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Effect and removal of bisphenol A by two extremophilic microalgal strains (Chlorophyta)

Sabrine Ben Ouada^{1,2,3} · Rihab Ben Ali² · Christophe Leboulanger³ · Hatem Zaghdien¹ · Sirine Choura¹ · Hatem Ben Ouada² · Sami Sayadi¹

Abstract

The effects and the removal efficiency of bisphenol A (BPA) on two extremophilic Chlorophyta strains, an alkaliphilic *Picocystis* and a thermophilic *Graesiella*, were assessed. BPA was shown to inhibit the growth and photosynthesis of both species, but to a greater extent for *Graesiella*. The growth IC_{50} (4 days) was 32 mg L^{-1} for *Graesiella* and higher than 75 mg L^{-1} for *Picocystis*. Oxidative stress was induced in both strains when exposed to increasing BPA concentrations, as evidenced by increased malondialdehyde content. BPA exposure also resulted in an over-expression of antioxidant activities (ascorbate peroxidase, glutathione *S*-transferase and catalase) in *Picocystis* whereas they were repressed in *Graesiella*. Both species exhibited high BPA removal efficiency, reaching 72% for *Picocystis* and 52.6% for *Graesiella* at 25 mg L^{-1} . BPA removal was mostly attributed to biodegradation for both species. Overall, according to its extended tolerance and its removal capacity, *Picocystis* appeared to be a promising species for the BPA bioremediation even at high contamination levels.

Keywords Microalgae · Extremophiles · Chlorophyta · Bisphenol a · Toxicity · Biodegradation

Introduction

Bisphenol A (BPA) is a synthetic chemical used as an intermediate in the production of polycarbonate plastics, epoxy resins, and certain polymers used in dental sealants or composites which are considered as one of the most used materials in daily life (Michałowicz 2014). BPA-based material production has increased continuously during the last decade to reach about 7 million tons per year (Jandegian et al. 2015). BPA can migrate from BPA-based products into the aquatic environment and can leach into the water, especially at high temperature and high pH, which induce additionally an increase in its solubility and then its bioavailability and mobility (Biedermann-Brem and Grob 2008;

Le et al. 2008; Nam et al. 2010; Hoekstra and Simoneau 2013; Si et al. 2014). High levels of BPA ranging from 0.7 to 17.2 mg L^{-1} have been identified in wastewater treatment plants and landfill sites (Yamamoto and Yasuhara 1999; Yamamoto et al. 2001; Filho et al. 2003).

BPA has become a serious environmental problem as large amounts have been dispersed into the aquatic environment, whether by direct disposal or by rainwater and drainage systems discharge (Kang et al. 2007; Hoekstra and Simoneau 2013; Michałowicz 2014). BPA has been detected at concentrations of $150 \text{ } \mu\text{g L}^{-1}$ in industrial wastewaters (Lee and Peart 2000), $21 \text{ } \mu\text{g L}^{-1}$ in rivers (Belfroida et al. 2002), and has been found in freshwater and seawater samples at concentrations ranging from 0.01 to $0.268 \text{ } \mu\text{g L}^{-1}$ (Yamamoto et al. 2001). BPA can migrate through the food chain causing toxic and endocrine-disruptive effects on many aquatic organisms (Kang et al. 2007; Webb et al. 2012). BPA concentration less than $1 \text{ } \mu\text{g L}^{-1}$ interfere with the endocrine system leading to adverse effects on the reproductive, neurological, and immunological systems in both humans and animals (United States Environmental Protection Agency 2010).

Several studies have reported that many species of plants and microorganisms, including bacteria, fungi, and algae, are capable of accumulating (Saiyood et al. 2010; Asadgol et al. 2014; Guo et al. 2017) and transforming or/and degrading BPA (Nakajima

✉ Sami Sayadi
sami.sayadi@cbs.mrt.tn

¹ Laboratory of Environmental Bioprocesses, Center of Biotechnology of Sfax, BP 1177, 3018 Sfax, Tunisia

² Laboratory of Blue Biotechnology and Aquatic Bioproducts, National Institute of Marine Sciences and Technology, 5000 Monastir, Tunisia

³ UMR 248 MARBEC (IRD-University Montpellier-CNRS-Ifremer), CS30171, 34203 Sète, France

et al. 2002; Gulnaz and Dincer 2009; Sun et al. 2012; Arboleda et al. 2013).

As the primary producers in aquatic food chains, microalgae play a crucial role in the structure and functioning of most aquatic ecosystems and are the first targets to be affected by water pollutants including BPA. BPA can cause the inhibition of many physiological processes, including cell division and photosynthesis (Ji et al. 2014; Zhang et al. 2014). Microalgae have also high ability to concentrate, accumulate, and biodegrade environmental BPA (Hirooka et al. 2005; Li et al. 2009). They appear promising as an ecologically sound and sustainable technology of BPA removal (Xiong et al. 2016).

However, the bioremediation of BPA by microalgae is dependent on the tolerance limit of microalgal strains and their removal efficiency. Increased attention is being paid to the isolation and characterization of tolerant algal strains able, not only to tolerate and remove high concentrations of pollutants, but also to maintain sustainable growth under variable environmental conditions (Wang et al. 2016). Such strains, maintained in continuous culture, may be useful for the fast and sustainable purification of the aquatic environment.

Accordingly, extremophilic microalgae thriving at harsh environmental conditions combining with a high anthropogenic pollution seem to be suitable candidates, as they are already known to possess wide possibilities for physiological adaptation and may possess genetic modifications that allow them to tolerate high concentrations of contaminants (Seckbach et al. 2007; Varshney et al. 2014).

Taking into consideration the lack of studies dealing with the BPA effect and removal by extremophilic organisms, the aim of the present work is to evaluate and compare, under laboratory culture conditions, the effects and the removal efficiency of BPA by two extremophilic microalgae strains isolated from two polluted ponds in Tunisia. The first strain, *Picocystis* sp., was isolated from alkaline, residual water, and household sewage pond. The second strain, *Graesiella* sp., was isolated from a hot-spring-fed basin exposed to high levels of plastic pollution. Primarily, the effect of BPA on the physiological and biochemical responses of these strains such as growth, photosynthesis, lipid peroxidation, and antioxidant activities were investigated. The ability of these strains to adsorb, accumulate, biodegrade, and remove BPA is also presented.

