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Effects of the sunscreen ultraviolet filter, oxybenzone, on green microalgae

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Abstract Ultraviolet (UV) filters are widely used in sunscreen and personal care products due to their ability to give protection to our skin from UV radiation. Oxybenzone, commonly known as benzophenone-3, is one type of UV filter found as the active ingredient in many pharmaceutical products. Although oxybenzone has been extensively studied as an environmental toxicant in the ecosystem, little is known about its toxicity effects on microalgae. The effects of oxybenzone on growth (measured as $OD_{620\ nm}$, chl *a* and carotenoids) and macromolecular composition of polar microalgae (*Chlorella* UMACC 400 and *Chlorella* UMACC 401) and temperate microalgae (*Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*) were investigated. These microalgae were cultured in triplicate and exposed to different oxybenzone concentrations (0, 100, 200, 300 and 400 mg L^{-1}), at 4 °C and 18 °C for polar and temperate species respectively, for 96 h. The oxybenzone concentrations used represent a range from environmental to extreme concentrations to understand the impact of this toxicant on microalgae. The results showed that the highest concentration of oxybenzone (300 and 400 mg L^{-1}) had adverse effects on growth rate and biomass of these microalgae. However, exposure to oxybenzone concentrations ranging from 200 mg L^{-1} to 400 mg L^{-1} did not have significant effects on *S. quadricauda* growth. The exposure to oxybenzone at higher concentrations also led to changes in cell structure after 96 h. Generally, protein and carbohydrate content of all microalgae except *S. quadricauda* increased with increasing oxybenzone concentrations. Protein content increased significantly when cells were exposed to oxybenzone, though effects were greater in the polar species, suggesting that it could be one of the adaptive strategies that enabled these microalgae to tolerate oxybenzone. Further investigation is required to determine the effects of oxybenzone on other features of microalgal performance at relevant environmental concentrations.

Keywords **polar algae, temperate algae, *Chlorella*, benzophenone-3, emerging contaminants**

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1 Introduction

Ultraviolet (UV) filters are individual compounds or mixtures that screen out damaging effects of UV radiation. 'Sunscreen products' commonly refer to the products that can protect human skin from exposure to deleterious wavelengths found in sunlight (Gao et al., 2013). The risks associated with the exposure of skin to UV radiation have led to increased usage of cosmetic products. Cosmetic products such as sunscreen lotions and personal care products usually contain organic and inorganic chemical

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UV filters to fulfill their function of UV protection for users (Tovar-Sánchez et al., 2013). UV filters generally function by absorbing UV photons and returning to the ground state by thermally emitting the energy through a series of vibrational transitions, thus providing protection against both UV-A and UV-B in sunlight (Serpone et al., 2007; Gao et al., 2013).

Oxybenzone, commonly known as benzophenone-3 (BP-3), is a type of UV filter found as the active ingredient in many sunscreen products and has been on the market for almost half a century (Downs et al., 2016; Mao et al., 2017). However, residues of UV filters such as oxybenzone have been reported to be found from various water bodies, wastewater, soil and sediment (Weisbrod et al., 2007). Due to the widespread use of UV filters in personal care products, these chemicals are directly input into aquatic environment by swimmers and municipal residents, in addition to input as wastewater treatment plant effluent (Gao et al., 2013; Downs et al., 2016). In environmental waters, reported oxybenzone concentrations are normally relatively low (in the ng·L^{-1} level), but in wastewater effluents or recreational water bodies affected by swimmers, the concentrations can be higher (reaching up to μg·L^{-1} or mg·L^{-1}) (Downs et al., 2016). Given that oxybenzone is a persistent contaminant in water bodies, the concentrations may further increase in the future.

The introduction of oxybenzone into environmental matrices has raised concern as this has led to high environmental concentrations. For instance, it has been reported that 210 ng·g^{-1} dry mass of oxybenzone was found in treated sludge (Langford et al., 2015), while 125 ng·L^{-1} of oxybenzone has also been detected in surface water (Poiger et al., 2004). In America, an extremely high concentration of oxybenzone (1.395 mg·L^{-1}) was detected at Trunk Bay in the Virgin Islands (Downs et al., 2016). More alarmingly, a study by Tsui et al. (2014) reported the presence of oxybenzone in the Arctic (<250 ng·L^{-1}), which is likely due to long-range oceanic transport. Recently, oxybenzone has been included in the group of "high production volume (HPV)" chemicals due to their increasingly common use in textiles and clothing, as well as their presence in high amounts in both imports and domestic production (US EPA, 2020). Oxybenzone has recently been banned as an active ingredient in sunscreen products in Hawaii, USA due to its known toxicity towards biota (Mao et al., 2019). Consequently, oxybenzone is also seen as an emerging contaminant of concern in marine environments (Downs et al., 2016; Mao et al., 2019).

Studies have shown that UV filters are stable against biodegradation or absorption, due to their lipophilicity. This has caused accumulation of chemical compounds in the food chain which may result in an imbalance of aquatic ecosystems (Tovar-Sánchez et al., 2013). For example, a study has shown that oxybenzone affected the morphology, growth rate and chlorophyll *a* (chl *a*) concentration of planulae of the coral *Stylophora pistillata* (Downs et al., 2016). The LC_{50} (LC_{50}: standard measure of toxicity – the concentration of the substance that causes the death of 50% of the test subjects) of planulae exposed to oxybenzone in the light for an 8- and 24-h exposure was 3.1 mg·L^{-1} and 139 μg·L^{-1}, respectively. The LC_{50}s for oxybenzone in darkness for the same time points were 16.8 mg·L^{-1} and 779 μg·L^{-1}. This confirms that oxybenzone can act as a photo-toxicant with adverse effects exacerbated in the light versus in darkness. It has also been reported that oxybenzone affected growth of the alga *Desmodesmus subspicatus* as this compound could increase damage to DNA, especially when cells were concurrently exposed to light, thus having a mutagenic effect (Tovar-Sánchez et al., 2013).

