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# Detailed methods

## Sample collection and field analyses

Water was collected using an acid-washed PTFE tube connected to a sterile HSW NORM-JECT syringe to suction water from the spring. 1.5 μm and 0.2 μm Thermo Scientific Target 2 brand nylon filters were used for filtration. Prior to collection, the syringe, the filter, and the sample container were rinsed with 15 mL of spring water in this order: syringe; syringe and filter; syringe, filter, and container. Temperature and pH were measured and then separate samples were collected with a syringe for H2O2, anions, cations, nitrate (NO3) and ammonia (NH3), Fe(II), and alkalinity. Nitrate (NO3), ammonia (NH3), and Fe(II) were determined spectrophotometrically and alkalinity were determined by titration using Hach kits (Hach, Loveland, CO) in the field. The peroxide sample was passed immediately to the analyst who put them in a cooler on ice and in the dark.

Cation concentrations from Elk Spring and Octopus Spring were analyzed by a commercial laboratory (SGS, Lakeland, Ontario, Canada) by inductively coupled plasma argon emission spectrometry (ICP-AES) with a reported error of 7 %. Anions were analyzed at the Environmental Biogeochemistry Lab at the University of Montana using a Thermo Scientific Dionex ICS-2100 Ion Chromatography System. Sample anion concentrations were determined from a standard curve generated from 7 standards made by serial dilutions. An additional sulfate internal standard curve was run with an accuracy of 10%. Analytical replicates were within 3% and collection replicates were within 6%.

The DO sample was collected with a 60 mL BOD bottle (i.e. Winkler bottle; Wheaton, Shanghai, China) mounted on the end of a painter’s pole and submerged and inverted three times prior to retraction and addition of the manganese sulfate and alkali-iodide-azide reagents (LaMotte, Chestertown, MD). The bottles were then inverted 10 times and stored for acidification and titration in the field laboratory. We have used this method to collect samples and achieved low or at detection levels of DO (0 mM; Gonsior et al., 2018). We did not use a DO electrode because, although rated to 60**°**C, in our experience such electrodes fail at temperatures above 40**°**C due to the formation of bubbles under the membrane; the temperatures of hot springs in this study exceeded 40**°**C.

## Scopoletin fluorescence-quenching analysis of H2O2

Reagents were prepared in H2O2-free MilliQ water. 10 mg of scopoletin solid (Sigma-Aldrich) was added to 1 L of water to make a scopoletin stock (50μM), which was diluted 1:2 v/v just before use. The concentration of this scopoletin solution is 40 % higher than the concentration used by Zhang and Wong (1999). This change was made to enable sufficient scopoletin fluorescence for the method as determined in the field. Phosphate buffer (pH 7) was made using phosphate salt capsules (pHydrion Buffers). 10 mg horseradish peroxidase (HRP) (Sigma P8125, type I, 100 purpogallin units (p.u.)/ mg solid) and 1.5 mg of the toxin, sodium azide (NaN3), were added to 10 mL of water (100 p.u./mL) to make a HRP stock solution and prevent degradation, respectively (Holm et al., 1987). Holm et al. (1987) report the addition of NaN3 preserved the horseradish peroxidase reagent for 8 hours without refrigeration but up to two months if stored properly. Here, the horseradish peroxidase reagent was made fresh daily and was stored on ice in a dark cooler in the field. Laboratory tests of the stability of the scopoletin and HRP after mixing with the sample were conducted in the laboratory showed variation of scopoletin activity of 8-11 % with no trend and of hrp activity of 5-12% with a slight (p>0.01) downward trend after one hour; fluorescence measurements were made within 5 minutes for each sample after mixing. Stock H2O2 standard was made from serial dilutions of reagent grade H2O2 (Fisher Scientific 30% solution reagent ACS) and further diluted in the field. The concentration of H2O2 was measured by iodometric titration with sodium thiosulfate. All reagents and standards were stored in opaque HDPE containers. All reagent and standard diluents were made daily in the field to minimize potential degradation during the analyses. Additionally, scopoletin and HRP reagents were stored on ice in a dark cooler in the field or in the refrigerator in the laboratory.

