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Research Article
Araştırma Makalesi

Determination of Oxidative Stress Responses in *Dreissena polymorpha* Exposed to Rare Earth Elements (Terbium, Lanthanum, Gadolinium and Praseodymium) with Temperature

Sıcaklıkla Birlikte Nadir Toprak Elementlerine (Terbium, Lantan, Gadolinyum ve Praseodimyum) Maruz Bırakılan *Dreissena polymorpha*'da Oksidatif Stres Tepkilerinin Belirlenmesi

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Abstract: The continuous development of industry pushes people to search for new resources, and for this reason, the usage areas of Rare Earth Elements (REEs) are increasing day by day. Increasing concentrations of REEs, as a result of increased use, create pollution in the environment and harm living organisms. This pollution interacts with increasing temperature and causes more negative synergistic effects of the pollutant in the environment and in the living body. In this study, sublethal concentration values were determined by literature review and the concentration value was determined as 125 mg/L. In the present study tt was aimed to investigate some oxidative stress and antioxidant responses of Terbium, Lanthanum, Gadolinium and Praseodymium REEs in *Dreissena polymorpha* at 125 mg/L concentration at 3 different temperatures (16, 18, 20 ^{0}C) with biomarkers. For this purpose, 24 and 96 hour experimental trial design was created and 7 *D. polymorpha* were used in each trial group, and the application experiments were carried out with 3 replications. The samples at the end of the experimental phase were stored at -80 degrees Celsius until they were analyzed. In this study, Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme activities and glutathione (GSH) and Thiobarbituric acid (TBARS) level biomarker responses were determined by ELISA test microplate reader. CAYMAN brand SOD (Catalog No 706002), CAT Catalog No 707002) and GPx (Catalog No 703102), GSH (Catalog No 703002) and TBARS (Catalog No 10009055) were used in the study. SPSS 24.0 package program one-way ANOVA (Duncan 0.05) was used for the evaluation of biochemical analyzes. According to the study data, statistically significant decreases were observed in SOD and CAT activities in the oxidative stress responses of REEs on *D. Polymorpha* with increasing temperature, while there was no significant change in GPx activities. It was determined that there were increases in TBARS levels and decreases in GSH levels. It is thought that the temperature factor, application concentration and application time are effective in the formation of these changes. It can be said that temperature change and pollutants cause oxidative stress in organisms and cause cell damage.

Keywords
- *Dreissena polymorpha*
- Temperature
- Oxidative stress
- Rare Earth Elements

Özet: Sanayi ve endüstrinin sürekli gelişmesi insanları yeni kaynak arayışlarına itmekte ve bu amaçla kullanım alanları her geçen gün artan Nadir Toprak Elementlerinin (NTE) kullanım alanları hızla artmaktadır. Artan kullanım sonucu NTE'lerin artan konsantrasyonları çevrede kirlilik yaratmakta ve canlı organizmalara zarar vermektedir. Bu kirlilik artan sıcaklıkla etkileşime girerek kirleticinin çevrede ve

Anahtar kelimeler
- *Dreissena polymorpha*
- Sıcaklık

canlı vücudunda daha olumsuz sinerjik etkilere neden olmaktadır. Bu çalışmada, subletal konsantrasyon değerleri literatür taraması ile belirlenmiş ve konsantrasyon değeri 125 mg/L olarak tespit edilmiştir. *Dreissena polymorpha*'da Terbium, Lanthanum, Gadolinium ve Praseodymium NTE'lerin 125 mg/L konsantrasyonda 3 farklı sıcaklıkta (16, 18, 20 0C) bazı oksidatif stres ve antioksidan tepkilerinin biyobelirteçler ile araştırılması amaçlanmıştır. Bu amaçla 24 ve 96 saatlik deneme deseni oluşturularak her deneme grubunda 7 adet *D. polymorpha* kullanılmış ve uygulama deneyleri 3 tekrarlı olarak gerçekleştirilmiştir. Deneme aşaması biten örnekler analiz edilene kadar -80 derecede muhafaza edilmiştir. Bu çalışmada Süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPx) enzim aktiviteleri ile glutatyon (GSH) ve Tiyobarbitürik asit (TBARS) düzeyi biyobelirteç yanıtları ELISA testi mikroplaka okuyucu ile belirlenmiştir. Çalışmada CAYMAN marka SOD (Katalog No 706002), CAT Katalog No 707002) ve GPx (Katalog No 703102), GSH (Katalog No 703002) ve TBARS (Katalog No 10009055) kullanılmıştır. Biyokimyasal analizlerin değerlendirilmesi için SPSS 24.0 paket programı tek yönlü ANOVA (Duncan 0.05) kullanılmıştır. Çalışma verilerine göre, *D. Polymorpha* üzerinde NTE'lerin oksidatif stres tepkilerinde artan sıcaklıkla birlikte SOD ve CAT aktivitelerinde istatistiksel olarak anlamlı düşüşler gözlenirken, GPx aktivitelerinde anlamlı bir değişiklik olmamıştır. TBARS seviyelerinde artışlar, GSH seviyelerinde ise düşüşler olduğu tespit edilmiştir. Bu değişikliklerin oluşumunda sıcaklık faktörü, uygulama konsantrasyonu ve uygulama süresinin etkili olduğu düşünülmektedir. Sıcaklık değişiminin ve kirleticinin organizmalarda oksidatif strese neden olduğu ve hücre hasarına yol açtığı söylenebilir.

