosate in natural water environments (Rueppel et al., 1977). While microbially mediated degradation is effective (Zaranyika and Nyandoro, 1993), the availability of glyphosate-degraders in natural water environments may limit the effect of microbial path on the degradation of water-borne herbicides.

A previous piece of work (Qin et al., 2013) reported the preliminary experimental results showing that hydrogen peroxide at a concentration range frequently encountered in rainwater could lead to degradation of diuron, butachlor and glyphosate. However, the work was limited to the observation on the effects of Fenton process on the individual herbicides. In field conditions, different types of herbicides along with other organic molecules may occur concurrently. Since different organic molecules have different composition of functional groups, it is unclear how they compete for the available hydroxyl radical. In this study, further laboratory experiments were conducted to observe the changes in herbicides in the scenarios where multiple herbicides or humic acid are present. The objective was to obtain further insights into the interactive processes of hydroxyl radical, various herbicides and humic acid.

### 2. Materials and methods

#### 2.1. Materials

Three commonly used herbicides (diuron, butachlor and glyphosate) were selected for the experiments. The analytical standards of these selected herbicides were purchased from the Shanghai Anpel Scientific Instrument Co., Ltd. The purity of the diuron, butachlor and glyphosate standards was 98%, 98% and 97%, respectively. The humic acid was purchased from the Shanghai Jufeng Scientific and Chemical Supplies Ltd. (purity: 97.4%).

#### 2.2. Experimental design

The experiment was to observe the change in each of the three herbicides (diuron, butachlor and glyphosate) in the presence of humic acid at 5 concentration levels: 0, 5, 10, 20 and 50 mg/L (labelled as HA0, HA5, HA10, HA20, HA50, respectively). The experimental set-up is shown in Table 1. For each level of humic acid, several levels of hydroperoxide and ferrous iron were used to conduct the experiments. The details on experimental set-up for Experiment 1 are shown in Table 1.

#### Table 1

<table>
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<th>Humic acid dose</th>
<th>Treatment</th>
<th>H\textsubscript{2}O\textsubscript{2} (µM)</th>
<th>Fe\textsuperscript{2+} (µM)</th>
<th>Herbicide\textsuperscript{*} (mg/L)</th>
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\* Either diuron, butachlor or glyphosate.
Means of each herbicide (diuron, butachlor or glyphosate) with different letters for the control and the treatments for each humic acid dose level are significantly different at p < 0.05.

2.3.2. Extraction and determination of butachlor

The following procedure was used for extracting residual butachlor in the solution after 1-h reaction: place 1 mL of the sample in a 250 mL separatory funnel and then add 30 mL of methanol, 30 mL of NaCl (25%) and 30 mL of dichloromethane; shake for 2 min and release the water; repeat the above procedure twice; the organic-dominated solvent was further dehydrated by adding anhydrous sodium sulfate and then condensed on a rotary evaporator (with temperature set at 40 °C) to 2 mL. The extract was further purified on a florisil cartridge and then frozen at −25 °C prior to analysis.

The instrumentation is the same as that for measurement of diuron except that a volume ratio of 80 (methanol) to 20 (water) was used for the mobile phase and the detector was set at a wavelength of 236 nm.

2.3.3. Determination of glyphosate

The solution after 1-h reaction was passed through a 0.22 μm membrane filter and stored at 4 °C in a refrigerator prior to analysis. Glyphosate in the solution was determined using a Dionex ICS-900 ion chromatography system, fitted with an electrical conductivity detector, a 250 μL sample injection loop, an IonPac AG11-HC (4 mm × 50 mm) guard column and an IonPac AS11-HC (4 mm × 250 mm) analytical column. KOH was used as the mobile phase and the eluent flow rate was set at 1.00 mL/min with an injection volume of 250 μL.
2.3.4. Determination of total organic carbon (TOC)

The concentrations of total organic carbon (TOC) was measured using a TOC analyzer (Vario TOC Elementar, Germany).

2.4. QA/QC and statistical method

All experiments were performed in triplicate. All chemical
3. Results

3.1. Herbicide removal in the presence of humic acid

For all the scenarios, there was a generally consistent trend showing the solution TOC in the following decreasing order: Ck > H2O > H50 > F20 > F50 (Table 2).

For the control (Ck, no added H2O2 or Fenton reagent), there was a general trend that herbicide in the solution decreased with increasing dose of humic acid. However, the magnitude of herbicide removal differed among the three herbicides: for diuron, significant difference was only observed between HA0 and the higher doses of humic acid treatments (H20 and H50); for butachlor, there was a significant difference between HA0 and all the added humic acid treatments; for glyphosate, the significant difference was only observed between HA0 and HA50, and there was no significant difference among H5A, HA10 and HA20, which was also significantly different from either HA0 or HA50 (Fig. 1).

In the presence of H2O2 only (H20 and H50), the removal rate of diuron tended to be higher (as compared to Ck) with significant difference being observed between HA0 and all added humic acid treatments. For butachlor, the removal rate tended to be lower under higher humic acid doses (H20 and H50) than under lower humic doses (HA5 and HA10). For glyphosate, there was significant difference between HA0 and any added humic acid treatment for H20 but no significant difference among HA0 and all added humic acid treatments (Fig. 1).

