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Some Physiological Effects of Bisphenol A on *Lemna gibba* L., A Free-Floating Aquatic Macrophyte

Muhittin DOĞAN[1], Şeyda YILMAZ[2]✍, Serap ŞAHİN YİĞİT[3]
[1,2,3] Department of Biology, Faculty of Arts and Sciences, Gaziantep University, Gaziantep-Türkiye
[1]https://orcid.org/0000-0001-5400-8065, [2]https://orcid.org/0000-0002-2262-432X, [3]https://orcid.org/0000-0002-2508-7275
✉: yilmazzseyda@gmail.com

ABSTRACT
The present study was carried out to evaluate the effect of bisphenol A (BPA) on *Lemna gibba*, a free-floating aquatic macrophyte, in a climate cabinet under controlled conditions. *L. gibba* was collected from natural water sources in Gaziantep (Türkiye) and acclimatized for two weeks in containers containing 10% nutrient solution. Macrophytes were treated with 1.5, 17.2, and 50 mg/L BPA for 96 hours. Chlorophyll a, chlorophyll b, carotenoid, protein, and total soluble carbohydrate contents were declined following BPA application. Contrary to this, an elevation in the contents of NP-SH, H_2O_2, and malondialdehyde were detected. In conclusion, correlation analyses showed that the changes may be related to BPA-induced oxidative stress.

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Bisfenol A'nın Serbest Yüzücü Makrofitlerden *Lemna gibba* L. Üzerindeki Bazı Fizyolojik Etkileri

ÖZET
Bu çalışma, kontrollü koşullar altında bir iklimlendirme dolabında BPA'nın *Lemna gibba* üzerindeki etkisini belirlemek amacıyla yapıldı. Makrofitler Gaziantep'teki (Türkiye) doğal su kaynaklarından toplandı ve %10 besin çözeltisi içeren kaplarda iki hafta boyunca aklimatize edildi. Makrofitler 96 saat boyunca 1.5, 17.2 ve 50 mg/L BPA ile muamele edildi. BPA'nın klorofil a, klorofil b, karotenoid, protein ve toplam çözünür karbonhidrat içeriğinde azalmaya neden olduğu belirlendi. Bunların aksine, protein olmayan sülfidril gruplar (NP-SH), H_2O_2 ve malondialdehit (MDA) içeriklerinde artışlar tespit edildi. Sonuç olarak, korelasyon analizleri bu değişikliklerin BPA kaynaklı oksidatif stresle ilişkili olabileceğini göstermektedir.

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INTRODUCTION
Industrially important monomer, bisphenol A (BPA: 2,2-Bis (4- hidroksifenil) propane; Fig. 1) is a synthetic chemical used extensively to synthesize epoxy resins, polymer materials, and polycarbonate plastics. For example, BPA is found in baby bottles, water bottles, dental fillings, thermal paper, toys, medical devices, etc. Therefore, since it has widespread use in many products, it causes a wide distribution of BPA in the environment, thus it appears as a potential pollutant (Manzoor et al., 2022).

The duckweed contains the smallest flowering plants. Therefore, it can be considered as a model plant. Due to their wide tolerance range, they have a high potential to be used in bioremediation studies. Due to their high protein content, they can be used as feed for fish, other animals, and even humans. Additionally, they may be important to use as a biofuel due to their high starch content (Coskun et al., 2018). *L. gibba* can continue to grow in eutrophied waters with low oxygen and excess carbon dioxide, low light, and very salty waters. On the other hand, it is considered as a potential indicator for the aquatic ecosystems (Thingujam et al., 2024).
Pollution of the environment due to anthropogenic activities causes negative effects on living things. The deterioration of environmental health caused by rapid industrialization, urbanization, and increasing population pressure brings along many environmental problems. In addition, although studies on the structure, amount,

physicochemical behavior, and effects of pollutants have increased greatly in recent years, more research is still needed on the effects of these pollutants on living things. Therefore, this study was carried out to determine the effects of BPA, which is an important environmental pollutant, on some physiological properties of *L. gibba*, a free-floating macrophyte.

