



Biochemical Responses of Zebra Mussel *Dreissena polymorpha* (Pallas, 1771) (Bivalvia: Dreissenidae) against Cadmium

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Abstract: The biochemical reactions of zebra mussels *Dreissena polymorpha* (Pallas, 1771) exposed to different concentrations of cadmium (Cd) in the acute period (24 and 96 hours after treatment) were studied. Four variants of experimental treatment were examined: 0 µg/L Cd (control), 10 µg/L Cd, 20 µg/L Cd and 40 µg/L Cd. Changes in the enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) levels were determined using ELISA kits. In *D. polymorpha* exposed to the three experimental concentrations of cadmium, there was a statistically significant decrease ($p < 0.05$) in SOD enzyme activity with increasing concentration or exposure times. No change was observed in CAT enzyme activity with increasing concentrations. The decrease in the activity depending on the exposure times was not statistically significant ($p > 0.05$). As for GPx enzyme activity, a significant increase was detected after Cd application compared to the control ($p < 0.05$). TBARS and GSH levels increased significantly in all Cd application groups compared to the control at 24 and 96 hours. The effects of different concentrations of Cd on *D. polymorpha* in the acute period were associated with changes in SOD, CAT, GPx enzyme activities. TBARS and GSH levels were effective parameters in determining the oxidative damage caused by Cd.

Key words: *Dreissena polymorpha*, Cadmium, Antioxidant, Oxidative stress, Biomarker

Introduction

Chemicals used in industry, agriculture and animal husbandry as well as available in domestic waste pollute a large part of vital water resources. For this reason, it is essential to reveal the risks for water resources and to take the necessary precautions. In recent years, industry, agriculture and animal husbandry have been developing rapidly to meet the needs of the increasing population around the world,

resulting in increased emission of by-products and waste in the environment, thus threatening the entire ecosystem. These pollutants mix with surface water and groundwater, disrupt the ecosystems and affect the organisms living in them, thus causing also economic losses. Therefore, it is important to determine the effect of these chemicals on the ecosystems.

The half-life of cadmium (Cd) in the environment varies between 10 and 25 years. Cadmium is found in nature in the form of CdS, which is fre-

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quently present in zinc ore. Metallic cadmium has a wide use because it is resistant to corrosion. Cadmium is the heavy metal with the highest solubility in water. Due to this solubility, its diffusion rate is relatively high. Cd^{2+} ions are taken by organisms and accumulate in biological systems (Duffus 1980). Cadmium, as a heavy metal pollutant, has toxic effects and is harmful even at low concentrations (Katalay & Parlak 2002, Asri et al. 2007).

Antioxidants provide a crucial mechanism for protecting against harmful reactive oxygen species (ROS) or healing oxidative damage (Dorval & Hon-tela 2003). Pollution of water with metal ions is a worldwide environmental problem. Heavy metals such as cadmium are released into the environment by various industries, reaching streams and rivers through runoff from unregulated waste disposal (Nriagu et al. 1998). Cadmium is a highly toxic heavy metal causing harmful effects on organisms, even at low-level concentrations (Cope et al. 1994). Besides various toxic effects of cadmium, its accumulation in the liver (Novelli et al. 1998), kidney (Klaassen & Liu 1997, Novelli et al. 1998) and testicles (Shen & Sangiah 1995) has also been reported. Oxygen is an essential element for aerobic metabolism as it is the terminal acceptor of electrons in oxidative phosphorylation. However, in cases of cadmium exposure (Novelli et al. 1998), the electron flow can be disrupted, resulting in the production of reactive oxygen species (ROS). Reactive oxygen species, superoxide anion (O_2^-), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) can cause widespread damage to cells, such as lipoperoxidation of polyunsaturated membrane lipids. Lipoperoxidation is a chain reaction mediated by free radicals. Oxidative stress, defined as an imbalance between oxidants and antioxidant defences (Nishiyama et al. 1998), may be associated with both cadmium exposure and tissue damage. Additionally, it serves as a catalyst for xenobiotics, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes. Typically, the cell antioxidant balance is upheld by a mixture of diminutive antioxidant molecules, such as SOD, GST and CAT enzymes (Glass et al. 1985). Heavy metal-derived pollutants allow oxidative stress to occur by disrupting the normal functioning of the damage to antioxidant enzyme systems in organisms (Dimitrova et al. 1994). Reactive oxygen forms are harmful for the body because they react with cellular components, resulting in the oxidation of lipids, proteins and DNA. This process leads to the deactivation of enzymes, tissue damage, rupture of cell membranes, mutations and, ultimately, cell death (Vutukuru et al. 2006).

Mussels accumulate pollutants and potentially toxic substances such as heavy metals in their bodies. When the pollutant levels in their bodies are measured, they reflect the pollution of the environment (Phillips 1980). Zebra mussels, which are also considered an invasive species, have a very wide habitat tolerance. They can adapt to hunger, drought, high-temperature differences and changes in the physicochemical properties of water (dissolved oxygen, pH, hardness, etc.) (Bobat et al. 2001). Due to these characteristics, mussel species in particular are used extensively in determining aquatic environment pollution (UNEP 1993). They feed by filtering water, respond quickly to pollutants, have no economic value and are easy to access. For all these reasons, *Dreissena polymorpha* was chosen as a model organism and was investigated in terms of oxidative stress and antioxidant against cadmium (Cd).

