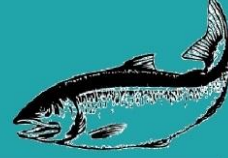




SU ÜRÜNLERİ FAKÜLTESİ
Faculty of Fisheries



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**DETERMINATION OF ACCUMULATION, ELIMINATION AMOUNTS AND SOME BIOMARKER
RESPONSES OF CHLORPYRIFOS PESTICIDE IN NAVICULA CRYPTOCEPHALA VAR. VENETA**

**NAVİCULA CRYPTOCEPHALA'DA KLORPİRİFOS PESTİSİTİNİN BİRİKME, ELİMİNASYON
MİKTARLARI VE BAZI BİYOBELİRTEÇ YANITLARININ BELİRLENMESİ**

ABSTRACT

Pesticides such as chlorpyrifos (CPF), which are used to combat pests in agricultural areas, mix with the aquatic environment in various ways and pose a danger to the aquatic environment. In this study, chlorpyrifos was used in *Navicula cryptocephala var. veneta* it was aimed to examine the bioaccumulation, elimination amounts and some biomarker responses. EC₅₀ values were determined as 0.19 mg/L and acute toxicity values were realized at 1/8, 1/4 and 1/2 of EC₅₀ values. *N. cryptocephala var. veneta* were exposed to CPF for 24, 48, 72, 96 and 120 days (elimination period). Accumulation amounts from the samples taken at 24 and 96 hours were measured using Atomic Absorption Spectrophotometry (AAS) mass spectrometry. With the supernatants obtained, lipid peroxidation (TBARS) and reduced glutathione (GSH) levels, superoxide dismutase (SOD) enzyme activity, glutathione peroxidase (GPx) enzyme activity, catalase (CAT) enzyme activity were determined with an ELISA microplate reader. According to research data, it was determined that the bioaccumulation amount of the CPF active ingredient in microalgae increased as the application concentration and duration increased. It was determined that the biomarker parameters showed statistically significant changes in the control and elimination groups compared to the application groups.

Keywords: *Navicula cryptocephala var. veneta*, chlorpyrifos, bioaccumulation, biomarker

Öz

Tarımsal alanlarda zararlılarla mücadele için kullanılan klorpirifos (CPF) gibi pestisitler, çeşitli yollarla sucul çevre ile karışarak sucul çevreye tehlike oluşturmaktadır. Bu çalışmada, *Navicula cryptocephala var. veneta*'da klorpirifos kullanılarak biyobirikim, eliminasyon miktarları ve bazı biyomarker tepkileri incelenmiştir. EC₅₀ değerleri 0,19 mg/L olarak belirlenmiş ve akut toksisite değerleri EC₅₀ değerlerinin 1/8, 1/4 ve 1/2'sinde gerçekleştirilmiştir. *Navicula cryptocephala var. veneta* bireyleri 24, 48, 72, 96 ve 120 gün (eliminasyon süresi) boyunca CPF'ye maruz bırakılmıştır. 24 ve 96 saatte alınan numunelerden birikim miktarları Atomik Absorpsiyon Spektrofotometrisi (AAS) kütle spektrometrisi kullanılarak ölçülmüştür. Elde edilen süpernatantlarla, lipid peroksidasyon (TBARS) ve indirgenmiş glutatyon (GSH) seviyeleri, süperoksit dismutaz (SOD) enzim aktivitesi, glutatyon peroksidaz (GPx) enzim aktivitesi, katalaz (CAT) enzim aktivitesi ELISA mikroparka okuyucu ile belirlendi. Araştırma verilerine göre, mikroalglerde CPF aktif maddesinin biyobirikim miktarının, uygulama konsantrasyonu ve süresi arttıkça arttığı belirlenmiştir. Biyomarker parametrelerinin, uygulama gruplarına kıyasla kontrol ve eliminasyon gruplarında istatistiksel olarak anlamlı değişiklikler gösterdiği belirlenmiştir.

