Research Paper

Biodegradation of triclosan in diatom *Navicula* sp.: Kinetics, transformation products, toxicity evaluation and the effects of pH and potassium permanganate

Tengda Ding\textsuperscript{a,b}, Kunde Lin\textsuperscript{b}, Mengting Yang\textsuperscript{a}, Lianjun Bao\textsuperscript{c}, Juying Li\textsuperscript{a,*}, Bo Yang\textsuperscript{a}, Jay Gan\textsuperscript{d}

\textsuperscript{a} Shenzhen Key Laboratory of Environmental Chemistry and Ecological Remediation, College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China
\textsuperscript{b} State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen 361005, China
\textsuperscript{c} School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
\textsuperscript{d} Department of Environmental Sciences, University of California, Riverside, CA 92521, United States

**HIGHLIGHTS**

- Triclosan posed high toxicity to *Navicula* sp.
- The pH had a significant influence on bioaccumulation and toxicity of triclosan.
- Higher unionized triclosan at pH 7.5 contributed to its higher toxicity.
- Presence of KMnO\textsubscript{4} reduced bioaccumulation and toxicity of triclosan in *Navicula* sp.
- Transformation pathways of triclosan are proposed based on 7 identified metabolites.

**GRAPHICAL ABSTRACT**

**ABSTRACT**

Triclosan (TCS) is one of the most widely used pharmaceutically active compounds and frequently detected in treated wastewater and the impacted aquatic environment. However, the fate and toxicity of TCS in aquatic organisms is poorly known, including in particular the potential for the formation of incomplete biological transformation products. In this study, TCS posed high toxic effects (e.g., growth inhibition and damage of photosynthesis) to typical freshwater diatom *Navicula* sp., with the 24 h and 72 h \textit{EC}_{50} \textit{values} of 173.3 and 145.6 μg L\textsuperscript{-1}, respectively. The bioaccumulation of TCS in diatom cells increased with the increasing exposure to TCS and showed to be time-dependent. The higher intracellular TCS lead to higher toxicity on *Navicula* sp. The intracellular TCS concentration and the growth inhibition of TCS in *Navicula* sp. at pH 7.5 was obviously higher than that at pH 8.3, which was likely due to the higher
1. Introduction

The occurrence and fate of pharmacologically active compounds (PhACs) in the aquatic environment was paid special attention in the last few decades due to their potential undesirable ecological and human health effects. Triclosan (5-chloro-2-(2, 4-dichlorophenoxy)-phenol, TCS) is widely used in medical and personal care products because of its high antimicrobial effectiveness. For example, up to 1000 tons of TCS was produced in Europe per year [1]. The widespread use of TCS over the last 40 years results in a massive discharge to wastewater treatment plants (WWTPs) and then in surface waters [2,3]. TCS was widely detected in wastewater (8.05 μg L\(^{-1}\)) [4], sludge (1965 μg kg\(^{-1}\)) [5], river (0.282 μg L\(^{-1}\)) [6], groundwater (0.03 μg L\(^{-1}\)) [6] and sediments (41.7 μg kg\(^{-1}\)) [7]. TCS possesses a relatively high octanol-water partition coefficient (log\(K_{ow}\) of 4.8) [8,9], which can lead to its bioaccumulation in biota and biomagnification via food chain, ultimately threatening the safety of organisms. TCS was found to be highly toxic to Daphnia magna, fish (zebrafish, fathead minnows, bluegill sunfish) and green algae [10,11]. For example, Dann and Hotenta [12] found that TCS showed toxic effects to green algae Selenastrum capricornutum, Scenedesmus subspicatus, and Anabaena flosaquae with EC\(_{50}\) values ranging from 1.4 to 4.7 μg L\(^{-1}\). The bioaccumulation factors of TCS in Cladophora sp. was as high as 2100 [13]. At the base of the trophic food chain, algae such as diatoms represent a source of food for numerous organisms, and these microalgae are particularly relevant or seriously affected by exposure of xenobiotic pollutants in aquatic ecosystems [14]. Navicula sp. is one of the most common and occurring diatoms in freshwater and is often used to predict the toxicity and the bioavailability of xenobiotics in aquatic environments [15]. For example, Magnusson et al. [16] found that the growth of Navicula sp. was significantly inhibited by the herbicides (e.g., diuron, tebuthiuron, atrazine, simazine, and hexazinone) with the EC\(_{50}\) ranging from 2.6 to 157 μg L\(^{-1}\). In addition, once released into the aquatic environment, transformations of TCS may occur, producing metabolites with different environmental behavior and ecotoxicological profile. For instance, Tohidi and Cai [17] reported that over 60% of TCS was biotransformed during the wastewater treatments, and three toxic/persistent metabolites (i.e., methyltriclosan, 2, 4-dichlorophenol, and 2, 8-dichlorodibenzo-p-dioxin) were found. Therefore, a simple exposure analysis of TCS on algae is not sufficient, the fate (e.g., biotransformation and its transformation products) of triclosan in the environment is required.

