Toxicity of bisphenol A and its structural congeners to microalgae

*Chlorella vulgaris* and *Desmodesmus armatus*

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Abstract

Bisphenol A and its structural congeners are increasingly recognized as emerging contaminants with toxic and estrogenic potential that have been widely used in many consumer products. Due to their widespread occurrence in aquatic environment, they could pose risks to the primary producers, such as microalgae. Therefore, the objective of this study was to examine the toxicity of bisphenol A, its six structural congeners, and their mixture towards the green algae *Chlorella vulgaris* and *Desmodesmus armatus*. Bisphenol A (average 14 days, EC$_{50}$: 42.29 mg L$^{-1}$) exhibited less harmful effect than structural congeners, such as bisphenol AF, bisphenol G, bisphenol X for *C. vulgaris* (average 14 days, EC$_{50}$: 22.39 mg L$^{-1}$) and bisphenol AF, bisphenol G, bisphenol M, bisphenol X for *D. armatus* (average 14 days, EC$_{50}$: 27.16 mg L$^{-1}$), respectively. Moreover, exposure to combined bisphenol A and its structural congeners leads to synergistic effects. Thus, the increased adverse effect caused by complex chemical mixture poses a greater risk to microalgae. The order of toxic effect (14 days, EC$_{50}$) of individual and combined structural congeners was: bisphenol G > bisphenol X > mixture > bisphenol AF > bisphenol A > bisphenol Y > bisphenol M > bisphenol P for *C. vulgaris* and mixture > bisphenol G > bisphenol X > bisphenol M > bisphenol AF > bisphenol A > bisphenol Y > bisphenol P for *D. armatus*, respectively. This is the first time that the toxicity of structural congeners of bisphenol A and its mixture to microalgae is described. Furthermore, these results were conducted to assess potential ecological risk of these compounds in the aquatic environment.

Keywords *Chlorella vulgaris* · *Desmodesmus armatus* · Chlorophyta · Bisphenol A · Structural congeners · Toxic effects

Introduction

Bisphenol A (BPA), a synthetic chemical substance belonging to phenols, has been widely used for more than 50 years as an additive or monomer in the manufacture of polycarbonate plastics, thermal paper, and epoxy resins in a wide range of consumer products, such as the lining of food cans, printing inks, compact discs, flame retardants, plastic bottles, thermal receipts, toys, pesticides, automobile parts, electronic and medical equipment (Makowska et al. 2021; Mustieles et al. 2021; Ramirez et al. 2021). To date, BPA is one of the world’s highest volume-produced chemical compounds. Global production of this endocrine-disrupting compound reached 7.7 million tonnes in 2015 and is expected to exceed 10.6 million in 2022 (Bousoumah et al. 2021; Catenza et al. 2021; Ohore and Zhang 2021). Due to the widespread use of BPA in many consumer products, it can migrate into the environment mainly through leaching from BPA-based materials (Bhatnagar and Anastopoulos 2017; Bjornsdotter et al. 2017). Consequently, BPA has been detected in different environmental media such as surface water (0.04–8300 ng L$^{-1}$ (Huang et al. 2012)), ground water (51–207 ng L$^{-1}$ (Huang et al. 2012)), tap water (15–63 ng L$^{-1}$ (Shao et al. 2008)), wastewater treatment plant effluents (10–1080 ng L$^{-1}$ (Lee and Part 2000)), wastewater treatment plant influents (193–2440 ng L$^{-1}$ (Drewes et al. 2005)), industrial effluents (220–370000 ng L$^{-1}$ (Lee and Part 2000; Fukazawa et al. 2001)), sediment (329–10500 ng L$^{-1}$ (Lin et al. 2006)), and air (1–17400 pg m$^{-3}$ (Fu and Kawamura 2010)).

Previous studies have reported that BPA was toxic to the aquatic organisms and can cause endocrine-disrupting effects, which are potentially associated with a variety of negative health consequences including developmental
Pollutants, such as BPA and its structural congeners, may play a pivotal role in preserving aquatic systems and their importance in aquatic communities. Due to the increasing concern about the harmful effects of BPA exposure in living organisms and its ubiquity in the environment, several countries like the USA, China, Japan, Canada, and the European Union have enacted law regulations aiming to reduce the use of BPA in some consumer products (Frankowski et al. 2020; Catenza et al. 2021; Liu et al. 2021). For instance, its use was restricted in thermal paper since 2020 (less than 0.02% by weight) (EU, 2016) and banned in the production of baby bottles in 2011 (EU, 2011; EU, 2013) in the European Union.

