Effect of hydroxyl radical on harmful microalgae: a potential technology for treatment of ship’s ballast water

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In this study, the effects of hydroxyl radicals on harmful microalgae in ballast water were investigated experimentally. Using an improvised ballast water treatment system, large concentrations of ·OH were produced and subsequently dissolved in ballast waters of different salinities which are high salinity seawater (HS) and low salinity seawater (LS) in order to eliminate the microalgae. The results show that the outcome of the treatment system fully meets the requirements of G8. At total residual oxidant (TRO) concentrations of 0.41 mg/L and 0.93 mg/L, the maximum concentration of killed algae was observed as 0.5×10³ cells/mL and 1×10⁴ cells/mL, respectively. Furthermore, the ·OH efficiently decomposed most organic matter, resulting in an improvement of the ship’s ballast water quality. These results conform to the D-2 ballast water discharge standard of IMO and validate the ·OH as an effective, rapid way of killing algae in the course of conveying the ship’s ballast water.

[Keywords: Ballast water treatment, Hydroxyl radical, Strong ionization discharge, Harmful microalgae, Water quality]

Introduction

Every year, around 100 million tons of ballast water are transported by ships around the world¹. Each cubic meter of ballast water contains over 100 million planktons. Therefore, there are thousands of species of plankton spread around the world every day with ballast water². With the growing problem of biological invasion caused by ballast water, research begun into the species of phytoplankton present in ballast water. The earliest research was conducted in the 1970s³. Researches then progressed to the study of phytoplankton that had the ability to survive for long periods in ballast water. One such study investigated ballast water in nine ships which reached Morehead port, North Carolina. The ballast water originated from Japan, Spain, the Dominican Republic, Belgium, and America. A total of 342 kinds of phytoplankton were detected alive, of which up to 145 species were from Belgian ports⁴. One study investigated phytoplankton activity in seven ships’ ballast water, which arrived into Tasmania, Australia. Their results showed that after 17 to 20 days of voyage, there were still 31 kinds of phytoplankton alive⁵. From September 2002 to July 2004, Burkholder et al. (2007) analyzed 28 ships’ ballast water arriving into U.S. ports. One hundred kinds of phytoplankton were discovered, including 23 types of harmful algae⁶. Between April and November 2007, a total of 67 ballast water samples from the ports of Canada and Detroit were tested. Overall, 52 varieties of Chaetoceros were detected and most of them were still alive⁷.

The method of ultraviolet light is usually combined with a filtering device; after filtration, the ballast water is treated by UV⁸. Ultraviolet light can effectively kill a variety of microorganisms, but it has poor penetration and efficiency can be impacted by color, turbidity,
organic matter, and salts in ballast water. Ozone is a strong oxidant and a concentration of 10 mg/L can kill single-celled organisms as well as some invertebrates with high tolerance. In addition, a concentration of 100 mg/L of ozone leads to the inactivation of cysts. However, ozone accelerates the corrosion of ballast tanks and is difficult to maintain at certain residual bactericidal concentrations. Therefore, ozone is deemed unsuitable for shipboard ballast water treatment. Electrolysis produces chlorine and sodium hypochlorite, which kill invading microbes. Chlorine concentrations greater than 10 mg/L kill algae and other aquatic vertebrates, unicellular microorganisms as well as some invertebrates. Electrolysis also results in the production of trihalomethanes and other carcinogenic organic matter, causing secondary pollution. The exchange of ballast water in open seas is considered to be the most feasible method for reducing the invasion of alien species. It works on the assumption that the offshore species will not survive in coastal waters and vice versa. However, this method also suffers from certain limitations, such as management of the sludge, safety of ship during rough weather condition and requirement of high energy consumption and operational time.

In addition to the above methods, hydroxyl radical (OH) production using advanced oxidation technology (AOT) is a new method for the treatment of ballast water. By using a strong electric field, oxygen and \( \text{H}_2\text{O}_{\text{gas}} \) are ionized. A mass of active particles (with OH as the main component) are produced. Using OH, bacteria and plankton are killed within a few seconds with no pharmaceutical residues. In the present study this method was used to evaluate the effect of OH ions on phytoplankton cells in the test water and the results of the same are presented.