Water was collected and filtered through a 0.2 μm Thermo Scientific Target 2 brand nylon filter, as described above, to minimize decay prior to analysis (Wilson et al., 2000b; Wong et al., 2015) and stored in the dark on ice until analysis, which commenced within 10 minutes. Next, 1 mL of sample was added to the cuvette and buffered to pH 7 (optimal pH). Buffer volume varied from 10-80 μL depending on sample pH and temperature. The buffer to sample ratio was determined by addition of known increments of the buffer to a known volume of filtered spring water until a pH of 7 was achieved; the ratio was calculated by dividing the buffer volume by the spring water volume. This determination was made at the beginning of each sample day. Fluorescence was read before and after buffer addition and showed no statistical change in fluorescence. Next, scopoletin was added in variable amounts to read ~90% of max PMT (photomultiplier tube) saturation. This volume varied from about 22-48 μL. 200 μL HRP was always added. The sample cuvette was pipette-mixed for 30 seconds and fluorescence intensity was read immediately after mixing.

H2O2 concentrations were calculated with a standard curve made each day in each hot spring matrix by adding known concentrations of H2O2 to separate cuvettes of the same sample in the field before addition of any reagents, and a linear regression analysis performed. Standard curves were analyzed in duplicate in each sample matrix. In addition spikes of 10-50 μL of 10 μM H2O2 stock were added to each sample in the field throughout the day to assure quality. The use of natural water samples for standardization obviated the potential for interferences due to ionic composition or elevated temperature. Standard curves for laboratory experiments were prepared by diluting the primary H2O2 standard to known concentrations with H2O2-free MilliQ (for method testing) or Elk Spring water (for experiments) prior to addition of reagents. Fluorescence intensity of each standard, whether prepared in hot spring water or in H2O2-free water, was plotted against concentration in a linear regression analysis and subsequent sample concentrations were determined using this standard curve. Use of standards prepared before the addition of any reagents obviates the concerns of Wu et al. (2017), who found that addition of the spikes after the reagents were added proved unreliable.

*Error Analysis*: Most measurements were analyzed in duplicate to determine error. Some samples were spiked with 1-3 standard additions as a method check to ensure reagent reactivity was consistent throughout each experiment. A minimum of 1 duplicate and 1 spiked sample were run along with each sample set analyzed together. Replicate analyses had an error of ±25% for concentrations less than 50 nM, ±15% for concentrations between 50-100 nM, and ±8% in experiments and ±12% for concentrations greater than 100 nM. The minimum detection limit (MDL) (20 nM) was determined by analyzing the H2O2 concentrations in H2O2-free MilliQ 10 times and taking their mean, plus 3 standard deviations.

## Field experiments with added Fe

The Fe-Si slides were prepared in the laboratory in a Class 100 clean hood by dipping petrographic slides in a solution of 0.125 Fe:Si (mole:mole; FeCl2 and LUDOX® colloidal Si) 9 times, allowing each coat to dry before dipping the slide again. Fe(II) oxidized to Fe3+ during drying periods, as evidenced by the formation of rust-colored solids. Fe was complexed in select experiments through the addition of sodium fluoride (NaF) at a ratio of 1,000:1 with Fe. Fluoride is a strong inorganic chelator of Fe (Table 2, Parkhurst and Appelo, 2016) that is naturally present in hydrothermal systems due to its common association with igneous minerals. In addition, F adsorbs to iron oxide surface, further removing reactive Fe from the system (Ding et al., 2012). This ratio was chosen so that F concentrations were sufficient to potentially complex nearly all Fe. Other analyses, i.e. dissolved O2 and Fe(II), although desirable, were not performed on experiments due to the difficulties in managing the H2O2 analyses in the field; i.e. requirement for immediate H2O2 analyses in Whirlpak bags or due to low volume (Fe experiments) of the experiments in tubes.

## Laboratory experiment with added Fe, F, superoxide dismutase, and catalase

Experiments conducted in the field were replicated in the laboratory to gauge the extent to which laboratory findings can be applied to natural systems. Differences between laboratory and field conditions likely include water composition, irradiance consistency, turbulence, and mixing/replenishing of water.