The potential toxicity and estrogenic activity of BPA and its gradual removal from some consumer products have led to its replacement by congeners, which have similar physicochemical properties (Barboza et al. 2020). BPA and its most replacements have a similar chemical structure with two p-hydroxyphenyl functional moieties (Siracusa et al. 2018; Usman et al. 2019). Therefore, bisphenol congeners (BPs) may potentially have a similar endocrine-disrupting capacity and may exert similar or even worse adverse effects on the reproductive system (Zhang et al. 2016; Wang et al. 2017a). Several studies show that structural congeners, such as bisphenol AF (BPAF), bisphenol G (BPG), bisphenol M (BPM), bisphenol P (BPP), bisphenol X (BPX), and bisphenol Y (BPY), exert estrogenic activities and displayed toxicological effects at concentrations similar to bisphenol A (Chen et al. 2016; Pelch et al. 2019; Lin et al. 2021; Sauer et al. 2021). For instance, compared to BPA, BPAF was more toxic to Daphnia magna and the obtained No Observed Effect Concentrations (NOEC) (21 days) were 5 and 0.23 mg L⁻¹, respectively (Tisler et al. 2016). The acute toxicity of BPAF, BPP, BPY, and BPX to adult zebrafish was at high toxic level (96 h, LC₅₀: 2.47, 0.37; 2.63 and 2.28 mg L⁻¹, respectively), lower than that of BPA (96 h, LC₅₀: 8.09 mg L⁻¹) (Liu et al. 2021). The extensive use of BPs in the production of BPA-free products leads to their widely detection in the aquatic environment at concentration from ng L⁻¹ to μg L⁻¹ level (Chen et al. 2016; Zhao et al. 2019).

Microalgae, as primary producers that can be found in most aquatic systems, play a pivotal role in preserving their ecological balance (Lemley et al. 2016; Czarny et al. 2017). Pollutants, such as BPA and its structural congeners, may cause changes in the species composition of the phytoplankton community and affect the proper functioning of the entire ecosystem (Ma 2005). Therefore, a lack of research data about possible toxic effects of BPs on microalgae, as an important part of aquatic community, is concerning. To the best of our knowledge, the adverse effects of exposure of microalgae to structural congeners of BPA have not been published so far. In this study, the freshwater microalgae Chlorella vulgaris and Desmodesmus armatus were used to determine the toxic effects of BPA, its six congeners, and their mixture. Moreover, the present investigation aimed to assess environmental risk of individual compound and mixture of bisphenol A and its congeners, which occur together in the environment.

Material and methods

Chemicals

Bisphenol A (> 97%) and bisphenol G (> 98%) were purchased from Sigma-Aldrich (USA). Bisphenol AF (> 99%), bisphenol M (> 98%), bisphenol P (> 98%), bisphenol X (AP) (> 98%), and bisphenol Y (Z) (98%) were supplied by TCI (Japan) (Table 1). Stock solutions containing 10 mg mL⁻¹ of BPA and its congeners were prepared daily in methanol (Chemsolve, Poland). All other chemical compounds were of analytical or HPLC grade.

Tested organisms and culture conditions

Cultures of Chlorella vulgaris (BA-0002) and Desmodesmus armatus (BA-0006) were acquired from the University of Gdansk. Algal species were cultivated in the phytotron chamber (Pol-Eko, Poland) in 250 mL Erlenmeyer flask on a rotatory shaker (100 rpm) in 100 mL BG-11 mineral medium (pH 7.64 ± 0.02) (Rippka et al. 1979). Algae were grown in the photoperiod in the day/night cycle 14/10 h, temperature 26/21 °C with a constant humidity of 30% under white light (60 μmol photons m⁻² s⁻¹). All experiments were performed using cultures at the logarithmic growth phase. The cell densities at the beginning of the experiment were 7.6 × 10⁴ for C. vulgaris and 4.2 × 10⁴ cells mL⁻¹ D. armatus.

