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# Application of a microbial toxicity assay for monitoring treatment effectiveness of pentachlorophenol in water using UV photolysis and $TiO_2$ photocatalysis

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**Abstract**

Conventional approaches for monitoring the effectiveness of wastewater treatment processes include evaluating the degradation of the target compound and/or generation of its nontoxic byproducts. These approaches are, however, limited because routine chemical analyses alone are neither able to fully address potential hazard to biological receptors nor characterize potential synergistic interactions. This study was carried out to investigate the degradation effectiveness of pentachlorophenol (PCP) by treatment with UV-A, UV-B photolysis, sunlight, $TiO_2$ photocatalysis, and/or their combinations. Chemical analyses of the parent compound and its selected byproducts, as well as acute toxicity assessment using the luminescent bacteria *Vibrio fischeri* (Microtox®), were conducted during and after the various photolytic and photocatalytic treatments. In general, the toxicity reduction pattern observed after treatment corresponded well with the chemical degradation data. However, it should be noted that there were occasions that acute microbial toxicity was observed even from the treated water samples, some of which showed complete removal of the parent compound. This post-treatment toxicity might be due to toxic PCP byproducts, which may include polychlorinated dibenzodioxins/furans, tetrachloro-*p*-benzoquinone, and other intermediates. The $TiO_2$ photocatalysis with UV-B photolysis was the most effective method to remove both PCP and its toxic derivatives in the water. The Microtox assay is an easy to use and promising approach for evaluating the effectiveness of wastewater treatment processes.


*Keywords:* Pentachlorophenol; Microtox; Ultraviolet; Photolysis; Photocatalysis

## 1. Introduction

Pentachlorophenol (PCP) a highly chlorinated hydrocarbon, has been widely employed in various applications in agriculture and especially as a wood preservative and as a broad spectrum biocide [1,2]. It is persistent in water and soil, and may migrate to groundwater aquifers to pose a potential risk to human beings [3,4]. Due to its handling and disposal practices in the past, PCP is still of concern in the soil and groundwater of many sites around world [1,5], and is listed as one of the U.S. EPA's priority pollutants. PCP is acutely toxic and acts as an uncoupler of oxidative phosphorylation: this compound may alter the electrical conductivities of biomembranes and inhibits cellular enzymes [6]. This compound may produce mutations in animal and human cells and may exhibit teratogenic, carcinogenic, and reproductive effects [7]. PCP adversely affects aquatic and terrestrial flora and fauna [8]. Even at low levels ranging from 0.1 to 1 μg/L in water, PCP can affect sensitive organisms and potentially damage aquatic ecosystems [9]. Due to the adverse effects of PCP on human and ecological receptors, it is important to remove PCP from contaminated media/site.

Advanced oxidation processes (AOPs) have been employed to treat chlorinated phenols. Because of its chemical stability and low cost, titanium dioxide ($TiO_2$) is the most commonly used for this purpose. The $TiO_2$ photocatalysis has been constituted a promising technology for the treatment of wastewaters containing refractory organic compounds including PCP [8,10–13]. Barbeni et al. [14] reported that $TiO_2$ was the most effective treatment option for PCP compared to other semiconductors such as $SnO_2$, $WO_3$, CdS, and ZnO.

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The photooxidation of PCP proceeds via hydroxyl radical attack on the para position of the PCP ring to form a semiquinone radical which in turn disproportionates to yield byproducts such as *p*-chloranil and tetrachlorohydroquinone [15]. In addition, polychlorinated biphenylethers, hydroxylated polychlorobiphenylethers, polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) may also be formed by dimerization process during the photolysis of PCP [16]. Vollmuth et al. [17] reported that PCDD/Fs were formed as intermediates when PCP was treated with UV (6 W peaking at 254 nm). Therefore, one must assure the complete removal of toxic byproducts as well as the target compounds after the treatment. However, it is not always feasible to analyze all the toxic intermediates after the treatment. Existing analytical techniques are limited for wastewater characterization, and comprehensive chemical characterization of wastewater is cost prohibitive. In addition, it is hard to predict the interactions among multiple chemical constituents derived from the target compounds. For these reasons, it may be desirable to employ toxicity bioassay techniques to evaluate the overall effectiveness of wastewater treatment.

