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Editor

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Dear Editor,

I submit a manuscript entitled “**Potential Effects of Rainwater-borne Hydrogen Peroxide on Pollutants in Stagnant Water Environments**” for possible publication in your journal.

Thank you in advance for your consideration.

Yours sincerely,

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AUTHORS' RESPONSE TO FURTHER COMMENTS FROM REVIEWERS

Reviewer #2: Since the authors have made the revision according to the reviewers' comments, the quality of the paper was improved. However, prior to publication, the followings need to be corrected:

Authors' Reply

We sincerely thank the reviewer's comments and further changes have been made in response to the reviewer's points as follows.

Reviewer's Comment

1. Page 2, Ln 31: Ln Please delete "(PAH)" in the abstract.

Authors' Reply

Done as per the reviewer's suggestion.

Reviewer's Comment

2. Page 5, Ln 100-101: Please use the abbreviation, i.e., PAH, herein.

Authors' Reply

Changes have been made as per the reviewer's suggestion (highlighted in green).

Reviewer's Comment

3. Page 6, Ln 113-116: These sentences were repeated twice.

Authors' Reply

Many thanks for pointing out this error. The repeated sentences have now been deleted.

Reviewer's Comment

4. Page 9, Ln 204-205: Please combine this sentence into other paragraphs since only one sentence was presented in this paragraph.

Authors' Reply

Accepted and change has been made accordingly.

Reviewer's Comment

5. Tables 2, 3 and 4: Please check the significant figures of mean and standard error for each measurement.

Authors' Reply

We have carefully checked these tables and made corrections accordingly.

Reviewer's Comment

6. Page 23, Ln 574 and 578: Please be consistent for "change in the concentration" and "change in the concentrations".

Authors' Reply

Thanks and correction has been made.

Reviewer's Comment

7. Figure 1: It suggests that different colors or matrix styles can be used for bars to distinguish the experiment characteristics.

Authors' Reply

Accepted and the figure has been redrawn to use different matrix styles for the bars representing different types of treatments (see the revised Fig. 1).

1 **Potential Effects of Rainwater-borne Hydrogen Peroxide on Pollutants in Stagnant**
2 **Water Environments**

3
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19 **ABSTRACT**

20 Microcosm experiments were conducted to examine the effects of rainwater-borne H₂O₂ on
21 inactivation of water-borne coliforms, oxidation of ammonia and nitrite, and degradation of organic
22 pollutants in canal and urban lake water. The results show that the soluble iron in the investigated
23 water samples was sufficiently effective for reaction with H₂O₂ in the simulated rainwater-affected
24 stagnant water to produce •OH (Fenton reaction), which inactivated coliform bacteria even at a H₂O₂
25 dose as low as 5 µM within just 1 min of contact time. Coliform inhibition could last for at least 1 h
26 and repeated input of H₂O₂ at a 30 min interval allowed maintenance of microbial inhibition for at
27 least 3 h. Nitrification was also impeded by the Fenton process. The resulting inhibition of ammonia-
28 oxidizing microbes reduced the removal rate of NH₄⁺ and the emission of gaseous N species. In the
29 presence of H₂O₂ at a dose of 20 µM, Fenton-driven chemical oxidation appeared to outplay the
30 impediment of biodegradation caused by inhibited microbial activities in terms of removing total
31 polycyclic aromatic hydrocarbons from the water column. The findings point to a potential research
32 direction that may help to explain the dynamics of water-borne pollutants in ambient water
33 environments.

34 **Key words:** Stagnant water, coliform, ammonia, organic pollutant, rainwater

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41 **1 Introduction**

42 Stagnant water environments such as lakes, ponds, canals act as sinks for organic matter and
43 various inorganic, organic and microbial contaminants (Pal et al., 2010; DeLorenzo et al., 2012;
44 Sales-Ortells et al., 2015). The organic-rich status could enhance the growth of microbial pathogens
45 (Sato et al., 2013; 2016). This poses a health risk to people who are exposed to water-borne
46 microbial pathogens e.g. during open water swimming (Wade et al., 2003; Schets et al., 2011;
47 McBride et al., 2013). Ammonia (NH_3) is a gaseous product of organic matter mineralization. In
48 non-alkaline water environments, ammonia tends to be converted into ammonium ion (NH_4^+) via a
49 reversible reaction with water molecule (Karthikeyan et al., 2008). Therefore, ammonium acts as an
50 immediate source of ammonia in the water environments. Stagnant water environments could receive
51 ammonium from agricultural, urban and industrial discharges (Erisman et al., 2007; Reche et al.,
52 2012; Wang et al., 2016). Ammonia can also be produced in-situ through in-water protein
53 metabolism by aquatic biota (Walsh and Wright, 1995). Therefore, ammonia is commonly present in
54 stagnant water environments. Due to its high toxicity to aquatic life, ammonia is viewed as one of the
55 major inorganic pollutants in aquatic ecosystems (Parker et al., 2012; Collos et al., 2014). Potentially
56 toxic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and various pesticides
57 from urban or agricultural runoffs can also enter into and accumulate in stagnant water environments
58 (Kong et al., 2005; Kanzari et al., 2014; Hijosa-Valsero et al., 2016).

59 Ammonia/ammonium can be transformed into nitrite and eventually nitrate through microbially
60 mediated nitrification (Bernhard et al., 2007; Stahl and de la Torre, 2012). It is believed that in
61 natural environments, organic pollutants are mainly degraded through microbial metabolism (Glick,
62 2003; Liste and Prutz, 2006; Khan et al., 2013). The activity of water-borne microbes in a given
63 water environment can be affected by many factors (Morens et al., 2004; Braks and de Roda Husman,
64 2013; Nguyen et al., 2016). While the effects of some environmental parameters such as pH,

65 temperature, salinity etc. on activities of water-borne microbes have been well recognized
66 (Wadowsky et al., 1985; Manas et al., 2003; Spinks et al., 2006), others are literally overlooked.

67 Hydrogen peroxide (H₂O₂) is commonly present in rainwater with a molar concentration
68 frequently ranging from 5 to 70 µM (Willey et al., 1996; Kieber et al., 2001; Gonçalves et al., 2010).
69 H₂O₂ is a strong oxidant, which can cause oxidative stress in cells (Schoonen et al., 2010; Matthijs et
70 al., 2012; Ma et al., 2013). Due to relatively anoxic and organic-rich conditions, iron compounds in
71 the benthic sediments under stagnant water layers are subject to reductive dissolution (Espana et al.,
72 2007; Anna and Jacek, 2014; Shamsul Haque et al., 2016). This could lead to release of ferrous iron
73 (Fe²⁺) into water column (Díez et al., 2007; Sanders et al., 2012; Willey et al., 2015). During rainfall
74 events, the Fe²⁺ in stagnant water can react with rainwater-borne H₂O₂ to trigger Fenton reaction, as
75 expressed in the following chemical equation:

77 Fenton reaction produces hydroxyl radical (·OH), which is an even stronger oxidant, as
78 compared to H₂O₂. Hydroxyl radical has the capacity to destroy cells and organic molecules within a
79 short period of time (Winterbourn, 1995; Lee et al., 2010). It is therefore likely that rainwater-borne
80 H₂O₂ has a role to play in affecting the activity of microbial communities in stagnant water
81 environments. Qin et al. (2013) showed that Fenton reaction triggered by H₂O₂ and Fe²⁺ (Fenton
82 reagent) at a concentration range encountered in rainwater-affected surface water was capable of
83 chemically degrading some herbicides (diuron, butachlor and glyphosate) in synthetic solutions. But
84 there have not been further tests on other organic pollutants. Brito et al. (2010) demonstrated that
85 hydroxyl radical generated from Fenton reaction could chemically oxidize ammonia in landfill
86 leachate. However, the dose of H₂O₂ they used was much higher than what can be encountered in
87 rainwater. It is likely that in natural water, Fenton reaction has a dual role to play in terms of
88 oxidizing ammonia and degrading organic pollutants; the chemical reaction of hydroxyl radical with

89 ammonia and organic pollutants may accelerate the oxidation of ammonia and decomposition of
90 organic pollutants while the oxidative stress in ammonia-oxidizing and organic pollutant-degrading
91 microbes caused by $\cdot\text{OH}$ is likely to impede ammonia oxidation and biodegradation of the organic
92 pollutants. Consequently, the net oxidation rate of ammonia and degradation rate of organic
93 pollutants by Fenton reagent is determined by the combined effects of these two counteractive
94 processes.

95 Understanding the behaviour and fate of water-borne pollutants is important for evaluating
96 aquatic ecosystem health and the risk to human exposure, which can inform development of
97 appropriate management strategies. To understand the potential role of rainwater-borne H_2O_2 in
98 affecting the behaviour and fate of some key pollutants in stagnant water environments, a multi-stage
99 research project has been set. This work reports on the results from the stage of microcosm
100 experiments with a focus on coliform bacteria, ammonia and PAHs. The aim of conducting these
101 microcosm experiments was to establish the dose-response relationships between the added H_2O_2
102 and the selected water-borne pollutants under controlled environmental conditions in order to inform
103 the design of field-based investigations in the follow-up phase of the project. The specific objectives
104 for this part of the project were to examine the effects of H_2O_2 at a concentration range that is likely
105 to be encountered in rainwater on (a) inactivation of potential microbial pathogens, as indicated by
106 coliform bacteria, (b) oxidation of ammonia and nitrite, and (c) degradation of PAHs in synthetic
107 solutions and/or selected natural stagnant water samples.

