Spatial and temporal variations and factors controlling the concentrations of hydrogen peroxide and organic peroxides in rivers

by

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Environmental context. Hydrogen peroxide (H_2O_2) and organic peroxides (ROOH) are ubiquitously present in natural waters and primarily essential for a number of redox reactions. This study examined the effects of various dissolved organic substances on the formation of H_2O_2 and ROOH and their relationships with water quality parameters in two Japanese rivers. This study suggests that fulvic acid is primarily responsible for production of H_2O_2 and ROOH in river waters.

Abstract

Hydrogen peroxide (H_2O_2) and organic peroxides (ROOH) were examined in water samples collected from the upstream and downstream sites of two Japanese rivers (the Kurose and the Ohta). H_2O_2 concentrations during monthly measurements varied 6-213 nM in the Kurose river and 33-188 nM in the Ohta river. ROOH varied 0-73 nM in the Kurose river and 1-80 nM in the Ohta. Concentrations of peroxides were higher during the summer months as compared to those in winter. H_2O_2 concentrations correlated well with the measured content of dissolved organic carbon and/or fluorescence intensity of the fluorescent dissolved organic matter (FDOM) in the water from these rivers, suggesting that the dissolved organic matter and FDOM are the major sources of H_2O_2. Further characterization of FDOM components by 3-D excitation emission matrix spectroscopy indicated that fulvic acid is a dominant source of H_2O_2 in river waters, which accounted for 23-70% of H_2O_2 production in the Ohta river, 25-61% in the upstream and 28-63% in the downstream waters of the Kurose river, respectively. Fluorescent whitening agents and its photoproduct (4-Biphenyl carboxaldehyde) together contributed 3-7% of H_2O_2 production in the downstream waters of the Kurose river. Tryptophan-like substances were a minor source of H_2O_2 (<1%) in both rivers. An increase in the H_2O_2 concentration was observed in the diurnal samples collected at noon compared to the samples collected during the period before sunrise and after sunset, thus indicating that H_2O_2 was produced photochemically. This study demonstrates that H_2O_2 is produced mainly from the photodegradation of FDOMs, such as fulvic acid, whereas the production mechanism of ROOH needs further clarifications.

Additional Keywords: Hydrogen peroxide; organic peroxides; dissolved organic carbon; fluorescent dissolved organic matter; upstream and downstream rivers.

Introduction

H_2O_2 is frequently present in natural waters and is an indicator of photochemical[1,2] and biological[3,4] processes of aquatic matters. Surface concentrations of H_2O_2 typically varies in natural waters from 20-320 nM in rivers[5,6], 10-800 nM in lakes[3,5,7], 0-1700 nM in coastal waters[1,8,9], and 10-300 in oligotrophic waters[1,10]. It has been reported that H_2O_2 may be produced as a final product through a chain reaction of chromophoric or colored dissolved organic matter (CDOM) with dissolved oxygen under natural sunlight (eqs 1-4)[1,11-13].

$$DOM + h\upsilon \longrightarrow DOM^* \quad \text{.....(1)}$$

$$DOM^* + O_2 \longrightarrow DOM^{\cdot+} + O_2^{\cdot-} \quad \text{.....(2)}$$

$$O_2^{\cdot-} + H^+ \longrightarrow HO_2^- + O_2^{\cdot-} \longrightarrow HO_2^- + O_2 \quad \text{.....(3)}$$

$$HO_2^- + O_2 + H_2O \longrightarrow H_2O_2 + O_2 \quad \text{.....(4)}$$

Humic substances (fulvic and humic acids), CDOM or FDOM are primarily susceptible to photochemical absorption of photons from natural sunlight.

A few studies have previously conducted by our group and others to detect organic peroxides (ROOH) in natural waters. Their concentrations were determined to vary from 33 to 200 nM in water samples from six rivers in Hiroshima prefecture[6] and from 1 to 389 nM in seawater[2,6,9]. Determinations of the concentrations of ROOH and H_2O_2 may be crucial for improving the understanding of the photochemical processes occurring in the water, particularly in freshwater ecosystems. Previous work revealed that ROOH may be formed (eqs 5-10) by the photodegradation of DOM (FDOM or CDOM) following the formation of H_2O_2 in the aquatic environment [14-16].

$$H_2O_2 + h\nu \longrightarrow 2\,{}^{\bullet}OH \quad \text{.....(5)}$$

$$DOM^{+} + {}^{\bullet}OH \longrightarrow R^{\bullet}/RO_2^{\bullet} + \text{products} \quad \text{.....(6)}$$

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet} \quad \text{. ..... (7)}$$

$$RO_2^{\bullet} + H^{+} \longrightarrow ROOH \quad \text{.....(8)}$$

$$RO_2^{\bullet} + R^{\bullet} \longrightarrow ROOR \quad \text{.....(9)}$$

$$RO_2^{\bullet} + RO_2^{\bullet} \longrightarrow ROOR + O_2 \quad \text{.....(10)}$$

First, the photodecomposition of H_2O_2 may generate ${}^{\bullet}OH$ (eq 5), which subsequently oxidizes DOM^{+} to form an organic radical $R^{\bullet}$ and/or an organo peroxide radical $RO_2^{\bullet}$ (eq 6). Secondly, organic radical ($R^{\bullet}$) may react with molecular O_2 to form the organo peroxide radical ($RO_2^{\bullet}$) (eq 7). $RO_2^{\bullet}$ may then combine with a proton (H^{+}) to form ROOH in natural waters (eq 8). Organic radicals ($R^{\bullet}$ and $RO_2^{\bullet}$) can rapidly associate with each other (eq 9) and organo peroxide radicals can combine (eq 10) to terminate the chain reactions. Terminated reactions (eqs 9-10) are competitive with reactions in eqs 7-8, which lead to complicated reaction kinetics [16].

Production of ROOH could be a marker of microbial changes in bulk DOM under dark conditions [9,17]. It can be noted that chromophores in CDOM or fluorophores in FDOM are considered to be equivalent components with respect to photosensitization due to solar radiation [12,18,19]. However, the fluorophores in FDOM are clearly distinguishable by their excitation-emission (Ex/Em) wavelengths in fluorescence spectra [20-22], whilst it is not possible to identify chromophores in CDOM in absorption spectra[19,23]. The excitation emission matrix spectroscopy (EEMS) of samples collected from freshwater has identified three characteristic peaks, indicating the presence of fulvic acid-like substances (peak C), fluorescent whitening agents (FWAs) (peak W),

and tryptophan-like substances (peak T)[20,24,25]. The fluorescence peak positions are at Ex/Em = 325-340/450-475 nm for standard fulvic acid (peak C), 350/436 nm for standard distyryl biphenyl, DSBP (peak W), 335-355/438-449 nm for standard diaminostilbene type, DAS1 (peak W), 275-280/342-356 nm for standard tryptophan, and 305/410 nm for 4-biphenyl carboxaldehyde, 4BCA (this study) in aqueous solutions[19,22,24]. The components of FWAs (DSBP and DAS1) are frequently detected in water samples from other rivers in Japan, USA, and Europe[24]. It has been revealed that fulvic acid is the dominant fraction (40-80%) in riverine DOM[26], as well as the fulvic acid along with FWAs is photochemically reactive[24,27,28]. Therefore, these substances can play a significant role in the production of peroxides in aquatic environments.

DOM in the upstream has some unique properties having dominant presence of fulvic acid due to its distinguishable source of origin, such as the forest or swamp ecosystems[24,26]. Conversely, the origins of DOM in the downstream are of different sources, namely mixtures of the upstream waters with effluents from various anthropogenic sources, such as habitations, agriculture, and industry, which depend on the surrounding environments along the banks of the river[22,26]. Our previous study on water samples from the Ohta and the Kurose rivers has shown that fulvic acid-like substances are dominant in the upstream waters of both rivers and in the downstream of the Ohta river, while the downstream of Kurose river are characterized by the presence of FWAs[24]. Due to characteristic differences of DOM composition and sources in upstream and downstream, it is of interest to examine the H_2O_2 and ROOH concentrations, their sources, and the causes of the variations in the upstream and downstream rivers. Moreover, H_2O_2 and its precursor superoxide (O_2^-) can be both an

120 oxidant and a reductant and is therefore potentially important for a number of redox
121 reactions in natural waters[29-31]. Despite the important role that H_2O_2 and ROOH may
122 play in the dynamics of DOM in aquatic environments, but the researchers did not
123 investigate to show what fractions of DOM are susceptible to photochemical production
124 of peroxides in aquatic ecosystems. Previous studies often examined the spatial and
125 temporal distributions of H_2O_2 concentrations, and rarely examined the distributions of
126 ROOH in natural waters.

127 This study uses a synoptic-sampling approach to examine relationships between
128 DOM, inorganic anions and other water quality parameters and the concentrations of
129 H_2O_2 and ROOH in two Japanese rivers. DOMs in river waters have various sources,
130 ranging from natural forest and wetland sources to anthropogenic sources. This
131 difference in DOM sources allows investigating the effect of DOM type, measured by
132 fluorescence, on the peroxide formation. Water sampling was conducted at a number of
133 upstream and downstream sites at monthly intervals over the course of two years. A
134 combination of sampling and laboratory experiments were performed to examine the
135 role of light in the variation in peroxide concentrations. This work included diurnal
136 sampling in the rivers and laboratory incubations of water samples in order to examine
137 the effect of various light conditions on the peroxide production with standard DOM
138 components.

139 **Experimental methods**

140 *Site Description*

141 The samples of the river water were collected from the upstream and the downstream
142 areas in the Ohta river (6 sites: OR1−OR6) and the Kurose river (6 sites: KR1−KR6)
143 located in Hiroshima prefecture, Japan (34°26′ N, 132°28′ E) in the summer (26 and 27

144 June) and the winter (18 and 20 December) of 2002 (Fig. A1). Water samples were also
145 collected monthly during the period of May 2002 to April 2003 from 2 sites in the Ohta
146 river and 4 sites in the Kurose river. On all the days when sampling took place, the
147 weather conditions were good and sunny with no rainfall that could potentially affect
148 the results. The 103 km long Ohta river is very wide, and fluxes of water are supposed
149 to occur mostly due to water flow from the Chugoku Mountains (up to 1350m
150 elevations) situated along the entire river side. Conversely, the 43 km Kurose river is
151 narrow and short. The middle sections of the Kurose river, where the city of
152 Higashi-Hiroshima is situated, are highly polluted due to the fluxes of household and
153 sewage effluents from the densely populated city areas. The water at the upstream (sites
154 KR1, KR2, and OR1) originates from the mountains, and the surrounding regions are
155 densely covered by forests (coniferous, deciduous or mixed-type) and are thus free of
156 pollution. The description of the two rivers and each sampling site provided
157 elsewhere[24].
158 *Sampling*
159 To understand the type of DOM fractions that are susceptible to production of H_2O_2 and
160 ROOH in rivers in general, photoexperiments using a solar simulator were conducted
161 using water samples from two upstream sites (KR1 and KR2) and two downstream sites
162 (KR5 and KR6) in the Kurose river. Samples were collected on May 12, 2004 and
163 August 26, 2004. The same photoexperiments using samples from the Ohta river were
164 conducted collecting samples from two upstream areas (OR1 and OR2) and two
165 downstream sites (OR5 and OR6) on August 10, 2004. Samples were collected
166 throughout the day beginning before sunrise (5:30 JST, Japan standard time) until after
167 sunset (7:00 JST) from upstream site KR2 of the Kurose river on August 21, 2003 and