In the presence of Fenton reagent, HA0 had significant lower diuron in the solution, as compared to any of added humic acid treatments. However, for butachlor, this was only observed at higher doses of humic acid (H20 and H50). There were different patterns between F20 and F50 for glyphosate; the former showed no significant difference between HA0 and most of the added humic acid treatments with HA5 even having significantly higher solution glyphosate, as compared to HA0; the latter showed a general trend that glyphosate decreased with increasing dose of humic acid though there was no significant difference among H10, H20 and H50 (Fig. 1).

3.2. Competitive removal of various herbicides

In the scenario where diuron and butachlor co-existed in the system, a significant difference in either diuron or butachlor was only observed in Treatments F50 and F100 though the control tended to had a higher concentration of herbicide, as compared to any of the treatments (Fig. 2).

In the combined diuron and glyphosate system, the same as the diuron-butachlor system was observed for the diuron in the solution with F50 and F100 having significant lower residual diuron than the control while there was no significant difference between the control and any other treatments. However for glyphosate, all the treatments with Fenton reagent showed significantly lower solution glyphosate, as compared to the control while there was no significant difference in solution glyphosate between the control and any of the H2O2 treatments (Fig. 3).

It is interesting to note that in the butachlorglyphosate system, both herbicides in the solutions were significantly higher in the control than in H100, F5, F10, F20 and F50 though the removal rate for glyphosate was markedly higher, as compared to that for butachlor (Fig. 4).
H$_2$O$_2$- or Fenton-driven degradation of butachlor was absent in the control, the removal of butachlor from the solution can be attributed to adsorption to the humic acid. In contrast, only less than 50% of the water-borne diuron was removed in the control when humic acid was at a dose of 20 mg/L (Fig. 1b). For glyphosate, only less than 30% was immobilized in the control when the humic acid dose was greater than 20 mg/L (Fig. 1c). Humic acid is also capable of scavenging free radicals (Wang et al., 2001; Goldstone et al., 2002; Romera-Castillo and Jaffé, 2015). Therefore, the presence of humic acid could enhance the consumption of hydroxyl radical generated from Fenton reaction and consequently had an effect on impeding degradation of herbicides. This was evident for diuron and butachlor in Treatments F20 and F50, showing that the removal rate of either herbicide was significantly higher in the absence of humic acid than in the presence of humic acid at any dosage levels. However, the same effect was not observed for glyphosate in Treatments F20 and F50; humic acids had no significant effects on reducing glyphosate removal rate (former) or even enhanced the removal of glyphosate (latter). This appears to suggest that glyphosate could compete strongly with the humic acid for the available hydroxyl radical in the reaction systems. It is not clear why more glyphosate was removed from the solution for F50 in the presence of humic acid. The capacity of humic acid itself to absorb glyphosate, as shown in the control (Ck) was limited. Therefore, the enhanced removal of glyphosate in F50 was likely to be associated with Fenton process. Reaction of humic acid with hydroxyl radical could result in formation of low-molecular-weight organic acids (Goldstone et al., 2002; Brinkmann et al., 2003). Since Fenton reaction produces ferric iron, this may lead to formation of iron-organic complexes that have the stronger capacity to bind glyphosate, as Piccolo et al., 1992; Undabeytia et al., 1996; Arroyave et al., 2016, Complexation of glyphosate with iron (III) was thought to be an important mechanism responsible for the immobilization of glyphosate (McBride and Kung, 1989).

The results obtained from Experiment 2 clearly demonstrated that the reactivity of glyphosate was much higher than those of diuron and butachlor. This may be explained by the relatively simpler chemical structure of glyphosate (Franz et al., 1997), as compared to either diuron or butachlor, which are aromatic compounds with higher chemical stability. It is interesting to note that degradation of butachlor was much weaker in the combined diuron and butachlor system than in the combined glyphosate and butachlor system. Possibly, the relatively low reactivity of diuron and butachlor with hydroxyl radical in combination with the strong competition between both herbicides for available hydroxyl radical resulted in their low removal efficiency. In the glyphosate-butachlor scenario, the consumption rate of hydroxyl radical by glyphosate was relatively smaller, as compared to that of diuron for the diuron-butachlor system. This made the hydroxyl radical more accessible to butachlor, allowing a higher removal rate of butachlor being observed.

The findings obtained from the current laboratory experiments have moved another step forward to understanding the potential role of rainwater-borne H$_2$O$_2$ in degrading the herbicides in open water environments, which inform the design of future microcosm, mosocosm and field-based experiments.

5. Conclusion

Under the set experimental conditions, the presence of humic acid could impede degradation of the diuron and butachlor by hydroxyl radical. However, humic acids had no significant effects on reducing glyphosate removal rate. Glyphosate could compete strongly with the humic acid for the available hydroxyl radical in the reaction systems. The reactivity of glyphosate with hydroxyl radical was much higher than those of diuron and butachlor due possibly to its relatively simpler chemical structure, as compared to either diuron or butachlor. Butachlor degradation was much weaker in the combined diuron and butachlor system than in the combined glyphosate and butachlor system. In the glyphosate-butachlor system, the opposite was observed. The findings obtained from the current experiments have moved another step forward to understanding the potential role of rainwater-borne H$_2$O$_2$ in degrading the herbicides in open water environments.

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