Materials and methods

Algal strains and culture conditions

Two Chlorophyta species were investigated in this study: *Picocystis* sp. CINS 23 and *Graesiella* sp. CINS 60. The strains have been deposited in the National Institute of Marine Technology Collection. *Picocystis* sp. was isolated from a household sewage “Essed valley” located in the

Center East of Tunisia (35° 59' 23" N, 10° 30' 10" E) at water pH 11. *Graesiella* sp. issued from a hot water catchment basin emerging from “Ain Echfa,” which is a hot spring located in northern Tunisia (36° 49' N, 10° 34' E) at a water temperature of 60 °C.

Samples collected were treated by filtration, centrifugation, and dilution techniques according to standard microbiological protocols (Stanier et al. 1971). After purification, the unialgal strains were identified morphologically and further confirmed by 18S rRNA gene analysis (Mezhoud et al. 2014).

The strains were cultivated separately in batch culture under sterile conditions: *Graesiella* in Bold's basal medium (BBM) (Bischoff and Bold 1963) modified according to the Elser concept for freshwater microalgae with C/N/P ratio equal to 166:20:1 (Elser et al. 2000) and *Picocystis* in Zarrouk medium (Zarrouk 1966). The initial pH was adjusted to 6.8 and 8.4 for BBM and Zarrouk medium, respectively. Each strain was initially subjected to different temperature and light conditions to define the optimal autotrophic growth conditions. These preliminary experiments showed that both species had the highest growth rate at temperature of 30 °C and light intensity of 75 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ under 8/16-h illumination cycle. Experimental cultures were conducted at these optimal growth conditions for all the designed experiences.

Exponentially, growing cultures of *Picocystis* and *Graesiella* were individually inoculated in a series of 500-mL Erlenmeyer flasks containing 300 mL Zarrouk or BBM media with an initial optical density of 0.07 at 680 nm. Cultures were exposed for 5 days to BPA at 0, 1, 10, 25, 50, and 75 mg L^{-1} initial concentrations.

A stock solution of 1000 mg L^{-1} BPA (Sigma-Aldrich) was prepared in anhydrous ethanol. Ethanol concentration in the medium including the control groups did not exceed 0.5% (v/v), which showed nontoxic effects on both Chlorophyta strains. The testing solutions were diluted to the desired concentrations during the experiments.

For each experimental BPA concentration and separately for each tested strain, a series of six Erlenmeyer flask cultures were used in triplicate. Daily, the culture volume of one Erlenmeyer flask (in triplicate) served for the determination of the optical density, the photosynthetic activity, the lipid peroxidation, the and antioxidant activities. The determination of the residual, adsorbed, accumulated, and biodegraded BPA amounts was carried out at the end of the experiment (day 5).

Growth inhibition test

Growth was followed spectrophotometrically by measuring the daily changes in OD₆₈₀. The growth curves were obtained by plotting OD₆₈₀ values and incubation time. The areas under each growth curve were evaluated using Origin 8.5 software (OriginLab, USA). Inhibition of algal activity was expressed

as the percentage inhibition ($I\%$) defined according to de Orte et al. (2013) by the following equation:

$$I\% = \left[\frac{\text{Control area} - \text{Treated area}}{\text{Control area}} \right] \times 100$$

where Control area and Treated area are the integrated areas under the growth curve of control and treated culture, respectively.

Photosynthetic activity

Net photosynthetic oxygen evolution

Oxygen evolution was measured using a fiber-optic oxygen micro-sensor (Firesting O₂; Pyro-Science, Germany). For the measurement of O₂ evolution rate in the light, cultures were collected and placed in a flask containing a microsensor spot with red flash dye at the same culture conditions (30 °C and 75 μmol photons m⁻² s⁻¹) and oxygen signal was recorded for 30 min. Then, the samples were kept in darkness for 30 min to record the respiratory O₂ consumption rate in the dark. The concentration of dissolved O₂ expressed as nanomoles per milliliter was calculated from the percentage of air saturation by correcting for the temperature and salinity of the medium according to Quicker et al. (1981). The sum of O₂ evolution rate in the light and the respiratory O₂ consumption rate in the dark was considered as the net photosynthetic activity and normalized to the chlorophyll *a* (Chl*a*) concentration for the corresponding culture (nmol O₂ min⁻¹ μg⁻¹ Chl*a*) under the assumption that the respiration rate was equal in light and in darkness.

Chl*a* content was determined spectrophotometrically after extraction in 100% ethanol according to the equation of Ritchie (2006).

Photochemical efficiency of PSII (F_v/F_m)

Chlorophyll *a* fluorescence transient (OJIP) of each sample was measured every day using the Aquapen fluorometer (Photon Systems Instruments, Czech Republic). A sample of 2 mL was collected from each experimental culture and diluted with fresh medium to a final optical density at 680 nm of 0.49. Before the measurement, each micro-algal sample was dark adapted for 15 min at room temperature to complete re-oxidation of PSII reaction centers. The chlorophyll fluorescence transients (OJIP) were induced by a fixed excitation wavelength at 650 nm and light intensity of 3000 μmol photons m⁻² s⁻¹ and recorded for up to 1 s on a logarithmic time scale. The emission was measured at wavelengths beyond 720 nm, and the initial time resolution was 50 μs. The OJIP-test parameters were determined from a

transient analysis according to Strasser et al. (2000), and the maximal PSII photochemical efficiency (F_v/F_m) was derived.

Lipid peroxidation and antioxidant activities

A volume of 250 mL of each treatment was centrifuged and collected; cells were mixed with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 mM Na₂EDTA and 1 mM ascorbic acid and ruptured by sonication three times for 30 s (Ben Ali et al. 2017). The homogenate was then centrifuged at 10,000×*g* for 10 min at 4 °C. The supernatant was considered as a cell-free enzyme extract and was stored at 80 °C for lipid peroxidation and antioxidant activity analyses.