TCS is a chlorinated phenoxyphenol with a \(pK_a\) value of 8.1 [18]. The pH in surface waters (common range from 7 to 9) can thus influence on the speciation and the following toxicity and fate of TCS in aquatic organisms. Rowett et al. [19] indicated that TCS with neutral species are more toxic than the corresponding anionic TCS in crustacean Gammarus pulex. Lipnick [20] pointed out that the un-ionized species of TCS were more permeable than its ionized species to lipid membranes. Additionally, potassium permanganate (KMnO\(_4\)) is commonly used in wastewater treatments with high removal efficiency, comparative stability, relatively low cost, and ease of operation [21,22], resulting in its distribution in natural waters and potential toxic effects on non-target organisms. However, little information is available on the effects of pH and KMnO\(_4\) on the toxicity and fate of TCS in algae.

The present study is to determine the toxicity and fate of TCS in a typical freshwater diatom Navicula sp. Transformation products are identified by liquid chromatography-mass spectrometric analysis, and the degradation pathways of TCS are proposed. Specific attention was also given to characterizing the influence of pH and KMnO\(_4\) on toxicity and fate of TCS.

2. Materials and methods

2.1. Chemicals

Triclosan was purchased from Sigma-Aldrich (China). HPLC-grade methanol were obtained from Fisher Scientific (China). The hydrochloric acid (HCl), sodium hydroxide (NaOH) and potassium permanganate (KMnO\(_4\)) were purchased from the Sinopharm Chemical Reagent Co. Ltd. (China). A stock solution of TCS (100 mg L\(^{-1}\)) was prepared by mixing the TCS in methanol. A 10 mg L\(^{-1}\) stock solution of KMnO\(_4\) was prepared. The initial pH was adjusted by 0.1 M HCl or 0.1 M NaOH. All chemicals used in this study were of analytical or HPLC grade.

2.2. Toxicity assay

The diatom Navicula sp. cultures were inoculated into 500 ml sterile D1 medium. The constituents in D1 medium and cultivation procedure were described in our previous study [23]. Algal bioassays were conducted with the addition of 0, 10, 50, 100, 200, 400 and 800 μg L\(^{-1}\) TCS for 72 h in triplicates. To explore the main environmental factors on toxicity of TCS, two pH values (7.5 and 8.3) were adjusted in the culture, and the toxicity of 400 μg L\(^{-1}\) TCS with different KMnO\(_4\) addition (0, 0.5, 1, 2, and 4 mg L\(^{-1}\)) was also investigated. The pH values commonly range from 7 to 9 in surface waters, two initial pH values (7.5 and 8.3) were demonstrated to be favorable conditions to culture freshwater algae Navicula sp. In addition, given that TCS has a \(pK_a\) value of 8.1, the pH of 7.5 and 8.3 were consequently selected for better elucidating the influence of the dissociated or pranoned status of TCS on toxicity and fate of TCS in Navicula sp. Batch experiments were conducted in 100 ml Erlenmeyer flasks containing 50 ml D1 medium. The optical densities of the algal suspensions were determined to indicate the algal density at 680 nm in a UV-2550 spectrophotometer (Shimadzu, Japan). The cellular growth rates (d\(^{-1}\)) were calculated by fitting the cell numbers to an exponential function and the chlorophyll content of Navicula sp. was analyzed using hot methanol extraction as reported in our previous work [23].