Anahtar Kelimeler: *Navicula cryptocephala var. veneta*, klorpirifos, biyobirikim, biyomarker

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Introduction

Approximately one-fifth of the world's population does not have access to clean water, and two-fifths suffer the consequences of unacceptable sanitation due to contamination of water resources with various synthetic and geogenic compounds leached from agricultural, industrial and domestic activities (He et al., 2011). Therefore, it is clear that chemical pollution of freshwater reserves is one of the most important ecological concerns facing humanity in almost every part of the world. The agricultural sector is one of the most common sources of water pollution, with 140 million tonnes of fertilizer and several million tonnes of pesticides and herbicides applied each year (Schwarzenbach et al., 2006). The chemical stability and wide application of these compounds have resulted in serious pollution of natural waters (Singh, 2009; Fenner et al., 2013).

It has been determined that pesticides, as one of the changes caused by human beings in nature, affect microalgae and other higher trophic organisms in water bodies. Some pesticides have been found to be very harmful to certain types of algae but not to others. Therefore, their presence induces changes in the species composition, production rates and biomass of the algal community. These chemicals can be considered an economical, labor-saving and effective means of pest management (Cooper & Dobson, 2007). Chlorpyrifos (CPF) is a widely used organophosphorus chemical that acts as an insecticide and acaricide. Originally, CPF was used to combat a range of pests in both agricultural and non-agricultural environments and has also been used as the active ingredient in a wide variety of pesticide formulations (Eaton et al., 2008). However, when exposed to this compound in early pregnancy, off-target use has tended to be restricted due to toxicological and epidemiological information suggesting a role for CPF in neurodevelopmental damage, including autism (Eaton et al., 2008; Landrigan, 2010). Irregular and haphazard application of pesticides has caused their frequent occurrence in water resources (Aydın et al., 2022).

Exposure to such pollutants leads to the generation of reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydroxyl radicals ($OH^\cdot$), and hydrogen peroxide (H_2O_2), which have high biological activity and lipids (peroxidation of unsaturated fatty acids in membranes), causing ecotoxicity through oxidative damage to cellular components including proteins (denaturation), DNA

(Gibbons et al., 2014) and carbohydrates (Imlay et al., 1998; Vandana et al., 2001; Olga et al., 2003). Reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are constantly formed in oxygen-consuming organisms. Like many other vertebrates, microalgae try to reduce damage caused by oxidative stress by using the antioxidant defense system; the first line of defense consists of antioxidant molecules such as glutathione (GSH), vitamins C and E, and carotenoids (Alvarez et al., 2005). Another defense mechanism consists of antioxidant enzymes, which include radical scavenging enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), etc. (Valavanidis et al., 2006).

Diatoms (*Bacillariophyta*) are single-celled photosynthetic organisms that make a significant contribution to more than 20% of the primary production of oxygen and organic substances (Tréguer et al., 1995). Diatoms are photosynthetic organisms that contain chlorophyll a (chl a) and also have carotenoid pigments that give them their brown/gold color (Debenest et al., 2013). Diatoms have various defense mechanisms to cope with stress from heavy metals, such as producing phytochelatins, immobilizing extracellular metals, and neutralizing metal ions inside the cell (Branco et al., 2010). Since green algae and diatoms are primary producers at the beginning of freshwater trophic chains, pesticides can disrupt the balance of the entire ecosystem if microalgal communities are affected (Ghosh & Gaur, 1998; Debenest et al., 2008). However, a productive and stable aquatic ecosystem includes a food web consisting of phytoplankton (primary producers), invertebrates (secondary consumers), and vertebrates (top consumers), all of which are extremely important for maintaining aquatic ecological balance.

This study, on the *N. cryptocephala* var. *veneta* microalgae species of CPF insecticide, is aimed at evaluating the accumulation and elimination amounts and the oxidative stress response TBARS and GSH levels and SOD, CAT and GPX activities

Methods

Acute Toxicity (EC_{50})

Microalgal pure cultures purchased from the University of Texas Algal Culture Collection (UTEX) were grown to sufficient volume for experiments in the laboratory of Munzur University Faculty of Fisheries. 15 ml of pure *N. cryptocephala* (5×10^4 cells/ml) in Ag diatom medium (autoclave sterilized) in a growth chamber with $20 \pm 1^\circ C$ and

12:12 h light:dark ($43,24 \mu\text{molm}^{-2}\text{s}^{-1}$) cycle for 250 ml. A starter culture was obtained by propagation in ml conical flasks. *N. cryptocephala* var. *veneta* cultures were harvested at the logarithmic growth stage (mean $2\text{--}3 \times 10^5$ cells/ml).

