KSÜ Tarım ve Doğa Derg 26 (5), 1120-1134, 2023
KSU J. Agric Nat 26 (5), 1120-1134, 2023
https://doi.org/10.18016/ksutarimdoga.1174954

Evaluation of Oxidative Stress And Growth Alterations on Arthrospira Platensis Gomont and Chlorella Vulgaris Beijerinck (Beijerinck) by Cambio

Şükrüye ER[1], Hatice TUNCA[2], Ali DOĞRU[3], Tuğba ONGUN SEVİNDİK[4]
[1]Adapazarı ENKA Schools, Sakarya, Turkiye [2,3,4] Sakarya Unıversity Science Faculty Biology Department, Sakarya, Turkiye
[1]https://orcid.org/ 0000-0002-0815-8430, [2]https://orcid.org/ 0000-0003-3724-5215, [3]https://orcid.org/ 0000-0003-0060-4691
[4]https://orcid.org/ 0000-0001-7682-0142
✉: esukriye@gmail.com

ABSTRACT

This study aims to evaluate the toxicity effects of different concentrations of Cambio on *Chlorella vulgaris* (0-500 µg mL^{-1}) and *Arthrospira platensis* (0-50 µg mL^{-1}) algae by determining the changes in chlorophyll-*a* amount, OD 750 (biomass) and antioxidant parameters (the activities of Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Glutathione reductase (GR) and the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline). *A. platensis* is being a cyanobacterium used commercially because of its high nutrient content. *C. vulgaris* used for medical and commercial purposes due to the capability of bioremediation, the structure of drug raw material, and nutrient compound. Ecotoxicological studies on these cosmopolitan algae are important for determining the harmful effects of chemicals on freshwater ecosystems. Cambio was toxic to *A. platensis* cells at the highest concentration, however, it stimulated the growth of *C. vulgaris*. For *A. platensis* application, the activity of Superoxide dismutase significantly decreased at moderate concentrations ($p<0.05$), while the activity of Ascorbate peroxidase decreased at the highest concentration ($p<0.05$). Moreover, the activity of Glutathione reductase rose at 20 µg mL^{-1} concentration. Malondialdehyde and H_2O_2 did not show significant changes, but the proline content showed significant increases in all Cambio concentrations compared to the control ($p<0.05$). However, for *C. vulgaris* application the antioxidant parameters did not show any alterations. These results are indicated that the effects of Cambio on *A. platensis* are more destructive than *C. vulgaris*.

Ecotoxicology

Research Article

Article History
Received : 14.09.2022
Accepted : 31.03.2023

Keywords
Herbicide
Stress
Antioxidant
Growth parameters
Algal ecotoxicology

Cambio'nun Arthrospira Platensis Gomont ve Chlorella Vulgaris Beijerinck (Beijerinck) Üzerinde Oluşturduğu Oksidatif Stresin Ve Büyüme Değişimlerinin Değerlendirilmesi

ÖZET

Bu çalışma, Cambio'nun farklı konsantrasyonlarının *Chlorella vulgaris* (0-500 µg mL^{-1}) ve *Arthrospira platensis* (0-50 µg mL^{-1}) alglerinde oluşturduğu biyokütle (klorofil-*a* miktarı, OD 750) ve antioksidan parametrelerindeki (Süperoksit dismutaz (SOD), Askorbat peroksidaz (APX), glutatyon peroksidaz (GR) enzim aktiviteleri ve malondialdehit (MDA), hidrojen peroksit (H_20_2) ve prolin içerikleri) değişimleri belirlemeyi amaçlamaktadır. *A. platensis*, yüksek besin içeriği nedeniyle ticari olarak kullanılan bir siyanobakteridir. *C. vulgaris*, biyoremediasyon kabiliyeti, ilaç hammaddesinin yapısı ve besin bileşimi nedeniyle tıbbi ve ticari amaçlar için kullanılmaktadır. Cambio, en yüksek konsantrasyonda *A. platensis* hücreleri için toksiktir, ancak *C. vulgaris*' in büyümesini uyarmıştır. Kozmopolit olan bu iki algin üzerinde yapılan ekotoksikolojik çalışmalar kimyasalların tatlı su ekosistemlerinde meydana getirdiği zararlı etkilerin belirlenmesi adına önemlidir. *A. platensis* uygulamasında, süperoksit dismutaz aktivitesi orta konsantrasyonlarda; Askorbat peroksidaz aktivitesi ise en yüksek konsantrasyonda anlamlı olarak azalmıştır ($p<0.05$). Ayrıca,

Ekotoksikoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 14.09.2022
Kabul Tarihi : 31.03.2023

Anahtar Kelimeler
Herbisit
Stres
Antioksidan
Büyüme parametreleri
Alg ekotoksikolojisi

Glutatyon redüktazın aktivitesi, 20 µg mL^{-1} konsantrasyonunda artış göstermiştir. Malondialdehit ve H_2O_2 miktarında önemli değişiklikler görülmemiştir. Ancak prolin içeriği, kontrole kıyasla tüm Cambio konsantrasyonlarında önemli artışlar göstermiştir ($p<0.05$). Ancak *C. vulgaris* uygulaması için antioksidan parametrelerde herhangi bir değişiklik gözlenmemiştir. Bu sonuçlar Cambio'nun *A. platensis* üzerindeki etkilerinin *C. vulgaris* üzerindeki etkilerine göre daha yıkıcı olduğunu göstermektedir.

Atıf İçin: Cambio'nun *Arthrospira platensis* Gomont ve *Chlorella vulgarıs* Beıjerınck (Beıjerınck) Üzerinde Oluşturduğu Oksidatif Stresin ve Büyüme Değişimlerinin Değerlendirilmesi (2023). *KSÜ Tarım ve Doğa Derg 26* (5), 1120-1134. https://doi.org/10.18016/ksutarimdoga.Vi.1174954.

To Cite: Evaluation of Oxidative Stress and Growth Alterations on *Arthrospira platensis* Gomont and *Chlorella vulgaris* Beijerinck (Beijerinck) By Cambio (2023). *KSU J. Agric Nat 26* (5), 1120-1134. https://doi.org/10.18016/ksutarimdoga.vi. 1174954.

INTRODUCTION

Cambio, which is widely used as a pesticide in corn fields in Turkey, belongs to the chemical family of the sulfonylurea group and its active ingredient is nicosulfuron. It is a selective herbicide used after the emergence against annual and perennial grasses and some broad-leaved weeds that are problematic in corn fields. This herbicide inhibits the synthesis of leucine, isoleucine, and valine amino acids by inhibiting the plant's acetolactate (ALS) enzyme. It halts the production of other plant components by blocking these essential amino acids (Rey-Caballero et al. 2016; Wu et al. 2022). In this way, it stops the development of target weeds by preventing cell division and consequently causes the death of the plants (PMRA-ARLA, 1996; Serim et al., 2017).

Cellular homeostasis is characterized by a baseline level of ROS that depends on the organism developmental stage, circadian clock, and environmental and physiological conditions. Different biotic and abiotic stresses such as temperature, heavy metals, pesticides, light intensity and nutrition restrictions can disrupt this homeostasis, uncouple metabolic pathways and lead to the accumulation of ROS in different cell compartments (Bhatnagar-Mathur et al., 2008; Hasanuzzaman et al., 2012; Mittler et al., 2022). The increase of free radicals in the cell is an important reason for cell damage. Especially, cell membranes may be affected by free radicals and may lose their fluidity and permeability through lipid peroxidation (Tunca et al., 2022). When oxidative stress progress, living things try to get rid of this situation by using antioxidant defense mechanisms (Xu et al., 2022; Özyurt et al., 2021). Superoxide dismutase (SOD) is one of the most important antioxidant enzymes that acts as the first step of free radical detoxification. While catalyzing the formation of molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) from superoxide radicals, it can make the superoxide anions less harmful (Valentine et al., 1998; Ighodaro et al., 2017). Ascorbate peroxidase (APX) is an enzymatic antioxidant that defends the cell from ROS in many organisms such as high plants, algae, and flagella. It uses an ascorbic electron donor and catalyzes the reduction of H_2O_2 to water. The task is very similar to catalase but has a higher affinity to H_2O_2. Glutathione reductase (GR) is an enzyme that is highly conserved in bacteria, yeasts, algae, plants, animals, and humans. It sustains the cell against ROS by forming a GSH pool by oxidation of NADPH (Anjum, 2010). MDA is generated by lipid peroxidation as an aldehyde metabolite, (Cao et al. 2022; Tang et al., 2022).