2.3. TCS uptake in diatom cultures

The uptake of TCS by Navicula sp. was investigated in 100 ml Erlenmeyer flasks containing 50 ml sterilized D1 mediums amended with different concentrations of TCS (50, 200 and 800 μg L\(^{-1}\)). The pH values usually range from 7 to 9 in surface waters, therefore, the influence of two pH values (7.5 and 8.3) was investigated. Different addition of KMnO\(_4\) (0.5 and 2 mg L\(^{-1}\))
was also individually tested. At 0, 24, 48, 72 and 144 h after treatment, three replicates of 10 mL solution were transferred to 10 mL centrifuge tubes and centrifuged at 2795 × g for 10 min. The algal pellets were washed twice by distilled water and centrifuged at 2795 × g for 10 min. After the supernatant was discarded, the pellet left was extracted with 3 mL dichloromethane: methanol (1:2, v/v) by sonication for 1 h, followed by centrifugation at 2795 × g for 10 min. The level of TCS in the extracts was determined to represent its accumulation amount within the microalgal cells. The TCS-spiked medium controls without *Navicula* sp. were included to assess the abiotic removal of TCS in the medium. All samples were filtered through 0.22 μm membrane filters before analysis.

### 2.4. Chromatographic analysis

All samples were subjected to a Waters ACQUITY ultra-performance liquid chromatography (UPLC) in tandem with a micromass triple quadrupole detector (MS/MS) (Xevo-TQD, Waters, Milford, MA) for quantification and identification of TCS and its metabolites. The MS/MS was equipped with an electrospray ionization source (ESI) operating in the negative-ion mode. An Aquity UPLC BEH C18 column (50*2.1 mm, 1.7 μm, Waters) was used with a column temperature of 35 °C. The mobile phase consisted of isocratic methanol and water (90:10 v/v). The injection volume was 10 μL and the flow rate was 0.2 mL min⁻¹. The interface voltage was 3.0 kV, nebulizing gas flow rate of 1.1 L min⁻¹, drying gas flow rate of 15 L min⁻¹, desolvation line temperature of 325 °C. The samples were analyzed using scan and product ion monitoring mode. A multiple reaction monitoring (MRM) method was used for quantification of TCS and its metabolites, and the data were acquired and processed using the MassLynx 4.1 software.

### 2.5. Statistical analysis

All experiments in this study were conducted in triplicates. One-way ANOVA was used to evaluate the significance of differences
in growth rate, chlorophyll and carotenoid content of *Navicula* sp. between controls and TCS treatments. A difference was considered statistically significant at a level of 0.05. The TCS species were calculated based on the pH and its pKa (8.1) according to the method as reported by Rowett et al. [19].

3. Results and discussion

3.1. Effects of TCS on *Navicula* sp

The toxicity of *Navicula* sp. cultivated in D1 amended with different TCS concentrations (0, 10, 50, 100, 200, 400, 800 μg L⁻¹) at pH 7.5 was monitored in terms of growth rate and chlorophyll contents for 72 h (Fig. 1). TCS showed high toxic effect to the diatom *Navicula* sp., with the 24 h and 72 h EC₅₀ values of 173.3 and 145.6 μg L⁻¹, respectively. Previous studies have reported that the 96 h EC₅₀ of TCS to the growth of green algae *Microcystis aeruginosa* and *Scenedesmus subsipicus* was 9.2 μg L⁻¹ at pH 7.0 and 2.8 μg L⁻¹ at pH 7.5, respectively [18,24], implying that the diatom may be more tolerant to TCS than green algae. The dramatic decrease of algal growth rate with the increasing TCS concentrations was observed during the 72 h of exposure, especially when the initial concentration of TCS was higher than 200 μg L⁻¹ (Fig. 1a). For example, the growth rate was 7.18 × 10⁻⁴ h⁻¹ under the exposure of 200 μg L⁻¹ TCS at 24 h and decreased to −5.32 × 10⁻⁴ h⁻¹ at 72 h, as compared to 7.40 × 10⁻³ h⁻¹ and 4.35 × 10⁻³ h⁻¹ at 24 h and 72 h, respectively, in the control without TCS. Chlorophyll plays an important role in light harvesting and energy transduction in the photosynthesis of microalgae, and thus the chlorophyll content is usually used as an indicator in ecophysiological and toxicological studies to examine the effect of pollutants on algal photosynthetic system [25,26]. For example, chlorophyll-a (Chl-a) could scavenge the accumulated reactive oxygen species in chloroplast, whereas the decreased Chl-a content could be an indicator for the damage of chloroplast [27]. In the present study, the Chl-a content of *Navicula* sp. decreased when TCS was >50 μg L⁻¹ after 72 h of exposure (Fig. 1b). For instance, the chlorophyll-a (Chl-a) content was 2.66 and 0.34 mg g⁻¹, respectively, in the control without TCS and the medium added with 800 μg L⁻¹ TCS, implying the photosynthesis of *Navicula* sp. was significantly damaged by addition of TCS.