The EC_{50} value of inhibition in microalgae was based on counting viable cells. Microalgae Inhibition Test was applied for 24, 48, 72 and 96 hours as recommended in OECD (2011). Test environment; Sterile glass tubes with a volume of 15 ml were used. The test setup was created by taking 9 ml of CPF solution and 1 ml of microalgae culture, making the total volume 10 ml. To detect dead/live microalgae cells, 1 ml of microalgae CPF sample taken from each test container after 24, 48, 72 and 96 hours was stained with 0.1 ml of trypan blue dye and incubated for 10 minutes in a dark environment. At the end of the period, the samples were counted under a light microscope using a hemocytometer (Neubauer). Counts were repeated three times for each sample and average values were calculated (Yoon et al., 2007; OECD 2011; Erdem et al., 2014; Özkaleli & Erdem 2017).

Experiment Design

As result of acute (EC_{50}) tests, EC_{50} values of CPF for microalgae were determined. A control group was created with application groups at 3 different concentrations defined below, according to the EC_{50} values of CPF.

Control Group; Microalgae not exposed to any pollutants, **Group 1;** Microalgae exposed to pollutants at 1/8 of the EC_{50} value, **Group 2;** Microalgae exposed to pollutants at 1/4 of the EC_{50} value, **Group 3;** Microalgae exposed to pollutants at 1/2 of the EC_{50} value, application groups have been established.

The created application groups were exposed to CPF pollutant at specified concentrations for 120 hours. In order to determine the accumulation and change of biomarker parameters, 1 liter sample was taken every 24 hours for 96 hours. In order to determine the effect of elimination amount and elimination time on biomarker parameters after the 96th hour samples were taken, the microalgae in the application medium were rapidly precipitated by centrifugation and the ambient water contaminated with CPF was removed. After microalgae samples were washed with pure water, UV-sterilized water was added and the elimination process was waited for 24 hours (120th hour in total) and samples were taken as stated below.

Ambient water (culture media) and microalgae samples were taken for bioaccumulation, labeled for processing and stored at -18°C . After the samples taken for biomarker analyzes were dried on blotting paper, they were placed in Eppendorf tubes with the help of a spatula, labeled and stored in a -80°C ultra-freezer for biochemical analysis. All these operations were carried out simultaneously with 3 replicates.

Determination of CPF Accumulation Amount

15 ml of the microalgae samples obtained were placed in falcon tubes (50 ml) and 15 ml of pure water was added. It was centrifuged at 4500 rpm for 15 minutes and the water accumulated on top was removed and 15 ml of pure water was added again. It was centrifuged at 4500 rpm for 15 minutes and the water formed on top was poured out again and washed twice. 12 ml of methanol chloroform (2:1) and 2 ml of 2% sodium chloride (NaCl) were added to the samples remaining at the bottom of the tube and sanitation (ultrasonic bath) was performed for 1 hour. Filter paper was placed on the volumetric flask and the samples were filtered, and the samples in the flask were evaporated in the evaporator. After evaporation in the evaporator, the remaining samples were dissolved by adding 2 ml of n-hexane. The dissolved samples were taken into ependrop tubes and the accumulation amounts of the samples were measured in the Gas Chromatography (GC) device.

Biomarker Analysis

In the bioassays carried out within the scope of the study, to determine the biochemical responses in the samples taken and preserved at 24, 48, 72, 96 and 120 hours, 0.5 g of sample was weighed and added to it with 1/10 w/v PBS buffer (phosphate buffered saline) (pH 7) was added and mixed and homogenized using a homogenizer. These homogenized samples were centrifuged in a refrigerated centrifuge at 17000 rpm for 15 minutes, and the resulting supernatants were stored in the deep freezer at -86°C until the measurement process was completed. Quantitative data and change amounts of TBARS, GSH, SOD, GPx, CAT biomarker parameters depending on the CPF pesticide in the obtained supernatants were determined with an ELISA microplate reader.