108 **2 Materials and Methods**

109 **2.1 Natural Water Samples used in the Experiment**

110 Canals and urban lakes were used as example stagnant water environments in this study. The
111 water samples were collected from the selected locations in the Bridgewater canal and Salford Quays
112 in Manchester City (Latitude: $53^\circ 28' 56.6''\text{N}$; Longitude: $2^\circ 14' 21.8''\text{W}$) and the Clarence Dock in

113 Leeds City (Latitude: 53°48'05.8"N; Longitude: 1°32'59.8"W). Both cities are located in England,
114 The United Kingdom. The Bridgewater canal is arguably the first canal in England, which was built
115 in mid-1700 to transport coal from the mine sites to the industrial areas in Manchester. Salford Quays
116 is an urban lake area regenerated from the former ship docks, which form part of the Manchester
117 Ship Canal that connected Manchester with Port of Liverpool. Part of the Salford Quays is used for
118 regular water sport activities, including swimming. The open water in Leeds Clarence Dock is an
119 urban lake surrounded by shops, office buildings and residential apartments with heavy population.
120 These water areas were heavily contaminated during the period of Industrial Revolution (Douglas et
121 al., 2002; Williams et al., 2010). The benthic sediments contained substantial amounts of organic and
122 inorganic pollutants. Although pollutant loads from the upper catchment areas have been
123 significantly reduced for the past decades as a result of de-industrialization in the region, water
124 quality remains poor in the urban lakes and canal sections adjacent to urban areas due largely to
125 pollutant inputs from urban runoff during rainfall events and re-entry of bed sediment-borne
126 pollutants into water column (Rees et al., 1999; Quesada et al., 2014). Water quality monitoring data
127 (our unpublished data) indicated that the canal water frequently contained elevated levels of coliform
128 bacteria, nitrogen and certain potentially toxic organic pollutants.

129 Due to the practical difficulties in satisfactorily quantifying all the intended physical, chemical
130 and microbial parameters in a single experiment, three sets of separate experiments were conducted
131 to individually examine the effects of H₂O₂ in synthetic rainwater-affected stagnant water on the
132 inhibition of coliform bacteria, oxidation of ammonia and nitrite, and degradation of organic
133 pollutants. Some major physical and chemical characteristics of the five water samples used in the
134 experiments are given in Table 1. The water samples WS-EXP1A, WS-EXP1B and WS-EXP1C used
135 for Experiment 1A-1C (coliform experiments) were collected from the Bridgewater canal, Salford
136 Quays and Leeds Clarence Dock, respectively. The sample WS-EXP2C used for Experiment 2C

137 (ammonia experiment) was taken from Salford Quays. The sample WS-EXP3 used for Experiment 3
138 (organic pollutants) was collected from Bridgewater canal.

139 **2.2 Experiments 1A-1C: Inhibition of Coliforms in Natural Water**

140 Experiments 1A was to test whether the iron present in the canal water was effective for
141 triggering Fenton reaction upon addition of H_2O_2 at a concentration range of 0-50 μM , which is
142 frequently encountered in rainwater (Willey et al., 1996; Kieber et al., 2001; Gonçalves et al., 2010).
143 One control and eight treatments were set and the details of experimental set-up are given in
144 Supplementary Table S1.

145 Experiments 1B and 1C were conducted to observe temporal variation in abundance of
146 coliforms in canal water upon repeated addition of H_2O_2 . To minimize the workload, only two H_2O_2
147 doses were used: (a) 20 μM , and (b) 50 μM . Therefore, there were one control (Ck) and four
148 treatments being set in a way exactly the same as H20, H50, F20, and F50 in Experiment 1A. The
149 experiment lasted for 3 h with an appropriate amount of H_2O_2 being injected into the canal water at
150 the 60th, 90th, 120th, and 150th min following initial addition of H_2O_2 . The amount of H_2O_2 added
151 each time was to achieve a theoretical concentration of H_2O_2 equivalent to what was set for each
152 treatment at the time of addition, assuming that the H_2O_2 from previous addition was completely
153 consumed. During the first stage of the experiment (60 min), samples were taken at 1st, 10th, 30th and
154 60th min before the second addition of H_2O_2 . This was to more closely observe the variation in
155 coliform population over time upon a single addition of H_2O_2 during a period of 1 h. The subsequent
156 addition of H_2O_2 at a 30-min interval was to simulate a scenario in which the canal water received
157 H_2O_2 from intermittent rainfall. Following each additional H_2O_2 addition, a water sample was taken
158 immediately before the next H_2O_2 addition (i.e. every 30 min).

159 Plastic bottles with a capacity of 125 mL were used as batch reactors. Upon addition of H_2O_2
160 and for Treatments 5-8, Fe^{2+} as well, an aliquot (2 mL) of the canal water was taken from each

reactor twice; one after 1 min of reaction and the other one at the end of the experiment (60 min).
The water sample was immediately used for determination of the concentration of coliforms.

2.3 Experiments 2A-2C: Oxidation of Ammonia in Synthetic Solutions and Natural Water

Experiments 2A and 2B were to observe the reaction of ammonia or nitrate, respectively, with H_2O_2 or Fenton reagent in abiotic system in order to understand whether water-borne ammonia and nitrate can be chemically oxidized by either H_2O_2 or Fenton Reagent. Synthetic NH_4^+ and NO_2^- solutions were prepared using NH_4Cl and NaNO_3 , respectively. The concentration of synthetic NH_4^+ solution was fixed at 556 μM of NH_4^+ -N and the concentration of synthetic NO_2^- solution was set at 217 μM of NO_2^- -N. For Experiment 2C, natural canal water (WS-EXP2C) was used with an appropriate amount of NH_4Cl (equivalent to 556 μM of NH_4^+ -N, as set for Experiment 2A) being added to the canal water sample to simulate an ammonia input from runoff. For all the three experiments, one control and four treatments were set: (a) control: solution only; (b) H_2O_2 at a dose of 20 μM ; (c) H_2O_2 at a dose of 50 μM ; (d) H_2O_2 at a dose of 20 μM plus Fe^{2+} at a dose of 20 μM ; (e) H_2O_2 at a dose of 50 μM plus Fe^{2+} at a dose of 20 μM .

Plastic bottles with a capacity of 1 L were used as batch reactors. Upon addition of H_2O_2 or Fenton reagent, an aliquot (10 mL) of the solution was taken from each reactor at the following times: 1, 5, 10, 20 and 30 min. After collection, the water samples were stored at -25 °C prior to analysis.

2.4 Experiment 3: Degradation of Organic Pollutants in Natural Water

This experiment was to test the potential effects of H_2O_2 at a dose of 20 μM on a range of potentially toxic organic pollutants. An appropriate amount of H_2O_2 was added to 1 L of the water sample to obtain the set treatment dose. The original sample (without added H_2O_2) was used as the control.

2.5 Analytical Methods

The concentration of coliforms in the water samples was determined by a membrane filtration method using Oxoid Membrane Lauryl Sulphate Broth (MLSB) as a culture medium (Okotto-Okotto et al., 2015). The diluted water sample was passed through a sterile membrane filter that was placed face upwards on a MLSB-saturated pad held by a culture petri dish. After completion of vacuum-enhanced filtration, the petri dish was placed in an incubator with temperature set at 37 °C for 48 h to promote growth of bacterial colonies. The total colonies were then counted with filter gridlines being used to facilitate the enumeration of the colonies.

The concentration of NH_4^+ , NO_2^- and NO_3^- in the water solutions were determined by ion chromatography. The concentration of NH_4^+ , NO_2^- and NO_3^- in the water solutions were determined by ion chromatography (DIONEX ICS-1000). For the determination of NH_4^+ , IonPac® CS12A analytical column (4 mm×250 mm), IonPac® CG12A guard column (4 mm×50 mm), and RFIC cation *self-regenerating suppressor 300* (4 mm) were used. 20 mM methanesulphonic acid was used as mobile phase. Flow rate was set at 1.0 mL/min with 20 µL injection volume. For the measurements of NO_2^- and NO_3^- , IonPac® AS14 anion analytical column (4 mm×250 mm), IonPac® AG14 guard column (4 mm×50 mm), and ULTRA II *anion self-regenerating suppressor* (4 mm) were used. A mixed 0.8 mM sodium carbonate (Na_2CO_3) and 1 mM sodium bicarbonate solution was used as mobile phase. Flow rate was set at 1.0 mL/min with 20 µL injection volume. Various PAHs were determined using gas chromatography mass spectrometry (GC/MS) by the accredited Scientific Analysis Laboratories Ltd. (Manchester, England).

2.6 QA/QC and Statistical Analysis

Analytical grade chemicals were used for all experiments. All the experiments except for Experiment 3 were performed in triplicate. Repeatability analysis shows that the mean relative standard deviation (RSD) was less than 6% for coliforms, 9% for NH_4^+ -N, 7% for NO_2^- -N, 10% for NO_3^- -N. Statistical analysis was performed by using one-way ANOVA (SPSS17.0).