In order to determine the effect of increasing industrial pollution on aquatic organisms, the present study aims to determine the effect of Cd on organisms in laboratory conditions using *D. polymorpha* as a model organism. In addition, it is expected that the effect of the biochemical response studied in the model organism used in the present study may help in prognoses of the effect of the pollutant on various organisms exposed to Cd in the environment, thus allowing planning future studies.

Materials and Methods

Model organism

Model organisms (*Dreissena polymorpha*) were collected from the Euphrates River (38°48' 09"N, 38°43' 51"E). They were brought to the aquaculture laboratory in ventilated plastic containers. During adaptation, organisms were observed daily for one month. To optimize environmental conditions, the bioanalysis environment was kept at constant temperature ($20 \pm 1^\circ C$) with a thermostat air conditioner. Ambient lighting was set to 14 : 10 hours dark : light. In order to be used in the study, we selected specimens of the similar size out of those in the stock tank. Morphometric data of the mussels used in the study were as follows: weight = 1.092 ± 0.27 g, length = 20.276 ± 2.09 mm, width = 10.13 ± 0.94 mm and height = 9.741 ± 1.07 mm).

Adaptation of the model organism

Deissena polymorpha samples brought to the laboratory alive were placed in pools prepared to resemble their natural environment. Under laboratory lighting, a photoperiod of 12 hours of light and 12 hours of darkness was applied (Serdar 2021). The ambient

temperature was kept constant at 23°C during both the adaptation and test phases using the thermostat air conditioner. Cultured phytoplankton was used to feed *D. polymorpha*.

Experimental design

For biochemical experiments, the concentrations applied to the trial groups were determined taking into account the availability of Cd in natural waters according to the Intra-Continental Water Quality Regulation (Su Kirliliği Kontrolü Yönetmeliği, SKKY, Türkiye). Four groups were formed, one of which was the control group. These groups were: C0 group 0 µg/L Cd; C1 group 10 µg/L Cd; C2 group 20 µg/L Cd; C3 group is 40 µg/L Cd. The organisms were exposed to the determined Cd concentrations in the formed groups. The samples taken to determine their biochemical responses at 24 and 96 hours were stored at -80 °C.

Biomarkers

To determine the biochemical response, SOD, CAT, and GPx enzyme activities as well as thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels were determined. SOD (Cat. No 706002), CAT (Cat. No 707002), GPx (Cat. No 703102), TBARS (Cat. No 10009055) and GSH (Cat. No 703002) kits were purchased from Cayman Chemical Company, USA. Statistical analysis was carried out using the SPSS 24.0 statistical program. The statistical difference between different groups (C0 – control, C1, C2, C3) within the same application period was determined by the DUNCAN test. The difference between application times (24 and 96 hours) was de-

termined by two independent tests (Sümbüloğlu & Sümbüloğlu 1998, Kalaycı et al. 2010).

Results

Morphometric data of the mussels used in the study were as follows: weight = 1.092 ± 0.27 g, length = 20.276 ± 2.09 mm, width = 10.13 ± 0.94 mm and height = 9.741 ± 1.07 mm).

Changes in SOD activity were determined in *D. polymorpha* exposed to different Cd concentrations (Fig. 1). Statistically significant differences were detected in SOD activity at the 24th and 96th hours compared to the control ($p < 0.05$). Due to the increase in concentration, the maximum decrease was found to be at 96 hours and 20 µg/L Cd concentration. Concerning the CAT activity (Fig. 2), there was no statistically significant difference of the activities between the control at the 24th and 96th hours experiments ($p > 0.05$) as well as between the increasing concentrations. Changes in GPx activity (Fig. 3) showed a statistically significant increase ($p < 0.05$) after 24 h. However, there was no statistically significant change ($p > 0.05$) in the increasing concentration groups compared to the control during the 96-hour exposure period. Changes in TBARS levels as a marker for lipid peroxidation (LPO) were determined in *D. polymorpha* exposed to different Cd concentrations (Fig. 4). It was determined that TBARS levels increased statistically significantly at both the 24th and 96th hours compared to the control ($p < 0.05$). It increased due to the increase in concentration. The highest increase was detected at 40 µg/L, i.e. in the most concentrated group. Changes