The pH in the culture had a significant effect on toxicity of TCS to *Navicula* sp. Generally, the toxicity of TCS increased with an increase in TCS concentrations and a decrease in alkalinity (Fig. 2). The algal growth rate of *Navicula* sp. was consistently lower in the medium added with 400 μg L⁻¹ TCS at a pH of 7.5 than the treatment with a pH of 8.3 (Fig. 2b). For example, the growth rate was −6.51 × 10⁻³ h⁻¹ exposed to 400 μg L⁻¹ TCS at 48 h with a pH value of 7.5 in the algal cultures, whereas the growth rate increased to 9.39 × 10⁻³ h⁻¹ when the initial pH was adjusted to be 8.3. The 72 h EC₅₀ value of TCS to *Navicula* sp. at pH 8.3 increased to 447.6 μg L⁻¹. In addition, the Chl-a contents of *Navicula* sp. at pH 8.3 were higher than that in treatments at pH 7.5 when the concentration of TCS was
higher than 50 µg L\(^{-1}\) (Fig. 2c). Similarly, TCS was found to show higher toxicity to crustacean *Gammarus pulex* at pH 7.3 than that at pH 8.4, with 24 h and 48 h EC\(_{50}\) of 1.42 and 1.22 mg L\(^{-1}\), respectively [19]. However, Goldman et al. [28] reported that pH had no significant effect on the growth and photosynthesis of diatom *Thalassiosira weissflogii*. Based on the pKa value of TCS (8.1) and the pH values in the medium, proportion of ionized TCS and unionized TCS in all treatments were calculated and shown in Table 1. In general, levels of ionized and unionized TCS were found to be pH dependent. At an initial pH of 7.5, most of TCS was present in the form of unionized TCS, whereas ionized TCS is the dominant form of TCS when an initial pH of the culture was adjusted to be 8.3. In addition, the concentration of ionized and unionized TCS changed with the initial TCS levels and incubation time. The TCS in the presence of ionized form decreased as the initial concentration of TCS increased. For instance, after 72 h of exposure, 48.1% of TCS was present in its ionized form when 10 µg L\(^{-1}\) TCS was initially added to the medium at pH 7.5, whereas the ionized TCS decreased to 9.75% when the initial concentration of TCS was 800 µg L\(^{-1}\) (Table 1). Furthermore, ionized TCS was consistently lower in medium with a lower pH value. For example, the proportion of ionized TCS was 9.75% and 29.95%, respectively, in the treatment with the same initial level of TCS (800 µg L\(^{-1}\)) at pH 7.5 and 8.3, respectively. These observations were in agreement with the toxicity assay measurements, suggesting that the toxicity of TCS in *Navicula* sp. was closely related to the unionized TCS content. Thus, the higher contents of unionized TCS in the medium resulted in a more toxic effect on *Navicula* sp. in the medium with higher TCS exposure and a lower pH (7.5). This speculation was confirmed by the negative linear correlation ($r^2 = 0.80, p < 0.01$) between abundance of unionized TCS and the growth rate of *Navicula* sp. (Fig. 2d). The result is in agreement with the observations reported by Rowett et al. [19] that neutral TCS are more toxic than the corresponding anionic TCS in crustacean *Gammarus pulex*.