- Oksidatif stres
- Nadir Toprak Elementleri

1.INTRODUCTION

Rare Earth Elements (REE) are considered as strategic elements because they are used in many different sectors in the production of advanced technological materials resistant to high temperature, abrasion and corrosion (Celep et al., 2021). It consists of 15 elements of the lanthanide group with similar chemical properties, a total of 17 elements including scandium and yttrium by Krishnamurthy and Gupta (2016). REEs are widely used in the production of many advanced technological devices (such as mobile phones, computers, TVs), rechargeable batteries (NiMH batteries), modern medical devices (such as MRI equipment), catalytic converters, engines (aircraft, hybrid vehicles, wind turbines), glass and ceramics, oil refinery, solar panels (Binnemas et al., 2013; Krishnamurthy & Gupta 2016; USGS, 2020). Terbium (Tb) is one of the rare earth elements, although it is still twice as much as silver in the earth's crust. It cannot be found in nature as a free element, but is found in many minerals. Gadolinium (Gd) is used in control rods for nuclear reactors and nuclear power plants. It is used to make garnet for microwave applications, and its compounds are used to make phosphorus for color TV tubes. Praseodymium (Pr) metal darkens slowly in air, forming a green oxide layer that flakes off like iron rust. It reacts slowly with most acids and cold water, more quickly with hot water (URL 1, 2023).

There are several release pathways through which anthropogenically derived REE can enter the aquatic environment or be transferred across environmental segments where they can have potentially adverse effects on organisms and ecosystems. Researchers investigating such potential adverse effects have reported a range of effects including changes in survival, reproduction and growth rates in freshwater zooplankton, echinoderms and fish, as well as changes in neural and cardiac activities in embryonic development (Blaise et al., 2018; Cui et al,. 2012; Dubé et al., 2019; Lürling & Tolman 2010; Zhao et al., 2021). These effects can be attributed to cellular inhibition, homeostasis, Ca^{2+} signaling and alteration of gene transcription involved in DNA repair processes. Chronic exposure to REE may adversely affect hepatic, respiratory and neural functions. They can affect a range of organisms starting from the most primitive living things in the environment to more evolved organisms such as humans. REE may be released into the environment as particulate matter or dust during processing and use. They enter the aquatic environment as a result of atmospheric transport and precipitation through urban and industrial wastewater flow, rivers, groundwater seepage (Olmaz et al., 1991;; Klaver et al., 2014; Morgan et al., 2016; Brito et al., 2018; Trifuoggi et al., 2018).

For many aquatic organisms, temperature is an important environmental variable that can affect physiological mechanisms at enzymatic and cellular levels and cause changes in metabolic rates (Cairns et al., 1975; Ward & Stanford 1982). Such temperature effects can alter an organism's ability to detoxify xenobiotics by altering pollutant uptake, elimination or biotransformation rates, ultimately affecting toxicokinetic and toxicodynamic processes and toxicity (Hooper et al., 2013). Water bodies can harbor an increasing number of agricultural and industrial chemicals that can disrupt free radical processes taken up by organisms. Uptake of these pollutants by hydrobionts can occur from water, sediments, suspended particulate matter, and food sources. Aquatic organisms also have special systems for the production and breakdown of free radicals. Current knowledge and recent developments in general toxicology and especially in the toxicology of hydrobionts provide a fertile field for aquatic toxicology studies (Lushchak 2011).