168 downstream site KR4 on September 26, 2003 to clarify the photochemical processes of
169 DOM in water from these rivers by day-time sunlight irradiation. The day-time water
170 samplings included eight samples from each site. The weather was clear throughout the
171 day on September 26, but was cloudy on August 21 with a shower at around 14:30
172 (about 10 minutes in duration). To measure H_2O_2 and ROOH concentration, samples
173 were filtered at the sampling site using an Ekicrodisc 25 mm syringe filter with a 0.45
174 μm HT-Tuffryn Membrane (PALL, Gelman Laboratory). Samples were stored in capped
175 30 ml brown glass bottles under ice and measured within 6 hours and under such
176 conditions measurements of H_2O_2 are effective in natural waters[8]. Polycarbonate
177 bottles (1 liter) were used to collect the samples for measurements of the other
178 parameters. The collected samples were then filtered using combusted 0.50 μm glass
179 fiber filters (type GF/F) within 5 h of collection. For measurements of EEM properties,
180 duplicate filtered samples of 6 ml each were aliquoted into 10 ml brown bottles. Glass
181 bottles were sealed using a Teflon coated butyl-rubber stopper and an aluminum cap.
182 They were then stored in a refrigerator and analyzed within three days. All
183 polycarbonate and brown glass bottles were cleaned before use with alkaline medium,
184 acid medium followed by tap water, de-ionized water and finally MQ water (TOC,
185 Millipore). The glass bottles were heated at 450 °C for two hours to remove any organic
186 substances from the glass surface.
187 *Analytical methods*
188 H_2O_2 was measured with a fluorometric method using a flow injection analyzer (auto
189 sampler: TOSOH, model AS8020; plunger pump: Sanuki Ind. Co., model 4P2U-4016;
190 fluorescence detector: Shimadzu: RF-10AXL, recorder: Shimadzu: C-R5A
191 Chromatopac) developed and described elsewhere [8]. In summary, 1 ml sample was first

192 treated with catalase (20 μl 500 unit ml^{-1}) for six minutes that is used as a blank and
193 correspondingly 1 ml of a sample replacing catalase with 20 μl MQ water was
194 performed for getting the signal from H_2O_2. The reactions were stopped using
195 peroxidase mixed *p*-hydroxy phenyl acetic acid (PHPAA). The difference in the
196 fluorescence values (Excitation/Emission = 320/400 nm) between samples treated with
197 catalase and without the enzyme provided the estimate of H_2O_2 concentrations. The
198 concentrations of the standards for H_2O_2 determinations used were 0, 100, 200, 300, 500
199 and 1000 nM and calibration curve was linear over the entire range. The H_2O_2
200 concentrations were measured in triplicate, and were averaged for each sample.
201 ROOH was measured using the same procedure [32] as that employed for
202 measuring H_2O_2 [8]. In this measurement, 50000 units ml^{-1} of the catalase solution was
203 used to decompose nearly all of the ROOH in water samples from these rivers during a
204 six minute reaction providing only the signal of the background DOM or water
205 fluorescence. The difference between the fluorescence measurements using 500 and
206 50000 units ml^{-1} of catalase gave an estimate of the ROOH concentrations in samples
207 analyzed. The concentrations of the standards for ROOH determinations used were 0,
208 100, 200, 300, 500 and 1000 nM of peracetic acid solution. The peroxide concentrations
209 were measured in triplicate, and were averaged for each sample. We note that the
210 concentrations of H_2O_2 and ROOH detected in water from these rivers are very low,
211 particularly during the winter period, sometimes falling under the detection limit (a few
212 nM) of the analytical method employed in this study. We also note that the
213 concentration of the ROOH as defined in this study refers to the total concentration of
214 all organic hydroperoxides present in water samples and no information on individual
215 organic hydroperoxides can be obtained from the analytical values.

Considering the volume of the manuscript, we have ~~been~~ discussed in details about chemicals; experimental design; analytical methods such as DOC, EEMS and others; H_2O_2 photoproduction rates and source contribution; statistical analysis; and parallel factor (PARAFAC) modeling as well as few Tables (Tables A1-A2) and Figures (Figs. A1-A4) in Accessory Publication.

Results and discussion

Spatial and temporal variations in HOOH and ROOH concentrations

H_2O_2 concentrations in water samples from the upstream of the Kurose river were low and varied from 6 to 33 nM during monthly measurements (mean 16±8 nM, n=12) at site KR1 and from 16 to 68 nM (mean 30±16 nM, n=12) at site KR2 (Fig. 1a). Similar H_2O_2 concentrations were found at sites KR3 and KR4 (18 to 49 nM, n=4). Slightly higher H_2O_2 concentrations ranging from 9 to 142 nM (mean 62±46 nM, n=12) and from 33 to 213 nM (mean 108±50 nM, n=12) were detected at sites KR5 and KR6, respectively. The results demonstrated that H_2O_2 concentrations are largely varied between upstream and downstream, as well as are gradually increased during transportation of waters from upstream to downstream locations in Kurose river, particularly during the summer period (Table A1). H_2O_2 concentrations in the Ohta upstream and midstream waters varied from 33 to 108 nM (n=8) at sites OR1−OR4. H_2O_2 in the Ohta downstream varied from 40 to 154 nM (mean 105±43 nM, n=12) at site OR5 and from 38 to 171 nM (mean 102±49 nM, n=12) at site OR6. In the Ohta river spatial variation of the H_2O_2 concentration were not significant.

The concentrations of ROOH during monthly readings varied from 9 to 73 nM (mean 31±20 nM) at site KR1 and from 11 to 65 nM (mean 28±15 nM) at site KR2 in

240 the Kurose river (Fig. 1b). The concentrations of ROOH in the Kurose midstream water
241 varied from 9 to 66 nM (n=4) at sites KR3 and KR4. ROOH concentrations were low at
242 site KR5, ranging from 0 to 39 nM (mean 12±14 nM), and at site KR6 where the ROOH
243 concentrations ranged from 0 to 67 nM (mean 24±19 nM) than Kurose upstream. The
244 concentrations of ROOH during monthly reading varied in the Ohta downstream from 1
245 to 61 nM (mean 28±19 nM) at site OR5 and from 3 to 80 nM (mean 30±25 nM) at site
246 OR6. These results showed that ROOH concentration was almost identical in water
247 samples collected from the upstream and downstream in the both rivers except the site
248 KR5 in Kurose River.
249 The seasonal variations in peroxide concentrations in the Kurose and the Ohta
250 rivers are depicted in Fig. 1. The results showed that the concentration of H_2O_2 was
251 sometimes lower during the winter season in both rivers and the differences between the
252 H_2O_2 concentrations in winter and during other seasons were statistically significant.
253 Similarly, the concentrations of ROOH were significantly higher during summer months
254 than during other seasons. These results suggest that the concentrations of both H_2O_2
255 and ROOH may display a similar seasonal trend; their concentrations are higher in
256 summer and lower in winter. Sunlight intensity effects on H_2O_2 production during the
257 summer and winter period can be understood from the significant relationship between
258 H_2O_2 concentrations and solar intensity estimated in $MJm^{-2}h^{-1}$ (r^2=0.80, P<0.01, n=32)
259 or water temperature (r^2=0.80, P<0.01, n=32) in Ohta river (Fig. A2).
260
261 *DOM and its optical properties*
262 The DOC concentrations during monthly readings were low (43−146 μMC) in the
263 upstream (sites KR1 and KR2) of the Kurose river. The DOC concentrations gradually

264 increased at the downstream locations to 123-154 μM C at sites KR3 and KR4, and
265 were found to be significantly higher (130−383 μM C) at sites KR5 and KR6,
266 suggesting that organic matter loading greatly increased at the mid- and downstream
267 locations. The variations were not as significant in the Ohta river, where DOC
268 concentration in water samples was low (59−67 μM C) at the upstream (sites OR1 and
269 OR2) and then gradually increased to 67-109 μM C at OR3 and OR4, and to 40-164 μM
270 C at OR5 and OR6. The organic matter pollution at the Kurose downstream is mostly
271 due to the input of untreated and partly treated sewerage effluents from the city of
272 Higashi-Hiroshima situated along the Kurose river[24].
273 The EEM spectra of DOM of water samples from the two rivers generally
274 exhibited some of the three characteristic peaks such as Peak C at Ex/Em =
275 305-335/426-487 nm in samples from sites KR1 and KR2 and OR1-OR6, Peak W at
276 Ex/Em = 340-350/427-450 nm in samples from sites KR5 and KR6, and Peak T at
277 Ex/Em = 275-285/321-368 nm in all samples from both rivers (Table A1). The peaks C,
278 W and T were identified by comparing their Ex/Em ratios with the Ex/Em ratios of the
279 standards: SRFA (315-340/442-475 nm), FWAs such as DAS1 (345-350/436-449 nm)
280 and DSBP (350/433-437 nm), and tryptophan (275-280/351-355 nm), as well as by
281 comparing their short-term (5 to 30 minutes) photo-irradiated fluorescence properties
282 with those of the standard compounds[24]. PARAFAC analysis on EEM spectra typically
283 identified the two components, indicating the occurrence of fulvic acid in all samples
284 from the two river, and FWAs in the downstream waters of the Kurose river (Fig. 3).
285 Moreover, PARAFAC model was not capable of identifying the tryptophan-like
286 components in riverine samples due to its low fluorescence intensity. The fluorescence

of peak W in the downstream samples of Kurose river was significantly higher (224-666 QSU at KR5 and KR6) than the fluorescence of peak C in the upstream samples of the Kurose river (27-68 QSU at KR1 and KR2) and in the six sampling sites of the Ohta river (39-101 QSU at six sites) (Table A1). These results indicate that the fulvic acid-like substances (Peak C) were dominant in the upstream of the Kurose river and all the water samples from the Ohta river, whilst FWAs (Peak W), an indicator of anthropogenic organic pollution, were mainly present in the downstream of the Kurose river. Tryptophan-like substances (Peak T) was detected in the EEM spectra of all water samples collected from both rivers. Photoirradiation of the standard DSBP solution showed a characteristic peak at Ex/Em = 305/410 nm (Fig. A3), indicating the presence of 4-biphenyl carboxaldehyde (4BCA), a photodegradation product of DSBP. This compound has been identified by comparison with fluorescence properties of a standard 4BCA solution, and other spectroscopic data[33]. It indicates that the photo-degradation products of FWAs, as well as FWAs themselves, may play an important role in the DOM dynamics in the rivers.