Statistical Analysis

The EC_{50} value from the acute toxicity experiments of CPF pesticide on *N. cryptocephala* var. *veneta* was calculated by probit analysis. SPSS 24.0 (IBM SPSS Corp., Armonk, NY, USA) package program One-Way Anova

(Duncan 0.05) was used to evaluate the data between the designed denial application groups

Results

EC₅₀ Values in *N. cryptocephala* var. *veneta*

EC₅₀ values on *N. cryptocephala* var. *veneta* exposed to the CPF active ingredient are given in Table 1.

Table 1. EC₅₀ values on *N. cryptocephala* var. *veneta* exposed to CPF active ingredient

	EC ₅₀ mg/l
Recurrence 1	0.23
Recurrence 2	0.11
Recurrence 3	0.23
Average value	0.19
Standard deviation	0.07

Accumulation Amounts in *N. cryptocephala* var. *veneta*

Within the scope of the study, bioaccumulation amounts were determined in *N. cryptocephala* organisms exposed to the pesticide pollutant containing the active ingredient CPF for 24, 48, 72, 96 and 120 (elimination) hours. As the application concentration and duration increased, the amount of pesticide bioaccumulation with the active ingredient CPF in microalgae also increased (Figure 1). Decreases in bioaccumulation amounts were recorded in the elimination group.

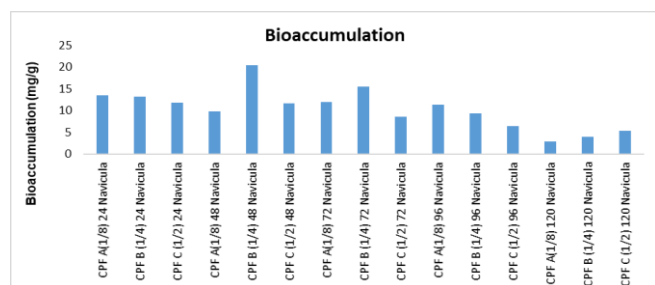


Figure 1. Accumulation amounts of pesticides containing CPF active ingredient and their by-products in *N. cryptocephala* var. *veneta* (mg/g).

Biochemical Responses of CPF on *N. cryptocephala* var. *veneta*

Within the scope of the study, samples were taken every 24 hours from three different concentration groups created in proportion to the control group and EC₅₀ value to determine the biomarker changes of the CPF-caused

pesticide on *N. cryptocephala* var. *veneta* microalgae. Among the biomarker parameters, TBARS level (Figure 2a), GSH level (Figure 2b), SOD activity (Figure 3c), CAT activity (Figure 2d) and GPx activity (Figure 2e) are given.

Although the TBARS level increased in the groups exposed to CPF compared to the control, no statistically significant difference was found ($p>.05$).

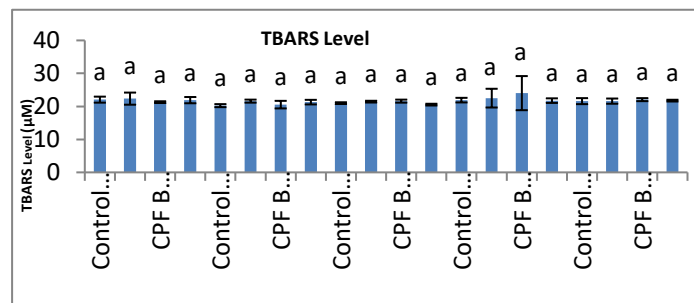


Figure 2a. TBARS level values of CPF pesticide active ingredient on *N. cryptocephala* var. *veneta*. Different letters on the bar indicate statistical differences $p<.05$ according to experimental groups. Values represent the mean.

A statistically significant difference was found in GSH level between the samples in the 96th hour CPF exposed groups and the control group samples ($p<.05$). The difference between the other groups and the control group was not statistically significant ($p>.05$).

