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RESEARCH PAPER

Determination of Toxic Effects of Copper in *Navicula cryptocephala var. veneta* by Biomarkers and Bioaccumulation Quantification

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Abstract

As a result of the increased use of metals such as copper (Cu), copper pollution in the environment is also increasing. In order to investigate the effect of copper mixed into the water environment and on aquatic biota, the microalga *Navicula cryptocephala var. veneta* was chosen as a model organism. *N. cryptocephala var. veneta* it was aimed to determine the accumulation and elimination amounts and some biomarker responses. Metal accumulation amounts in the organisms used in the study. The amount of metal accumulated in relation to the wet weight mass of the samples was determined using Atomic Absorption Spectophotometry (AAS) mass spectrometry. In the bioassays conducted within the scope of the study, lipid peroxidation (TBARS) and reduced glutathione (GSH) levels, superoxide dismutase (SOD) enzyme activity, glutathione peroxidase (GSH-Px) enzyme activity and catalase (CAT) enzyme activity were determined with an ELISA microplate reader at 24, 48, 72, 96 and 120 hours.
According to the study data, it was observed that Cu metal accumulated in *Navicula cryptocephala var. veneta* and caused oxidative stress in the organism, affecting the organism by causing changes in TBARS, GSH levels and antioxidant enzymes SOD, CAT and GPx activities. Additionally, it was observed that different elimination levels occurred in the application groups.

Introduction

The limited availability of fresh water resources in many parts of the world and the increasing pollution of these resources require studies to remove pollutants (Aydın et al., 2022). The discharge of heavy metals by metal processing industries is known to have adverse effects on the environment. Disposal of wastewater containing high levels of Cr, Pb, Cu and other heavy metals is a growing concern. The toxicity and health hazards associated with heavy metals have been established beyond any doubt (Volesky, 2001). For example, metal pollution can have many biological effects on the structure of freshwater planktonic communities. In particular, it can change their density and diversity and reduce the growth rate of microalgae species, with potentially harmful effects on aquatic ecosystems (Gagneten and Paggi, 2009; Gutiérrez et al., 2010; Gagneten et al., 2012). The effects of heavy metals on microalgae growth and metal deposition efficiency have been extensively studied, but little information is available on the relationships between the two processes.

Releasing excess amounts of heavy metals such as Cu in wastewater into the environment poses harm to organisms. These ions can cause toxic and harmful effects for living organisms in water and water consumers (Aksu and Holy, 1990). Copper (Cu) is an essential trace metal and micronutrient for cellular metabolism in living organisms as it is an essential component of metabolic enzymes. Fish require copper (Cu) as a micronutrient and obtain it from either water

or diet. Copper sulfate is an effective algaecide, but is toxic to many fish species at or near the concentration necessary for algal control (Farhangi et al., 2022). However, it may be extremely toxic to intracellular mechanisms at concentrations higher than normal levels in aquatic animals. With its widespread use, copper is an abundant element that occurs as a natural mineral. Copper pollution results from the widespread use of fungicides, algaecides, molluscides, insecticides, and discharge of waste (Pak 2019).

Microalgae are a group that is highly sensitive to heavy metals, and heavy metal ions directly affect many physiological and biochemical processes such as photosynthesis, respiration, mineral uptake, as well as growth of the organism, reflecting the picture of metabolism within the cell. Therefore, the growth of microalgae is often used as a barometer to study the toxicity of heavy metals (El-Naggar and Sheikh 2014; Duque et al. 2019). Navicula cryptocephala is a widespread, cosmopolitan benthic diatom of medium size (20–40 µm in length) that is well described and sampled in standard freshwater diatom floras (Poulíčková vd., 2010). To evaluate the responses of algae to the toxicity of heavy metals, lethal concentration EC_{50} values can be used effectively, although they are generally prioritized for statistical accuracy (Mohy El-Din et al., 2020).

In any organism, adverse environmental conditions trigger the formation of Reactive Oxygen Species (ROS), which severely damages proteins, lipids and nucleic acids. ROS affect both free radicals and non-radical (molecular) forms. This suggests that ROSs have a dual role (Luis 2015). At low doses, ROS serve as important signals in cells. It affects the expression of a number of genes and signal transduction pathways and therefore serves as key regulators in processes such as growth, development, response to biotic and environmental stimuli, plant metabolism, programmed cell death. On the other hand, environmental stress conditions trigger excessive production of ROS, which damages the detoxification ability of cells and causes oxidative cell injuries (Vanderauwera et al., 2009; Gill and Tuteja 2010). The cell defense mechanism clears excess oxidants and eliminates the harmful effects of ROS. This includes enzymatic scavengers such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase, etc. and non-enzymatic antioxidant molecules such as GSH, pigment, polysaccharides, polyphenols, proline, carotenoids, flavonoids, etc. (Cirulis et al., 2013). Whether ROS will harm, protect, or act as a signaling factor depends on the balance between their production and their scavenging at the appropriate place and at the appropriate time (Gratão et al., 2005).

The aim of this study was to examine the amount of accumulation occurring as a result of Cu exposure in the microalga *N. cryptocephala*, the amount of elimination occurring at the 120^{th} hour, and the oxidative stress response using biomarkers.