The chemical residues from UV sunscreens detected in aquatic environments are known to have adverse effects towards fish, corals and protozoa but little is known about their effects on microalgae (Weisbrod et al., 2007; Gao et al., 2013; Downs et al., 2016). A study has shown that exposure to 33 μL·L^{-1} of oxybenzone resulted in the release of large proportion (96%) of zooxanthellae by *Acropora* sp. within 48 h, and exposure to 50 μL·L^{-1} resulted in the release of 79% of zooxanthellae in *A. pulchra* within 96 h (Danovaro et al., 2008).

In aquatic environments, microalgae are important as they are major primary producers in food chains. As microalgae form the base of food webs in aquatic ecosystems, any adverse impact of oxybenzone on these organisms will eventually affect those higher in the food chain. Therefore, there has been increased concern regarding the potential negative impacts of oxybenzone towards microalgae and ecosystems in the future. The primary aim of this study was to determine the growth responses, cell size, pigments (chl *a* and carotenoid content), and biochemical composition (protein and carbohydrate) of green microalgae exposed to different oxybenzone concentrations.

2 Materials and methods

2.1 Algal culture

The polar microalgae strains, namely *Chlorella* UMACC 400 and *Chlorella* UMACC 401, were obtained from the University of Malaya Algae Culture Collection (UMACC), while the temperate microalgae, *Chlorella* sp. (152069), *Chlamydomonas reinhardtii* (152040) and *Scenedesmus quadricauda* (152510), were purchased from the Carolina Biological Supply Company, USA. The polar and temperate microalgae were grown in Bold's Basal Medium (Phang and Chu, 1999) and maintained in a temperature-controlled growth chamber (Protech Growth Chamber, Tech-Lab Scientific Sdn. Bhd., Malaysia) set at 4 ℃ and 18 ℃, respectively. Illumination was provided by cool fluorescent lamps (42 μmol photons·m^{-2}·s^{-1}) with a 12 h : 12 h light-dark cycle. To ensure homogenous cell growth, flasks

were shaken and randomly relocated twice or three times a day to eliminate any effect of slight variations in light intensity at different positions in the chamber.

2.2 Experimental design

A stock solution of oxybenzone (2282.4 mg L^{-1}) was prepared in ethanol (solubility of oxybenzone in 95% ethanol was 50 mg mL^{-1}) by dissolving 2.2824 g of BP-3 in 45.65 mL ethanol. The stock solution was topped up to 1000 mL with Bold's Basal Medium and stored at −20 ℃ in the dark (Mao et al., 2017). The working solutions were diluted to the desired concentrations in the experimental flasks. At a constant temperature (4 ℃ for polar strains, 18 ℃ for temperate strains), triplicate flasks containing algal cultures were exposed to different oxybenzone concentrations (0, 100, 200, 300 and 400 mg L^{-1}) for 96 h to study their growth responses and biochemical composition. The dilution of stock oxybenzone resulted in minimal concentrations of ethanol in the flasks that were too low to influence algal growth.

2.3 Growth measurements

The inoculum (10%) used was from exponential phase cultures standardized to an optical density at 620 nm ($OD_{620\ nm}$) of 0.2. The algal cultures were grown in 500 mL flasks and were exposed to different oxybenzone concentrations as described above. The cultures were sampled every 24 h for 96 h. Two parameters were used to monitor growth of cultures, $OD_{620\ nm}$ and chl *a* concentration. The latter was measured using a spectrophotometric method after extraction of the filtered cells (glass-fibre filters, 0.45 μm) in acetone (Parsons and Strickland, 1963).

The carotenoid content was calculated using the following formula:

$$\text{Carotenoid } (\mu g\ mL^{-1}) = OD_{452\ nm} \times 3.86 \times V_a/V_c,$$

where V_a is the volume of acetone (mL) and V_c is the volume of culture (mL).

Specific growth rate (μ, unit: d^{-1}) based on $OD_{620\ nm}$ was calculated using the following formula:

$$\mu\ (d^{-1}) = (\mathrm{Ln}N_2 - \mathrm{Ln}N_1)/(t_2 - t_1),$$

where N_2 is $OD_{620\ nm}$ at t_2, N_1 is $OD_{620\ nm}$ at t_1, and t_2 and t_1 are times within the exponential phase. The cells were harvested at the end of the experiment by filtration for determination of the protein and carbohydrate content.

2.4 Percentage inhibition of growth

The following equation was used to calculate the percentage of inhibition of specific growth rate (μ) by the treatments.

$$I_{\mu i} = \frac{\mu_c - \mu_i}{\mu_c} \times 100\%,$$

where $I_{\mu i}$ = percentage of inhibition for test concentration *i*, μ_i = mean specific growth rate for test concentration *i* and μ_c = mean specific growth rate for control.

2.5 Protein analysis

Proteins were extracted in 0.5 mol L^{-1} NaOH (80 ℃, 20 min) and the concentration was determined by the dye-binding method (Bradford, 1976).

2.6 Carbohydrate analysis

Carbohydrates were extracted in 2 mol L^{-1} HCl (80 ℃, 60 min) and the concentration quantified by the phenol-sulphuric acid method (Kochert, 1978) using glucose as a standard.

