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Original Research

Assessment of Paracetamol (Acetaminophen) Toxicity in Microalgae

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Abstract

There has been concern over the ecotoxicity of residual pharmaceuticals detected in the aquatic environments. Paracetamol (acetaminophen) is one the most extensively used over-the-counter drugs and its residues have been detected in the aquatic environment. There have been contradictory reports on the sensitivity of microalgae to paracetamol (PCM). The primary aim of this study was to assess the toxicity of PCM in five microalgae, namely *Pseudokirchneriella subcapitata*, *Scenedesmus dimorphus*, *Stichococcus bacillaris*, *Chlorella vulgaris,* and *Chlamydomonas reinhardtii* based on 96 h test at concentrations ranging from 0, 30, 60, 120, to 240 mg L^{-1}. Results showed that the microalgae were very resistant to PCM as the EC_{50} values based on OD_{620} were beyond the highest concentration (>240 mg L^{-1}) tested. However, *P. subcapitata* was more sensitive than the other species when compared using EC_{10} (91.4 mg L^{-1}) based on chlorophyll a (chl a) concentration. Both chl a and total carotenoid concentrations of *Psedokirchneriella subcapitata* and *Scenedesmus dimorphus* exposed to the highest PCM concentration (240 mg L^{-1}) were significantly ($p<0.05$) lower than the control. In comparison, the car:chl a ratio of *Chlorella vulgaris* increased with increasing PCM concentrations.

Keywords: microalgae, paracetamol (acetaminophen), toxicity, *Chlamydomonas reinhardtii, Chlorella vulgaris, Pseudokirchneriella subcapitata*

Introduction

Paracetamol (acetaminophen) [PCM] is one of the most extensively used pharmaceuticals in the world, with a consumption of 620 tons in Germany in 2001 alone [1], and a consumption of 390 tons in England in 2000 [2]. It is one of the most commonly used analgesic and antipyretic drugs, especially for the relief of fever, headache, and other minor aches and pains. It is considered a non-steroidal anti-inflammatory drug (NSAID) that inhibits the cyclooxygenase enzyme [3].

Due to the huge production and extensive consumption, residues of PCM have been detected in the aquatic environment. For instance, a survey conducted in the US showed that 24% of water samples from streams contained up to 10 µg L^{-1} of PCM [4]. Paracetamol reaching 79.2 µg L^{-1} has been detected in surface waters of Serbia [5]. The drug also has been detected at average concentrations of 12-61 ng L^{-1} in Korea [6], 52-289 ng L^{-1} in the UK [7], and up to 66 ng L^{-1} in the Elbe River, Germany [8]. In addition, PCM has been detected up to 1.83 µg L^{-1} in groundwater used for public drinking supply in California [9], and up to 211 ng L^{-1} in a number of reservoirs tapped for drinking water monitored along the Lergue River in southern France [10]. It has also been found that the removal of PCM in sewage treatment plants (STP) was only 1.8% [11]. As the levels of PCM detected in surface waters exceed the predicted no-effect concentration (PNEC) of 9.2 µg L^{-1}, the drug might be a threat to non-target organisms [3].

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The presence of PCM in water means that a host of aquatic organisms are exposed to the drug. Consequently, PCM being designed for human physiology may cause unwanted and unforeseen effects in aquatic organisms and be bioaccumulated within these organisms and transferred within the food web to higher animals, threatening the ecosystem [3]. Ultimately, public health may be affected through the consumption of PCM accumulated within food, and the drinking of PCM-contaminated water. Thus there is a need to investigate the possible toxicity of paracetamol to the ecosystem and human health.

Toxicity testing of PCM has been conducted using organisms from different trophic levels. For instance, Kim et al. reported that EC_{50} values of the drug for *Daphnia magma* and *Moina macrocopa* were 11.85 and 56.34 mg L^{-1}, respectively, while in crustaceans the values ranged from 9.2 to 50 mg L^{-1}[12]. In chronic fish toxicity testing, significant reduction in juvenile survival was observed at 30 d post-hatch after exposure to 95 mg L^{-1} PCM. In higher plants, PCM was shown to inhibit root elongation of wheat (*Triticum aestivum*) (EC_{50} = 668.8 mg L^{-1}) and the damage was more evident after chronic exposure for 21 days, where soluble protein synthesis was affected [13]. It was also demonstrated that PCM was taken up and detoxified through the formation of glutathione and glucose conjugates in root cell culture of horseradish [14].