208 **3 Results**

209 **3.1 Experiments 1A-1C: Coliform Responses**

210 At the 1st min of the experiment, the concentration of coliforms was significantly ($P < 0.05$)
211 lower in all the treatments (either H₂O₂ or Fenton reagent treatments) than in the control (Fig. 1a).
212 After another 59 min. (i.e. at the 60th min of the experiment), the same trend was observed except for
213 H20, which showed no significant ($P > 0.05$) difference from the control (Fig. 1b). For the H₂O₂-only
214 treatments at the 1st min, the abundance of coliforms was significantly ($P < 0.05$) greater in the two
215 lower dosed treatments (H5 and H10) than in the two higher dosed treatments (H20 and H50) and
216 there was no significant ($P > 0.05$) difference in coliform abundance between H5 and H10 or between
217 H20 and H50 (Fig. 1a). However, at the 60th min, there was no significant ($P > 0.05$) difference in
218 coliform abundance among all the treatments (Fig.1b). At the 1st min, there was no significant
219 ($P > 0.05$) difference in coliform abundance between the H₂O₂ only treatment and the Fenton reagent
220 treatment at the same H₂O₂ dosage level except for the H20 vs F20 pair, which showed a
221 significantly ($P < 0.05$) lower concentration of coliforms in H20 than in F20 (Fig. 1a). At the 60th min,
222 there was also insignificant ($P > 0.05$) difference in coliform abundance between each of the H₂O₂ vs
223 Fenton reagent pairs except for the H20 vs F20 pair. However, the opposite was observed: F20 had
224 significantly ($P < 0.05$) lower concentration of coliforms, as compared to H20 (Fig. 1b). Overall, the
225 concentration of coliforms tended to increase from the 1st min to the 60th min (Fig. 1).

226 From Fig. 2, it is clear that, for Sample WS-EXP1B, coliform population growth continued
227 from the 1st min to the 60th min with a rapid increase being observed from the 1st min to the 60th min
228 for either the control or the treatments. On any sampling occasions during this period, the abundance
229 of coliforms was always greater in the control than in the treatments. While the concentration of
230 coliforms tended to be slightly higher in H20 than in H50, there was no significant ($P > 0.05$)
231 difference in coliform abundance between the two treatments.

232 The second addition of H₂O₂ at the 60th min did not markedly affect the concentration of
233 coliforms in H20 but significantly reduced the abundance of coliforms in H50, as measured at the
234 90th min. After the fourth addition of H₂O₂ at the 120th min, the concentration of coliforms in the
235 treatments, as measured at the 150th min, all sharply dropped and then re-increased until the end of
236 the experiment after the fifth addition of H₂O₂ at the 150 min. However, the coliform concentration
237 in the control also showed the same variation pattern during the same period with the coliform
238 abundance at all sampling occasions being consistently in the following decreasing order:
239 H50 > H20 > Ck.

240 For Sample WS-EXP1C, the concentration of coliforms was always higher in the control than in
241 the treatments, which was the same as what was observed for WS-EXP1B except that in the latter,
242 coliform abundance was slightly smaller in the control than in the treatments at the 120th min. The
243 variation pattern in coliform concentration in WS-EXP1C was markedly different from that in WS-
244 EXP1B. Within the initial 60 min, the concentration of coliforms in the control tended to decreased
245 from the 1st min to the 30th min and then increased from the 30th min to the 60th min. Unlike the
246 control, the coliform concentration in H20 maintained at a relatively stable level while H20 exhibited
247 a trend that coliform population increased over time during the same period (60 min). During the
248 period of intermittent H₂O₂ addition (i.e. from the 60th min to the 180th min of the experiment),
249 coliform population in the control showed a relatively narrow range of fluctuation. While also
250 fluctuating, the abundance of coliforms in the treatments showed a decreasing trend during the same
251 period.

252 3.2 Experiments 2A-2C

253 In Experiment 2A, there was no significant difference in ammonium-N among the control
254 and treatments on any sampling occasions (Table 2). For all the samples collected, no nitrite and
255 nitrate were detected.

256 In Experiment 2B, there was no significant difference in nitrite-N among the control and the
257 treatments at the 1st, 5th, 10th, and 20th min of the experiment. At the 30th min, significant difference
258 was observed with nitrite-N in the following decreasing order: Ck > H₂O₂ treatment group (H20 and
259 H50) > Fenton treatment group (F20 and F50). Corresponding with nitrite-N, there was an opposite
260 trend for nitrite-N at the 30th min: Ck < H₂O₂ treatment group (H20 and H50) < Fenton treatment
261 group (F20 and F50). While there was no significant difference in nitrite-N among the control and
262 the treatments at the 1st min, the concentration of nitrite-N in F50 tended to be higher than the control
263 and other treatments (at significant level $P < 0.05$ in most circumstances). For the sum of nitrite-N
264 and nitrate-N, there was no significant difference among the control and the treatments on any
265 sampling occasion except at the 30th min when the sum of nitrite-N and nitrate-N was significantly
266 higher in the control than the treatment with F50 having the lowest sum value (Table 3).

267 In Experiment 2C, there was no significant difference in NH₄⁺-N among the control and the
268 treatments at the 1st min. However, after the 5th min NH₄⁺-N was all significantly lower in the control
269 than in the treatments for any sampling occasions except at 30th min when there was no significant
270 difference in NH₄⁺-N between Ck and H20. An opposite trend was observed for the NO₃⁻-N, showing
271 that NO₃⁻-N was always higher in the control than any treatments at any sampling occasions and in
272 the latter part of the experiment (20-30 min), the Fenton treatment group (F20 and F50) even had
273 significantly lower NO₃⁻-N, as compared to the H₂O₂ treatment group (H20 and H50). Except at the
274 1st min when there was no significant difference in the sum of NH₄⁺-N and NO₃⁻-N among the
275 control and the treatments, the sum tended to be significantly lower in the control than in the
276 treatments. Overall, despite that the NH₄⁺-N in the control or each of the treatments decreased over
277 time, there was no clear trend showing the increase in NO₃⁻-N over time in the control or each of the
278 treatments (Table 4).

279 3.3 Experiment 3

Most of the investigated organic pollutants were under detection limits. The organic pollutants that were detected are shown in Table 5. Mixed results were observed in terms of the treatment effects on the toxic organic pollutants. Addition of H₂O₂ into the canal water caused a reduction in the concentration of fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene, benzo(ghi)perylene and total PAH while the concentration of naphthalene, acenaphthene, phenanthrene was lower in the control than in the treatment. Chrysene and isophorone diisocyanate showed no difference between the control and the treatment.

4 Discussion

4.1 Inactivation of Coliforms

The results obtained from Experiment 1A suggest that H₂O₂ at a concentration as low as 5 µM could inactivate coliforms in the canal water within just 1 min of contact time and such an effect lasted for at least 1 h. The similar effects on the coliform bacteria between the H₂O₂-only and Fenton reagent treatments suggest that the iron present in canal water was sufficiently effective for reacting with H₂O₂, leading to production of hydroxyl radical that significantly inactivated the coliform bacteria in the canal water.

The results obtained from Experiments 1B and 1C are generally consistent with those observed in Experiment 1A, further confirming that H₂O₂ at a concentration range encountered in the rainwater could inactivate coliform bacteria in canal water. However, the marked difference in the variation pattern of coliform population between WS-EXP1B and WS-EXP1C for the first reaction cycle (60 min) does suggest that coliform dynamics in the canal water are highly site-specific. Microbial growth is usually controlled by availability of nutrient substrate (Kovářová-Kovar, 1998; Fontaine et al., 2003). WS-EXP1B was relatively nutrient-rich, as compared to WS-EXP1C (Table 1). This may explain the growing trend of coliform population in WS-EXP1B during the earlier part of the experiment, which was not observed in WS-EXP1C. Lim and Flint (1989) showed that the

304 growth of *Escherichia coli* by enhanced nutrient supply was selective. The reasons responsible for
305 the sudden drop in coliforms from the 120th min to the 150th min and then re-increase in coliforms
306 until the end of the experiment for either the control or the treatments in Fig. 2 are not clear. Perhaps
307 this was caused by a systematic error introduced during water sample dilution or/and coliform
308 culturing. Nevertheless, like other sampling occasions, the trend showing Ck > H20 > H50 was clear.
309 Given the high complexity of the open water systems, substantial further work is required to
310 understand the actual role of H₂O₂ in complicating the dynamics of water-borne coliform bacteria.
311 The current experiment was limited to evolution of total coliforms upon addition of synthetic
312 rainwater containing H₂O₂. Future work should be focussed on specific pathogenic microbes and the
313 use of natural rainwater as a source of H₂O₂ for the experiments.

314 Nevertheless, the preliminary results obtained so far shed some light on the possible role of
315 rainwater-borne H₂O₂ in inactivating coliform bacteria and potentially pathogenic microbes in
316 stagnant water environments. It points to a potential research direction that may help to explain the
317 dynamics of harmful microbes in ambient water environments that are subject to microbial
318 contamination. It also provides fundamental knowledge that can be used to assist in assessing human
319 health risk in recreational water sites.