Preliminary studies have shown that the growth rate and chlorophyll contents were not affected by individual KMnO\(_4\) (1 mg L\(^{-1}\)) ($p > 0.05$) (Fig. 3). Franca et al. [29] found significant reductions in the cell numbers of *Pseudokirchneriella subcapitata* after 72 h of exposure to KMnO\(_4\) (0.25–4 mg L\(^{-1}\)), indicating that diatom frustules were likely evolved as mechanical protection for the diatom cells. The growth of *Navicula* sp. exposed to 0.4 mg L\(^{-1}\) TCS was accelerated by KMnO\(_4\) at an addition level of >0.5 mg L\(^{-1}\) (Fig. 3a). For example, the growth rate of *Navicula* sp. increased to 0.022 h\(^{-1}\) in the presence of KMnO\(_4\) (2 mg L\(^{-1}\)) as compared to 0.013 h\(^{-1}\) in the treatment with the same amount of TCS (0.4 mg L\(^{-1}\)) and without KMnO\(_4\). The Chl-a content of *Navicula* sp. exposed to 0.4 mg L\(^{-1}\) TCS also increased with the presence of 2 mg L\(^{-1}\) KMnO\(_4\). When exposed to 0.4 mg L\(^{-1}\), the Chl-a contents were 0.68 mg g\(^{-1}\) and 3.73 mg g\(^{-1}\), respectively, in the medium without KMnO\(_4\) and with 2 mg L\(^{-1}\) KMnO\(_4\), respectively. A complete degradation of TCS (0.4 mg L\(^{-1}\)) by 1 mg L\(^{-1}\) KMnO\(_4\) was observed by Chen et al. [22]. Wu et al. [30] also found that KMnO\(_4\) can remove TCS in water at pH 7.0 and 8.6 with a removal rate of up to 100%. Thus, the increased growth rate of *Navicula* sp. at KMnO\(_4\) concentrations of >1 mg L\(^{-1}\) may be caused by the degradation of TCS by KMnO\(_4\).

### 3.2. Bioaccumulation of TCS in diatom *Navicula* sp

During 144 h of incubation, the intracellular TCS varied with different initial concentrations (Fig. 4a). Generally, the concentration of TCS in diatom cells increased with the increased initial TCS concentration. For instance, the intracellular TCS concentration was 0.25, 2.57 and 20.63 mg g\(^{-1}\), respectively, after 48 h of exposure to 50, 200 and 800 µg L\(^{-1}\), respectively. The intracellular concentration of TCS showed to be time-dependent. At pH 7.5, the intracellular TCS concentration increased from 1.10 mg g\(^{-1}\) at 24 h to 2.57 mg g\(^{-1}\) at 72 h and then decreased to 1.28 mg g\(^{-1}\) at 144 h with the initial TCS concentration of 200 µg L\(^{-1}\). This observation was consistent with the trends observed for the toxicity measurements in this study. The higher intracellular TCS lead to higher toxicity on *Navicula* sp., indicating that bioaccumulation or intracellular TCS played an important role in its toxicity to *Navicula* sp. The dramatic decrease of intracellular TCS at 144 h may be attributed to the biotransformation of TCS in diatom or its excretion to the medium. Similarly, Bai and Acharya [31] reported that over 42% of TCS removal was related to the uptake of *Nannochloris* sp. cells, implying the bioaccumulation played an important role in the removal of TCS in aquatic waters.

The initial pH values had a significant influence on bioaccumulation of TCS. The intracellular TCS concentration in *Navicula* sp. at pH 7.5 was obviously higher than that at pH 8.3 when the initial TCS concentration was 200 µg L\(^{-1}\) (Fig. 4b). For example, the intracellular TCS concentration was 2.57 mg g\(^{-1}\) at pH 7.5, as compared to that of 0.59 mg g\(^{-1}\) at pH 8.3 after 48 h of exposure. Similarly, Rowett et al. [19] reported that TCS can easily pass the biological membranes at pH <8, leading to a higher bioaccumulation of TCS in crustacean *Gammarus pulex*. Additionally, the intracellular TCS concentration decreased with exposure time as the addition of KMnO\(_4\) increased. For instance, the intracellular TCS concentration decreased from 2.18 to 0.05 mg g\(^{-1}\), when the concentration of KMnO\(_4\) increased from 0.5 to 2 mg L\(^{-1}\) at 48 h. With the addition of 0.5 mg L\(^{-1}\) KMnO\(_4\), the intracellular TCS concentration decreased from 3.62 mg g\(^{-1}\) at 24 h to 0.27 mg g\(^{-1}\) at 144 h (Fig. 4c). It is likely due to the oxidation of TCS by KMnO\(_4\) [22], resulting in the reduced level of TCS in the algal cultures, especially in the medium with higher KMnO\(_4\) concentrations.