In the present study, we employed various AOP treatments to remove PCP from the contaminated water: that is, (1) UV-A; (2) UV-B; (3) sunlight; and/or (4) photocatalysis using $TiO_2$. In order to determine the most effective treatment system, we conducted a simple microbial toxicity assay to measure the reduction of acute toxicity as well as chemical analyses to ensure the removal of the parent compound, its intermediates, and the generation of mineralized products. The Microtox assay using a marine bacterium *Vibrio fischeri* was chosen for monitoring toxicity reduction for evaluating treatments effectiveness. That is because of its quick response time, widespread use, and the commercial availability of suitable instrumentation and quality controlled reagents [18]. By comparing chemistry data and toxicity results, we also aimed at showing the value of this simple toxicity assay technique in evaluating the effectiveness of wastewater treatment.

## 2. Materials and methods

### 2.1. Chemicals

Vendor certified PCP (99% purity; Sigma Chemical Co., St. Louis, MO, USA), and tetrachloro-*p*-benzoquinone (TCBQ, 99% purity; Sigma Chemical Co.) were employed as standards in manufacturing sample solutions or for chromatographic analyses. Solutions of PCP were prepared by adding 50 mg of PCP in 5 L milli-Q water in the dark on a heated magnetic stirrer (~50 °C) for several days. The PCP solution was used within a day of preparation to avoid potential decomposition. The initial concentration of PCP used with ultra-pure water was 40 μM (10 mg/L). The initial concentration was chosen since the solubility limit of PCP is approximately 0.05 mM at 20 °C. The $TiO_2$ powder employed in this study was Degussa model P-25 (Frankfurt, Germany), of which surface area was reported $50 \pm 15\ m^2/g$ (Degussa). This $TiO_2$ product was used without any pre-treatment in this study.

### 2.2. Photoreactor

The indoor photocatalytic degradation experiments were performed in a circulating photoreactor system, as shown in Fig. 1a. The reservoir was a 2 L glass bottle placed on a magnetic stirrer. The reactor system consisted of a temperature-controlled reservoir, a metering pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) for circulating the reactor contents, and a photoreaction chamber, all of which were connected with flexible Teflon tubing. The PCP solutions were circulated with a metering pump at a flow rate of 1 L/min. Reactor stirring was sufficient to maintain uniformity of the reaction solution. All the experiments were carried out in a continuous flow photoreactor containing six-columns (each diameter 12-mm) with recirculation of the suspension. The photoreactor has 6 UV-$A_{365nm}$, $B_{315nm}$, and $C_{254nm}$ lamps (G20T10, 20 W, 580-mm length, 32.5-mm-diameter, Sankyo Electrics Co., Japan). The UV intensity was measured with a radiometer (VLX-3W, Cole-Parmer Instrument Co.). The external surface of the reactor around the quartz columns was covered with aluminum foil for UV safety and energy concentration. Reactions were performed under atmospheric pressure at 20 °C. The reaction solutions were prepared by diluting stock solutions, and the aqueous phase was then introduced into the reservoir. The reaction solution was transferred to the sample collector and solution storage using a three-way valve.

We also designed and constructed a solar outdoor system, as shown in Fig. 1b. The UV-concentrating radiation system had eight quartz tube modules connected together, each module being 1.5 m (length) × 0.8 m (width), with UV-transparent tubular receivers and a radiation area of $1.2\ m^2$. Polished aluminum was used as the reflective material because it is highly reflective in the UV range (300–380 nm). Quartz tubes were used owing to their excellent UV stability and transmission. The PCP solution flowed directly from one module to another, and finally to the reservoir tank. In a $TiO_2$ slurry system, a metering pump continuously re-circulated the $TiO_2$ suspension to a batch vessel and solar reactor. The solar light intensity was measured using a radiometer with a UV-A region chip (365 nm), mounted at the same inclination angle (37°) to the plate. All the experiments, unless specified, were performed under random weather conditions at Seoul National University campus located in Seoul (37°34′52″N, 127°00′03″E, elevation 55 m). An average ratio of photoreactor residence time to accumulated photons can be derived from the standard value for UV radiation which is $2.2\ mW/cm^2$, equivalent to a photon flux of $6 \times 10^{-5}$ mol of photons $m^{-2}\ s^{-1}$.