Toxicity tests

Chlorella vulgaris and D. armatus were cultured in the presence of the following BPs: BPA, BPAF, BPG, BPM, BPP, BPX, BPY. The cultures were also exposed to a mixture of BPA and its six congeners (MIX). The MIX was prepared by mixing of each pollutants together at equal proportion (1:1:1:1:1:1:1) to obtain the same final concentration as for single substance. The final experimental concentration of examined BPs was chosen based on the results of the range-finding test. After preliminary assays, tested compounds were added to the 100 mL cultures of C. vulgaris and D. armatus (in the range of 5 to 100 mg L⁻¹). The control...
samples were cultured on the same medium and in the same conditions, but without the presence of BPs. The concentration of methanol in the tested and control sample was lower than 1% (v/v) and had no toxicity effects towards both microalgae species. The biomass productivity and chlorophyll a content were determined after 1, 2, 3, 4, 7, 8, 10, 11, and 14 days of exposure to the tested pollutants and in control samples. pH and concentration of BPs in medium were monitored at the beginning of the experiment and after 4, 7, 11, and 14 days. Each treatment had five replications.

**Measurement of pH, biomass, and chlorophyll a content**

The growth of *C. vulgaris* and *D. armatus* was assessed by pH, biomass productivity and chlorophyll a content

### Table 1  Physical and chemical properties, and predicted toxic effect of bisphenol A and its structural congeners

| Chemical compound | Chemical name / IUPAC name* | CAS number | Molecular formula | Molecular weight [g mol\(^{-1}\)] | Log \(K_{ow}\) estimated/ measured (25°C) | Water solubility** [mg L\(^{-1}\)] estimated/measured (25°C) | Predicted toxic effect (QSAR model)*** | Structural formula |
|-------------------|-----------------------------|------------|-------------------|-----------------------------------|---------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------|
| Bisphenol A       | 2,2-Bis(4-hydroxyphenyl)propane / 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol | 80-05-7 | \(\text{C}_6\text{H}_8\text{O}_2\) | 228.29 | 3.64 / 3.32 | 172.7 / 120.0 | 1.33 / 0.23 | ![Structural formula](image) |
| Bisphenol AF      | 2,2-Bis(4-hydroxyphenyl)hexafluoropropane / 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenol | 1478-61-1 | \(\text{C}_9\text{H}_5\text{F}_6\text{O}_2\) | 336.23 | 4.47 / (n/a) | 4.30 / (n/a) | 1.03 / 0.2 | ![Structural formula](image) |
| Bisphenol G       | 2,2-Bis(4-hydroxy-3-isopropylphenyl)propan-4'-[1-Methyl]ethyldiene)biphenyl | 127-54-8 | \(\text{C}_8\text{H}_{12}\text{O}_2\) | 312.45 | 6.56 / (n/a) | 0.0996 / (n/a) | 0.189 / 0.049 | ![Structural formula](image) |
| Bisphenol M       | 1,3-Bis[4-(4-hydroxyphenyl)-2-propyl]benzene / 4,4'-[1,3-Phenylenedimethylidenedione]biphenol | 13595-25-0 | \(\text{C}_9\text{H}_{14}\text{O}_2\) | 346.46 | 6.25 / (n/a) | 0.113 / (n/a) | 0.265 / 0.066 | ![Structural formula](image) |
| Bisphenol P       | α,α'-Bis(4-hydroxyphenyl)-1,4-diisopropylbenzene / 4,4'-[1,4-Phenylenediisopropylidenedione]biphenol | 2167-51-3 | \(\text{C}_{14}\text{H}_{18}\text{O}_2\) | 346.46 | 6.25 / (n/a) | 0.113 / (n/a) | 0.265 / 0.066 | ![Structural formula](image) |
| Bisphenol X       | α,α'-Bis(4-hydroxyphenyl)ethylbenzene / 4,4'-[1-Phenyldiethylidenedione]biphenol | 1571-75-1 | \(\text{C}_{14}\text{H}_{18}\text{O}_2\) | 290.36 | 4.86 / (n/a) | 3.76 / (n/a) | 0.657 / 0.134 | ![Structural formula](image) |
| Bisphenol Y       | 1,1-Bis(4-hydroxyphenyl)cyclohexane / 4,4'-Cyclohexyldienebiphenol | 843-55-0 | \(\text{C}_{19}\text{H}_{22}\text{O}_2\) | 268.35 | 5.48 / (n/a) | 1.47 / (n/a) | 0.373 / 0.084 | ![Structural formula](image) |