---

**Table 1**

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>Treatment</th>
<th>Species of TCS</th>
<th>Initial TCS concentration (µg L(^{-1}))</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>pH=7.5</td>
<td>Ionized</td>
<td>11.91</td>
<td>6.97</td>
<td>19.12</td>
<td>17.44</td>
<td>14.34</td>
<td>14.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH=8.3</td>
<td>Ionized</td>
<td>88.09</td>
<td>93.03</td>
<td>80.88</td>
<td>82.56</td>
<td>85.66</td>
<td>85.35</td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>pH=7.5</td>
<td>Ionized</td>
<td>84.11</td>
<td>9.32</td>
<td>14.29</td>
<td>17.30</td>
<td>12.39</td>
<td>11.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH=8.3</td>
<td>Ionized</td>
<td>61.55</td>
<td>90.68</td>
<td>85.71</td>
<td>82.70</td>
<td>87.61</td>
<td>88.35</td>
<td></td>
</tr>
<tr>
<td>72 h</td>
<td>pH=7.5</td>
<td>Ionized</td>
<td>12.73</td>
<td>51.66</td>
<td>61.56</td>
<td>52.87</td>
<td>70.56</td>
<td>31.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH=8.3</td>
<td>Ionized</td>
<td>27.27</td>
<td>48.34</td>
<td>38.44</td>
<td>47.13</td>
<td>29.44</td>
<td>68.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unionized</td>
<td>51.90</td>
<td>62.38</td>
<td>89.60</td>
<td>91.28</td>
<td>91.09</td>
<td>90.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unionized</td>
<td>93.26</td>
<td>80.01</td>
<td>57.86</td>
<td>94.86</td>
<td>95.99</td>
<td>29.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unionized</td>
<td>6.74</td>
<td>19.99</td>
<td>42.14</td>
<td>5.14</td>
<td>4.01</td>
<td>70.05</td>
<td></td>
</tr>
</tbody>
</table>
3.3. The metabolic fate of TCS in Navicula sp

3.3.1. Identification of TCS metabolites

Mass spectrometric analysis by the UPLC–MS² system was employed to identify the transformation products of TCS in the diatom *Navicula* sp. A total of 7 metabolites were detected in algal cells during 72 h of incubation. These metabolites are designated herein as TP 163, TP 174, TP 190, TP 206, TP 228, TP 256, and TP 486 (Fig. S1). Because of the lack of authentic standards for reference, structural identification of the metabolites was based on the analysis of the total ion chromatogram (TIC) and the corresponding mass spectrum (Figs. S2–8). The structural and fragmentation information for TCS and its transformation products in algal cells are shown in Table 2.