Material Method

N. cryptocephala Cultivation and Sample Preparation

Microalgae pure cultures were propagated in the laboratory of Munzur University Faculty of Fisheries. Pure *N. cryptocephala* (3.5-4×10^6 cells/ml) purchased from the University of Texas Algal Culture Collection (UTEX) was grown in proteose nutrient medium (autoclave sterilized) at 24±1°C and 12:12 h light:dark (A starter culture was obtained by propagating it in 250 ml conical flasks in a growing medium (3200 lux) cycle. In this process, the conical flasks were shaken with an orbital shaker (Ashraf et al., 2011). The starter culture (0th generation) was inoculated by adding commercial fertilizer (Gübretaş 20:20:20 (N:P:K)+Trace Element) at a rate of 40 mg/L (Ammar, 2016) as a nutrient. *N. cryptocephala* cultures were harvested at the logarithmic growth phase (average 1-2×10^6 cells/ml) (Regaldo et al., 2013).

Acute Toxicity of Copper (Cu) Heavy Metal on *N. cryptocephala*

The inhibition occurring in microalgae was established based on the counting of living cells. Microalgae Inhibition Test was applied for 24, 48 and 72 hours as recommended in OECD (2011). Sterile glass tubes with a volume of 15 ml were used as test media. A test setup was created by taking 9 ml of Cu solution at different concentrations and 1 ml of microalgae culture, making the total volume 10 ml. To detect dead/live microalgae cells, 1 ml of microalgae Cu sample taken from each test tube at the end of 24, 48 and 72 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). The counts were repeated three times for each sample and calculated by obtaining average values (Yoon et al., 2007; Erdem et al., 2014; Özkaleli and Erdem 2017; OECD 2011).

Compared to the EC_{50} values of Cu pollutants,

Group A; Not exposed to any pollutants (control)

Group B; pollutant 1/8 of the pollutant EC_{50} value,

Group C; pollutant at 1/4 of the EC_{50} value,

Group D; pollutant is pollutant at 1/2 of the EC_{50} value,

N. cryptocephala was directly exposed to the Cu in 3 replicates by determining the concentrations. A 1 liter sample was taken from the application groups every 24 hours for 4 days and precipitated by centrifugation. For bioaccumulation, ambient water was taken, microalgae samples were taken, passed through pure water, labeled for pre-treatment and stored at -18°C. After the samples taken for biochemical analysis 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. As for the

elimination samples, after the 96th hour samples were taken, the microalgae in the application medium were rapidly precipitated by centrifugation to remove the Cu concentration from the medium. After microalgae samples were washed with pure water, UV-sterilized water was added and they were kept for 24 hours (120th hour), and the elimination samples were preserved using the same methods (Çiçek Çimen and Serdar, 2022).

It was aimed to measure TBARS and GSH levels, which are indicators of oxidative stress and antioxidant enzymes SOD, CAT and GPx activities.

Biochemical Analysis

In the bioassays conducted within the scope of the study, to determine the biochemical responses in the samples taken at 24, 48, 72, 96 and 120 hours, 0.5 g of sample was weighed and mixed with PBS buffer (phosphate-buffered salt solution) (pH 7.4) at a ratio of 1/10 w/v) was added and homogenized using a homogenizer with ice. These homogenized samples were centrifuged in a refrigerated centrifuge at 17000 rpm for 15 minutes, and the resulting supernatants were stored in a deep freezer at -86°C until the measurement process was completed (Serdar et al., 2024; Aydın and Serdar, 2024). With the supernatants obtained, lipid peroxidation (TBARS) and reduced glutathione (GSH) levels, superoxide dismutase (SOD) enzyme activity, glutathione peroxidase (GSH-Px) enzyme activity, catalase (CAT) enzyme activity and metallothionein (MT) were determined by using ELISA microplate reader.

Cu Metal Deposition Measurement

After *N. cryptocephala exposed to Cu* in the logarithmic growth period at three different sublethal concentrations (24, 48, 72, 96 and 120 (elimination) hours), a 15 ml portion of each microalgae culture was added to 10 per 6000 g for each repetition (Şişman-Aydın et al., 2013). Algal biomass was filtered using an MF-Millipore filter (0.45 micron). The algae were washed three times with 10 ml of 0.001 M EDTA (Ethylene diamine tetraacetic acid disodium salt dihydrate, Sigma Ultra grade, Sigma-Aldrich) solution to remove metals adsorbed on the cell walls. After filtering the microalgae, the filters were oven dried at 103°C for 2 h.

In the digest process; 1 ml pure nitric acid (70% HNO_3) and 125 µl hydrogen peroxide (H_2O_2) were added to the samples in the tubes with the help of an automatic pipette (Esmaeili, 2015). After the amount of acid in the teflon tubes was increased to 2 ml and the tubes were carefully closed, the samples were burned in a microwave oven (180-150°C, 5-15 min) with the appropriate protocol. After the dissolution process, the samples were allowed to cool, and the teflon tubes were washed with ultrapure water and made up to 15 ml. 2 ml of the samples obtained were transferred to eppendorf tubes. Determination of the amount of metal bioaccumulation on the surface of algal cells; it was calculated by subtracting the intracellular metal concentration from the total metal concentration.