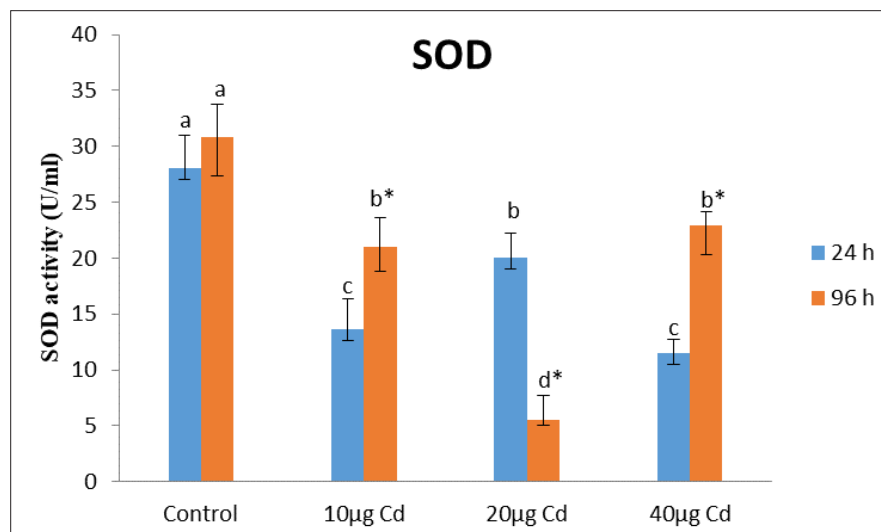


Fig. 1. Effect of Cd on SOD activity in *Deissena polymorpha*. The letters above the bars are statistically significant ($p < 0.05$). Duncan 5% confidence interval.

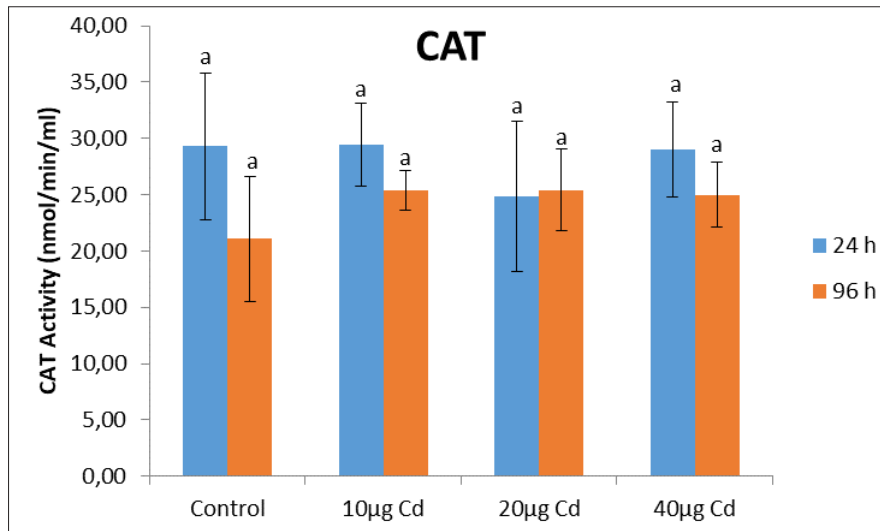


Fig. 2. Effect of Cd on CAT activity in *Deissena polymorpha*. The letters above the bars are statistically significant ($p < 0.05$). Duncan 5% confidence interval.

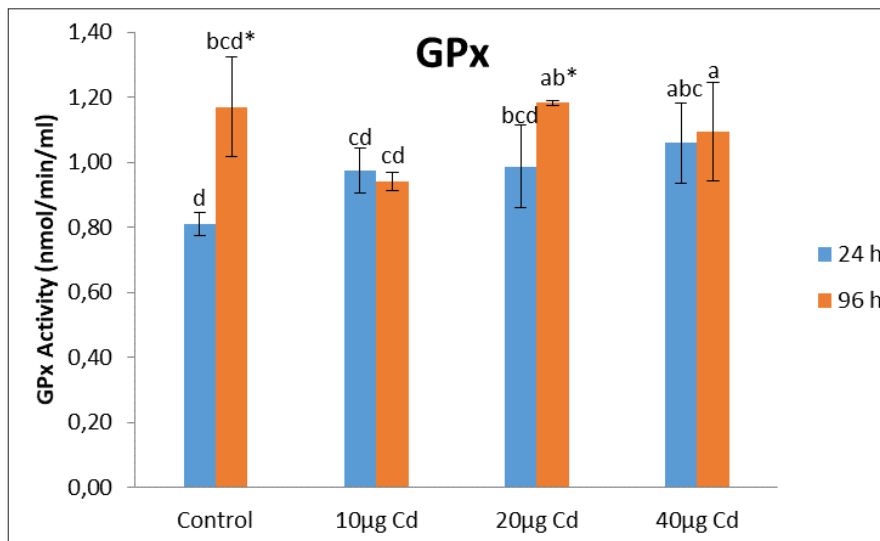


Fig. 3. Effect of Cd on GPx activity in *Deissena polymorpha*. The letters above the bars are statistically significant ($p < 0.05$). Duncan 5% confidence interval.

in GSH levels in *D. polymorpha* exposed to different Cd concentrations (Fig. 5) demonstrated that GSH levels increased statistically significantly at the 24th and 96th hours compared to the control ($p < 0.05$). The highest increase was detected at 40 µg/L Cd (the most concentrated group).

Discussion

Since mussels are filter-feeders, they can accumulate high levels of xenobiotic substances in their tissues. For this reason, they are a group of organisms used as biomarkers in monitoring studies in the field of ecotoxicology. These effects can be observed in

the form of physiological, behavioural, cellular, biochemical and molecular changes (Faggio et al. 2018). Aquatic toxicity studies in this context are conducted to ascertain the concentration at which a contaminant causes harm to aquatic organisms (Karataş 2005).