Reactive oxygen species (ROS) are an indispensable part of aerobic life. Steady-state concentrations are a balance between production and elimination providing a certain steady-state level of ROS. The dynamic balance can be disrupted, leading to increased levels of ROS and damage to cellular components called "oxidative stress". Changes in temperature, oxygen levels, and salinity can cause stress in natural and artificial conditions by inducing an imbalance between ROS production and elimination (Serdar et al., 2024a). Catalase (CAT) is one of the antioxidant enzymes and has been implicated as an essential defense against the potential toxicity of superoxide anions such as the hydroxyl free radical. Therefore, it is thought to act as a cellular defense against the potentially harmful effects of the superoxide anion produced by a wide variety of biological reactions (David et al., 2008). Superoxide dismutase (SOD) is an important antioxidant enzyme that catalyzes the conversion of superoxide to oxygen and hydrogen peroxide in aerobic organisms (Kim et al., 2011). Among the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GSH) have been widely used as effective biomarkers of environmental contamination in aquatic organisms and have been identified as effective protective barriers against ROS formation (Figueiredo et al., 2018). Depletion has an imbalance in the redox state and ability to cope with organic xenobiotics metabolized by glutathione S-transferase (GST) and glutathione peroxidases (GPx) (Aydın & Serdar, 2024). Lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS), has been frequently used as a marker of oxidative stress in response to different environmental pollutants in various studies (Roméo et al., 2019; Serdar, 2019; Choi & Oris 2000; Oakes & Van Der Kraak 2003; Almroth et al. 2005).

In the examination of pollution in aquatic ecosystems, the longevity, limited mobility and non-selective filter feeding of freshwater mussels ensure their widespread and reliable use in toxicological studies with biomarkers (Serdar et al., 2021). *D. polymorpha* may cause the imbalance of the very sensitive food chain to deteriorate and the aquatic ecosystem may be adversely affected by them (Serdar 2021). In addition, the sticking behavior of mussels to hard surfaces also causes problems. In addition, bivalve mollusks are susceptible to heat stress and water quality due to their sedentary lifestyle, inability to regulate body temperature and bioaccumulation of pollutants (Serdar et al., 2024b). Since *D. polymorpha* has a strong oxidative defense and a relatively high resistance to xenobiotics, it is widely used to conduct ecotoxicological experiments (Faria et al., 2009). The invasive behavior of the zebra mussel is seen as a disadvantage in its widespread use. This apparent disadvantage may represent one of the important reasons to ensure the conservation of native species by sampling *D. polymorpha*, which is both invasive and widely used in biomonitoring and toxic impact assessment studies (Binelli et al., 2015).This makes them excellent watchdogs for ecosystem health in freshwater ecosystems and also good model organisms for studying the interactive effects of temperature and pollution stress in the field (Negri et al., 2013).

In this study, it is aimed to examine the oxidative stress responses of SOD, CAT, GPx activities and TBARS and GSH levels in living organisms as a result of the application of REEs in mixed form to *D. Polymorpha* individuals with increasing temperature.

2.MATERIAL METHOD

2.1.Model Live Supply and Adaptation

D. polymorpha individuals were collected from the Euphrates River (38º 48 '25 ”N, 38º 43' 51” E). The organisms were quickly brought to the laboratory in plastic bottles. Before being used in the experiments, stock ponds were created by feeding microalgae in 500 L aerated reinforced tanks for at least 30 days in an environment similar to natural living conditions in a 12:12 hours light:dark cycle for adaptation to laboratory conditions. Healthy-appearing organisms at similar developmental stages were selected for the experimental study and were not fed during the experimental study.

2.2.Experiment Design

Model organisms were exposed to a 1:1:1:1 mixture of REE (Tb, Gd, La, Pr) at 3 different temperatures (16, 18, 20℃) and 125 mg/L concentrations for 24 and 96 hours. In particular, the global average surface temperature is expected to increase from 1.0 °C to 5.7 °C by the end of this century, depending on different CO_2 emission scenarios (IPCC 2022). In line with this information given in the literature, the study was designed by determining temperature values (16, 18, 20℃) close to the specified temperature averages.

125 mg/L Mix REE at 16 ^{o}C
125 mg/L Mix REE at 18 ^{o}C
125 mg/L Mix REE at 20 ^{o}C

Experiments were carried out with 3 replications.