In recent years, increasing usage of herbicides in agricultural activities has indirectly affected aquatic ecosystems (Relyea, 2005; Schmitt-Jansen & Altenburger, 2005; Schuler & Rand, 2008; Vervliet-Scheebaum et al., 2010). Unfortunately, the toxicity and environmental destruction of these compounds acting on non-target organisms are ignored by laws and policies (Niedobova et al., 2022). Microalgae are the non-target organism for herbicides but these creatures are the most sensitive due to being primary producers of food-chain in aquatic ecosystems (Cedergreen & Streibig, 2005, Yang et al., 2022, Walsh, 1978). These features cause to transport of the xenobiotics to higher trophic levels. Primary producers being indicators provide us with important information about pollution in these systems (Tunca et al., 2022). *Arthrospira platensis* is an alkaliphilic cyanobacterium used commercially because of its high nutrient content. *Arthrospira* sp. has been recorded in Turkish algal flora (Aysel, 2005). The studies about the biology and physiology of this algae are important, due to being cosmopolitan. The fact that this alga exhibits its metabolic responses to environmental stimuli in the natural environment makes it a reliable microorganism in ecotoxicological studies (Sili et al. 2012). *C. vulgaris* also used for medical and commercial purposes due to the capability of bioremediation, the structure of drug raw material, and nutrient compound. *C. vulgaris* is unicellular and coccoid (Kookal et al. 2023; Tamil Selvan et al. 2023).

This study aims to determine the effects of Cambio on the development of *C. vulgaris* and *A. platensis* algae and to evaluate the changes in antioxidant and growth

parameters.

MATERIAL and METHOD

Algae culture and treatment

A. platensis M2 (Culture collection No: SLSP01) and *C.* vulgaris were obtained from Soley Microalgae Institute California (USA) and Çukurova University (Turkey), respectively. Spirulina Medium (Aiba & Ogawa, 1977) was used in the culture of *A. platensis* and BG11 Medium (Rippka et al., 1979) was used in the culture of *C. vulgaris*. The 50 mL of cultures were grown under the conditions of 93 µmol photons m^{-2} s^{-1}, in 12:12 h circadian rhythm at 30±1 ºC and 25±1 ºC for *A. platensis* and *C. vulgaris,* respectively. Various concentrations of Cambio compound (40 g/L, nicosulfuron EC, İstanbul, Turkey) for *C. vulgaris* (100, 200, 300, 400, 500 µg mL^{-1}) and *A. platensis* (10, 20, 30, 40, 50 µg mL^{-1}) were added to the culture medium. The concentration ranges were determined according to preliminary laboratory experiments.

Cell growth assay

Optic density (OD) of the algae was measured for 7 days under control and stressed conditions by using a spectrophotometer taking absorbance at 750 nm. Cultures were diluted at a 1/10 ratio with BG11 Medium for *C. vulgaris* and Spirulina Medium for *A. platensis*. At the same time, BG11 Medium and Spirulina Medium were used as curves, and each measurement was conducted every 24 h for 7 days. To determine the Chlorophyll-*a* contents, methanol extractions were performed and the samples were measured at the absorbance of 665 nm wavelength for 7 days (MacKinney, 1941).

Antioxidant enzyme activities

On the 7th day, the centrifugation was applied to 2 mL culture solutions of the control and each Cambio-exposed algae medium, at 15.000 rpm for 20 min at 4 °C. Pellets resulting from centrifugation were stored at -20 °C until the enzyme assays. The Bradford (1976) method was performed to determine the protein concentrations of algal cell extracts, using bovine serum albumin (BSA) as a standard. The modified method of Beyer and Fridovich (1987) was performed to determine the SOD (EC 1.15.1.1.) activity, while the Sgherri et al. (1994) method was modified to determine the GR (EC 1.6.4.2) activity and the modified method of Wang et al. (1991) was used to determine the APX (EC 1.11.1.11) activity, by estimating the decreasing rate of ascorbate oxidation at 290 nm. Detailed information about methods was given in Kılıç et al. (2019), Günsel et al. (2018) and, Önem et al. (2018).

Nonenzymatic parameters

The modified method of Heath and Packer was performed to determine the MDA content. For determination of the H_2O_2 content, 0.5 mL of supernatant was mixed with 1 mL of 1 M KI and 0.5 mL of 0.1 M Tris–HCl (pH 7.6) solutions. After 90 min, the absorbance of the samples was measured at 390 nm according to explained in Tunca et al., (2020). The method of Weimberg (1987) was followed for the determination of proline content. Detailed information about methods was given in Tunca et al. (2020).

Statistical analysis

The one-way ANOVA was used to analyze the differences between the control and treated samples, according to LSD. The confidence interval was selected as 95%. Three biological replicate cultures were used for each treatment. The mean values and standard errors (SE) of each application were given in the Figures.

RESULTS

Biomass and chlorophyll-a content

OD750 measurements and chlorophyll-*a* content of *C. vulgaris* and *A. platensis* cultures in Cambio application are given in Figures 1 and 2, respectively. In *A. platensis* cultures, the application caused a significant increase in biomass accumulation in a dose-dependent manner during the first 2 days ($p < 0.05$). However, at the end of 4th day, the continuous decrease was observed in biomass accumulation in a dose-dependent manner ($p < 0.05$). Generally, significant decrease was seen in chlorophyll-*a* content of *A. platensis* cells in a dose-dependent manner at the end of 2nd day ($p < 0.05$). In *C. vulgaris* cultures, Cambio added to the culture medium for 7 days caused a significant increase in biomass accumulation (OD750 absorbance) in a dose-dependent manner at the beginning of first day ($p < 0.05$). However, during progressive days, there was a significant increase in the amount of chlorophyll-*a* in a dose-dependent manner after the 4th day ($p < 0.05$).

Antioxidant enzyme activities

The activity of SOD significantly increased at 20 and 50 µg mL^{-1} concentrations in *A. platensis* cultures, while it did not change significantly at all Cambio concentrations in *C. vulgaris* cultures ($p < 0.05$) (Figure 3). SOD, APX, and GR activities of *C. vulgaris* cultures did not show significantly changes compared to control (Figures 4a and 5a). APX activity of *A. platensis* significant decreased at 50 µg mL^{-1} concentration ($p < 0.05$) (Fig. 4b), while GR activity significantly increased at 20 µg mL^{-1} concentration ($p < 0.05$) (Fig. 5b).

Nonenzymatic parameters

The MDA amounts of *A. platensis* and *C. vulgaris*

cultures exposed to different Cambio concentrations did not show significant changes compared to the control ($p > 0.05$) (Figures 6 and 7). H_2O_2 amount did not alter in either organism. On the other hand, the proline content displayed increases at 20, 30, 40, and 50 µg mL^{-1} concentrations in *A. platensis* application but it did not change in *C. vulgaris* at all Cambio concentrations compared to the control ($p< 0.05$) (Figure 8).