2.7 Cell size

The cell size was measured every 24 h until the end of the experiment (96 h) using a microscope (Nikon Inverted Microscope Eclipse Ti-E, Japan) and analysed using imaging software (Imaging Software NIS-Elements Version 3.2). For each replicate, ten cells were randomly selected and measured for cell size under 100× magnification.

2.8 Statistical analyses

IBM SPSS Statistics 20.0 was used to conduct statistical analysis. Analysis of Variance (ANOVA) was used to test for overall differences between treatments, followed if significant by pairwise Tukey comparisons. The different concentrations of oxybenzone were used as the independent variable while specific growth rate, percentage inhibition of growth rate, cell size, chl *a* concentration, carotenoid, chl *a* ∶ carotenoid, protein and carbohydrate were used as dependent variables. Differences were considered significant at $p < 0.05$.

3 Results

3.1 Growth trends

Specific growth rate (μ) calculated based on the $OD_{620\ nm}$ for the five microalgae is shown in Figure 1. Among the five microalgae, higher μ was observed in the three temperate microalgae compared to the two polar microalgae. Oxybenzone did not affect either *Chlorella* sp. or *Chlorella* UMACC 400 as there was no significant difference in μ when the cultures were exposed to different oxybenzone concentrations. Highest μ was observed in *C. reinhardtii* grown at 100 mg L^{-1} oxybenzone. In general, similar trends were observed for most microalgae where the μ decreased when exposed to higher oxybenzone concentrations (200 to 400 mg L^{-1}). However, the opposite trend was observed in *S. quadricauda* where μ increased with increasing oxybenzone concentrations.

3.2 Percentage inhibition of growth rate

The percentage inhibition of growth rate due to exposure to oxybenzone for the five microalgae is shown in Figure 2. *S. quadricauda* showed a negative percentage inhibition

(=stimulation of growth) from 0 mg Ł$^{-1}$ to 400 mg Ł$^{-1}$ (–63.6% to –24.2%). This indicated that oxybenzone stimulated the growth of this chlorophyte. However, the reverse trend was observed in *Chlorella* sp. and *Chlorella* UMACC 401. Both *C. reinhardtii* and *Chlorella* UMACC 400 responded in the same way towards the oxybenzone. The growth of both chlorophytes was stimulated when grown at 100 mg Ł$^{-1}$ oxybenzone, but inhibited at higher concentrations. Among the microalgae studied, *Chlorella* UMACC 401 was most sensitive to oxybenzone with the percentage inhibition of growth rate ranging from 48.0% to 72.0%.

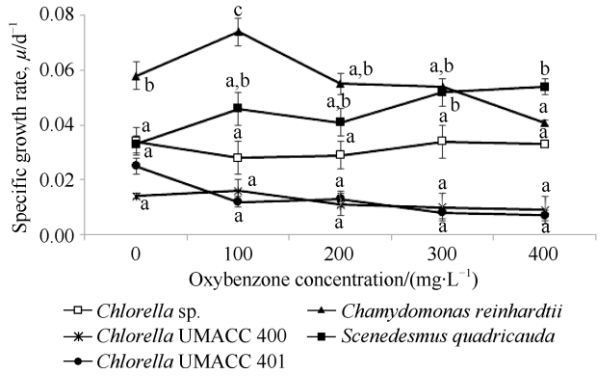

Figure 1 Specific growth rate (μ, unit: d^{-1}) of *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations. The data are presented as means ± S.D., n = 3 and error bars are standard deviations. Different letters represent significant differences between concentrations with p-values < 0.05, ANOVA, Tukey multiple comparison.

3.3 Biomass

The final biomass attained by the end of the experiment (96 h) based on the dry weight of the five microalgae is shown in Figure 3. Both polar *Chlorella* strains (UMACC 400 and 401) showed similar trends where the final biomass decreased when exposed to higher oxybenzone (300 and 400 mg Ł$^{-1}$). In contrast, the final biomass of *S. quadricauda* increased with increasing oxybenzone from 100 to 400 mg Ł$^{-1}$. Highest biomass was attained by *C. reinhardtii* (223.3±8.82 mg Ł$^{-1}$, DW) grown at 100 mg Ł$^{-1}$ oxybenzone.

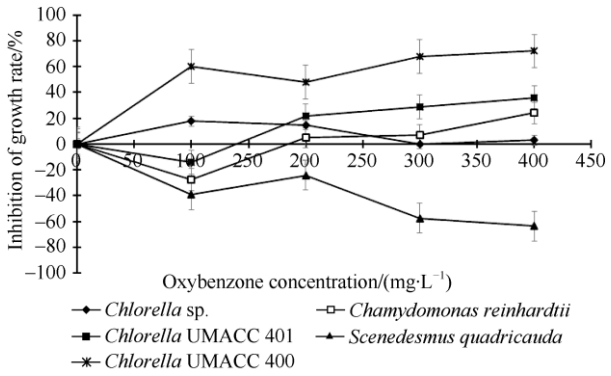

Figure 2 The percentage inhibition of growth for *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations.