*Calculated by LexiChem 2.6.6. **Calculated by EPI Suite KOWWIN v.1.68 (US EPA). ***Calculated by ECOSAR Class Program v2.0 (US EPA)
determinations. pH was measured with a pH meter (Multi 3430 SETF, WTW, Germany). The biomass concentration was assessed by gravimetric method (Czarny et al. 2019a; b). In order to measure the dry weight, 10-mL algal suspension aliquots were filtered through pre-weighed and pre-combusted nylon membrane filters. Afterwards, the filters were dried at 105 °C for 1 h to a constant weight and then weighed again. The biomass concentration was determined by calculating the difference between the initial and final dry weight of filters. The chlorophyll a content was evaluated by AlgaeChek Ultra fluorometer (Modern Water, UK) (Czarny et al. 2019c). The number of the algal cells at the beginning of experiment was measured using a Fuchs-Rosenthal haemocytometer.

**Statistical analyses**

Data were analyzed using Q-Dixon test for detection of outliers (Dixon 1953). All results were expressed as mean values with standard deviation (SD) of five independent replicates. To determine significant differences among the control sample and BPs treatments, the obtained results were statistically assessed by one-way ANOVA using the Statistica v.13.3 software (StatSoft, USA). Values of P lower than 0.05 were considered statistically significant.

Percentage inhibition (PI) was calculated according to the following formula (Eq. 1) (Czarny et al. 2021):

\[
\%PI = \left( \frac{b_c - b_r}{b_c} \right) \times 100
\]

where \(b_c\), biomass content of control sample; \(b_r\), biomass content of test sample.

The concentration–response curve, concentration–response equation, and effective concentration at which 50% inhibition occurs (EC\(_{50}\)) were calculated by Microsoft Excel (Microsoft, USA) using linear log method based on the biomass content. The combined toxicity of BPA and its six structural congeners was achieved using the Keplinger evaluation system, in which the expected EC\(_{30}\) values were compared with the obtained EC\(_{50}\) values (14 days) and expressed as a ratio (Keplinger and Deichmann 1967).

**Results**

In most cases BPs showed a strong inhibition activity against *C. vulgaris* and *D. armatus* (Fig. 1, Fig. 2 Table S1, Table S2, Table S3, Table S4). Compared to control samples, the biomass and chlorophyll a content of the algae species were increased in a time- and concentration-dependent manner. The percentage inhibition values in response to BPs treatment were calculated for different concentrations and times (1–14 days) of incubation based on the measured biomass content. As shown in Fig. 2, compared with the control samples, BPA, BPAF, BPG, BPX, and MIX have strongly negative effects on *D. armatus* and lead to algal cell death. The maximum percentage inhibition value for BPG and BPX was 100% at 50 mg L\(^{-1}\) treatments on the 3rd day, while bisphenol G exerted in lethal effect at 25 mg L\(^{-1}\) on the 11th day of incubation. During the 2nd and 10th day, MIX and BPA exerted lethal effect at concentrations higher than 75 mg L\(^{-1}\), respectively. Similar effect was observed for BPAF at 100 mg L\(^{-1}\) treatments on day 4. For *C. vulgaris*, 100% of the cells were killed under BPG and BPX concentrations of 50–100 mg L\(^{-1}\) on the 11th day of exposure, while BPG showed lethal effect also at 75 mg L\(^{-1}\) already within 8th day of treatment. Compared with the control samples, BPM and BPA, BPAF, MIX significantly (\(P<0.05\)) inhibited the growth of *D. armatus* and *C. vulgaris* (even to 74.37% compared to the control sample). The growth of *C. vulgaris* was moderately inhibited by BPM and BPY, for which the maximum percentage inhibition values were 40.38–55.21 and 55.63–59.64% at 25–100 mg L\(^{-1}\) after the 14th day of incubation. BPY was moderately toxic to *D. armatus*, for which the growth of cultures was inhibited (29.92–44.94%) at concentrations higher than 25 mg L\(^{-1}\) on 3rd day of treatment. BPP had a negligible adverse effect on *C. vulgaris* and *D. armatus* and no lethal or growth stimulating effects were observed. However, in some cases, a hormesis (stimulating) effect on the growth of *C. vulgaris* and *D. armatus* was observed at low concentrations of BPs (5–25 mg L\(^{-1}\)) or on the 1st day of incubation. The maximum stimulating effect was 6.56% at 25 mg L\(^{-1}\) BPX treatment (1 day) for *C. vulgaris* and 40.81% at 25 mg L\(^{-1}\) BPP treatment (14 days) for *D. armatus*, respectively.