Lipid peroxidation

Five hundred microliters of cell-free enzyme extract were added to an equal volume of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 10% (w/v) trichloroacetic acid (TCA). The mixture was heated at 95 °C for 15 min and quickly cooled in an ice bath. After centrifuging at 10,000×*g* for 5 min, the absorbance at 532 nm was recorded and the value at 600 nm was subtracted. The concentration of malondialdehyde (MDA), as a main product of lipid peroxidation, was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹ according to Heath and Packer (1968) and expressed as micromoles of MDA per milligram of protein.

Antioxidant activities

Catalase (CAT) activity was determined according to Claiborne (1985). The rate of enzymatic decomposition of hydrogen peroxide (H₂O₂) was determined by following the absorbance decrease at 240 nm. The assay mixture consisted of 750 μL of sodium phosphate buffer (0.1 mol L⁻¹, pH 7.8 and 25 °C), 100 μL of 500 mM H₂O₂, and 150 μL of cell-free enzyme extract.

Glutathione *S*-transferase (GST) activity was assayed by the method described by Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Thirty microliter of CDNB (20 mM in ethanol) was added to 60 μL glutathione (GSH) (50 mM) and followed by the addition of 450 μL of potassium phosphate buffer (133 mM, pH 6.5). The above combined solution was mixed well and incubated at 25 °C for 5 min. The reaction was then initiated by adding 60 μL of cell-free enzyme extract to the assay solution. The absorbance at 340 nm increased linearly with time over a 5-min duration. Absorbance values were converted to units using 9.6 mM⁻¹ cm⁻¹ as a molar extinction coefficient.

Ascorbate peroxidase (APX) activity was assayed spectrophotometrically according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H₂O₂, 10 mM ascorbic acid, and cell-free

enzyme extract, with a 1-mL total volume. The reaction was started by adding H₂O₂ at 25 °C. The oxidation rate of ascorbate was determined by the decrease in the absorbance at 290 nm.

Enzymatic activities of CAT, GST, and APX were expressed as unit per milligram of protein. One unit is defined as the amount of enzymes that catalyze the conversion of 1 µmol of substrate per minute.

The protein contents of the samples were determined according to the Hartree method (Hartree 1972), using bovine serum albumin as a standard.

Determination of BPA in the medium and adsorbed and accumulated by cells

After 5 days of exposure, 150 mL of algal culture form of 0, 25, 50, and 75 mg L⁻¹ BPA-treated groups was centrifuged at 10,000×g for 10 min. The supernatant S1 was used for the determination of the residual BPA in the medium. The cell pellets were washed three times with de-ionized water and then centrifuged. The supernatant S2 was used for the determination of BPA adsorbed on the microalgae surface as described by Li et al. (2009). The pellets obtained after centrifugation were mixed with 3 mL of dichloromethane-methanol (1:2 v/v) and digested by sonication for 45 min (Ji et al. 2014). After centrifugation for 10 min at 4500×g, the resulting supernatant S3 was conserved to determine the accumulated BPA within the microalgal cells.

The supernatants S1, S2, and S3 were individually transferred to a separation glass funnel, and BPA was extracted in 5 mL of dichloromethane. The extraction step was repeated three times to ensure total extraction of the BPA amount. The extract was evaporated to dryness under nitrogen and resuspended in 2 mL of methanol.

The same extraction procedure was applied for media without algae cells exposed to the same culture conditions during 5 days and under different experimental BPA concentrations to determine the abiotic removal of BPA.

All samples (S1, S2, and S3) were stored at 4 °C in the dark prior to HPLC analysis.

HPLC analysis

BPA analysis was carried out using Agilent series 1260 HPLC-DAD instrument (Agilent Technologies, Germany) equipped by a Zorbax Eclipse XDB-C18 column, serial number USNH027266 (4.6 mm I.D. × 250 mm × 3.5 µm particle size). Acetic acid (A) at 0.01 and 100% acetonitrile (B) was used as the mobile phases at a 0.5 -mL min⁻¹ flow rate. The elution conditions were as follows: 0–20 min from 25 to 50% B, 20–25 min from 50 to 100% B, 25–30 min 100% B, and 30–40 min from 100 to 75% B. The injection volume was 5 µL, and the detection was performed using a diode array

detector (DAD) at λ = 280 nm. Quantification was performed by external calibration with standards. The limit of detection (LOD) and the limit of quantification (LOQ) were 2.131 and 6.458 mg L⁻¹, respectively.

The residual, adsorbed, and accumulated amounts of BPA were determined in S1, S2, and S3 by HPLC analysis. The abiotic removal of BPA in the medium (without microalgae) at each tested initial concentration was also determined during the experimental period.

The bio-concentration factor (BCF) of BPA by microalgae was calculated according to the following equation (Li et al. 2009):

$$BCF = \frac{(Ac/Fw)}{Ar}$$

where *Ac* is the amount of BPA accumulated within algal cells (µg), *Fw* is the microalgal fresh weight (g mL⁻¹), and *Ar* is the residual amount of BPA in the medium (µg mL⁻¹).

Biodegradation percentage (*Bp*) of BPA by microalgae was calculated according to the following equation (Li et al. 2009):

$$Bp(\%) = (CI - Ar - Aa - Ad - Ac) \times 100 / CI$$

where *CI* is the initial amount of BPA, *Ar* is the residual BPA amount in the medium, *Aa* is the amount of abiotic BPA removal, *Ad* is the amount of BPA adsorbed by the algal cells, and *Ac* is the amount of BPA accumulated within the algal cells.

Statistical analysis

Mean and standard deviation values of the three biological replicates were calculated for each treatment and for the control. In all cases, data are given as mean value with standard deviation.

To determine significant differences among test concentrations, data were statistically analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistic 21.0.0 software. A *P* value < 0.05 was considered statistically significant. When significant differences were observed, means were compared using the multiple-range Duncan's test at a level of significance of 0.05 (*P* < 0.05).

