Elsevier Editorial System(tm) for
Chemosphere
Manuscript Draft

Manuscript Number: CHEM42979R1

Title: Potential Effects of Rainwater-borne H2O2 on Competitive Degradation of Herbicides and in the Presence of Humic Acid

Article Type: Research paper

Section/Category: Environmental Chemistry (including Persistent Organic Pollutants and Dioxins)

Keywords: Herbicide, hydrogen peroxide, free radical, chemical degradation, rainwater, Fenton reaction

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Cover Letter

Editor

Chemosphere

September 6, 2016

Dear Editor,

I submit a manuscript entitled "**Potential Effects of Rainwater-borne H_2O_2 on Competitive Degradation of Herbicides and in the Presence of Humic Acid**" for possible publication in your journal.

Thank you in advance for your consideration.

Yours sincerely,

Chuxia Lin

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Authors Responses to Comments from Editor and Reviewers

Dear Editor,

Thank you for your invitation to submit a revised manuscript for further consideration.

We would like to thank the two reviewers for their constructive comments and suggestions. Changes have been made accordingly in the revised manuscript. The point-by-point responses to the reviewers' comments are provided below.

Kind regards,

Chuxia Lin

Reviewer #2:

Reviewer's Comments
The authors describe in their manuscript how the presence of hydrogen peroxide in natural waters can affect the degradation of three herbicides in the aqueous environment. The topic is interesting and novel, therefore, the manuscript may interest wider community of researchers who are working on the environmental fate of man-made organic chemicals, such as pesticides. However, the manuscript requires revisions because it lacks of details in experimental design and interpretation of the obtained results.
Authors' Reply
We thank the reviewer's comments and changes have been made to provide details in experimental design and further interpretation of data, as suggested by the reviewer.

Reviewer's Comments
1. Introduction should be extended about the degradation mechanisms of diuron, butachlor and glyphosate in waters, what was done previously about degradation of these herbicides in waters, factors affecting their dissipation in waters, persistence, etc. I am sure that main degradation mechanisms for these organic molecules should be hydrolysis and photo degradation. Additionally, it would be interesting to know the concentrations of H2O2 and Fe2+ in surface waters, not only to state that they are present in waters.
Authors' Reply
We thank the reviewer's constructive suggestions. Additional information on degradation mechanisms of diuron, butachlor and glyphosate in waters (Page 3, Lines 56-72) and the concentrations of H_2O_2 and Fe^{2+} in surface waters have now been provided in the revised manuscript (Page 4, Lines 74-76; Lines 78-81).

Reviewer's Comments
2. In Materials and Methods, Part 2.2.1 Experiment 1, you describe the treatments with five levels of humic acid (HA), however cited Table 1 does not correspond with this.
In Table, I can see very different treatments. One may deduce from the text that "Experiment 1" was done by adding HA to tubes with individual herbicides dissolved in water with no H2O2 and Fe2+

but Table 1 says different story. Be precise in the description of experimental protocol. In addition, it is not clear whether you measured pH of your treatments and how the experiment was performed. Under direct Sun? Or under simulated light?

Authors' Reply

We accept the reviewer's points and changes have been made to improve the clarity of presentation. These include revision of Table 1 to provide much more detailed information on the experimental set-up and make further explanation in the text (Page 5, Lines 105-112).

Reviewer's Comments

3. It should be explained in Materials and Methods section why the experiments lasted only 1 hour. I think that this has no relevance to the natural conditions in waters. It is possible that the effect of Fenton reaction induced by H2O2 and Fe2+ on the degradation of herbicides in water will disappear, considering longer times due to consumption of OH radicals.

Authors' Reply

Pre-experiment testing showed that the reaction time for various herbicides under different treatments varied but all reactions were completed within a period of 1 hour (as indicated by no significant further drop in the herbicide concentration). While this is obviously an operationally defined reaction time (as pointed out by the reviewer), it was the best practice that we could use under the laboratory conditions when the experiments were performed. To better reflect the nature of the current experiment, we have also taken Reviewer #3's point and replaced the term "microcosm experiment" by "laboratory experiment". The reason for setting the experiment for 1 hour have now been provided in the revised manuscript (Page 5, Lines 111-112).

Reviewer's Comments

4. You state that combination of all three herbicides was also investigated (Page 5, Line 87). However, I cannot find any results and discussion for this treatment.

Authors' Reply

The results of this component were presented in " **3.2 Competitive Removal of Various Herbicides**" under "**Results**" section (Page 9, Lines 188-200) and the discussion of these results is made under the "**Discussion**" section (Pages 10-11, Lines 229-239):

"*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 butchlor 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.*"

Reviewer's Comments

5. Did you consider a possibility of forming insoluble complexes of glyphosate with Fe3+ in treatments F50? Could they contribute to the increase in glyphosate degradation in F20 and F50 treatments?