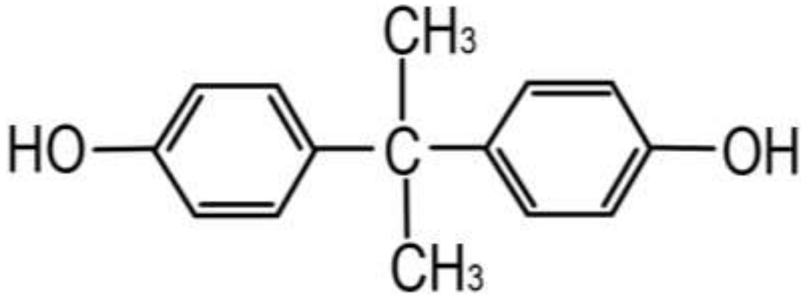

Fig. 1. Structure of BPA
Şekil 1. BPA'nın yapısı

MATERIALS and METHODS

Plant material and BPA treatment

L. gibba was collected from water bodies in the province of Gaziantep (Türkiye) and acclimated to 10% nutrient solution in a climate chamber (Snijders Scientific, Netherlands) in determined conditions (light/dark regimes of 16/8 h, light level 120 µE.m^{-2}.s^{-1}, temperature 23±1 °C) for two weeks before BPA treatments. Three different BPA concentrations were applied in the study. The BPA concentration of 1.5 mg/L was chosen because it is the upper safe limit for drinking water according to the US EPA (Geens et al., 2011). The concentration of 17.2 mg/L is the hazardous landfill leachate concentration (Yamamoto et al., 2001). The concentration of 50 mg/L was preferred because it causes possible contamination by BPA and this concentration is often used to indicate the toxicity of BPA in plants (Dogan et al., 2010; Cinar & Dogan, 2020). Thus, healthy macrophytes were treated with concentrations of 1.5, 17.2, and 50 mg/L of BPA with 10% nutrient solution (Öztürk et al. 2002) in triplicate in 100 ml glass vessels. A 10% nutrient solution (without BPA) was used as a control. The macrophytes were harvested after 96 hours. Ultrapure water was used in all applications and analyses. The reagents used in the study were of analytical grade.

Physiological analysis

Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid contents were determined spectrophotometrically according to Lichtenthaler & Wellburn (1985). The anthrone method was used to measure total soluble carbohydrate content (Plummer, 1978) using glucose as standard. The method introduced by Lowry et al. was used to determine the protein content using bovine serum albumin as standard (Lowry et al., 1951). Non-protein sulfhydryl groups (NP-SH) were determined according to Ellman's method (Ellman, 1959). Malondialdehyde (MDA) level was determined by Zhou (2001). Hydrogen peroxide was determined according to Sergiev et al. (1997).

Data Analysis

Statistical analyses were performed using the SPSS software program. The least significant difference (LSD) test was used to compare the data. Pearson correlation was applied to evaluate the relationship among physiological changes.

RESULTS and DISCUSSION

Plants need the synthesis and accumulation of organic substances for growth and development, which in turn depends on their photosynthesis. Chlorophyll molecules bound to the proteins of photosynthetic membranes harvest sunlight (Von Wettstein et al., 1995) Carotenoids are photosynthetic pigments that play an important role in physiological processes such as light harvesting and energy transfer, photoprotection, and stabilization of light-harvesting pigment-protein complexes (Sutherland et al., 2022) Therefore, since the chlorophyll content is directly related to photosynthesis, respiration, and energy (Qiu et al., 2013) the effect of BPA applications on the photosynthetic pigment content of *L. gibba* was determined (Fig. 2). Chlorophyll content was dose-dependent manner decreased at 1.5, 17.2 and 50 mg/L BPA concentrations by 3.1% (p=0.631), 7.5% (p=0.228) and 43.7% (p=00006), respectively, when compared to the control. Similarly, chlorophyll a and carotenoid contents at 50 mg/L BPA decreased by up to 34.3% (p=0.02) and 40.5% (p=0.01), respectively, when compared to the control. Photosynthesis is strongly affected by stress factors, including BPA (Qiu et al., 2013). Previous studies have reported that BPA causes oxidative stress by triggering the formation of reactive oxygen species (ROS) in plants (Dogan et al., 2020; Qiu et al., 2013; Dogan et al., 2012). There was a positive relationship between H_2O_2 content and photosynthetic pigments (r=0.703 and p=0.011 for Chl a; r=0.552 and p=0.063 for Chl b; and r=0.629 and p=0.028 for carotenoids). Besides, regression analysis showed a positive correlation between MDA content and photosynthetic pigments (r=0.708 at p=0.010 for Chl a, r=0.563 at p=0.057 for Chl b, and r=0.632 at p=0.027 for carotenoids). According to the findings, BPA reduced the photosynthetic pigment content in *L. gibba* cells, which may be due to the oxidative stress of BPA. Furthermore, the decrease in BPA-treated *L. gibba* may be due to peroxidation of chloroplast membrane lipids (Cinar & Dogan, 2020; Qiu et al., 2013).