320 **4.2 Oxidation of Ammonia and Nitrite**

321 Ammonia can be oxidized in the presence of H₂O₂ and catalysts such as copper or titanium
322 (Mir, 2011; Sirijaraensre and Limtrakul, 2013). It was also showed that hydroxyl radical generated
323 from photochemical process using H₂O₂ (at a concentration of 10%) could effectively remove
324 ammonia from landfill leachate (Brito et al., 2010). The ineffectiveness of H₂O₂ on ammonium
325 removal (H20 and H50) observed in this study was in agreement with Mir (2011) that H₂O₂ was not
326 capable of oxidizing ammonia without mediation by a catalyst. However, the insignificant effect of
327 Fenton reagent on oxidation of ammonia in this experiment suggests that hydroxyl radical produced

328 from Fenton reaction under the current experimental conditions was not able to oxidize ammonia to a
329 significant degree. This may be attributed to its much lower dosage level ($<50\ \mu\text{M}$), as compared to
330 that ($2.9 \times 10^6\ \mu\text{M}$) in the experiment by Brito et al. (2010).

331 In contrast with ammonia, nitrite was chemically oxidized by either H_2O_2 or Fenton reagent,
332 resulting in formation of nitrate. However, this was only becoming significant after a period of 30
333 min. It is clear that Fenton reagent had much stronger capacity to drive nitrite-nitrate conversion, as
334 compared to H_2O_2 treatment only. The differential reactivity with H_2O_2 and hydroxyl radical can be
335 explained by the difference in thermodynamic stability of these two different compounds; ammonia
336 is thermodynamically more stable than does nitrite (Zumdahl and Zumdahl, 2003). The significant
337 lower sum of nitrite and nitrate in the treatments than in the control at the 30th min of the experiment
338 indicates that some of the nitrogen in the system was lost during nitrite-nitrate transformation.
339 Formation of gaseous nitrogen compounds during microbially mediated nitrification was well
340 established (Spott et al., 2011; Lam and Kuypers, 2011). However, the current work suggests
341 chemical oxidation of nitrite by H_2O_2 and hydroxyl radical could also lead to generation of gaseous
342 nitrogen species.

343 In the presence of ammonia-oxidizing and nitrifying microbes in the natural water, addition
344 of either H_2O_2 or Fenton reagent significantly impeded oxidation of ammonia. This is attributable to
345 the inhibition of ammonia-oxidizing and nitrifying microbes by hydroxyl radical. Since the natural
346 water sample contained soluble iron, Fenton reaction could take place even in the treatments with
347 H_2O_2 only. This has been demonstrated in Experiments 1A-1C.

348 Nitrite was not detected in the samples collected at any sampling occasions, which reflects
349 the fact that the nitrite consumption rate was much higher than its production rate from ammonia
350 oxidation in the reaction systems set for this study. The sum of ammonia-N and nitrate-N sharply
351 decreased from 666 mg/L at the 1st min to 353 mg/L at the 30th min in the control, indicating that

nearly half of the nitrogen escaped as gases from the system. The significant higher sum value in the treatments than in the control suggests that rainwater-borne H₂O₂ could potentially reduce emission of gaseous nitrogen species from stagnant waters by impeding microbially mediated nitrification.

4.3 Degradation of Organic Pollutants

The organic pollutants detected in the canal water are largely the constituents of coal tar, which is a by-products of coal gasification and coke production that took place in Manchester during the period of Industrial Revolution (Redford, 1956). Coal tar was also widely used by the chemical industries in Manchester during the same period (Kargon, 1977). The presence of these PAHs in the canal water despite that the coal tar-related industrial operations have been discontinued in the area for over a century suggests that the water-borne organic pollutants were of sediment sources.

The mixed results on the responses of different PAHs to H₂O₂ treatment suggest that the integrative effect of chemical and microbial processes on the organic pollutants varies from PAH type to PAH type. It appears that PAHs with simple structure (e.g. naphthalene) are more susceptible to biodegradation while others are more prone to chemical oxidation by hydroxyl radical. Overall, Fenton-driven decomposition seems to outplay biodegradation in terms of removing the PAHs from the canal water. Due to relatively limited work being performed here, a solid conclusion cannot be reached before further work is undertaken. However, the preliminary findings obtained so far do shed some light on the likely impacts of rainwater-borne H₂O₂ on the degradation of water-borne organic pollutants.

5 Conclusion

The chemical and microbial pollutants in the investigated stagnant water samples were significantly affected by the H₂O₂ in the synthetic rainwater-affected stagnant water systems. The soluble iron in the canal and urban lake waters was sufficiently effective for reaction with H₂O₂ in

the synthetic rainwater to produce hydroxyl radical, which inactivated the water-borne coliform bacteria. Significant effect on coliform inactivation was observed even at a H₂O₂ dose as low as 5 µM within just 1 min of contact time. There was a consistent trend that the inhibition of coliforms could last for at least 1 h. Repeated input of H₂O₂ at a 30 min interval allowed maintenance of microbial inhibition for at least 3 h. In the abiotic systems, H₂O₂ and hydroxyl radical was capable of chemically oxidizing nitrite but not ammonia. In the biotic system, microbially mediated nitrification was impeded in the presence of H₂O₂, which reacted with water-borne iron to trigger Fenton reaction to produce hydroxyl radical. The resulting inhibition of ammonia-oxidizing microbes reduced the removal rate of ammonium ion and the emission of gaseous nitrogen species. In the presence of H₂O₂, decomposition of water-borne PAHs appeared to be affected by both Fenton-driven chemical oxidation and impeded biodegradation. Overall, the former seemed to outplay the latter in terms of removing total PAH from the water column. The research findings obtained from this study have implications for understanding the complication of water-borne pollutant behaviour by rainwater. It points to a potential research direction that may help to explain the dynamics of water-borne pollutants in ambient water environments.

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544 **Caption**

545 Fig. 1 Abundance of coliforms at the 1st min (a) and 60th min (b) of the incubation experiment. Ck:
546 control; H5, H10, H20 and H50: addition of H₂O₂ at a concentration of 5, 10, 20 and 50 µM,
547 respectively; F5, F10, F20 and F50: addition of H₂O₂ at a concentration of 5, 10, 20 and 50
548 µM, respectively, plus a fixed concentration of Fe²⁺ at 20 µM for each treatment. All values
549 are presented as mean ± standard error (n=3) and bars with different letters indicate
550 significantly different (*P* < 0.05).

551 Fig. 2 Change in the concentration of coliforms during the entire period of the microcosm
552 experiment for the control (Ck), Treatment H20 (H₂O₂ dose at 20 µM) and Treatment H50
553 (H₂O₂ dose at 50 µM) using canal water collected from Salford Quays (SQ). All values are
554 presented as mean ± standard error (n=3).

555 Fig. 3 Change in the concentration of coliforms during the entire period of the microcosm
556 experiment for the control (Ck), Treatment H20 (H₂O₂ dose at 20 µM) and Treatment H50
557 (H₂O₂ dose at 50 µM) using canal water collected from Leeds Clarence Dock (LCD). All
558 values are presented as mean ± standard error (n=3).

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Highlights

- Stagnant water environments are subject to chemical and microbial contamination
- Microcosm experiments using canal and urban lake water as example stagnant water
- Synthetic rainwater-borne H_2O_2 reacted with water-borne Fe^{2+} to trigger Fenton reaction
- Coliforms, ammonia-oxidizing microbes and organic pollutant-degraders were inactivated
- Nitrite and PAHs can be chemically decomposed by $\cdot\text{OH}$ generated from the reaction system

1 **Potential Effects of Rainwater-borne Hydrogen Peroxide on Pollutants in Stagnant**
2 **Water Environments**

3
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ABSTRACT

Microcosm experiments were conducted to examine the effects of rainwater-borne H_2O_2 on inactivation of water-borne coliforms, oxidation of ammonia and nitrite, and degradation of organic pollutants in canal and urban lake water. The results show that the soluble iron in the investigated water samples was sufficiently effective for reaction with H_2O_2 in the simulated rainwater-affected stagnant water to produce $\bullet\text{OH}$ (Fenton reaction), which inactivated coliform bacteria even at a H_2O_2 dose as low as $5\text{ }\mu\text{M}$ within just 1 min of contact time. Coliform inhibition could last for at least 1 h and repeated input of H_2O_2 at a 30 min interval allowed maintenance of microbial inhibition for at least 3 h. Nitrification was also impeded by the Fenton process. The resulting inhibition of ammonia-oxidizing microbes reduced the removal rate of NH_4^+ and the emission of gaseous N species. In the presence of H_2O_2 at a dose of $20\text{ }\mu\text{M}$, Fenton-driven chemical oxidation appeared to outplay the impediment of biodegradation caused by inhibited microbial activities in terms of removing total polycyclic aromatic hydrocarbons from the water column. The findings point to a potential research direction that may help to explain the dynamics of water-borne pollutants in ambient water environments.