Light intensity factor for H_2O_2 production

Concentrations of H_2O_2 in the upstream waters (site KR2) gradually increased from 9 (5:30 JST, Japan standard time) to 43 nM (12:00 JST) during the period before sunrise to noon and then gradually decreased to 9 nM (19:00 JST) after sunset (Fig. 2). In the downstream waters (site KR6), the concentrations of H_2O_2 also gradually increased from 4 (5:30 JST) to 69 nM (13:00−14:00 JST) and then decreased to 20 nM (19:00 JST) after sunset (Table A2). These results suggested that light intensity is an important factor in the generation of H_2O_2 in these rivers. We note that, although data of water

311 flow rate (WFR) was only available from the downstream site (site KR6), almost no
312 fluctuation of WFR during the study period was observed, e.g. 2.2 m^3 s^{-1} during the
313 peak H_2O_2 production from 11:00 JST to 19:00 JST and slightly higher WFR from 2.5
314 to 3.0 m^3 s^{-1} was detected in the morning from 5:30 to 10:00 JST. Thus, the diurnal
315 change in the concentration of H_2O_2 observed was not due to change in WFR. The
316 magnitude of the diurnal variations comparing the H_2O_2 concentration before sunrise
317 was about 35 nM (79% higher) in the upstream waters and 65 nM (94% higher) in the
318 downstream waters. A decrease in the fluorescence of FWAs (maximum 28% decrease
319 as compared to the sample collected before sunrise) occurred in the mid-day samples in
320 the downstream waters[24]. However, almost no change in the fluorescence intensity of
321 fulvic acid-like substances was observed in the upstream waters. The difference may be
322 due to the photo-resistant nature of fulvic acid-like substances, which are relatively slow
323 to photodegradation, judging by the photoirradiation experiments using standard DOM
324 samples, and FWAs, which are rapidly photodegraded[24]. Rapid decay of H_2O_2 (within
325 several hours) in natural waters has been shown to occur by biological processes[1,4,7].
326 Moreover, apparent low concentrations (nM levels) of peroxides in the water from these
327 rivers, in spite of large supply of anthropogenic DOM, are thought to be caused by rapid
328 microbial decay of peroxides that was beyond the scope for this study. Therefore, more
329 efforts are needed to examine the dynamics of production of peroxides and
330 decomposition processes of the peroxides in the water from these rivers.
331
332 *Peroxides production related to factors of DOM sources*
333 Variations observed in the concentrations of H_2O_2 in water samples from upstream and
334 downstream of the two rivers studied (Fig. 1a) can be explained by photo-induced

productions of H_2O_2 from river (Fig. 4a-b) and standard samples (Fig. 5a). In the case of both rivers, the concentration of H_2O_2 gradually increased with increase in the length of the irradiation period (Figs. 4 a-b). Higher H_2O_2 concentrations observed in the downstream waters of the Kurose river were likely caused by higher photoproduction rates of H_2O_2 from various DOM components that are more predominant in the downstream waters of the Kurose river as compared to the upstream waters of the Kurose and the entire Ohta river. Photo irradiation experiments showed that production rates of H_2O_2 were high for various standard FDOM compounds, such as SRFA, DSBP, SRHA, tryptophan and so on (Table 1). The concentration of H_2O_2 gradually increased with increase in the length of the irradiation period when various standard FDOM substances were used in the experiment (Fig. 5a). Fulvic acid-like substances were dominant in our study sites and humic acid-like substances were not found at all (Fig. 3)[24]. Photo experiments conducted using an aromatic amino acid, tryptophan, indicated that tryptophan show a significantly high H_2O_2 production potential (Table 1). The production of H_2O_2 obtained from the irradiation of standard tryptophan and DSBP (Fig. 5a) were similar to the values reported elsewhere[34]. Other aromatic amino acids, such as tyrosine and phenylalanine, showed similar production potentials of H_2O_2. We, however, note that tryptophan-like compounds were the only amino acid compounds detected in the rivers studied, apparently due to much higher fluorescence of tryptophan-like compounds (5016 QSU mg^{-1}) than other aromatic amino acids studied (13-41 QSU mg^{-1}) (Table 1).

ROOH concentrations in the rivers studied were very low compared to the H_2O_2 concentration (Figs. A5c-d). However, we observed that ROOH, as well as H_2O_2, were

358 more abundant in downstream water samples from the Kurose river than in any other
359 samples we analyzed. These results suggest that quantity of DOM is also an important
360 factor in the production of ROOH in water from these rivers. Production of ROOH
361 using standard DOM samples was examined by photo irradiation experiments (Fig. 5b).
362 Unlike uniform generation of H_2O_2 in these standard samples, which followed a regular
363 trend of increasing concentration with increase in the length of the irradiation, the
364 concentration of the produced ROOH was very low and fluctuated heavily without any
365 observable trends. This may be due to the inherently unstable chemical nature of ROOH,
366 which are sensitive to acid, alkali, redox and light in aqueous solutions.
367 The correlation study between hydrogen peroxide and DOC concentrations
368 showed a good correlation between the concentration of H_2O_2 and DOC (r^2=0.65,
369 P<0.01) in the waters of the Kurose river, whilst no such relation was found in the
370 samples collected from the Ohta river (Table 2). This can be understood with relatively
371 large production of photo-induced H_2O_2 in Kurose river, particularly at sites KR5 and
372 KR6 than Ohta river (Figs. A5a-b) and is subjected to be caused by various DOC
373 subgroups present in Kurose river that might be photochemically more potential. The
374 concentrations of ROOH did not show any correlation with the concentration of DOC in
375 either the Kurose or the Ohta rivers. The fluorescence intensity of fulvic acid-like (peak
376 C), FWAs (peak W), and tryptophan-like (peak T) substances were detected in water
377 samples collected from both the Kurose and the Ohta rivers (Table A1). The relationship
378 between H_2O_2 and fluorescence of various FDOM showed that H_2O_2 concentrations
379 correlated well with the measured fluorescence intensity of peak C in water samples
380 from the Ohta river (r^2=0.64, P<0.01), whereas, in samples from the Kurose river, the
381 fluorescence intensity of combined peaks C + W correlated with the concentration of

382 H_2O_2 (r^2=0.61, P<0.01) (Table 2). This suggests that fulvic acid-like component (and
383 plus fluorescent whitening agent) may be a major source of H_2O_2 production in rivers.
384 This is typically resulted due to predominant presence of fulvic acid (40-80% of total
385 DOM) in rivers[22,26] and its relatively high production rate of H_2O_2 (69×10^{-12} Ms^{-1},
386 Table 1). The concentration of H_2O_2 correlated well with the fluorescence of peak T
387 observed in water samples from the Ohta river (r^2=0.62, P<0.05), but such a correlation
388 was not observed in water samples from the Kurose river.
389 The concentrations of ROOH correlated with the measured fluorescence intensity of
390 peak C in water samples from the Ohta river (r^2=0.62, P<0.01), but a similar
391 relationship was not found between the observed fluorescence of the combined peaks C
392 + W in the waters of the Kurose river (Table 2). The concentrations of ROOH did not
393 correlate with the fluorescence of peak T in the samples from the Ohta river, but
394 correlated inversely in the Kurose river. These results suggest that the fulvic acid-like
395 (peak C) and tryptophan-like (peak T) substances may control the concentrations of
396 peroxides in the Ohta river, whilst concentrations of peroxides in the water from the
397 Kurose river are controlled by fulvic acid-like (peak C) and FWAs (peak W) substances,
398 but tryptophan-like substances (peak T) have a mixed relation on the concentrations of
399 peroxides.
400
401 *Peroxides production related to factors of water quality parameters*
402 The water quality parameters, such as pH, water temperature, solar intensity, content of
403 the inorganic anions (NO_2^- and NO_3^-) and total iron (total Fe and Fe^{2+}) contents greatly
404 varied in the two rivers (Table A1). The concentrations of the inorganic anions, such as
405 NO_2^- and NO_3^- and total Fe varied significantly at various sites of collection, exhibiting

406 higher concentrations in the polluted downstream of the Kurose river and lower
407 concentrations at the upstream of the Kurose as well as in the Ohta river. The correlation
408 between the concentration of peroxides and solar intensity showed that both H_2O_2 and
409 ROOH are strongly affected by solar intensity in the waters of the Ohta river (Fig. A2),
410 but are only slightly correlated in samples collected from the Kurose river. This suggests
411 the photochemistry as a major source of H_2O_2 and ROOH in Ohta river, and there is
412 some organic substances, plausibly fulvic acid that is present in relatively similar
413 amounts in all water samples from the Ohta. The measured water temperature
414 significantly affected the concentrations of H_2O_2 and ROOH in water samples collected
415 from both rivers. The concentration of NO_2^- was well correlated with concentrations of
416 both H_2O_2 and ROOH in water samples from the Kurose river. The concentration of
417 NO_3^- was partly correlated with the concentration of H_2O_2 in the Kurose river but no
418 correlation was observed in water samples from the Ohta river. These results indicate
419 that NO_2^- and NO_3^- may be involved in H_2O_2 and ROOH production in Kurose river.
420 Photoirradiation experiments on aqueous solutions of NO_2^- demonstrated that NO_2^- is
421 susceptible to production of H_2O_2 during the irradiation period (Fig. A4). It can be
422 proposed that photo-induced generation of $O^{\bullet-}$ from both NO_2^- and NO_3^-[35], the most
423 potential mechanism for production of $^{\bullet}OH$ ($O^{\bullet-} + H_2O \rightarrow {}^{\bullet}OH + OH^-$), may likely
424 susceptible to accelerate simultaneously the high production of H_2O_2 through
425 production of hydroxyl radicals ($^{\bullet}OH$ and OH^-) in aqueous media. This should be the
426 focus for potential further study.
427 The concentrations of H_2O_2 and ROOH were not correlated with total dissolved Fe

and Fe^{2+} concentrations studied, suggesting that Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) or photo Fenton reaction may not be an important factor in the generation of peroxides in water samples studied. No clear evidence has been produced for involvement of total Fe and Fe^{2+} in production of H_2O_2 in field observations and this should be the focus for further research. While, the concentrations of ROOH were well correlated with the concentrations of H_2O_2 in water samples from the Ohta river, no such relationship was observed in the samples from the Kurose river. These results suggest that H_2O_2 and ROOH may originate from the same sources or be generated by similar production mechanisms in the waters of the Ohta river, but that such similarity in the origin of the two types of peroxide does not occur in the Kurose river.

Relative contributions of various classes of DOM to H_2O_2 production

The sources of H_2O_2 in the upstream and downstream waters in both rivers were estimated using the EEM data and summarized in Table 3. PARAFAC model was applied to isolate the overlapping of various peaks of DOM compositions at the same peak positions in EEM spectra in both river waters (Fig. 3). This result showed that two components (fulvic acid and FWAs) were identified in the downstream waters of the Kurose river (sites KR5 and KR6) and one component (fulvic acid) was often detected in all other sampling sites in both rivers. Contribution percentage demonstrated that the major sources of H_2O_2 are the fulvic acid-like substances present in the waters of the Ohta river (23−70%) and in the upstream (25−61%) as well as downstream (28−63%) of the Kurose river (Table 3). It is generally considered that the humic substances (fulvic

451 and humic acids) act as a photosensitizer that are responsible for production of H_2O_2 in
452 natural waters[1,8,11]. This study firstly estimated the contribution of fulvic acid in H_2O_2
453 production in rivers that may pave the way to examine H_2O_2 contributions from various
454 DOM fractions in a variety of natural waters. Tryptophan-like substances are a minor
455 source of H_2O_2 (~1%) in both rivers. Even though the FWAs (DAS+DSBP) were
456 dominant FDOMs in the downstream waters of the Kurose, their contribution to the
457 H_2O_2 production was only minor at 1−2%. Contribution percentage of fulvic acid was
458 significantly higher (62−63%) in summer (August) samples collected from the
459 downstream waters of Kurose river (sites KR5 and KR6) than in spring (May) samples
460 (28−37%) (Table 3). This is likely to be caused as a result of relatively large in fulvic
461 acid-like fluorescence intensity observed in summer (225-228 QSU) than in spring
462 samples (128-144 QSU) (Table 3). Unknown sources of H_2O_2 (other than fulvic
463 acid-like and tryptophan-like substances, and FWAs) accounted for 37−72% of H_2O_2 in
464 the upstream waters of the Kurose (sites KR1 and KR2), 51−77% at the upstream areas
465 of the Ohta (sites OR1 and OR2), 29−32% at the downstream sites of the Ohta (sites
466 OR5 and OR6), and 33−64% at the downstream sites of the Kurose (sites KR5 and
467 KR6). The unknown sources of H_2O_2 may be other fluorescent and non-fluorescent
468 substances including humic acid that can originate from forests ecosystem in the
469 upstream regions of a river and various anthropogenic sources affecting the downstream
470 regions[33] (Fig. A3). Although, this study did not identify the humic acid using
471 PARAFAC model presumably due to its low concentration, but it is reported that the
472 ratio of fulvic acid to humic acid in DOM is generally 9:1 for waters having low DOC
473 concentration and it decreases to 4:1 or less for waters having high DOC[21,26]. This