In comparison to other pharmaceuticals, there have been very few toxicity studies of PCM on microalgae. Such studies are important as adverse effects of PCM on microalgae may have an impact on organisms at the higher trophic levels as they form the basis of aquatic food webs. Toxicity testing conducted on *Scenedesmus subspicatus* showed that EC_{50} of PCM was 134 mg L^{-1}, much higher than another drug, clobrifinic acid (89 mg L^{-1}) [15]. Paracetamol was less toxic to the microalga compared to fish cell cultures and *Daphnia*. Another study showed that the total phytoplankton community and two microalgal isolates, namely *Chlorella ellipsoidea* and *Oscillatoria* sp. were highly sensitive to PCM based on EC_{50} of 4 mg L^{-1} after only 24 h exposure [16]. With such contradictory findings from the two studies, are microalgae sensitive to PCM? In attempts to answer this question, we assessed the effect of PCM on the growth and pigmentation of five common microalgae.

Materials and Methods

Test Materials and Test Organisms

Paracetamol (acetaminophen) of analytical standard grade (purity>98.0%) with molecular formula of $CH_3CONHC_6H_4OH$ and molecular weight of 151.16 g mol^{-1} was purchased from Sigma-Aldrich Chemicals (CAS-No 103-90-2). The PCM solution was prepared using ultrapure water and sterilized using a cellulose nitrate filter (Sartorius, 50 mm, 0.2 µm).

Five species of chlorophytes (green microalgae) were used for the testing, namely *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

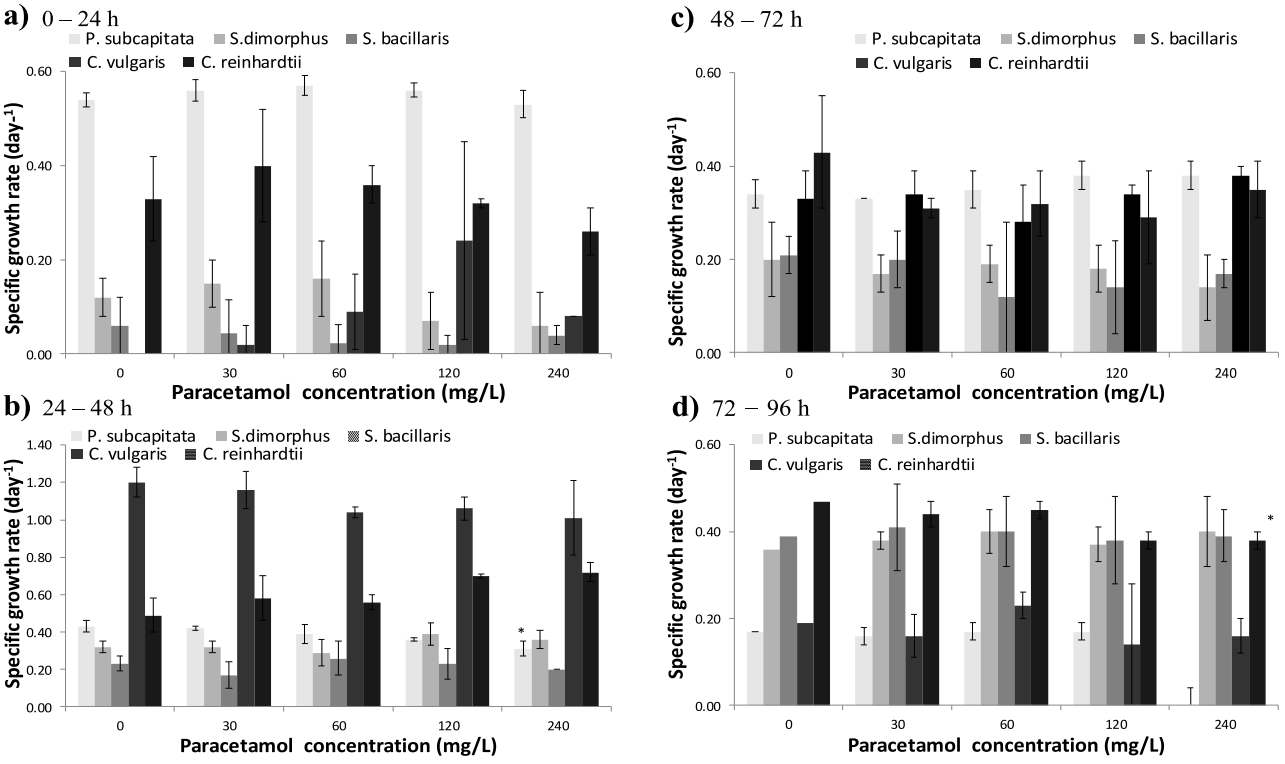

Fig. 1. Specific growth rates (µ, day $^{-1}$) at different growth periods of the five microalgae exposed to different concentrations of paracetamol. a) 0-24 h; b) 24-48 h; c) 48-72 h; d) 72-96 h. Vertical bars denote standard deviations from the mean values of three replicate cultures. * indicates significant difference from the control (0 mg/L) at $p<0.05$.