Pollution in aquatic environments can lead to harmful effects, such as lipid peroxidation by increasing the generation of reactive oxygen species (ROS) due to an imbalance between ROS concentration and the antioxidant defence system (Regoli et al. 2004). The activity of important antioxidant enzymes and the levels of non-enzymatic antioxidants are affected by several individual pollutants

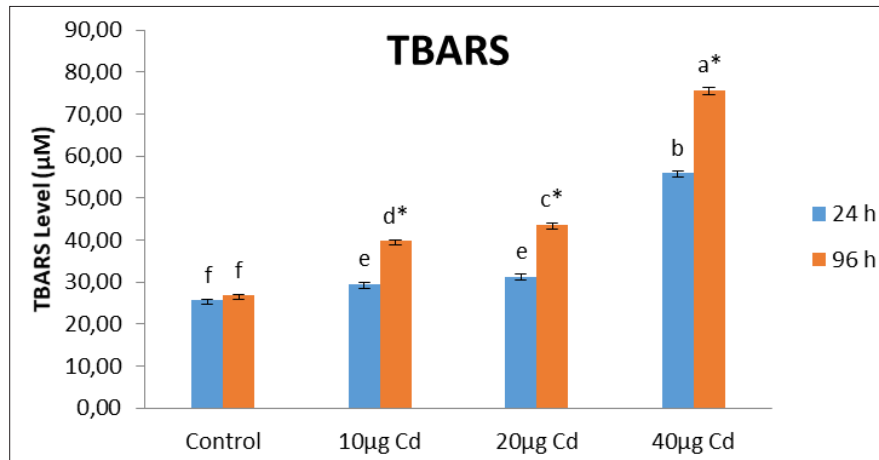


Fig. 4. Effect of Cd on the TBARS levels in *Dreissena polymorpha*. The letters above the bar are statistically significant ($p < 0.05$). Duncan 5% confidence interval.

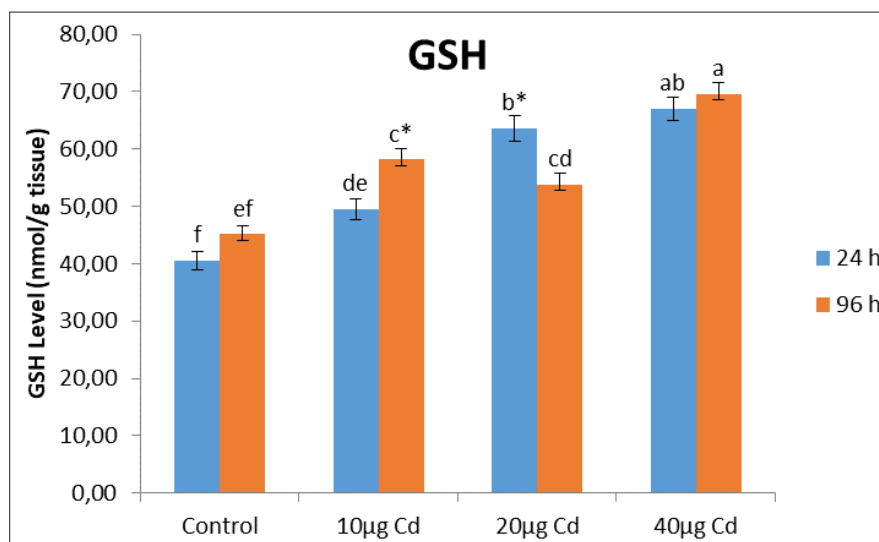


Fig. 5. Effect of Cd on the GSH levels in *Dreissena polymorpha*. The letters above the bar are statistically significant ($p < 0.05$). Duncan 5% confidence interval.

that are known to raise levels of ROS (Valko et al. 2006, Rytter et al. 2007, Serdar et al. 2021). Oxidative stress is defined as the imbalance in the antioxidant defence mechanism of the aerobic organism due to excessive ROS production caused by xenobiotics. Antioxidant and biotransformation enzymes, including SOD, CAT, GPx and Glutathione S-transferase (GST), protect organisms against ROS. Various environmental pollutants can exert toxicity through the induction of oxidative stress.

Lipid peroxidation is a reliable measure of oxidative harm to cellular components and is the initial stage in the degradation of cellular membranes. It is triggered by pesticides, metals and other substances

(Gamble et al. 1995, Regoli et al. 1998). Duman & Kar (2015) reported that the accumulation of Cd in organisms led to an increase in TBARS content, which was dependent on the concentration and period of exposure. Serdar et al. (2019) reported that there was an increase in TBARS levels in the model organism *Gammarus pulex* exposed to Cd with increasing concentration and exposure time. Chandran et al. (2005) examined the methods for altering the TBARS levels of Cd and Zn in *Achatina fulica*; according to their data, the levels of TBARS rose as the concentration of Cd increased.