Results and discussion

Effect of BPA on microalgae growth

The growth of *Picocystis* sp. and *Graesiella* sp. as a function of BPA concentrations during 5 days of exposure is shown in Fig. 1. For both studied species, growth was inhibited at different extents depending on the BPA initial concentration and the exposure period. The inhibition percentage (*I*%) (Table 1)

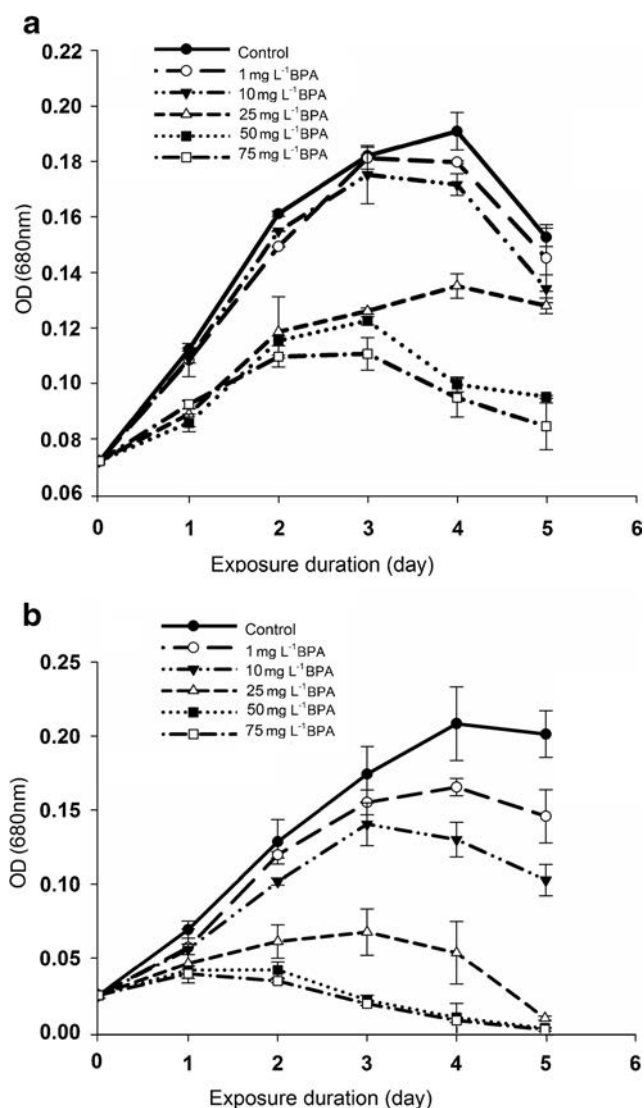


Fig. 1 The variation of the OD (680 nm) of **a** *Picocystis* sp. and **b** *Graesiella* sp. as a function of BPA concentration after 1, 2, 3, 4, and 5 days of BPA exposure. Data are fitted using a logistic regression model ($R^2 > 0.95$)

showed that the growth of both species declined remarkably with increasing BPA concentrations and especially after 2 days of BPA exposure. However, for the same concentration, $I\%$ was higher for *Graesiella* than for *Picocystis* cultures. During all the exposure period, the growth inhibition did not exceed 10, 32, and 43% at 10, 25, and 75 mg L⁻¹ BPA, respectively, for *Picocystis* while it reached 20, 62, and 80% for *Graesiella*. These results demonstrate the high tolerance of *Picocystis* to BPA as compared to *Graesiella*.

Toxic effects of pollutants on algae are generally evaluated by the determination to the 50% growth inhibitory concentrations (IC₅₀). The reported IC₅₀ (4 days) of BPA for microalgae usually ranges between 1 and 10 mg L⁻¹ (Staples et al. 1998), and it can reach a maximum of 63 mg L⁻¹ for some tolerant Chlorophyta species (Zhang et al. 2014). It is obvious that

Table 1 Growth inhibition percentage ($I\%$) of *Picocystis* sp. and *Graesiella* sp. exposed during 5 days to initial concentrations of 1, 10, 25, 50, and 75 mg L⁻¹ BPA

BPA initial concentration (mg L ⁻¹)	Exposure duration				
	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Picocystis</i>					
1	7.78e-3	0.10	0.027	0.022	0.091
10	2.30	4.19	4.73	6.47	8.43
25	11.41	20.20	25.14	29.23	31.63
50	15.98	27.86	34.65	39.21	41.36
75	16.65	28.98	35.89	40.60	43.85
<i>Graesiella</i>					
1	-0.61	0.19	0.17	4.86	6.16
10	1.53	6.43	10.78	15.54	19.17
25	7.35	22.57	39.94	51.81	62.02
50	18.74	37.80	59.31	72.94	80.42
75	22.75	43.40	63.78	76.02	82.66
BPA IC ₅₀ (mg L ⁻¹)	Day 4		Day 5		
<i>Picocystis</i>	>75		>75		
<i>Graesiella</i>	24		19.5		

both microalgae investigated in this study, but chiefly *Picocystis*, were far more tolerant to BPA compared to the species mentioned in the literature. Indeed, the IC₅₀ (4 days) of BPA was 24 mg L⁻¹ for *Graesiella* sp., while for *Picocystis* sp., we did not attain 45% of growth inhibition even after 5 days of exposure to 75 mg L⁻¹ BPA. Probably, the high tolerant behavior of *Picocystis* could be inherent, due to its wastewater origin. Aguilera and Amils (2005) and Rehman et al. (2007) have reported that algal strains isolated from highly polluted rivers possess a great resistance to several contaminants.

It has to be emphasized that, for both studied species, the BPA IC₅₀ values were much higher than the BPA concentrations measured in the isolation sites, which were lower than the HPLC limit detection (data not shown). This fact has been observed in many Chlorophyta species exposed to several contaminants such as antibiotics and herbicides (Costas et al. 2001; Lopas-Rodas et al. 2007; Marvá et al. 2010), algacides (Garcia-Villada et al. 2004), and heavy metals (Ben Ali et al. 2017). The continuous exposure to low contaminant concentrations could induce an inherent resistance probably through the selection of rare spontaneous mutations (García-Balboa et al. 2013).