NIES-35, *Scenesdesmus dimorphus* NIES-93, *Stichococcus bacillaris*, *Chlorella vulgaris,* and *Chlamydomonas reinhardtii.* The first two microalgae were purchased from the National Institute of Environmental Studies (NIES) in Japan, while the other three species were isolated from the soil of potted plants in an office building in Kuala Lumpur [17].

Growth Characterization

A preliminary characterization of the growth of the five microalgae was conducted before toxicity testing. The cultures were grown in Bold Basal Medium (BBM) [18] with 10% inoculum from exponential phase pre-cultures standardized at an optical density at 620 nm (OD_{620}) of 0.2. The cultures (50 mL) in conical flasks (100 mL) were placed in a controlled-environment incubator set at 28±2ºC with irradiance of 42 µmol m^{-2} s^{-1} on 12:12 h light-dark cycle. The cultures were grown in triplicate for each species. Growth was monitored daily by measuring OD_{620} with a spectrophotometer (Perkin Elmer, USA), and cell counting with a Double-Neubauer haemocytometer until the cultures attained stationary phase. The specific growth rate (µ) was calculated using the following formula: $\mu\ (day^{-1}) = (\ln N_2 - \ln N_1) / (t_2 - t_1)$, where N_2 and N_1 correspond to OD_{620} at times t_2 and t_1 within the exponential phase.

Toxicity Testing

The toxicity testing (96 h) of PCM was conducted using static microalgal cultures (50 mL) grown in 100 mL conical flasks. The inoculum was obtained from exponential phase cultures standardized at OD_{620} of 0.2. Five mL of inoculum was added to 45 mL BBM with final nominal concentrations of PCM ranging from 0 (control), 30. 60, 120, to 240 mg L^{-1}. The cultures were grown in an incubator as described above. The algal growth was monitored daily by measuring OD_{620} for four days. The µ were determined for every 24 h growth period for 96 h.

At the end of the experiment (day 4), the cells were harvested on glass fibre filters (Whatman GF/C, 47 mm, 20 µm) for the extraction of chlorophylls and carotenoids

