The University of Tehran Press

Pollution

Print ISSN: 2383-451X
Online ISSN: 2383-4501

https://jpoll.ut.ac.ir/

Toxic Effect of Bisphenol A Causes Oxidative Stress in cyanobacterium *Gloeocapsopsis crepidinum*

Raad Hassan Hakim | Haider A. Alghanmi ✉

Biology Department, College of Education, University of Al-Qadisiyah, Iraq

Article Info

Article type:
Research Article

Article history:
Received: 20 March 2024
Revised: 18 May 2024
Accepted: 30 May 2024

Keywords:
Cyanobacteria
BPA
Oxidative Stress
G. crepidium

ABSTRACT

Microalgae are good tools for toxicity indicators in aquatic habitats. The present study was carried out to evaluate the toxicity of bisphenol A at different concentrations (1, 5, 10, 20, 50, 75, 100) mg/l and the oxidative response induced by this exposure using the cyanobacterium *Gloeocapsopsis crepidinum*. The results showed a decrease in the algal biomass rate with increasing concentrations of bisphenol A, while the Half inhibition concentration (IC50) of BPA was 2.68 mg/l. The chlorophyll-a and carotenoids recorded highest value in the control group, which were 0.96 and 0.56 µg/ml, while the concentrations of these pigments decreased with increasing concentrations of BPA, their lowest value being recorded at (0.54 and 0.35) µg/ml at a concentration of 100 mg/l.

The results showed that Catalase (CAT) and Ascorbate peroxidase (APX) enzymes recorded a higher value of (0.34 and 4.66) U/g at 100 mg/l BPA, while the lowest values of these enzymes recorded 0.10 and 3.7 U/g in the control group respectively. While the Superoxide dismutase (SOD) enzyme recorded a high value of 22.22 U/g at 1 mg/l BPA and decreased with a lower value of 19.46 U/g at 100 mg/l. In addition, Glutathione (GST) showed lower values of 5.413 µmole/g in the control group and increased at higher values at a concentration of 100 mg/l which reached to 18.68 µmole/g. Nevertheless, the indication of cell damage such as Malondialdehyde (MDA) and Reactive Oxygen Species (ROS) recorded lowest values of 0.13 and 14.153 µmole/g in the control group, while higher values recorded with increasing concentrations of BPA were recorded at 3.487 and 74.4 µmole/g at 100mg/l BPA, respectively. All treatments were statistically analyzed with $p \leq 0.05$ as significant differences were found between all treatments. This study concluded that cyanobacteria *G. crepidinum* have the ability to resist the toxic effects of bisphenol A by increasing antioxidant production in their bodies, so they can be considered biological tools to eliminate toxic compounds in aquatic environments.

Cite this article: Hassan Hakim, R., & Alghanmi, H.A. (2024). Toxic Effect of Bisphenol A Causes Oxidative Stress in cyanobacterium Gloeocapsopsis crepidinum. *Pollution*, 10 (3), 997-1006.
https://doi.org/10.22059/poll.2024.374177.2295

 Publisher: The University of Tehran Press.
DOI: https://doi.org/10.22059/poll.2024.374177.2295

INTRODUCTION

Bisphenol A (BPA) is a synthetic compound commonly used in the manufacture of plastics such as epoxy resins and polycarbonate plastics. Its widespread use in various industries, including food packaging, consumer products and medical devices, has raised concerns about its potential negative effects on both the environment and human health. Recent studies have shown that in addition to posing risks to human health, BPA also poses a significant threat to aquatic organisms, especially algae (Sharma *et al.*, 2023). Algae are one of the primary and main producers in aquatic ecosystems and is highly sensitive to changes in their environment. Exposure to bisphenol A can produce reactive oxygen species (ROS), highly reactive molecules that can induce oxidative stress and disrupt cellular functions in algae. Due to its widespread industrial use, BPA has been detected in aquatic environments around the world. Recent research

*Corresponding Author Email: *haider.alghanmi@qu.edu.iq, haideralghanmi@yahoo.com*

has focused on investigating the potential toxicity of BPA to algae, which plays a crucial role as major producers in aquatic ecosystems. Studies have shown that BPA can interfere with many physiological and biochemical processes in various types of algae, including cell growth, photosynthesis and the production of light-harvesting pigments (Azizullah *et al.*, 2022; Kearney, 2023).