### 2.3. Procedures and analysis

All the liquid samples were filtered through 0.2 μm MCE membrane filters (Advantec MFS Co., Dublin, CA, USA) to remove the $TiO_2$ particles before analysis. PCP and TCBQ were determined by a Summit$^{TM}$ high performance liquid chromatography (HPLC) system, equipped with a UVD340S detector, and an RP C-18 silica column (25 cm × 4.6 mm i.d., 5 μm particles, Supelco, Bellefonte, PA, USA). The mobile phase was a mix-

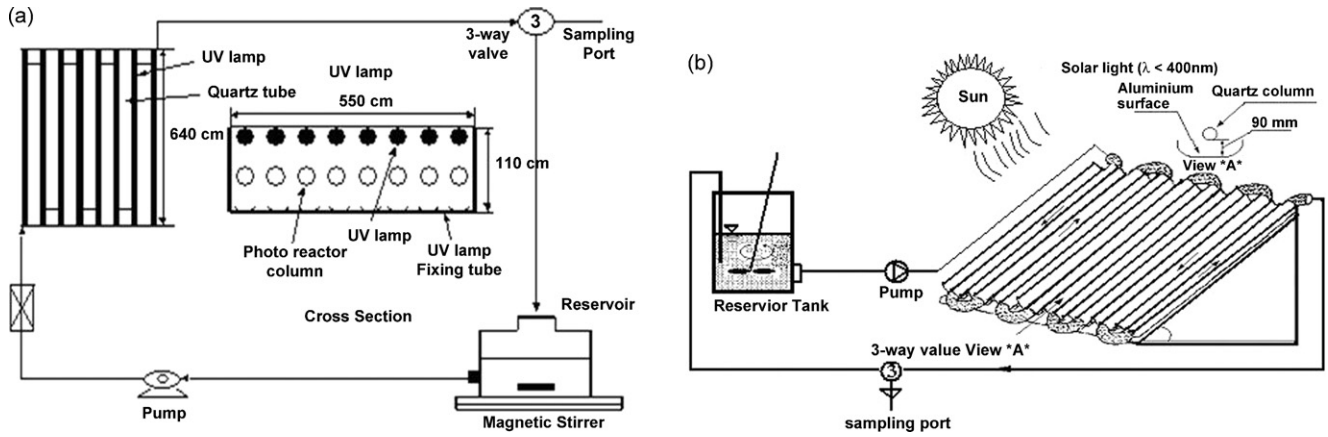


Fig. 1. Schematic diagram of photochemical reactor for: (a) indoor and (b) outdoor experiments.

ture of 1% acetate acid aqueous solution and methanol/water with ratio of 80:20 (v/v). The flow rate of the mobile phase was 1.0 mL/min. Detection was carried out at 220 nm for TCBQ and 280 nm for PCP, respectively. The volume of injection was 20 μL. Calibration curves were prepared for the quantitative analysis of PCP and the intermediate products formed during UV irradiation. The intermediates were identified by comparing retention time. Chloride was analyzed using a DX-120 ion chromatograph (Dionex Co., Sunnyvale, CA, USA). The column was an Ion-Pac AS-14 column, and the eluent was a mixture of 1 mM $NaHCO_3$ and 3.5 mM $Na_2CO_3$. Total organic carbon (TOC) analysis was performed on a TOC analyzer (TOC-5000A; Shimadzu Co., Kyoto, Japan).

### 2.4. Toxicity assay and statistical analyses

The Microtox® (Strategic Diagnostics Inc., Newark, DE, USA) toxicity assay using a marine bacterium *V. fischeri* was conducted to evaluate acute toxicity during the various treatments. The bioassay is based on detecting these changes in light output. Modified 81.9% basic test protocol was followed for the toxicity determination, with 5 and 15 min of exposure. The median inhibitory concentrations ($IC_{50s}$) for each sample were calculated using a vendor-provided software, Microtox Omni (Azur Environmental, Carlsbad, CA, USA). The $IC_{50}$ is a calculated toxicity value representing the sample concentration (%) estimated to cause a 50% response by the exposed test organisms. Details of the test procedure were in accordance with the manufacturer provided test protocol [19].