Key words: Stagnant water, coliform, ammonia, organic pollutant, rainwater

41 **1 Introduction**

42 Stagnant water environments such as lakes, ponds, canals act as sinks for organic matter and
43 various inorganic, organic and microbial contaminants (Pal et al., 2010; DeLorenzo et al., 2012;
44 Sales-Ortells et al., 2015). The organic-rich status could enhance the growth of microbial pathogens
45 (Sato et al., 2013; 2016). This poses a health risk to people who are exposed to water-borne
46 microbial pathogens e.g. during open water swimming (Wade et al., 2003; Schets et al., 2011;
47 McBride et al., 2013). Ammonia (NH_3) is a gaseous product of organic matter mineralization. In
48 non-alkaline water environments, ammonia tends to be converted into ammonium ion (NH_4^+) via a
49 reversible reaction with water molecule (Karthikeyan et al., 2008). Therefore, ammonium acts as an
50 immediate source of ammonia in the water environments. Stagnant water environments could receive
51 ammonium from agricultural, urban and industrial discharges (Erisman et al., 2007; Reche et al.,
52 2012; Wang et al., 2016). Ammonia can also be produced in-situ through in-water protein
53 metabolism by aquatic biota (Walsh and Wright, 1995). Therefore, ammonia is commonly present in
54 stagnant water environments. Due to its high toxicity to aquatic life, ammonia is viewed as one of the
55 major inorganic pollutants in aquatic ecosystems (Parker et al., 2012; Collos et al., 2014). Potentially
56 toxic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and various pesticides
57 from urban or agricultural runoffs can also enter into and accumulate in stagnant water environments
58 (Kong et al., 2005; Kanzari et al., 2014; Hijosa-Valsero et al., 2016).

59 Ammonia/ammonium can be transformed into nitrite and eventually nitrate through microbially
60 mediated nitrification (Bernhard et al., 2007; Stahl and de la Torre, 2012). It is believed that in
61 natural environments, organic pollutants are mainly degraded through microbial metabolism (Glick,
62 2003; Liste and Prutz, 2006; Khan et al., 2013). The activity of water-borne microbes in a given
63 water environment can be affected by many factors (Morens et al., 2004; Braks and de Roda Husman,
64 2013; Nguyen et al., 2016). While the effects of some environmental parameters such as pH,

65 temperature, salinity etc. on activities of water-borne microbes have been well recognized
66 (Wadowsky et al., 1985; Manas et al., 2003; Spinks et al., 2006), others are literally overlooked.

67 Hydrogen peroxide (H₂O₂) is commonly present in rainwater with a molar concentration
68 frequently ranging from 5 to 70 µM (Willey et al., 1996; Kieber et al., 2001; Gonçalves et al., 2010).
69 H₂O₂ is a strong oxidant, which can cause oxidative stress in cells (Schoonen et al., 2010; Matthijs et
70 al., 2012; Ma et al., 2013). Due to relatively anoxic and organic-rich conditions, iron compounds in
71 the benthic sediments under stagnant water layers are subject to reductive dissolution (Espana et al.,
72 2007; Anna and Jacek, 2014; Shamsul Haque et al., 2016). This could lead to release of ferrous iron
73 (Fe²⁺) into water column (Díez et al., 2007; Sanders et al., 2012; Willey et al., 2015). During rainfall
74 events, the Fe²⁺ in stagnant water can react with rainwater-borne H₂O₂ to trigger Fenton reaction, as
75 expressed in the following chemical equation:

77 Fenton reaction produces hydroxyl radical (·OH), which is an even stronger oxidant, as
78 compared to H₂O₂. Hydroxyl radical has the capacity to destroy cells and organic molecules within a
79 short period of time (Winterbourn, 1995; Lee et al., 2010). It is therefore likely that rainwater-borne
80 H₂O₂ has a role to play in affecting the activity of microbial communities in stagnant water
81 environments. Qin et al. (2013) showed that Fenton reaction triggered by H₂O₂ and Fe²⁺ (Fenton
82 reagent) at a concentration range encountered in rainwater-affected surface water was capable of
83 chemically degrading some herbicides (diuron, butachlor and glyphosate) in synthetic solutions. But
84 there have not been further tests on other organic pollutants. Brito et al. (2010) demonstrated that
85 hydroxyl radical generated from Fenton reaction could chemically oxidize ammonia in landfill
86 leachate. However, the dose of H₂O₂ they used was much higher than what can be encountered in
87 rainwater. It is likely that in natural water, Fenton reaction has a dual role to play in terms of
88 oxidizing ammonia and degrading organic pollutants; the chemical reaction of hydroxyl radical with

89 ammonia and organic pollutants may accelerate the oxidation of ammonia and decomposition of
90 organic pollutants while the oxidative stress in ammonia-oxidizing and organic pollutant-degrading
91 microbes caused by $\cdot\text{OH}$ is likely to impede ammonia oxidation and biodegradation of the organic
92 pollutants. Consequently, the net oxidation rate of ammonia and degradation rate of organic
93 pollutants by Fenton reagent is determined by the combined effects of these two counteractive
94 processes.

95 Understanding the behaviour and fate of water-borne pollutants is important for evaluating
96 aquatic ecosystem health and the risk to human exposure, which can inform development of
97 appropriate management strategies. To understand the potential role of rainwater-borne H_2O_2 in
98 affecting the behaviour and fate of some key pollutants in stagnant water environments, a multi-stage
99 research project has been set. This work reports on the results from the stage of microcosm
100 experiments with a focus on coliform bacteria, ammonia and PAHs. The aim of conducting these
101 microcosm experiments was to establish the dose-response relationships between the added H_2O_2
102 and the selected water-borne pollutants under controlled environmental conditions in order to inform
103 the design of field-based investigations in the follow-up phase of the project. The specific objectives
104 for this part of the project were to examine the effects of H_2O_2 at a concentration range that is likely
105 to be encountered in rainwater on (a) inactivation of potential microbial pathogens, as indicated by
106 coliform bacteria, (b) oxidation of ammonia and nitrite, and (c) degradation of PAHs in synthetic
107 solutions and/or selected natural stagnant water samples.

108 **2 Materials and Methods**

109 **2.1 Natural Water Samples used in the Experiment**

110 Canals and urban lakes were used as example stagnant water environments in this study. The
111 water samples were collected from the selected locations in the Bridgewater canal and Salford Quays
112 in Manchester City (Latitude: $53^\circ 28' 56.6''\text{N}$; Longitude: $2^\circ 14' 21.8''\text{W}$) and the Clarence Dock in

113 Leeds City (Latitude: 53°48'05.8"N; Longitude: 1°32'59.8"W). Both cities are located in England,
114 The United Kingdom. The Bridgewater canal is arguably the first canal in England, which was built
115 in mid-1700 to transport coal from the mine sites to the industrial areas in Manchester. Salford Quays
116 is an urban lake area regenerated from the former ship docks, which form part of the Manchester
117 Ship Canal that connected Manchester with Port of Liverpool. Part of the Salford Quays is used for
118 regular water sport activities, including swimming. The open water in Leeds Clarence Dock is an
119 urban lake surrounded by shops, office buildings and residential apartments with heavy population.
120 These water areas were heavily contaminated during the period of Industrial Revolution ([Douglas et](#)
121 [al., 2002](#); [Williams et al., 2010](#)). The benthic sediments contained substantial amounts of organic and
122 inorganic pollutants. Although pollutant loads from the upper catchment areas have been
123 significantly reduced for the past decades as a result of de-industrialization in the region, water
124 quality remains poor in the urban lakes and canal sections adjacent to urban areas due largely to
125 pollutant inputs from urban runoff during rainfall events and re-entry of bed sediment-borne
126 pollutants into water column ([Rees et al., 1999](#); [Quesada et al., 2014](#)). Water quality monitoring data
127 (our unpublished data) indicated that the canal water frequently contained elevated levels of coliform
128 bacteria, nitrogen and certain potentially toxic organic pollutants.

129 Due to the practical difficulties in satisfactorily quantifying all the intended physical, chemical
130 and microbial parameters in a single experiment, three sets of separate experiments were conducted
131 to individually examine the effects of H₂O₂ in synthetic rainwater-affected stagnant water on the
132 inhibition of coliform bacteria, oxidation of ammonia and nitrite, and degradation of organic
133 pollutants. Some major physical and chemical characteristics of the five water samples used in the
134 experiments are given in Table 1. The water samples WS-EXP1A, WS-EXP1B and WS-EXP1C used
135 for Experiment 1A-1C (coliform experiments) were collected from the Bridgewater canal, Salford
136 Quays and Leeds Clarence Dock, respectively. The sample WS-EXP2C used for Experiment 2C

137 (ammonia experiment) was taken from Salford Quays. The sample WS-EXP3 used for Experiment 3
138 (organic pollutants) was collected from Bridgewater canal.

139 **2.2 Experiments 1A-1C: Inhibition of Coliforms in Natural Water**

140 Experiments 1A was to test whether the iron present in the canal water was effective for
141 triggering Fenton reaction upon addition of H_2O_2 at a concentration range of 0-50 μM , which is
142 frequently encountered in rainwater (Willey et al., 1996; Kieber et al., 2001; Gonçalves et al., 2010).
143 One control and eight treatments were set and the details of experimental set-up are given in
144 Supplementary Table S1.