The pH of *C. vulgaris* and *D. armatus* cultures exposed to BPs increased in a concentration-dependent manner (Fig. 3 and Fig. 4). pH varied following treatment with BPs at different concentrations (5–100 mg L\(^{-1}\)) during the 14-day test periods. The pH of the algal cultures ranged from the initial value of 7.64 to a final value between 8.14–9.66 and 7.74–9.83, respectively. The pH values at high concentrations were significantly lower than the control sample, indicating that increasing concentration had an inhibiting effect on photosynthesis.

Table 2 summarizes the calculated EC\(_{50}\) values (4, 7, and 14 days) for *C. vulgaris* and *D. armatus* exposed to seven different BPs and their mixture based on the measured biomass content. The toxic effects of BPA and its structural congeners were dependent on both concentration and exposure time. With respect to the determined EC\(_{50}\) values (14 days), a highly toxic effect on *D. armatus* cells was observed for BPA, BPAF, BPG, BPM, and BPX (EC\(_{50}\): 42.06, 34.76, 18.84, 28.24, 26.80 mg L\(^{-1}\), respectively), moderate for BPY (EC\(_{50}\): 82.65 mg L\(^{-1}\)) and the weakest for BPP (no
Fig. 1 The percentage of inhibition of *C. vulgaris* exposed to individual and combined compounds (a BPA, b BPAF, c BPG, d BPM, e BPP, f BPX, g BPY, and h MIX) for 14 days. Error bars indicate SD (*n* = 5)
Fig. 2 The percentage of inhibition of *D. armatus* exposed to individual and combined compounds (a BPA, b BPAF, c BPG, d BPM, e BPP, f BPX, g BPY, and h MIX) for 14 days. Error bars indicate SD (n = 5).
Fig. 3  Effect of individual and combined compounds (a BPA, b BPAF, c BPG, d BPM, e BPP, f BPX, g BPY, and h MIX) on pH of *C. vulgaris*. Error bars indicate SD (*n* = 5)
Fig. 4 Effect of individual and combined compounds (a BPA, b BPAF, c BPG, d BPM, e BPP, f BPX, g BPY, and h MIX) on pH of *D. armatus*. Error bars indicate SD (*n* = 5)
lethal and growth stimulating effect). The toxicity of BPA, BPAF, BPG, and BPX (EC$_{50}$: 42.52, 28.99, 15.76, 22.42 mg L$^{-1}$, respectively) suggested that these BPs induced stronger harmful effect to *C. vulgaris* than BPM and BPY (EC$_{50}$: 62.12 and 48.76 mg L$^{-1}$, respectively). Toxic effects of mixture to *C. vulgaris* and *D. armatus* were higher than the sum of individual effect of each mixture component (14 days, EC$_{50}$: 28.56 and 18.22 mg L$^{-1}$, respectively). Generally, it can be concluded that *D. armatus* was more sensitive to tested BPs than *C. vulgaris* (mean EC$_{50}$ value of all tested BPs, 14 days: 38.89 and 46.52 mg L$^{-1}$, respectively). With respect to EC$_{50}$ values (14 d), BPs toxicity decreases in the order: BPG > BPX > MIX > BPAF > BPA > BPY > BPM > BPP for *C. vulgaris* and MIX > BPG > BPX > BPM > BPA for *C. vulgaris* and MIX > BPG > BPX > BPM > BPA for *D. armatus*.