**Materials and Methods**

Five red-tide causing species, belonging to 3 phyla, were procured from the Liaoning Marine Fisheries Research Institute, China. The cell size of the algae was between 10–50 \( \mu \text{m} \) (Table 1). The algae were cultivated in 5 liter Erlenmeyer flasks, using nutrient enriched F/2 medium. The cultures were maintained at 20±1 °C, pH of 7.2, with light intensity of 2,600 Lux, and a light to dark ratio of 10:14. All algal species used in the experiments were in their exponential growth phase. The cell density ranged from \( 5\times10^5 \) to \( 2\times10^6 \text{cells/mL} \).

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Species</th>
<th>Body size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyta</td>
<td><em>Skeletonema costatum</em></td>
<td>20 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Thalassiosira rotula</td>
<td></td>
<td>40–50 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Pyrrophyta</td>
<td><em>Alexandrium tamarense</em></td>
<td>40 ( \mu \text{m} )</td>
</tr>
<tr>
<td></td>
<td><em>Karenia mikimotoi</em></td>
<td>30–40 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Xanthophyta</td>
<td><em>Heterosigma akashiwo</em></td>
<td>15 ( \mu \text{m} )</td>
</tr>
<tr>
<td></td>
<td><em>Hada</em></td>
<td></td>
</tr>
</tbody>
</table>

Original seawater was obtained from Guanglu Island, Dalian, China. According to the Guidelines for approval of Ballast Water Management Systems (G8), the challenge water should have dissolved and particulate content in one of the combinations with the given salinities (Table 2).

<table>
<thead>
<tr>
<th>Item</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved</td>
<td>&gt; 32 PSU</td>
</tr>
<tr>
<td>Organic Carbon (DOC)</td>
<td>&gt; 1 mg/L</td>
</tr>
<tr>
<td>Organic Carbon (POC)</td>
<td>&gt; 1 mg/L</td>
</tr>
<tr>
<td>Total Suspended Solids (TSS)</td>
<td>&gt; 1 mg/L</td>
</tr>
</tbody>
</table>

Note: Sets was separated by practical salinity unit (PSU).

In the test, at least two sets should be conducted, each with a different salinity range and associated dissolved and particulate content. Tests under adjacent salinity ranges in the above table should be separated by at least 10 PSU.

Keeping this in view, in the present study the challenge water with salinity of 33.7 PSU,
meeting the G8 requirements was used. The original seawater (stock) was labeled as high salinity seawater (HS) in the experiment. Subsequent lower salinity (23.1 PSU) was obtained by diluting the HS with fresh/distilled water and labeled as LS. Meanwhile, sea bottom deposits acquired from the Yellow Sea, Dalian, China, were added to LS samples to meet the particulate organic carbon (POC) requirements (10 g sea bottom deposits for every 1 m$^3$ of LS). Humic acid, procured from the Tianjin Guangfu Fine Chemical Research Institute, China, was added to the LS set to raise dissolved organic carbon (DOC) concentrations above 5 mg/L (6 g humic acid for every 1 m$^3$ of LS). Diatomite procured from Taixiang Company, China, was also added to LS to raise the TSS above 50 mg/L (60 g diatomite for every 1 m$^3$ of LS).

Total organic carbon (TOC), DOC, and POC were measured using a TOC analyzer (liquid TOC II, Elementar Analysensysteme GmbH, Germany) following the US EPA 415.3 guidelines. TSS was estimated gravimetrically according to GB 17378.4–2007. The other water quality parameters such as, salinity, temperature, pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), and turbidity were measured by multi-parameter water quality sondes (YSI-6600 V2, USA).

In order to meet the requirements of G8 (cell density, 1,000 - 10,000 cells/liter), the test water was seeded with algal culture. Five different test waters were formulated with two different salinities. Two of the test waters contained all five algal species with concentrations of 10,000±1,000 cells/mL (the concentration of each algal species was 2,000±200 cells/mL) and 5,000±500 cells/mL (the concentration of each algal species was 1,000±100 cells/mL). The three remaining test waters contained a single species of algae with a concentration of 10,000±1,000 cells/mL. The three single algal species were *Skeletonema costatum*, *Alexandrium tamarense*, and *Heterosigma akashiwo* Hada.