145 Experiments 1B and 1C were conducted to observe temporal variation in abundance of
146 coliforms in canal water upon repeated addition of H_2O_2 . To minimize the workload, only two H_2O_2
147 doses were used: (a) 20 μM , and (b) 50 μM . Therefore, there were one control (Ck) and four
148 treatments being set in a way exactly the same as H20, H50, F20, and F50 in Experiment 1A. The
149 experiment lasted for 3 h with an appropriate amount of H_2O_2 being injected into the canal water at
150 the 60th, 90th, 120th, and 150th min following initial addition of H_2O_2 . The amount of H_2O_2 added
151 each time was to achieve a theoretical concentration of H_2O_2 equivalent to what was set for each
152 treatment at the time of addition, assuming that the H_2O_2 from previous addition was completely
153 consumed. During the first stage of the experiment (60 min), samples were taken at 1st, 10th, 30th and
154 60th min before the second addition of H_2O_2 . This was to more closely observe the variation in
155 coliform population over time upon a single addition of H_2O_2 during a period of 1 h. The subsequent
156 addition of H_2O_2 at a 30-min interval was to simulate a scenario in which the canal water received
157 H_2O_2 from intermittent rainfall. Following each additional H_2O_2 addition, a water sample was taken
158 immediately before the next H_2O_2 addition (i.e. every 30 min).

159 Plastic bottles with a capacity of 125 mL were used as batch reactors. Upon addition of H_2O_2
160 and for Treatments 5-8, Fe^{2+} as well, an aliquot (2 mL) of the canal water was taken from each

reactor twice; one after 1 min of reaction and the other one at the end of the experiment (60 min).
The water sample was immediately used for determination of the concentration of coliforms.

2.3 Experiments 2A-2C: Oxidation of Ammonia in Synthetic Solutions and Natural Water

Experiments 2A and 2B were to observe the reaction of ammonia or nitrate, respectively, with H_2O_2 or Fenton reagent in abiotic system in order to understand whether water-borne ammonia and nitrate can be chemically oxidized by either H_2O_2 or Fenton Reagent. Synthetic NH_4^+ and NO_2^- solutions were prepared using NH_4Cl and NaNO_3 , respectively. The concentration of synthetic NH_4^+ solution was fixed at 556 μM of NH_4^+ -N and the concentration of synthetic NO_2^- solution was set at 217 μM of NO_2^- -N. For Experiment 2C, natural canal water (WS-EXP2C) was used with an appropriate amount of NH_4Cl (equivalent to 556 μM of NH_4^+ -N, as set for Experiment 2A) being added to the canal water sample to simulate an ammonia input from runoff. For all the three experiments, one control and four treatments were set: (a) control: solution only; (b) H_2O_2 at a dose of 20 μM ; (c) H_2O_2 at a dose of 50 μM ; (d) H_2O_2 at a dose of 20 μM plus Fe^{2+} at a dose of 20 μM ; (e) H_2O_2 at a dose of 50 μM plus Fe^{2+} at a dose of 20 μM .

Plastic bottles with a capacity of 1 L were used as batch reactors. Upon addition of H_2O_2 or Fenton reagent, an aliquot (10 mL) of the solution was taken from each reactor at the following times: 1, 5, 10, 20 and 30 min. After collection, the water samples were stored at -25 °C prior to analysis.

2.4 Experiment 3: Degradation of Organic Pollutants in Natural Water

This experiment was to test the potential effects of H_2O_2 at a dose of 20 μM on a range of potentially toxic organic pollutants. An appropriate amount of H_2O_2 was added to 1 L of the water sample to obtain the set treatment dose. The original sample (without added H_2O_2) was used as the control.

2.5 Analytical Methods

The concentration of coliforms in the water samples was determined by a membrane filtration method using Oxoid Membrane Lauryl Sulphate Broth (MLSB) as a culture medium (Okotto-Okotto et al., 2015). The diluted water sample was passed through a sterile membrane filter that was placed face upwards on a MLSB-saturated pad held by a culture petri dish. After completion of vacuum-enhanced filtration, the petri dish was placed in an incubator with temperature set at 37 °C for 48 h to promote growth of bacterial colonies. The total colonies were then counted with filter gridlines being used to facilitate the enumeration of the colonies.

The concentration of NH_4^+ , NO_2^- and NO_3^- in the water solutions were determined by ion chromatography. The concentration of NH_4^+ , NO_2^- and NO_3^- in the water solutions were determined by ion chromatography (DIONEX ICS-1000). For the determination of NH_4^+ , IonPac® CS12A analytical column (4 mm×250 mm), IonPac® CG12A guard column (4 mm×50 mm), and RFIC cation *self-regenerating suppressor 300* (4 mm) were used. 20 mM methanesulphonic acid was used as mobile phase. Flow rate was set at 1.0 mL/min with 20 µL injection volume. For the measurements of NO_2^- and NO_3^- , IonPac® AS14 anion analytical column (4 mm×250 mm), IonPac® AG14 guard column (4 mm×50 mm), and ULTRA II *anion self-regenerating suppressor* (4 mm) were used. A mixed 0.8 mM sodium carbonate (Na_2CO_3) and 1 mM sodium bicarbonate solution was used as mobile phase. Flow rate was set at 1.0 mL/min with 20 µL injection volume. Various PAHs were determined using gas chromatography mass spectrometry (GC/MS) by the accredited Scientific Analysis Laboratories Ltd. (Manchester, England).

2.6 QA/QC and Statistical Analysis

Analytical grade chemicals were used for all experiments. All the experiments except for Experiment 3 were performed in triplicate. Repeatability analysis shows that the mean relative standard deviation (RSD) was less than 6% for coliforms, 9% for NH_4^+ -N, 7% for NO_2^- -N, 10% for NO_3^- -N. Statistical analysis was performed by using one-way ANOVA (SPSS17.0).

208 **3 Results**

209 **3.1 Experiments 1A-1C: Coliform Responses**

210 At the 1st min of the experiment, the concentration of coliforms was significantly ($P < 0.05$)
211 lower in all the treatments (either H₂O₂ or Fenton reagent treatments) than in the control (Fig. 1a).
212 After another 59 min. (i.e. at the 60th min of the experiment), the same trend was observed except for
213 H20, which showed no significant ($P > 0.05$) difference from the control (Fig. 1b). For the H₂O₂-only
214 treatments at the 1st min, the abundance of coliforms was significantly ($P < 0.05$) greater in the two
215 lower dosed treatments (H5 and H10) than in the two higher dosed treatments (H20 and H50) and
216 there was no significant ($P > 0.05$) difference in coliform abundance between H5 and H10 or between
217 H20 and H50 (Fig. 1a). However, at the 60th min, there was no significant ($P > 0.05$) difference in
218 coliform abundance among all the treatments (Fig.1b). At the 1st min, there was no significant
219 ($P > 0.05$) difference in coliform abundance between the H₂O₂ only treatment and the Fenton reagent
220 treatment at the same H₂O₂ dosage level except for the H20 vs F20 pair, which showed a
221 significantly ($P < 0.05$) lower concentration of coliforms in H20 than in F20 (Fig. 1a). At the 60th min,
222 there was also insignificant ($P > 0.05$) difference in coliform abundance between each of the H₂O₂ vs
223 Fenton reagent pairs except for the H20 vs F20 pair. However, the opposite was observed: F20 had
224 significantly ($P < 0.05$) lower concentration of coliforms, as compared to H20 (Fig. 1b). Overall, the
225 concentration of coliforms tended to increase from the 1st min to the 60th min (Fig. 1).

226 From Fig. 2, it is clear that, for Sample WS-EXP1B, coliform population growth continued
227 from the 1st min to the 60th min with a rapid increase being observed from the 1st min to the 60th min
228 for either the control or the treatments. On any sampling occasions during this period, the abundance
229 of coliforms was always greater in the control than in the treatments. While the concentration of
230 coliforms tended to be slightly higher in H20 than in H50, there was no significant ($P > 0.05$)
231 difference in coliform abundance between the two treatments.

232 The second addition of H₂O₂ at the 60th min did not markedly affect the concentration of
233 coliforms in H20 but significantly reduced the abundance of coliforms in H50, as measured at the
234 90th min. After the fourth addition of H₂O₂ at the 120th min, the concentration of coliforms in the
235 treatments, as measured at the 150th min, all sharply dropped and then re-increased until the end of
236 the experiment after the fifth addition of H₂O₂ at the 150 min. However, the coliform concentration
237 in the control also showed the same variation pattern during the same period with the coliform
238 abundance at all sampling occasions being consistently in the following decreasing order:
239 H50 > H20 > Ck.

240 For Sample WS-EXP1C, the concentration of coliforms was always higher in the control than in
241 the treatments, which was the same as what was observed for WS-EXP1B except that in the latter,
242 coliform abundance was slightly smaller in the control than in the treatments at the 120th min. The
243 variation pattern in coliform concentration in WS-EXP1C was markedly different from that in WS-
244 EXP1B. Within the initial 60 min, the concentration of coliforms in the control tended to decreased
245 from the 1st min to the 30th min and then increased from the 30th min to the 60th min. Unlike the
246 control, the coliform concentration in H20 maintained at a relatively stable level while H20 exhibited
247 a trend that coliform population increased over time during the same period (60 min). During the
248 period of intermittent H₂O₂ addition (i.e. from the 60th min to the 180th min of the experiment),
249 coliform population in the control showed a relatively narrow range of fluctuation. While also
250 fluctuating, the abundance of coliforms in the treatments showed a decreasing trend during the same
251 period.

252 3.2 Experiments 2A-2C

253 In Experiment 2A, there was no significant difference in ammonium-N among the control
254 and treatments on any sampling occasions (Table 2). For all the samples collected, no nitrite and
255 nitrate were detected.