Batmaz (2019) investigated the biochemical effect of the zinc pyrithione (ZnPT) on the model organ-

ism *Dreissena polymorpha*. He reported that TBARS levels as a marker for lipid peroxidation in the gills and digestive gland were higher in the experimental group than in the control group. It was concluded that ZnPT taken from the water environment caused lipid peroxidation in gill cells. Since the digestive gland is responsible for the detoxification of xenobiotic substances taken into the body, they concluded that lipid peroxidation occurs in the digestive gland cells when the cells are exposed to this substance.

In the present study, we found that the TBARS level in Cd concentration data in *D. polymorpha* increased depending on time and concentration. Similar studies have shown that metals can overproduce ROS through depletion of excess sulfhydryl groups, LPO and DNA damage (Stohs & Bagchi 1995). However, antioxidant enzymes (SOD and GPx) can reduce ROS and, therefore, ultimately reduce oxidative damage, which is consistent with this study. SOD and GPx activities in organisms are significantly stimulated by pollutant exposure (Wang & Wang 2010). In this study, Cd exposure may force *D. polymorpha* to suffer from oxidative stress through ROS excess and the organism may initiate antioxidant systems to counteract this stress in turn. This suggests that lipid peroxidation damage occurs in membrane lipids due to the oxidative stress.

Batmaz (2019) determined that the glutathione (GSH) levels in the digestive gland and gill tissues of the freshwater mussel *Unio mancus eucirrus*, which was exposed to sublethal zinc pyrithione for 96 hours, increased significantly ($p < 0.05$) compared to the control. He stated that the increase in reduced glutathione levels, which is the main antioxidant and cell defence mechanism, showed the negative effect of ZnPT on cellular antioxidant defence mechanisms. During metal exposure, GSH is in an inhibitory state. Moreover, its antioxidant and detoxifier response is faster than enzymes such as SOD, GPx and GST (Wang & Wang 2010). GSH can undertake the initial defence against metal attack by direct complexation with metal or by the participation of GPx or GST in the detoxification process (Sies 1999). Similarly, some authors attribute the toxic effects of Ni to a decrease in cellular GSH and a concomitant increase in GSSG, altering the redox state of cells. Thus, GSH/GSSG may be a suitable biomarker for oxidative stress or injury in biological organisms (Hwang et al. 1992). Moreover, the strong positive correlation of GSH level with the GSH/GSSG ratio suggests that the remarkable depletion of GSH may reflect the oxidative state caused by pollutant attack associated with interfering with the cellular redox state (Wang & Wang 2010).

SOD is a group of metalloenzymes that play a key role in protecting aerobic organisms from the harmful effects of superoxide radicals. SOD catalyses the conversion of superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen (O_2), which is essential for the antioxidant system. This process is crucial for maintaining cellular health and preventing oxidative damage (Kappus 1985, Kohen & Nyska 2002). The study by Cheung et al. (2001) confirmed that the presence of xenobiotics leads to an increase in antioxidant activity. Chandran et al. (2005) examined the enzyme activities of *Achatina fulica* by exposing it to Cd and Zn; they found that there were decreases in the SOD activities of organisms exposed to Cd compared to the control. Duman & Kar (2015) reported that there were significant changes in SOD activity in the *Gammarus pulex* exposed to Cd. Pandey et al. (2008) reported that SOD activity decreased in *Channa punctata* exposed to multiple trace metals applied in increasing concentration groups and compared to the control group. Many studies reported decreases in SOD activity in aquatic organisms exposed to various pollutants (Tutuş 2016, Tunca 2017, Tatar et al. 2018, Serdar et al. 2018). In this study, it was observed that SOD activity decreased in *D. polymorpha* after exposure to Cd and this depended on the concentration increase and the exposure time.

The CAT enzyme is ubiquitous in aerobic organisms. It facilitates the breakdown of hydrogen peroxide, leading to the production of water and oxygen (Chelikani et al. 2004). The upregulation of these antioxidant enzymes is essential for reducing cellular damage (Rajeshkumar et al. 2013). Conversely, the activity of CAT may either rise or fall in polluted surroundings, depending on the specific material present (Sobjak et al. 2017). Prior research has indicated that reactive oxygen species (ROS) can impede catalase (CAT) activity (Kono & Fridovich 1982, Escobar et al. 1996, Duman & Kar 2015). The investigation revealed that the activity of CAT rose as the concentration increased. However, it was determined that this rise was not statistically significant ($p > 0.05$). The antioxidative stress activity can differ based on factors such as gender, physiological stage and species (Felten et al. 2008, Zhang et al. 2011). Nevertheless, it was discovered that the concentration and duration of Cd exposure also modify the activity of antioxidative stress (Duman & Kar 2015). Additionally, it was shown that brief exposure to organic chemical contaminants results in the activation of antioxidant enzymes in aquatic creatures. Nevertheless, the activity of CAT was adversely impacted by compounds that stimulate re-

dox cycling (Pandey et al. 2008, Rajeshkumar et al. 2013). Serdar et al. (2019) reported that statistically significant increases were detected in CAT activity in the model organism *Gammarus pulex* exposed to Cd compared to the control with increasing concentration and exposure time. The study found that the activity of the CAT enzyme was hindered in organisms that were subjected to stress from exposure to Cd. The literature on the activity of this enzyme reveals that potential antioxidant variations can be explained by factors such as species and environments (Gluszczak et al. 2007).