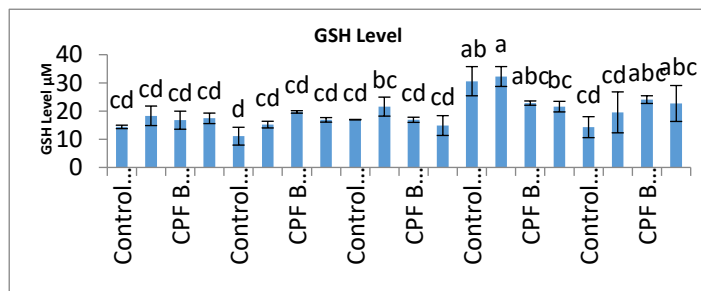


Figure 2b. GSH level values of CPF pesticide active ingredient on *N. cryptocephala* var. *veneta*. Different letters on the bar indicate statistical differences $p<.05$ according to experimental groups. Values represent the mean.

The changes determined in SOD enzyme activity between the application groups exposed to CPF and the control group samples weren't found to be statistically significant ($p>.05$).

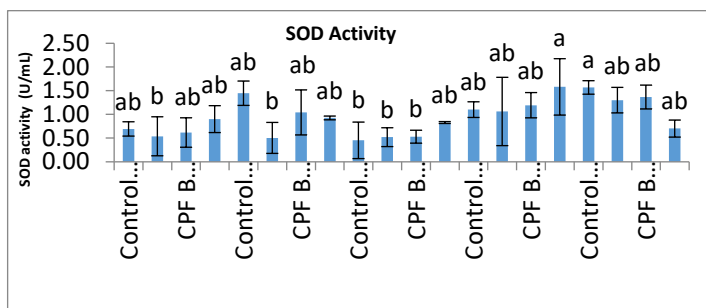


Figure 2c. SOD enzyme activity values of CPF pesticide active ingredient on *N. cryptocephala* var. *veneta*. Different letters on the bar indicate statistical differences $p < 0.05$ according to experimental groups. Values represent the mean.

The changes in CAT enzyme activity between the elimination (120s) application groups exposed to CPF and the control group samples were found to be statistically significant ($p < 0.05$).

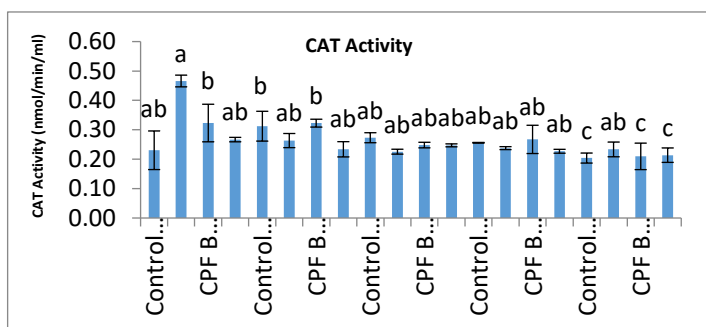


Figure 2d. CAT enzyme activity values of CPF pesticide active ingredient on *N. cryptocephala* var. *veneta*. Different letters on the bar indicate statistical differences $p < 0.05$ according to experimental groups. Values represent the mean $\pm$ SE

Changes in GPx enzyme activity between the treatment groups exposed to CPF and the control group samples were found to be statistically significant ($p < 0.05$).

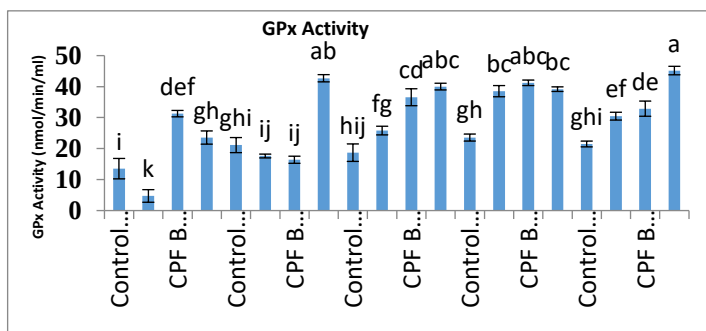


Figure 2e. GPx enzyme activity values of CPF pesticide active ingredient on *N. cryptocephala* var. *veneta*. Different letters

on the bar indicate statistical differences $p < 0.05$ according to experimental groups. Values represent the mean.