2.3.Biochemical response

All application experiments were carried out with 3 repetitions and 7 *D. polymorpha* were used for each experimental group. Samples were taken from each group at 24 and 96 hours, and the soft tissues of the *D. polymorpha* individuals were collected by dissection, following the collection process they were stored at -80 degrees until they were analyzed. In this study, SOD, CAT and GPx enzyme activities and TBARS and GSH levels were determined with CAYMAN kits and ELISA test microplate reader to determine biochemical responses (Aydın & Serdar, 2023). CAYMAN brand SOD (Catalog No 706002), CAT Catalog No 707002) and GPx (Catalog No 703102), GSH (Catalog No 703002) and TBARS (Catalog No 10009055) were used in the study. The kits used in the study were purchased from CAYMAN.

2.4.Dissection Procedures and Preparation of Supernatants

Test organism individuals were separated from their shells with the help of scalpel and forceps. An average of 0.5 g from each organism was carefully weighed, then placed into 1:2 PBS (phosphate-buffered saline) buffer and homogenized with DAIHAN brand ultra turrax homogenizer while keeping everything on ice. The homogenized samples were then centrifuged at 4°C, at 17000 rpm for 15 minutes. The resulting supernatants were kept at -80 °C until the measurement was performed.

2.5.Statistical Analysis

SPSS 24.0 package program one-way ANOVA (Duncan 0.05) was used for the evaluation of biochemical analyzes.

3.RESULTS

3.1.Determination of Biochemical Response TBARS Level

Time-dependent TBARS levels at increasing temperature values at 125 mg/L concentrations of Mix REE are given in Figure 1. It was determined that the increases in TBARS levels in all exposure groups due to increasing temperatures compared to the control group were statistically significant ($p<0.05$).

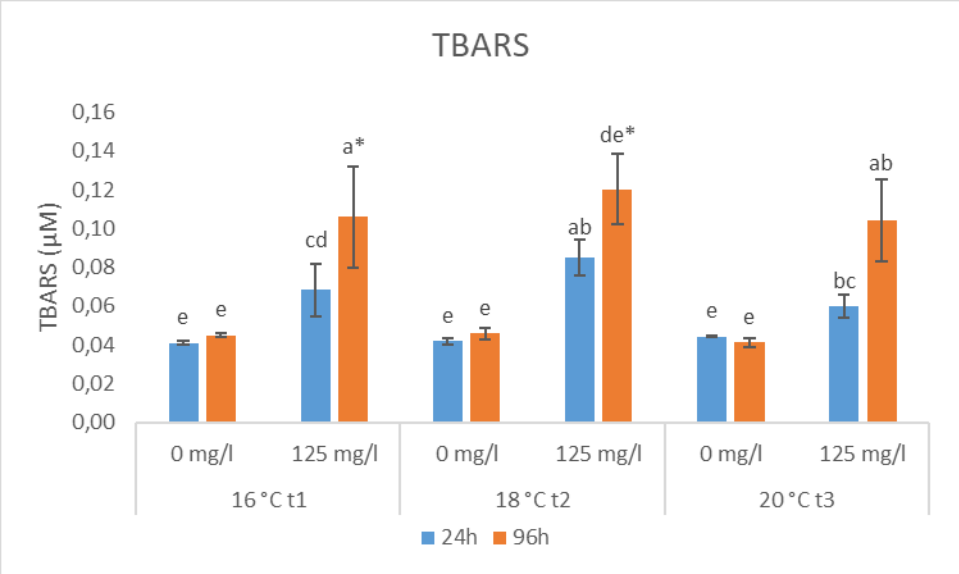

Figure 1: TBARS (µM tissue) levels of *D. polymorpha* exposed to Mix REE, different letters of the bar are statistically significant ($p<0.05$).

3.2.GSH Level

The time-dependent GSH levels of REE mixture at increasing temperatures at a concentration of 125 mg/L are given in Figure 2. While a statistically significant decrease was observed in GSH levels with 125 mg/L REE mixture at 16 and 20 ^{0}C compared to the control groups ($p<0.05$), no significant difference was observed at 18^{0}C ($p>0.05$).