The particulate matter laboratory study explored the effect of particulate matter on photochemical H2O2 formation and dark H2O2 decay rates. This laboratory study also explored the effect of water type (i.e. different types of particulates) on H2O2 cycling, but the effect of oxygen was not explored. The particulate matter photochemical formation study exposed filtered and unfiltered waters (Elk Spring, Octopus Spring, and Purple Spring) to UVA (760-770 uW/cm2) and UVB (0.73-1.00 uW/cm2). The particulate matter dark decay study included filtered and unfiltered waters (Elk Spring, Octopus Spring, and a MilliQ control). Waters were spiked with 500 nM of H2O2 at the start and allowed to decay in dark conditions for 24 hours. Experiments were sampled at 0 hours, 4 hours, 6 hours, and 24 hours.

The iron-added formation study explored the effect of increasing iron concentrations on photochemical H2O2 formation in various hydrothermal water types (Elk Spring, Octopus Spring, and a MilliQ control). In Elk Spring waters, the effect of filtration and iron-addition on H2O2 formation was explored as well. Waters were exposed to UVA (763-770 μW/cm2) and UVB (0.80-1.00 μW/cm2). Iron was added in the form of sterile iron-silicate slides (preparation described in Field Study Methods). Total soluble iron was also analyzed at the end of the experiment.

The iron-complexation formation study explored fluoride’s ability to photochemically inactivate iron through complexation in various hydrothermal water types (Elk Spring and Octopus Spring). Photochemical inactivation should shut down photo-Fenton reactions that H2O2 formation. Filtered waters were exposed to UVA (767-772 μW/cm2) and UVB (0.83-1.02 μW/cm2). Iron was added to some experiments in the form of sterile iron-silicate slides and fluoride was added to other experiments in the form of sodium fluoride at a ratio of 1,000:1 with iron. This ratio was chosen so that fluoride concentrations were sufficient to potentially complex all iron. Total soluble iron was also analyzed at the end of the experiment.

Catalase experiments explored the effect of catalase on H2O2 formation in various hydrothermal water types (Elk Spring, Octopus Spring, and MilliQ control). Filtered waters were exposed to UVA (765-770 μW/cm2) and UVB (0.79-1.00 μW/cm2). Catalase was added in excess at 1,000 mg/L.

Superoxide dismutase (SOD) experiments explored the affect of this enzyme on H2O2 formation in natural hydrothermal water types (Elk Spring, Octopus Spring, and a MilliQ control). Filtered waters were exposed to UVA (760-770 μW/cm2) and UVB (0.73-1.00 μW/cm2). SOD was added in excess at 20 kU L-1.

## References

Ding, X., Song, X., and Boily, J.-F. (2012). Identification of Fluoride and Phosphate Binding Sites at FeOOH Surfaces. *Journal of Physical Chemistry C* 116**,** 21939-21947.

Holm, T.R., George, G.K., and Barcelona, M.J. (1987). Fluorometric-Determination of Hydrogen-Peroxide in Groundwater. *Analytical Chemistry* 59**,** 582-586.

Zhang, L.-S., and Wong, G.T. (1999). Optimal conditions and sample storage for the determination of H2O2 in marine waters by the scopoletin–horseradish peroxidase fluorometric method. *Talanta* 48**,** 1031-1038.

# Tables

## Sample Collection and Storage

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Analyte | Bottle Type | Bottle Rinse | Filtration | Acidification | Storage |
| H2O2 | HDPE | 1 x | filtered, 0.2 μm | none | dark, ice |
| Total soluble Fe | HDPE | 1x | filtered, 0.2 μm | hydrochloric acid | dark |
| DO | Glass vial | none | none | none | upright, dark |
| Alkalinity | HDPE | none | none | none | dark, ice |
| NH3 | HDPE | 3 x | filtered, 0.2 μm | sulfuric acid | dark, ice |
| NO3 | HDPE | 3 x | filtered, 0.2 μm | sulfuric acid | dark, ice |
| Metals/Cations | HDPE | 3 x | filtered, 0.2 μm | nitric acid | dark, ice |
| Anions | HDPE | 3 x | filtered, 0.2 μm | none | dark, ice |