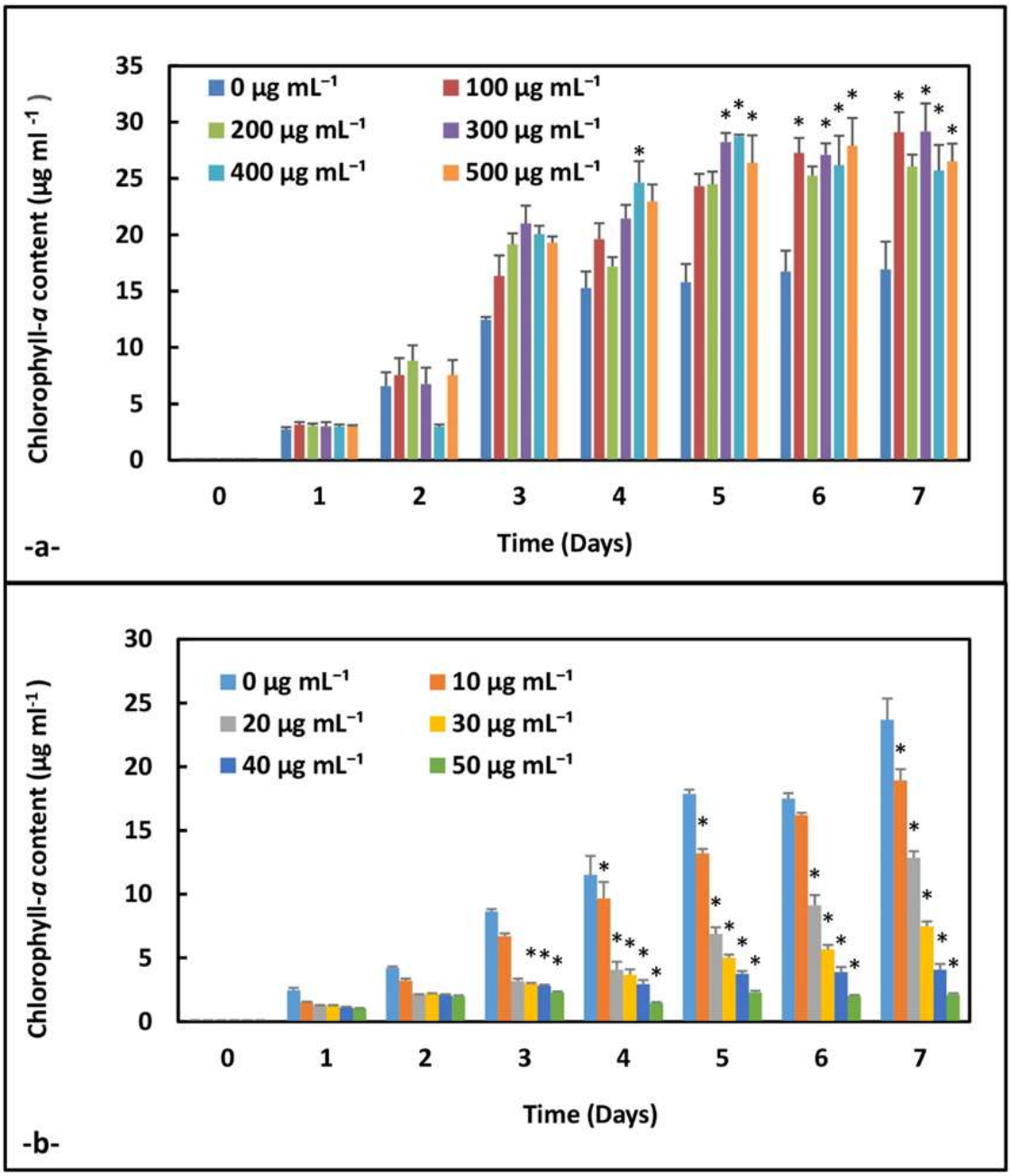

Figure 1. Alterations of chlorophyll-*a* content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment for 7 days. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 1. *(a) C. vulgaris ve (b) A. platensis alglerinde 7 gün boyunca Cambio uygulaması ile klorofil-a içeriğindeki değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

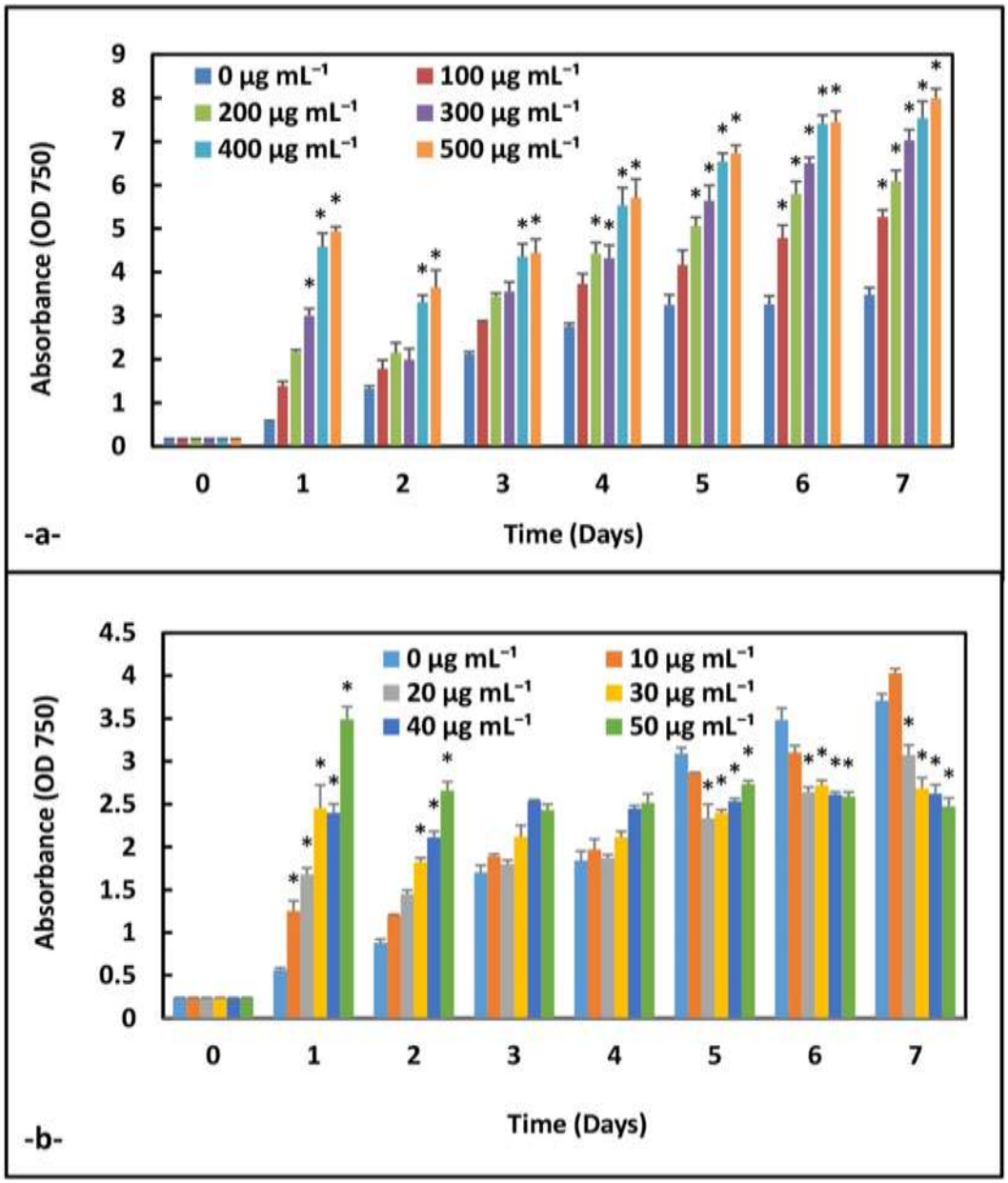

Figure 2. Alterations of OD 750 absorbances in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment for 7 days. The asterisks show statistical differences at a 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 2. *(a) C. vulgaris ve (b) A. platensis alglerinde 7 gün boyunca Cambio uygulaması ile büyüme eğrilerindeki değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