Several studies have reported that BPA exposure stimulates DNA production in algae. ROS, including O2-, H2O2-, and OH-, are natural by-products of cellular metabolism. However, overproduction of ROS can overwhelm algae antioxidant defense systems, leading to oxidative stress. Fan *et al.* (2021) studied the effects of BPA exposure on *Microcystis aeruginosa*, the results showed that ROS levels increased significantly in BPA-exposed blue cells at all stages of development. Furthermore, ROS production was higher in cells in the stationary phase compared to cells in the lag or exponential growth phase in both BPA control and treatment groups. MDA levels decreased, and SOD and GSH activity did not show significant differences between controlled groups and low BPA concentration. However, exposure to higher concentrations of BPA significantly increased SOD, GSH activity and intracellular oxygen levels. Another study looked at the toxicity of bisphenol A (BPA) in micro-sea algae *Stephanodiscus hantzschii* and its ability to accumulate and remove BPA from contaminated water. The results showed that the 96-hour effective concentration of BPA for *S. hantzschii* was 8.65±0.26 mg/l and that both cell count and chlorophyll content were significantly reduced when exposed to BPA concentrations above 3 mg/L. The study concluded that *S. hantzschii* is a solid strain that can tolerate BPA and has the potential to be used to remove BPA from contaminated water (Li *et al.*, 2009).

A similar result was achieved by Zhang *et al.* (2014) in acute and chronic toxic effects of bisphenol A (BPA) on two algae species, C*hlorella pyrenoidosa* and *Scenedesmus obliquus*, acute test results showed that BPA significantly inhibits the growth of both algae. However, chronic exposure did not show a similar trend. The activities of SOD and CAT enzymes in both algae were stimulated in all treatments, suggesting that the cells attempted to counter oxidative stress caused by BPA. In both algae, the synthesis of chlorophyll A, a necessary pigment for photosynthesis, showed a similar inhibitory trend in short-term treatments. However, in chronic tests, *C. pyrenoidosa* showed no visible effect, while *S. obliquus* showed dose-dependent inhibitory effects. Another study investigated the effects of fluorine 9-bisphenol (BHPF), a substitute for bisphenol A (BPA), on common chlorella. Results showed that common *chlorella* was sensitive to BHPF concentrations above 1 mg L-1 resulting in a significant increase in lipid peroxidation, suggesting oxidative stress in algae. In addition, antioxidant enzyme activities in algal cells were significantly reduced when exposed to BHPF concentrations above 0.5 mg/l (Zhang *et al.*, 2021).

Czarny-Krzymińska *et al.* (2022) investigated the toxicity of bisphenol A (BPA) and its six structural congeners, as well as their mixture, on green algae Chlorella vulgaris and Desmodesmus armatus. The study showed that BPA exhibited fewer damaging effects than its structural congeners, with a 14-day EC50 of 42.29 mg/L for C. vulgaris and its structural congeners with average 14-day EC50 values of 22.39 mg/L and 27.16 mg/L for *C. vulgaris* and D. armatus, respectively. These results provide important insights into the toxicity of structural congeners of BPA and their mix to microalgae and contribute to the assessment of potential environmental risks of these compounds in aquatic environments. According to the study by Li *et al.* (2022), the effects of bisphenol A (BPA) and bisphenol S (BPS) on growth, chlorophyll, and the oxidative content and stress of *Chlorella pyrenoidosa* were investigated. yours results showed that both BPA and BPS, Either alone or combined, showed dose-dependent inhibition of *C. pyrenoidosa* growth. The highest inhibition rate was observed on Day 6 of the trial. In addition, the combination of BPA and bps increased the production of ROS in algae and increased the activity of antioxidant enzymes such as SOD and POD.

The study aims to investigate the effects of bisphenol A (BPA) on cyanobacteria *Gloeocapsopsis crepidinum*, as exposure to BPA increases oxidative stress in these particular cyanobacteria, contributing to a broader understanding of the toxicity of BPA and its potential impact on cyanobacterial populations in aquatic ecosystems.

MATERIALS AND METHODS

Algal Cultivation

The isolate of *Gloeocapsopsis crepidium* was cultured in the BG-11 medium under laboratory conditions at 25 °C, 60 µmol m^{-2} s^{-1} light intensity, pH 7.2 and a dark light cycle of 16:8. The effect of different concentrations of BPA (1, 5, 10, 20, 50, 75, 100) mg/l was studied (Hakim and Alghanmi, 2024).

Experimental Design

To examine the toxicity of BPA to cyanobacterium, seven concentrations of BPA (1, 5, 10, 20, 50, 75, 100) mg/L were made using BG-11 media in addition to the Control group without adding BPA, inculcated with 1% (v/v) of *Gloeocapsopsis crepidium* cultures, and incubated at 60 µmol m-2 s-1, 26 °C, pH 7, and photoperiod 16 light: 8 dark (Hakim and Alghanmi, 2024). To determine the antioxidant enzyme activity the samples were collected after 12 days of growth in the stationary phase.