3.4 Cell size

The cell size of the five microalgae at 96 h is shown in Table 1 and Figure 4. There was a significant increase in the cell size of *Chlorella* sp. when exposed to increasing concentrations of oxybenzone. The highest concentration of oxybenzone (400 mg Ł$^{-1}$) caused an increase of 65.5% (from 5.1±0.4 μm to 8.4±0.6 μm) in cell size for this chlorophyte. A similar trend was observed for *S. quadricauda* where the smallest cell diameter (26.1±3.8 μm)

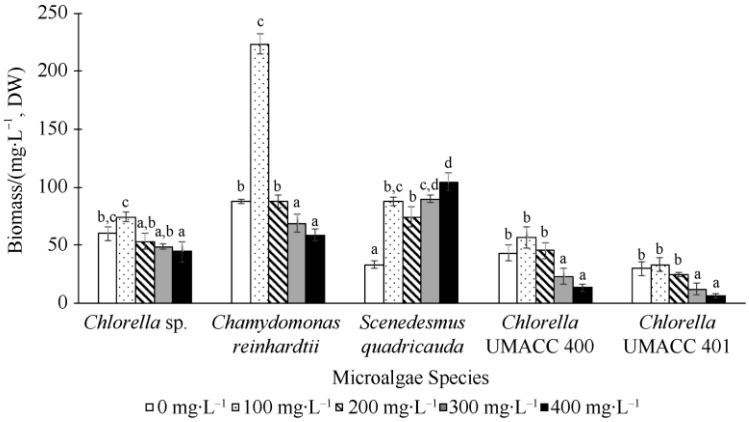

Figure 3 Biomass after 96 h growth for *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations. The data are presented as mean ± S.D., n = 3 and error bars are standard deviation. Different letters represent significant differences between concentrations with p-values < 0.05, ANOVA, Tukey multiple comparison.

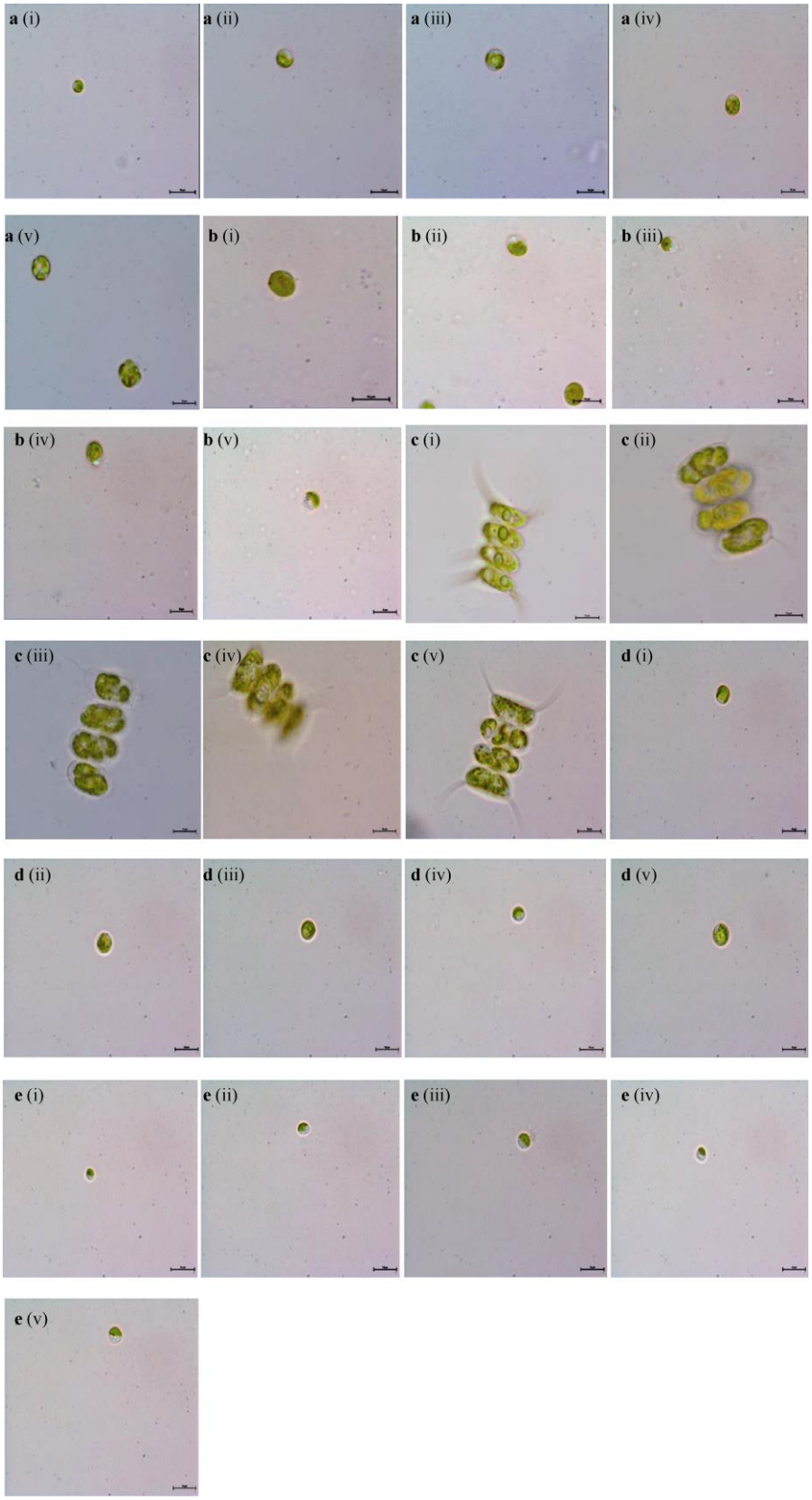

Figure 4 Morphological changes of the microalgae *Chlorella* sp. (**a**), *Chlamydomonas reinhardtii* (**b**), *Scenedesmus quadricauda* (**c**), *Chlorella* UMACC 400 (**d**) and *Chlorella* UMACC 401 (**e**) exposed to different concentration of oxybenzone: (i) 0 mg Ł$^{-1}$, (ii) 100 mg Ł$^{-1}$, (iii) 200 mg Ł$^{-1}$, (iv) 300 mg Ł$^{-1}$ and (v) 400 mg Ł$^{-1}$ for 96 h.