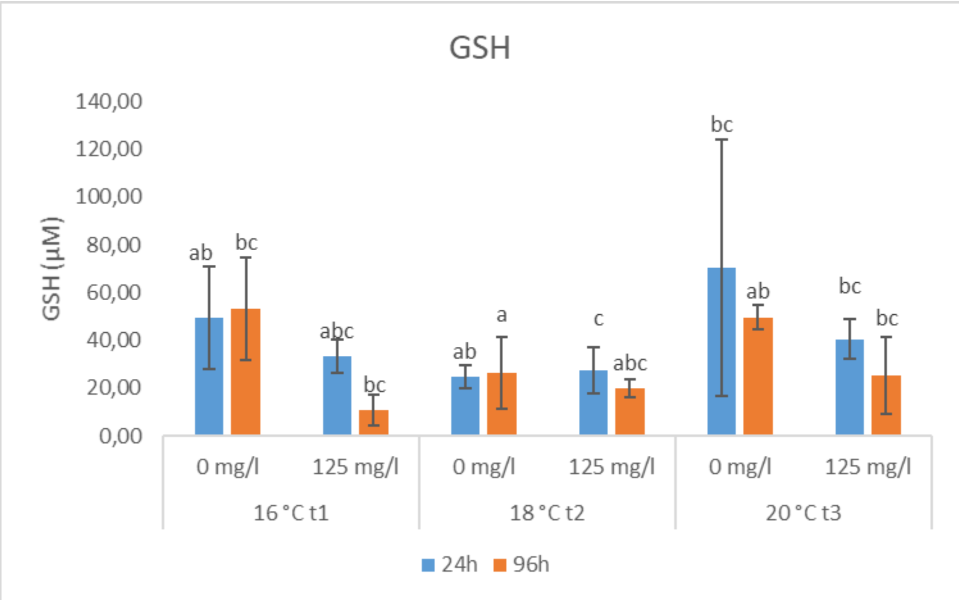

Figure 2: GSH (µM tissue) levels of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant ($p<0.05$).

3.3.CAT Activity

CAT activities in *D. polymorpha* exposed to 125 mg/L concentration of REE mixture and time dependent increase for tested temperatures are given in Figure 3. While there was a decrease in CAT activities at 16 ^{0}C in 24 hours, the decrease at the 96th hour was not statistically significant ($p>0.05$). While there was a decrease in CAT activities at 16 ^{0}C in 24 hours, the decrease at the 96th hour was not statistically significant ($p>0.05$).

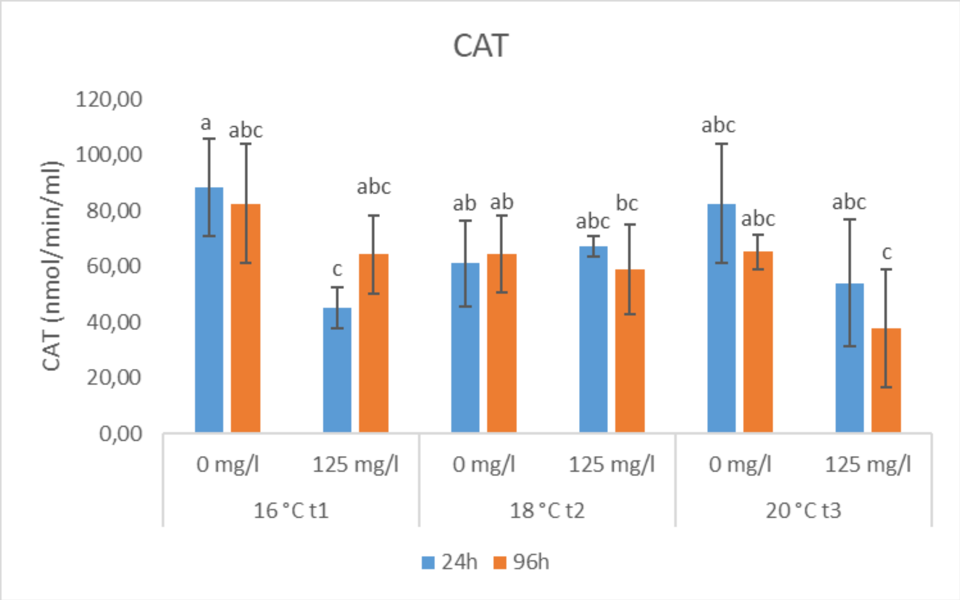

Figure 3: CAT (nmol/min/ml) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant (p<0.05).

3.4.GPx Activity

GPx activities in *D. polymorpha* exposed to 125 mg/L of REE mixture at different temperatures over time are given in Figure 4. It was stated that the changes in all temperature groups after 24 and 96 hours compared to the control group were not statistically significant (p>0.05).