## Analytical Methods Summary

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analyte | Method | Reference | Completion Time | LOD |
| Temperature/pH | Orion electrode (model 91-57BN) |  |  |  |
| H2O2 | Fluorescence quenching | Holm et al. (1987) | <30 minutes | 10-700 nM |
| Total soluble Fe | Ferrozine Method | Lovely and Phillips (1987) | <30 minutes | 0.1-10 mg/L |
| DO | Winkler Titration with LaMotte DO test kit | Helm et al. (2012) | <3 days | - |
| Alkalinity | HACH Titration Method 8203 |  | <7 days | - |
| NH3 | HACH Method 8155 |  | <7 days | 0.03-0.5 mg/L |
| NO3 | HACH Method 8192 |  | <15 days | 0.03-0.5 mg/L |
| Metals/Cations | ICP-AES, SGS Canada |  | <90 days | see Table 5 |
| Anions | IC, University of Montana Biogeochemistry Lab |  | <17 days | see Table 6 |

## **Schematic diagram showing experimental setup for** in situ **measurements, field (bag and tube) experiments, and laboratory experiiments with Elk Spring water.** In situ samples were taken from the spring and analyzed after filtration. Water was collected from the spring and treated as per each condition and placed in bags incubated in the spring or tubes incubated beside the spring. Water was collected from the spring and transported to the laboratory. The water was filtered prior to laboratory experiments.

Diagram

Description automatically generated

## Elk Spring *in situ* study summary

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | July 17 | | July 18 | | September 29 | |
| Analyte | Average | Maximum | Average | Maximum | Average | Maximum |
| H2O2 (nM) | 76 | 284 | 217 | 644 | 158 | 431 |
| UVA (mW/cm2) | 2.1 | 3.9 | 2.2 | 3.9 | 1.4 | 2.9 |
| UVB (μW/cm2) | 98.2 | 212.0 | 121.1 | 215.0 | 61.4 | 124.8 |
| Total soluble Fe (mg/L) | <0.1 | - | 0.4 | - | 0.2 | - |
| Total Fe (ICP) (mg/L) | <0.2 | - | - | - | 1.2 | - |
| pH | 3.93 | - | 4.05 | - | 3.31 | - |
| Air Temperature (°C) | - | 25.6 | - | 25.6 | - | 12.8 |
| Est. Wind Speed | - | 17 | - | 9 | - | 11 |

## Summary of field experiments. Values are averages of H2O2 formation rate in each experiment.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Site | Filtered? | Water | None | Fe |
| Light | Elk | Yes | *in situ* | 214 |  |
| Light | Elk | No | Exp | 27 | 87 |
| Light | Elk | Yes | Exp | 188 | 91 |
| Light | Elk | Yes | MQ |  | b.d. |
| Dark | Elk | No | Exp | b.d. |  |
| Dark | Elk | Yes | Exp | b.d. |  |
| Dark | Elk | Yes | MQ |  | b.d. |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| <50 |  | 50-100 |  | 100-150 |  | 150-200 |  | 200-300 |  | no data |

## Weather observations at Elk Spring.

|  |  |  |
| --- | --- | --- |
|  | Morning | Afternoon |
| July 17, 2017 | Steam cover persisted until ~08:00. Skies were overcast from sunrise to 08:00 and remained partly cloudy until 11:00. Skies were clear from 11:00-11:30 when heavy winds blew in dense clouds.  Average wind speed estimate: 2 mph.\* | Skies remained partly cloudy throughout the day. Clouds covered the spring about 75% of the time. The remaining 25% consisted of sunny patches above the spring between cloud cover. The last measurement was collected at 16:00 due to intense cloud cover, heavy winds, and equipment malfunction.  Average wind speed estimate: 17 mph.\* |
| July 18, 2017 | Steam cover and overcast skies until ~08:00 when the sky and steam both cleared. It remained sunny until about 12:00.  Average wind speed estimate: 1 mph.\* | Skies became partly cloudy ~12:00 and remained that way for the rest of the afternoon. *Cloud cover was less dense than July 17.*  Average wind speed estimate: 9 mph.\* |
| September 29, 2017 | Light steam persisted until ~10:00. Skies were clear and sunny the rest of the morning.  Average wind speed estimate: 0 mph.\* | Skies were clear and sunny until ~13:00 when scattered clouds blew in. Partial cloud cover persisted through the afternoon with prolonged sunny patches between cloud cover. *Cloud density was comparable to that of July 18.*  Average wind speed estimate: 11 mph.\* |