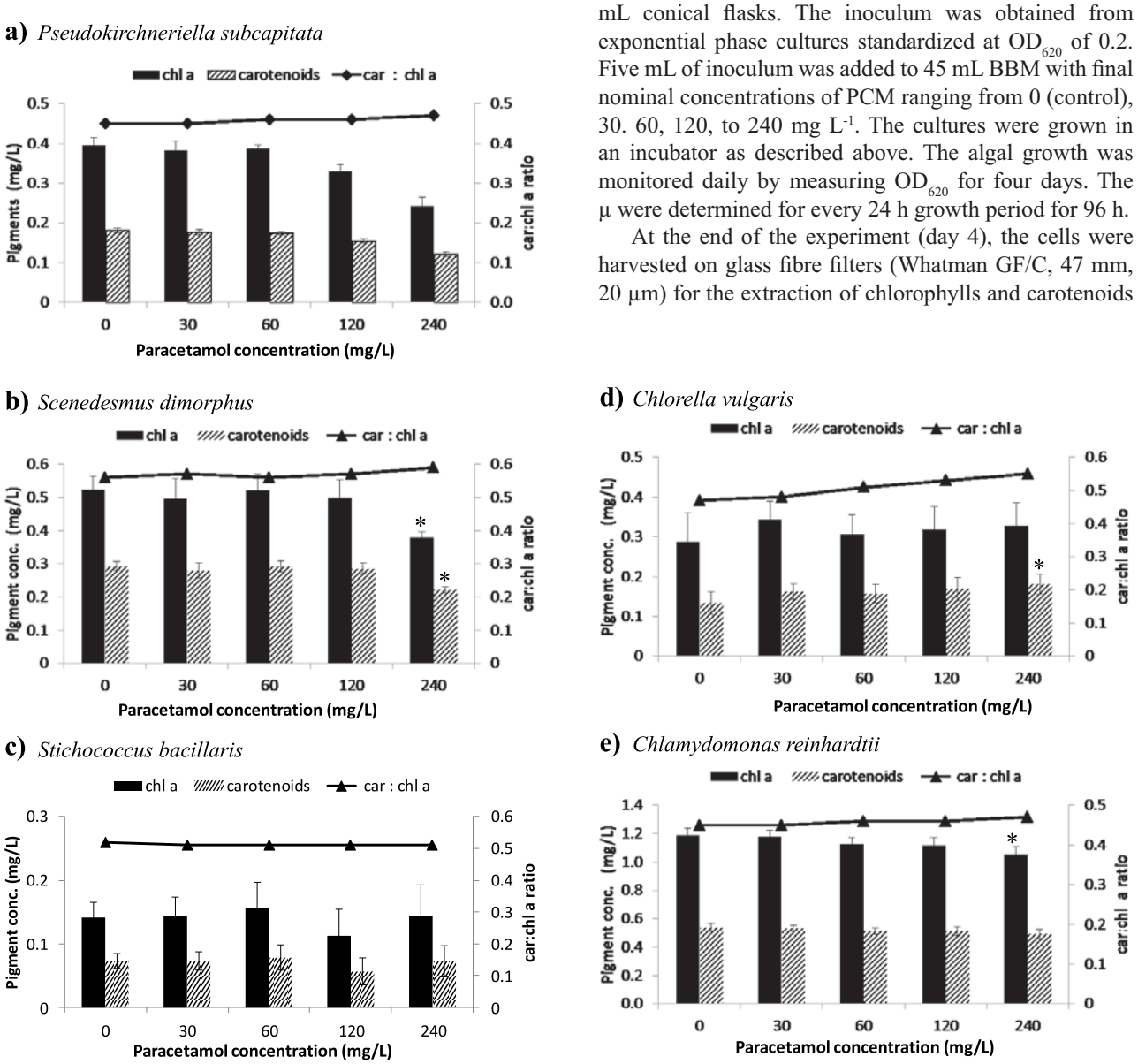

Fig. 2. Chlorophyll a and total carotenoid concentrations, and carotenoid to chlorophyll a ratios (car:chl a) of the five microalgae exposed to different concentrations of paracetamol. a) *Pseudokirchneriella subcapitata*, b) *Scenedesmus dimorphus*, c) *Stichococcus bacillaris*, d) *Chlorella vulgaris,* and e) *Chlamydomonas reinhardtii.* Vertical bars denote standard deviations from mean values of three replicate cultures. * indicates significant difference from the control (0 mg/L) at $p<0.05$.

Table 1. Characteristics of the test microalgae used in this study.

Species	Origin	Dimension	Morphology	Specific growth rate (μ, day^{-1}) based on:		Final OD_{620}	Final cell number (x 10^4/mL)	Regression equation (r^2) - cell number versus OD_{620}
				OD_{620}	Cell number			
Pseudokirchneriella subcapitata NIES-35	Nitelva River, Norway	Length: 6 – 12 μm Width: 2 –5 μm	Crescent-shaped, non-motile, unicellular	0.37±0.03	0.03±0.03	0.27±0.11	199.7±5.5	y = 926.47x + 6.3496 r^2 = 0.9099
Scenedesmus dimorphus NIES-93	Lake Kasumigaura, Ibaraki, Japan	Length: 5 – 33 μm Width: 3 -11 μm	Arcuate, single cell to coenobia of 4 cells, pyrenoids present; non-motile	0.24±0.02	0.28±0.01	0.18±0.01	108.2±4.5	y = 556.17x + 0.5916 r^2 = 0.958
Stichococcus bacillaris	Soil from potted plants in an office building in Kuala Lumpur	Length: 8 – 14 μm Width: 8 – 11 μm	Short filaments of 2-4 cells, usually fragmented into unicells	0.24±0.02	0.28±0.14	0.14±0.02	26.4±1.9	y = 199.76x + 0.7987 r^2 = 0.7556
Chlorella vulgaris	Soil from potted plants in an office building in Kuala Lumpur	Diameter: 6 – 12 μm	Unicellular, coccoid, pyrenoid present	0.43±0.03	0.45±0.07	0.28±0.01	144.9±11.4	y = 601.02x + 4.4897 r^2 = 0.9336
Chlamydomonas reinhardtii	Soil from potted plants in an office building in Kuala Lumpur	Length: 7 – 15 μm Width: 3 – 11 μm	Unicellular, flagellate, formed aplanospores	0.44±0.02	0.57±0.05	0.44±0.01	143.5±0.5	y = 348.5x + 0.191 r^2 = 0.9871

in acetone. The concentrations of chlorophyll a (chl a) and total carotenoids were determined by spectrophotometry using the equations given by Strickland and Parsons [19] and Hansmann [20], respectively.