## 3. Results and discussions

### 3.1. Chemical analyses of PCP degradation

Degradation of PCP under various UV or $TiO_2$ treatments was observed (Fig. 2). The pH of test water with PCP was initially 4.5, and while the photocatalytic degradation of PCP took place, the pH of the test water gradually increased up to 6.5. No pH adjustment was made, since it was technically impossible to harmonize the pH throughout all the treatments. As shown in Fig. 2a–c, when only UV-A, UV-B, or solar light was applied, respectively, the yield of chloride ion after 180 min of treatments was approximately 3.1, 8.4, and 2.7% of the total theoretical concentration of chloride (200 mM) released from the amount of PCP photodegraded, while >90% of PCP was removed within 120 min of all treatments. This observation suggests significant amount of chlorinated intermediates were formed during the UV or solar light only treatments. In a similar study with PCP, Ho and Bolton [20] also implied that significant levels of toxic chlorinated byproducts were formed during the direct photolysis, as evidenced by acute toxicity to *Daphnia* and fish.

When PCP contaminated water was treated with UV-A or UV-B together with $TiO_2$ slurry, PCP was completely removed within 60 min of reaction (Fig. 2e and f). In general, the photocatalytic reaction of $TiO_2$ with UV, compared to the UV only reaction, results in faster and more complete removal of target organic compounds, with which our results are in agreement. Goutailler et al. [21] showed that photolysis and photocatalysis involved the same degradation pathway, and the difference in the reaction rates between the two processes is due to the hydroxyl radical concentration.

The $TiO_2$-only reaction was not effective at removing PCP to any meaningful extent. Formation of chloride ion was also negligible (Fig. 2d). This lack of PCP removal with $TiO_2$-only treatment is inconsistent with previous reports showing that PCP and 2,4,6-trinitrotoluene (TNT) were removed to some extent by the $TiO_2$-only reaction [11,22]. At pH 6.5, the initial condition of the PCP-$TiO_2$ mixture in the present study, Pecchi et al. [11] showed that >55% adsorption of PCP with $TiO_2$ catalysis. This apparent inconsistency may be in part due to the fact that the amount of $TiO_2$ catalyst (1 g/L) applied in this study was far less than Pecchi's (15 g/L), and unlike Pecchi's no oxygen was provided in our experiment.

The results of the degradation of PCP and the formation of its toxic derivative, TCBQ, as a function of the reaction time are given in Fig. 2. TCBQ was formed and removed more rapidly by the UV-$TiO_2$ reactions (Fig. 2e and f) compared to the UV only treatments (Fig. 2a and b). This observation is similar to Ho and Bolton [20] which showed that the combined treatment of UV photolysis + $H_2O_2$ and photocatalysis produced and removed the intermediates of PCP faster than the UV photolysis only treatment.