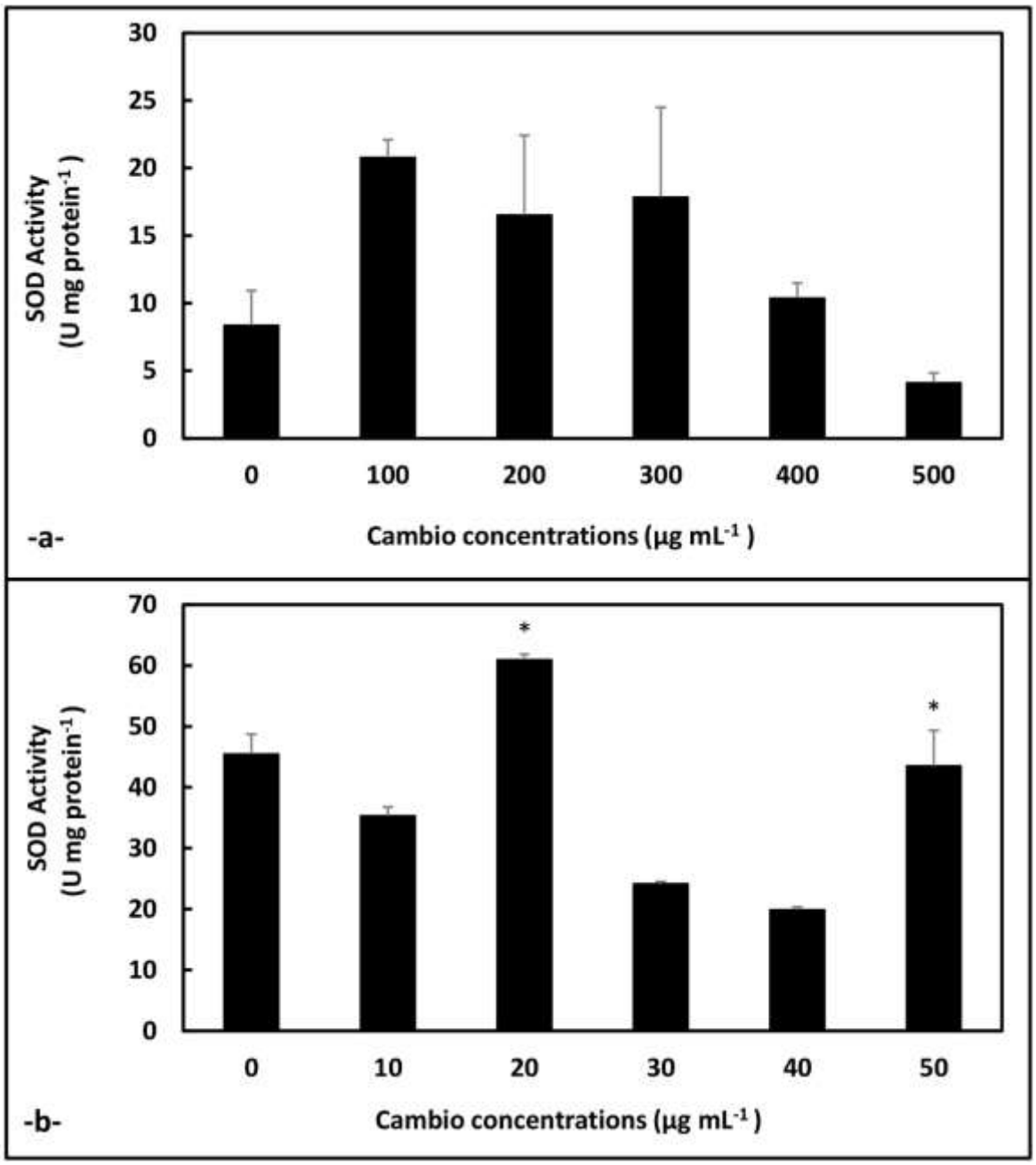

Figure 3. Alterations of SOD activities of (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at a 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 3. *(a) C. vulgaris ve (b) A. platensis'in Cambio uygulamasıyla SOD aktivitelerinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

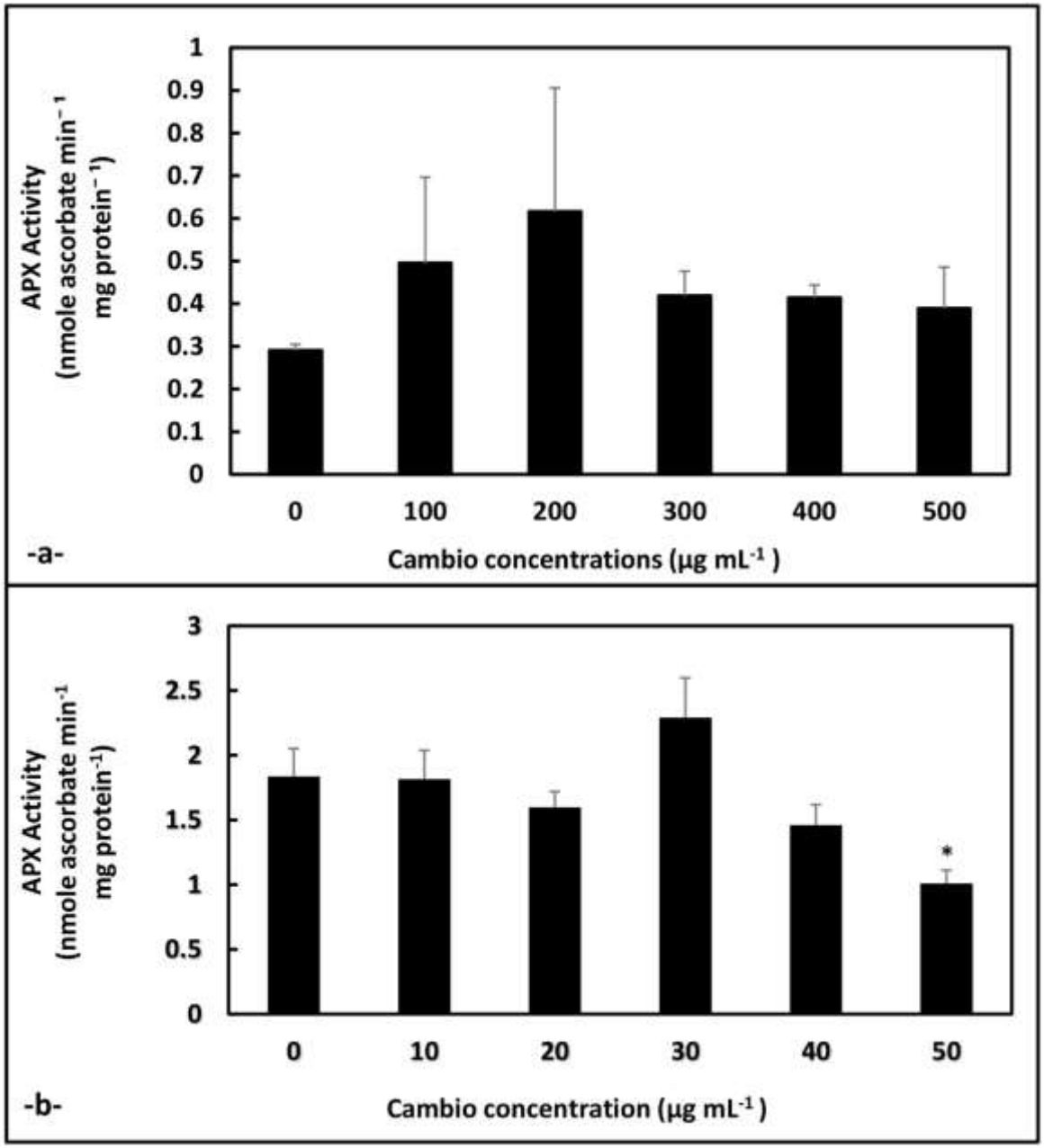

Figure 4. Alterations of APX activities of (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 4. *(a) C. vulgaris ve (b) A. platensis'in APX aktivitelerinde Cambio uygulaması ile meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak göstermektedir.*