**Discussion**

For some decades BPs have been intensively introduced into the terrestrial and aquatic ecosystems without previous research about their potential harmful impact on organisms (Michałowicz 2014; Tisler et al. 2016). In this study, the adverse effects of BPAF, BPG, BPM, BPP, BPX, and BPY to *C. vulgaris* and *D. armatus* were investigated for the first time and the results were compared to the toxicity of bisphenol A. Several studies have shown that BPA was toxic to green algae (Staples et al. 1998; Liu et al. 2010; Seoane et al. 2021). In the present study, the EC$_{50}$ values (14 d) for *C. vulgaris* and *D. armatus* were 42.52 and 42.06 mg L$^{-1}$, respectively. Similar findings were reported by Ji et al. (2014) that the EC$_{50}$ (120 h) of bisphenol A for *C. vulgaris* was 39.80 mg L$^{-1}$. Esperanza et al. (2020) also found that bisphenol A was harmful to *Chlamydomonas reinhardtii* with EC$_{50}$ of 32.40 mg L$^{-1}$ (3 days). The EC$_{50}$ values (3 days) for *Scenedesmus quadricauda* was 13.23 mg L$^{-1}$) by (Xiang et al. 2018). The results achieved in the present study showed that in most cases, BPAF, BPG, BPM, and BPX (14 days, EC$_{50}$: 15.76–34.76 mg L$^{-1}$) had higher toxicities to *C. vulgaris* and *D. armatus* in comparison to BPA (14 days, EC$_{50}$: 42.52 and 42.06 mg L$^{-1}$, respectively). Therefore, the industrial replacement of BPA with congeners, such as BPAF, BPG, BPM, and BPX, is questionable. Unfortunately, there are only few available data about toxic effects of structural congeners of BPA to microalgae. For instance, Tisler et al. (2016) reported that BPAF (3 days, IC$_{50}$: 3.00 mg L$^{-1}$) was more harmful to *Desmodesmus subspicatus* than BPA (3 days, IC$_{50}$: 19.60 mg L$^{-1}$). Ding et al. (2020) found that bisphenol S showed stronger toxicity to *C. vulgaris* than bisphenol A, and the obtained EC$_{50}$ values (2 d) were 3.16 and 41.43 mg L$^{-1}$, respectively. Unfortunately, no data are available for adverse effects of BPG, BPM, BPP, BPX, and BPY to microalgae.

BPs have been widely used in industry as alternatives of BPA, which have been widely detected in different environmental and biological samples (Wang et al. 2020; Liu et al. 2021). As a result, variety of this substitutes coexisting in aquatic environment; thus, the microalgae are exposed to these chemicals simultaneously. In aquatic ecosystems, the combined effect of chemical compounds may be stronger (e.g. synergistic) or weaker (e.g. antagonistic) as they would be predicted on the basis of response addition (Gebara et al. 2020). Thus, the investigation of adverse impact of bisphenol A and its structural congeners in complex mixtures is highly important to assess their environmental risk in a more realistic way. Hence, this study was designed to investigated the toxic effect of mixture of seven BPs (BPA, BPAF, BPG, BPM, BPP, BPX, and BPY) on the microalgae *C. vulgaris*.

### Table 2 The effective concentration values (EC$_{50}$ (14 d)) of bisphenol A and its structural congeners for microalgae *C. vulgaris* and *D. armatus*

| BPs         | *Chlorella vulgaris* | *Desmodesmus armatus* |
|-------------|----------------------|------------------------|
|             | Dose response equation ($R^2$) | EC$_{50}$ ± SD (mg L$^{-1}$) | Dose response equation ($R^2$) | EC$_{50}$ ± SD (mg L$^{-1}$) |
| BPA         | $y = -0.624x + 1.516$ (0.9016) | 42.52 ± 2.13 | $y = -1.056x + 2.215$ (0.8561) | 42.06 ± 2.10 |
| BPAF        | $y = -0.609x + 1.391$ (0.8225) | 28.99 ± 1.45 | $y = -0.946x + 1.958$ (0.9196) | 34.76 ± 1.74 |
| BPG         | $y = -0.751x + 1.399$ (0.9465) | 15.76 ± 0.79 | $y = -0.966x + 1.732$ (0.8451) | 18.84 ± 0.94 |
| BPM         | $y = -0.451x + 1.308$ (0.8271) | 62.12 ± 3.11 | $y = -0.287x + 0.917$ (0.8539) | 28.24 ± 1.41 |
| BPP         | $y = -0.154x + 1.160$ (0.8420) | 19.93 ± 0.56 | $y = -0.226x + 1.615$ (0.8426) | 84.73 ± 0.72 |
| BPX (BPAP)  | $y = -0.922x + 1.745$ (0.8351) | 22.42 ± 1.12 | $y = -1.032x + 1.974$ (0.9211) | 26.80 ± 1.34 |
| BPY (BPZ)   | $y = -0.591x + 1.498$ (0.8105) | 48.76 ± 2.44 | $y = -0.744x + 1.927$ (0.8175) | 82.65 ± 4.13 |
| MIX         | $y = -0.586x + 1.353$ (0.8996) | 28.56 ± 1.43 | $y = -0.786x + 1.490$ (0.9342) | 18.22 ± 0.91 |