## H2O2 concentrations *in situ* and for diel field experiments.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time | Date | T | pH | H2O2 | H2O2 (ave) | Fe(II) | | NH4 | UVA | UVB | Comments |
|  |  | C |  | nM/L | nM/L | mg/L | | mg/L | (mW/cm2) | (uW/cm2) |  |
| 0:00 | 7/17 |  |  |  |  |  | |  |  |  | foggy, and overcast, thick cloud cover |
| 6:35 | 7/17 |  |  |  |  |  | |  | 0.1405 | 2.7 | foggy, overcast |
| 6:50 | 7/17 | 72.5 | 3.93 |  |  |  | |  |  |  |  |
| 7:00 | 7/17 |  |  | <20 | 0 |  | |  |  |  |  |
| 8:15 | 7/17 |  |  |  |  |  | | 0.07 |  |  | max- partly cloudy |
| 8:45 | 7/17 |  |  |  |  |  | |  | 1.633 | 51.6 | min- when clouds were covering |
| 8:45 | 7/17 |  |  |  |  |  | |  | 1.488 | 40.5 |  |
| 8:50 | 7/17 |  |  | <20 | 0 |  | |  |  |  |  |
| 9:20 | 7/17 |  |  | <20 | 0 |  | |  |  |  |  |
| 11:00 | 7/17 |  |  | 268, 227 | 247.5 |  | |  | 2.93 | 153.1 | partly cloudy, winds picking up around noon. Cloud cover increased around noon as well |
| 12:50 | 7/17 |  |  |  |  |  | |  | 3.88 | 212 |  |
| 13:45 | 7/17 |  |  | 74, 66 | 70 |  | |  |  |  | eagle scouts took all syringes and im still not over it |
| 14:05 | 7/17 |  |  |  |  |  | |  | 3.04 | 196 |  |
| 15:00 | 7/17 |  |  |  |  |  | |  | 0.5 | 10 | sunny, partly cloudy, windy |
| 16:00 | 7/17 |  |  |  |  |  | |  | 2.77 | 119.5 | cloudy and overcast, foggy |
| 6:40 | 7/18 |  |  | <20 | 0 |  | | 0.15, 0.14 |  |  |  |
| 7:00 | 7/18 | 71.3 | 4.01 |  |  |  | |  | 0.295 | 4.6 |  |
| 9:05 | 7/18 |  |  |  |  |  | |  | 2.04 | 56.4 | sunny, no clouds |
| 9:40 | 7/18 |  |  | 66, 61 | 63.5 |  | |  |  |  | tube experiments put out at this time |
| 11:00 | 7/18 |  |  |  |  |  | |  | 3.09 | 159.7 | sample collected from bags. Sunny conditions just until I got to the spring, then clouds came out and covered sun just above the spring. Clouds cleared and sun came back out just as I finished sampling bags |
| 12:00 | 7/18 |  |  |  |  |  | |  |  |  | bags sampled from 12-12:30 |
| 12:30 | 7/18 |  |  | 652, 636 | 644 |  | |  |  |  | max- when sun is uncovered by clouds |
| 12:40 | 7/18 |  |  |  |  |  | |  | 2.57 | 212.2 | min- when clouds are overhead. Conditions were partly cloudy, rapidly fluctuating between max and min conditions at this time |
| 12:40 | 7/18 |  |  |  |  |  | |  |  | 207.3 | sunny with light cloud coverage |
| 13:20 | 7/18 |  |  |  |  |  | |  | 3.93 | 215.1 | cloudy conditions- cloud cover over spring area |
| 13:35 | 7/18 |  |  |  |  |  | |  | 1.28 | 92.5 | sunny, partly cloudy. Sun out at time of sample collection-- sampled tubes and spring at this time |