Authors' Reply

Thank you for the point here. This has been incorporated into the discussion of the results in the "Discussion" section (Page 10, Lines 227-228). The reviewer's help here is also acknowledged in the Acknowledgements section.

Reviewer's Comments
Figures 1-3 and their captions - revise "diruon" to "diuron" in all figures and captions.
Authors' Reply
Corrections made

Reviewer #3:

Reviewer's Comments
The current submission is the continuation of a work published in Chemosphere in 2013 (volume 92, issue 8, pp 1048-1052) describing the individual degradation of diuron, butachlor and glyphosate by Fenton reaction driven by hydrogen peroxide and Fe(II) concentrations typically found in rain waters and surface waters, respectively. The present work described the degradation of mixtures of two or three of the same herbicides in absence and presence of humic acid, which is the main component of natural organic matter found in soils and waters. The content of the manuscript is of interest for environmental scientists, but the manuscript needs major revisions before being considered for publication. The major points that should be addressed by the authors are as follows:
Authors' Reply
We thank the reviewer's comments and revision has been made in response to the reviewer's comments, as detailed below

Reviewer's Comments
Line 26: The results were obtained from essentially bench-top laboratory experiments, so that I think the term "microcosm experiments" is not suitable to describe the content of the manuscript.
Authors' Reply
We accept the reviewer's criticism and the term "microcosm experiment" has been changed to "laboratory experiment" throughout the entire manuscript (Page 2, Line 33; Page 11, Line 240).

Reviewer's Comments
Line 32: Glyphosate is not more reactive than diuron or butachlor just because its molecule is simpler (how do you define a simpler molecule in this case?). I guess diuron and butachor are more stable because they have an aromatic ring in their structure, conferring greater chemical stability compared with glyphosate.
Authors' Reply
We agree with the reviewer's point and this has now been incorporated into the manuscript (Page 2, Line 40) (with acknowledgement being made in the Acknowledgement section of the revised manuscript).

Reviewer's Comments
Line 61 - 62: Herbicides do not compete for hydroxyl radicals; functional groups of herbicides are chemically attacked by hydroxyl radicals.
Authors' Reply
We have re-written the sentence and it now reads "Since different organic molecules have different composition of functional groups, it is unclear how they compete for the available hydroxyl radical" (Page 4, Line 88-90).

Reviewer's Comments
Line 72: How can you determine the purity of a humic acid? Did you determine the organic carbon content?
Authors' Reply
The information on purity of the humic acid used was provided by the manufacturer. We measured the organic carbon using a TOC analyzer (Vario TOC Elementar, Germany).

Reviewer's Comments

Section 2.3 - Analytical methods: The chromatograph and chromatographic column used for separation of diuron and butachlor are exactly the same. You should describe the instrumentation once.

Authors' Reply

The relevant paragraph under **Section 2.3.2 Extraction and determination of butachlor** has now re-written as follows:

"*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*." (Page 7, Lines 144-146)

Reviewer's Comments

Section 2.3.3, line 121: According to the text, the detection of glyphosate was made by an electron capture detector (ECD). This is the first time I see this detector being used for liquid chromatography (although the paper published in 2013 also claims the use of this detector). As long as I know ECD is a typical detector used in gas chromatography. Are you sure that detection was made using an ECD? If so I very curious about the conditions used for detection.

Authors' Reply

We made a mistake here. It should an electrical conductivity detector (ECD) rather than an electron capture detector (ECD). We thank the reviewer for pointing out this error and correction has now been made in the revised manuscript (Page 7, Line 150).

Reviewer's Comments

Table 2: The TOC values and their respective standard deviation should be given with the correct number of significant figures. There are standard deviations with three digits; there are no physical meaning in these numbers. For example, TOC for diuron in absence of HA should read as 33.6 ± 0.9; 25.9 ± 0.6; 23 ± 1; 14 ± 1 and 7.1 ± 0.8.

Authors' Reply

We have made corrections accordingly.

Reviewer's Comments

Table 2 and Figures: For me it is not clear the meaning of the letters after the numbers (Table 2) or on the error bars (Figures).

Authors' Reply

We have re-written the table footnote and also created a space between two different humic acid dose blocks in an attempt to improve the clarity:

"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 $p < 0.05$."

The captions of the figures have also been re-written to improve the clarity of expression.

Reviewer's Comments

Lines 216 - 217: Why the results obtained from Experiment 1 suggest that butachlor had a stronger affinity towards the humic acids compared to diuron and glyphosate?

Authors' Reply

We have now explained this in the revised manuscript (Pages 9-10, Lines 204-210):

"*The results obtained from Experiment 1 suggest that butachlor had a stronger affinity towards the humic acid, as compared to diuron and glyphosate. For the control (no added* H_2O_2 *or Fenton reagent), at a humic acid dose greater than 20 mg/L, >70% of the water-borne butachlor disappeared after 1 h (Fig. 1a). Since* H_2O_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)*"

Reviewer's Comments
Line 218 - 219: Humic acid does not weaken the strength of the hydroxyl radical. Humic acids probably consume these radicals, perhaps preferentially to the herbicides.
Authors' Reply
We have re-written the sentences in response to the reviewer's comments (Page 10, Lines 212-214).