*Growth-stimulating effect
"y percentage inhibition [%], x log(chemical concentration [mg L$^{-1}$])
and *D. arnatus* for the first time. Keplinger and Deichmann (1967) showed that the ratio of expected to observed EC$_{50}$ values of 1.75 and higher indicated a synergistic effect. The results showed that the expected EC$_{50}$ values of mixture were 2878.70 and 12138.44 mg L$^{-1}$ and the EC$_{50}$ values were 28.56 and 18.22 mg L$^{-1}$ for *C. vulgaris* and *D. arnatus*, respectively. Thus, the mixture of BPA and its six structural congeners exhibited synergistic effects, because the ratio was higher than 1.75. Synergistic toxicity for BPs is of high concern, because the predictable harmful effect for the green algae based on the individual chemicals would underestimate the mixture impact. Moreover, the results of our study indicated that mixture of bisphenol A and its six structural congeners were more harmful to the tested microalgae than BPA (average 14 day, EC$_{50}$: 42.29 mg L$^{-1}$). Elersek et al. (2021) assessed the toxicity of the binary mixture of BPA and BPF in the green algae *Pseudokirchneriella subcapitata*, and the results showed an additive effect at a lower concentration and antagonism at a higher concentration. Unfortunately this study is the only published report on the toxicity of mixture of bisphenol A and its six congeners in microalgae.

The investigations on toxic effects of BPs on algae are not available. Therefore, the mechanisms of their action are not well understood yet and may be related to several factors occurring simultaneously. The molecular structure and physicochemical properties of BPs may affect environmental behavior and fate of BPs. In this work, the US Environmental Protection Agency (US EPA) EPI Suite KOWWIN version 1.68 was used to estimate physicochemical properties such as octanol–water partition coefficient (log$_{K_{ow}}$) and water solubility. The calculated log$_{K_{ow}}$ values of BPs were 3.64–6.56 (Table 1). Thus, due to their hydrophobic character (log$_{K_{ow}}$ > 3), they can easily cross the cell wall of microalgae and bioaccumulate. Moreover, ECOSAR Class Program v2.0, which employs quantitative structure–activity relationship (QSAR) models, was used to estimate the toxic effect of BPs (Czarny et al. 2021). The calculated toxicity (the EC$_{50}$, chronic value (ChV) for algae) (Table 1) indicates that BPA shows relatively lower toxic effect than the other tested BPs. The experimental data obtained in this study show that BPY, BPM, BPP (*C. vulgaris*) and BPY, BPP (*D. arnatus*) demonstrated lower toxicity than BPA.