Table 1 Cell sizes of *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different concentrations of oxybenzone after 96 h growth. Different letters indicate significant differences at $p < 0.05$, ANOVA, Tukey multiple comparison

Concentration /(mg Ł$^{-1}$)	Cell diameter/μm				
	Chlorella sp.	*Chlamydomonas reinhardtii*	*Scenedesmus quadricauda*	*Chlorella* UMACC 400	*Chlorella* UMACC 401
0	5.10 ±0.44^{a}	8.00 ±0.61^{a,b}	26.14 ±3.78^{a}	5.78 ±0.76^{a}	4.91 ±0.42^{a}
100	6.08 ±0.70^{b}	7.94 ±0.73^{a}	37.89 ±2.22^{c}	6.18 ±0.46^{a}	4.97 ±0.41^{a}
200	6.49 ±0.91^{b}	7.94 ±0.67^{a}	30.07 ±4.93^{a,b}	6.15 ±0.47^{a}	5.25 ±0.36^{a}
300	6.72 ±0.44^{b}	8.93 ±1.02^{b}	33.29 ±1.86^{b}	5.89 ±0.76^{a}	4.92 ±0.39^{a}
400	8.44 ±0.56^{c}	8.76 ±0.79^{a,b}	30.61 ±3.90^{a,b}	6.05 ±0.84^{a}	5.43 ±0.80^{a}

was obtained when the microalgae were grown under control conditions (0 mg Ł$^{-1}$ oxybenzone) and there was a 15.0% to 45.0% increase in cell size (30.1–37.9 μm) when the cultures were exposed to increasing concentrations of oxybenzone (Figure 4c). In contrast, no significant difference was observed for both polar *Chlorella* strains exposed to different oxybenzone concentrations (Figure 4d and 4e).

3.5 Chl *a*, carotenoid and chl *a* ∶ carotenoid ratio

The final cellular chl *a*, carotenoid contents (as measured on a dry weight basis) and chl *a* ∶ carotenoid ratio (chl *a* ∶ car ratio) attained by the five microalgae are shown in Figure 5. Generally, a similar trend was observed for the chl *a* and carotenoid content in all microalgae tested (Figures 5a and 5b). At 100 mg Ł$^{-1}$ oxybenzone, the chl *a* content of *Chlorella* sp. and *C. reinhardtii* increased by 61.3% and 25.1%, respectively in regard to control (Figure 5a). The same trend was observed for the carotenoid content where the increase was 39.5% and 9.5%, respectively (Figure 5b). Higher concentrations of oxybenzone (300 to 400 mg Ł$^{-1}$) led to a significant decrease in the final chl *a* and carotenoid content of *C. reinhardtii* and *S. quadricauda*. However, the opposite trend was observed for both polar *Chlorella* strains where higher chl *a* and carotenoid content per mg dry weight were found at 400 mg Ł$^{-1}$ oxybenzone. As for the chl *a* ∶ car ratio (Figure 5c), higher values were obtained for *Chlorella* sp. at 400 mg Ł$^{-1}$ oxybenzone. A similar trend was observed for *Chlorella* UMACC 400 at lower oxybenzone concentrations (100 mg Ł$^{-1}$ and 200 mg Ł$^{-1}$). In contrast, no significant difference was observed for *C. reinhardtii*, *S. quadricauda* and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations.

3.6 Protein and carbohydrate content

The protein and carbohydrate content of the five microalgae are shown in Figure 6. In general, all microalgae, except *S. quadricauda*, showed a similar trend when exposed to increasing oxybenzone concentrations. The protein and carbohydrate content per dry weight increased with increasing oxybenzone concentrations. However, an inverse trend was observed in *S. quadricauda* where oxybenzone caused a significant decrease in protein and carbohydrate content by 54.3%–67.6% and 54.3%–67.5%, respectively. Of the microalgae tested, the two polar *Chlorella* strains (*Chlorella* UMACC 400 and *Chlorella* UMACC 401) produced significantly higher protein (56.1% to 75.2%, DW) and carbohydrate (4.1% to 6.0%, DW) at 400 mg Ł$^{-1}$ oxybenzone.

4 Discussion

Widespread use of oxybenzone in various personal care products including sunscreens and cosmetics has led to the release of this compound and its derivatives into aquatic environments around the world (Poiger et al., 2004; Langford et al., 2015; Downs et al., 2016). Its frequent detection in both water and biota have raised concerns about the ecological risks of this compound. Several studies have reported adverse effects of oxybenzone on the reproduction and development of aquatic organisms. Oxybenzone concentrations that have been reported as causing adverse acute and chronic effects on algae and invertebrates were 0.36–0.96 mg Ł$^{-1}$ and 0.50–1.90 mg Ł$^{-1}$, respectively (Rodil et al., 2009; Fent et al., 2010; Sieratowicz et al., 2011; Li, 2012).