Biomass And Ic50 Estimation

The wet weight method (50 mg) was used to estimate the toxicity of bisphenol A (IC50) by sampling the alga after 12 days at the beginning of the stationary phase. The samples were then centrifuged at 6000 rpm for 15 minutes. The centrifugation was repeated once more to completely remove the water. The weight of the alga was then calculated using a sensitive balance and the EC50 was calculated based on the wet weight of the alga (Sebaugh, 2011).

Antioxidant Parameters Determination

Chlorophyll-A And Total Carotenoid Estimation

The chlorophyll-a and carotenoid content of alga was determined by the method of (Zavřel *et al.*, 2015) using the spectrophotometer (Apple / Japan) according to the following equations:

Chla [µg/ml] = 12.9447 (A665 − A720)

Carotenoids [µg/ml] = [1000 (A470 − A720) − 2.86 (Chl-a [µg/ml])] / 221

Superoxide Dismutase Enzyme (SOD) Activity

The efficacy of the enzyme to block the autoxidation of pyrogallol was used to determine SOD activity using a simple and quick technique. (Marklund and Marklund, 1974).

Catalase (CAT) Activity

For determining catalase activity of alga *G. crepidinum*, a precise colourimetric technique was developed (Hadwan and kadhum, 2018). This assay is based on reactions of ammonium metavanadate with $H2O_2$ under acidic conditions, and H2O2 dissociation rates are directly proportional to catalase activity.

Ascorbate Peroxidase (APX) Activity

APX activity was assessed by monitoring ascorbate reduction and measuring the change in absorbance at 290 nm over 1 minute in a 2ml reaction mixture containing 50mM potassium phosphate buffer(Nakano and Asada, 1987).

Malondialdehyde (MDA) Estimation

Lipid oxidation in plants has been assessed using reactive acidic thiobarbituric acid compounds. The TBA test provides a basic, repeatable and standardized tool for measuring fat oxidation. The MDA-TBA compound, formed by the reaction of MDA and 1,3-diethyl-2-thiobarbituric acid (DETBA) at high temperatures of 90-100 °C under acidic conditions, is measured colourimetrically at a wavelength of 530-540 nm or fluorometrically at an emission wavelength of 555 nm with an excitation wavelength of 515 nm (Jo and Ahn, 1998).

Glutathione (GSH) Estimation

The reagent used is dithiobis (2-nitrobenzoic acid) (DTNB). DTNB is readily reduced by glutathione compounds to give a very intense yellow compound. It has a maximum absorbance at 412 nm and directly proportional to the glutathione concentration (Hadwan and kadhum, 2018).

Reactive Oxygen Species (ROS) Estimation

A unique method developed by Erel (2005) was used to measure the reactive oxygen species (ROS) in the sample. The oxidants present in the sample cause the ferrous ion-o-dianisidine complex to be oxidized to a ferric ion. The oxidation reaction is further enhanced by glycerol molecules, which are abundant in the reaction medium.

Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) with less significant differences (LSD) to compare the mean of toxic effect of bisphenol concentrations and oxidative stress, all treatments were performed in triplicate.

RESULTS AND DISCUSSION

There are two main types of antioxidants, enzymes and non-enzymes. Enzymatic antioxidants, such as CAT, SOD and APX, serve as the primary defense against ROS. Non-enzymatic antioxidants, including glutathione (GSH), carotenoids, act as a secondary defense against ROS, While MDA and ROS indicate cell damage. (Kim *et al.*, 2020).

The results showed a decrease in the algal biomass rate with increasing concentrations of bisphenol A. The highest concentration was recorded in the untreated control group and started to decrease with increasing concentration, while the IC50 of BPA was 2.68 mg/l. (Figure 1, 2).

The results of the current study showed that the photosynthetic pigments chlorophyll-a and carotenoids recorded their highest value in the control group, which amounted to 0.96 and 0.56 µg/ml, while the concentrations of these pigments decreased with increasing concentrations of BPA, their lowest value being recorded at 0.543 and 0.356 µg/ml at a concentration of 100 mg/liter, respectively. The results of this study showed that CAT and APX enzymes activity recorded a higher value of 0.345 and 4.660 U/g at 100 mg/l BPA, While the lowest values of these enzymes recorded 0.104 and 3.7 U/g in the control group, respectively. While the SOD enzyme recorded a high value of 22.227 U/g at 1 mg/l BPA and decreased with increasing concentrations of BPA with a lower value of 19.467 U/g at 100 mg/l. In addition, GST recorded lower values of 5.413 µmole/g in the control group and increased with increasing concentrations of BPA until it recorded higher values at a concentration of 100 mg/l, reaching 18.680 µmole/g. Nevertheless, the indication of cell damage such as MDA and ROS recorded lowest values 0.13 and 14.153 µmole/g in the control group, while higher values were recorded with increasing concentrations of BPA 3.487 and 74.4 µmole/g at 100mg/l BPA, respectively. (Table 1 and Figure 3, 4). The statistical analysis supported the results of this study at probability level $p \leq 0.05$.