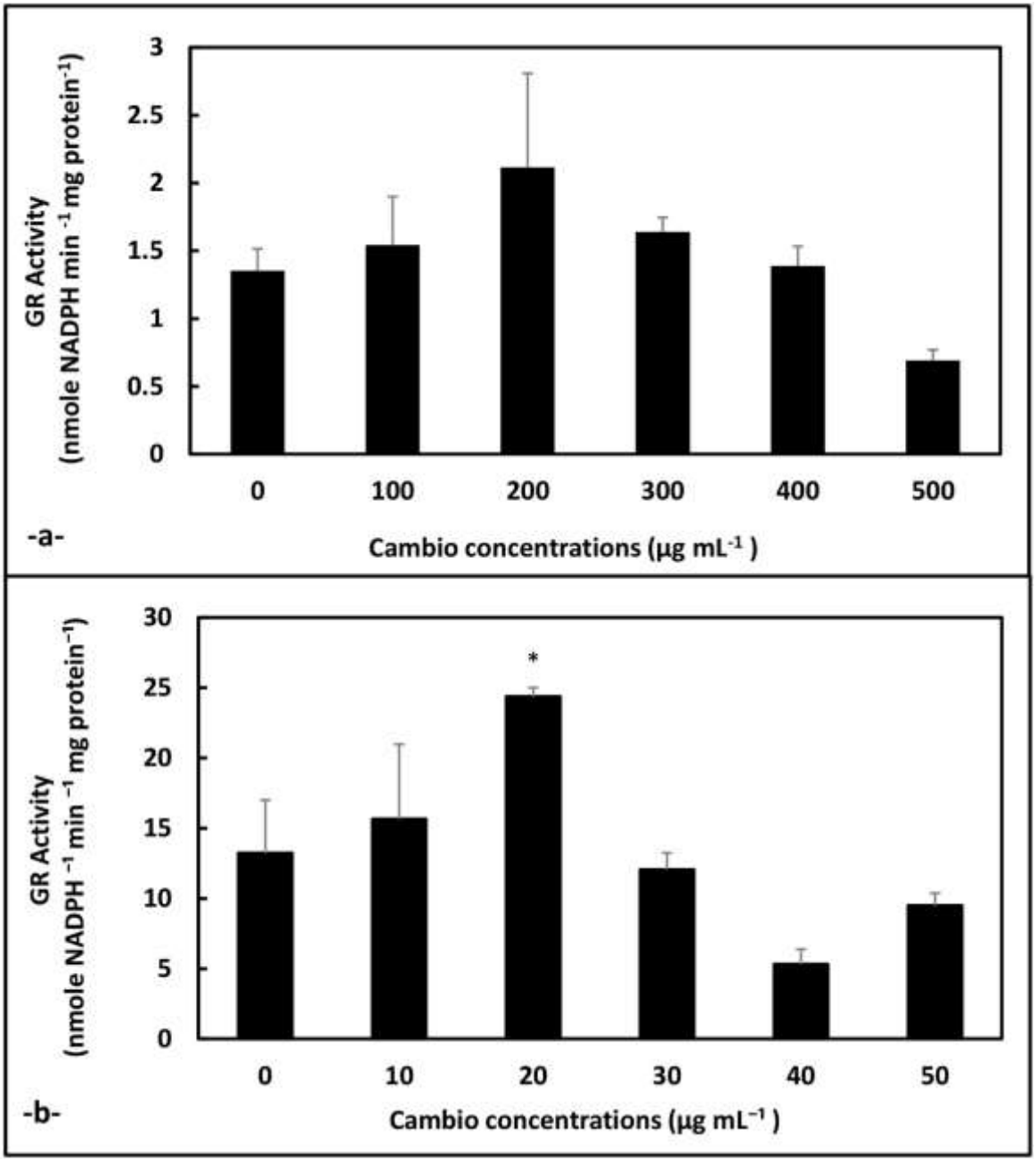

Figure 5. Alteration of GR activities of (a) *C. vulgaris* and (b) *A. platensis* Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 5. *(a) C. vulgaris ve (b) A. platensis Cambio uygulaması ile GR aktivitelerinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

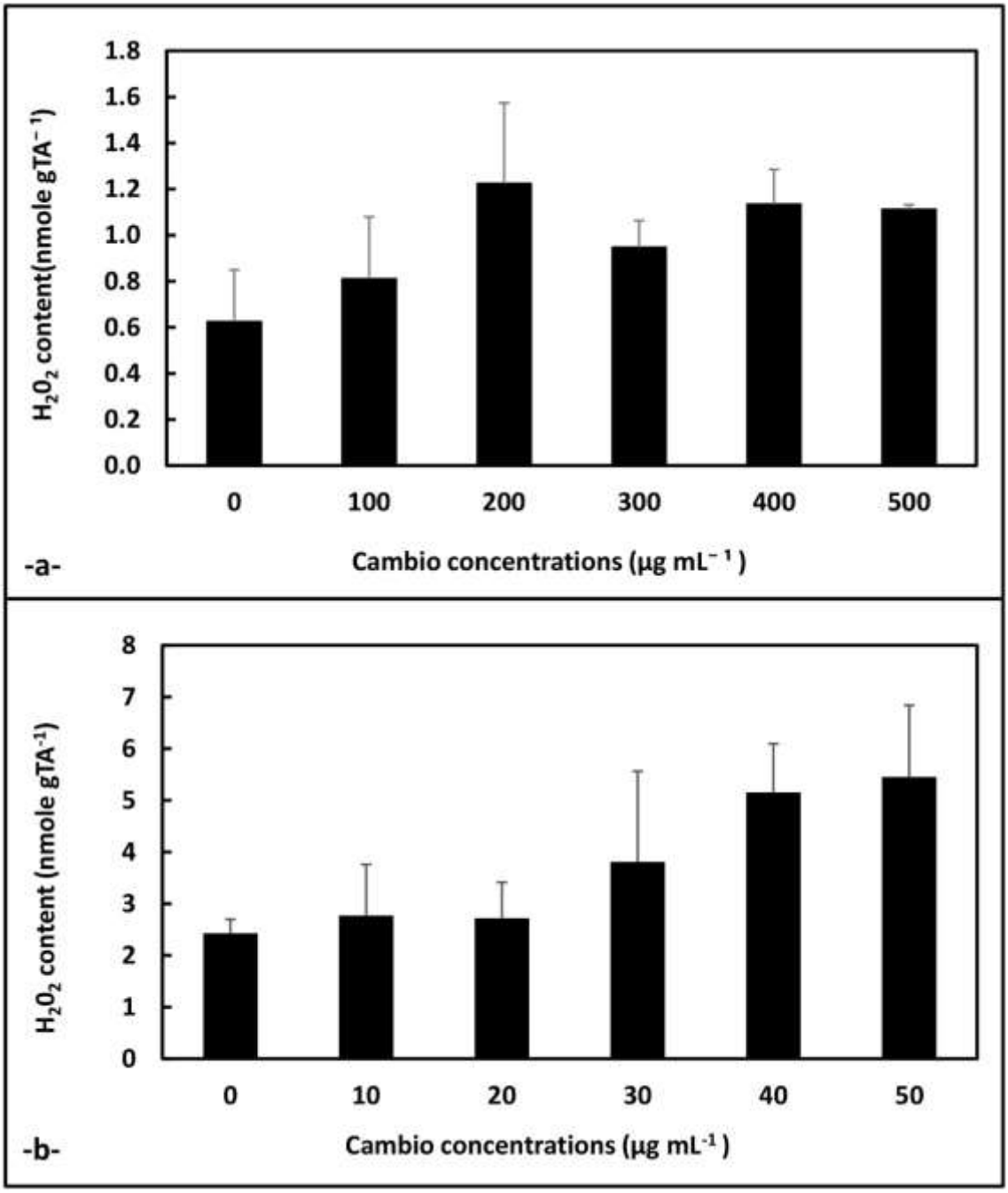

Figure 6. Alterations of H_2O_2 content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 6. *Cambio uygulamasıyla (a) C. vulgaris ve (b) A. platensis alglerindeki H_2O_2 içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

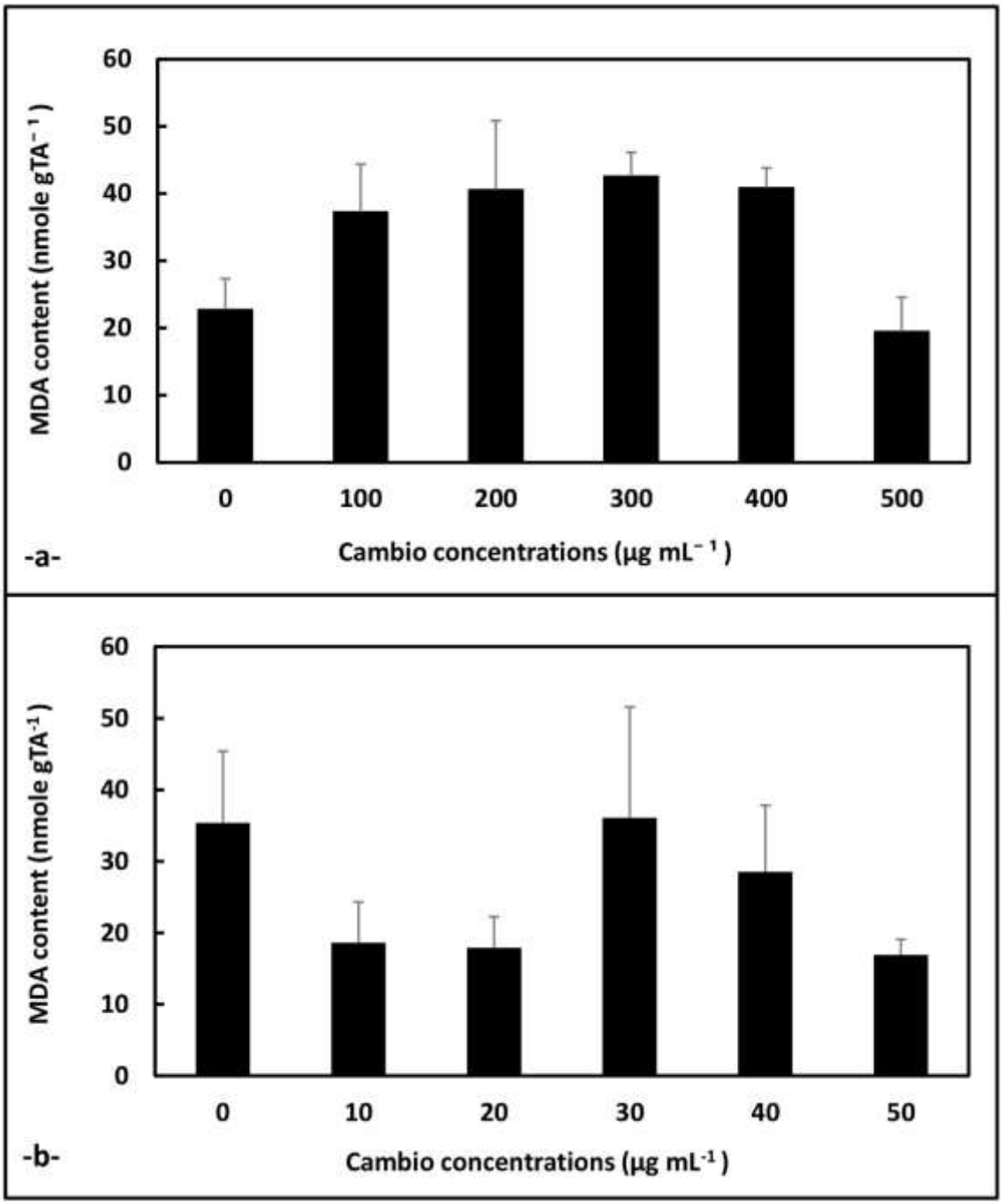

Figure 7. Alterations of MDA content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 7. *(a) C. vulgaris ve (b) A. platensis'te Cambio işlemi ile MDA içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