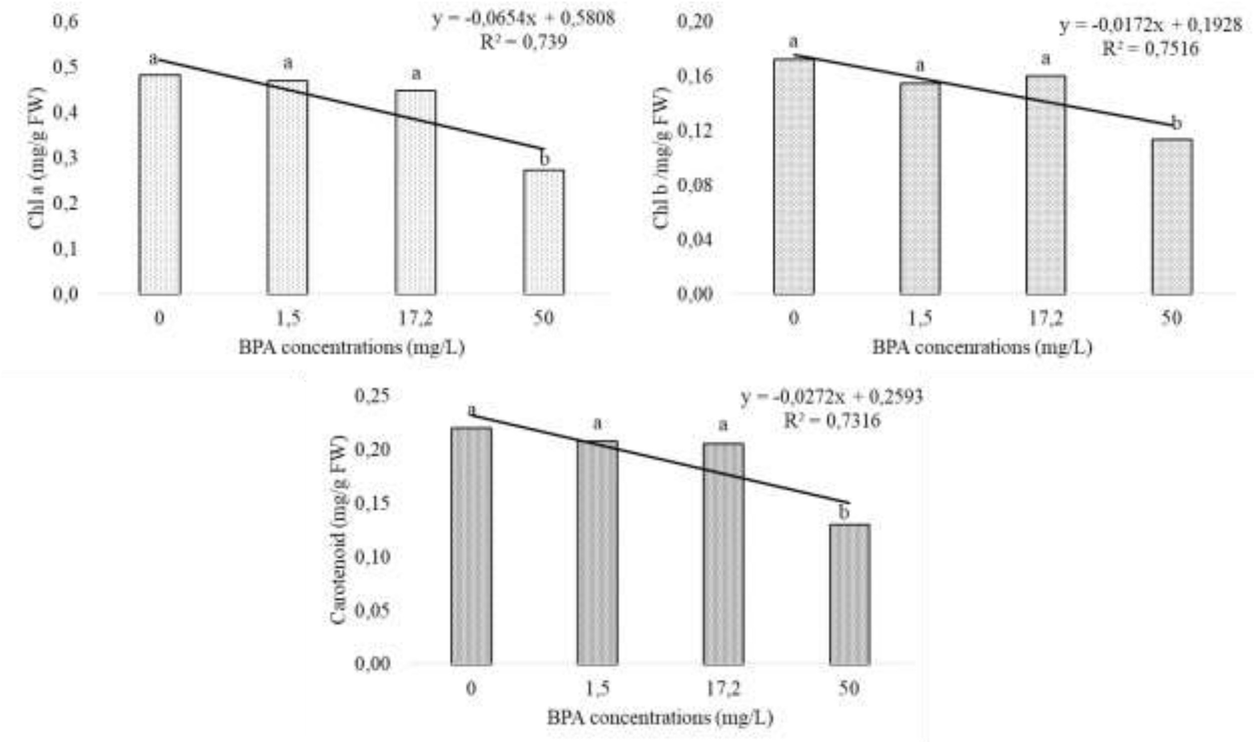

Fig. 2. Photosynthetic pigment contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test (p<0.05).

Şekil 2. BPA uygulamaları sonrası L. gibba'nın fotosentetik pigment içerikleri. Farklı harfler LSD testine göre istatistiksel önemi göstermektedir (p<0.05).