Reviewer's Comments
Lines 236 - 237: Again, what is a simpler structure?
Authors' Reply
We have further clarified this (Page 10, Lines 231-232).

Reviewer's Comments
Line 247 and 248: Despite the large number of measurements, the present work cannot be considered a microcosm experiment. Important parameters such as light, pH, ionic strength, longer reaction times were not evaluated. Thus, I do not agree with the statement that results will be useful to design mesocosm and macrocosm experiments.
Authors' Reply
We agree with the reviewer's point and the term "microcosm experiment" has now been changed to laboratory experiment, as stated previously (Page 2, Line 33; Page 11, Line 240).

Reviewer's Comments
Conclusion: The content of the manuscript is totally descriptive. From a molecular point of view, there is no explanation for the results found. Thus, I do not agree with the statement that these results have improved our understanding the potential role of rainwater-borne H2O2 in degrading the herbicides in aquatic environments.
Authors' Reply
We agree with the reviewer that the results were not explained from a molecular perspective, which has been considered in our ongoing work. However, we do think that the set research objective in this study (to obtain further insights into the interactive processes of hydroxyl radical, various herbicides and humic acid) has been satisfactorily achieved with appropriate critical analysis and evaluation of the data collected. Further in-depth investigations are currently underway.

*Revised manuscript with changes marked
Click here to view linked References

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.

Key word: Herbicide, hydrogen peroxide, free radical, chemical degradation, rainwater, Fenton reaction

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 et al., 1999; Salvestrini et al., 2002; Fenoll et al., 2013). Diuron, butachlor and glyphosate are among the most common herbicides in open water environments (Solomon et al., 2003; Yu et al., 2003; Giacomazzi et al., 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 et al., 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 (Guérit 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, photodegration of butchlor 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 glyphosate (Ghassemi, 1982).

Hydrogen peroxide (H_2O_2) is commonly present in rainwater (Willey et al., 1996; Gonçalves et al., 2010). For example, our monitoring data collected at a location in Guangzhou, southern China (unpublished) shows that H_2O_2 was detected in the rainwater samples taken on any sampling occasions (n=103) with the concentration of rainwater-borne H_2O_2 ranged from 1 to 93 μM. Trace amount of ferrous iron (Fe^{2+}) is also encountered in open water especially stagnant water environments (Mackey and Mackay, 1996; Díez et al., 2007; Sanders et al., 2012). For example, our unpublished data shows that Fe^{2+} concentration in canal water in Manchester and Leeds in the UK ranged from 0.2 to 0.5 mg/L. Testa et al. (2002) found a concentration of Fe^{2+} up to nearly 20 μM in the estuarine water of Waquoit Bay. Consequently Fenton reaction may take place in open water environments during heavy rainfall events. The hydroxyl radical ($^{\bullet}OH$) generated from Fenton reaction is likely to act as a powerful oxidant to decompose water-borne herbicides.

In a previous piece of work (Qin et al., 2013), we 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

2.2.1 Experiment 1: Concurrent Presence of Herbicide and Humic Acid

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 addition, one control and four treatments were set: (a) control: no added H_2O_2 and Fe^{2+}; (b) Treatment H20: H_2O_2 at 20 μM and no added Fe^{2+}; (c) Treatment H50: H_2O_2 at 50 μM and no added Fe^{2+}; (d) Treatment F20: H_2O_2 at 20 μM and Fe^{2+} at 20 μM; and (e) Treatment F50: H_2O_2 at 50 μM and Fe^{2+} at 20 μM. Centrifuge tubes with 15 mL capacity were used as batch reactors with each tube containing 10 mL of either diuron, butachlor or glyphosate solution at 1 mg/L. The experiment lasted for 1 h (under natural light) to allow sufficient time for complete consumption of hydroxyl radical or hydrogen peroxide. Solution samples were taken at the end of the experiment for determination of total organic carbon (TOC) and the relevant herbicide. After collection, the solution samples were stored at -25 °C prior to analysis.

2.2.2 Experiment 2: Concurrent Presence of Multiple Herbicides

For the mixed herbicide experiment, four combinations are set (with the concentration of each herbicide all being fixed at 1 mg/L): diuron+butachlor, diuron+glyphosate, butachlor+glyphosate, and diuron+butachlor+glyphosate. For each combination, one control and eight treatments were set:

Control (Ck): no added H_2O_2 and Fe^{2+}; Treatments 1-4 (H5, H20, H50, and H100): H_2O_2 at 5, 20, 50 and 100 µM, respectively; Treatments 5-8 (F5, F20, F50, and F100): a fixed concentration of Fe^{2+} at 20 µM, in combination with H_2O_2 at 5, 20, 50 or 100 µM, respectively.