The obtained results showed that BPs at low concentrations may lead to stimulation of the growth of *C. vulgaris* and *D. arnatus* but exhibited inhibitory effect at higher concentrations. Therefore, the response of tested microalgae exposed to increasing dose of BPs can be regarded as hormetic. Similar findings were reported by Zhu et al. (2021), and they found that trans-4-hydroxy-3-methoxycinnamic acid promoted the growth of freshwater microalgae *Euglena gracilis* at low concentrations (0.5 g L$^{-1}$) and show toxic effect at high concentration (> 1 g L$^{-1}$). Hormesis is defined in toxicological sciences as a biphasic dose–response phenomenon characterized by low concentration promotion and high concentration inhibitory effect, which may be characterized by a U- or inverted U-shaped dose–response curve (Calabrese 2008; Mattson 2008; Nweke and Ogbonna 2017, He et al. 2020). Several studies have shown that this phenomenon is related to the activation of adaptive responses required to protect cells from pollutants (Zhang 2008; Zhang et al. 2009). In turn, de Morais et al. (2014) reported that low dose of toxic substance might promote cell division. Therefore, in this study, stimulation of the growth of *C. vulgaris* and *D. arnatus* may be an attempt to provide a dilution effect of BPs as its dose will be divided for a larger amount of biomass. The occurrence of growth promotion effect phenomenon may have unexpected implications for ecological risk assessment. Therefore, due to phenomenon of hormesis, actual measurements from laboratory studies are more reliable for environmental risk assessments of aquatic contaminants than estimated by QSAR model. The accurate quantification of chlorophyll $a$ content is a parameter which is used to examine the adverse effect of environmental pollutants on algae (Kasahara et al. 2002; Ding et al. 2020). In this study, with respect to the control sample, chlorophyll $a$ content decreased in a concentration dependent manner of BPs for both algae species. A reduction in chlorophyll $a$ content might be caused to the stress response in algae that can be owing by the degradation of PSII complex or the peroxidation of thylakoids lipid (Xiong et al. 2016). These results were in agreement with the observations reported by Li et al. (2009), who showed significant reductions in the chlorophyll $a$ content of *S. hantzschii* exposed to BPA at concentrations higher than 1 mg L$^{-1}$. In addition, they showed that at a concentration higher than 5 mg L$^{-1}$, cell division was inhibited and the degradation of chloroplasts and chlorophyll occurred. During photosynthesis green algae consume dissolved carbon dioxide which is responsible for an increase in pH (Gerardi and Lytle 2015). The pH values of the *C. vulgaris* and *D. arnatus* cultures with higher concentration of BPs (> 25 mg L$^{-1}$) were almost the same as the initial value due to low growth (7.74–9.00). Therefore, lower pH levels compared to the control sample might have appeared due to the photosynthesis inhibition. A similar trend was noticed by Ji et al. (2014), who found that after 10 days of exposure to 50 mg L$^{-1}$ of bisphenol A, the pH of the *C. vulgaris* culture was almost the same as the initial level (6.6). Wang et al. (2017b) found that pH levels of *Desmodesmus* sp.WR1 cultures exposed to BPA were lower with respect to the control sample at concentrations higher than 3 mg L$^{-1}$ BPA.

Zhang et al. (2014) noticed that *Chlorella pyrenoidosa* has lower sensitivity to BPA than *Scenedesmus obliquus* with EC$_{50}$ (4 days) of 63.53 and 26.72 mg L$^{-1}$, respectively. Similar findings have been reported for *C. pyrenoidosa*...
of bisphenols (14 days, EC\textsubscript{50}: 18.22–28.56 mg L\textsuperscript{-1}) showed stronger toxic effects compared to BPA. The results indicated that mixture of BPA and its six congeners show synergistic effect. Primary producers, such as green algae in aquatic environments are usually exposed to a complex mixture of BPs. Thus, the increased toxicity of combined contaminants may lead to inhibition of their growth and consequently to changes in the species composition of the phytoplankton community, creating a serious threat to proper functioning of the entire ecosystems. Moreover, \textit{D. armatus} (average 14 days, EC\textsubscript{50}: 38.89 mg L\textsuperscript{-1}) was more sensitive than \textit{C. vulgaris} (14 days, mean EC\textsubscript{50}: 46.52 mg L\textsuperscript{-1}) to the adverse effect of bisphenol A and its structural congeners. Based on EC\textsubscript{50} values (14 days), the order of the toxicity of BPs was BPG > BPX > MIX > BPAF > BPA > BPY > BPM > BPP for \textit{C. vulgaris} and MIX > BPG > BPX > BPM > BPAF > BPA > BPY > BPP for \textit{D. armatus}. Although a large number of studies have proved the adverse impact of bisphenol A on green algae, studies about potential risk of BPs on these aquatic microorganisms are still lacking. Thus, the occurrence of variety of BPs in aquatic ecosystems, their interactions in a mixture and potential adverse effect merit further investigation.

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**Data availability** All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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