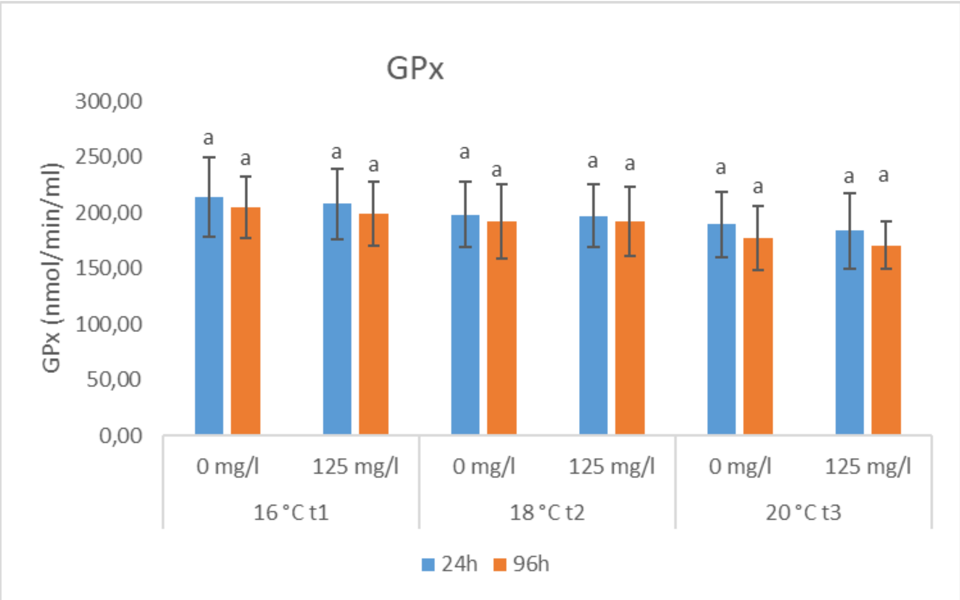

Figure 4: GPx (nmol/min/ml) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant (p<0.05).

3.5.SOD Activity

Time dependent SOD activities at increasing temperature ratios at 125 mg/L of REE mixture are given in Figure 5. The reductions in SOD activities at 16, 18 and 20 ^{o}C temperatures compared to the control group were statistically significant (p<0.05).

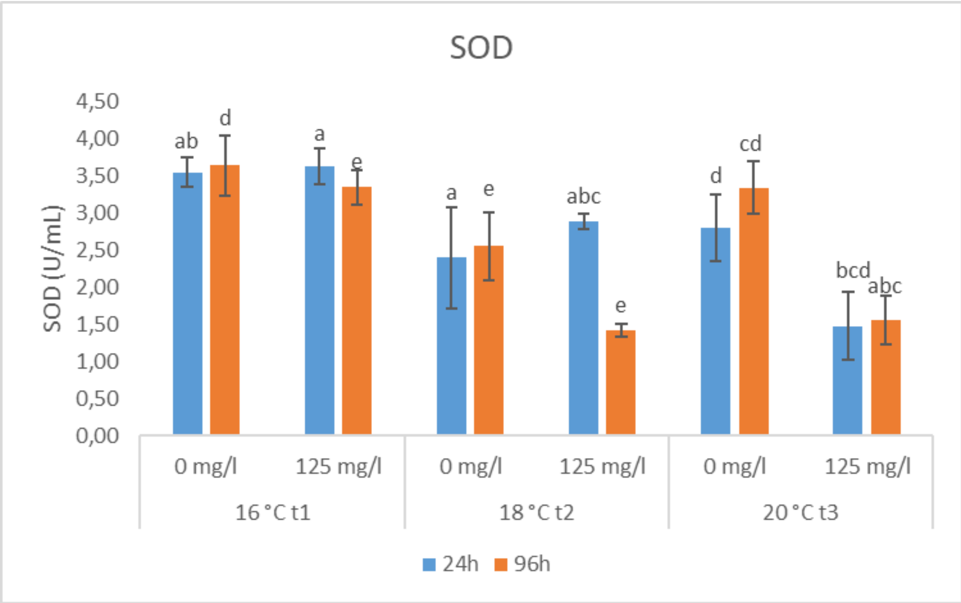

Figure 5: SOD (U/mL) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant (p<0.05).