Accumulation amounts in the organisms used in the study was determined by measuring the amount of accumulated metal in relation to the wet weight of the samples. Metal concentration analysis was performed using AAS mass spectrometry through service. The confirmability of AAS was achieved with its standard curve.

Statistical Analysis

Cu heavy metal was found in *N. cryptocephala var. veneta* acute toxicity on (Table 1) was calculated by probit analysis. In this study, SPSS 24.0 package program One-Way Anova (Duncan 0.05) was used to evaluate the data of biomarker parameters.

Results

EC_{50} Values

ED_{50} (Effective Dose 50) refers to the dose of a drug or substance that produces the desired effect in 50% of a population or sample under a specific set of experimental conditions or in a group of test subjects. EC_{50} values on *N. cryptocephala* exposed to the active ingredient Cu are given in Table 1.

Cu Heavy Metal Bioaccumulation in *N. cryptocephala*

Cu accumulation amounts occurring in *N. cryptocephala* due to the effect of Cu heavy metal are given in Figure 1.

Table 1. EC_{50} values of Cu heavy metal on *N. cryptocephala* calculated by probit analysis

	EC_{50} mg/l
Recurrence 1	2,76
Recurrence 2	2,03
Recurrence 3	1,17
Average value	1,99
Standard deviation	0,80

Determination of TBARS Levels of Cu Heavy Metal on *N. cryptocephala*

To determine the TBARS level values of Cu heavy metal on *N. cryptocephala* microalgae, the TBARS level was measured by taking samples every 24 hours from three different concentration groups created in proportion to the EC_{50} value and one control group (Figure 2). TBARS level increased compared to the control, but only a statistically significant difference was found between the Cu heavy metal exposed group samples and the control group samples at the 96th hour ($p<0.05$). In the elimination group, decreases in TBARS levels were observed.

The Effect of Cu on GSH Levels in *N. cryptocephala*

The effect of Cu on GSH level in *N. cryptocephala* microalgae was measured (Figure 3). A statistically significant difference was found between the some application groups and the control group ($p<0.05$).

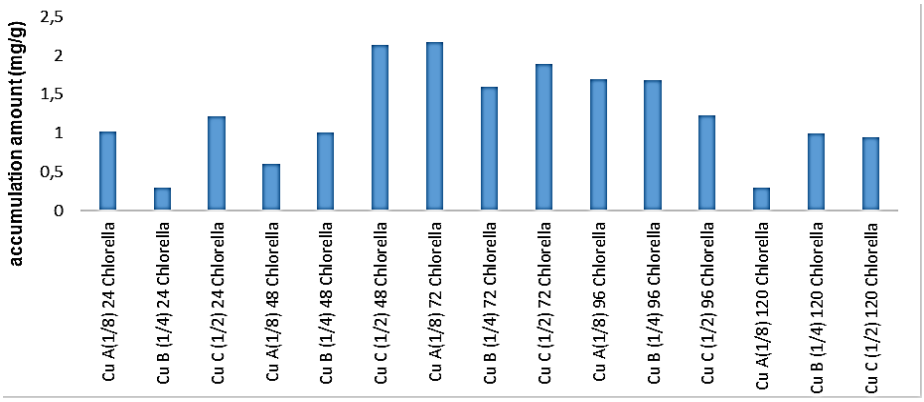

Figure 1. Cu heavy metal bioaccumulation amounts in *N. cryptocephala* (mg/g).

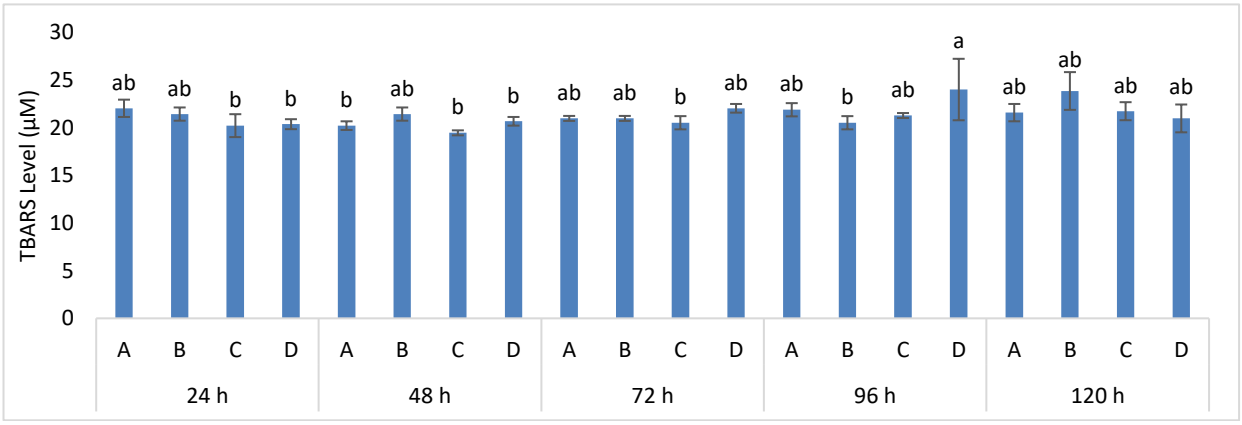

Figure 2. TBARS level values of Cu heavy metal on *N. Cryptocephala.*
Different letters on the column within the same time shows differences