Discussion

Pesticides can enter the food chain and bioaccumulate in higher trophic level organisms and even the human body through the process of biomagnification (Nie et al., 2020). Pérez-Legaspi et al. (2016), stated in their study that while the lindane concentration in the environment decreased in *Nannochloris oculata*, which they exposed to the lindane pesticide, *N. oculata* biomass increased, and in this case, *N. oculata* accumulated the pesticide. Rioboo et al. (2007), in their study, examined the accumulation amounts of microalgae (*C. vulgaris*) cultures by exposing them to different concentrations of the triazine herbicide terbutryin, and as a result, they stated that as the herbicide concentrations in the environment increased, terbutrin accumulated in microalgae cells increased significantly. Kováčik et al. (2018), in their research, five hexachlorocyclohexane (α , β , γ , δ and ϵ -HCH, total 159.4 $\mu\text{g/L}$) and two trichlorobenzene (TCB, total 65.2 $\mu\text{g/L}$) isomers and two microalgae (*Scenedesmus quadricauda* and *Coccomyxa subellipsoidea*) and stated that *S. quadricauda* had more accumulation than *C. Subellipsoidea* due to its higher concentration. Du et al. (2023), in their study, *Chaetoceros neogracile* (T-CN; CCMP1425), *Micromonas bravo* (T-MB; CCMP1646) and two Arctic species strains *Micromonas polaris* (A-MP; CCMP2099), *Chaetoceros neogracilis* (A-CN; RCC2279) investigated the pesticides atrazine and simazine at certain concentrations and concluded that all species tended to increase cell biovolume by 2-12%. Zhang et al. (2011) examined the bioaccumulation of fluroxypyr in *Chlamydomonas reinhardtii* and concluded that intracellular accumulation increased with increasing fluroxypyr concentration, with a saturation point around 0.75-1.00 mg/L. In this study, in parallel with the literature information, it was determined that the CPF active ingredient pesticide and its by-products accumulated in model live microalgae organisms *N. cryptocephala* var. *veneta*.

Redox balance is an important form of innate immunity and is common in all algal phyla (Nikkanen et al., 2021). Microalgae will produce reactive oxygen species (ROS) during the normal growth process, while their antioxidant systems are activated to maintain redox balance in vivo. When the cell ages or abiotic stress acts chronically on the cells, ROS production will exceed the capacity of the scavenging system, thus oxidative damage occurs (Zhu et al., 2023). Basically, the increase in MDA levels is shown as a sign of increased oxidative stress and increased lipid

peroxidation of cells (Yagi, 1984). Hernández-García, and Martínez-Jerónimo (2020) reported in their study that the TBARS level values of *C. vulgaris* microalgae they exposed to glyphosate pesticide increased at all increasing concentrations. Baruah and Chaurasia, (2020) stated that there were increases in MDA levels caused by alpha-cypermethrin (ACy) in *Chlorella* sp. Tunca et al. (2023) stated that there were decreases in MDA levels due to the oxidative stress effect caused by dimethoate and chlorpyrifos in *Arthrospira platensis* Gomont. Qian et al. (2009), observed that paraquat increased the MDA content in *C. vulgaris*. Romero et al. (2011) stated in their study that there were increases in MDA levels in *Chlorella kessleri* due to the effect of glyphosate. Zhao et al. (2017), stated in their study that topramezone increased MDA levels in *C. vulgaris*. Deng et al. (2022), observed that there was no significant effect on MDA levels with the neonicotinoid effect in *Chlorella* sp. Guo et al. (2020), observed in their study that there were increases in MDA levels on *Raphidocelis subcapitata* (*R. subcapitata*) and *C. vulgaris* as a result of exposure to clarithromycin (CLA). Wang et al. (2012) stated that a general increase in MDA levels of cypermethrin on species of *Keletonema costatum* (*Bacillariophyceae*), *Scripsiella trochoidea* (*Dinophyceae*) and *Chattonella marina* (*Raphidophyceae*) occurred. Xi et al. (2019), stated in their study that triflumizole caused increases in MDA levels in *C. vulgaris*. Li et al. (2020) observed in their study that there were increases in MDA levels in *Chlorella pyrenoidosa* with the effect of Roxithromycin (ROX). Narra et al. (2017), in their study, it was determined that there were increases in MDA levels of *Clarias batrachus* due to the effect of chlorpyrifos and monocrotophos. Serdar et al. (2023), stated that dimethoate (DI) and malathion (MA) pesticides increased TBARS levels in *Dreissena polymorpha*. Serdar (2019) examined the effect of dimethoate on *Gammarus pulex* in his study and stated that there were changes in MDA levels. Söylemez et al. (2021) stated that Beta-Cyfluthrin increased MDA levels in *D. polymorpha*. It is thought that the reason why there are no significant increases in TBARS levels with the CPF effect in *N. cryptocephala* var. *veneta* depends on the CPF concentration and effect value.