4.DISCUSSION AND CONCLUSION

Many researchers have contributed to the literature with their studies investigating the effects of various pollutants on the environment as a result of the pollutants' interaction with increasing temperature. Vergauwen et al. 2013, they exposed zebrafish acclimated to 12, 18, 26 (standard temperature) and 34 °C to 5 µM cadmium for 4 or 28 days at the respective adaptation temperature and reported that oxidative stress parameters increased and mortality rates increased depending on the temperature. Abdel-Tawwab et al., 2017 examined the oxidative stress responses of Nile tilapia by co-exposing them to 0.0 or 0.5 mg Cd/L for 8 weeks at 20, 24, 28 and 32°C and they noted that SOD, CAT, GPx and GST activities were significantly induced due to Cd exposure and water temperatures reflecting the direct effect of Cd as a cell signaling molecule. Gholamhosseini et al., 2023 investigated the physiological response of the freshwater crayfish *Astacus leptodactylus* exposed to polyethylene microplastics at different temperatures (17 and 22 °C) and observed increases in SOD and CAT activities as a result. Zhang et al., 2023 investigated the antioxidant system against the combined effects of ammonia and temperature in *Procambarus clarkii* in their study and stated that the interaction between ammonia and temperature was significant in SOD, GPX, but not significant in CAT. Figueiredo et al., 2022 examined the single and combined ecotoxicological effects of ocean warming (15 and 19℃) on lanthanum exposure in *Spisula solida*, and stated that there were decreases in SOD, GPx and CAT activities as a result. It is thought that the changes in the enzyme activities that occur with REE mixture and increasing temperature in *D. polymorpha* are caused by the temperature values and concentration, and the results are thought to be in parallel with the studies in the literature.

Oxidative stress develops due to excessive accumulation of reactive oxygen species (ROS). It controls the physiological and chemical events that perform roughly all biotic and abiotic stresses (Demidchik, 2015). The role of various REEs in the redox imbalance leading to oxidative stress has been demonstrated in a number of independent studies in both plant and animal models, and many REEs have been reported to cause oxidative stress (Tseng et al., 2012; Wang et al., 2012; Zhao et al., 2013). Verlecar et al., 2007 they investigated the biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and heat, and as a result, they stated that there were increases in TBARS levels and increases in SOD and CAT activities. Banni et al., 2004 examined the biomarker responses in *Mytilus galloprovincialis* exposed to nickel and heat stress in their study and stated that as a result, CAT, SOD and GST levels increased significantly compared to the control. Park et al., 2020 examined the antioxidant defense system responses of cadmium and high temperature combined stressors in zebrafish (*Danio rerio*) embryos and stated that there was an increase in SOD and CAT activities. Ihunwo et al., 2022 investigated the oxidative stress responses of young *Oreochromis niloticus* to some heavy metals under the simulation of increasing temperature and stated that there were decreases in GSH level, SOD and GPx activity. Mlouka et al., 2019 examined the biological responses of *M. galloprovincialis* to copper with increasing temperature and stated that there were increases in CAT and SOD activity. Lannig et al., 2006 investigated the co-effects of temperature and cadmium in *Crassostrea virginica* and determined decreases in GSH levels as a result. Additional stressors such as pollution may further sensitize mollusks to temperature-induced oxidative stress Falfushynska et al., 2014 Therefore, increased temperature and combined exposure to REE mixture are thought to affect the antioxidant capacity of *D. polymorpha* and caused differences in oxidative stress responses.

The increase in intracellular ROS due to HO overproduction was associated with a decrease in CAT expression (Venkatesan et al., 2006). Dubé et al., 2019 investigated the effect of 7 different REEs on the *Rainbow trout* and found that Yttrium (Y), Samarium (Sm), Erbium (Er) and Gadolinium (Gd) were the most toxic elements in fish, CAT and GST in Ce. They stated that its activity was down-regulated, and the most sensitive for the 7 elements examined were HSP72, GST, CYAP1A1, GADD45 and SOD for Y, Nd, Ce, Gd, Sm, La and Er, respectively. Hanana et al., 2021a five (cerium (Ce, 280 μg/L), lanthanum (La, 140 μg/L), neodymium (Nd, 120 μg/L), praseodymium (Pr, 28 μg/L) in *Oncorhynchus mykiss* and samarium (Sm, 23 μg/L) rare earth elements, investigated the toxicity of the mixture state and stated that there were increases in CAT activities at all concentrations of the mixture, SOD activity was not affected and GSH levels increased. Liu et al., 2023 examined the effects of neodymium in zebrafish and stated that CAT activity decreased. Hanana et al., 2021b examined the biomarkers of *rainbow trout* exposed to dysprosium (Ds) and lutetium (Lu), and there were significant changes in Dy exposure, CAT and SOD activity compared to controls. They stated that exposure to Lu was lower than control, and best differentiated it from SOD, CAT and MT. Andrade et al., 2023 examined the effect of yttrium (Y) in *Mytilus galloprovincialis* and observed decreases in CAT activity. Figueiredo et al., 2018 study, examined the effects in *Anguilla anguilla* under lanthanum exposure and stated that there were decreases in CAT activities in the internal organs. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *Drosophila melanogaster* and observed reductions in CAT activities. Similar to Huang et al.'s data, the reduction in CAT activities that occurred in this study, as a result of exposing *D. polymorpha* to the REE mixture (Tb, La, Gd, Pr), proves the effectiveness of determined REE mixture concentration and the applied temperature increments.