In the ballast water treatment system used (Figure 1), the core part is the apparatus for producing ·OH. In this device, the plasma discharge of a strong electric-filed in a whole micro-gap consists of large numbers of micro-streamers. This process involves a well-distributed, diffuse, and stable discharge phenomenon akin to glow discharge (Figure 2). In the micro-streamer channel, a large number of active particles are produced resulting in a series of plasma chemistry reactions\textsuperscript{17}. The handling capacity of the system is 10 ton/h. The challenge water is pumped into the main line. The mechanical filter separates plankton larger than 50 μm. Next, part of the challenge water passes through the improvised equipment to generate a solution of high ·OH concentration, which is injected into the liquid-liquid dissolver. Now, the fully miscible and diluted ·OH solution is of a suitable concentration for killing algae in the
main line. The surplus ·OH is decomposed into H₂O and O₂, thus, there are no negative effects on the marine environment using the principles of AOT.

During the ·OH treatment, all radicals are converted into other active oxygen particles, which are mainly ·OH radicals but also include HO₂, O₂, HO₂, O₂H₂O, etc. These chemical species are able to kill harmful aquatic organisms and pathogens in nanoseconds. TRO measurements were used as a measure of the ·OH concentrations. The TRO accounts for the hypobromous acid/hypobromite (HOBr/OBr⁻) and bromamines (NH₂Br, NHBr₂, NBr₃) present, which are produced by ·OH radicals reacting with the bromide ion (Br⁻) in seawater. These species have particular toxic effects on algae during the storage periods. The TRO concentrations were measured using CE2501 spectrophotometer (BioQuest, UK) with the N,N-diethyl-p-phenylenediamine method (DPD method, US EPA Method 8016). In this system, the TRO concentration was controlled by the flow of oxygen and H₂O gas.

![Figure 2. Plasma reactor with strong ionization discharge](image)

The experimental set up was divided into two steps. The first step was to determine the minimum TRO concentration required for killing algae. Part of the test water was made into a solution of high ·OH concentration. It was then added directly into the remaining test waters in different proportions. The total volume of test water was 1 L, thus, treatment effects on algae at different TRO concentrations were obtained. Finally, the minimum TRO concentrations for killing different concentrations of algae were found. The second step relied on the experimental results obtained in the first step. This was needed to set the required TRO concentration in the ballast water treatment system. The test water contained a mixture of five kinds of algae with a concentration of 10,000±1,000 cells/mL. The volume of the test water was 1 m³. After the system was stabilized (1 min), samples (5 L of each sample) were taken at 30 sec. intervals to verify the efficiency of the experimental system. A total of 5 replicate samples were taken for analyses untreated samples were used as the control.

Algal cells were enumerated following the direct count method according to GB17378-2007. The treated seawater was first screened through a 35 μm mesh sieve (50 μm diagonal) and then concentrated on a 7 μm sieve (10 μm diagonal). Thus a 5 L sample of treated water was concentrated to 10 mL. Cells were then examined for their destruction under a Olympus microscope (CX31). Morphological changes of algae, before and after treatments, were also studied and pictures of the same were taken.

Algal chlorophyll was measured using fluorescence technique with chlorophyll a standard as reference material. Water samples of 500 ml were passed through GF/F (47 mm) glass fiber filters under reduced pressure. Filters were then subjected to extraction in acetone at 4°C in dark for 15 hrs and used for estimation of chlorophyll a. The extract was excited at 432 nm and the resulting emission was recorded at 666 nm wavelength using fluorescence spectrophotometer (Hitachi F-4500).