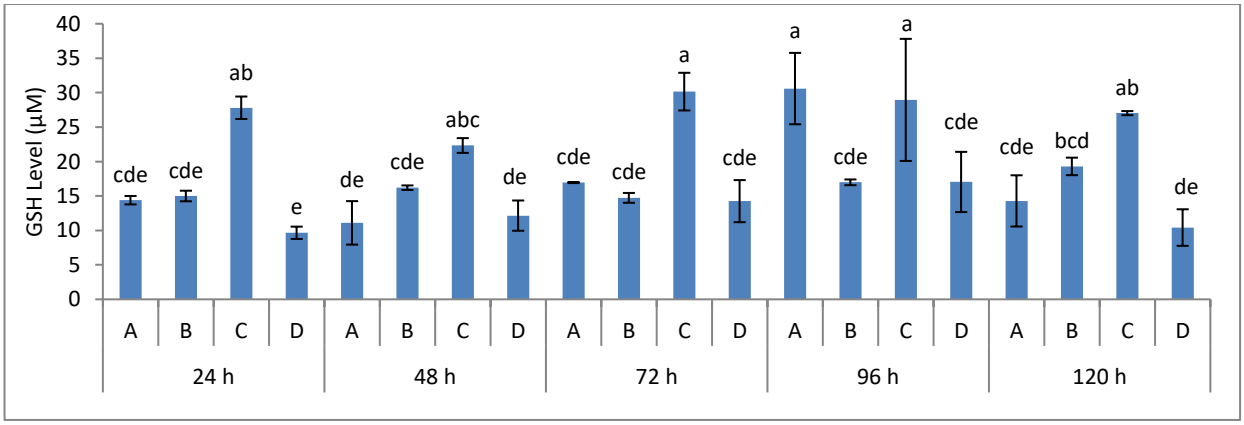

Figure 4. SOD enzyme activity values of Cu heavy metal on *N.cryptocephala.* There are differences at the $p<0.05$ level between the data shown with different letters on the column within the same time group. Different letters on the column within the same application time shows diffferences.

Effect of Cu on SOD Enzyme Activity in *N. cryptocephala*

The effect of Cu on SOD enzyme activity in *N. cryptocephala* microalgae was measured (Figure 4). A statistically significant difference was found in SOD enzyme activity between the application groups and the control groups at the 24th and 48th hour ($p<0.05$). Data similar to the control group were obtained in the elimination groups.

Effect of Cu on CAT Enzyme Activity in *N. cryptocephala*

The effect of Cu on CAT enzyme activity in *N. cryptocephala* microalgae was measured (Figure 5). Changes in CAT enzyme activity the exposure and control groups were not found to be statistically significant ($p>0.05$).

Effect of Cu Heavy Metal on GPx Enzyme Activity of *N. cryptocephala*

The effects of Cu on GPx enzyme activity in *N. cryptocephala* microalgae was measured (Figure 6). The increases in GPx enzyme activity in all exposure and control group were found to be statistically significant ($p<0.05$). It was determined that there were decreases in the elimination group compared to other application groups.

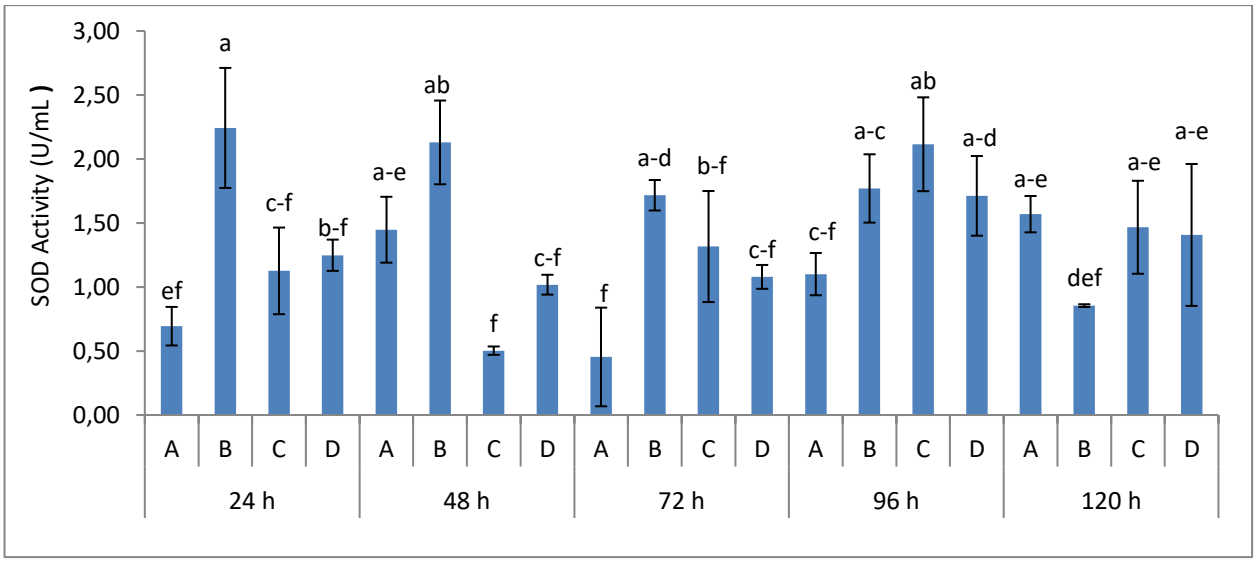

Figure 4. SOD enzyme activity values of Cu heavy metal on *N.cryptocephala.* There are differences at the $p<0.05$ level between the data shown with different letters on the column within the same time group. Different letters on the column within the same application time shows diffferences