Microalgae serve as valuable bioindicators for monitoring environmental changes and

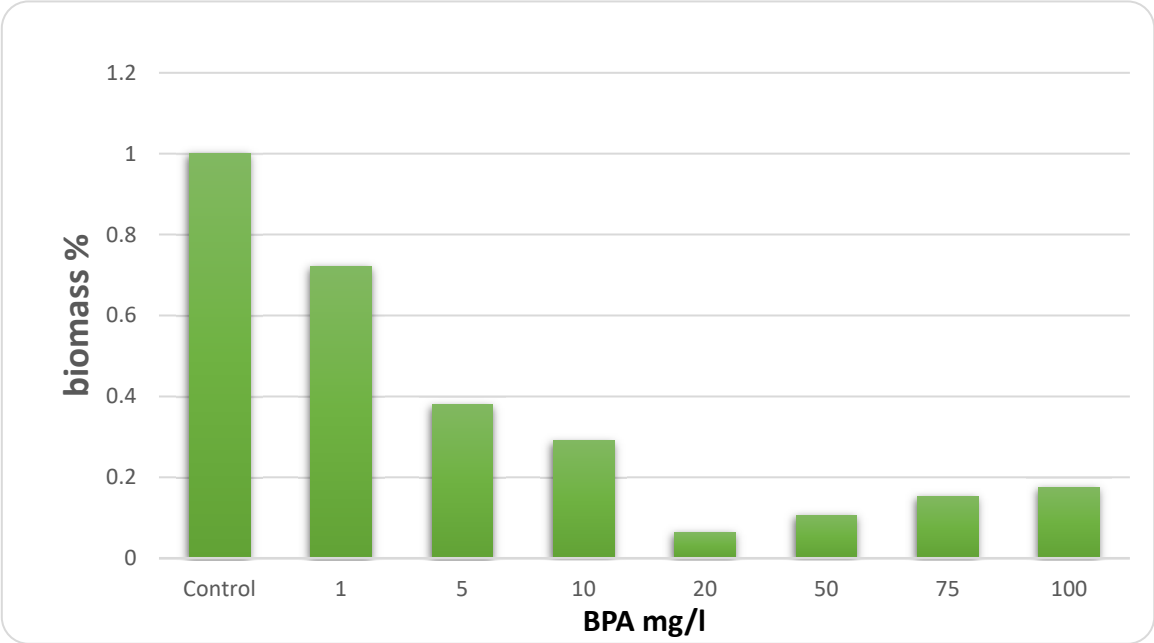

Fig. 1. Biomass of alga *G. crepidinum* treated with different concentrations of BPA in comparison with the control group