The efficiency with which ·OH kills algae in HS and LS waters was analyzed using one way ANOVA through SPSS 14.0 software. A fixed model was adopted for the effects of salinity on the survival of algae using the following equation:

\[ y_{ij} = \mu + G_i + e_{ij} \]

where \( y_{ij} \) is the observed value of \( j \)th algae concentration of salinity \( i \), \( \mu \) is the mean of observed values, \( G_i \) is the effective value of the salinity \( i \), and \( e_{ij} \) is the random effect corresponding to the observed values. In this
study, the effect of the surrounding environment was included as random error.

The samples were sealed at 4 °C, avoiding exposure to light. Based on G8 requirements, the treated challenge water should be stored for 5 days. During the 5 day storage period, biological availability experiments of the treated and untreated challenge water were performed at 48 and 120 hours to ensure that the algae were not regenerate.

**Results and Discussion**

During the experiment, TRO concentrations varied in different salinities of seawater with the same flow of oxygen and H$_2$O$_{gas}$. TRO concentrations in LS were lower than that in HS. This indicated that the components of seawater affect the production of TRO. The factors effecting TRO are TOC (including POC and DOC), salinity, and TSS. During the experiment with LS, due to the large amount of fresh water reactive gas particles containing ·OH were not completely dissolved. Meaning they were unavailable to form ·OH in solution. A portion of the reactive particles remained in the gaseous form. Therefore, the actual amounts of reactive particles in LS were less than what was available in HS. Meanwhile POC, DOC, and TSS, which were added to LS, consumed part of ·OH and lowered the amount of generated TRO.

*S. costatum*, *A. tamarense*, and *H. akashiwo* Hada, with an initial concentration of $1 \times 10^4$ cells/mL, were studied in different TRO concentrations. As seen in Figure 3 (a) and Figure 3 (b), as the concentration of TRO increases there is a continual decline in the concentration of the three kinds of algae. In HS and LS, the tolerance of the algae to ·OH was different. *H. akashiwo* Hada declined with the fastest speed indicating that it had the lowest tolerance to ·OH. The differences in the lethal thresholds of ·OH concentrations on algal species was caused by variances in the cell structures. For example, *S. costatum* has siliceous cell walls with slender spines around the cell. The cell structure is compact, and therefore, predisposed to fewer outside interferences and is more difficult to kill.

On the contrary, *H. akashiwo* Hada has no hard cell wall and is covered by a membrane, thus making it more susceptible to external pressure, such as low concentration of ·OH in the present case. These three types of algae belong to three phyla and have different shapes and sizes. The lethal TRO concentrations lie between 0.9–1.0 mg/L. This threshold is representative for killing algae with a size range of 10–50 μm in challenge water. When the TRO concentrations were low, the decrease in algal concentration was slower in LS than in HS. This indicates that chemical substances in LS had an effect on ·OH, but this did not affect the final result. With the increase of the concentration of TRO, the influence of chemical substances became weaker and the treatments in HS and LS fail to reflect any differences. According to the analysis of the remaining concentrations of algae treated by ·OH in HS and LS, using one-way ANOVA (P <0.01), the results show that there were no significant differences in the algae concentration between the HS and LS.

Biological conditions in ballast water are complex and diverse. There is usually a variety of algae that coexist. In order to realistically assess the efficacy of ·OH solution on challenge water, five kinds of algae were mixed in the same concentrations. The two final concentrations were 5,000±500 and 10,000±1,000 cells/mL. As showed in Figure 4, when mixed algal concentrations were 5,000±500 and 10,000±1,000 cells/mL, the lethal threshold concentrations of TRO were 0.41 mg/L and 0.93 mg/L, respectively. The results agreed well with the killing effect on single species of algae. For different concentrations of algae, the lowest TRO concentrations for killing algae did not follow a linear relationship. This indicated that when the ·OH concentration was relatively low, the algae had a certain tolerance to ·OH and the concentrations were insufficient for killing algae. Only when the ·OH concentration was over a
specific value, a large number of algae can be killed. Further research is required to determine the specific value for the lethal threshold. In summary, when the TRO concentration was 0.93 mg/L, the killing effect on mixed algae was maximum and can meet the requirements of G8.

Figure 3—Relationship between concentrations of single algae species and TRO concentration

Note: (a) algae concentrations in HS; (b) algae concentrations in LS.