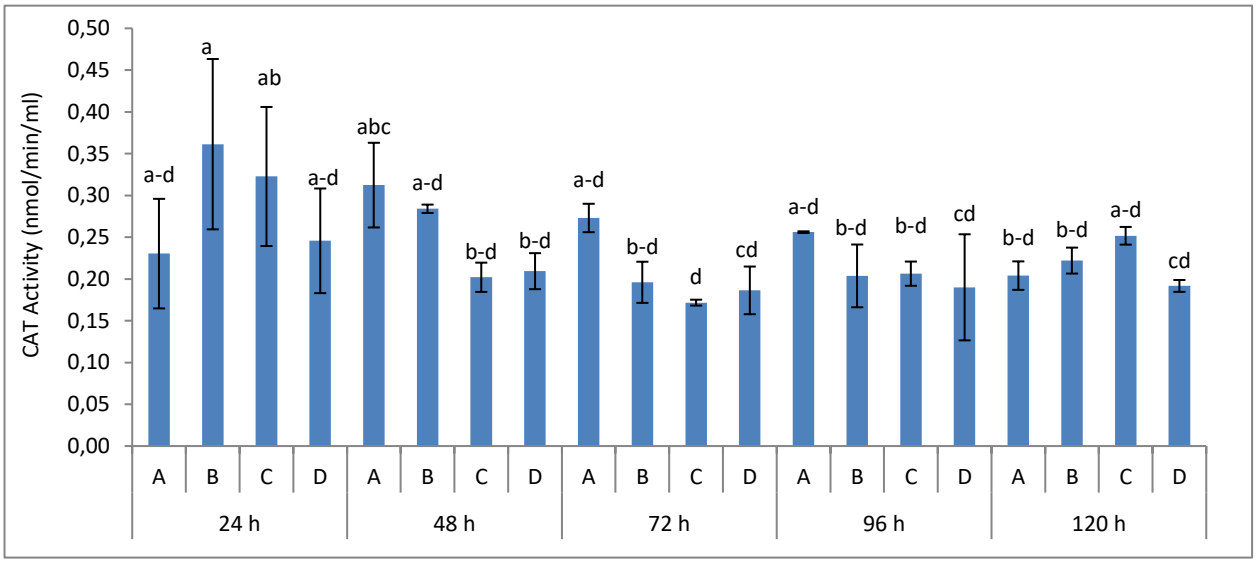

Figure 5. CAT enzyme activity values of Cu heavy metal on *N. Cryptocephala*. There are differences at the $p<0.05$ level between the data shown with different letters on the column within the same time group.

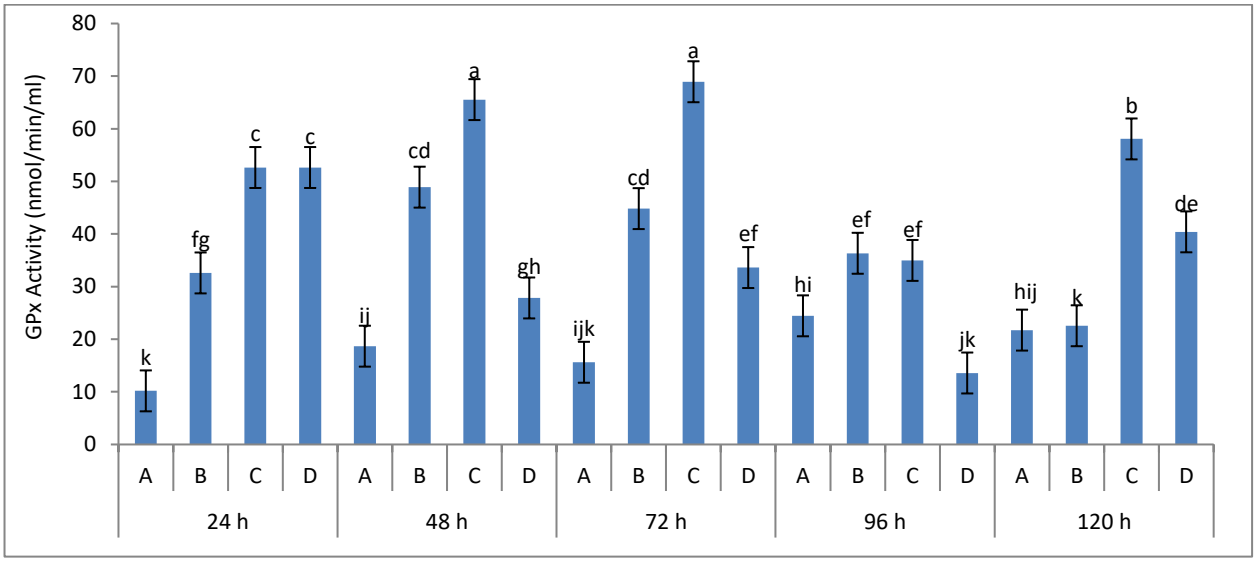

Figure 6. GPx enzyme activity values of Cu heavy metal on *N. cryptocephala*. There are differences at the p<0.05 level between the data shown with different letters on the column within the same time group.