2.3 Analytical methods

2.3.1 Extraction and determination of diuron

The following procedure was used for extracting residual diuron 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 purified by passing through an Anpelclean Alumina-N SPE column and frozen at -25 °C prior to analysis.

An Agilent 1100 HPLC equipped with an ultraviolet absorption detector was used to determine diuron in the extracts. A LiChrospher C18 reversed-phase column (250 mm×4.6 mm) was used for separation and the mobile phase was a mixture of methanol and water at a volume ratio of 65 to 35. The flow rate was set at 1.0 mL/min. The column temperature was maintained at 25 °C, and the detector was set at a wavelength of 245 nm.

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 99:1 hexane/acetone mixed solution; shake for 2 min and release the water; repeat the above procedure for twice, the organic-dominated solvent was further dehydrated by repeatedly adding 30 mL of anhydrous sodium sulfate solution (5%) and releasing aqueous phase for three times. The dehydrated sample was 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 reagents used in the experiments were of analytical reagent grade. Ultrapure water (18.2 MΩ/cm) was used throughout the entire course of all the experiments. Repeatability analysis shows that the RSD for TOC, diuron, butachlor and glyphosate was <11.3%, <4.44%, <8.33%, and <8.79%, respectively. The statistical significance of difference between the treatment means was determined by IBM SPSS software Version 17.0.

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 > H20 > H50 > F20 > F50 (Table 2).

For the control (Ck, no added H_2O_2 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 HA5, HA10 and HA20, which was also insignificantly different from either HA0 or HA50.

In the presence of H_2O_2 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.

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.

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.

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.

It is interesting to note that in the butachlor-glyphosate 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.

4 Discussion

It has been long recognized that humic acid is an effective sorbent for herbicides (Khan, 1973; Martin-Neto et al., 1994; Arroyave et al., 2016). The results obtained from Experiment 1 suggest that butachlor had a stronger affinity towards the humic acid, as compared to diuron and glyphosate. For the control (no added H_2O_2 or Fenton reagent), at a humic acid dose greater than 20 mg/L, >70% of the water-borne butachlor disappeared after 1 h (Fig. 1a). Since H_2O_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 adsorb 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 (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 butchlor 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_2O_2 in degrading the herbicides in open water environments, which informs 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_2O_2 in degrading the herbicides in open water environments.

Acknowledgements

This work was partly supported by the Natural Science Foundation of China (Project No. 41271469). The authors would like to thank the two anonymous reviewers for their constructive comments and suggestions that contributed to the improvement of the manuscript.

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Captions

Fig 1. Comparison of diuron (a), butchalor (b) and glyphosate (c) in the solution after 1-h reaction at different doses of humic acid. Ck: control; H20 and H50: addition of H_2O_2 at a concentration of 20 and 50 µM; F20 and F50: addition of H_2O_2 at a concentration of 20 and 50 µM plus a fixed concentration of Fe^{2+} at 20 µM for each treatment; HA0: no added humic acid; HA5, HA10, HA20 and HA50: addition of humic acid at a concentration of 5, 10, 20 and 50 mg/L, respectively. All values are presented as mean ± standard error (n=3) and bars with different letters for each treatment (Ck, H20, H50, F20 or F50) indicate significantly different ($p < 0.05$).

Fig 2. Comparison of residual concentration of diuron and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either diuron or butachlor indicate significantly different ($p < 0.05$).

Fig. 3 Comparison of residual concentration of diuron and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100:

addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either diuron or glyphosate indicate significantly different ($p < 0.05$).

Fig. 4 Comparison of residual concentration of diruon and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either butachlor or glyphosate indicate significantly different ($p < 0.05$).

Highlights

- Potential role of rainwater-borne H_2O_2 in degrading herbicides in open water
- Previous work was limited to individual herbicides.
- Further microcosm experiments were conducted for more complex systems
- Humic acid impeded degradation of the diuron and butachlor but not glyphosate
- The reactivity of glyphosate with $^{\bullet}OH$ was much higher than that of other herbicides

*Revised manuscript with no changes marked
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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.

Key word: Herbicide, hydrogen peroxide, free radical, chemical degradation, rainwater, Fenton reaction

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 et al., 1999; Salvestrini et al., 2002; Fenoll et al., 2013). Diuron, butachlor and glyphosate are among the most common herbicides in open water environments (Solomon et al., 2003; Yu et al., 2003; Giacomazzi et al., 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 et al., 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 (Guérit 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, photodegration of butchlor 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 glyphosate (Ghassemi, 1982).