Oxybenzone, among all the UV sunscreen compounds, is considered the most dominant in water bodies and it acts as a pseudo-persistent pollutant. Also, it is photochemically transformed and tends to be constantly renewed, thereby causing the ecological receptors to experience prolonged exposure (Vione et al., 2013). Oxybenzone can be metabolized into benzophenone-1 in organisms, leading to more harmful effects as this compound was found to be more toxic than oxybenzone (Kim et al., 2014). Previous studies confirmed various toxicological impacts of oxybenzone towards a range of organisms from the molecular level to multi-organ systems (Gilbert et al., 2013). Oxybenzone is a reproductive toxicant where exposed mice were found to show a reduction in immunocompetence, and

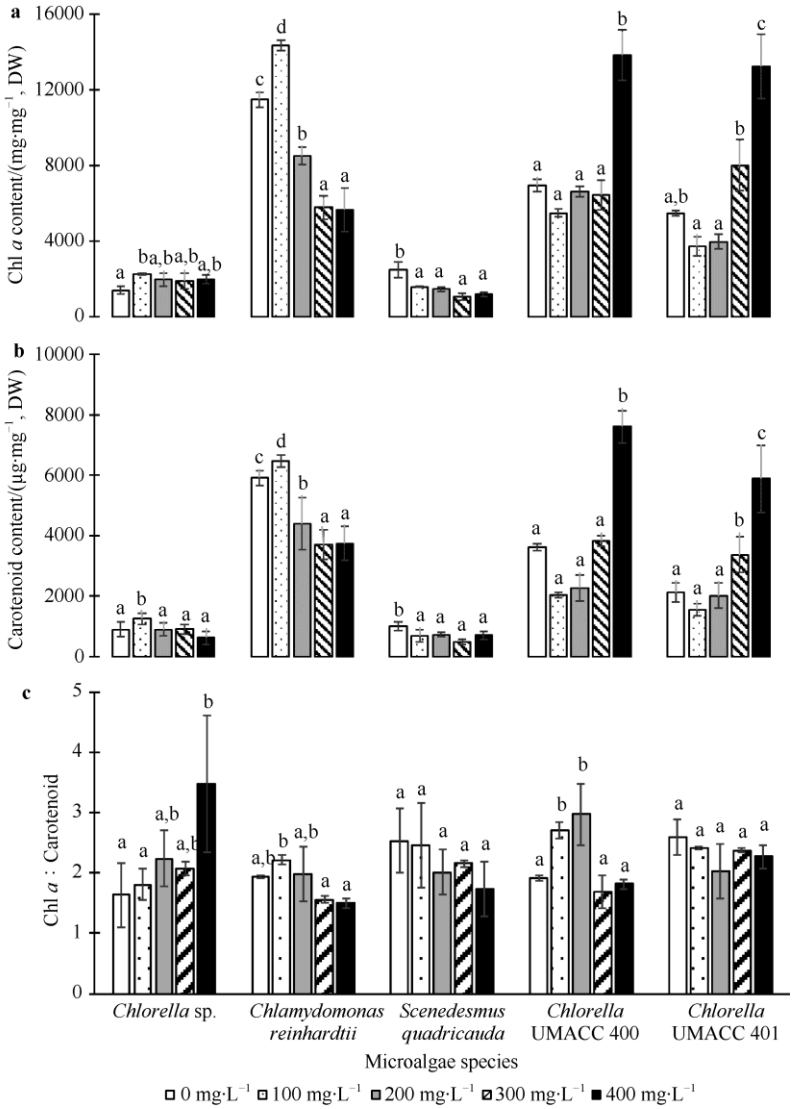

Figure 5 The final chl *a* content (**a**), carotenoid content (**b**) and chl *a* ∶ car ratio (**c**) of *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations on 96 h. The data presented as mean ± S.D., n = 3 and error bars are standard deviation. Different letters on columns represent significant differences between concentrations with p-value < 0.05, ANOVA, Tukey multiple comparison.

significant increase in uterine weight (Ranchon et al., 2006; Downs et al., 2016). Oxybenzone is also an endocrine-disrupting agent which caused reduction in egg production in fish after chronic exposure (Coronado et al., 2008). Other organisms such as coral planulae have even higher sensitivities towards this toxicant, and it caused an increased rate of coral bleaching with increasing concentration of oxybenzone (Downs et al., 2016).

Oxybenzone tends to bioaccumulate, and hence organisms such as microalgae which acts as the basis of the food chain require extensive study as any effect at this level will critically affect the entire food chain (Mao et al., 2019). In this study, we quantified the response of five green microalgae from different regions (temperate and polar) to oxybenzone stress. This report serves as the first report of oxybenzone effects on polar microalgae.

4.1 Effect of oxybenzone on microalgae growth

In this study, *S. quadricauda* showed high tolerance towards oxybenzone with increasing oxybenzone concentrations (Figure 1) where increased specific growth rate was observed. However, *C. reinhardtii* sp. and the polar *Chlorella* UMACC 401 exhibited a significant decreased in specific growth rate with increasing concentrations of oxybenzone. Different species of microalgae exhibit different stress responses towards toxicant stress. This phenomenon was also observed in another study where the specific growth rate of *M. aeruginosa* was moderately affected by benzophenone-3, whereas the specific growth rate of *C. reinhardtii* showed a significant decrease when