At pH 8.3, five metabolites of TCS were detected. TP 190 was tentatively identified as (4-chloro-2-hydroxyphenoxy) methanediol. The fragment ions at m/z 174, 157 and 141 corresponded to the consecutive losses of –OH (Fig. S2). The hydroxylation of TP 190 can result in the formation of TP 206, which may correspond to 4-chloro-6-(dihydroxymethoxy) benzene-1, 3-diol. The fragment ion at m/z 189 for TP 206 corresponded to the loss of a hydroxyl group, and the fragment ion at m/z 149 was indicative of the fracture of benzene and the loss of a hydroxyl group (Fig. S3). TP 228 was likely a valine-conjugated metabolite, which was determined to be 2-amino-1-(3-chlorophenoxy)-3-methylbut-1-en-1-ol. Fragmentation experiments (MS²) revealed the formation of fragments m/z 209 (H₂O), 152 (CH₃OH, –NH₂) and 43 (CH₃, –NH₂, –C₆H₅Cl) (Fig. S4). TP 256 was a proline-conjugated metabolite. The fragment ions at m/z 237 and 116 corresponded...
Table 2
LC–MS/MS data for the identification of TCS and its metabolites in *Navicula* sp. under the different pH and KMnO₄ conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Product</th>
<th>Mₘw</th>
<th>Chemical Structure</th>
<th>Retention time (min)</th>
<th>ESI⁻–MS² (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan</td>
<td>–</td>
<td>289.5</td>
<td></td>
<td>0.91</td>
<td>287 &gt; 35</td>
</tr>
<tr>
<td>2,4-dichlorophenol</td>
<td>TP 162</td>
<td>161.9</td>
<td></td>
<td>0.39</td>
<td>161 &gt; 126 &gt; 102</td>
</tr>
<tr>
<td>(4-chloro-2-hydroxyphenyl)(hydroxy)methanolate</td>
<td>TP 174</td>
<td>174</td>
<td></td>
<td>0.48</td>
<td>173 &gt; 125 &gt; 112</td>
</tr>
<tr>
<td>(4-chloro-2-hydroxyphenoxy)methanediol</td>
<td>TP 190</td>
<td>190</td>
<td></td>
<td>0.63</td>
<td>189 &gt; 174 &gt; 157</td>
</tr>
<tr>
<td>4-chloro-6-(dihydroxymethoxy)benzene-1,3-diol</td>
<td>TP 206</td>
<td>206</td>
<td></td>
<td>0.75</td>
<td>205 &gt; 189 &gt; 149</td>
</tr>
<tr>
<td>2,4,6-trichloro-3-methoxyphenol</td>
<td>TP 228</td>
<td>227.5</td>
<td></td>
<td>1.24</td>
<td>227 &gt; 209 &gt; 152</td>
</tr>
<tr>
<td>5-chloro-2-methoxyphenoxy(pyrrrolidine-2-ylidene)methanol</td>
<td>TP 256</td>
<td>255.5</td>
<td></td>
<td>2.17</td>
<td>255 &gt; 237 &gt; 116</td>
</tr>
<tr>
<td>1-(5-chloro-2-(2,4-dichlorophenoxy)-4-hydroxyphenoxy)hexane-1,2,3,4,5,6-hexaol</td>
<td>TP 486</td>
<td>485.5</td>
<td></td>
<td>0.51</td>
<td>485 &gt; 469 &gt; 453 &gt; 413 &gt; 280</td>
</tr>
</tbody>
</table>

to the loss of H₂O and proline, respectively (Fig. S5). The proposed structure for this product is 5-chloro-2-methoxyphenoxy (pyrrrolidine-2-ylidene) methanol. TP 486 has a greater molecular weight than parent TCS and the mass difference of 197 are suggestive of a glucuronide molecular. The fragment ions at m/z 469, 453 and 413 were indicative of the consecutive losses of H₂O, –OH, and the loss of two chloride ions, respectively. Thus, TP 486 was identified as 1-(5-chloro-2-(2, 4-dichlorophenoxy)-4-hydroxyphenoxy)hexane-1, 2, 3, 4, 5, 6-hexaol (Fig. S6). At pH 7.5, four metabolites with the exception of TP 486 were also detected in the algal cells of *Navicula* sp., suggesting that TCS showed certain similar metabolisms in *Navicula* sp. at the two pH tested.

In the presence of KMnO₄, TP 206, TP 228, and TP 256 were detected with two additional metabolites (TP163 and TP174) of TCS in *Navicula* sp. TP 163 was identified as 2, 4-dichlorophenol (2, 4-DCP). The fragment ions at m/z 126 was likely due to the loss of a chloride ion. The ion at m/z 102 was an indicative of the successive loss of hydroxyl group and the subsequent cleavage of benzene (Fig. S7). TP 174 was tentatively identified as (4-chloro-2-
On incubation, the possible losses of hydroxyl groups in algae were identified by the esterification of 4-chlorobenzene-1, 2-diol and the hydroxylation by hydroxylases like CPA 450, which are widespread enzymes in algae [32]. Since 4-chlorobenzene-1, 2-diol was not detected in the present study, it was likely a transient intermediate that rapidly underwent further transformation. Further hydroxylation of TP190 could lead to the formation of TP206. Various amino acids are also present in algae and can be conjugated with TCS or its intermediates, leading to the formation of a series of conjugated metabolites. TP228 was metabolites of TCS generated from the conjugation of valine and 3-chlorophenol, and TP256 was a transformation product of proline conjugated 5-chloro-2-methoxyphenol. The formation of such amino-conjugated metabolites may affect the function of amino acids in diatom cells, which may have a potential threat to the synthesis of protein and the growth of diatom. The relative detection levels of TP228 and TP256 at pH 7.5 were obviously higher than that in medium with pH = 8.3 (Fig. S10a, b), implying that amino-conjugated metabolites may be associated to the higher toxicity of TCS to Navicula sp. at pH 7.5. TP486, which may be formed by the esterification of TCS and glucuronic acid, was only detected in the diatom cells of Navicula sp. at pH 8.3. Previous studies have found that TCS could be transformed by glucuronidation in fish and edible plants [33,34]. Ding et al. [23] demonstrated that the glucuronide-conjugated metabolites may be considered the detoxification mechanism to ibuprofen in diatom. Thus, the detection of TP486 in Navicula sp. at pH 8.3 may contribute the lower toxicity of TCS to diatom Navicula sp. than that at pH 7.5.