256 In Experiment 2B, there was no significant difference in nitrite-N among the control and the
257 treatments at the 1st, 5th, 10th, and 20th min of the experiment. At the 30th min, significant difference
258 was observed with nitrite-N in the following decreasing order: Ck > H₂O₂ treatment group (H20 and
259 H50) > Fenton treatment group (F20 and F50). Corresponding with nitrite-N, there was an opposite
260 trend for nitrite-N at the 30th min: Ck < H₂O₂ treatment group (H20 and H50) < Fenton treatment
261 group (F20 and F50). While there was no significant difference in nitrite-N among the control and
262 the treatments at the 1st min, the concentration of nitrite-N in F50 tended to be higher than the control
263 and other treatments (at significant level $P < 0.05$ in most circumstances). For the sum of nitrite-N
264 and nitrate-N, there was no significant difference among the control and the treatments on any
265 sampling occasion except at the 30th min when the sum of nitrite-N and nitrate-N was significantly
266 higher in the control than the treatment with F50 having the lowest sum value (Table 3).

267 In Experiment 2C, there was no significant difference in NH₄⁺-N among the control and the
268 treatments at the 1st min. However, after the 5th min NH₄⁺-N was all significantly lower in the control
269 than in the treatments for any sampling occasions except at 30th min when there was no significant
270 difference in NH₄⁺-N between Ck and H20. An opposite trend was observed for the NO₃⁻-N, showing
271 that NO₃⁻-N was always higher in the control than any treatments at any sampling occasions and in
272 the latter part of the experiment (20-30 min), the Fenton treatment group (F20 and F50) even had
273 significantly lower NO₃⁻-N, as compared to the H₂O₂ treatment group (H20 and H50). Except at the
274 1st min when there was no significant difference in the sum of NH₄⁺-N and NO₃⁻-N among the
275 control and the treatments, the sum tended to be significantly lower in the control than in the
276 treatments. Overall, despite that the NH₄⁺-N in the control or each of the treatments decreased over
277 time, there was no clear trend showing the increase in NO₃⁻-N over time in the control or each of the
278 treatments (Table 4).

279 3.3 Experiment 3

Most of the investigated organic pollutants were under detection limits. The organic pollutants that were detected are shown in Table 5. Mixed results were observed in terms of the treatment effects on the toxic organic pollutants. Addition of H₂O₂ into the canal water caused a reduction in the concentration of fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene, benzo(ghi)perylene and total PAH while the concentration of naphthalene, acenaphthene, phenanthrene was lower in the control than in the treatment. Chrysene and isophorone diisocyanate showed no difference between the control and the treatment.

4 Discussion

4.1 Inactivation of Coliforms

The results obtained from Experiment 1A suggest that H₂O₂ at a concentration as low as 5 µM could inactivate coliforms in the canal water within just 1 min of contact time and such an effect lasted for at least 1 h. The similar effects on the coliform bacteria between the H₂O₂-only and Fenton reagent treatments suggest that the iron present in canal water was sufficiently effective for reacting with H₂O₂, leading to production of hydroxyl radical that significantly inactivated the coliform bacteria in the canal water.

The results obtained from Experiments 1B and 1C are generally consistent with those observed in Experiment 1A, further confirming that H₂O₂ at a concentration range encountered in the rainwater could inactivate coliform bacteria in canal water. However, the marked difference in the variation pattern of coliform population between WS-EXP1B and WS-EXP1C for the first reaction cycle (60 min) does suggest that coliform dynamics in the canal water are highly site-specific. Microbial growth is usually controlled by availability of nutrient substrate (Kovárová-Kovar, 1998; Fontaine et al., 2003). WS-EXP1B was relatively nutrient-rich, as compared to WS-EXP1C (Table 1). This may explain the growing trend of coliform population in WS-EXP1B during the earlier part of the experiment, which was not observed in WS-EXP1C. Lim and Flint (1989) showed that the

304 growth of *Escherichia coli* by enhanced nutrient supply was selective. The reasons responsible for
305 the sudden drop in coliforms from the 120th min to the 150th min and then re-increase in coliforms
306 until the end of the experiment for either the control or the treatments in Fig. 2 are not clear. Perhaps
307 this was caused by a systematic error introduced during water sample dilution or/and coliform
308 culturing. Nevertheless, like other sampling occasions, the trend showing Ck > H20 > H50 was clear.
309 Given the high complexity of the open water systems, substantial further work is required to
310 understand the actual role of H₂O₂ in complicating the dynamics of water-borne coliform bacteria.
311 The current experiment was limited to evolution of total coliforms upon addition of synthetic
312 rainwater containing H₂O₂. Future work should be focussed on specific pathogenic microbes and the
313 use of natural rainwater as a source of H₂O₂ for the experiments.

314 Nevertheless, the preliminary results obtained so far shed some light on the possible role of
315 rainwater-borne H₂O₂ in inactivating coliform bacteria and potentially pathogenic microbes in
316 stagnant water environments. It points to a potential research direction that may help to explain the
317 dynamics of harmful microbes in ambient water environments that are subject to microbial
318 contamination. It also provides fundamental knowledge that can be used to assist in assessing human
319 health risk in recreational water sites.

320 **4.2 Oxidation of Ammonia and Nitrite**

321 Ammonia can be oxidized in the presence of H₂O₂ and catalysts such as copper or titanium
322 (Mir, 2011; Sirijaraensre and Limtrakul, 2013). It was also showed that hydroxyl radical generated
323 from photochemical process using H₂O₂ (at a concentration of 10%) could effectively remove
324 ammonia from landfill leachate (Brito et al., 2010). The ineffectiveness of H₂O₂ on ammonium
325 removal (H20 and H50) observed in this study was in agreement with Mir (2011) that H₂O₂ was not
326 capable of oxidizing ammonia without mediation by a catalyst. However, the insignificant effect of
327 Fenton reagent on oxidation of ammonia in this experiment suggests that hydroxyl radical produced

328 from Fenton reaction under the current experimental conditions was not able to oxidize ammonia to a
329 significant degree. This may be attributed to its much lower dosage level ($<50\ \mu\text{M}$), as compared to
330 that ($2.9 \times 10^6\ \mu\text{M}$) in the experiment by Brito et al. (2010).

331 In contrast with ammonia, nitrite was chemically oxidized by either H_2O_2 or Fenton reagent,
332 resulting in formation of nitrate. However, this was only becoming significant after a period of 30
333 min. It is clear that Fenton reagent had much stronger capacity to drive nitrite-nitrate conversion, as
334 compared to H_2O_2 treatment only. The differential reactivity with H_2O_2 and hydroxyl radical can be
335 explained by the difference in thermodynamic stability of these two different compounds; ammonia
336 is thermodynamically more stable than does nitrite (Zumdahl and Zumdahl, 2003). The significant
337 lower sum of nitrite and nitrate in the treatments than in the control at the 30th min of the experiment
338 indicates that some of the nitrogen in the system was lost during nitrite-nitrate transformation.
339 Formation of gaseous nitrogen compounds during microbially mediated nitrification was well
340 established (Spott et al., 2011; Lam and Kuypers, 2011). However, the current work suggests
341 chemical oxidation of nitrite by H_2O_2 and hydroxyl radical could also lead to generation of gaseous
342 nitrogen species.

343 In the presence of ammonia-oxidizing and nitrifying microbes in the natural water, addition
344 of either H_2O_2 or Fenton reagent significantly impeded oxidation of ammonia. This is attributable to
345 the inhibition of ammonia-oxidizing and nitrifying microbes by hydroxyl radical. Since the natural
346 water sample contained soluble iron, Fenton reaction could take place even in the treatments with
347 H_2O_2 only. This has been demonstrated in Experiments 1A-1C.

348 Nitrite was not detected in the samples collected at any sampling occasions, which reflects
349 the fact that the nitrite consumption rate was much higher than its production rate from ammonia
350 oxidation in the reaction systems set for this study. The sum of ammonia-N and nitrate-N sharply
351 decreased from 666 mg/L at the 1st min to 353 mg/L at the 30th min in the control, indicating that

nearly half of the nitrogen escaped as gases from the system. The significant higher sum value in the treatments than in the control suggests that rainwater-borne H_2O_2 could potentially reduce emission of gaseous nitrogen species from stagnant waters by impeding microbially mediated nitrification.

4.3 Degradation of Organic Pollutants

The organic pollutants detected in the canal water are largely the constituents of coal tar, which is a by-products of coal gasification and coke production that took place in Manchester during the period of Industrial Revolution (Redford, 1956). Coal tar was also widely used by the chemical industries in Manchester during the same period (Kargon, 1977). The presence of these PAHs in the canal water despite that the coal tar-related industrial operations have been discontinued in the area for over a century suggests that the water-borne organic pollutants were of sediment sources.

The mixed results on the responses of different PAHs to H_2O_2 treatment suggest that the integrative effect of chemical and microbial processes on the organic pollutants varies from PAH type to PAH type. It appears that PAHs with simple structure (e.g. naphthalene) are more susceptible to biodegradation while others are more prone to chemical oxidation by hydroxyl radical. Overall, Fenton-driven decomposition seems to outplay biodegradation in terms of removing the PAHs from the canal water. Due to relatively limited work being performed here, a solid conclusion cannot be reached before further work is undertaken. However, the preliminary findings obtained so far do shed some light on the likely impacts of rainwater-borne H_2O_2 on the degradation of water-borne organic pollutants.