474 suggests that humic acid may also contribute a little to H_2O_2 production in rivers. In
475 addition, the EEM of 2-sulfonic acid benzaldehyde (2SAB), a photoproduct of the
476 FWAs-DSBP[33], showed no significant fluorescence properties, but its H_2O_2 production
477 rate was 37×10^{-12} M s^{-1}, estimated by the photochemical degradation of 2SAB using a
478 solar simulator (Table 1). To identify various sources of H_2O_2 in the downstream waters
479 of the Kurose, we conducted the photo irradiation experiments on DSBP using a solar
480 simulator. The EEM data of DSBP showed the appearance of three characteristic
481 fluorescence peaks at Ex/Em=305/408 nm, 260/317 nm and 260-265/366-367 nm (after
482 20 h, Fig. A3b), along with concomitant disappearance of the DSBP peak (0 h, Fig.
483 A3a). The fluorescence peak at Ex/Em=305/408 nm was identified to be 4-biphenyl
484 carboxaldehyde (4BCA), which has a fluorescence peak at Ex/Em=305/410 nm. 4BCA
485 was previously identified as a degradation product of DSBP by a HPLC/DAD
486 method[33]. The production rate of H_2O_2 estimated from the photo experiments using a
487 4BCA standard was 34×10^{-12} M s^{-1} and 2−5% of contribution to total H_2O_2 production
488 in the downstream waters of the Kurose river (Table 3).
489
490 **Conclusions**
491 In this study, variations in the concentrations of peroxides in the Kurose river and
492 the Ohta river were shown to result from differences in quantity and optical nature of
493 DOM and also change in water quality parameters. The results are summarized below:
494 • Spatial distribution of H_2O_2 showed low concentrations in the upstream waters
495 and high concentrations in the downstream waters in both rivers while the
496 concentration of ROOH was almost identical at the upstream and downstream
497 sites.

- A clear diurnal variation in the H_2O_2 concentration, which follows variations in the solar radiation, was found at the study sites of the Kurose river. This indicated that photo-production of H_2O_2 occurs rapidly under natural solar irradiation of the water from the Kurose and the Ohta rivers.
- The concentrations of the ROOH did not show any correlation with the concentrations of DOC in either the Kurose or the Ohta rivers. The concentrations of the ROOH were only correlated with the concentration of the fulvic acid-like substances in the waters of Ohta river. This suggests that concentrations of ROOH in these rivers are only partly controlled by the quantity and optical nature of DOM and that other parameters, such as water temperature and solar intensity, have more influence on the concentration of the organic peroxides in the water.

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Table 1. The production rates of H_2O_2 estimated as a result of photo irradiation on various standard substances (1 mg L^{-1}) and their corresponding dissolved organic carbon (DOC) concentrations as well as fluorescence intensity (FI) in aqueous solutions.

Samples	Production rate[#] H_2O_2 $\times 10^{-12}$ (Ms^{-1})	DOC (μM C)	FI (QSU)
Suwannee River Fulvic Acid (SRFA)	69	49	65
Suwannee River Humic Acid (SRHA)	179	49	22
Tryptophan	155	69	5016
Tyrosine	73	33	13
phenylalanine	4.4	47	41
DAS1	46	38	1454
DSBP	244	34	75200
Phenol	27	21	5150
4-Biphenyl carboxaldehyde (4BCA)	34	74	735
2-Sulfonic acid benzaldehyde (2SAB)	37	80	NFP

[#] production rate calculated for initial 60 min irradiation period and normalized to sunlight intensity (noon time) at the Campus of Hiroshima University, Japan.
NFP: No fluorescence properties.

Table 2. The Pearson correlation coefficients between the concentrations of peroxides (H_2O_2 and ROOH) and other water quality parameters studied in the Kurose and the Ohta river waters.

	H_2O_2		ROOH	
Coefficients	r	P-value	r	P-value
Parameters				
		Kurose River:		
SI	0.33	<0.05 (n=52)	0.33	<0.05 (n=48)
WT	0.58	<0.01 (n=52)	0.43	<0.01 (n=48)
DOC	0.65	<0.01 (n=48)	0.00	NS (n=44)
Peaks (C + W)	0.61	<0.01 (n=51)	-0.27	NS (n=47)
Peat T	0.14	NS (n=17)	-0.51	<0.05 (n=17)
NO_3^-	0.60	<0.01 (n=38)	-0.28	NS (n=34)
NO_2^-	0.65	<0.01 (n=18)	0.82	<0.01 (n=16)
Fe^{2+}	0.11	NS (n=25)	-0.31	NS (n=25)
total Fe	0.30	NS (n=37)	-0.52	<0.01 (n=33)
pH	-0.09	NS (n=52)	-0.61	<0.01 (n=48)
ROOH	0.16	NS (n=48)	1	1
		Ohta River:		
SI	0.80	<0.01 (n=32)	0.61	<0.01 (n=30)
WT	0.80	<0.01 (n=32)	0.72	<0.01 (n=30)
DOC	0.00	NS (n=28)	-0.14	ns (n=26)
Peak C	0.64	<0.01 (n=31)	0.62	<0.01 (n=30)
Peat T	0.62	<0.05 (n=12)	0.43	NS (n=12)
NO_3^-	-0.24	NS (n=22)	-0.41	NS (n=20)
NO_2^-	ndc	ndc	ndc	ndc
Fe^{2+}	-0.20	NS (n=16)	-0.05	NS (n=16)
total Fe	0.35	NS (n=22)	0.31	20 (n=20)
pH	-0.07	NS (n=32)	-0.22	NS (n=30)
ROOH	0.74	<0.01 (n=30)	1	1

Statistical significance is reported as either NS (p>0.05, (0.05>p0.01, or (p<0.01).
n=Number of monthly samples studied in river waters.
NS=Not significant, and (-) = negative corelation.
ndc= Below detection limit of concentration.

Table 3. Production rates of H_2O_2, DOC, fluorescence intensities (FI) of peaks (C, W and T) and 4-biphenyl carboxaldehyde(4BCA), estimated H_2O_2 production rates of fulvic acid (FA)-like components, FWAs, 4BCA and tryptophan-like substances and their contribution percentages to the net H_2O_2 production rates in the Kurose and the Ohta river waters. Estimation of H_2O_2 photoproduction rates and source contribution has been explained in details in Accessory Publication.

Samples	Production rate of H_2O_2	DOC	FI[#]			Estimated production rate of H_2O_2 from				F_i				
			Peak C and (Peak C) + (Peak W)*	Peak T	4BAC	FA-like components	Tryptophan-like compounds	FWAs-like (DAS1+DSBP)	4BAC	FA-like components	Tryptophan-like compounds	FWAs-like (DAS1+DSBP)	4BAC	Unknown
	$\times 10^{-12}$ (Ms^{-1})	(μM C)	(QSU)			$\times 10^{-12}$ (Ms^{-1})				(%)				
Kurose River														
Namitakiji (KR1)	100 - 248 (n=2)	111 - 152	58 - 59	33 - 34	NP	61 - 63	1.0 - 1.1	NP	NP	25 - 61	0.4 - 1.1	ND	ND	37 - 74
Shouri (KR2)	209 - 214 (n=2)	106 - 134	55 - 59	16 - 18	NP	58 - 63	0.5 - 0.6	NP	NP	28 - 29	0.2 - 0.3	ND	ND	71 - 72
Izumi (KR5)**	386 - 536 (n=2)	310 - 505	(144 - 228) + (218 - 593)*	119 - 146	288 - 377	152 - 241	3.7 - 4.5	7.2 - 9.4	10 - 27	28 - 62	0.8 - 1.0	1.0 - 2.0	3.0 - 5.0	33 - 64
Hinotsume (KR6)**	366 - 379 (n=2)	276 - 445	(128 - 225) + (199 - 381)*	124 - 135	272 - 278	136 - 238	3.8 - 4.2	6.2 - 6.3	9 - 17	37 - 63	1.0 - 1.1	1.0 - 2.0	2.0 - 5.0	33 - 55
Ohta River														
Miwaku (OR1)	211	101	46	12	NP	49	0.37	NP	NP	23	0.2	ND	ND	77
Shintagiri (OR2)	109	88	50	13	NP	53	0.40	NP	NP	49	0.4	ND	ND	51
Takase (OR5)	116	115	76	29	NP	80	0.90	NP	NP	70	0.8	ND	ND	29
Asa (OR6)	127	124	81	31	NP	86	0.96	NP	NP	67	0.8	ND	ND	32

[#]Fluorescence intensity (FI) determined at Ex/Em=320/450 nm for isolated FA-like component and 350/437 nm for FWAs-like component in riverine and standard samples using PARAFAC model.

* indicates the FI of peak W (FWAs-like components) in rivers, ** River waters only having both peak C (FA-like) and peak W (FWAs-like) components;

NP means no peak, and n = number of samples analyzed.

628 **Figure captions**

629 Fig. 1

630 Seasonal variations of the H_2O_2 (a) and ROOH (b) concentrations in the waters of
631 Kurose river and Ohta river in Hiroshima prefecture, Japan. The error bar indicates the
632 standard deviation of seasonal average value of peroxides. Mean values labeled with
633 different letters are significantly differed at $p<0.05$ (Fisher's LSD analysis).

634 Fig. 2

635 Diurnal variations of H_2O_2 concentrations in the upstream waters (site KR2) on August
636 21, 2003 and in the downstream waters (site KR6) on September 26, 2003, in the
637 Kurose river.

638 Fig. 3

639 Various fluorescent components of riverine samples and aqueous solutions of standard
640 substances identified using the PARAFAC model. The isolated components are a) fulvic
641 acid-like (one component, Site KR1), b) fluorescent whitening agents (FWAs)-like
642 (component 1, Site KR5), c) fulvic acid-like (component 2, Site KR5), d) fulvic
643 acid-like (one component, Site OR5), e) Standard Suwannee River Fulvic Acid (one
644 component), f) Standard DSBP (one component) and g) standard DAS1 (one
645 component).

646 Fig. 4

647 Production of H_2O_2 (Figs. a-b) and ROOH (Figs. c-d) due to solar irradiation on the
648 Kurose river waters (collected from sites KR1, KR2, KR5 and KR6) and the Ohta river
649 waters (sites OR1, OR2, OR5 and OR6) in photo experiments conducted using solar
650 simulator.

651 Fig. 5

652 Production of H_2O_2 (a) and ROOH (b) due to light irradiation on various standard
653 substances in photo experiments conducted using solar simulator.