Table 3—Killing effect on algae by ballast water treatment system in HS

<table>
<thead>
<tr>
<th>Item</th>
<th>0h (Day 0)</th>
<th>48h (Day 2)</th>
<th>120h (Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>TRO (mg/L)</td>
<td>0</td>
<td>0.93</td>
<td>0</td>
</tr>
<tr>
<td>T. rotula (cells/mL)</td>
<td>0.20×10⁴</td>
<td>1</td>
<td>0.12×10⁴</td>
</tr>
<tr>
<td>S. costatum (cells/mL)</td>
<td>0.21×10⁴</td>
<td>0</td>
<td>0.14×10⁴</td>
</tr>
<tr>
<td>A. tamarense (cells/mL)</td>
<td>0.21×10⁴</td>
<td>5</td>
<td>0.12×10⁴</td>
</tr>
<tr>
<td>K. mikimotoi (cells/mL)</td>
<td>0.20×10⁴</td>
<td>1</td>
<td>0.11×10⁴</td>
</tr>
<tr>
<td>H. akashiwo Hada (cells/mL)</td>
<td>0.21×10⁴</td>
<td>0</td>
<td>0.15×10⁴</td>
</tr>
<tr>
<td>Total (cells/mL)</td>
<td>1.03×10⁴</td>
<td>7</td>
<td>0.64×10⁴</td>
</tr>
</tbody>
</table>

The effects of the improvised treatment system were thoroughly tested. The results in HS waters are shown in Table 3 (due to the similarity in the results of LS and HS water, only HS results are reported in this paper). No regenerative phenomenon was observed for the algae within five days. The results show that the outcome of the treatment system fully meets the requirements of G8. The control samples were retained in a dark, closed environment, and the water lacked a fresh supply of oxygen. The essential oxygen and light for the survival and growth of phytoplankton were difficult to obtain, thus resulting in a gradual reduction in algae. However, after five days, the concentration of remaining algae in the control water was still much higher than the requirements of G8.

This supported the findings that the treatment system tested was effective in killing the algae in ballast water.

Chlorophyll pigment is used as an index of biomass for microalgae. The ·OH particle owing to its fast response and strong oxidative capacity causes algal pigment bleaching and prohibits photosynthesis, resulting cell death. Figure 5 shows that in HS, different concentrations of ·OH had significant effects on the chlorophyll a of S. costatum, A. tamarense, and H. akashiwo Hada. Figure 6 shows that ·OH had a strong effect on the decomposition of chlorophyll a.
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**Figure 4**—Relationship between killing efficiency of algae and TRO concentration

**Figure 5**—Relationship between chlorophyll a concentrations in single algae species and TRO concentration

Note: the corresponding concentration of each kind of algae is $1 \times 10^4 \pm 1000$ cells/mL.

Chlorophyll a concentrations decreased sharply with the increased concentration of ·OH. At the same algal concentrations, chlorophyll a concentrations varied in different algae. Chlorophyll a concentrations of *A. tamarense* were significantly higher than that of *S. costatum* and *H. akashiwo* Hada. The main reason for this difference is the inconsistency in the size of algae, as larger algae cells contain more chlorophyll a. For HS; the decline in chlorophyll a concentrations was gradual in *S. costatum* than the other two kinds of algae. The outer cell of *S. costatum* has a thick siliceous protective shell, which protects the cell contents, including pigments, against ·OH. However, this was not the case of *A. tamarense* and *H. akashiwo* Hada. Although the size of *A. tamarense* is much larger than *H. akashiwo* Hada, there was no significant difference of the decline trend of chlorophyll a of the two algae species after treatment of ·OH, indicating that the impact of ·OH on chlorophyll a is not related with algal sizes. Moreover, certain phytoplankton cells can produce exopolymeric materials to protect the inner cell from external stresses. Therefore, it is also an explanation the differences in response to ·OH by different algal species.

The decline trend of chlorophyll a in HS and LS is different, showing that the chemicals added in LS impact the effect of ·OH on algae.