Effect of BPA on the photosynthetic activity

The observed inhibitory effect of BPA on both microalgae growth may be due to damage in many physiological processes including photosynthesis. The effects of BPA on the net

photosynthetic activities (NPS) of *Picocystis* (Fig. 2a) and *Graesiella* (Fig. 2b) were determined, based on oxygen evolution measurements. Compared to the control, the NPS decreased gradually with the increase of BPA concentration and exposure duration, especially for *Graesiella*. At low BPA concentrations (1–25 mg L⁻¹), the decrease of the NPS did not exceed 40% in *Picocystis* during the exposure period; whereas it reached 80% in *Graesiella* after 5 days of BPA exposure. At higher BPA concentrations (50–75 mg L⁻¹), the NPS decreased by a maximum of 82% in *Picocystis* and was nullified in *Graesiella* after 5 days of BPA exposure.

Interestingly, it was notable that for *Picocystis*, the NPS and the growth were inhibited to different extents. Even when the NPS activity decreased dramatically (more than 80% after 5 day exposure to 75 mg L⁻¹ BPA), the *I*% did not exceed 50%. This observation suggests that the photosynthesis was not the unique source of carbon-based metabolic pathway. Several Chlorophyta species shift to photo-heterotrophic metabolism using organic carbon when mineral carbon medium is complemented by organic substrates (Ogawa and Aiba 1981; Ratha et al. 2013). Similar behavior could be supposed when *Picocystis* cells were exposed to high BPA

concentrations. Such assumption needs to be confirmed by further investigations.

The main cause of the reduced photosynthetic capacity may be damage to photosynthesis components. PSII is generally considered as the major target of environmental stress compared to the rest of the photosynthetic machinery (Ashraf and Harris 2013). The effect of BPA on the PSII photochemical efficiency was monitored by the F_v/F_m ratio. Compared to the control, F_v/F_m decreased in both micro-algae species when the BPA concentration increased (Fig. 3a, b), suggesting that a proportion of PSII reaction centers were inactivated or photodamaged (Elsheery and Cao 2008), which might contribute to the inhibition of NPS. However, this decrease was significantly less pronounced for *Picocystis* than for *Graesiella*. For *Picocystis* the reduction of F_v/F_m was insignificant below 50 mg L⁻¹ BPA during the whole exposure duration, and it reached at most 73% after 5 days of exposure to 75 mg L⁻¹ BPA. However, for *Graesiella*, this reduction was about 60% even at low BPA concentrations and reached 90% after five exposure days to 75 mg L⁻¹ BPA.

It has been reported that cells having ability to grow heterotrophically under sufficient light intensity might use a part of the O₂ produced by photosynthesis, decreasing dissolved oxygen concentration (Ashton and Ziegler 1987; Wu et al. 1991). Such metabolic pathway protects the photosynthetic

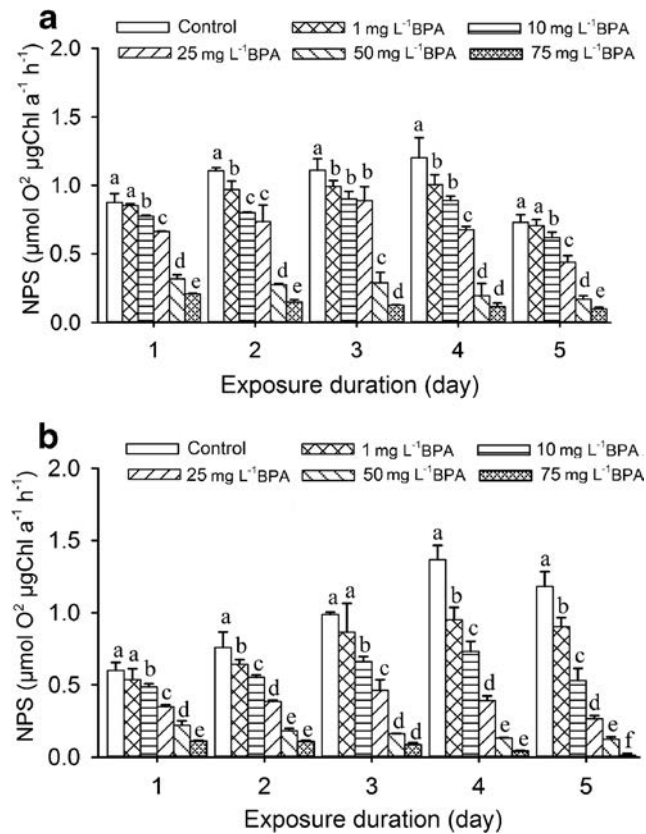


Fig. 2 Changes in the net photosynthetic activities (NPS) of **a** *Picocystis* sp. and **b** *Graesiella* sp. under exposure to 0, 1, 10, 25, 50, and 75 mg L⁻¹ BPA for 5 days. Same letters indicate no significant difference according to Duncan's test ($P \geq 0.05$)

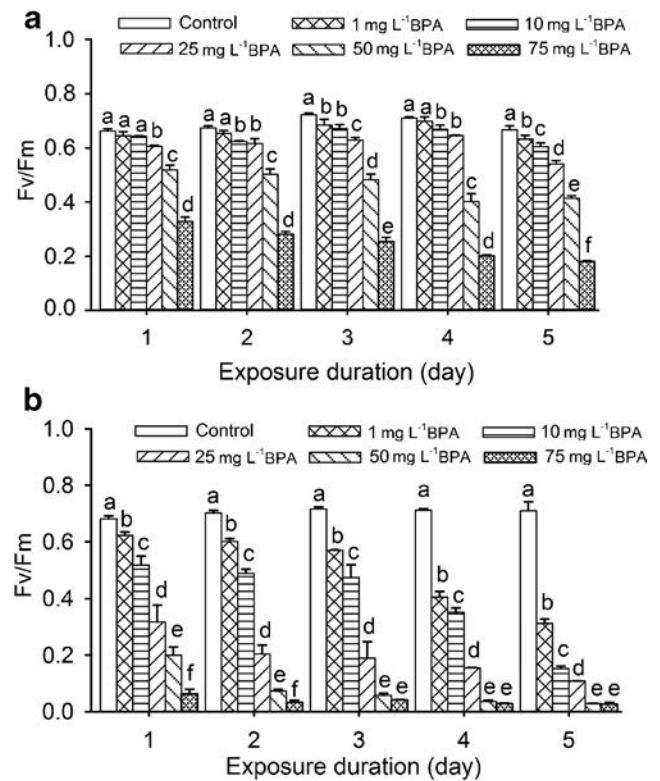


Fig. 3 Variation in the PSII photochemical efficiency (F_v/F_m) of **a** *Picocystis* sp. and **b** *Graesiella* sp. during 5 days of BPA exposure. Same letters indicate no significant difference according to Duncan's test ($P \geq 0.05$)

apparatus by reducing photo-oxidative damage occasioned by excess of reactive oxygen species (ROS) induced by BPA contamination. The lower PSII damage in *Picocystis* could be then eventually attributed to its ability to undergo photo-heterotrophic metabolic pathway.