Discussion

Interactions between metals such as copper and water chemistry can affect metal bioavailability, which is an index of the rate and extent to which the metal reaches the toxic effect zone and can vary up to 100-fold across a range of water chemistries in surface waters (Nikjoo et al., 2023). Smaller cells have relatively larger surface area and more sites for binding metals than larger cells, so the amount of Cu accumulation in small cells is generally greater than that in larger cells (Khoshmanesh et al., 1997). There are some studies in the literature that examine the accumulation and effects of pollutants such as Cu on microalgae. The study conducted by Yan and Pan 2002, examined the effect and accumulation of Cu on the growth of *Scenedesmus obliquus, Chlorella pyrenoidosa* and *Closterium lunula*, and observed that Cu accumulated in algal species. Debelius et al., (2009), examined the accumulation amounts and effects of Cu and lead on 5 marine microalgae. Rugnini et al., (2017), investigated the effects of Cu and nickel (Ni) on two green microalgae species, *Chlorella vulgaris* and *Desmodesmus* sp., and stated that microalgae growth decreased with increasing metal concentrations and metal accumulation occurred. Vojvodić et al., (2020), examined the reactions of *Chlorella sorokiniana* to Cu and observed that changes in lipid structures and polyphosphate accumulation occurred. Huang et al., (2021) examined the combined pollution of Arsenic (As) and copper (Cu) in *Cyanophyta* and *Chlorophyta* and it was stated that the absorption and accumulation affected by algae. Li et al., (2021) in their research, examined the effect of sulfonamide (SA) and Cu on *C. vulgaris* and stated that C. Vulgaris's growth was inhibited by SAs and Cu. Sun et al., (2020), in their research they examined the effects and accumulation amounts of Cu, cadmium (Cd) and zinc (Zn) on *Scenedesmus obliquus*. Aydın and Serdar, (2024) examined probiotic protection against Cu accumulation in their study and stated that probiotics helped to reduce the Cu accumulati in *D. polymorpha* It was observed that the amount of elimination occurring at the 120th hour (After 96 hours, the water was exposed to a habitat compatible with the environment that was not exposed to the pollutant for 24 hours) in *N. cryptocephala* due to the Cu effect was reduced compared to the control. Environmental levels of copper (Cu) and other heavy metals are increasing due to human activities. Cu is a trace element vital for the development of photosynthetic species. High levels are phytotoxic to cells, can significantly inhibit growth and also lead to cell death (Yan and Pan, 2002; Sabatini et al., 2009). Cu stress reduces the growth rate and pigment content in microalgae (Sáeza et al., 2015; Machado and Soares, 2016), while increasing the ROS produced through the interference of Cu ions in the Fenton reaction (Okamoto et al., 2001). Increased levels of ROS can rapidly attack nucleic acids, proteins, and lipids, leading to permanent metabolic dysfunction and cell death (Gill and Tuteja, 2010; Pandey et al., 2015).

Malondialdehit (MDA) is the product of lipid peroxidation, and its content can explain oxidation and lipid damage under stress (Qian et al., 2012). The increase in free radicals causes excessive production of MDA (Aydın et al., 2023). It is thought that the increases in TBARS levels as a result of the effects of Cu heavy metal on *N. cryptocephala* indicate that free radicals increase in cells under stress and this is why the increases in TBARS levels occur. There are studies in the literature supporting increases in TBARS levels, and our results are supported by the literature.. Danouche et al. 2020, in their study, Cu, Cr, Pb and Cd metals were detected in *C. vulgaris,* C. *ellipsoidea, C. sorokiniana, C. pyrenoidosa, Scenedesmus dimorphus, S. obliquus, Chlamydomonas reinhardtii* and *Aphanothece* sp. they