Protein content and their statistical evaluations of *L. gibba* tissues are presented in Fig. 3. Protein content was decreased at 1.5, 17.2, and 50 mg/L BPA as 3.1% (p=0.811), 29.0% (p=0.047) and 58.3% (p=0.02), respectively, when compared to the control. Correlation analyses showed that there was a positive relationship between H_2O_2 content and protein content (r=0.600; p=0.039). The findings may indicate that BPA is mediated by oxidative stress resulting in ROS production in *L. gibba* cells, thus may be a reason for the decreased protein content (Dogan et al., 2012; Cinar & Dogan, 2020; Halliwell, 1987).

Total soluble carbohydrate contents at 1.5, 17.2, and 50 mg/L BPA were found to be decreased by 26.9% (p=0.023), 35.8%(p=0.006), and 36.9% (p=0.005), respectively, when compared to the control (Fig. 3). The positive correlation was found between the H_2O_2 and total carbohydrate contents suggesting BPA elicited oxidative stress (r=0.015; p=0.964). Chronic exposure to BPA has been reported to cause oxidative stress and impair carbohydrate metabolism through the downregulation of carbohydrate metabolizing enzymes (Ul Haq et al., 2020).

There is evidence that non-protein thiols contribute to plant stress tolerance. In the majority of aerobic cells, the main non-protein thiol is glutathione (GSH), which is an important antioxidant, playing a central role in ROS scavenging in the GSH-ascorbate cycle (Noctor et al., 2012). In *L. gibba*, NP-SH content increased up to 79.9% by BPA (p=0.00007) (Fig. 4). Positive correlation was determined between NP-SH and H_2O_2 contents (r= 0.543; p=0.088). This may indicate that increased GSH content under stressful conditions is related to the tolerance of *L. gibba* to BPA stress.

As previously mentioned, BPA causes oxidative stress by inducing ROS. For this purpose, H_2O_2 and MDA contents were determined to evaluate whether BPA caused oxidative stress in *L. gibba* cells. H_2O_2 content increased by 17.1% (p=0.058) and 20.1% (p=0.031) in 1.5 and 17.2 mg/L BPA, respectively, compared to control, but decreased by 32.4% (p=0.003) in 50 mg/L BPA (Fig. 4). MDA is an important biomarker of oxidative stress (Dogan et al. 2010; Akbulut et al., 2020). Similar findings were also found in MDA content (Fig. 4). Correlation analysis revealed a significant and positive relationship between H_2O_2 and MDA (r=0.994; p<0.00001), confirming the status of oxidative stress.

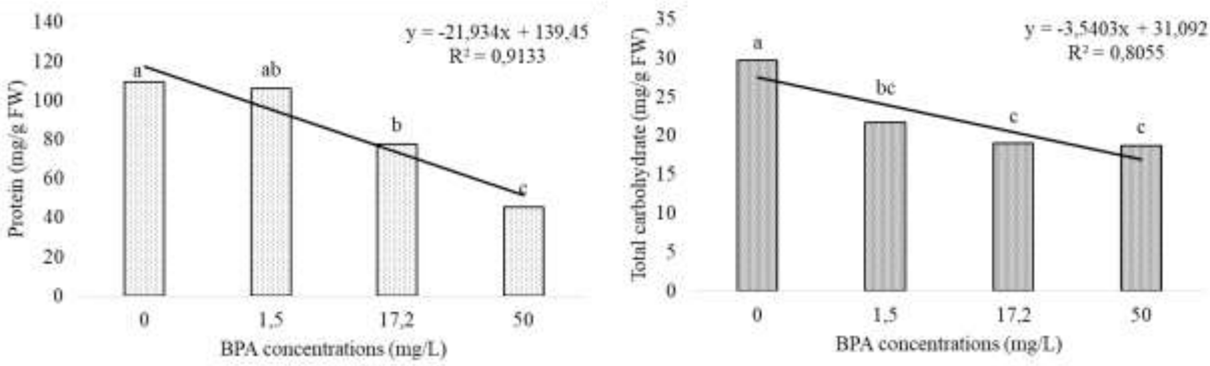

Fig. 3. Protein and total carbohydrate contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test (p<0.05).

Şekil 3. BPA uygulamalarından sonra L. gibba'nın protein ve toplam karbonhidrat içerikleri. Farklı harfler LSD testine göre istatistiksel önemi göstermektedir (p<0.05).