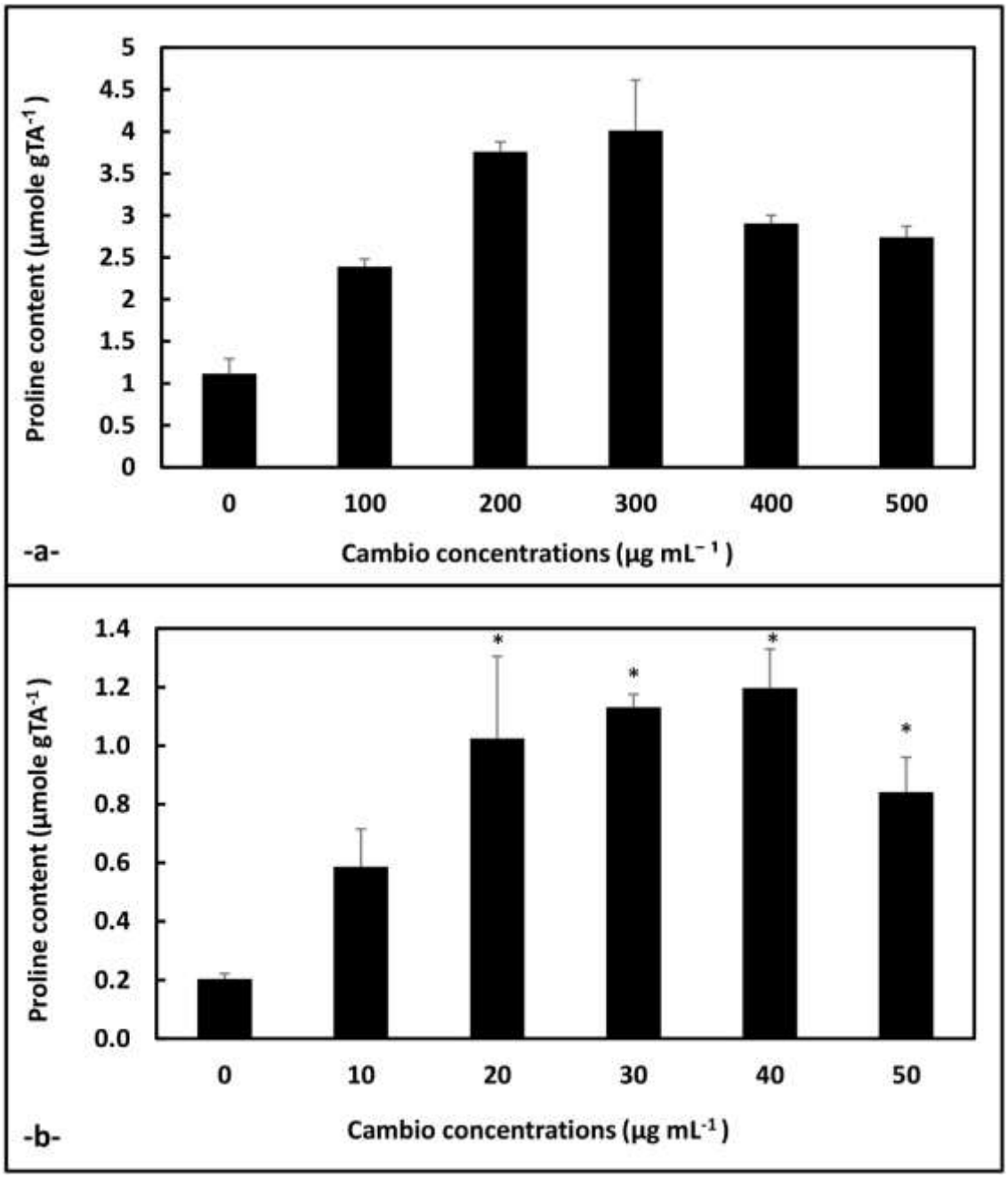

Figure 8. Alterations of Proline content in (a) *C. vulgaris* and (b) *A. platensis* Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 8. *(a) C. vulgaris ve (b) A. platensis Cambio tedavisinde Prolin içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

DISCUSSION

Until environmental toxicology studies scientifically contradict, Cambio has been considered a herbicide that can be used in agriculture (PMRA-ARLA, 1996; EPA, 2004). In the literature, there is no study on the effect of this herbicide on aquatic system organisms. Therefore, the effect of Cambio on two different algae (one is eukaryotic and the other is prokaryotic) was investigated in this study. The effective concentrations of Cambio for *C. vulgaris* were around 10 times higher than for *A. platensis*. It is found that higher concentrations (> 20 µg mL^{-1}) of Cambio have growth inhibitory effects on *A. platensis* cyanobacteria according to OD750 nm absorbance and chlorophyll-a content. These different effect of two application may arise from the detoxification capability of the organisms having different cell types (Lynch and Marinov, 2018).

Bozic et al. (2016) observed that chlorophyll production increased due to herbicide stress when they used

nicosulfuron in sunflower breeding. They have attributed this to the inhibition of acetolactate synthase (ALS) and different defense responses of plants to nicosulfuron. Wang et al. (2022) found that nicosulfuron led to the disruption in the function of PSII in sugar beet leaf. It is obvious that nicosulfuron caused to the decrease in photosynthetic parameters in lower by significant photoinhibition. Leboulanger et al., (2001) have examined the effect of Atrazine and Nicosulfuron herbicides on the growth rate of *Pseudokirchneriella subcapitata*, *C. vulgaris*, *Navicula accomoda*, and *Oscillatoria limnetica* algae. They have observed that EC50s for Atrazine arranged between 40 and 100 µg L^{-1}, whereas overall, nicosulfuron inhibited only the growth of *O. limnetica.* Similarly, Seguin et al. (2001) have reported that the EC50 of Atrazine range between 4 and 400 µg L^{-1} concentrations on different microalgae and nicosulfuron have been found less toxic atrazine. Nicosulfuron have been grouped in sulfonylurea herbicides, and in some studies, these herbicide group has been found less toxic to algae (Soares et al., 2022). Cambio is also a sulfonylurea herbicide, however, its toxicity was very effective on *A. platensis.* On the other hand, it stimulated the growth of *C. vulgaris* in the study. This can be attributed to either the perception of the herbicide by *C. vulgaris* as a stress factor or the use of the herbicide as a substrate. Nyström et al., (1999) investigated that the sulfonylurea sensitivity displayed differences according to algae species. Since these pesticides are capable of blocking the synthesis of essential amino acids (Seguin et al., 2001; Ma et al., 2002), Neilson and Larsson (1980) have suggested that the sulfonylurea sensitivity in the algae arises from the different properties of the incorporation of amino acids.

SOD enzyme activity in *A. plantensis* cultures firstly showed a tendency to increase at 20 and 50 µg mL^{-1} concentrations but it did not change at 30 and 40 µg mL^{-1} concentrations. It may have been occurred due to running out of the cellular concentrations of the enzyme at these concentrations but it may have triggered the gene expression of the enzyme at 50 µg mL^{-1} concentration. Qian et al. (2008) have exposed different concentrations of glufosinate to *C. vulgaris* cultures and they have observed a similar increase in SOD activity. They explained this situation that glufosinate produced superoxide radicals ($\cdot O^{2-}$) in the cells. To explain the enhancement of SOD activity, it can be suggested that the herbicide may be effect directly the SOD gene or, it may increase the $\cdot O_2$ radicals in the medium (Wang and Ki, 2020). Moreover, Cypermethrin insecticide has been tested on *Scenedesmus obliquus* algae, and it has been found that this insecticide has enhancing effect at low concentrations on SOD activity (Wang et al., 2012). This sensitivity could be affected by the blocking of a specific enzyme which plays a role in the production essential amino acids. In this way, the production of chlorophyll may decrease. And also, SOD activity reductions may arise from a deficiency of photosynthetic metabolism (Önem et al., 2018). The activity of SOD significantly decreased at moderate concentrations and Cambio caused decreases in chlorophyll-*a* content of *A. platensis*.