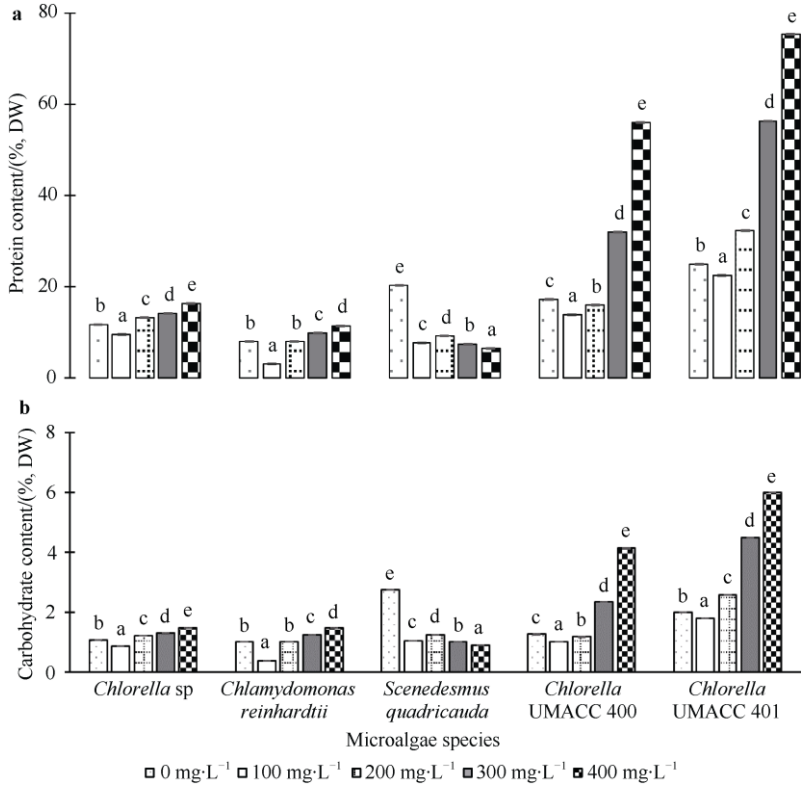

Figure 6 The protein content (**a**) and carbohydrate content (**b**) of *Chlorella* sp., *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Chlorella* UMACC 400 and *Chlorella* UMACC 401 exposed to different oxybenzone concentrations on 96 h. The data presented as mean ± S.D, $n = 3$ and error bars are standard deviation. Different letters on columns represent significant differences between concentrations with p-value < 0.05, ANOVA, Tukey multiple comparison.

exposed to the toxicant (Mao et al., 2017).

The ability of *S. quadricauda* to tolerate the increase of oxybenzone concentrations might be due to their tendency to form colonies compared to other microalgae (Pickett-Heaps and Staehelin, 1975). Earlier studies showed that *Scenedesmus* sp. showed lesser induction of SOD activity as compared to *Chlorella*, when exposed to TiO_2 nanoparticles (Roy et al., 2016). The high tendency of *Scenedesmus* to become colonial (Pickett-Heaps and Staehelin, 1975) resulted in lesser damage incurred to *Scenedesmus* sp. as compared to other microalgae tested (Roy et al., 2016). However, the fact that *S. quadricauda* growth rate actually increased under the range of oxybenzone concentrations used here is unexpected and requires further investigation.

Both Antarctic and Arctic polar regions provide unique ecosystems due to the extreme environment and differ the most from every other habitat on the planet. It has been reported that organisms in the polar regions are much more susceptible to extremely low levels of toxicants compared to those of temperate latitudes (CARC, 1990). For example, some studies have reported that the temperate microalgae, able to adapt to higher temperatures, are more able to reduce toxicity to PSII reaction centres (Kottuparambil et al., 2017). The D1 protein which is a key subunit of photosystem II, is usually present as single copy in the chloroplast genome of algae (Komenda, 2000). Compared to other species such as cyanobacteria, which contain three genes encoding two different forms of D1 protein (Golden et al., 1986; Golden, 1995), there are fewer copies of the gene in the genome. Therefore, the turnover rate of this protein and the recovery rate of the PSII may be lower at lower temperature which may contribute to the sensitivity difference between the polar and temperate algae towards the toxic effects of oxybenzone.

Slower growth of *Chlorella* sp. was observed at higher concentrations of oxybenzone (Figure 1). This could be due to the inhibition of photosynthetic activities, suggesting impairment of photosynthesis as shown by Dao and Beardall (2016) for lead toxicity. However, introducing a low concentration of oxybenzone (100 mg·L^{-1}) resulted in the highest growth of *C. reinhardtii*. This might be because these cells responded towards oxybenzone by increasing their photosynthetic activity compared to the other microalgae species as was observed when the two Arctic microalgae (*Chlamydomonas* and *Chlorella*) were exposed to Irgarol 1051 and diuron. It was found that the toxicants act on PS II, resulting in increased photosynthetic activity at low concentration and only appearing toxic at higher

concentration (Kottuparambil et al., 2017).

4.2 Effect of oxybenzone on biomass

The biomass achieved in microalgal cultures is affected by environmental factors such as light, pH, temperature, etc. *Scenedesmus* and *Chlorella* have been found to be able to survive well in extreme environments (Maity et al., 2014; Ravindran et al., 2016). Studies have shown that the biomass of benthic microalgal mats was significantly higher under toxicant stress whereas planktonic microalgae exhibit a significant decrease of dry weight under toxicant stress. Light dependent functions which includes photosynthetic oxygen production were found to be affected when the microalgae were exposed to the toxicant stress (Alsterberg and Sunbäck, 2013).

By comparing the five microalgae species tested, the final biomass of all microalgae decreased at the higher concentrations compared to the control at 96 h, except for *S. quadricauda* where higher biomass was attained at 300 mg Ł$^{-1}$ and 400 mg Ł$^{-1}$ oxybenzone concentrations compared to the control conditions. Different microalgae species exhibit different responses towards stressors as their defence systems vary, and this leads to different amount of biomass being reached. The data presented here showed that oxybenzone is only toxic to some algae, and for others it is either entirely neutral or even beneficial.

4.3 Effect of oxybenzone on cell size

In general, there was an increase in the cell size for *Chlorella* sp. and *S. quadricauda* exposed to different concentrations of oxybenzone (Figure 4; Table 1). The cell size of *Chorella* sp. was significantly increased when exposed to a minimum concentration of oxybenzone at 100 mg Ł$^{-1}$. This result could be supported by a study on the effects of phenol on *Dunaliella salina*, where smaller cells were more sensitive towards the toxicity of copper in the presence of phenolic compounds. The reason could be that small cell size leads to higher absorption and an increased tendency to biotoxicity (Duan et al., 2017). In contrast, no significant difference was observed in the cell size of both polar *Chlorella* strains as compared to the control conditions. This suggested that the effect of oxybenzone on cell size might be species specific. Similarly, the cell size of *S. quadricauda* also increased significantly at higher oxybenzone concentrations. However, this is not in accordance to a study by Pham (2019) where the cell size of *Scenedesmus* sp. did not show significant differences when exposed to silver nanoparticles.