For non-enzymatic antioxidants, GSH is an important component that can eliminate oxidative damage. Increased GSH, which provided more substrate, removed hydrogen peroxide by catalysis of glutathione peroxidase (Machado & Soares, 2019; Wang et al., 2019; Guo et al., 2020;). It is supported by many studies in the literature that GSH levels are accepted as oxidative stress response in various organisms. Almeida et al. (2019), investigated the effects of

bifenox and metribuzin on the microalga *Chlamydomonas reinhardtii* in their study and observed that there were changes in GSH content. Zhang et al. (2021), examined the effects of sulfamethoxazole (SMX) and erythromycin (ERY) on *Raphidocelis subcapitata* in their study and stated that GSH levels decreased with the SMX effect and GSH levels decreased with the ERY effect. Romero et al. (2011), stated in their study that there were concentration-dependent increases in GSH levels in *C. kessleri* due to the effect of glyphosate. Guo et al. (2020), observed in their study that there were increases in GSH levels on *R. subcapitata* and *C. vulgaris* as a result of CLA exposure. Liu et al. (2015), stated that there were changes in GSH levels as a result of the effect of azoxystrobin (AZ) on *C. vulgaris*. Narra et al. (2017), in their study, it was determined that there were increases in the GSH levels of *Clarias batrachus* due to the effects of chlorpyrifos and monocrotophos. Serdar et al. (2023), stated that DI and MA pesticides decreased GSH levels in *D. polymorpha*. Serdar (2019), examined the effect of dimethoate on *G. pulex* in his study and stated that there were changes in GSH levels. Söylemez et al. (2021) stated that Beta-Cyfluthrin decreased GSH levels in *D. polymorpha*. Changes in GSH levels in *N. cryptocephala* var. *veneta* due to the effect of CPF show similar results that are supported by the literature.

Changes in antioxidant systems and altered macromolecules have served as biomarkers for various xenobiotics (Gil & Pla, 2001). To prevent damage caused by free radicals (products of cellular metabolism) in cells under oxidative stress, aerobic organisms have developed antioxidant enzyme defenses such as superoxide dismutase (SOD). SOD plays a role in the reduction of superoxide radical to hydrogen peroxide (H_2O_2) (Van Camp et al., 1994), which is easily broken down into water and oxygen by CAT (De Zwart et al., 1999). Failure of these defenses to detoxify excess reactive oxygen species (ROS) can lead to significant oxidative damage, including lipid peroxidation (LPO) (Soto et al., 2011).

Both SOD and CAT are important antioxidant enzymes involved in ROS scavenging and are a potential biomarker of environmental pollution. The increase in CAT activity in *N. cryptocephala* exposed to CPF is thought to indicate the onset of cellular tolerance to oxidative stress to cope with excessive H_2O_2 production. Baruah and Chaurasia, (2020) stated that there are increases in SOD and CAT activities caused by alpha-cypermethrin (ACy) in *Chlorella* sp. Kumar et al. (2015), stated in their study that SOD and CAT activities were induced by the effect of acephate and imidacloprid on *Chlamydomonas mexicana*. Chen et al.