In *A. platensis* cultures exposed to Cambio concentrations, only 20 µg mL^{-1} showed a significant increase in GR activity compared to control. Bajguz (2010) observed that when heavy metals such as cadmium, copper, and lead were applied to *C. vulgaris*, GR activity increased. Geoffroy et al. (2002) suggested that when they applied the different concentrations of diuron on *S. obliquus*, they obtained poor GR stimulation results. The antioxidant defense system and also production of ROS were decreased due to the phenylurea (diuron).

In the study, it was observed that *A. platensis* cultures exposed to different concentrations of Cambio did not change the MDA and H_2O_2 amounts compared the control. Lipid peroxidation end- product known as MDA, which is the indicator of oxidative stress in the cells (Freire et al. 2023). H_2O_2 is a weak reducing compound and causes the formation of hydroxyl radical, and thus cell damage (lipid peroxidation) (Özcan et al., 2015). The absence of significant changes in the amount of H_2O_2 in the cell suggests that it may be due to the effectiveness of enzymes that consume H_2O_2 content, such as the APX (Mallick and Mohn, 2000). In the study, SOD and APX enzyme activities conduct to the H_2O_2 and MDA levels at the same concentration. Likewise, the MDA content was balanced with protective effect of the proline content. In other words, these antioxidant defense systems were related with the levels of oxidant biomarkers. The free proline amount of *A. platensis* cultures displayed increases between 20-50 µg mL^{-1} concentrations with the Cambio application. It has been reported that ROS, which occurs due to the toxicity of endosulfan during photosynthesis, causes proline accumulation due to membrane peroxidation and oxidation problems (Kumar et al., 2008). In addition, studies with *Spirulina* and *Anabaena* species suggest that proline, which has a metal chelating feature due to metal ion stress, increases with metal toxicity and consequently, it increases the amount of protein in the cells (Sultan & Fatma 1999; Kumar et al., 2004; Choudhary et al., 2007). It is known proline prevents the formation of ROS in stress conditions (Phetchuay et al., 2019), and works as a function and structure stabilizer by interacting with macromolecules such as protein and cell membrane as an OH-ion trap (Rezayian et al. 2019).

The activities of SOD, APX, GR enzymes, and the amount of MDA, H_2O_2, and proline did not show a significant change in *C. vulgaris*. In the study on *C.*

vulgaris, the results indicated that Cambio did not cause oxidative stress to increase at all concentrations. Moreover, it was seen that this herbicide promotes growth in this algae, and thus lipid peroxidation and oxidative damage prevents.

CONCLUSION

The study indicated that the effects of Cambio on *A. platensis* are more destructive than *C. vulgaris.* This situation can be explained by the different cell structures of these organisms. Since that the advanced detoxification mechanisms of *C. vulgaris* can tolerate higher levels than the concentrations in the *A. platensis* application. Moreover, it should be noted that one freshwater ecosystem is not exposed to a uniform herbicide/pesticide, and any pesticide entering the system can also degrade and forms different products. Therefore, different combinations of pesticides on microorganisms should be studied (Delorenzo et al., 2001). Because, in sites polluted with solvents, two or more organic compounds are often found together in the environment (Wu et al., 2014). The use of these small single-celled organisms as quick and reliable indicators in the detection of environmental contamination compared to the other plants will be a pioneer for further studies.

Declaration of Contribution

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.

REFERENCES

Aiba, S. & Ogawa, T. (1977). Assessment of growth yield of a blue-green alga, *Spirulina platensis,* in axenic and continuous culture. *Journal of General Microbiology, 102*, 179-182.

Anjum, N.A., Umar, S. & Chan, M.T. (2010). Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Springer science- Bussiness Media. 91-113.

Aysel, V. (2005). Checklist of the Freshwater Algae of Turkey. *Journal of the Black Sea/Mediterranean Environment*, *11*, 1-124.

Bajguz, A. (2010). An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in *Chlorella vulgaris* cultures under heavy metals stress. *Environmental Experimental Botany, 68 (2),* 175-179.

Beyer, W. F. & Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analytical Biochemistry, 161*, 559–566.

Bhatnagar- Mathur, P., Vadez, V. & Sharma, K.K. (2008). Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Reports, 27*, 411–424.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry, 72*, 248-254.

Bozic, D., Vrbnicanin, S., Stojicevic, D. & Pavlovic, D. (2016). Effect of nicosulfuron on the populations of invasive weedy sunflower. 27. Deutsche Arbeitsbesprechung uber Fragen der Unkrautbiologie und -bek.mpfung, 23.-25. February 2016 Braunschweig, Germany.

Cao, Q., Sun, W., Yang, T., Zhu, Z., Jiang, Y., Hu, W., ... & Yang, H. (2022). The toxic effects of polystyrene microplastics on freshwater algae *Chlorella pyrenoidosa* depends on the different size of polystyrene microplastics. *Chemosphere*, *308*, 136135.

Cedergreen, N. & Streibig, J.C. (2005). The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. *Pest Management Science 61(12).* 1152-1160.

Choudhary, M., Jetley, U.K., Khan, M.A., Zutshi, S. & Fatma, T. (2007). Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. *Ecotoxicology and Environmental Safety, 66*, 204–209.

Delorenzo, M.E., Scott, G.I., & Ross, P.E. (2001). Toxicity of pesticides to aquatic microorganisms: A Review *Environmental Toxicology and Chemistry, 20(1),* 84–98.

EPA. (2004) – US Environmental Protection Agency. Report of the Food Quality Protection Act (FQPA) Tolerance Reassessment Progress and Risk Management Decision (TRED) for Nicosulfuron.

Freire, V. A. F., de Melo, A. D., de Lima Santos, H., & de Barros Pinheiro, M. (2023). Evaluation of oxidative stress markers in subtypes of preeclampsia: A systematic review and meta-analysis. *Placenta*, *132*, 55-62.

Geoffroy, L., Teisseire, H., Couderchet, M. & Vernet. G. (2002). Effect of oxyfluorfen and diuron alone and in mixture on antioxidative enzymes of *Scenedesmus obliquus*. *Pesticide Biochemistry and Physiology, 72,* 178–185.

Gökpınar, S., Koray, T., Akcicek, E., Göksan, T. & Durmaz, Y. (2006). Algal antioksidanlar. *Ege Üniversitesi Su Ürünleri Dergisi, 23,* 85-89.

Hasannuzzaman, M., Hossain, M.A., Da Silva, J.A.T. & Fujita, M. (2012). Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor. Crop Stress and its Management: Perspectives ans Strategies. Springer.

Heath, R.L. & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry*

*and Biophysics, 1086 (125),*189-198.

Ighodaro, O.A. & Akinloye, O.A. (2017). First-line defense antioxidants-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. *Alexandria Journal of Medicine, 54 (4),* 287-293

Kookal, S. K., Nawkarkar, P., Gaur, N. A., & Kumar, S. (2023). Bioremediation of ethanol wash by microalgae and generation of bioenergy feedstock. *Journal of Applied Phycology*, *35(1)*, 183-194.

Kumar, S., Jetley, U.K. & Fatma, T. (2004). Tolerance of *Spirulina platensis*- S5 and Anabaena sp., to endosulfan an organochlorine pesticide. *Annual Plant Physiology,* 18*(2),* 103-107.

Kumar, S., Habib, K. & Fatma, T. (2008). Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. *Science of The Total Environment, 403 (1–3),* 130-138.

Lynch, M., & Marinov, G. (2018). Correction: membranes, energetics, and evolution across the prokaryote-eukaryote divide. *Evolutionary Biology,*1-30.

Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S. & Huang, Y. (2002). Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. *Ecotoxicology and Environmental Safety, 51*, 128-132.

Mackinney, Q. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry, 140,* 315-322.

Mittler, R., Zandalinas, S. I., Fichman, Y., & Van Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. *Nature Reviews Molecular Cell Biology*, *23*(10), 663-679.

Mallick, N. & Mohn, F. H. (2000). Reactive oxygen species: response of algal cells. *Journal of Plant Physiology, 157*, 183-193.

Niedobová, J., Ouředníčková, J., Michalko, R., & Skalský, M. (2022). The toxicity of the glyphosate herbicide for Pardosa spiders' predatory activity depends on the formulation of the glyphosate product. *Environmental Chemistry Letters*, *20*(2), 983-990.