4.4 Effect of oxybenzone on final chl *a* and carotenoid content

There have been intensive studies examining microalgal photosynthetic activity. Therefore, by studying their photosynthetic physiology we can better understand the adaptations towards stressors and any further impacts down the food chain. Chlorophylls and carotenoids are the main photosynthetic pigment used by microalgae for their photosynthetic activity.

In our study, there was a marked increase in chl *a* per mg dry weight for *Chlorella* sp. and *C. reinhardtii* at 100 mg Ł$^{-1}$. This may indicate that low oxybenzone concentration (100 mg Ł$^{-1}$) is beneficial to this chlorophyte. However, higher oxybenzone concentrations led to decreased production of chl *a* for *Chlorella* sp. and *C. reinhardtii* suggesting that the photosynthetic cells are under potential stress from oxybenzone. This might be due to the stressor having interfered with the synthesis of photosynthetic pigments and/or damage the chloroplast ribosomes (Sendra et al., 2017). A decrease in chl *a* could also be associated with inhibition of the electron transport chain in the donor center (Sendra et al., 2017). This result was also supported by a study on cadmium toxicity on *Chlorella* sp. where high concentration of cadmium inhibited PS II due to the damage to thylakoid membranes and reaction centers.

Carotenoids serve as accessory pigments and also act to provide protection from photo-oxidative damage. Our results showed that the carotenoid content for *Chlorella* sp. and *C. reinhardtii* was lowest at high oxybenzone concentrations. This is in accordance with the findings of Shirazi et al. (2015), where the carotenoid content decreased with increasing concentration of aluminium nanoparticles in *D. salina*. This could be due to carotenoid functioning as an anti-oxidant and thus the exposure towards high levels of the stressor led to disruption of these pigments and reduced carotenoid content.

Pigment ratios such as chl *a* to carotenoids are good indicators for detecting stress (Zhang et al., 2008). A lowered chl to carotenoid ratio would indicate an increase in the carotenoid content and subsequent protection of the photosynthesis apparatus and enhancement of the antioxidant ability under oxybenzone stress (Zhang et al., 2008). However, the changes in cellular pigment content with increasing oxybenzone concentrations did not show a consistent trend across all species tested, though both polar species showed very significant increases in chlorophyll and carotenoid levels at the highest concentration tested. The reasons for the differences between temperate and polar species' responses are not clear at present and require further, more detailed, investigations into the physiological changes (such as alterations to photophysiology and possible generation of reactive oxygen species) induced by oxybenzone.

4.5 Effect of oxybenzone on protein and carbohydrate content

There were significant differences, compared to the control, in the protein and carbohydrate content at the highest concentration (400 mg Ł$^{-1}$) among all microalgae tested, except for *S. quadricauda* (Figure 5). The opposite trend

was shown by *S. quadricauda* where the highest concentration showed the lowest protein and carbohydrate content. Overall, the protein content produced by these microalgae was relatively higher than the carbohydrate content.

The protein content in *S. quadricauda* decreased with increasing oxybenzone concentrations. This might be due to the high energy demands of protein synthesis being unmet as a result of the stress caused by oxybenzone, therefore leading to a deficiency in protein synthesis. Low protein content in *S. quadricauda* grown under stress conditions could be the result of changes in gene expression allowing them to cope with the stressor (Salman et al., 2016). In contrast, the increase in protein content observed in the other microalgae tested might be due to the oxidative stress caused by oxybenzone and the cellular synthesis of enzyme systems such as SOD and catalase, which would be induced in response to such stress (Gao et al., 2013).

The high levels of carbohydrate produced at the highest concentration (400 mg Ł$^{-1}$) by all the microalgae (except *S. quadricauda*) could be due to the overproduction of starch as storage material in response to the stressor. These results are in accordance with the findings of Wu et al. (2014) where exposure to selenite caused an increase in the starch content of the algae. There was also an accumulation of carbohydrate under N and P limitation in *S. costatum* and *P. donghaiense* (Zhao et al., 2009). As for *S. quadricauda*, the lowest carbohydrate content produced at highest concentrations of oxybenzone could be due to impaired energy transfer to the photosystem II, from the photo-oxidation of chl *a* induced by oxybenzone (Salman et al., 2016). Since protein and carbohydrate contents were the only parameters measured in this study, a fuller analysis of macromolecular composition (including lipid and nucleic acids) as well as measurements of other parameters such as photosynthesis and respiration rates should be included in future work in order to fully understand and predict their ability to adapt to this emerging contaminant in the environment.

5 Conclusions

It has been hypothesised that microalgae from different geographical regions will respond differently towards oxybenzone because of the effect of temperature on tolerance mechanisms. Of the five microalgae tested, *S. quadricauda* (temperate region) was the most tolerant strain, while the two polar *Chlorella* strains were most sensitive to oxybenzone. The highest concentration of oxybenzone (400 mg Ł$^{-1}$) had significant effects on growth and biomass of all microalgae tested, except *S. quadricauda*. Thus, widely used personal care products that contain oxybenzone could affect microalgae growth and eventually the organisms higher up the food chain. The sensitivity of the polar strains to oxybenzone is of concern in relation to reports of increasing oxybenzone concentrations in polar waters.

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