examined the changes in the species and observed increases in MDA levels. Gao et al. (2022), in their study, they examined the effect of Cu and two types of polystyrene nanoplastics on *Platymonas helgolandica* and stated that the MDA content was not significantly different from that of the control. Li vd., (2020) in their study, examined the effects of 17β-estradiol (E2) and Cu(II) on the growth and oxidative responses of *S. dimorphus* and stated that MDA content was significantly stimulated as a result. High MDA levels were also observed in the marine microalgae *Isochrysis galbana* when exposed to Oxytetracycline and Cu (Wu and He ., 2019). Wang et al., (2021) examined the effects of microplastic, Cu and Cd on *C. vulgaris* and stated that single and combination pollutants caused cell damage in microalgae with an increase in the MDA content. Lu et al., (2021) stated that increases in MDA levels occurred in *C. vulgaris* due to the effect of Cr. Mao et al., (2021) stated that azithromycin decreased the MDA content in the *Chlorella pyrenoidosa* species. Jin et al., (2020) stated that there were increases in MDA levels in *I.s galbana* due to the effect of Cr. Fang et al., (2022) examined the effects of carbon nanotubes and copper oxide nanoparticles (CuO NPs) on *Tetradesmus obliquus* and noted increases in MDA levels. Xu et al., (2024) examined the toxicity of lithium (Li) on *Chromochloris zofingiensis* and stated that Li caused decreases in MDA content. Serdar et al., (2024) examined the effect of two different forms of aluminum oxide (γ-Al_2O_3 and α-Al_2O_3) on *Gammarus pulex* and stated that there were increases in TBARS levels. Hamed et al., (2024) examined the effects of mercury oxide on *Scenedesmus obliquus* and *Nostoc muscorum* species and observed that the MDA level increased in *N. Muscorum* and *S. obliquus*. Zhang et al., (2024) stated in their research that there were increases in MDA levels in *Chromochloris zofingiensis* due to the effect of cadmium. Cimen et al., (2020) stated that Cu caused increases and decreases in TBARS levels of *Artemia salina*. Wang et al., (2020) observed that there were increases in the levels of Cu and Zn in *Oreochromis niloticus* and MDA. It was observed that there was no significant change in the elimination amounts occurring at the 120th hour due to the Cu effect in *N. cryptocephala* compared to the control.

GSH is the most abundant low molecular weight thiol-containing compound in living cells. Since its reduced form (GSH) is a reducing agent for hydroperoxides and free radicals, the defense systems it secretes protect cells against oxidative damage (El-Rashidy et al., 1984; Ergüven et al., 2022). Wang et al., (2020) observed that there were decreases in GSH levels in *C. vulgaris* as a result of CuO exposure. Gao et al., (2022) examined the effects of Cu and two types of polystyrene nanoplastics on *Platymonas helgolandica* and stated that there were increases in GSH levels. Mao et al. (2021) stated that azithromycin increased GSH content in *C. pyrenoidosa*. Xu et al., (2024) examined the toxicity of Li on *C. zofingiensis* and stated that Li caused increases in GSH content. Serdar et al. (2024), examined the effects of γ-Al_2O_3 and α-Al_2O_3 in *G. pulex* and stated that there were decreases in GSH levels. Hamed et al., (2024) investigated the effects of mercury oxide on *S. obliquus* and *N. muscorum* species and they observed that the GSH level increased in *N. muscorum* and *S. obliquus*. Zhang et al., (2024) stated that there were increases in GSH levels in *C. zofingiensis* due to the effect of cadmium. Cimen et al., (2020) stated that Cu caused concentration-dependent increases in the GSH levels of *A. salina*. It has been observed that there are increases in the GSH levels of Cu heavy metal on *N. cryptocephala* compared to the control, and these increases are thought to depend on the concentration and exposure time. Moreover, the results are in line with the studies in the literature. It was observed that the elimination amounts occurring at the 120th hour in *N. cryptocephala* due to the effect of Cu reached values close to control.

SOD is one of the most important antioxidant enzymes, ubiquitously present in all subcellular compartments of aerobic organisms prone to ROS-mediated oxidative stress (Gill and Tuteja, 2010). It provides the first line of defense against the toxic effects of ROS by catalyzing the neutralization of 2 superoxide radicals by the addition of 2 hydrogen ions to generate H_2O_2 and O_2 at a very rapid rate (Gonçalves et al., 2007). When *N. cryptocephala* cells are exposed to low concentrations of Cu, they can increase the activity of antioxidant enzymes to remove excess free radicals, thus preventing or reducing oxidative damage. For this reason, it is thought that SOD and CAT activities increase in *N. cryptocephala*. The study results are seen to be compatible with literature studies. Hamed et al., (2017) observed in their study that *C. sorokiniana* and *S. acuminatus* increased SOD activities with the effect of Cu. Danouche et al., (2020), investigated the effects of Cu, Cr, Pb and Cd metals on *C. vulgaris, C. ellipsoidea, C. sorokiniana, C. pyrenoidosa, S. dimorphus, S. obliquus, C. reinhardtii* and *Aphanothece* sp. They examined the changes in their species and observed increases in SOD activities. Gao et al,. (2022) examined the effects of Cu and two types of polystyrene nanoplastics on *Platymonas helgolandica* and stated that there were increases in SOD activities. Gallo et al., (2020) examined the effects of Cu^{+2} on *D. salina* and *S. elongatus* species and stated that there were increases in SOD activities. Li et al., (2020) examined the effect of E2 and Cu(II) on the growth and oxidative responses of *S. dimorphus* and stated that there was little significant difference in SOD activity. Wang et al., (2021) examined the effects of microplastic, Cu and Cd on C. vulgaris and stated that single and combination pollutants caused cell damage in microalgae with an increase in SOD activity. Lu et al., (2021) stated that increases in SOD activities occurred in *C. vulgaris* due to the effect of Cr. Mao et al., (2021) stated that azithromycin increased SOD activity in the *C. pyrenoidosa* species. Jin et al., (2020) stated that there were increases in the activities of *I. galbana* due to the effect of Cr. Fang et al., (2022) examined the effects of