Neilson, A. H. & Larson, T. (1980). The utilization of organic nitrogen for growth of algae: physiological aspects. *Physiology Plantarum, 48*, 542–553.

Nyström, B., Björbsäter, B. & Blanck, H. (1999). Effects of sulfonylurea herbicides on non-target aquatic micro-organisms. Growth inhibition of micro-algae and short-term inhibition of adenine and thymidine incorporation in periphyton communities. *Aquatic Toxicology, 47*, 9–22.

Özyurt, M., Kopar, H., Özyurt, S., Demirhan, İ. & Belge Kurutaş, E. (2021). Menengiç, Işgın ve Çiriş Otu'nda Antioksidan Aktivitenin Araştırılması. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 24(4), 733-737. DOI: 10.18016/ ksutarimdoga.vi.819974*

Phetchuay, P., Peerakietkhajorn, S., Duangpan, S., & Buapet, P. (2019). Toxicity effects of copper and zinc on the photosynthetic efficiency and oxidative stress-related parameters of the green alga *Chlorella vulgaris* Beijerinck. *Journal of Fisheries and Environment*, *43*(2), 14-26.

PMRA-ARLA. (1996). Decision document: nicosulfuron. Canada: Pesticide Management and Regulation Agency. (42 pp.) http://www.hc-sc.gc.ca/ pmra-arla/e9601e.pdf. (Accessed: 23.20.2021)

Relyea, R. A. (2005). The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities. *Ecological Applications, 15 (2),* 618-627.

Rezayian, M., Niknam, V., & Ebrahimzadeh, H. (2019). Oxidative damage and antioxidative system in algae. *Toxicology reports*, *6*, 1309-1313.

Rey-Caballero, J., Menéndez, J., Giné-Bordonaba, J., Salas, M., Alcántara, R., & Torra, J. (2016). Unravelling the resistance mechanisms to 2, 4-D (2, 4-dichlorophenoxyacetic acid) in corn poppy (*Papaver rhoeas*). *Pesticide Biochemistry and Physiology*, *133*, 67-72.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology, 111*, 1-61.

Qian, H., Chen, W., Sheng, G. D., Xu, X., Liu, W. & Fu, Z. (2008). Effects of glufosinate on antioxidant enzymes, subcellular structure, and gene expression in the unicellular green alga *Chlorella vulgaris*. *Aquatic Toxicology, 88(4),* 301-307.

Saradhi, A. & Saradhi, P. P. (1991). Proline accumulation under heavy metal stress. *Journal of Plant Physiology, 138*, 554-558.

Schmitt-Jansen, M. & Altenburger, R. (2005). Toxic effects of isoproturon on periphyton communities – a microcosm study. *Estuarine, Coastal and Shelf Science, 62*, 539–545.

Schuler, L. J. & Gary, M. (2008). Aquatic Risk Assessment of Herbicides in Freshwater Ecosystems of South Florida. *Archives of Environmental Contamination Toxicology 54*, 571–583.

Seguin, F., Leboulanger, C., Rimet, F., Druart, J. C. & Bérard, A. (2001). Effects of atrazine and nicosulfuron on phytoplankton in systems of increasing complexity. *Archives Environmental Contamination Toxicology 40*, 198–208.

Serim, A. T., Koca, E., Güzel, N. P. & Asav, Ü. (2017). Vejetatif filtre şeritlerinde kullanılabilecek bazı dar yapraklı bitkilerin Rimnosulfuron ve Nicosulfuron' a toleransı. *Turkish Journal of Weed Science, 20* (1), 1-9.

Sgherri. C. L. M., Loggini, B., Puliga, S. & Navari-Izzo, F. (1994) Antioxidant system in Sporobolus stapfianus: changes in response to desiccation and rehydration.' *Phytochemistry, 35*, 561–565.

Sili, C., Torzillo, G., & Vonshak, A. (2012). Arthrospira (Spirulina). In Ecology of Cyanobacteria II (pp. 677-705) Dordrecht, Springer.

Soares, C., Fernandes, B., Paiva, C., Nogueira, V., Cachada, A., Fidalgo, F., & Pereira, R. (2023). Ecotoxicological relevance of glyphosate and flazasulfuron to soil habitat and retention functions–Single vs combined exposures. *Journal of Hazardous Materials*, *442*, 130128.

Sultan, S. & Fatma, T. (1999). Phytotoxicity of heavy metals on *Spirulina platensis*. *Phykos, 38(1-2)*, 87-92.

Tang, J., Liang, Q., Li, C., Huang, X., Xian, X., Li, J., ... & Zhang, R. (2022). Application of Marine Algae in Water Pollution Control. In *IOP Conference Series: Earth and Environmental Science* (Vol. 966, No. 1, p. 012001). IOP Publishing.

Tamil Selvan, S., Dakshinamoorthi, B. M., Chandrasekaran, R., Muthusamy, S., Ramamurthy, D., & Balasundaram, S. (2023). Integrating eco-technological approach for textile dye effluent treatment and carbon dioxide capturing from unicellular microalga Chlorella vulgaris RDS03: a synergistic method. International Journal of Phytoremediation, 25(4), 466-482.

Tunca, H., Doğru, A., Köçkar, F., Önem, B. & Sevindik, T. O. (2020). Evaluation of Azadirachtin on *Arthrospira plantensis* Gomont growth parameters and antioxidant enzymes. *Annales de Limnologie-International Journal of Limnology, 56*, 1-8.

Tunca, H., Özpınar, S. Ç., Tekbaba, A. G., Sevindik, T. O., Günsel, A., Bilgiçli, A. T., & Yaraşır, M. N. (2022). Algaecidal and oxidative effects of metal-free phthalocyanine beta tetra-substituted with sodium 2-mercaptoethanesulfonate. *Turkish Journal of Chemistry*, *46* (2), 367-377.

Valentine, J. S., Wertz, D. L., Ltons, T. J., Liou, L. L., Goto, J. J. & Gralla, E. B. (1998). The dark side of dioxygen biochemistry. *Current Opinion In Chemical Biology, 2*, 253-262.

Vervliet-Scheebaum, M., Alain Straus, A., Horst Tremp, H., Hamer, M., Maund, S. J., Edgar Wagner E & Schulz R (2010). A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental Pollution 158*: 615–623.

Walsh, G. E. (1978). Toxic Effects of Pollutants on Plankton. *Principles of Ecotoxicology*. 257-274, USA.

Wang, H., & Ki, J. S. (2020). Molecular identification, differential expression and protective roles of iron/manganese superoxide dismutases in the green algae Closterium ehrenbergii against metal stress. *European Journal of Protistology*, *74*, 125689.

Wang, L., Riaz, M., Song, B., Song, X., Huang, W., Bai, X., & Zhao, X. (2022). Study on phytotoxicity evaluation and physiological properties of nicosulfuron on sugar beet (Beta vulgaris L.). *Frontiers in Plant Science*, *13*, 998867.

Wang, S. Y., Jiao, H., Faust, M. (1991). *Changes in ascorbate, glutathione, and related enzyme activity during thidiazuron-induced bud break of apple.* Plant Physiology *82*: 231-236.

Wang, Z. H., Xie, J., Jiang, S., Shi, J., Liu, Y. & Gong, W. (2012). Effects of commercial cypermethrin on the growth of *Scenedesmus obliquus* and its physiochemical responses. *China Environmental Science 32 (4)*: 659-665.

Weimberg, R. (1987). Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. *Physiologia Plantarum 70*: 381-388.

Wu, S., Zhang, H. & Qiu, L. (2014). Toxicological Responses of *Chlorella vulgaris* to Dichloromethane and Dichloroethane. *Environmental Engineering Science 31*: 1.

Wu, Z. X., Xu, N. W., Yang, M., Li, X. L., Han, J. L., Lin, X. H., ... & Wang, J. (2022). Responses of photosynthesis, antioxidant enzymes, and related gene expression to nicosulfuron stress in sweet maize (Zea mays L.). *Environmental Science and Pollution Research*, *29*(25), 37248-37265.

Xu, L., Zhao, Z., Yan, Z., Zhou, G., Zhang, W., Wang, Y., & Li, X. (2022). Defense pathways of Chlamydomonas reinhardtii under silver nanoparticle stress: Extracellular biosorption, internalization and antioxidant genes. *Chemosphere*, *291*, 132764.

Yang, Y., Fan, X., Zhang, J., Qiao, S., Wang, X., Zhang, X., ... & Hou, J. (2022). A critical review on the interaction of iron-based nanoparticles with blue-green algae and their metabolites: From mechanisms to applications. *Algal Research*, *64*, 102670.