**Figure 6**—Relationship between chlorophyll a concentrations in mixed algae species and TRO concentration

Note: (a) algae concentrations were about $1 \times 10^4 \pm 1000$ cells/mL; (b) algae concentrations were about $5 \times 10^3 \pm 500$ cells/mL.

The morphological features of *S. costatum*, *A. tamarense*, and *H. akashiwo* Hada were examined under a microscope and recorded on a digital camera. As shown in Figure 7, before the treatment of ·OH, the edges of algal cells were smooth. The shapes of the cells were normal. Similarly, the cell walls were smooth and in good shape. Intracellular materials were uniformly distributed. Cytochromes were dense and bright, the membrane and internal organelles were clear.
After treatment, the algal cell morphology changed significantly. All cell bodies were found to be ruptured and deformed. A large amount of intracellular materials were spilled out, causing shrinkage of cells. The membranes were dissolved and some organelles were missing. The cell membrane is a fluid mosaic structure composed of lipid and proteins, which is an important barrier to separate the intracellular environment from the external environment. The permeability and selectivity of the membrane transport are important foundations for sustaining life, so any disturbance and destruction to the membrane will cause serious consequences to the physiology of algae. The ·OH ion, which is a strong oxidizer, might have influenced phospholipid molecules and causes lipid peroxidation, resulting in a rapid consumption and degradation of the lipid molecules. The photographs confirmed that ·OH had the characteristics of destroying, breaking down, and killing algal cells effectively.

Figure 8—TOC, POC, DOC concentrations before and after being treated

Note: the corresponding concentration of each kind of algae is $1 \times 10^4 \pm 1000$ cells/mL.

Quality of any water is assessed through measurement of TOC which is an index of pollution. After ·OH treatment, the TOC in HS and LS, with algal concentrations of $10,000 \pm 1,000$ cells/mL, was found to decrease significantly (Figure 8). Similarly, POC also exhibited sharp decline after ·OH treatment. With regard to DOC, the concentration was found to decrease, presumably due to oxidation and degradation of organic matter, including bacteria, into CO$_2$, H$_2$O, and inorganic salts. The results of seawater quality parameters of HS is shown in Table 4 (as above, the results from LS waters are not reported because they were similar to those in HS waters). The salinity, temperature, and pH were practically unaltered after the ·OH treatment with a TRO of 0.93 mg/L. The ORP increased three fold because of the injected ·OH and other oxygen active particles. Accordingly, the DO increased from 5.57 to 6.01 mg/L due to the decomposition of residual ·OH. The TSS was reduced from 20.62 to 5.85 mg/L. The turbidity decreased to 1.08 NTU from 3.53 NTU with a change rate of 69.4%.
Conclusion
In conclusion, the experimental results on the use of ·OH radical as water disinfectant were found to be encouraging and hence the technique stands a great potential for using it as a Ballast Water Treatment System, subject to up scaling of the same to meet the Ships' requirements.

Acknowledgements
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References
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Table 4—Changes of water quality in HS

<table>
<thead>
<tr>
<th>Item</th>
<th>0h (Day 0)</th>
<th>48h (Day 2)</th>
<th>120h (Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>TRO (mg/L)</td>
<td>0</td>
<td>2.23±0.04</td>
<td>0</td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>32.8±0.1</td>
<td>32.9±0.05</td>
<td>32.9±0.03</td>
</tr>
<tr>
<td>Conductivity (mv/cm)</td>
<td>50.3±0.3</td>
<td>50.5±0.6</td>
<td>50.5±0.4</td>
</tr>
<tr>
<td>pH</td>
<td>21.85±0.06</td>
<td>21.87±0.08</td>
<td>22.37±0.04</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>155.4±0.7</td>
<td>474.1±0.9</td>
<td>151.3±0.9</td>
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<tr>
<td>DO (mg/L)</td>
<td>5.57±0.03</td>
<td>6.01±0.02</td>
<td>5.53±0.04</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>3.53±0.02</td>
<td>1.08±0.04</td>
<td>2.96±0.04</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>20.62±1.03</td>
<td>5.85±0.92</td>
<td>15.14±0.97</td>
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</table>