Hydrogen peroxide (H_2O_2) is commonly present in rainwater (Willey et al., 1996; Gonçalves et al., 2010). For example, our monitoring data collected at a location in Guangzhou, southern China (unpublished) shows that H_2O_2 was detected in the rainwater samples taken on any sampling occasions (n=103) with the concentration of rainwater-borne H_2O_2 ranged from 1 to 93 µM. Trace amount of ferrous iron (Fe^{2+}) is also encountered in open water especially stagnant water environments (Mackey and Mackay, 1996; Díez et al., 2007; Sanders et al., 2012). For example, our unpublished data shows that Fe^{2+} concentration in canal water in Manchester and Leeds in the UK ranged from 0.2 to 0.5 mg/L. Testa et al. (2002) found a concentration of Fe^{2+} up to nearly 20 µM in the estuarine water of Waquoit Bay. Consequently Fenton reaction may take place in open water environments during heavy rainfall events. The hydroxyl radical ($^{\bullet}OH$) generated from Fenton reaction is likely to act as a powerful oxidant to decompose water-borne herbicides.

In a previous piece of work (Qin et al., 2013), we 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

2.2.1 Experiment 1: Concurrent Presence of Herbicide and Humic Acid

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 addition, one control and four treatments were set: (a) control: no added H_2O_2 and Fe^{2+}; (b) Treatment H20: H_2O_2 at 20 μM and no added Fe^{2+}; (c) Treatment H50: H_2O_2 at 50 μM and no added Fe^{2+}; (d) Treatment F20: H_2O_2 at 20 μM and Fe^{2+} at 20 μM; and (e) Treatment F50: H_2O_2 at 50 μM and Fe^{2+} at 20 μM. Centrifuge tubes with 15 mL capacity were used as batch reactors with each tube containing 10 mL of either diuron, butachlor or glyphosate solution at 1 mg/L. The experiment lasted for 1 h (under natural light) to allow sufficient time for complete consumption of hydroxyl radical or hydrogen peroxide. Solution samples were taken at the end of the experiment for determination of total organic carbon (TOC) and the relevant herbicide. After collection, the solution samples were stored at -25 °C prior to analysis.

2.2.2 Experiment 2: Concurrent Presence of Multiple Herbicides

For the mixed herbicide experiment, four combinations are set (with the concentration of each herbicide all being fixed at 1 mg/L): diuron+butachlor, diuron+glyphosate, butachlor+glyphosate, and diuron+butachlor+glyphosate. For each combination, one control and eight treatments were set:

Control (Ck): no added H_2O_2 and Fe^{2+}; Treatments 1-4 (H5, H20, H50, and H100): H_2O_2 at 5, 20, 50 and 100 μM, respectively; Treatments 5-8 (F5, F20, F50, and F100): a fixed concentration of Fe^{2+} at 20 μM, in combination with H_2O_2 at 5, 20, 50 or 100 μM, respectively.

2.3 Analytical methods

2.3.1 Extraction and determination of diuron

The following procedure was used for extracting residual diuron 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 purified by passing through an Anpelclean Alumina-N SPE column and frozen at -25 °C prior to analysis.

An Agilent 1100 HPLC equipped with an ultraviolet absorption detector was used to determine diuron in the extracts. A LiChrospher C18 reversed-phase column (250 mm×4.6 mm) was used for separation and the mobile phase was a mixture of methanol and water at a volume ratio of 65 to 35. The flow rate was set at 1.0 mL/min. The column temperature was maintained at 25 °C, and the detector was set at a wavelength of 245 nm.

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 99:1 hexane/acetone mixed solution; shake for 2 min and release the water; repeat the above procedure for twice, the organic-dominated solvent was further dehydrated by repeatedly adding 30 mL of anhydrous sodium sulfate solution (5%) and releasing aqueous phase for three times. The dehydrated sample was 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 reagents used in the experiments were of analytical reagent grade. Ultrapure water (18.2 MΩ/cm) was used throughout the entire course of all the experiments. Repeatability analysis shows that the RSD for TOC, diuron, butachlor and glyphosate was <11.3%, <4.44%, <8.33%, and <8.79%, respectively. The statistical significance of difference between the treatment means was determined by IBM SPSS software Version 17.0.

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 > H20 > H50 > F20 > F50 (Table 2).

For the control (Ck, no added H_2O_2 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 HA5, HA10 and HA20, which was also insignificantly different from either HA0 or HA50.

In the presence of H_2O_2 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.

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.

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.

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.

It is interesting to note that in the butachlor-glyphosate 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.

4 Discussion

It has been long recognized that humic acid is an effective sorbent for herbicides (Khan, 1973; Martin-Neto et al., 1994; Arroyave et al., 2016). The results obtained from Experiment 1 suggest that butachlor had a stronger affinity towards the humic acid, as compared to diuron and glyphosate. For the control (no added H_2O_2 or Fenton reagent), at a humic acid dose greater than 20 mg/L, >70% of the water-borne butachlor disappeared after 1 h (Fig. 1a). Since H_2O_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 adsorb 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 (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 butchlor 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_2O_2 in degrading the herbicides in open water environments, which informs 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_2O_2 in degrading the herbicides in open water environments.

Acknowledgements

This work was partly supported by the Natural Science Foundation of China (Project No. 41271469). The authors would like to thank the two anonymous reviewers for their constructive comments and suggestions that contributed to the improvement of the manuscript.