Data Analysis

The data were presented as mean ±standard deviation, and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett post hoc test using SPSS software (PASW Statistics Version 18) if there was significant difference ($p<0.05$). Correlation between cell number and OD_{620} was analyzed using the linear regression model and the regression coefficient (r^2) was calculated. The median inhibitory concentration (EC_{50}) based on the final day OD_{620} and chl a concentrations was determined using the ICPIN program [21].

Results and Discussion

All five test microalgae were chlorophytes, originating from freshwater and soil habitats (Table 1). Both *Pseudokirchneriella subcapitata* and *Scenedesmus dimorphus* were temperate aquatic species while the other three were tropical soil microalgae. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were unicellular while *Scenedesmus dimorphus* formed coenobia of 2-4 cells. *Stichococcus bacillaris* formed short filaments, which often fragmented into unicells. *Chlamydomonas reinhardtii* was a flagellate, which also formed aplanospores.

Preliminary growth characterization showed that *Chlamydomonas reinhardtii* and *Chlorella vulgaris* attained higher specific growth rates (μ) than the other species. Although *Pseudokirchneriella subcapitata* attained relatively low μ, the final cell number attained was the highest. The final cell number of this species was much higher than *Chlamydomonas reinhardtii* in spite of the lower final OD_{620}. There was good correlation between OD_{620} and cell number (r^2 = 0.91-0.99) for the test microalgae, except *Stichococcus bacillaris* (r^2 = 0.76). Therefore, subsequent toxicity testing was based on OD_{620}.

Of the five microalgae, *Pseudokirchneriella subcapitata* is a recommended species for toxicity testing [22] and widely used as a test organism for pharmaceuticals such as antibacterial agents [23, 24], but has not been tested on PCM. *Chlamydomonas* has been used for the testing of toxicants such as nanomaterials [25], heavy metals and pentachlorophenol [26], and oxytetracyline [3]. *Chlorella vulgaris* is a fast-growing microalga as indicated by the high μ attained. *Chlorella* is a cosmopolitan microalga widely used in bioassay of various toxicants, including pharmaceuticals such as antibiotics [27]. It is the only chlorophyte besides *Scenedesmus subspicatus* that has been used for the testing of PCM [16]. In comparison, *Stichococcus bacillaris* tends to form short filaments, making it a less suitable bioassay organism for ecotoxicity testing. In spite of its wide occurrence in soil, its sensitivity to pharmaceuticals has not been reported.

In general, there were no significant differences between μ of the test species grown in medium containing paracetamol and the control (Fig. 1). One exception was the lower μ ($p<0.05$) of *Chlamydomonas reinhardtii* grown in medium with PCM than the control during the 72-96 h period (Fig. 1(d)). Significant differences in pigment concentrations were only observed at the highest PCM concentration (240 mg L-1), where chl a concentrations were significantly lower ($p<0.05$) than the control in *Chlamydomonas reinhardtii*, *Pseudokirchneriella subcapitata,* and *Scendesmus dimorphus* (Fig. 2).

Decreases in chl a concentrations have been reported in microalgae exposed to pharmaceuticals. For instance, chl a concentration of *Chlorella ellipsoidea* decreased by 65% when exposed to 15 mg L^{-1} PCM [16]. Chlorophyll a concentration of *Chlorella vulgaris* decreased by more than 50% when exposed to the antibiotic ciprofloxacin (31.25 mg L^{-1}) for 96 h, but there was no difference after 48 h [27]. In another study, the chl a, chl b, and total chlorophyll contents decreased by more than 50% in *Chlorella vulgaris* exposed to streptomycin (10 mg L^{-1}) for 96 h [28]. In comparison, the chl a and carotenoid contents were not affected, but chl b content decreased in *Pseudokirchneriella subcapitata* exposed to a mixture of pharmaceutically-active compounds [29].

Total carotenoid concentrations were significantly lower ($p<0.05$) in *Pseudokirchneriella subcapitata* and *Scendesmus dimorphus,* but were significantly higher ($p<0.05$) in *Chlorella vulgaris* compared to the control (Fig. 2). The car:chl a ratio of *Chlorella vulgaris* increased with increasing PCM concentration. The increase in carotenoid content could be an adaptive mechanism against oxidative stress resulting from the exposure to PCM as carotenoids are antioxidant compounds. It has been demonstrated that exposure to PCM imposed oxidative stress in *Chlorella ellipsoidea* as indicated by the increase in thiobarbituric acid-reactive substances (TBAR) [16].