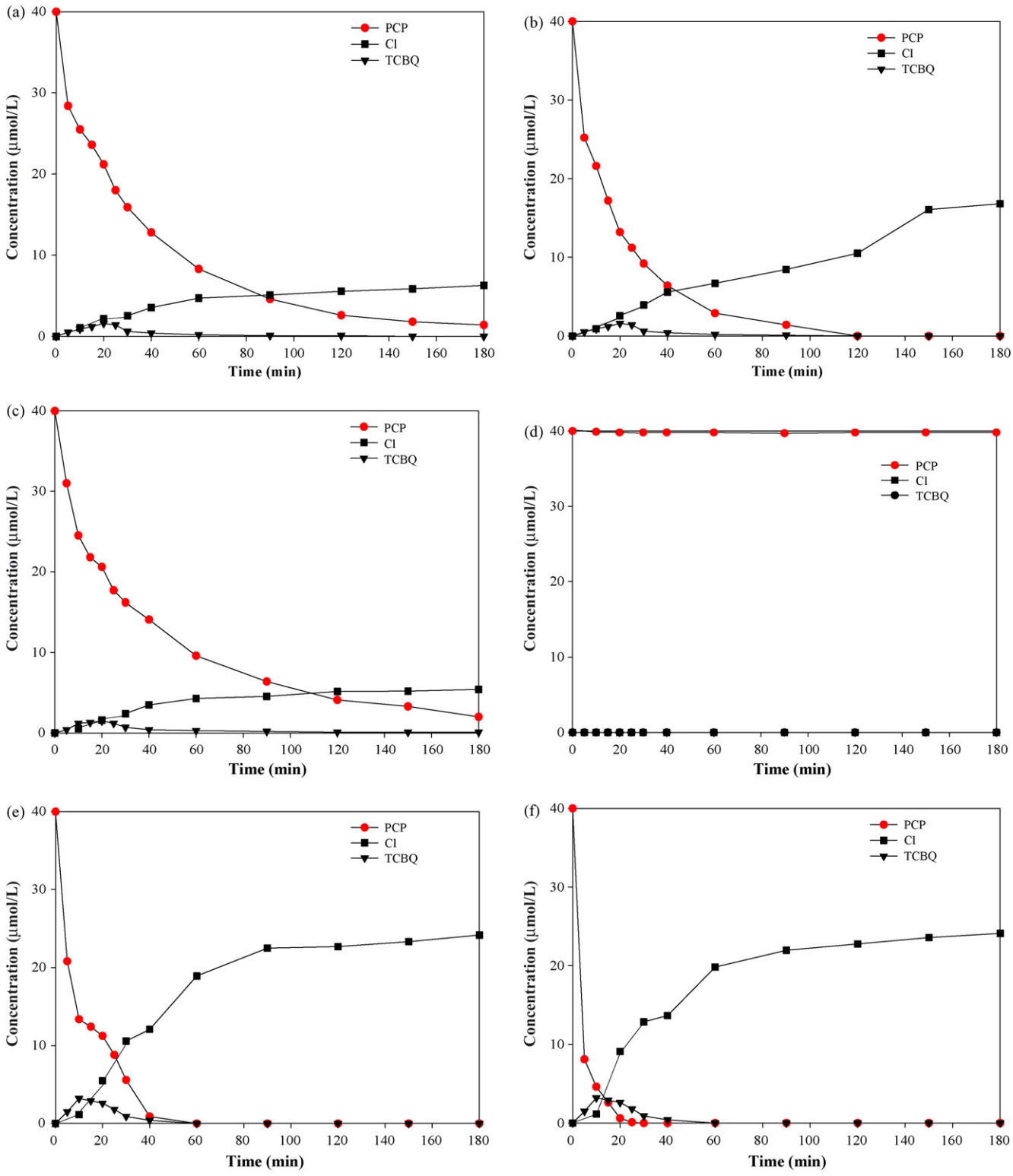


Fig. 2. Degradation of PCP and the formation of chlorine ion and TCBQ by: (a) UV-A photolysis, (b) UV-B photolysis, (c) solar photolysis, (d) $TiO_2$, (e) UV-A + $TiO_2$, and (f) UV-B + $TiO_2$ treatments. (Experimental condition: Initial concentration of PCP = 40 μM, pH 4.5, UV irradiance = 12 mW/cm$^2$, $TiO_2$ = 0.1 wt%, flow rate = 1 L/min.)

As shown in Fig. 3, the degradation efficiency of TOC, which indicates the mineralization of PCP by each treatment process, showed similar pattern with the degradation efficiency of PCP (Fig. 2). TOC was degraded more efficiently with photocatalysis compared with photolysis. By $TiO_2$-only reaction, TOC was degraded by around 16%, in which PCP appeared to be transferred from solution to $TiO_2$ catalysts basically by adsorption. Therefore, major degradation mechanism of PCP in $TiO_2$ pho-

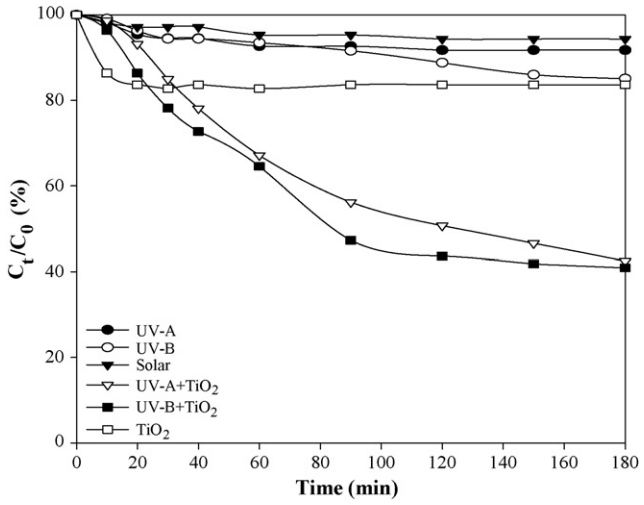


Fig. 3. The formation of TOC by various photolysis and/or photocatalysis treatments.

tocatalysis was likely to be mediated by the attack of OH radical generated from the reaction between $H_2O/OH^-$ and $TiO_2$ under UV light (<380 nm).