654

Figure 1

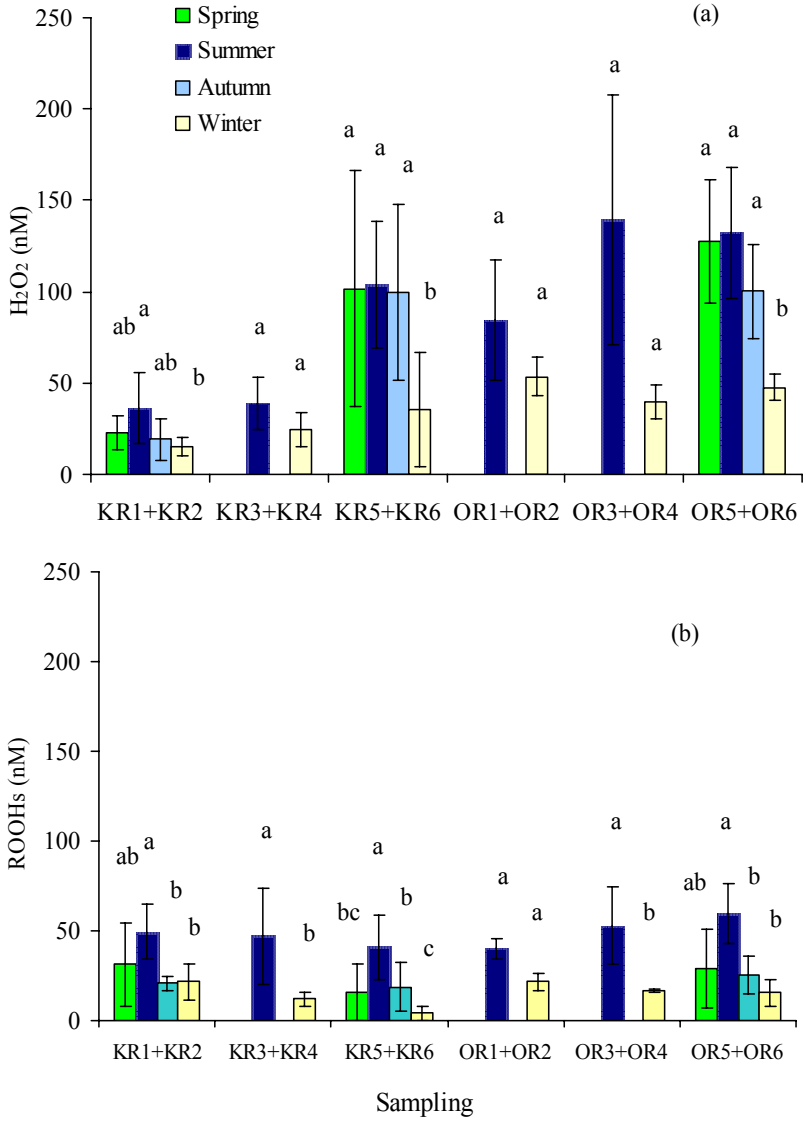

Spring
Summer
Autumn
Winter
(a)
(b)
H2O2 (nM)
ROOHs (nM)
250
200
150
100
50
0
KR1+KR2
KR3+KR4
KR5+KR6
OR1+OR2
OR3+OR4
OR5+OR6
Sampling

Fig. 2

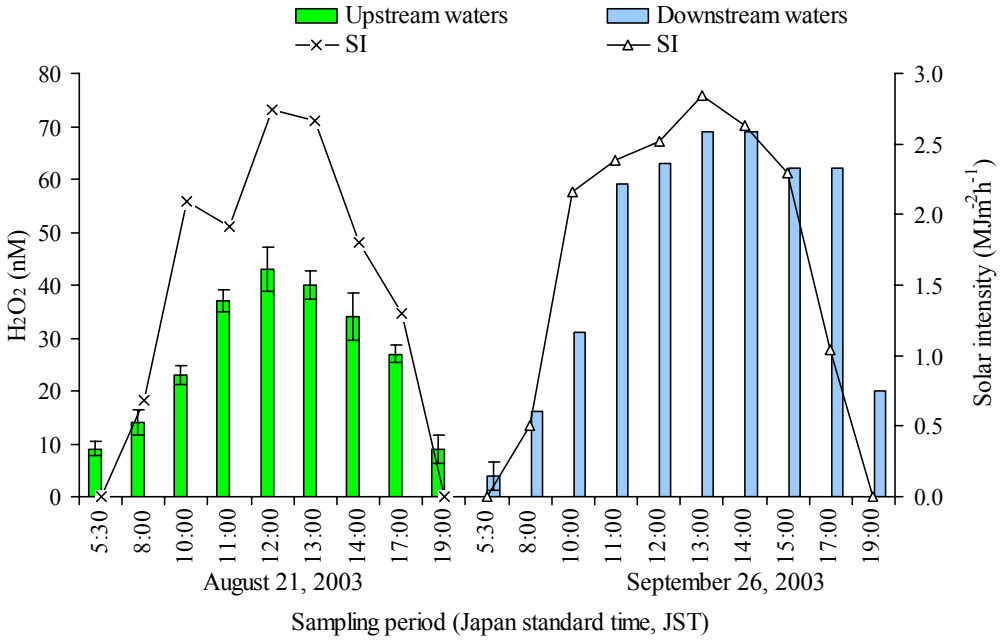

Upstream waters
SI
Downstream waters
SI
H2O2 (nM)
Solar intensity (MJm-2h-1)
0
10
20
30
40
50
60
70
80
0.0
0.5
1.0
1.5
2.0
2.5
3.0
5:30
8:00
10:00
11:00
12:00
13:00
14:00
17:00
19:00
5:30
8:00
10:00
11:00
12:00
13:00
14:00
15:00
17:00
19:00
August 21, 2003
September 26, 2003
Sampling period (Japan standard time, JST)

Fig. 3

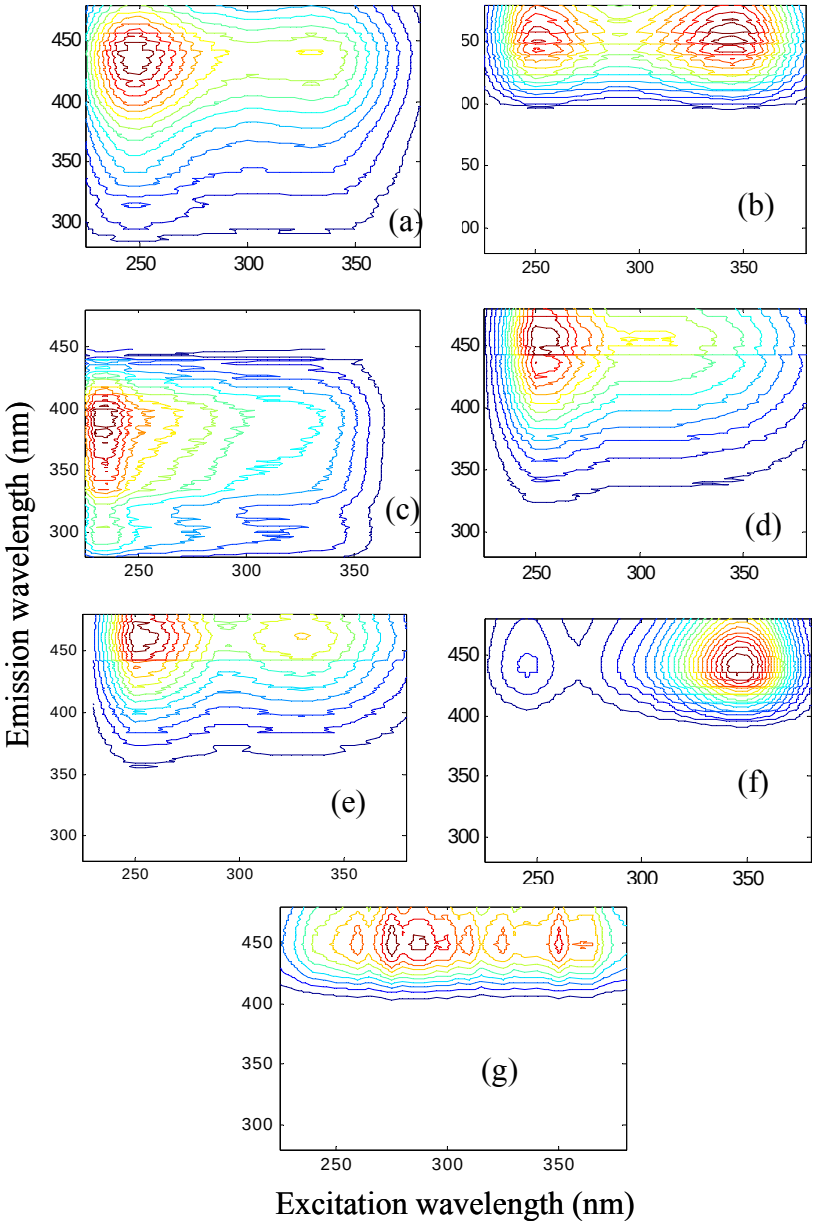


Fig. 4

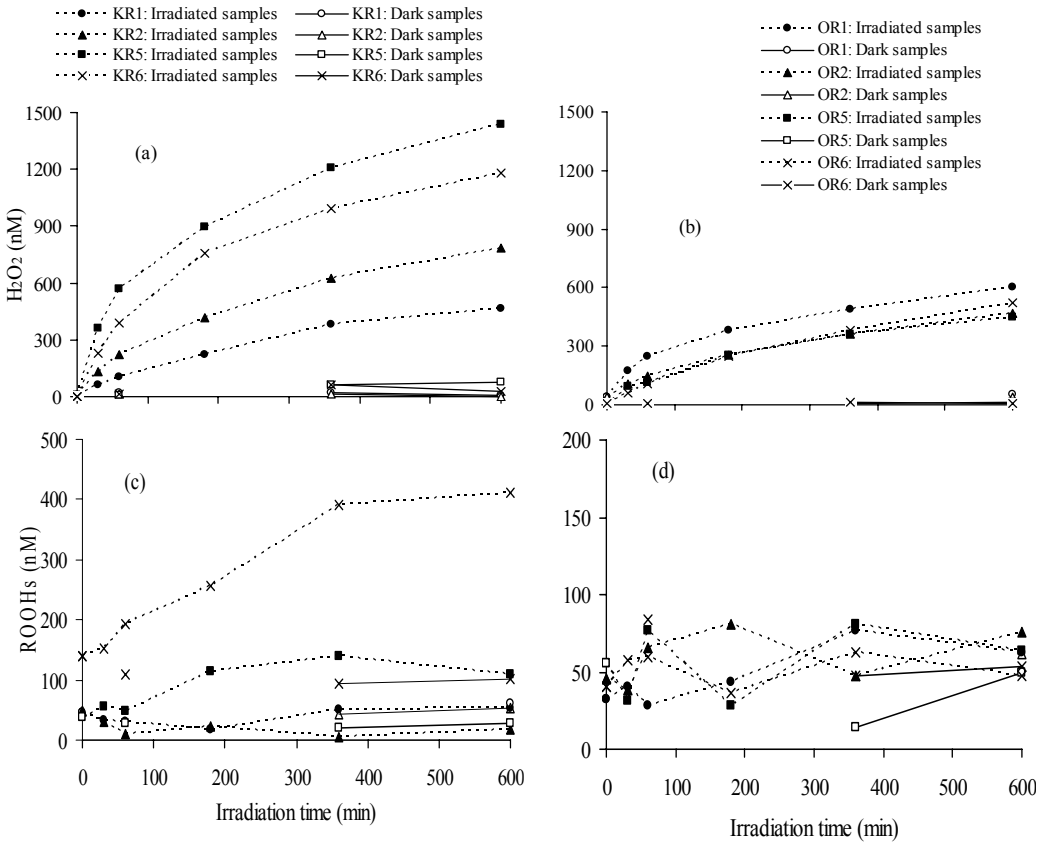
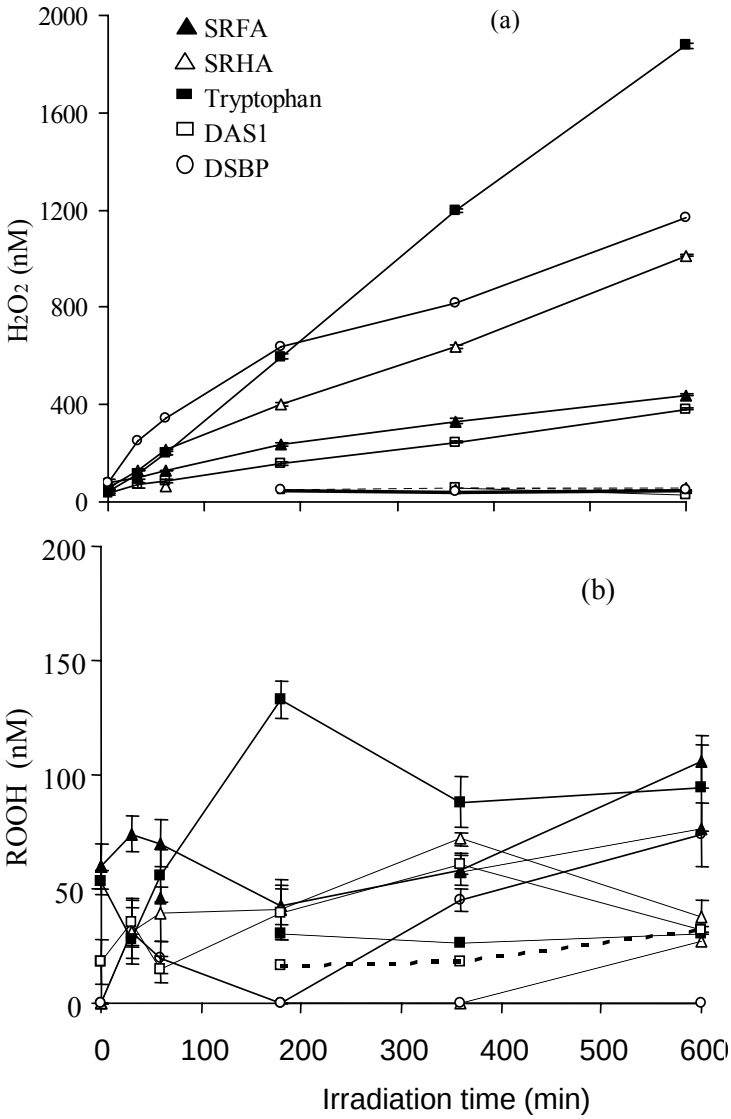