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Captions

Fig 1. Comparison of diuron (a), butchalor (b) and glyphosate (c) in the solution after 1-h reaction at different doses of humic acid. Ck: control; H20 and H50: addition of H_2O_2 at a concentration of 20 and 50 µM; F20 and F50: addition of H_2O_2 at a concentration of 20 and 50 µM plus a fixed concentration of Fe^{2+} at 20 µM for each treatment; HA0: no added humic acid; HA5, HA10, HA20 and HA50: addition of humic acid at a concentration of 5, 10, 20 and 50 mg/L, respectively. All values are presented as mean ± standard error (n=3) and bars with different letters for each treatment (Ck, H20, H50, F20 or F50) indicate significantly different ($p < 0.05$).

Fig 2. Comparison of residual concentration of diuron and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either diuron or butachlor indicate significantly different ($p < 0.05$).

Fig. 3 Comparison of residual concentration of diuron and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100:

addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either diuron or glyphosate indicate significantly different ($p < 0.05$).

Fig. 4 Comparison of residual concentration of diruon and butachlor in the solution after 1-h reaction among the control and the treatments. Ck: control; H5, H20, H50 and H100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively; F5, F20, F50 and F100: addition of H_2O_2 at a concentration of 5, 20, 50 and 100 µM, respectively, plus a fixed concentration of Fe^{2+} at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters for either butachlor or glyphosate indicate significantly different ($p < 0.05$).

Table 1
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Table 1 The details on experimental set-up for Experiment 1

Humic acid dose	Treatment	H_2O_2 (μM)	Fe^{2+} (μM)	Herbicide* (mg/L)	Water (mL)
HA0	Control	0	0	1	10
	H20	20	0	1	10
	H50	50	0	1	10
	F20	20	20	1	10
	F50	50	20	1	10
HA5	Control	0	0	1	10
	H20	20	0	1	10
	H50	50	0	1	10
	F20	20	20	1	10
	F50	50	20	1	10
HA10	Control	0	0	1	10
	H20	20	0	1	10
	H50	50	0	1	10
	F20	20	20	1	10
	F50	50	20	1	10
HA20	Control	0	0	1	10
	H20	20	0	1	10
	H50	50	0	1	10
	F20	20	20	1	10
	F50	50	20	1	10
HA50	Control	0	0	1	10
	H20	20	0	1	10
	H50	50	0	1	10
	F20	20	20	1	10
	F50	50	20	1	10

*Either diuron, butachlor or glyphosate

Table 2
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Table 2 Total organic carbon (TOC, mg/L) in the herbicide-containing solutions at the end of the experiment for the control and the treatments at various doses of humic acid

Dose of humic acid (mg/L)	Treatment	Diuron	Butachlor	Glyphosate
0	Ck	33.5±0.9a	7.6±0.5a	14.6±1.0a
	H20	25.8±0.6b	6.3±0.2ab	4.3±1.0b
	H50	22.5±1.2c	5.3±0.3b	1.8±0.4c
	F20	13.5±1.2d	4.8±0.6b	0.7±0.2c
	F50	7.1±0.8e	2.5±0.4c	0.1±0.0c
5	Ck	64.2±3.3a	24.1±0.6a	7.0±0.1a
	H20	33.7±2.9b	11.5±0.3b	6.1±0.8ab
	H50	22.8±0.8c	11.9±0.7b	4.0±0.6c
	F20	16.3±0.6d	10.1±0.5b	4.6±0.3bc
	F50	17.3±0.2d	4.6±0.8c	3.0±0.2c
10	Ck	54.3±0.4a	21.2±0.7a	13.0±1.1a
	H20	50.3±1.3b	18.4±0.7b	10.6±0.3b
	H50	30.1±0.8c	15.9±0.4c	6.9±0.9c
	F20	26.8±1.3d	14.4±0.5cd	4.0±0.0d
	F50	18.3±0.1e	12.8±0.9d	4.3±0.4d
20	Ck	73.5±1.8a	34.0±0.1a	20.6±2.1a
	H20	37.4±3.0b	28.7±0.8b	20.4±1.5a
	H50	36.4±1.0b	25.9±0.5b	16.9±0.3a
	F20	30.9±1.1c	20.3±0.9c	11.1±1.0b
	F50	27.4±0.2d	19.2±2.2c	1.4±0.2c
50	Ck	92.5±3.7a	79.2±5.4a	65.1±1.4a
	H20	88.1±0.9a	66.7±3.5b	37.8±3.0b
	H50	71.5±1.1b	60.2±4.7bc	28.0±2.6c
	F20	46.8±1.8c	52.5±1.9c	29.4±2.5c
	F50	32.2±3.0d	53.4±2.8c	9.1±1.1d

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 $p < 0.05$.