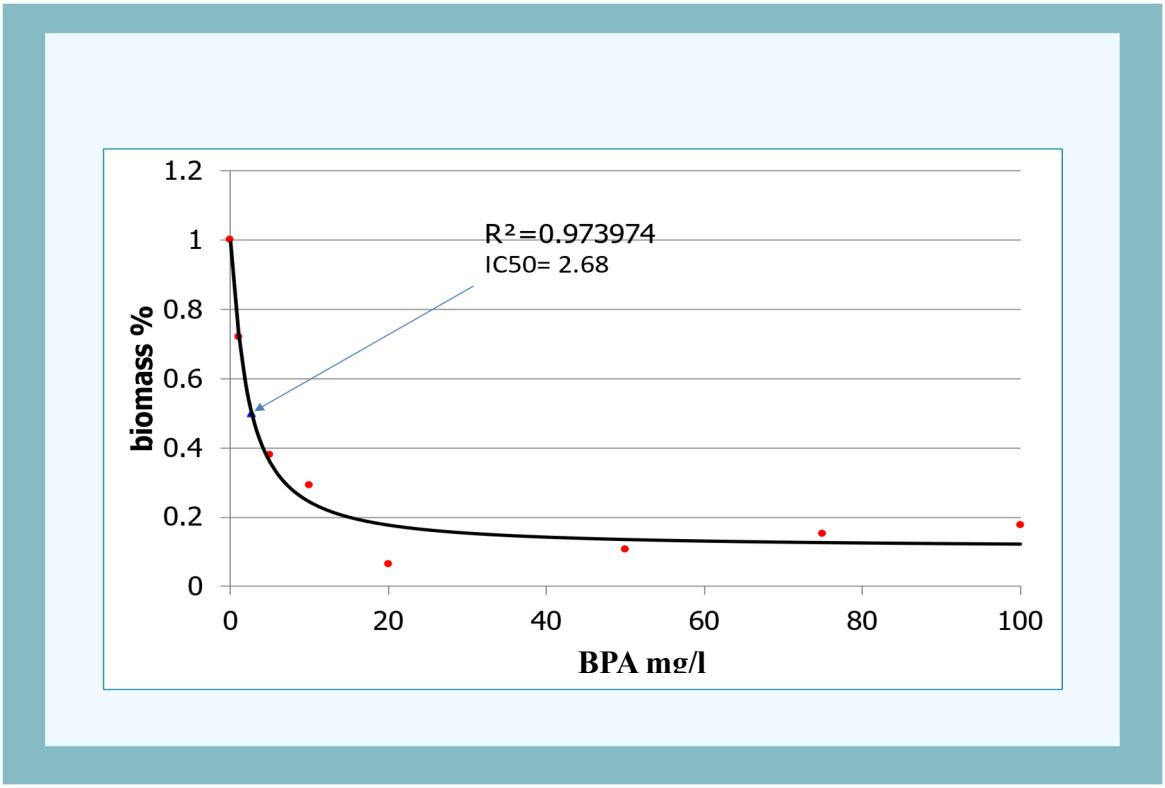

Fig. 2. IC50 of alga *G. crepidinum* treated with different concentrations of BPA in comparison with the control group

Table 1. Antioxidant response of alga comparison with the control group

Antioxidant Parameters	Chl-a µg/ml	Carotenoid µg/ml	CAT U/g	SOD U/g	APX U/g	GST µmole/g	MDA µmole/g	ROS µmole/g
BPA mg/l								
Control	0.9600±0.058 c	0.560±0.252 B	0.104±0.003 f	21.523±0.162 b	3.700±0.252 d	5.413±0.205 f	0.130±0.012 g	14.153±0.033 f
1	0.983467±0.23 a	0.6467±0.203 A	0.134±0.012 e	22.227±0.064 a	4.080±0.012 cd	7.230±0.065 e	0.450±0.023 f	14.163±0.033 f
5	0.8533±0.120 b	0.4700±0.153 C	0.130±0.006 e	21.157±0.030 b	4.070±0.012 cd	9.570±0.187 d	0.767±0.024 e	17.447±0.202 e
10	0.7433±0.203 d	0.4400±0.208 C	0.144±0.004 d	20.487±0.205 c	4.270±0.119 bc	15.597±0.250 c	0.757±0.024 e	19.507±0.210 d
20	0.7400±0.265 d	0.3567±0.240 D	0.145±0.005 d	20.313±0.116 c	4.327±0.146 bc	15.597±0.250 c	0.840±0.021 d	19.517±0.155 d
50	0.5433±0.203 e	0.3327±0.145 D	0.185±0.022 c	19.673±0.188 d	4.267±0.145 bc	15.447±0.179 c	1.077±0.019 c	30.470±0.203 c
75	0.4567±0.203 f	0.3267±0.233 D	0.236±0.012 b	19.540±0.178 d	4.477±0.204 a	16.490±0.159 b	2.577±0.091 b	31.600±0.058 b
100	0.3600±0.208 g	0.2300±0.351 E	0.345±0.042 a	19.467±0.176 d	4.660±0.239 ab	18.680±0.291 a	3.487±0.174 a	74.400±0.252 a
LSD	0.568	0.568	0.0069	0.373	0.406	0.514	0.401	0.406

Small letters indicate significant differences between all treatments at $P \leq 0.05$