| 14:30 | 7/18 |  |  | 31, 35 | 33 |  | |  | 3.09 | 178.2 | measurement taken during full sun |
| 15:35 | 7/18 |  |  |  |  |  | |  | 3.34 | 162.2 |  |
| 16:00 | 7/18 |  |  |  |  |  | | 0.25 |  |  | measured bags and spring |
| 16:30 | 7/18 |  |  | 531, 515 | 523 |  | |  |  |  | meas taken at full sun |
| 17:00 | 7/18 | 68 | 3.99 |  |  |  | |  | 2.14 | 90.3 |  |
| 18:00 | 7/18 |  |  |  |  |  | |  | 1.24 | 54.9 | measured tubs last time, took ph and fe samples and temp, kept fe slides but didn’t do anything with them. Still in 318 |
| 19:00 | 7/18 |  |  |  |  |  | |  | 0.76 | 20 | measured bags again, took pH and temp of bags and spring |
| 19:40 | 7/18 | 65.6 | 4.15 | <20 | 0 |  | |  |  |  |  |
| 7:12 | 9/29 |  |  |  | 19 | 0.35 | | 0.25 |  |  | add 60 ul scop and 40 ul buffer and 200 ul hrp |
| 9:10 | 9/29 |  |  |  |  |  | |  | 0.87 | 24.7 | low fog, with full sun |
| 10:16 | 9/29 |  |  |  |  |  | |  | 1.675 | 68.8 | clear, full sun |
| 11:00 | 9/29 | 70 | 3.31 |  |  |  | |  |  |  |  |
| 11:10 | 9/29 |  |  |  |  |  | |  | 2.28 | 98.9 | full sun, nitrate 0.086 mg/L |
| 11:30 | 9/29 |  |  |  | 430 |  | |  |  |  |  |
| 11:55 | 9/29 |  |  |  |  | 0.24 | |  |  |  |  |
| 12:00 | 9/29 |  |  |  |  |  | |  | 2.52 | 110.5 | full sun |
| 12:12 | 9/29 |  |  |  | 431 |  | |  |  |  |  |
| 12:21 | 9/29 |  |  |  |  | 0.35 | |  |  |  |  |
| 12:50 | 9/29 |  |  |  |  |  | |  | 2.89 | 124.8 | full sun |
| 12:56 | 9/29 |  |  |  |  |  | |  | 1.122 | 97 | partial cloud cover |
| 13:00 | 9/29 |  |  |  | 372 |  | |  |  |  |  |
| 13:40 | 9/29 |  |  |  |  |  | |  | 2.042 | 119.3 | mostly sunny |
| 13:44 | 9/29 |  |  |  |  |  | |  | 2.89 | 51.2 | full sun for UV-A, cloud cover for UV-B. UV-B increased to ~60 in full sun |
| 13:57 | 9/29 |  |  |  |  |  | |  | 1.118 | 61.5 | cloud cover |
| 14:00 | 9/29 |  |  |  | 133 |  | |  |  |  |  |
| 15:15 | 9/29 |  |  |  | 85 | 0.11 | |  |  |  |  |
| 15:18 | 9/29 |  |  |  |  |  | |  | 1.124 | 48.5 | cloud cover |
| 16:00 | 9/29 |  |  |  | 50 | <0.1 | |  |  |  |  |
| 16:10 | 9/29 |  |  |  |  |  | |  | 0.891 | 30.2 | full clouds |
| 17:00 | 9/29 |  |  |  | 21 | <0.1 | |  |  |  |  |
| 17:04 | 9/29 |  |  |  |  |  | |  | 0.46 | 15.6 | cloudy, overcast |
| 18:00 | 9/29 |  |  |  | <20 | <0.1 | |  |  |  |  |
| 18:06 | 9/29 |  |  |  |  |  | |  | 0.265 | 7.7 | cloudy |
| 18:53 | 9/29 |  |  |  |  |  | |  | 0.049 | 0.6 | cloudy |
| 19:00 | 9/29 |  |  |  | 27 | <0.1 | |  |  |  |  |
|  | | | | | | |
|  |  |  |  |  |  |  | |  |  |  |  |