Figure 1
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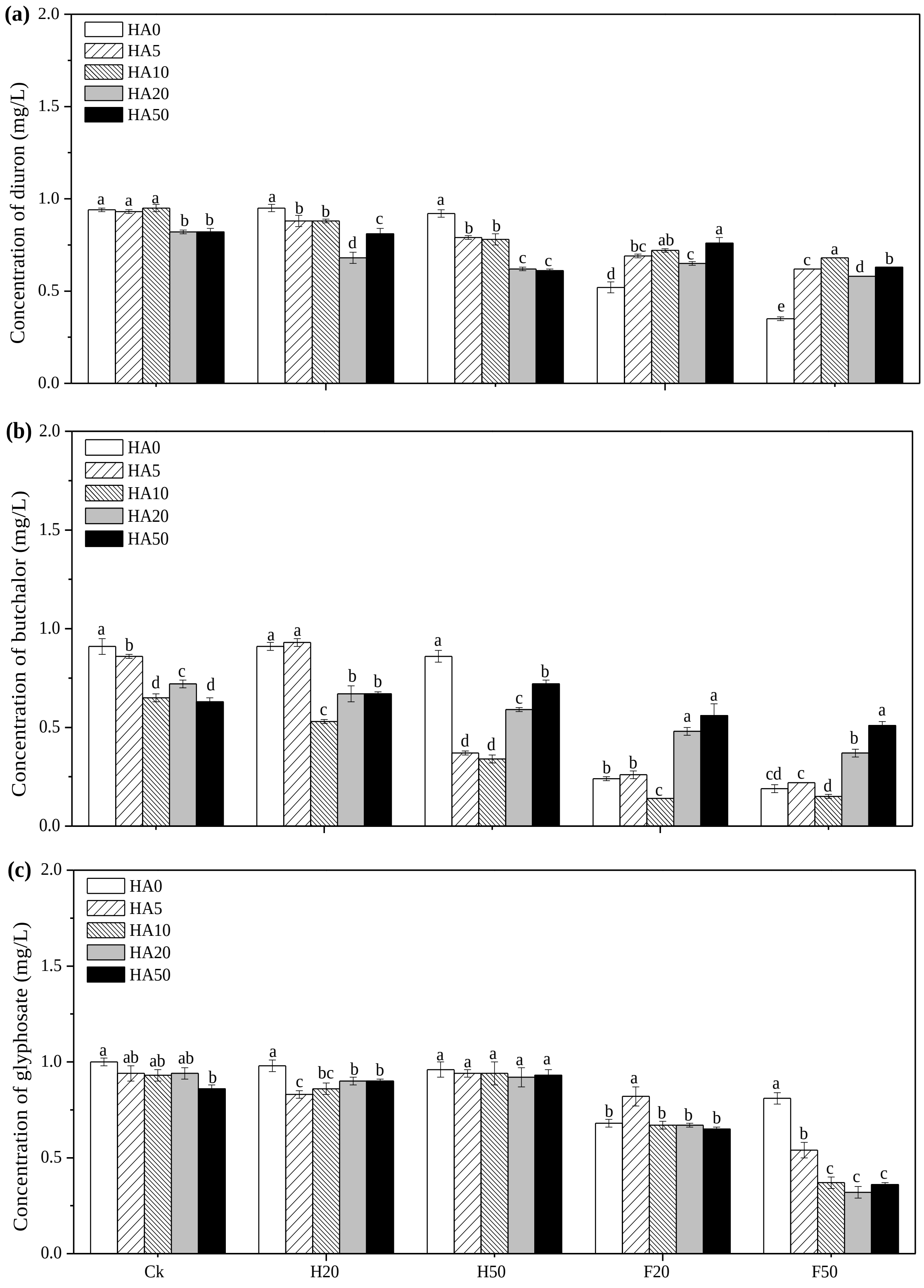

Fig 1.

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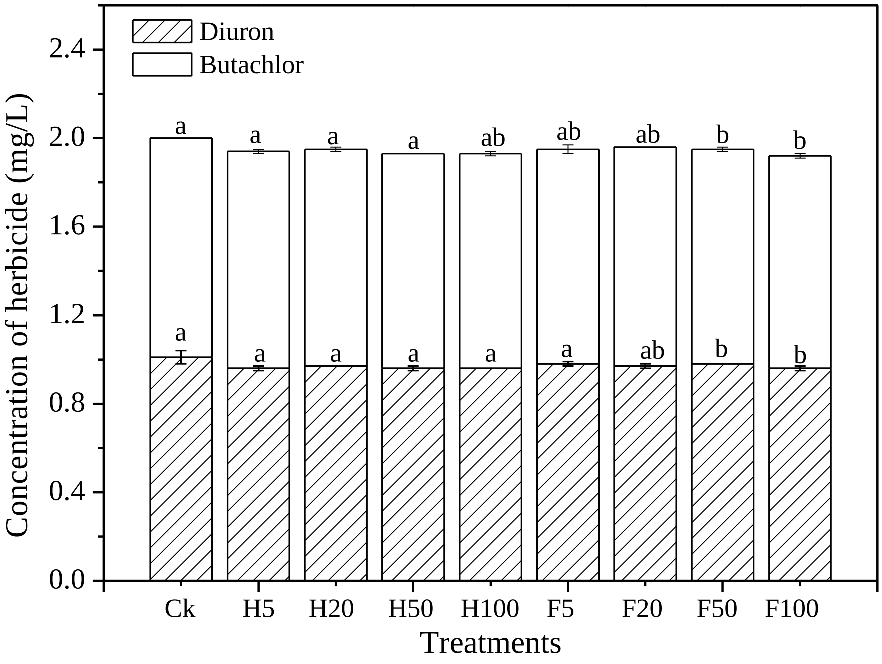