Effect of BPA on lipid peroxidation levels

The ROS excess in the cell induced by BPA exposure may attack the unsaturated fatty acids, which induces a lipid peroxidation of cell membranes (Wang et al. 2015). This peroxidation leads to the formation of several products. As a main product of lipid peroxidation, MDA was quantified at different BPA concentrations to gauge this effect (Fig. 4). The MDA content exhibited a significant increase in both *Picocystis* (Fig. 4a) and *Graesiella* (Fig. 4b) when the BPA concentration and the exposure period increased. For *Picocystis*, during the first 3 days, MDA did not exceed 2-fold of the control value regardless the BPA concentration. Then, it was enhanced to be at most five times higher than the control value at the fifth day of exposure to 75 mg L⁻¹ BPA. For *Graesiella*, although the increase in lipid peroxidation levels occurred mainly at BPA concentrations higher than 25 mg L⁻¹, it exceeded seven times the control levels since the first BPA treatment day. Based on these results, stimulated MDA formation clearly demonstrates

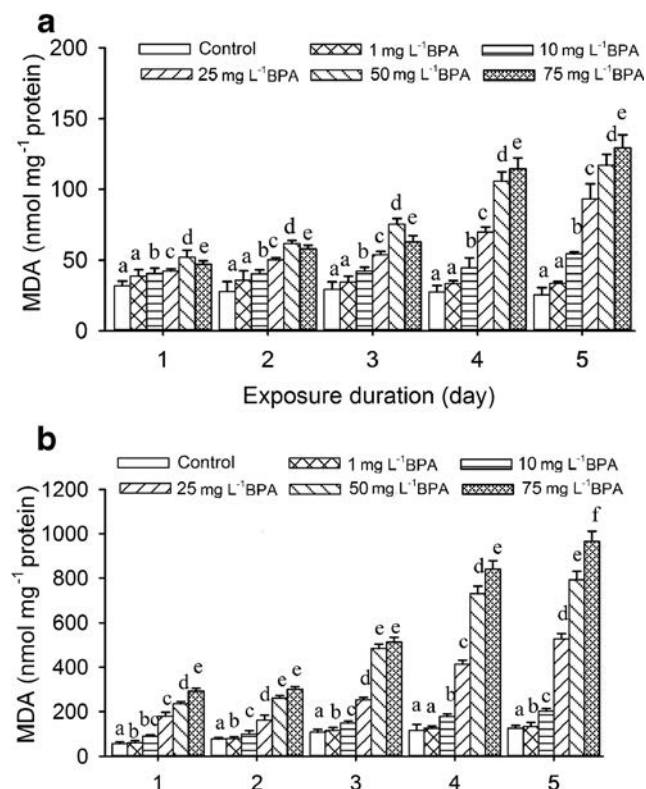


Fig. 4 Changes in MDA content of **a** *Picocystis* sp. and **b** *Graesiella* sp. during 5 days of exposure to 0, 1, 10, 25, 50, and 75 mg L⁻¹ BPA. Same letters indicate no significant difference according to Duncan's test ($P \geq 0.05$)

the BPA-induced oxidative stress in both microalgae strains with a higher extent for *Graesiella*. Such mechanism might contribute to the greater damage to the *Graesiella* photosynthesis apparatus (Nishiyama et al. 2001).

Effect of BPA on the antioxidant activities

To handle the induced oxidative stress, microalgae display several antioxidant enzymes that catalyze reactions to neutralize free radicals and ROS (Kabuto et al. 2003). This process was monitored in this work by the activity of APX, GST and CAT of both *Picocystis* and *Graesiella* measured after 1–5 days of exposure to 0–75 mg L⁻¹ BPA (Fig. 5).

APX is the unique enzyme scavenging the chloroplastic H₂O₂ (Nguyen-Deroche 2008), and CAT is in the peroxisome or mitochondria and similarly to APX plays an important role in the detoxification of H₂O₂ (Mittler 2002; Gill and Tuteja 2010). GST catalyzes the enzymatic conjugation of GSH to many hydrophobic substances for the purpose of detoxification (Hayes et al. 2005). It can be clearly seen that the two microalgae species handled differently the BPA stress, especially at high concentrations (25–75 mg L⁻¹ BPA) and at final exposure duration (days 4 and 5).

APX activity was over-expressed in *Picocystis* sp. when exposed to BPA and was about 1.5-fold higher than the control values after 5 days at 75 mg L⁻¹ BPA (Fig. 5a), whereas it was under-expressed since the first exposure day in *Graesiella* (Fig. 5b). The same trend was observed for GST activity with a significant increase in *Picocystis*, when BPA concentrations increased, reaching two times the control value after five exposure days to 75 mg L⁻¹ BPA (Fig. 5c). In contrast, an important decrease since the first exposure day was shown in *Graesiella* (Fig. 5d) in all tested BPA concentrations.

Conversely to the GST and APX activities, CAT activity was stimulated in both studied species during the first 3 days at BPA concentrations ranging from 1 to 25 mg L⁻¹ BPA.

At higher BPA concentrations (50–75 mg L⁻¹), CAT activity in *Picocystis* still over-expressed during all exposure days to be about 1.5 times the control value after 5 days of BPA exposure (Fig. 5e) while in *Graesiella* cells, CAT activity decreased significantly since the second day of BPA exposure to reach nearly one half after 5 days (Fig. 5f).