GPx is a constituent of an intricate system that defends against harmful oxidizing agents. Its reaction is probably linked to the reactions of other enzymes and chemicals that scavenge for these harmful agents. However, its activation may serve as a sign of protection against oxidative stress (Tsangaris et al. 2007). The decrease in GPx activity could indicate the ineffectiveness of the antioxidant system when exposed to pollution (Ballesteros et al. 2009) or could be attributed to the direct impact of superoxide radicals or pollutants on the production of the enzyme (Bainy et al. 1993). The study found that the level of Cd exposure in *D. polymorpha* rose as the exposure period and concentration of Cd increased compared to the control group. The observed variations in GPx activity in this study align with the findings by Kutlu & Susuz (2004). Serdar et al. (2019) reported that there was a decrease of GPx activity in *Gammarus pulex* exposed to Cd (compared to the control with increasing concentration and exposure time). Zhang et al. (2011) reported that CAT activity increased with Cd exposure and this increase suppressed the increase in GPx. Wang & Wang (2010) determined the response of GPx activity in the copepod *Tigriopus japonicus* exposed to Ni concentrations; they reported that a significant induction effect occurred with increasing Ni concentration exposure ($p < 0.05$). In this study, statistically significant differences were found in the changes in GPx activity of Cd on *D. polymorpha* compared to the control. The rise in GPx activity can be attributed to the alteration in CAT activity. In this respect, the study is similar to the other mentioned studies.

Organisms show behavioural and physiological responses to pollution in the ecosystem. The situation is more critical for the aquatic environment and aquatic organisms, which are the final stop of all ecosystem pollution. All kinds of physicochemical changes in water affect the vital activities of aquatic organisms such as reproduction, nutrition, shelter and migration. Researching, determining, and eliminating the effects of polluting factors on aquatic or-

ganisms is important for a clean environment and the well-being of aquatic creatures.

In aquatic ecosystem, bivalve molluscs (mussels), which are sediment-dependent sessile organisms, are the most affected animals by the pollution because they are filter-feeders. Metal pollution in water is caused by agricultural and industrial wastes and leaks from old mines. Rainwater also causes metals to leach from the surrounding soil. Metals with the most common pollution effects in studies on aquatic organisms physiology are Cu, Zn, Sn, Cd, Hh, Cr, Pb, Ni, As and Al. While the order of heavy metals in terms of toxic effects in salmon is $Hg \geq Cd > Cu$, the order in terms of accumulation in the body is $Hg \gg Pb > Cr$ and Cd (Atamanalp & Yanık 2003).

According to the present findings, Cd affects the oxidative status of *D. polymorpha*. It was concluded that SOD, CAT and GPx were useful markers in investigating the toxic effects of Cd on the water filter-feeding test organism *D. polymorpha*. The results obtained showed that the response of the test organism to the toxic substance varies with the concentration of the toxic substance and the duration of application.

Conclusion

This study has demonstrated that the exposure to sublethal Cd concentrations of *D. polymorpha*, which is used as an indicator in ecotoxicological evaluations, provoked a response to oxidative stress. It can be concluded that stress conditions provided by Cd exposure at sublethal concentrations evoked specific responses in *D. polymorpha*.

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References

- Asri F. Ö., Sönmez S. & Çıtak S. 2007. Kadmiyumun çevre ve insan sağlığı üzerine etkileri. *Derim* 24 (1): 32-39.
- Atamanalp M. & Yanık T. 2003. Salmonidlerde yapılan toksikolojik çalışmalar. *Atatürk Üniv. Ziraat Fak. Derg* 34 (1): 105-110.
- Bainy A. C. D., Arisi A. C. M., Azzalis L. A., Simizu K., Barros S. B. M., Videla L. A. & Junqueira V. B. C. 1993. Differential effects of short-term lindane administration on parameters related to oxidative stress in rat liver and erythrocytes. *Journal of Biochemical Toxicology* 8 (4):187-194.
- Ballesteros M. L., Wunderlin D. A. & Bistoni M. A. 2009. Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan. *Ecotoxicology and Environmental Safety* 72 (1): 199-205.