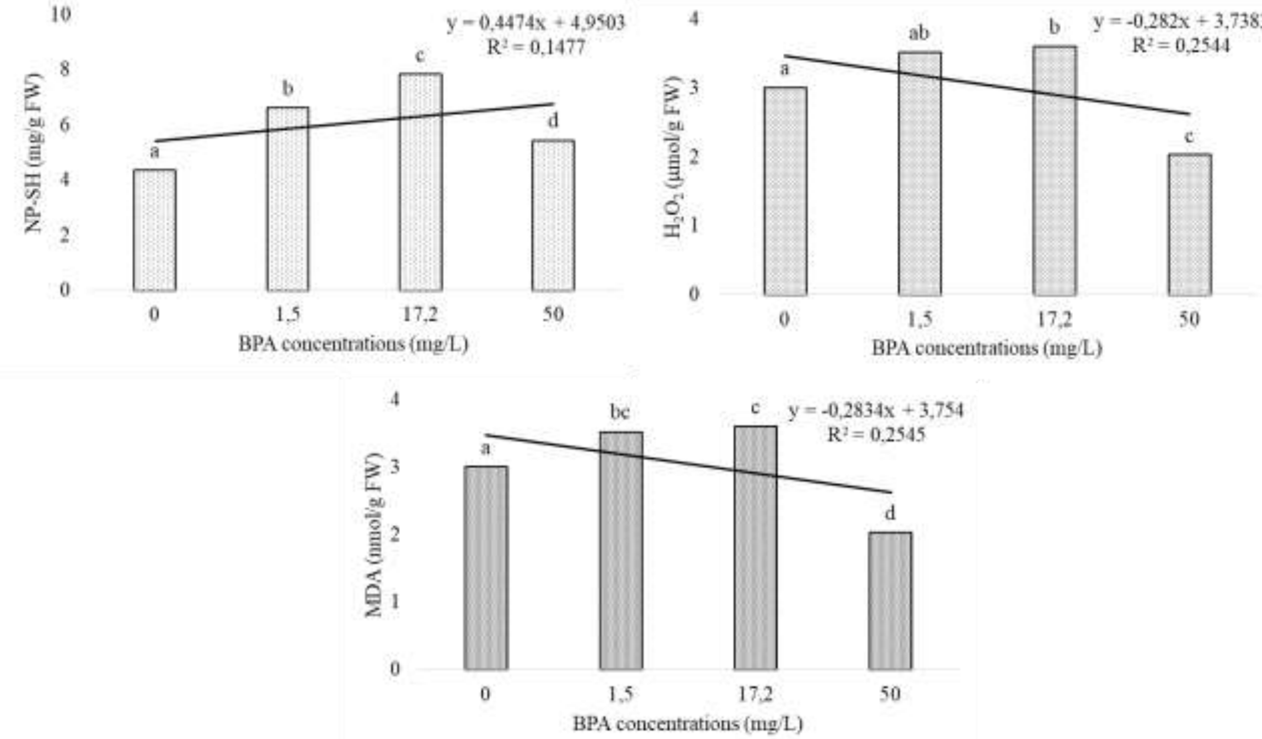

Fig. 4. NP-SH, H_2O_2, and MDA contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test (p<0.05).

Şekil 4. BPA uygulamaları sonrası L. gibba'nın NP-SH, H2O2 ve MDA içerikleri. LSD testine göre farklı harfler istatistiksel anlamlılığı göstermektedir (p<0,05).

CONCLUSION

This study investigated the effects of BPA on some physiological processes in *L. gibba* to clarify the effect of BPA on aquatic plants and to provide a reference for the assessment of the ecological risk of BPA in the environment. It was determined that BPA caused a decrease in photosynthetic pigments, protein, and total soluble carbohydrate contents. On the other hand, increased NP-SH contents may be related to their tolerance to BPA-induced stress. It was demonstrated by the increase in the content of H_2O_2, a type of ROS, that BPA triggered oxidative stress in *L. gibba* cells. In addition, an increase in MDA, which is both an indicator of oxidative stress and a marker of lipid peroxidation, clearly demonstrated this situation as well.

Contribution of Authors

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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