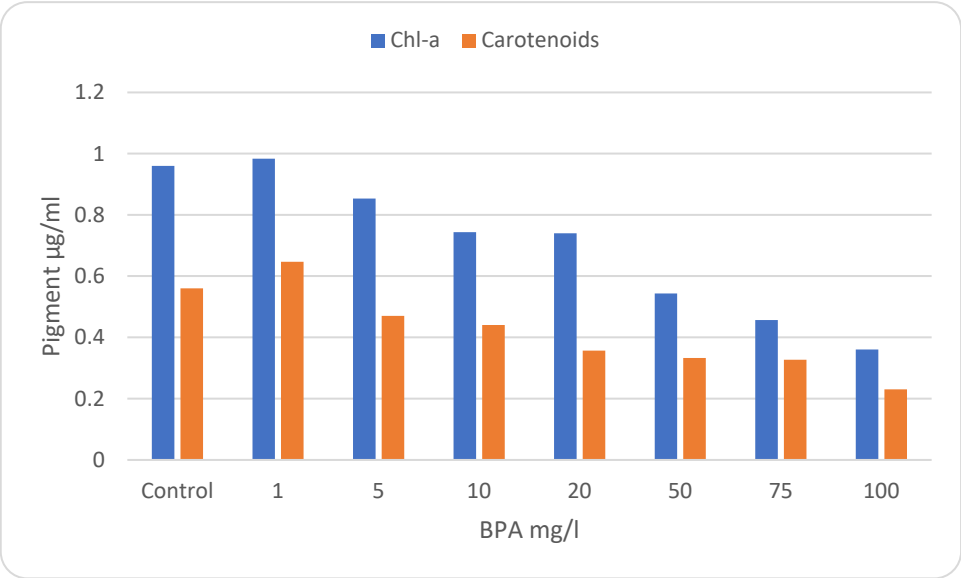

Fig. 3. Photosynthetic pigments Chl-a and Carotenoid of alga *G. crepidinum* treated with different concentrations of BPA in comparison with the control group

conducting environmental risk assessments, as well as for setting limits on the release of toxic chemicals into aquatic environments. The results of the current study are consistent with the work of Ebenezer and Ki (2016), who investigated the effects of bisphenol A (BPA) on the green alga *Tetraselmis suecica*, the diatom *Ditylum brightwellii* and the dinoflagellate *Prorocentrum minimum*. Each species showed varying degrees of sensitivity to BPA exposure, with the diatom D. brightwellii being the most sensitive. The 72-hour effective concentration (EC50) for BPA was calculated to be 0.037 mg/L for *D. brightwellii*, indicating greater sensitivity than the other species. This is also consistent with the study by Czarny-Krzymińska *et al.* (2022), who tested the green alga *Chlorella vulgaris* and *Desmodesmus armatus* for the toxicity of bisphenol A and its six structural congeners. Bisphenol A (with a mean EC50 of 42.29 mg L-1 over 14 days) was less toxic to *C. vulgaris* (with a mean EC50 of 22.39 mg L-1 over 14 days) than its structural congeners such as bisphenol AF, bisphenol G and bisphenol X, and bisphenol AF, bisphenol G, bisphenol M and bisphenol X to *D. armatus* (with a mean EC50 of 27.16 mg L-1 over 14 days).

Another study found that BPA inhibited the growth and photosynthesis of both algae, with *Graesiella* having a greater effect than *Picocystis*. The growth IC50 (4 days) was 32 mg L-1 for *Graesiella*, which was higher than 75 mg L^{-1} for *Picocystis*. Increased levels of oxidative stress were observed in both strains when exposed to increasing concentrations of BPA, as indicated by an increase in MDA levels. Furthermore, BPA exposure led to an up-regulation of antioxidant activities (APX, GST and CAT) in *Picocystis*, while these activities were suppressed in *Graesiella* (Ben Ouada *et al.*, 2018). Likewise, research results investigated the immediate and long-term adverse effects of bisphenol A (BPA) on *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. The results showed that BPA significantly inhibited the growth of both algae in short-term tests, but did not have the same effect on prolonged exposure. In addition, the activities of superoxide dismutase (SOD) and catalase (CAT) were increased in both algae in all treatments. Furthermore, the synthesis of chlorophyll a in both algae showed a similar inhibition pattern in the short-term experiments. This study contributes to a fundamental understanding of the toxicity of BPA to aquatic organisms (Zhang *et al.*, 2014).

The reasons for the decrease in biomass and photosynthetic pigments and the induction of