It is well known that organisms can increase ROS production in the presence of a stressful situation, including the presence of pollutants. To avoid damage (including lipid peroxidation, protein carbonylation, and DNA damage) caused by ROS, organisms can increase the activity of antioxidant enzymes. Among these enzymes is SOD, which has the capacity to remove ROS (i.e. superoxide anion, hydroxyl radical and hydrogen peroxide) and protects organisms from cellular damage. However, this response normally occurs when oxidative stress is not too high or too long-lasting (Freitas et al., 2020). In the study, it is thought that oxidative stress causes a decrease in the biomarker of SOD in the organism. Similarly, Pastorino et al., 2021 examined the effects of cerium (Ce), scandium (Sc), neodymium (Nd), lanthanum (La), yttrium (Y) and praseodymium (Pr) on *Barbus balcanicus*; They stated that SOD and GST were higher in gills. Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that there were decreases in SOD activity. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *D. melanogaster* and stated that decreases in SOD activities occurred.

GSTs are a superfamily of Phase II detoxification enzymes involved in the detoxification of ROS and toxic xenobiotics. These enzymes can catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for detoxification purposes, and therefore, in the presence of contaminants, GST activity is induced to achieve efficient cell protection. *D. polymorpha* was exposed to REE mixture in this study and it is thought that the change in the GSH level occurred due to the presence of the pollutant, its effective concentration and the effective temperature values tested. Similar to the results of the study, Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that GSH-Px values increased. Freitas et al., 2020 examined the effects of Neodymium (Nd) in *M. galloprovincialis* and reported that GSH levels decreased. Henriques et al., 2019 examined the effects of gadolinium on *M. galloprovincialis* and reported that there were decreases in GSH levels as a result.

Malondialdehyde levels are a reliable indicator of lipid peroxidation (LPO). Lipid peroxidation initiates the damaging process by increasing the stiffness of cellular membranes (Nagarani et al., 2011). Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that MDA content increased. Figueiredo et al., 2018 study, examined the effects in *A. anguilla* under lanthanum exposure and stated that there were significant differences in MDA levels in the internal organs. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *D. melanogaster* and stated that cerium increased the MDA content. Yang et al., 2016 study, investigated the effect of yttrium in *M. aeruginosa* and observed increases in MDA levels. Serdar et al., 2019 study, examined the biochemical effects of Gadolinium exposure on *D. polymorpha* and observed increases in TBARS levels. In this study, it is thought that the tested pollutant concentration and temperature increments are effective in these increases in TBARS levels.

Glutathione peroxidase plays an important role in antioxidant defense and the reduction of lipids, hydrogen peroxides and organic hydroperoxides to H_2O and related alcohols (Arthur, 2000). Freitas et al., 2020 study, examined the toxicological effects of neodymium in *M. galloprovincialis* and stated that GPx did not change compared to the control. In this study, as in previous studies in the literature, it is observed that REEs alone or in a mixture cause oxidative stress in the organism by affecting the biological activities of the living organism.

CONCLUSION

It has been observed that there is a parallelism between the present study's data and previous studies in the literature. It is an undeniable fact that temperature affects all living organisms at every stage of their lives. Considering that temperature is effective in all biological and physical events of the living things, it can be thought that the effects of the pollutant are also affected by the temperature increase. According to the results of the study, it is considered that the combined use of REEs may cause environmental and water pollution if they mix with the environment even in trace amounts. In this case, it can be thought that this pollution effect may culminate with the temperature increase. Therefore, it is suggested that all kinds of pollutants released directly or indirectly to the environment should be minimized.

ETHİCAL 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.

CONSENT TO PARTİCİPATE

All authors have confirmed that this has not been published elsewhere and is currently not considered to be published elsewhere.

PERMİSSİON TO PUBLİSH

All authors agree that the article can be published.

CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

Since no new data was created or analyzed in this study, data sharing is not applicable to this article**.**

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