This mechanism of reaction is similar to photolysis mechanism that involves OH radicals produced under UV light [23]. Consequently, photodegradation of PCP with $H_2O_2$ photocatalyst is expected to follow a similar mechanism as $TiO_2$ photocatalysis.

### 3.2. Removal of microbial toxicity

The Microtox test was used to monitor the toxicity changes during the photochemical treatment of PCP. The results of the Microtox median effective concentrations (EC50s) for each sample are summarized in Table 1. The 5- and 15-min EC50s of PCP, 4.16 and 3.52 μM, respectively, were in a similar range from another study [8,24]. The time-dependent trends of Microtox 5-min EC50 value of PCP contaminated water by UV-A, UV-B, and solar light only treatments are shown in Table 1, respectively. The 15-min EC50s showed similar patterns with one exception which will be discussed later.

With the photolysis with UV-A, UV-B, and solar light (Fig. 2a–c), the microbial toxicity initially decreased (Table 1); however, still significant levels of acute toxicity were noted in the samples where >90% of PCP was removed. Lower PCP mineralization as indicated by less formation of chloride ion may partly explain the residual toxicity. Fig. 2a and b shows that TCBQ, a toxic byproduct of PCP, was detected until up to 120 min of photolytic reactions, and it contributes in part to the observed microbial toxicity at the initial stage of reaction. It should be also noted that the microbial toxicity was noted even after complete degradation of TCBQ. Formation of other toxic intermediates may be responsible for the observed toxicity [17].

The $TiO_2$-only treatment was the least effective method of PCP removal in water among the systems we employed in this study. The microbial toxicity of samples was not reduced to any meaningful extent by the $TiO_2$-only treatment (Table 1).

The UV + $TiO_2$ combined treatments, especially the UV-B and $TiO_2$ reaction, could treat PCP contaminated water much more effectively. When UV-A was applied with $TiO_2$, the microbial toxicity was completely removed after 60 min of treatment (Table 1). With UV-B + $TiO_2$ treatment, the toxicity reduction and the mineralization of PCP was faster: PCP was completely degraded at 30 min and the acute toxicity was removed after 40 min of reaction. Ho and Bolton [20] showed good correlation between the photodegradation of PCP and toxicity reduction using a bacterial toxicity test (Toxichromo) and a 96 h fathead minnow toxicity test; the bacterial and fish toxicity decreased as the concentration of PCP or the total organic chlorine concentration fell. In the present work, however, the UV-B + $TiO_2$ reaction resulted in a 5-min EC50 value of 41.3% when PCP was completely removed at 20 min, which might be attributed to the toxic derivatives of PCP including TCBQ (Fig. 2d, Table 1).

Table 1
Changes in acute microbial toxicity (median inhibitory concentrations, i.e., EC50s) during the various photolysis and/or photocatalysis treatments