- Batmaz G. 2019. Zinc pyrithione'un iki tür tatlı su bivalvi üzerine ekotoksikolojik etkileri. Yüksek Lisans Tezi. Gazi Üniversitesi Biyoloji Anabilim Dalı. Ankara. 73 s.
- Bobat A., Hengirmen O. M. & Zapletal W. 2001. Tatlısu Ekosisteminde Teknik, Ekonomik ve Ekolojik bir Zararlı: Zebra Midye. Kırsal Çevre Yılığ 112-127.
- Chandran R., Sivakumar A. A., Mohandass S. & Aruchami M. 2005. Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica*. Comparative Biochemistry and Physiology, Series C. 140 (3-4): 422-426.
- Chelikani P., Fita I. & Loewen P. C. 2004. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences 61 (2): 192-208.
- Cheung C. C. C., Zheng G. J., Li A. M. Y., Richardson B. J., Lam P. K. S. 2001. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. Aquatic Toxicology 52 (34): 189-203.
- Cope W. G., Wiener J., Steingraeber M. T. & Atchison G. J. 1994. Cadmium, metal-binding proteins, and growth in bluegill (*Lepomis macrochirus*) exposed to contaminated sediments from the upper Mississippi River basin. Canadian Journal of Fish and Aquatic Science 51: 1356-1367.
- Dimitrova M. S., Tishinova V. & Velcheva V. 1994. Combined effect of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (*Cyprinus carpio*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 108 (1): 43-46. [https://doi.org/10.1016/1367-8280\(94\)90087-6](https://doi.org/10.1016/1367-8280(94)90087-6)
- Dorval J. & Hontela A. 2003. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Toxicology and Applied Pharmacology 192 (2): 191-200.
- Duffus J. H. 1980. Environmental toxicology. London: Edward Arnold, 164 p.
- Duman F. & Kar M. 2015. Evaluation of effects of exposure conditions on the biological responses of *Gammarus pulex* exposed to cadmium. International Journal of Environmental Sciences and Technology 12: 437-444.
- Escobar J. A., Rubio M. A. & Lissi E. A. 1996. SOD and catalase inactivation by singlet oxygen and peroxy radicals. Free Radical Biology and Medicine 20 (3): 285-290.
- Faggio C., Tsarpali V. & Dailanis S. 2018. Mussel digestive gland as a model tissue for assessing xenobiotics: An overview. Science of the Total Environment 636: 220-229.
- Felten V., Charmantier G., Mons R., Geffard A., Rousselle P., Coquery M., Garric J. & Geffard O. 2008. Physiological and behavioural responses of *Gammarus pulex* (Crustacea: Amphipoda) exposed to cadmium. Aquatic Toxicology 86 (3): 413-425.
- Gamble S. C., Goldfarb P. S., Porte C. & Livingstone D. R. 1995. Glutathione peroxidase and other antioxidant enzyme function in marine invertebrates (*Mytilus edulis*, *Pecten maximus*, *Carcinus maenas* and *Asterias rubens*). Marine Environmental Research 39 (1-4): 191-195.
- Glass M., Sutherland M. W., Forman H. J. & Fisher A. B. 1985. Selenium deficiency potentiates paraquat-induced lipid peroxidation in isolated perfused rat lung. Journal of Applied Physiology 59 (2): 619-622.
- Gluszczak L., dos Santos Miron D., Moraes B. S., Simões R. R., Schetinger M. R. C., Morsch V. M. & Loro V. L. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comparative Biochemistry and Physiology, Series C 146 (4): 519-524.
- Hwang C., Sinskey A. J. & Lodish H. F. 1992. Oxidized redox state of glutathione in the endoplasmic reticulum. Science 257: 1496-1502.
- Kalaycı Ö. A., Cömert F. B., Hazer B., Atalay T., Cavicchi K. A. & Cakmak M. 2010. Synthesis, characterization, and antibacterial activity of metal nanoparticles embedded into amphiphilic comb-type graft copolymers. Polymer Bulletin 65: 215-226.
- Kappus H. 1985. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. Oxidative Stress, pp. 273-310.
- Karataş M. 2005. Balık biyolojisi araştırma yöntemleri. Ankara: Nobel Yayınları, 498 p.
- Katalay S. & Parlak H. 2002. Su kirliliğinin, *Gobius niger* Linn., 1758 (Pisces: Gobiidae)'in kan parametreleri üzerine etkileri. E.Ü. Su Ürünleri Dergisi 19 (12): 115-121.
- Kohen R. & Nyska A. 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods of their quantification. Toxicological Pathology 30 (6): 620-650.
- Kono Y. & Fridovich I. 1982. Inhibition of catalase by superoxide radicals. Journal of Biological Chemistry 257: 5751-5754.
- Kutlu M. & Susuz F. 2004. Effects of lead as an environmental pollutant on EROD enzyme in *Gammarus pulex* (L.) (Crustacea: Amphipoda). Bulletin of Environmental Contamination and Toxicology 72(4): 750-755.
- Nishiyama Y., Ikeda H., Haramaki N., Yoshida N. & Imaizume T. 1998. Oxidative stress is related to exercise intolerance in patients with heart failure. American Heart Journal 135: 115-120.
- Novelli E. L., Vieira E. P., Rodrigues N. L. & Ribas B. O. 1998. Risk assessment of cadmium toxicity on hepatic and renal tissues of rats. Environmental Research 79 (2): 102-105.
- Nriagu J. O., Wong H. K., Lawson G. & Daniel P. 1998. Saturation of ecosystems with toxic metals in Sudbury basin, Ontario, Canada. Science of the Total Environment 233: 99-117.
- Pandey S., Parvez S., Ansari R. A., Ali M., Kaur M., Hayat F., Ahmad F. & Raisuddin S. 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chemo-Biological Interactions 174 (3): 183-192.
- Phillips D. J. H. 1980. Quantitative aquatic biological indicators. London: Applied Science Publishers, Pollution Monitoring Series.
- Rajeshkumar S., Mini J. & Munuswamy N. 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milkfish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. Ecotoxicology and Environmental Safety 98: 8-18.
- Regoli F., Nigro M. & Orlando E. 1998. Lysosomal and antioxidant responses to metals in the Antarctic scallop *Adamusium colbecki*. Aquatic Toxicology 40 (4): 375-392.
- Regoli F., Frenzilli G., Bocchetti R., Annarumma F., Scarcelli V., Fattorini D. & Nigro M. 2004. Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a