The over-expression of the antioxidant enzyme activities in *Picocystis* cells upon exposure to BPA can be regarded as a cellular defense mechanism to prevent additional damage to the photosynthetic apparatus (Wang et al. 2013) and explains the relative low level of lipid peroxidation. Conversely, the decrease of these activities in *Graesiella* reflects that BPA might be able to overwhelm the self-defense system. This may be due to the exhaustion of the enzymes in attempting to eliminate the ROS generated after the exposure to BPA or due to the enzyme inactivation caused by the excess ROS production (Pigeolet et al. 1990). Furthermore, the fact that the CAT activity was

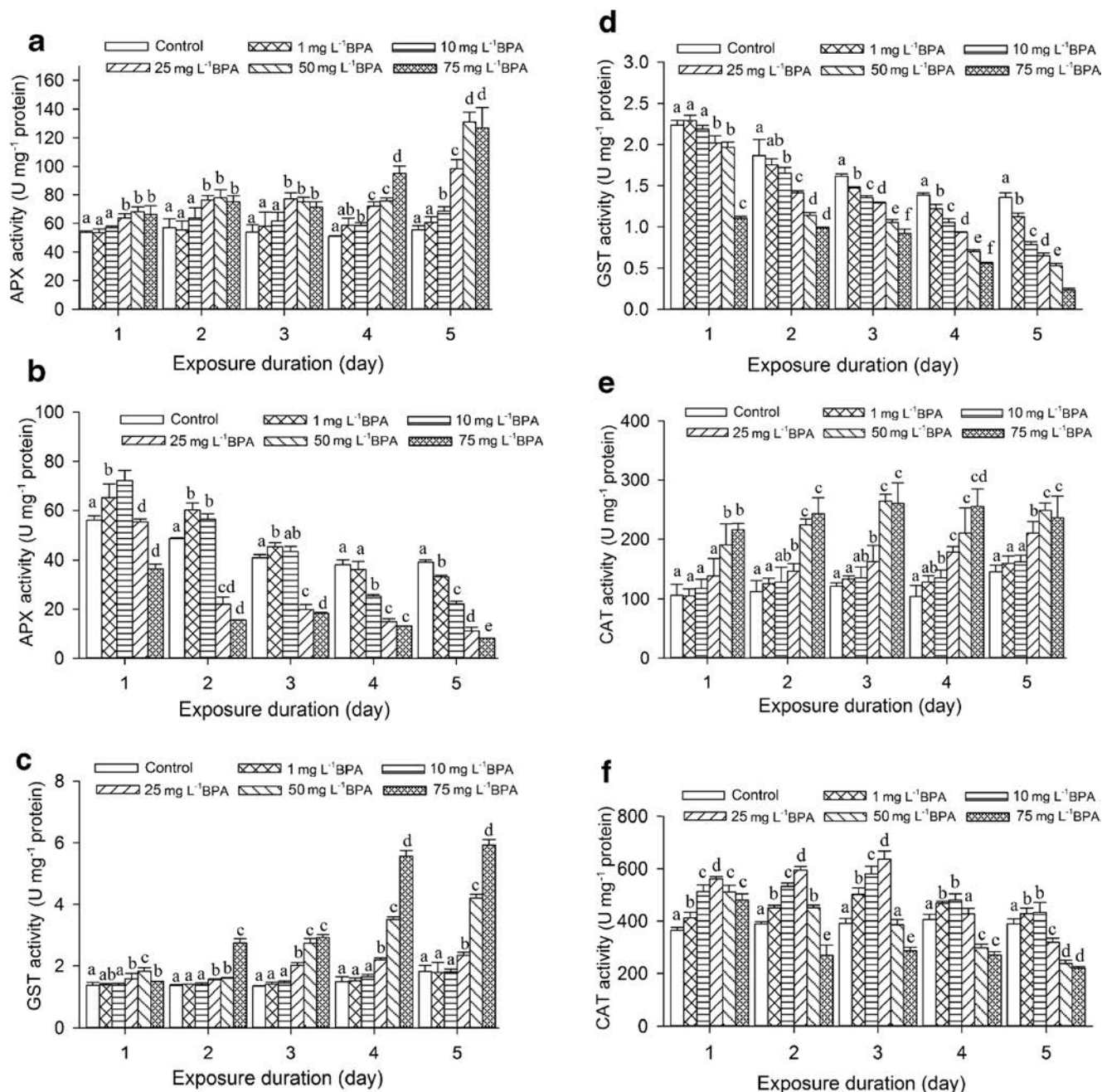


Fig. 5 Antioxidant enzyme activities: APX, GST, and CAT in **a**, **c**, and **e** of *Picocystis* sp. and **b**, **d**, and **f** of *Graesiella* sp. exposed to various BPA concentrations for 5 days. Same letters indicate no significant difference according to Duncan's test ($P \geq 0.05$)

stimulated since the first day of BPA exposure for the both studied species, while GST and APX activities occur later, suggests that among the tested enzymes, CAT is the first enzyme implied against BPA induced oxidative stress.

BPA removal, accumulation, and biodegradation

The removed BPA concentrations were investigated in the medium with and without microalgae (blank controls) (Table 2). After 5 days of exposure, 13 to 19.23% of 25 to

75 mg L⁻¹ BPA was removed from the medium without algae cells indicating that BPA could be removed by abiotic processes. According to several studies, the abiotic removal of BPA from aqueous solution is ensured mainly by photo-oxidation and it is in the same range as that found in this study (Ohko et al. 2001; Li et al. 2009; Ji et al. 2014).