The change in pigmentation due to exposure to pharmaceuticals may affect photosynthesis in microalgae, as this has been shown in studies on other toxicants. For instance, photosynthetic yield (quantum yield and electron transport rate) was suppressed in *Euglena gracilis* when exposed to chlorophenol compounds [30]. In addition, the expression of phothosynthesis-related genes such as *psaB* was suppressed in *Chlorella vulgaris* exposed to streptomycin [28]. Assessment of the effect of PCM on photosynthetic efficiency and expression of photosynthesis-related genes of *Pseudokirchneriella subcapitata* and *Scenedesmus dimorphus* is warranted for further studies.

Results showed that the five microalgae were highly resistant to PCM, as the EC_{50} were beyond the highest concentration tested (240 mg L^{-1}) (Table 2). When expressed on the basis of chl a, EC_{50} could only be obtained for *Pseudokirchneriella subcapitata* (230 mg L^{-1}). On the basis of EC_{10}, *Pseudokirchneriella subcapitata* was most sensitive (91 mg L^{-1}) while *Chlorella vulgaris* was most resistant (>240 mg L^{-1}). The findings were contradictory to that reported for *Chlorella pyrenoidosa*, *Oscillatoria* sp. and a mixed population of phytoplanktom [16]. In that study, there was 100% reduction in cell number of the microalgae after exposure to 15 mg L^{-1} PCM for 96 h. The EC_{50} for the total phytoplankton community and *Oscillartoria* sp. was 4 mg L^{-1} after only 24 h exposure to PCM. The drug also affected biochemical markers such as acylesterase activity, lipid peroxidation, and total thiol concentration, and the adverse effect was attributed to oxidative stress. In comparison with the present study, the commercial form of PCM tablets (500 mg) instead of pure acetaminophen was used by Touliabah et al. [16]. The excipient of commercial formulation of PCM tablets may contain the biocidal agent nipastat (0.2%) as a preservative [31], which might affect the sensitivity of the microalgae to PCM in toxicity testing. In the other report on *Scenedesmus subspicatus,* the EC_{50} (72 h) was 134 mg L^{-1} [15] -much higher than that reported by Touliabah et al. [16] although lower than the values obtained in the present study.

The resistance of microalgae to PCM could be due to several reasons. The drug may be adsorbed onto the cell wall or if it is taken into the cell, there may be mechanism that convert them to non-toxic metabolites. Although there have been no reports on the metabolism of PCM in microalgae, it has been shown in higher plant cell culture that detoxification of PCM is similar to the mammalian system, involving cytochrome P450 enzymes, glucosidation, and the formation of gluthatione conjugate [14]. Paracetamol has also been shown to cause oxidative stress in germinating wheat and the increased activities of peroxidase and SOD could serve as a defense mechanism against the toxic effect of the drug [13].

The sensitivity of aquatic plants, including microalgae, to pharmaceuticals is dependent on the molecular targets of the drug, as reviewed by Brain et al. [32]. The primary site of action of PCM is still under debate, but it is believed that it acts mainly by inhibiting cyclooxygenase-2 (COX-2) [33]. It is not well known whether the cyclooxygenase pathway exists in algae. However, a gene coding for cyclooxygenase has been cloned, from the red alga

Table 2. EC_{50} and EC_{10} (mg/L) of paracetamol based on OD_{620} and chlorophyll a (chl a) concentrations of the microalgae grown for 96 h.

Species	EC_{50} based on:		EC_{10} based on:	
	OD_{620}	Chl a	OD_{620}	Chl a
Pseudokirchneriella subcapitata	>240	230	>240	91.4
Scenedesmus dimorphus	>240	>240	>240	149.0
Stichococcus bacillaris	>240	>240	>240	106.8
Chlorella vulgaris	>240	>240	>240	>240
Chlamyodomonas reinhardtii	>240	>240	>240	205.5

Gracilaria vermiculophylla [34]. Thus, the resistance to PCM could be due to the lack of molecular target for the drug in microalgae. In comparison, microalgae are sensitive to other pharmaceuticals such as triclosan (antibacterial agent), which acts on the fatty acid synthesis pathway, a common target that exists in both bacteria and microalgae [32].