5 Conclusion

The chemical and microbial pollutants in the investigated stagnant water samples were significantly affected by the H_2O_2 in the synthetic rainwater-affected stagnant water systems. The soluble iron in the canal and urban lake waters was sufficiently effective for reaction with H_2O_2 in

the synthetic rainwater to produce hydroxyl radical, which inactivated the water-borne coliform bacteria. Significant effect on coliform inactivation was observed even at a H₂O₂ dose as low as 5 µM within just 1 min of contact time. There was a consistent trend that the inhibition of coliforms could last for at least 1 h. Repeated input of H₂O₂ at a 30 min interval allowed maintenance of microbial inhibition for at least 3 h. In the abiotic systems, H₂O₂ and hydroxyl radical was capable of chemically oxidizing nitrite but not ammonia. In the biotic system, microbially mediated nitrification was impeded in the presence of H₂O₂, which reacted with water-borne iron to trigger Fenton reaction to produce hydroxyl radical. The resulting inhibition of ammonia-oxidizing microbes reduced the removal rate of ammonium ion and the emission of gaseous nitrogen species. In the presence of H₂O₂, decomposition of water-borne PAHs appeared to be affected by both Fenton-driven chemical oxidation and impeded biodegradation. Overall, the former seemed to outplay the latter in terms of removing total PAH from the water column. The research findings obtained from this study have implications for understanding the complication of water-borne pollutant behaviour by rainwater. It points to a potential research direction that may help to explain the dynamics of water-borne pollutants in ambient water environments.

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Caption

Fig. 1 Abundance of coliforms at the 1st min (a) and 60th min (b) of the incubation experiment. Ck: control; H5, H10, H20 and H50: addition of H₂O₂ at a concentration of 5, 10, 20 and 50 µM, respectively; F5, F10, F20 and F50: addition of H₂O₂ at a concentration of 5, 10, 20 and 50 µM, respectively, plus a fixed concentration of Fe²⁺ at 20 µM for each treatment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly different ($P < 0.05$).

Fig. 2 Change in the concentration of coliforms during the entire period of the microcosm experiment for the control (Ck), Treatment H20 (H₂O₂ dose at 20 µM) and Treatment H50 (H₂O₂ dose at 50 µM) using canal water collected from Salford Quays (SQ). All values are presented as mean ± standard error (n=3).

Fig. 3 Change in the concentration of coliforms during the entire period of the microcosm experiment for the control (Ck), Treatment H20 (H₂O₂ dose at 20 µM) and Treatment H50 (H₂O₂ dose at 50 µM) using canal water collected from Leeds Clarence Dock (LCD). All values are presented as mean ± standard error (n=3).

Table 1 Some major physical and chemical characteristics of the three canal water samples used in the microcosm experiments

Parameter	WS-EXP1A	WS-EXP1B	WS-EXP1C	WS-EXP2C	WS-EXP3	Method used
Weather conditions	Sunny	Sunny	Sunny	Sunny	Cloudy	
Temperature (°C)	16.1	16.7	17.2	16.9	16.6	DO meter
pH	7.38	7.55	7.49	7.45	7.66	pH meter
EC (µs/cm)	224	248	232	253	228	EC meter
DO (mg/L)	6.77	8.70	7.60	8.60	7.80	DO meter
Ca ²⁺ (mg/L)	22.2	25.6	23.3	21.7	22.5	IC
K ⁺ (mg/L)	2.55	5.92	8.76	6.02	3.20	IC
Mg ²⁺ (mg/L)	3.30	4.08	3.50	4.10	3.83	IC
NH ₄ ⁺ (mg/L)	2.03	3.62	2.16	1.08	2.63	IC
Cl ⁻ (mg/L)	28.4	29.6	27.6	28.3	27.4	IC
NO ₃ ⁻ (mg/L)	6.02	8.05	5.75	6.29	6.35	IC
SO ₄ ²⁻ (mg/L)	20.7	22.4	19.5	21.7	22.9	IC
PO ₄ ³⁻ (mg/L)	0.12	0.75	0.25	0.62	0.14	IC
Fe (mg/L)	0.40	0.63	0.22	0.67	0.39	ICP-OES

Table 2 Concentrations (μM) of ammonia-N in the synthetic solution for the control and the treatments during the period of the incubation experiment

Treatment	1 min	30 min	60 min
Ck	566±1.4a	550±4.1a	546±6.3a
H20	565±1.7a	532±7.1a	535±8.5a
H50	562±2.0a	543±19.3a	540±20.5a
F20	565±2.2a	538±1.7a	536±2.1a
F50	566±4.8a	538±12.9a	535±11.5a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column are significantly different (*P* <0.05). Same in Tables 4 and 5.

Table 3 Concentrations of nitrite-N and nitrate-N in the synthetic solution for the control and the treatments during the period of the incubation experiment

Sampling time	Code	NO ₂ ⁻ -N (μM)	NO ₃ ⁻ -N (μM)	Sum (μM)
1 min	Ck	203±8.67a	23.3±4.00a	226±4.68a
	H20	207±6.83a	18.6±2.02a	226±5.74a
	H50	192±2.18a	22.7±2.30a	215±3.03a
	F20	204±8.49a	27.5±1.79a	232±7.05a
	F50	197±6.19a	32.4±8.25a	229±12.5a
5 min	Ck	192±3.28a	17.3±2.17ab	209±5.35a
	H20	198±1.19a	12.8±0.79b	211±1.73a
	H50	198±6.88a	14.1±1.42b	212±5.78a
	F20	193±2.37a	17.2±4.59ab	210±2.51a
	F50	193±3.85a	24.2±1.11a	217±4.95a
10 min	Ck	204±5.68a	13.0±1.68b	217±5.23a
	H20	198±2.63a	19.81±5.40ab	217±5.52a
	H50	196±4.44a	14.3±0.29b	210±4.44a
	F20	197±5.89a	19.7±0.89ab	216±6.67a
	F50	192±5.61a	25.5±2.98a	217±8.06a
20 min	Ck	193±2.09a	18.1±1.66ab	211±0.44a
	H20	200±4.24a	19.6±2.55ab	219±6.51a
	H50	197±1.66a	15.7±1.51b	212±3.12a
	F20	192±1.33a	18.8±1.33ab	211±0.91a
	F50	194±6.27a	23.2±2.64a	218±3.96a
30 min	Ck	198±2.23a	22.9±1.33c	221±1.03a
	H20	138±10.5b	47.5±2.88b	186±12.4bc
	H50	149±5.26b	45.0±4.48b	193±0.84b
	F20	57.7±4.19c	131.±6.89a	189±5.90bc
	F50	41.8±1.26c	126±3.75a	168±4.80c

Table 4 Concentrations of nitrite-N and nitrate-N in the urban lake water for the control and the treatments during the period of the incubation experiment

Time	Code	NH ₄ ⁺ -N (μM)	NO ₃ ⁻ -N (μM)	Sum (μM)
1 min	Ck	550±28.5a	115±3.40a	665±28.3a
	H20	553±22.4a	91.5±2.89b	645±24.8a
	H50	545±5.78a	68.1±4.86c	613±5.65a
	F20	564±4.42a	82.4±8.87bc	647±13.2a
	F50	543±10.7a	76.8±2.68bc	620±8.05a
5 min	Ck	430±4.64c	99.9±3.54a	530±8.10c
	H20	514±4.10b	72.8±4.19c	587±0.76b
	H50	527±8.91b	84.7±4.85b	612±12.2a
	F20	559±7.17a	67.3±2.10c	626±8.16a
	F50	563±2.22a	73.6±1.75c	636±3.21a
10 min	Ck	377±19.0c	81.9±2.55a	459±19.9b
	H20	486±1.80ab	55.6±5.76b	542±4.12a
	H50	472±5.61b	53.8±4.88b	526±5.92a
	F20	508±4.14a	51.6±3.57b	559±6.87a
	F50	509±5.61a	51.0±2.13b	560±3.89a
20 min	Ck	334±8.91c	92.4±5.23a	426±5.51b
	H20	385±12.4b	72.5±7.08b	457±18.0ab
	H50	399±15.4b	69.3±1.97b	468±17.3ab
	F20	401±6.13b	54.3±2.03c	455±6.07ab
	F50	438±7.92a	51.2±2.76c	489±8.49a
30 min	Ck	255±9.72c	97.7±1.36a	353±11.0c
	H20	277±3.40c	89.1±0.34b	366±3.72c
	H50	324±7.82b	79.5±4.56c	403±8.81b
	F20	352±11.0ab	69.8±0.94d	422±11.9ab
	F50	378±17.9a	66.6±1.27d	445±16.7a

Table 5 Concentration (ng/L) of various toxic organic pollutants detected in the control or/and the treatment

Types of organic pollutant	Ck	Treatment
Naphthalene	N.D.	30
Acenaphthene	N.D.	20
Phenanthrene	10	20
Fluoranthene	30	20
Benzo(a)anthracene	10	N.D.
Chrysene	20	20
Benzo(b)fluoranthene	40	20
Benzo(a)pyrene	20	N.D.
Indeno(123-cd)pyrene	20	N.D.
Benzo(ghi)perylene	30	10
Isophorone diisocyanate	60	60
Total PAH	190	160

Fig. 1

Fig. 2

Fig. 3

Supplementary Material

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