MWCNTs and CuO NPs on *T. obliquus* and stated that there were increases in SOD activities. Xu et al., (2024) examined the toxicity of Li on *C. zofingiensis* and stated that Li caused increases in SOD activity. Serdar et al., (2024) examined the effects of γ-Al_2O_3 and α-Al_2O_3 in *G. pulex* and stated that there were increases in SOD activities.There were increases in SOD activities in *C. zofingiensis* due to the effect of Cd Zhang et al., (2024). It was observed that the elimination amounts occurring at the 120th hour in *N. cryptocephala* due to the Cu effect increased compared to the control

CATs are tetrameric heme-containing enzymes with the potential to convert HO directly to HO and O_2 and are indispensable for ROS detoxification during stressful conditions (Romero-Puertas et al., 2006). CAT is important in the removal of H_2O_2 produced in peroxisomes by oxidases involved in b-oxidation of fatty acids, photorespiration, and purine catabolism. Danouche et al., (2020) investigated the effects of Cu, Cr, Pb and Cd metals on *C. vulgaris, C. ellipsoidea, C. sorokiniana, C. pyrenoidosa, S. dimorphus, S. obliquus, C. reinhardtii* and *Aphanothece* sp. They examined the changes it caused in its species and observed increases in CAT activities. Ameri et al., (2020) characterized the antioxidant response of aluminum (Al) of *Scenedesmus* sp. and stated that there was a gradual loss in CAT activities. Gallo et al., (2020) examined the effects of Cu^{+2} on *D. salina* and *S.s elongatus* species and stated that there were increases in CAT activities. Li et al., (2020) examined the effects of E2 and Cu(II) on the growth and oxidative responses of *S. dimorphus* and stated that there were significant increases in CAT activity as a result. León-Vaz et al., (2021) examined the effects of Cu^{2+}, Cd^{2+}, As (III) and As (V) in the *C. sorokiniana* species and stated that there were increases in CAT activity. Lu et al., (2021) stated that increases in CAT activities occurred in *C. vulgaris* due to the effect of Cr. Xu et al., (2024) examined the toxicity of Li on *C. zofingiensis* and stated that Li caused increases in CAT activity. Serdar et al., (2024) examined the effects of γ-Al_2O_3 and α-Al_2O_3 in *G. pulex* and stated that there were increases in CAT activities. Zhang et al., (2024) stated that there were increases in CAT activities in *C. zofingiensis* due to the effect of Cd. It was observed that the elimination amounts occurring at the 120th hour in *N. cryptocephala* due to the effect of Cu reached values close to control. The increases in CAT activities in existing studies in the literature and the study data support each other.

Inhibition of GPx activity may reflect the failure of the antioxidant system in contact with pollutants (Ballesteros et al., 2009; Aydin and Serdar, 2024) or may be related to the direct effect of superoxide radicals or the pollutant on enzyme synthesis (Bainy et al., 1993). Many studies in the literature report that GPx activity is triggered by pollutants. Aydın and Serdar, (2024) observed that Terbium (Tb) caused decreases in GPx activities in *Pontastacus leptodactylus.* Serdar et al., (2021) stated that there were decreases in GPx activities due to the effect of cadmium (Cd) on *G. pulex*. Hamed et al., (2024) examined the effects of mercury oxide on *S. obliquus* and *N. muscorum* species, and they observed increases in GPx activities in both species. Wang et al., (2020) observed that there were decreases in the GPx activities of Cu and Zn in *Oreochromis niloticus*. Raeeszadeh et al., (2023) examined the effects of Pb, As, Hg and Zn on trout, carp and shrimp and stated that GPx was highest in shrimp and lowest in carp. Zaidi et al., (2023) study examined the effects of polyethylene microplastics (MPs; <0.02 mm) and $CuSO_4$, alone and in combination, on the freshwater crayfish *P. leptodactylus* and observed that they caused increases in GPx activities in both application cases. Santos et al., (2021) stated that the combined effect of microplastic and Cu caused changes in GPx activities in *Danio rerio*. The increases in GPx activity due to the effect of Cu heavy metal on *N. cryptocephala* are thought to be related to concentration and application time, and it is thought that the cell increases GPx activity to defend itself. The increases in GPx activities in existing studies in the literature and the study data support each other. It was observed that there were variable values in the elimination amounts occurring at the 120th hour due to the Cu effect in *N. cryptocephala.*

Results

With the rapid development of industry, the use of metals such as Cu is increasing. In parallel with the increasing use, the diffusion of Cu into the environment is also increasing. The water environment, which is one of the primary systems that make up the environment, is the final stop for metals such as Cu and other pollutants.

Research aimed at a more comprehensive understanding of the relationships that occur between living organisms and their environment (in this case, water) in any case provides important information about the functioning of the aquatic ecosystem and its ability or failure to cope with stress. According to the data obtained from this study, the presence of Cu in the environment caused Cu accumulation and oxidative stress in *N. cryptocephala* cells; TBARS caused changes in GSH levels and SOD, CAT, GPx activities.

Considering the fact that algae constitute the lowest level of the food chain, it can be concluded that this situation may also affect the creatures at the upper level of the food chain.

Ethical Statement

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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Author Contribution

All authors involved in the manuscript contributed to the article. There is no conflict of interest between the authors.

Conflict of Interest

Not applicable.

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