The present study corroborated with other findings that predict the environmental risk of PCM is very low, with a hazard quotient (HQ) of less than 1 [32, 35]. The HQ is calculated based on the ratio of measured environmental concentration (MEC) over predicted no effect concentration (PNEC). If MEC is taken as 79.2 µg L^{-1} [5] and EC_{10} of 91,400 µg L^{-1} from the present study is used as the PNEC, the HQ is 0.0009. The extremely low HQ suggests that PCM has a very low environmental risk compared with other drugs such as ibuprofen and fluoroxetine [35]. Based on the toxicity data from the present study, PCM may not pose a significant threat to microalgae in the aquatic environment.

Conclusion

This study showed that the five microalgae tested were highly resistant to PCM. The presence of the drug in the environment may not pose a threat to the microalgae as indicated by the high HQ calculated from the toxicity data. If compared on the basis of EC_{10} (chl a), *Pseudokirchneriella subcapitata* was most sensitive while *Chlorella vulgaris* was most resistant among the test microalgae. In general, the effect of PCM on pigmentation of the microalgae was only evident at the highest PCM concentration tested (240 mg L^{-1}). As change in pigmentation may affect photosynthesis, assessment of the effect of PCM on photosynthetic efficiency and expression of photosynthesis-related genes of the microalgae, especially *Pseudokirchneriella subcapitata* and *Scenedesmus dimorphus* is warranted for further studies.

Acknowledgements

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References

1. HUSCHEK G., HANSEN P.D., MAURER H.H., KRENGEL D., KAYSER A., Environmental risk assesssment of medicinal products for human use according to European Commission recommendations. Environ. Toxicol. **19**, 226, **2004**.
2. JONES O.A., VOULVOULIS N., LESTER J.N., Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Res. **36**, 5013, **2002**.
3. SANTOS L.H.M.L.M., ARAÚJO A.N., FACHINI A., PENA A., DELERUE-MATOS C., MONTENEGRO M.C.B.S.M., Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. **175**, 45, **2010**.
4. KOLPIN D.W., FURLONG E.T., MEYER M.T., THURMAN E.M., ZAUGG S.D., BARBER L.B., BUXTON H.T., Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ. Sci. Technol. **36**, 1202, **2002**.
5. GRUJIC S., VASILJEVIC T., LAUˇSEVIC M., Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography-ion trap-tandem mass spectrometry. J. Chromatogr. A **1216**, 4989, **2009**.
6. CHOI K., KI P.G., PARK J., Pharmaceuticals in the environment and their implication in environmental health. J. Environ. Health Sci. **35**, 433, **2009**.
7. BOUND J.P., VOULVOULIS N., Predicted and measured concentrations for selected pharmaceuticals in UK rivers: implications for risk assessment. Water Res. **40**, 2885, **2006**.
8. WIEGEL S., AULINGER A., BROCKMEYER R., HARMS H., LOFFLER J., REINCKE H., SCHMIDT R., STACHEL B., VON TUMPLING W., WANKE A., 2004. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere **57**, 107, **2004**.
9. FRAM M.S., BELITZ K., Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. Sci. Tot. Environ. **409**, 3409, **2011**.
10. RABIET M., TOGOLA A., BRISSAUD F., SEIDEL J.L., BUDZINSKI H., ELBAZ-POULICHET F., Consequence of wastewater disposal on the contamination of the water resource by pharmaceuticals in a Mediterranean basin. Environ. Sci. Technol. **40**, 5282, **2006**.
11. BOTTONI P., CAROLI S., BARRA CARACCIOLO A., Pharmaceuticals as priority water contaminants. Toxicol. Environ. Chem. **92**, 549, **2010.**
12. KIM P.G., PARK Y., JI K., SEO J., LEE S., CHOI K., KHO Y., PARK J., CHOI K., Effect of chronic exposure to acetaminophen and linomycin on Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*, and potential mechanisms of endocrine disruption. Chemosphere **89**, 10, **2012**.
13. AN J., ZHOU Q., SUN F., ZHANG I., Ecotoxicological effects of paracetamol on seed germination and seedling development of wheat (*Triticum aestivum* L.). J. Hazard. Mater. **169**, 751, **2009**.
14. HUBER C., BARTHA B., HARPAINTNER R., SCHRODER P., Metabolism of acetaminophen (paracetamol) in plants – two independent pathways that result in the formation of a glutathione and a glucose conjugate. Environ. Sci. Pollut. Res. **16,** 206, **2009**.
15. HENSCHEL K.P., WENZEL A., DIEDRICH M., FLIEDNER A., Environmental hazard assessment of pharmaceuticals. Regul. Toxicol. Pharmacol. **25**, 220, **1997**.
16. TOULIABAH H.E., EL-BASSAT R.A., EL-SHIMY A., HARISA G.I., Oxidative stress of plankton community and some isolated species during paracetamol toxicity test. J. Biol. Sci. **8**, 13, **2008**.
17. CHU W.L., TNEH S.H., AMBU S., A survey of airborne algae and cyanobacteria within the indoor environment of an office building in Kuala Lumpur. Grana **52** (3), 207, **2013**.
18. NICHOLS H.W., BOLD H.C. *Trichorsarcina polymorphia*. J. Phycol. **1**, 34, **1965**.
19. STRICKLAND J.P.H., PARSONS T.R., A Practical Handbook of Seawater Analysis; Bulletin Fisheries Research Board of Canada: Canada, Bulletin 167, **1968**.