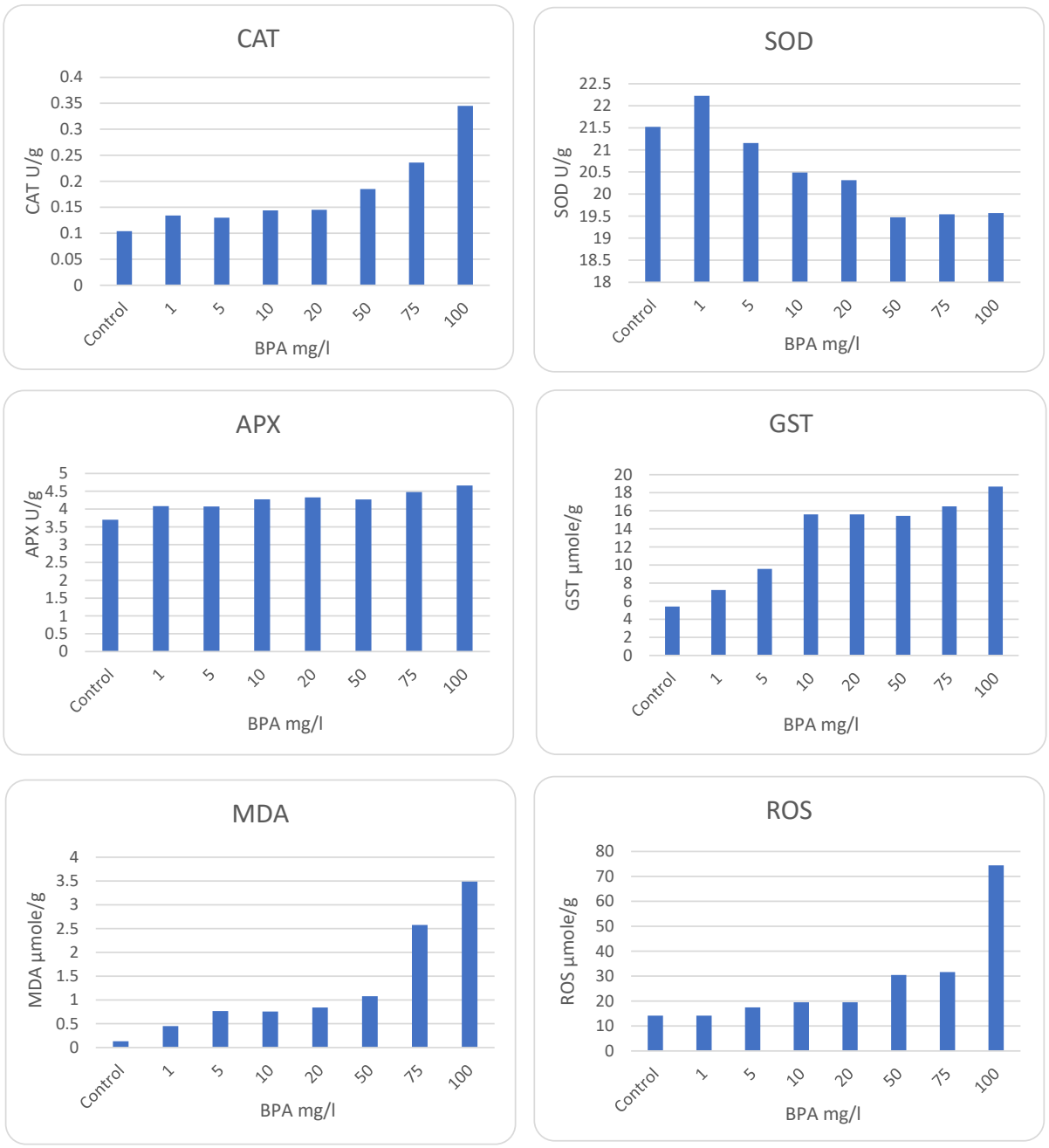

Fig. 4. Antioxidant enzymes (CAT, SOD and APX), GSH, MDA and ROS of alga G. *crepidinum* treated with different concentrations of BPA in comparison with the control group

some of the antioxidants studied may be due to the toxicity of bisphenol A to aquatic organisms, especially algae, as many of the above studies have shown that bisphenol A causes environmental stress and thus stimulates the production of many free radicals in algae, leading to an increase in reactive oxygen species, and this is what was observed in the current study, the antioxidant response increased with increasing concentrations of bisphenol, leading to deterioration and decrease in biomass, photosynthetic pigments, lipid peroxidation and super enzymes, While the enzymes CAT and APX also increased GSH to eliminate or limit ROS production and increase the toxicity of BPA, thus increasing the ability of algae to tolerate bisphenol toxicity.

CONCLUSIONS

Bisphenol A, a toxic substance to the aquatic environment due to its toxicity to aquatic algae, the toxic effect of bisphenol A on alga *G. crepidinum* induces oxidative stress as evidenced by an increase in the levels of reactive oxygen species and oxidative damage. This highlights the potential deleterious effects of bisphenol A on the cellular health and antioxidant defense mechanisms of this alga.

ACKNOWLEDGMENT

The authors of this study would like to thank Prof. Dr. Ahmed J. Hassan, Head of the Biology Department, University of Al-Qadisiyah, Iraq, for laboratory assistance and help with statistical analysis.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

REFERENCES

Azizullah, A., Khan, S., Gao, G., & Gao, K. (2022). The interplay between bisphenol A and algae–A review. *Journal of King Saud University-Science*, *34*(5), 102050.