| Treatment type | Microtox exposure time (min) | Treatment time (min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 20 | 40 | 60 | 120 | 180 |
| UVA | 5 | 10.6 (9.5–11.9) | 16.5 (13.6–19.9) | 22.4 (19.0–26.5) | 32.6 (31.5–33.8) | 44.4 (43.1–45.7) | 38.6 (25.3–58.7) |
| | 15 | 8.4 (6.5–11.0) | 14.6 (13.1–16.3) | 18.9 (15.9–22.3) | 23.7 (19.1–29.3) | 28.2 (27.1–29.3) | 26.4 (22.8–30.6) |
| UVB | 5 | 10.2 (7.7–13.5) | 24.5 (17.6–34.2) | 32.0 (25.0–40.8) | 34.9 (20.7–58.8) | 17.9 (6.6–46.7) | 19.9 (6.8–58.1) |
| | 15 | 7.4 (5.8–9.4) | 18.8 (16.1–21.8) | 21.1 (13.1–34.0) | 22.0 (8.5–56.8) | 15.2 (4.5–51.4) | 14.0 |
| Solar | 5 | 13.6 (11.8–15.8) | 22.9 (16.3–32.3) | 33.5 (23.4–47.8) | 49.8 (37.3–66.5) | 47.3 (27.1–82.3) | 54.1 (38.8–75.3) |
| | 15 | 13.0 | 21.0 (20.5–21.5) | 28.7 (24.4–33.8) | 40.1 (33.4–48.2) | 40.2 (29.7–54.3) | 42.5 (31.8–56.8) |
| $TiO_2$ | 5 | 9.0 (5.7–14.1) | 11.7 (10.5–13.0) | 11.5 (9.4–14.1) | 11.9 (8.4–16.9) | 11.1 (5.3–23.3) | 11.9 (5.8–24.2) |
| | 15 | 8.5 (6.5–11.0) | 10.9 | 10.5 | 11.8 | 10.8 (9.1–12.7) | 11.6 |
| UVA + $TiO_2$ | 5 | 8.7 (6.3–12.1) | 26.0 | 78.9 | >82 | >82 | >82 (48.1–179.8) |
| | 15 | 7.1 (6.0–8.4) | 21.4 (10.9–41.8) | 64.9 | >82 | >82 | 45.4 (28.6–72.0) |
| UVB + $TiO_2$ | 5 | 10.2 (7.9–13.2) | 41.3 (9.7–174.7) | >82 (3.4–2377.0) | >82 | >82 | >82 |
| | 15 | 8.5 (8.2–8.9) | 34.2 | 66.6 | >82 | >82 | >82 |

Unit in % sample dilution which shows 50% reduction in microbial metabolism. Values are median effective concentration (EC50s) of Microtox® assay; values in parentheses are 95% confidence intervals; EC50s without confidence intervals were estimated from two data points.

Bozzi et al. [25] showed in a similar study with melamine that the photocatalyzed solutions had higher toxicity than that of the solution before treatment, because of the intermediates formed during the UV/$H_2O_2$/$TiO_2$ photo-oxidation.

The results of the 15-min Microtox test for the sample treated with UV-A + $TiO_2$ showed an interesting pattern. While no microbial toxicity was observed from the sample treated for 180 min with 5-min exposure, an EC50 value of 45.4% (95% confidence interval, 28.6–72.0%) was obtained from 15-min exposure (Table 1). With UV-B + $TiO_2$, however, no such pattern was noted. This difference may be due to the UV-A's relatively less potent photolytic capacity. It is likely that certain PCP byproducts formed during the final stage of UV-A + $TiO_2$ treatment might exert a prolonged effect on *V. fischeri*.

Together with UV-B, $TiO_2$ photocatalysis could improve the removal of PCP and its toxic derivatives more effectively as evidenced by quick removal of PCP, fast mineralization of organic chlorines, and elimination of acute microbial toxicity.

## 4. Conclusions

Wastewater treatment provides an important protection for the public and the ecosystem from the toxic effects of contaminated water. In considering treatment technologies, it is important to ensure removal of potential hazards. In this study, we evaluated various AOP techniques for its effectiveness in hazard reduction from PCP contamination in water. For this purpose, the Microtox assay, a microbial toxicity test was carried out, in addition to chemical analyses of PCP and its byproducts. Among the various photolysis and/or photocatalysis treatment options tested in this study, PCP was best treated by the UV-B and $TiO_2$ combination, as evidenced by almost complete degradation of PCP (100% degradation of the parent compound, about 90% of chlorine ion yield, and 58% reduction in TOC (Figs. 2f and 3)), and rapid removal of the acute microbial toxicity.

There were occasions that acute microbial toxicity was observed from the treated water samples, where the parent compound was completely removed. This toxicity might be resulted from toxic byproducts, such as PCDD, PCDF, TCBQ, and other toxic intermediates. Therefore, determination of the parent compound only may not be a sufficient measure for evaluating an overall effectiveness of wastewater treatment. As evidenced by chemical analyses and toxicity assessment, the $TiO_2$ photocatalysis with UV-B photolysis was the most effective method to remove both PCP and its toxic derivatives in the water.

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