20. HANSMANN E., Pigment analysis. In: STEIN J.R.(ed.) Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press; Cambridge, pp 359-368, **1973**.
21. NORBERG-KING T.J., A Linear Interpolation Method for Sublethal Toxicity: the Inhibition Concentration (ICP) Approach. Version 2.0. National Effluent Toxicity Assessment Center Technical Report 03-93. Environmental Research Laboratory, Duluth, MN 55804, **1993**.
22. OECD (Organisation for Economic Co-operation and Development). OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development (OECD), pp 1-21, **2012**.
23. YANG L.H., YANG G.G., SU H.C., STAUBER J.L., ADAMS M.S., NINET M.T., Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. **27**, 1201, **2008**.
24. ISHIDORI M., LAVORGNA M., NARDELLI A., PASCARELLA L., PARRELLA A., Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci. Tot. Environ. **346,** 87, **2005**.
25. WANG J., ZHANG X., CHEN Y., SOMMERFELD M., HU Q., Toxicity assessment of manufactured nanomaterials using the unicellular green alga *Chlamydomonas reinhardtii*. Chemosphere **73**, 1121, **2008**.
26. DANILOV R.A., EKELUND N.G.A., Effects of Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and pentachlorophenol on photosynthesis and motility in *Chlamydomonas reinhardtii* in short-term exposure experiments. BMC Ecology **1**, 1, **2001**.
27. NIE X., WANG X., CHEN J., ZITKO V., AN T., Response of the freshwater alga *Chlorella vulgaris* to trochloroisocyanuric acid and ciprofloxacin. Environ. Toxicol. Chem. **27**, 168, **2008**.
28. QIAN H., LI J., PAN X., SUN Z.,YE C., JIN G., FU Z., Effects of streptomycin on growth of algae *Chlorella vulgaris* and *Microcystis aeruginosa*. Environ. Toxicol. **27** (4), 229, **2010**.
29. VANNINI C., DOMINGO G., MARSONI M., DE MATTIA F., LABRA M., CASTIGLIONI S., BRACALE M., Effects of a complex mixture of therapeutic drugs on unicellular algae *Pseudokirchneriella subcapitata*. Aquat.Toxicol. **101**, 459, **2011**.
30. AHMED H., HADER D., Short-term bioassay of chlorophenol compounds using *Euglena gracilis*. Scholarly Research Exchange Ecology Article ID 421950, doi:10.3814/2010/421950, **2010**.
31. ANONYMOUS. Paracetamol 500 mg- Lennon Tablets (Package Insert). Available at http://www.aako.nl/product-groups/personal-hair-care/products/preservative-biocide/nipastat, **1999** [accessed 21 February 2013].
32. BRAIN R.A., HANSON M.L., SOLOMON K.R., BROOKS B.W., Aquatic plants exposed to pharmaceuticals: effects and risks. Rev. Environ. Contam. Toxicol. **192**, 67, **2008**.
33. HINZ B., BRUNE K., Paracetamol and cyclooxygenase inhibition: is there a cause for concern? Ann. Rheumat. Dis. **71**, 20, **2012**.
34. KANAMOTO H., TAKEMURA M., OHYAMA K., Identification of a cyclooxygenase gene from the red alga *Gracilaria vermiculophylla* and bioconversion of arachidonic acid to PGF(2α) in engineered *Escherichia coli*. Appl. Microbiol. Biotechnol. **91**, 1129, **2011**.
35. GROS M., PETROVIC M., GINEBREDA A., BARCELO D., Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. **36**, 26, **2010**.