Fig 2.

Figure 3

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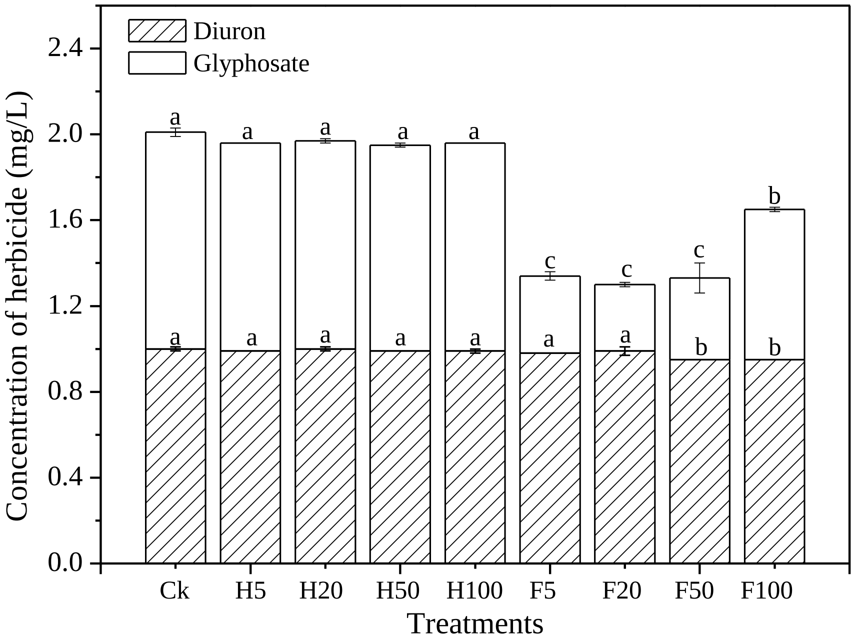

Fig. 3

Figure 4
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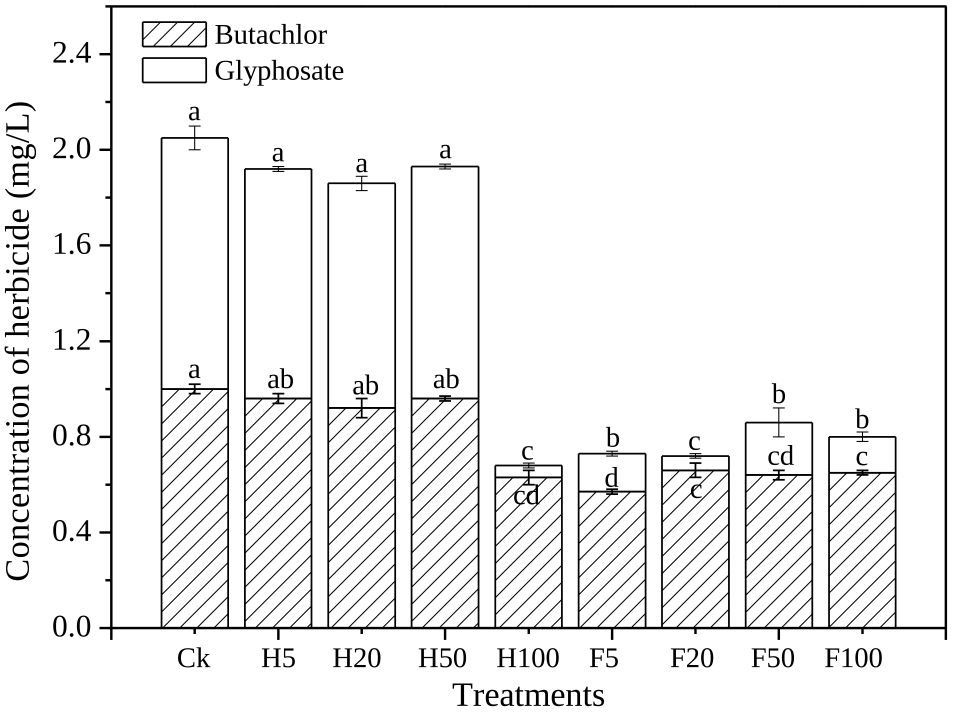

Fig. 4