The amount of BPA removed from culture media, at all BPA tested levels, was higher than the abiotic removal indicating that both *Picocystis* and *Graesiella* promoted significantly the BPA-removal at different degrees (Table 2). In

Table 2 Abiotic (Ab) and total removal (Tr), accumulated (Ac) and adsorbed (Ad) amount, bio-concentration factor (BCF), and biodegradation percentage (Bp) of BPA by *Picocystis* sp. and *Graesiella* sp. cells exposed for 5 days to initial concentrations of 25, 50, and 75 mg L⁻¹ BPA. Different letters within the same column indicate significant difference according to Duncan's test ($P \geq 0.05$)

Initial concentration (mg L ⁻¹)	Ab (%)	Tr (%)	Ac (%)	Ad (%)	BCF	Bp (%)
<i>Picocystis</i>						
25	19.23a	72.00a	01.29a	11.61a	06.86a	39.87a
50	16.72b	52.65b	01.56a	06.53b	05.49b	27.84b
75	13.81c	40.40c	01.23a	04.66bc	03.90c	20.70c
<i>Graesiella</i>						
25	17.59a	52.63a	03.82a	12.34a	09.88a	18.88a
50	13.32b	37.22b	02.38b	08.06b	08.68a	13.46b
75	12.99bc	27.77c	01.36c	06.13c	05.49b	07.29c

presence of *Picocystis* sp., the removed amounts of BPA were up to 72, 52, and 40% of 25, 50, and 75 mg L⁻¹, respectively, after 5 days of BPA exposure. However, only 52, 37, and 27% of 25, 50, and 75 mg L⁻¹ BPA, respectively, were removed in the presence of *Graesiella*. The observed low removal efficiency at higher BPA concentrations in both species might be attributed to the high growth inhibition observed in tested microalgae species.

The BPA removal efficiency of numerous Chlorophyta species tested by various authors (Li et al. 2009; Gattullo et al. 2012; Ji et al. 2014) did not exceed 17% at 25 mg L⁻¹ BPA and was lower than 8% at 50 mg L⁻¹ BPA. These values are more than 2-fold lower than those observed in the present study for both *Picocystis* and *Graesiella*. Higher removal levels were reported for *Chlorella sorokiniana* (43.9 and 39.5% at 20 and 50 mg L⁻¹ BPA, respectively) (Eio et al. 2015), exceeding the *Graesiella* removal ability when treated with 50 mg L⁻¹ BPA, but are still lower than that of *Picocystis*.

As illustrated in Table 2, after 5 days of BPA exposure, 5–12 and 6–12% of BPA removal from the culture media by *Picocystis* and *Graesiella*, respectively, were by adsorption processes. The BPA adsorption could be due to the hydrophobic binding effect of proteins on the microalgal cell wall (Endo et al. 2007) or physical trapping of BPA within or throughout the extracellular polymers (Kawaguti et al. 2006).

In all tested BPA concentrations, 2% BPA in *Picocystis* and 4% BPA in *Graesiella* were accumulated within the algal cells which confirm their ability to remove BPA from the medium by cellular uptake (Liu et al. 2010). The BPA bio-concentration factor (BCF) was 9.8 for *Graesiella* and 6.8 for *Picocystis* at 25 mg L⁻¹ BPA and did not exceed 5.4 for *Graesiella* and 3.9 for *Picocystis* at 75 mg L⁻¹ BPA. Lower BCFs were observed in other tolerant Chlorophyta species (*Chlamydomonas mexicana* and *Chlorella vulgaris*) not

exceeding 4.1 at 25 mg L⁻¹ BPA and 2.1 at 50 mg L⁻¹ BPA (Ji et al. 2014). Our results showed that the reduction in the amounts of BPA in the culture medium was significantly higher than the amounts of BPA accumulated and adsorbed by the microalgal cells (Table 2). These results suggest that both *Picocystis* and *Graesiella* were able to remove BPA from the medium by biodegradation processes. Several authors have reported BPA biodegradation by many microalgae species (Hirooka et al. 2005; Kang et al. 2006; Peng et al. 2009; Liu et al. 2010). Such processes could imply several pathways as the production of some active oxygenic species which could induce the photo-degradation of BPA (Liu et al. 2004) or as the synthesis of extracellular enzymes contributing to the breakdown of several endocrine-disrupting chemicals including BPA (Otto et al. 2015). Nakajima et al. (2007) asserted that several microalgae species could metabolize BPA in its original form and then release biodegraded metabolites into the media. The biodegradation products consist generally of non-toxic or less toxic compounds like monohydroxyl BPA (Hirooka et al. 2005) and BPA-glycosides (Nakajima et al. 2007).

The BPA biodegradation percentage (*Bp*) showed significant differences among the two algae species (Table 2). *Picocystis* was able to degrade 39.87% of 25 mg L⁻¹ BPA, while the biodegradation ability of *Graesiella* was more than 2-fold lower (18.88%) at the same initial BPA concentration. In both species, *Bp* decreased as initial BPA concentrations increased and reached 20.7 and 7.29% for *Picocystis* and *Graesiella* when exposed to 75 mg L⁻¹ BPA, respectively. The decrease in *Bp* at higher BPA levels might be correlated with the decrease in algae growth. The same trend was found by Li et al. (2009).

Conclusion

BPA showed toxic effects on both *Picocystis* and *Graesiella* strains. However, based on IC₅₀ values, especially *Picocystis*, showed high tolerance to BPA compared to the literature. *Picocystis* growth was inhibited by less than 50%, even at high tested concentrations. Upon BPA exposure, *Picocystis* showed an ability to promote simultaneously the activity of several antioxidant enzymes, which can be regarded as a defense mechanism to prevent additional damage to the photosynthetic apparatus.

Interestingly, *Picocystis* exhibited a high BPA removal efficiency insured mainly by biodegradation processes.

Such properties suggest the use of this species in bioremediation processes using a continuous flow culture system. Since the BPA concentrations in natural environment sites, including landfill leachates and wastewater treatment plants, are far lower than the tolerance limit of *Picocystis*, such systems could insure the fast BPA removal using a low culture volumes and additionally produce an efficient algal biomass.

The eventually accumulated BPA within *Picocystis* cells could be treated by chemical processes. Furthermore, our recent investigations revealed also the ability of *Picocystis* sp. to concentrate and/or degrade several pollutants at once, such as cadmium (Ben Ali et al. 2017), diclofenac, oxytetracycline, and sulfadiazine (under publication) which could lead to more efficiency in bioremediation processes.

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