KR1: Irradiated samples
KR1: Dark samples
KR2: Irradiated samples
KR2: Dark samples
KR5: Irradiated samples
KR5: Dark samples
KR6: Irradiated samples
KR6: Dark samples
OR1: Irradiated samples
OR1: Dark samples
OR2: Irradiated samples
OR2: Dark samples
OR5: Irradiated samples
OR5: Dark samples
OR6: Irradiated samples
OR6: Dark samples
(a)
(b)
(c)
(d)
H2O2 (nM)
ROOHs (nM)
Irradiation time (min)
Irradiation time (min)

Fig. 5


(a)
SRFA
SRHA
Tryptophan
DAS1
DSBP
H2O2 (nM)
2000
1600
1200
800
400
0
(b)
ROOH (nM)
200
150
100
50
0
0
100
200
300
400
500
600
Irradiation time (min)

Accessory publication

Spatial and temporal variations and factors controlling the concentrations of hydrogen peroxide and organic peroxides in rivers

By

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Accessory publication

1. Experimental Section

Chemicals

To examine the peroxide production in water from these rivers, we used Suwannee River Fulvic Acid (SRFA) (ref. No 1S101H), Suwannee River Humic Acid (SRHA) (ref No 1S101F) (International Humic Substances Society, USA), tryptophan (Nakalai Tesque Inc., Kyoto, Japan), FWAs standards, such as distyryl biphenyl, DSBP: 4,4'-bis[(2-sulfostyryl)biphenyl (Tinopal CBS-X, LOT: 112013R2EY) and

848 diaminostilbene type, DAS1: 4,4'-bis[(4-anilino-6-morphilino-s-triazine-2-yl)amino]
849 2,2'-stilbenedisulfonate (Tinopal AMS-GX, LOT 001288BOEK), 2-sulfonic acid
850 benzaldehyde (2SAB) and 4-biphenyl carboxaldehyde (4BCA) (Kanto Chemicals
851 company, Japan). One mg L^{-1} of each standard solution was prepared by dissolving into
852 MQ water. 30 % H_2O_2 (Wako Chemical Ltd, Japan) and peracetic acid (Aldrich, Japan)
853 were used as the standards for H_2O_2 and ROOH, respectively. Catalase and peroxidase
854 were purchased from Sigma, Japan. All the chemicals were of analytical grade.
855
856 *Experimental design*
857 The irradiation experiment was conducted using a solar simulator (Oriel, Model
858 81160-1000) equipped with the 150 W Xenon lamp (Ozone free, Oriel Model 81160)
859 and special glass filters restricting the transmission of wavelengths below 300 nm. Few
860 experiments were conducted with the more intense radiation using the 300 W Xenon
861 lamp by replacing the previous one. The light intensity of the lamp was calculated by
862 measuring the degradation rate of a 8 μM standard 2-nitrobenzaldehyde (2-NB) solution
863 in a 60 ml quartz cell. The degradation rates of 2-NB for a Xenon lamp employed in this
864 study were in the range of 0.00196-0.00214 s^{-1} and 0.00331-0.00338 s^{-1} whilst the
865 degradation rate for natural sunlight on 6 July 2004 at Hiroshima University Campus (at
866 noon under clear sky conditions) was 0.00783 s^{-1}. To examine photoproduction potential
867 of H_2O_2 and ROOH, each FDOM standard solution (1, 3 or 5 mg L^{-1} in Milli-Q water)
868 and river samples were prepared for light irradiation experiments. The exposure time
869 was 10 h for 1 mg L^{-1} samples and 1 h for 3 and 5 mg L^{-1} samples. All river samples
870 were pre-filtered and were exposed to total irradiation period of 10 h with aliquots taken
871 for peroxide determination at 0, 30, 60, 180, 360 and 600 minutes. The amounts of H_2O_2

872 and ROOH in the standard solutions and samples from the rivers were normalized as a
873 function of natural sunlight using the following equation (eq 5):

874
875 $$R_{(H_2O_2,Is)} = \frac{D_{(2-NB,Is)} R_{(H_2O_2,Ixe)}}{D_{(2-NB,Ixe)}} \quad \ldots\ldots\ldots\ldots(1)$$
876
877

878 where $R_{(H_2O_2, Is)}$ is the rate of H_2O_2 production corrected for the intensity of natural
879 sunlight (at noon under clear sky conditions on 6 July 2004 at Hiroshima University
880 Campus) in water samples from the river and standard DOM materials, $D_{(2\text{-}NB,Is)}$ and
881 $D_{(2\text{-}NB,Ixe)}$ are the degradation rates of 2-NB estimated using the intensity of natural
882 sunlight and the Xe lamp, respectively, and $R_{(H_2O_2,Ixe)}$ is the observed H_2O_2 production
883 rate produced under the conditions of Xe lamp.

884

885 *Other Analytical methods*

886 Dissolved organic carbon (DOC) concentration in water samples was measured
887 using a high temperature catalytic oxidation method (TOC 5000A, Shimadzu, Kyoto,
888 Japan). The standard potassium hydrogen phthalate was used as a reference organic
889 substance to determine DOC concentration. After removing dissolved inorganic carbon
890 (DIC) by bubbling pure air, 106 μl of each sample was injected into TOC analyzer.
891 DOC measurements were conducted for each sample 3 to 5 times under conditions of
892 <2% coefficient of variance or with the standard deviation being the area counted for
893 <200 (equivalent to 1.3 μM C). Triplicate measurements were performed for each
894 sample. The three-dimensional (3-D) excitation emission matrix (EEM) spectra of water
895 samples were obtained using a fluorescence spectrophotometer (F-4500, Hitachi, Japan).
896 The EEM spectra were constructed by scanning emission spectra from 225 to 500 nm as
897 a function of excitation wavelength from 225 to 400 nm. Readings were collected at

898 intervals of 5 nm for excitation with 1 nm emission wavelengths using a scanning speed
899 of 1200 nm min^{-1}. The wavelength accuracy was within ±2 nm. The fluorescence
900 spectra were measured in triplicate for each sample and were averaged. Fluorescence
901 readings (peaks C, W and T) were calibrated using the fluorescence intensity
902 (Ex/Em=350/450 nm) of a quinine sulphate standard. Quinine sulphate solution (4 μg
903 L^{-1}) was prepared in 0.01 N H_2SO_4 for fluorescence measurements. The fluorescence
904 intensity (FI) for 1 μg L^{-1} of quinine sulphate solution was equal to 1 QSU (quinine
905 sulphate unit) in this study. The concentrations of the NO_3^- and NO_2^- ions were
906 determined using a suppressor type ion chromatograph with the column Ion Pac AS11
907 (Yokogawa Analytical Systems, IC-7000II and Dionex, DX500). Dissolved Fe(II) and
908 total Fe content were measured using the 1,10-phenanthroline method. In this method,
909 Fe (III) was estimated as the difference between Fe(II) and total Fe after reduction of
910 Fe(III) by 5% hydroxyl amine. Ferrous ammonium sulfate was used as a standard. The
911 river and standard samples were processed followed by the earlier method[1]. The
912 absorbance of the samples then measured at wavelength ranges of 450-550 nm using
913 UV-VIS Spectrophotometer (Shimadzu UV-2401, Shimadzu, Japan). The maximum
914 absorbance at a specific wavelength was used for determination of Fe(II) and total Fe
915 concentrations in samples. The pH was measured using a portable pH meter (Horiba,
916 Japan). The solar intensity (SI) was measured using a pyranometer (MS62, Eikoseiki
917 Inc., Japan) located on the roof of the Faculty of Integrated Arts and Science in
918 Hiroshima University (HU), in proximity to KR5 site. The data from this instrument
919 provided meteorological data for the Kurose river. SI data at Misasa Primary School,
920 Nishi-ku Hiroshima City (Air Pollution Monitoring Center, Hiroshima prefecture,
921 Japan), located close to OR6 site, was used for the Ohta river meteorological data.

H_2O_2 photoproduction rates and source contribution

The rate of production of H_2O_2 in irradiated standard DOM solution and in water samples from the river was determined from the net production of H_2O_2 (final concentration minus initial concentration) measured for the initial 60 minutes of the irradiation period. The rate of generating H_2O_2 was then normalized to sunlight intensity at noon under clear sky conditions on 6 July 2004 at Hiroshima University Campus[2]. The normalized rate of production of H_2O_2 of an identified fluorescent substance is estimated on the basis of its fluorescence intensity observed in natural waters and can be determined using the following equation:

$$R_{Fi(river)} = \frac{FI_{Fi(river)} R_{RS}}{FI_{RS}} \qquad \text{……..……(2)}$$

where $R_{Fi\ (river)}$ is the normalized production rate of H_2O_2 of an identified fluorescent substance in natural waters, $FI_{Fi\ (river)}$ is the fluorescence intensity of the identified fluorescent substance in natural waters, FI_{RS} is the fluorescence intensity of the relevant standard substance in the aqueous solutions, and R_{RS} is the normalized production rate of H_2O_2 of the relevant standard substance in the aqueous solution. Finally, percentages of each identified fluorescent substance contributing to the rate of production of H_2O_2 are calculated using the following equation:

$$F_{i\ (river)} = \frac{R_{Fi(river)} \times 100}{R_{net(river)}} \qquad \text{……(3)}$$

where $F_{i\ (river)}$ is the contribution percentage of the normalized net H_2O_2 production rate in the water (%) for each identified fluorescent substance, $R_{Fi(river)}$ is the normalized H_2O_2 production rate generated by each identified fluorescent substance in water from these

947 rivers, and $R_{net(river)}$ is the normalized net H_2O_2 production rate of all FDOM in water
948 from these rivers. The percent contributions of unknown sources of H_2O_2 in water
949 samples from the river were estimated using a simple formula: $F_{unknown} = 100 - (F_{FA} +$
950 $F_{Tryptophan} + F_{FWAs})$, where the sum of the normalized H_2O_2 production rate of FA-like
951 substances, tryptophan-like substances and FWA-like substances is subtracted from the
952 normalized net H_2O_2 production rate of 100%.
953
954 *Statistical analysis*
955 Statistical analysis of the data obtained for peroxide measurements and other relevant
956 analytical data was conducted using a SPSS program (SPSS Inc., U.S.A). Significances
957 of the differences in average values among seasonal peroxide concentrations were
958 evaluated by one-way ANOVA and Fisher's LSD analysis ($P < 0.05$). The Pearson
959 correlation coefficients between peroxides concentrations and other water quality
960 variables were estimated using the same program.
961
962 *PARAFAC modeling*
963 Recently, parallel factor (PARAFAC) analysis has effectively applied on EEM data to
964 isolate the different components of DOM compositions into the organic compounds[3].
965 The PARAFAC model was performed in MATLAB using the "N-way toolbox for
966 MATLAB ver. 3.1" with methods described in earlier studies[3]. The data EEMs of the
967 samples were modeled with excitation wavelength ranging from 220 to 380 nm by
968 every 5 nm and emission wavelength from 280 to 480 nm by every 1 nm in this study.
969 Milli-Q water blank was subtracted from every sample before running in the PARAFAC
970 model. PARAFAC is a three-way multivariate method that can be applied on EEM

mathematical data of DOM in natural waters or in a mixture of model organic components in aqueous media, which is capable of the separation and quantification of specific fluorescent components. PARAFAC can often identify the major fluorescent components in DOM compositions, and it can not possible to isolate the minor fluorescent components occurrence in DOM fractions in natural waters[3].