Ben Ouada, S., Ben Ali, R., Leboulanger, C., Zaghden, H., Choura, S., Ben Ouada, H., & Sayadi, S. (2018). Effect and removal of bisphenol A by two extremophilic microalgal strains (Chlorophyta). *Journal of applied phycology*, *30*, 1765-1776.

Czarny-Krzymińska, K., Krawczyk, B., & Szczukocki, D. (2022). Toxicity of bisphenol A and its structural congeners to microalgae Chlorella vulgaris and Desmodesmus armatus. *Journal of applied phycology*, *34*(3), 1397-1410.

Ebenezer, V., & Ki, J.-S. (2016). Toxic effects of Aroclor 1016 and bisphenol A on marine green algae Tetraselmis suecica, diatom Ditylum brightwellii and dinoflagellate Prorocentrum minimum. *Korean Journal of Microbiology*, *52*(3), 306-312.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical biochemistry*, *38*(12), 1103-1111.

Fan, G., Chen, Z., Yan, Z., Du, B., Pang, H., Tang, D., Luo, J., & Lin, J. (2021). Efficient integration of plasmonic Ag/AgCl with perovskite-type LaFeO3: enhanced visible-light photocatalytic activity for removal of harmful algae. *Journal of Hazardous Materials*, *409*, 125018.

Hadwan, M. H., & kadhum, A. S. (2018). New spectrophotometric assay for assessments of catalase

activity in biological samples. *Analytical biochemistry*, *542*, 29-33.
Hakim, R. H., & Alghanmi, H. A. (2024). Bio-removal of bisphenol A by cyanobacterium Gloeocapsopsis crepidium. *Egyptian Journal of Aquatic Biology and Fisheries*, *28*(1), 1719-1726.
Jo, C., & Ahn, D. (1998). Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poultry science*, *77*(3), 475-480.
Kearney, P. (2023). *Removal of endocrine disruptor Bisphenol A from water* University of Bath].
Kim, T. Y., Leem, E., Lee, J. M., & Kim, S. R. (2020). Control of reactive oxygen species for the prevention of Parkinson's disease: the possible application of flavonoids. *Antioxidants*, *9*(7), 583.
Li, J., Wang, Y., Li, N., He, Y., Xiao, H., Fang, D., & Chen, C. (2022). Toxic Effects of Bisphenol A and Bisphenol S on Chlorella Pyrenoidosa under Single and Combined Action. *Int J Environ Res Public Health*, *19*(7). https://doi.org/10.3390/ijerph19074245
Li, R., Chen, G.-Z., Tam, N. F. Y., Luan, T.-G., Shin, P. K., Cheung, S. G., & Liu, Y. (2009). Toxicity of bisphenol A and its bioaccumulation and removal by a marine microalga Stephanodiscus hantzschii. *Ecotoxicology and Environmental safety*, *72*(2), 321-328.
Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry*, *47*(3), 469-474.
Nakano, Y., & Asada, K. (1987). Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant and cell physiology*, *28*(1), 131-140.
Sebaugh, J. (2011). Guidelines for accurate EC50/IC50 estimation. *Pharmaceutical statistics*, *10*(2), 128-134.
Sharma, V., Jain, D., Rai, A. R., Kumari, P., Nagar, V., Kaur, A., Singh, A., Verma, R. K., Pandey, H., & Sankhla, M. S. (2023). Toxicological assessment and concentration analysis of Bisphenol A in food grade plastics: A systematic review. *Materials Today: Proceedings*, *95*, 18-25. https://doi.org/https://doi.org/10.1016/j.matpr.2023.06.336
Zavřel, T., Sinetova, M. A., & Červený, J. (2015). Measurement of chlorophyll a and carotenoids concentration in cyanobacteria. *Bio-protocol*, *5*(9), e1467-e1467.
Zhang, H., Ding, T., Luo, X., & Li, J. (2021). Toxic effect of fluorene-9-bisphenol to green algae Chlorella vulgaris and its metabolic fate. *Ecotoxicol Environ Saf*, *216*, 112158. https://doi.org/10.1016/j.ecoenv.2021.112158
Zhang, W., Xiong, B., Sun, W. F., An, S., Lin, K. F., Guo, M. J., & Cui, X. H. (2014). Acute and chronic toxic effects of bisphenol a on Chlorella pyrenoidosa and Scenedesmus obliquus. *Environmental toxicology*, *29*(6), 714-722.