(2016), stated that there were increases in SOD and CAT activities with the effect of chlorpyrifos in *Chlorella pyrenoidosa* and *Merismopedia sp.* Zhang et al. (2021), examined the effects of sulfamethoxazole (SMX) and erythromycin (ERY) on *Raphidocelis subcapitata* in their study and stated that *R. subcapitata* showed resistance to SOD and CAT oxidative damage with the effect of SMX. Tunca et al. (2023), stated that there were concentration-dependent changes in SOD activities due to the oxidative stress effect caused by cimethoate and chlorpyrifos in *Arthrospira platensis gomont.* Romero et al. (2011), stated in their study that there were increases in SOD and CAT activities in *C. kessleri* due to the effect of glyphosate. Deng et al. (2022), observed increases in SOD activities with the effect of neonicotinoid in *Chlorella sp.* Guo et al. (2020), observed in their study that there were increases in SOD and CAT activities on *R. subcapitata* and *C. vulgaris* as a result of CLA exposure. Xi et al. (2019) stated in their study that triflumizole caused increases in SOD and CAT activities in *C. vulgaris*. Liu et al. (2015) stated that there were changes in SOD activities as a result of the effect of AZ on *C. vulgaris*. Li et al. (2020), observed in their study that there were increases in SOD and CAT activities due to the ROX effect in *C. pyrenoidosa*. Narra et al. In 2017 studies, it was determined that SOD and CAT increases in *Clarias batrachus* due to the effects of chlorpyrifos and monocrotophos. Serdar et al. (2023), stated that DI and MA pesticides increased SOD and CAT activities in *D. polymorpha*. Serdar (2019), examined the effect of dimethoate on *G. pulex* in his study and stated that there were changes in SOD and CAT activities. The changes observed in SOD and CAT activities due to the effect of CPF in *N. cryptocephala var. veneta* show that the cells defend against oxidative stress.

Reactive oxygen species such as superoxide, hydroxyl and hydrogen peroxide radical are formed during normal metabolism, especially as a result of oxidative events in the mitochondrial membranes. However, these radicals are inhibited by various antioxidants in the body. One of the most important of these antioxidants is glutathione peroxidase (GPx) (Piner, 2009). Liu et al. (2015), stated that there were changes in GPx activities as a result of the AZ effect on *C. vulgaris*. Esperanza et al. (2020), observed in their study that there were increases in GPx activities in microalgae *Chlamydomonas reinhardtii* and clam *Corbicula fluminea* species with the effect of Bisphenol A. Narra et al. (2017), in their study, it was determined that the GPx activities of *Clarias batrachus* were inhibited by the effects of chlorpyrifos and monocrotophos. Serdar (2019), examined the effect of dimethoate on *G. pulex* in his study and stated that there were changes in GPx activities. Serdar

(2021), stated that GPx activities decreased in *D. polymorpha* with the effect of cyfluthrin. Yildirim et al. (2018), stated in their study that there were increases in GPx activities in *G. pulex* with the effect of malachite green. It can be interpreted that increases in GPx activities occur in *N. cryptocephala var. veneta* as a result of CPF exposure and that the cell fulfills its defense duty against oxidative events.

Conclusion

The results of this study are supported by studies conducted in the literature. It is thought that pollutants such as pesticides can accumulate in microalgae and affect cellular events by causing oxidative stress in the cell. According to the results of the changes in TBARS and GSH levels, SOD, CAT and GPx activities in line with the biomarker results, it is thought that the CPF pesticide causes oxidative damage in the microalgae *N. cryptocephala var. veneta* and these parameters can be used as indicators. Since it is thought that CPF accumulated in microalgae will enter the food chain and affect the creatures at higher levels, it is recommended to impose restrictions on the use of pesticides and to impose restrictions on their areas of use.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVAL

All authors declare that there is no ethical violation in this manuscript. Also, this manuscript does not contain data belonging to others. The authors declare that they have no conflict of interest. The authors alone are responsible for the content and authoring of the present paper.

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CONSENT FOR PUBLICATION

The author has read and approved the final version of the manuscript and consent to its publication.

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