After the addition of 0.5 mg L⁻¹ KMnO₄, the levels of TP206 and TP256 were lower than that in the culture with no KMnO₄ (Fig. S10 c, d), suggesting that TP206 and TP256 may also be vulnerable to degradation by KMnO₄. Furthermore, the ether bond cleavage products TP163 and TP174 were found in diatom cells with the addition of KMnO₄, suggesting that the initial dihydroxylation occurred on both aromatic rings of triclosan. The metabolite 2, 4-dichlorophenol (TP163) was detected as the major monoaromatic metabolite of TCS by bacterial strain such as Sphingomonas sp. PH-07 and Sphingophyxis KCY1 [35,36]. The level of TP163 increased from 24 h to 144 h (Fig. S10 c, d), implying that TP163 was gradually formed or accumulated in the algal cells. Though the dioxin-like compounds were known to be toxic and carcinogenic [37], the overall toxicity of these products (e.g., 2, 4-dichlorophenol) to fish decreased as compared to fish [38]. It is consistent with the increased algal growth rate in the presence of KMnO₄, which may be partly attributed to the more accumulated 2, 4-DCP.

4. Conclusions

TCS showed high toxic effect to the diatom Navicula sp., with the 24 h and 72 h EC₅₀ values of 173.3 and 145.6 μg L⁻¹, respectively. The higher intracellular TCS lead to higher toxicity on Navicula sp., indicating that bioaccumulation played an important role in toxicity of TCS. The pH showed a significant effect on toxicity and bioaccumulation of TCS to Navicula sp. with higher toxicity and accumulation at a lower pH, which was likely due to the higher distribution of unionized TCS in the medium at an initial pH of 7.5. The addition of KMnO₄ (>1 mg L⁻¹) significantly reduced the toxicity and bioaccumulation of TCS to Navicula sp. UPLC–MS² analysis revealed that seven transformation products of TCS were detected and identified. TP174, TP189, TP206, TP228, TP256, and TP486 were reported for the first time. The conjugation products (e.g., TP486) may be included in the detoxification mechanism of TCS. The potential effects of TCS metabolites in aquatic environment should

---

**Fig. 4.** The intracellular TCS concentration in Navicula sp. at the pH value of 7.5 (a), and the pH effects (b), and KMnO₄ effects (c). Error bars represent standard error of the mean (n = 3).

hydroxyphenyl) (hydroxy) methanolate. The peak at m/z 125 may be attributed to the consecutive losses of hydroxyl groups (Fig. S8).

### 3.3.2. The proposed metabolic pathway of TCS in diatom Navicula sp.

On the basis of the identified metabolites and their kinetics during incubation, possible degradation pathways of TCS in Navicula sp. are schematically shown in Fig. 5. The degradation of TCS may lead to the cleavage of the ether bond, resulting in the formation of 4-chlorobenzene-1, 2-diol. Previous studies have reported that cytochrome P-450 (CYP 450) hydroxylases and the hydroxylation by hydroxylases like CPA 450, which are widespread enzymes in algae [32]. Since 4-chlorobenzene-1, 2-diol was not detected in the present study, it was likely a transient intermediate that rapidly underwent further transformation.
be further investigated. Given that TCS may continuously enter the aquatic environment, the long-term fate and toxicity of TCS must be evaluated in actual waters.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (Grants Nos. 21607106, 21777104, 21407108 and 41503082), China Postdoctoral Science Foundation (Grant Nos: 2017M612748), the Shenzhen Science and Technology Project (Grant Nos. KQJSCKJNYJ201609270069A and ZDSYS201605151750049), and the Natural Science Foundation of SZU (Grant Nos. 827-000077).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2017.09.033.

References