995 **Figure Captions**

996 Fig. A1

997 Water sampling sites in the Kurose river (sites KR1 to KR6) and the Ohta river (sites
998 OR1 to OR6) in Hiroshima prefecture, Japan. Oblique lined areas indicate urban areas,
999 Hiroshima or Higashi-Hiroshima.

1000 Fig. A2

1001 Relationship between hydrogen peroxide and solar intensity estimated as MJ m^{-2} h^{-2}
1002 (Fig. 3a) or water temperature (Fig. 3b) in the waters of Ohta river.

1003 Fig. A3

1004 The EEM of the standard DSBP before irradiation (a) and after 20-h irradiation using
1005 solar simulator (b). Fig. b shows a fluorescence peak for 4BCA-like substances and also
1006 unknown peaks in course of decomposition of DSBP.

1007 Fig. A4

1008 Production of H_2O_2 as a result of light irradiation on the aqueous solutions of NO_2^-
1009 using solar simulator.

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Fig. A1

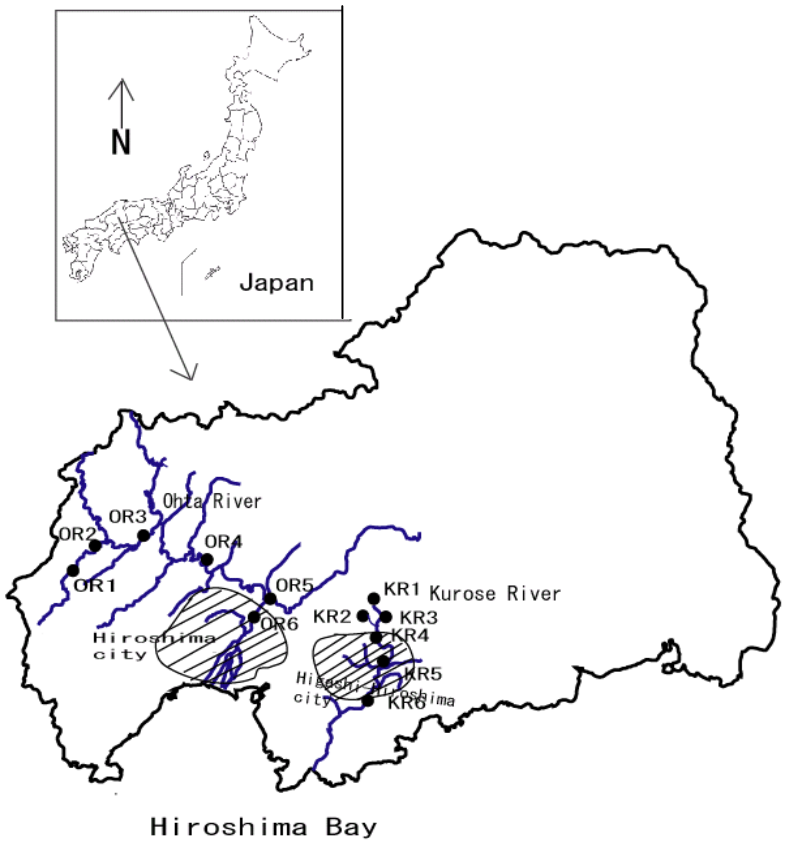

N
Japan
Ohta River
OR1
OR2
OR3
OR4
OR5
OR6
KR1 Kurose River
KR2
KR3
KR4
KR5
KR6
Hiroshima city
Higashi-Hiroshima city
Hiroshima Bay

Fig. A2

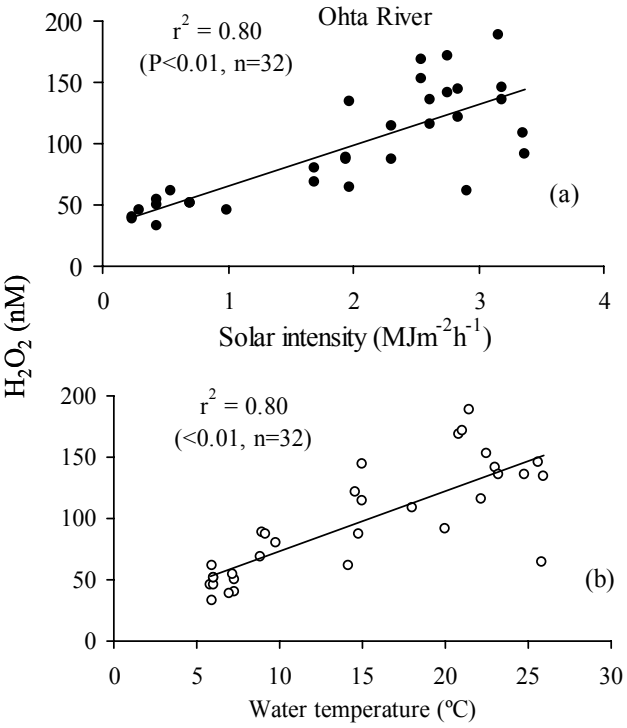
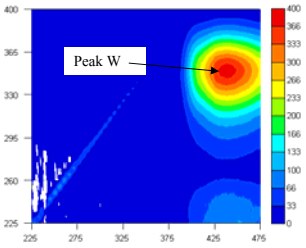
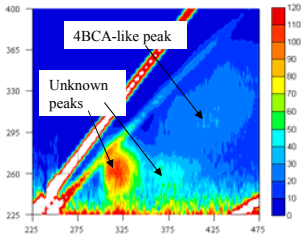

Ohta River
r² = 0.80
(P<0.01, n=32)
(a)
H2O2 (nM)
Solar intensity (MJm-2h-1)
r² = 0.80
(<0.01, n=32)
(b)
Water temperature (ºC)

Fig. A3



a. DSBP: unirradiated sample



b. DSBP: 20 h of irradiated sample

Fig. A4

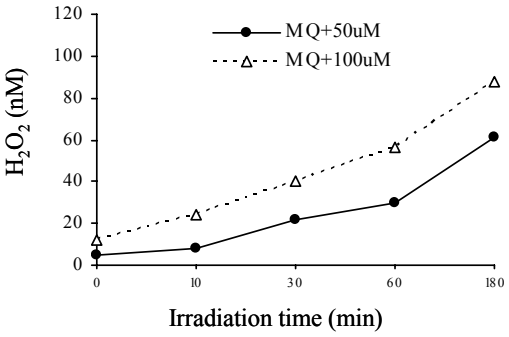

120
100
80
60
40
20
0
H_2O_2 (nM)
MQ+50uM
MQ+100uM
0
10
30
60
180
Irradiation time (min)

Table A1. Monthly variations of pH, water temperature (WT), solar intensity (SI), dissolved organic carbon (DOC), fluorescence peak intensity (FI) of fulvic acid-like substances (peak C), fluorescent whitening agents (peak W) and tryptophan-like substances (peak T), total Fe, Fe^{2+}, NO_3^-, NO_2^- H_2O_2 and ROOH at six smpling sites in the Kurose and the Ohta river waters in Hiroshima prefecture, Japan.

Sampling site and time	pH	WT	SI	DOC	FI		total Fe	Fe^{2+}	NO_3^-	NO_2^-	H_2O_2	ROOH
					Peaks (C or W)	Peak T						
		(^oC)	($MJm^{-2}h^{-1}$)	(μM C)	(QSU)			(μM)			(nM)	
Namitakiji (KR1)												
May 2002.	5.9	15.8	2.88	116±2.5	27	np	nd	nd	nd	nd	14±1.1	73±6.2
June 2002.	6.0	15.1	2.38	104±1.5	55	39	nd	nd	nd	nd	17±9.5	59±12.6
July 2002.	7.1	25.8	1.33	nd	68	67	nd	nd	nd	nd	21±4.2	44±8.4
August 2002.	7.4	25.4	1.12	146±3.9	55	np	54	nd	1.6	bd	33±9.8	nd
September 2002.	7.2	18.0	1.91	105±3.5	54	np	2	nd	8.8	bd	26±3.9	17±4.0
October 2002.	7.2	10.5	1.84	77±1.7	48	np	40	nd	5.5	bd	9±1.1	21±3.1
November 2002.	7.1	7.0	1.01	105±5.5	50	np	54	2.5	4.4	bd	6±0.6	25±2.0
December 2002.	6.9	6.0	0.76	87±6.4	52	np	20	0.2	6.4	bd	15±1.0	29±5.1
January 2003.	7.1	3.0	0.50	115±8.2	39	np	36	1.2	7.5	bd	11±0.5	9±1.0
February 2003.	7.3	5.6	1.01	47*	41	np	28	0.7	4.6	bd	9±1.1	20±5.8
March 2003.	7.0	8.7	1.91	51±3.9	51	np	8	2.8	14.2	bd	20±2.2	11±1.7
April 2003.	7.3	15.5	2.20	117±4.6	61	76	60	0.8	4.0	bd	16±4.9	30±12.6
December 2004.	8.0	9.0	0.53	nd	42	np	nd	nd	nd	bd	6±1.5	47±1.8
Mean	7.0	12.7±7.3	1.57±0.72	97±31	50±11	61±19	34±21	1±1	6±3		16±8	31±20
Shouriki (KR2)		12.7										
May 2002.	7.2	15.6	2.88	125±2.3	nd	np	nd	nd	nd	nd	29±1.6	41±1.8
June 2002.	7.1	16.1	2.38	88±5.9	59	np	nd	nd	nd	nd	68±9.0	65±5.3
July 2002.	7.1	22.3	1.33	nd	62	75	nd	nd	nd	nd	27±2.8	30±1.3
August 2002.	7.6	22.0	1.12	75±7.9	58	np	64	nd	7.4	bd	50±3.6	nd
September 2002.	7.3	19.0	1.91	66±2.0	45	np	20	nd	7.0	bd	37±1.4	19±0.6
October 2002.	7.7	11.5	1.84	124±2.5	39	np	48	nd	5.1	bd	19±1.6	17±2.4
November 2002.	7.7	7.5	1.01	146±2.6	44	34	48	2	4.3	bd	18±6.1	26±3.1
December 2002.	7.1	5.8	0.76	78±25	43	np	nd	nd	5.1	bd	21±3.3	35±7.0
January 2003.	7.3	2.0	0.50	53	32	19	10	1.3	7.7	bd	16±1.5	11±1.8
February 2003.	7.3	5.6	1.01	48±3	34	37	28	0.5	8.0	bd	21±7.7	26±7.1
March 2003.	7.2	8.9	1.91	43±3.9	40	np	40	3.2	8.7	bd	39±9.7	12±7.5
April 2003.	7.1	12.9	2.20	49±9.4	68	54	96.7	8.5	9.8	bd	19±3.4	22±10.9
December 2004.	7.5	9.0	0.53	nd	27	np	nd	nd	nd	bd	31±8.0	41±9.2
Mean	7.3	12.2±6.5	1.57±0.72	81±36	48±12	44±22	44±28	3±3	7±2		30±16	28±15
Sasa (KR3)												
June 2002.	6.5	18.0	2.74	123±7.8	145	np	nd	nd	nd	nd	49±8.6	66±9.4
December 2002.	7.3	7.0	0.97	143±9.5	117	np	6	0.0	28.7	nd	18±3.7	15±5.4
Tokumasa (KR4)												
June 2002.	7.3	19.5	2.27	154±1.3	245	86	nd	nd	nd	nd	29±3.1	28±0.7
December 2002.	7.2	7.0	1.22	146±25.8	380	93	30	0.0	71.4	1.7	31±2.4	9±2.7
Izumi (KR5)												
May 2002.	7.1	22.9	2.12	383±8.8	608	np	nd	nd	nd	nd	142±2.0	25±2.4
June 2002.	7.1	18.0	2.27	344±3.6	581	np	nd	nd	nd	nd	62±7.7	39±9.6
July 2002.	7.2	26.5	1.91	nd	666	np	nd	nd	nd	nd	76±6.6	32±11.1
August 2002.	7.6	27.2	0.94	349±0.7	659	np	176	nd	58.7	5.1	91±7.9	nd
September 2002.	7.4	22.2	2.16	215±2.1	606	207	88	nd	107.4	8.0	135±4.2	7±1.2
October 2002.	7.7	13.8	2.66	269±10.0	494	np	80	nd	84.3	6.1	77±1.2	1±1.0
November 2002.	7.4	8.2	1.3	244±6.0	548	182	60	3.2	131.9	4.7	16±2.5	14±1.1
December 2002.	8.4	7.0	1.22	319	589	np	180	0	110.9	1.4	14±3.2	3±1.7
January 2003.	7.2	2.0	0.76	212±23.9	504	240	158	6.5	127.9	2.5	9±1.5	3*
February 2003.	7.2	7.5	1.08	191±19.1	469	229	156	5.8	112.4	3.3	10±7.4	0
March 2003.	7.2	12.8	2.41	212±23.1	487	np	140	7.3	63.5	3.6	47±6.5	3±2.7
April 2003.	7.3	16.4	3.13	238±4.1	454	np	210	4.7	71.8	4.1	66±2.4	1±0.9
December 2004.	7.4	10.6	0.53	nd	263	np					47±3.2	5±3.5
Mean	7.4	15.0±8.0	1.83±0.75	271±67	555±73	215±25	139±51	5±3	97±28	4±2	62±46	12±14