- field translocation experiment. *Aquatic Toxicology* 68 (2): 167-178.
- Ryter S. W., Kim H. P., Hoetzel A., Park J. W., Nakahira K., Wang X. & Choi A. M. 2007. Mechanism of cell death in oxidative stress. *Antioxidants and Redox Signaling* 9 (1): 49-89.
- Serdar O. 2021. Determination of the effect of cyfluthrin pesticide on zebra mussel (*Dreissena polymorpha*) by Some Antioxidant Enzyme Activities. *Journal of Anatolian Environmental and Animal Sciences* 6 (1): 77-83.
- Serdar O., Yildirim N. C., Tatar S., Yildirim N. & Ogedey A. 2018. Antioxidant biomarkers in *Gammarus pulex* to evaluate the efficiency of electrocoagulation process in landfill leachate treatment. *Environmental Science and Pollution Research* 25: 12538-12544.
- Serdar O., Yildirim N.C., Tatar Ş. & Yildirim N. 2019. Kurşuna Maruz Bırakılan *Gammarus pulex*'de Antioksidan Enzim Yanıtları. *Journal of Anatolian Environmental and Animal Sciences* 4 (2): 216-220.
- Serdar O., Aydın R. & Çalta M. 2021. Determination of some biochemical parameters changes in *Gammarus pulex* exposed to Cadmium at different temperature and different concentration. *Journal of Limnology and Freshwater Fisheries Research* 7 (1): 69-76.
- Shen H. M. & Sangiah S. 1995. Na⁺, K⁺-ATPase, glutathione and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice. *Archives of Environmental Contamination and Toxicology* 29: 174-179.
- Sies H. 1999. Glutathione and its role in cellular functions. *Free Radical Biology and Medicine* 27 (9-10): 916-921.
- Sobjak T. M., Romão S., do Nascimento C. Z., dos Santos A. F. P., Vogel L. & Guimarães A. T. B. 2017. Assessment of the oxidative and neurotoxic effects of glyphosate pesticide on the larvae of *Rhamdia quelen* fish. *Chemosphere* 182: 267-275.
- Sümbüloğlu K. and Sümbüloğlu V. 1998. *Biostatistics*. Ankara, Hatiboğlu Yayınevi, pp. 76-86.
- Stohs S. J. & Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine* 18 (2): 321-336.
- Tatar Ş., Cıkcıkoglu Yildirim N., Serdar O., Yildirim N. & Ogedey A. 2018. The using of *Gammarus pulex* as a biomonitor in ecological risk assessment of secondary effluent from municipal wastewater treatment plant in Tunceli, Turkey. *Human and Ecological Risk Assessment: An International Journal* 24 (3): 819-829.
- Tsangaris C., Papathanasiou E., Cotou E. 2007. Assessment of the impact of heavy metal pollution from a ferronickel smelting plant using biomarkers. *Ecotoxicology and Environmental Safety* 66 (2): 232-243.
- Tunca H. 2017. Bazı Pestisitlerin *Arthrospira platensis* M2 Alginin Gelişimi ve Antioksidan Parametreler Üzerine Etkisi. Sakarya Üniversitesi, Fen Bilimleri Enstitüsü
- Tutuş R. 2016. *Oreochromis Niloticus*'un Karaciğer Dokusundaki Antioksidan Sistemler ve Lipid Peroksidasyonu Üzerine Chlorpyrifos, Emamectin Benzoate ve Abamectin Türü Pestisitlerin Etkileri. Adıyaman Üniversitesi, Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı.
- UNEP 1993. Guidelines for monitoring chemical contaminants in the sea using marine organisms, reference method for marine pollution studies.
- Valko M., Rhodes C. J., Moncol J., Izakovic M. & Mazur M. 2006. Free radicals, metals and antioxidants in oxidative stress induced cancer. *Chemico-Biological Interactions* 160 (1): 1-40.
- Vutukuru S. S., Chintada S., Radha Madhavi K., Venkateswara Rao J. & Anjaneyulu Y. 2006. Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. *Fish Physiology and Biochemistry* 32: 221-229. <https://doi.org/10.1007/s10695-006-9004-x>
- Wang M. & Wang G. 2010. Oxidative damage effects in the copepod *Tigriopus japonicus* Mori experimentally exposed to nickel. *Ecotoxicology* 19 (2): 273-284.
- Zhang Y., Sun G., Yang M., Wu H., Zhang J., Song S., Ma E. & Guo Y. 2011. Chronic accumulation of cadmium and its effects on antioxidant enzymes and malondialdehyde in *Oxya chinensis* (Orthoptera: Acridoidea). *Ecotoxicology and Environmental Safety* 74(5): 1355-1362.

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