Fe samples were collected, acidified and analyzed within 24 hours in lab trailer on colorimeter via ferrozine. H2O2 measured onsite. Last 2 samples analyzed at camp ~2-3 hrs later because batteries died.

## Experimental data for field experiments as a function of treatment.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Amendment | 9:00 | 12:30 | 14:18-14:30 | 16:30 | 19:00-19:30 | pH | Fe average | Formation rate | UVA+  UVB |
|  |  |  | [H2O2] nM | [H2O2] nM | [H2O2] nM | [H2O2] nM | [H2O2] nM |  | mg/L | (nM/W/s/cm2) | (W/cm2) |
| Elk Spring | in situ |  | 65 | 652 | 31 | 531 | <20 |  | - | 211.55 | 0.003083 |
| Elk Spring | in situ |  | 73 | 636 | 35 | 515 |  |  | - | 202.90 |  |
| Filtered | bag | None | 69 | 580 | - | 23 | <20 | 4.12 | - | 184.16 |  |
| Filtered | bag | None | 69 | 602 | - | 32 |  |  |  | 192.09 |  |
| Unfiltered | bag | None | 69 | 149 | - | 14 | <20 | 4.08 | - | 28.83 |  |
| Unfiltered | bag | None | 69 | 140 | - | - |  |  |  | 25.59 |  |
| Filtered, dark | bag | None | 69 | <20 | - | <15 | <20 | 4.19 | - | - |  |
| Filtered, dark | bag | None | 69 | <20 | - | <15 |  |  |  | - |  |
| Unfiltered, dark | bag | None | 69 | <20 | - | <15 | <20 | 4.07 | - | - |  |
| Unfiltered, dark | bag | None | 69 | <20 | - | - |  |  |  | - |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Elk Spring | in situ |  | 65 | 652 | 31 | 531 | <20 | 4.2 | 0.35 | 225.24 | 0.003385 |
| Elk Spring | in situ |  | 73 | 636 | 35 | 515 | - |  |  | 216.23 |  |
| Filtered | tube | Fe | 69 | - | 45 | nd | 282 | 3.9 | 1.896 | 94.42 |  |
| Filtered | tube | Fe | 69 | - | 22 | nd | 265 |  |  | 87.57 |  |
| Unfiltered | tube | Fe | 69 | - | 69 | nd | 306 | 4.9 | 2.791 | 85.41 |  |
| Unfiltered | tube | Fe | 69 | - | 77 | nd | 323 |  |  | 88.66 |  |
| MilliQ | tube | Fe | 69 | - | <20 | nd | 178 | 3.6 | 3.785 | - |  |
| MilliQ | tube | Fe | 69 | - | - | nd | 156 |  |  | - |  |
| Filtered MilliQ, dark | tube | Fe | 69 | - | <20 | nd | <20 | 3.9 | 1.626 | - |  |
| Filtered MilliQ, dark | tube | Fe | 69 | - | - | nd | <20 |  |  | - |  |

## Summary of laboratory experiments. Values are averages of H2O2 formation rate in each experiment.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Site | Filtered? | None | Fe | Fe + F | Catalase | SOD |
| Light | Elk | Yes | 0.265 | 0.411 | 0.113 | b.d. | 0.170 |
| Light | Octopus | Yes | 0.161 | 0.202 | 0.065 | b.d. | 0.030 |
| Light | Purple Pool | Yes | 0.167 |  |  |  |  |
| Light | MilliQ | Yes | 0.132 | 0.166 |  | b.d. | b.d. |
| Light | Elk | No | 0.284 | 0.435 |  |  |  |
| Light | Octopus | No | 0.187 |  |  |  |  |
| Light | Purple Pool | No | 0.277 |  |  |  |  |
| Dark | Elk | No | b.d. |  |  |  |  |
| Dark | MilliQ | Yes |  | 0.027 |  |  |  |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| <0.1 |  | 0.1-0.2 |  | 0.2-0.3 |  | 0.3-0.4 |  | 0.4-0.5 |  | No data |  |

## Direction of effect of three factors on H2O2 formation rates

|  |  |  |  |
| --- | --- | --- | --- |
| **Factor** | **Heat** | **Wind** | **Clouds** |
| **Effect** |  |  |  |

Bruskov et al. (2002a,b) reported an increase in H2O2 formation rate with increasing temperature, an effect that was not observed by Wilson et al. (2000b). Cooper et al. (1994) and Cooper and Lean (1989) reported a decrease in H2O2 formation rate with increasing wind speed. Cooper and Zika (1983) reported that H2O2 formation is a function of incident near-UV radiation.

## % Fe Speciation with and without F

|  |  |  |
| --- | --- | --- |
| Species | Elk Spring | Elk Spring + F |
|  | % | % |
| Fe+3 | <0.01 | <0.01 |
| FeOH+2 | 4.45 | <0.01 |
| Fe(OH)2+ | 36.34 | <0.00 |
| FeF3 | 13.53 | 96.96 |
| FeF2+ | 40.69 | 3.03 |
| FeF+2 | 4.71 | 0.01 |