Table A1. (Continued)

Sampling site and time	pH	WT	SI	DOC	FI		total Fe	Fe^{2+}	NO_3^-	NO_2^-	H_2O_2	ROOH
					Peak C or W	Peak T						
		(^oC)	($MJm^{-2}h^{-1}$)	(μM C)	(QSU)			(μM)			(nM)	
Hinotsume (KR6)												
May 2002.	5.8	22.3	2.12	323±8.3	445	210	nd	nd	nd	nd	213±2.5	43±4.0
June 2002.	7.1	21.0	2.27	287±3.6	421	np	nd	nd	nd	nd	135±8.0	67±7.9
July 2002.	7.3	27.0	1.91	nd	431	np	nd	nd	nd	nd	152±5.5	25±13.3
August 2002.	7.3	27.4	0.94	348±15	659	np	130	nd	97.8	47.3	105±4.0	nd
September 2002.	7.4	23.5	2.16	260±11.0	534	np	92	nd	128.8	57.3	149±4.1	29±2.0
October 2002.	7.9	15.0	2.66	273±5.3	500	np	74	nd	179.7	31.1	101±2.3	25±8.1
November 2002.	7.3	10.1	1.30	280±9.9	628	np	56	4.7	190.5	28.3	119±2.2	37±2.7
December 2002.	7.5	7.5	1.22	225	515	np	34	1.8	189.6	13.4	83±2.2	8±4.7
January 2003.	7.5	4.0	0.76	255±6.0	491	303	122	1.7	139	18.0	33±0.7	10±1.1
February 2003.	7.3	8.8	1.08	130±7.4	421	np	126	0.7	149.4	4.4	64±5.2	0
March 2003.	7.5	12.2	2.41	265±18.8	410	np	90	2.3	102.4	9.0	51±4.4	11±7.9
April 2003.	7.5	17.5	3.13	146±4.1	310	175	136.7	0.2	75.3	5.3	91±3.9	11±15.4
December 2004.	7.2	10.5	0.48	nd	224	np	nd	nd	nd	nd	80±7.7	45±4.1
Mean	7.3	15.9±7.8	1.83±0.75	254±66	481±97	229±66	96±36	2±2	139±42	24±19	108±50	24±19
Miwaku (OR1)												
June 2002.	5.97	14.2	2.91	59±1.5	43	np	nd	nd	nd	nd	61±7.2	36±4.6
December 2002.	6.90	5.9	0.55	nd	39	np	30	0.0	3.5	bd	61±5.1	25±7.6
Shintagiri (OR2)												
June 2002.	6.53	18.0	3.35	67±1.7	51	np	nd	nd	nd	nd	108±7.4	44±7.9
December 2002.	7.10	5.8	0.99	nd	36	15	16	2.0	17.7	bd	46±3.0	18±2.2
Doi (OR3)												
June 2002.	7.05	20.0	3.37	67±1.6	50	np	nd	nd	nd	nd	91±10.4	38±11.9
December 2002.	7.10	5.9	0.43	74±10.7	44	np	16	1.0	16.6	bd	33±2	17±3.1
Okawa (OR4)												
June 2002.	7.06	21.5	3.16	92±3.5	68	39	nd	nd	nd	nd	188±1.2	68±11.5
December 2002.	7.00	6.0	0.29	109±11.2	59	30	22	0.7	26.1	bd	46±7.3	16±7.1
Takase (OR5)												
May 2002.	5.5	22.5	2.54	123±8.3	84	44	nd	nd	nd	nd	153±5.3	51±1.1
June 2002.	7.1	23.0	2.75	101±1.1	101	np	nd	nd	nd	nd	142±1.8	61±6.2
July 2002.	7.2	24.8	3.19	nd	79	np	nd	nd	nd	nd	136±10.2	40±9.8
August 2002.	7.6	25.9	1.97	75±6.9	nd	np	76	nd	17.4	bd	135±6.2	nd
September 2002.	7.6	22.2	2.62	82±4.8	64	39	nd	nd	19.8	bd	116±6.6	32±4.5
October 2002.	7.3	15.0	2.31	164±6.0	53	44	40	nd	23.3	bd	114±1.3	19±0.7
November 2002.	7.6	8.9	1.69	104±1.9	47	np	40	3.5	17.7	bd	69±2.7	29±2.2
December 2002.	7.3	7.3	0.24	117±6.9	64	np	24	0	27.3	bd	40±2.7	9±3.5
January 2003.	7	6.0	0.70	133	44	40	14	5.3	34.9	bd	51±0.5	25±5.3
February 2003.	7.1	7.3	0.43	40±6.0	44	np	40	6.2	40.7	bd	50±7.7	23±1.9
March 2003.	7.1	9.0	1.94	70±7.7	46	np	16	7.3	64.6	bd	89±5.9	2±2.7
April 2003.	7.5	15.0	2.84	64±5.9	44	45	93.3	0.7	15.6	bd	144±1.6	33±15.5
Mean	7.2	15.6±7.7	1.94±0.99	98±36	66±25	42±3	38±30	4±3	29±16		105±43	28±19
Asa (OR6)												
May 2002.	6.6	20.8	2.54	137±1.5	75	np	nd	nd	nd	nd	168±6.9	52±5.5
June 2002.	7.1	21.0	2.75	94±1.2	82	np	nd	nd	nd	nd	171±7.7	80±5.0
July 2002.	7.3	25.6	3.19	nd	79	61	nd	nd	nd	nd	146±3.8	58±2.8
August 2002.	7.7	25.8	1.97	75±6.9	69	np	86	nd	23	bd	64±0.2	nd
September 2002.	7.3	23.2	2.62	76±12.9	65	45	10	nd	20.4	bd	136±0.6	29±1.6
October 2002.	7.3	14.8	2.31	114±3.9	59	np	18	nd	25.6	bd	87±3.1	8±3.2
November 2002.	8	9.8	1.69	83±4.0	47	31	32	2.7	26.6	bd	80±1.1	36±3.2
December 2002.	7	7.0	0.24	118±13.7	65	np	34	1.5	27.6	bd	38±0.7	10±2.8
January 2003.	7.2	6.0	0.70	96	50	np	48	4.2	30.7	bd	52±5.5	18±5.6
February 2003.	6.8	7.2	0.43	145	50	np	42	0.7	36.8	bd	55±7.8	8±3.0
March 2003.	7.5	9.2	1.94	66±3.6	45	np	8	2	20.3	bd	87±2.1	3±2.7
April 2003.	7.4	14.6	2.84	45±0.8	73	58	93.3	0	20.3	bd	121±2.5	31±13.7
Mean	7.3	15.4±7.6	1.94±0.99	95±31	63±13	49±14	41±31	2±1	26±6		102±49	30±25

'nd' means not detected; 'bd' mean concentration below detection limit.

± means the standard deviation among trilicate samples measured for each site.

* means data obtained only from single sample meansurement.

Table A2. Diurnal variations of pH, water temperature (WT), air temperature (AT), solar intensity (SI) and H_2O_2 in the upstream and downstream waters of the Kurose river.

Sampling	Upstream waters (site KR2)					Downstream waters (site KR6)				
	pH	WT (°C)	AT	SI (MJm^{-2})	H_2O_2 (nM)	pH	WT (°C)	AT	SI (MJm^{-2})	H_2O_2 (nM)
5:30 a.m.	7.0	19.2	22.5	0.00	9±1.3	7.1	19.5	18.5	0.00	4±1.3
8:00 a.m.	7.5	19.2	22.5	0.68	14±2.5	7.1	20.2	22	0.50	16±1.5
10:00 a.m.	7.4	20.0	24.8	2.09	23±1.9	7.0	22.0	19	2.16	31±4.5
11:00 a.m.	7.4	20.5	28.7	1.91	37±2.2	7.2	22.0	30.9	2.38	59±3.8
12:00 p.m.	7.4	20.8	28.5	2.74	43±4.1	7.1	23.0	30.5	2.52	63±3.1
13:00 p.m.	7.6	21.0	28.0	2.66	40±2.6	7.2	24.0	29.5	2.84	69±2.7
14:00 p.m.	7.5	21.0	28.0	1.80	34±4.5	7.2	24.5	29.5	2.63	69±5.5
15:00 p.m.	7.4	21.0	26.5	1.33	113±1.7	7.3	25.0	29	2.30	62±7.2
17:00 p.m.	7.5	21.0	26.5	1.30	27±2.6	7.3	24.5	27	1.04	62±12.5
19:00 p.m.	7.2	20.5	25.0	0.00	9±1.3	7.2	21.5	23.5	0.00	20±2.7

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