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ARAŞTIRMA MAKALESİ — RESEARCH PAPER

Effect of Synthetic Wastewater on Antioxidant Defence System in *Dreissena polymorpha*

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Abstract: The effects of the synthetic wastewater on antioxidant defence system in *Dreissena polymorpha* was evaluated. For this purpose, SOD, CAT, GP_X activities and TBARS and GSH levels in tissues were determined by using commercial kits. The activities of SOD and CAT were in sythtetic group were determined lower than the control group in *D. polymorpha* during 24 and 96 h ($p<0.05$). After exposure of the cells of *D. polymorpha* samples to synthetic wastewater, depletion in GSH levels and subsequent increase in TBARS levels were observed, reflecting their unstable state. According to the data obtained from the study, It can be inferred that the biomarkers being domestic monitored are beneficial and effective in evaluating the biochemical mechanisms of wastewater as early warning indicators.

Keywords: Antioxidant, biochemical mechanism, biomarkers.

Sentetik Atıksuyun *Dreissena polymorpha*'da Antioksidan Savunma Sistemine Etkisi

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Öz: Sentetik atık suyun *Dreissena polymorpha*'da antioksidan savunma sistemi üzerine etkileri değerlendirildi. Bu amaçla ticari kitler kullanılarak dokulardaki SOD, CAT, GPX aktiviteleri ile TBARS ve GSH düzeyleri belirlendi. Sentetik grupta SOD ve CAT aktivitelerinin *D. polymorpha*'da 24 ve 96 saat boyunca kontrol grubuna göre daha düşük olduğu belirlendi ($p<0.05$). *D. polymorpha* örneklerinin hücrelerinin sentetik atık suya maruz bırakılmasından sonra, GSH seviyelerinde azalma ve ardından TBARS seviyelerinde artış gözlendi ve bu durum onların kararsız durumlarını yansıtıyordu. Çalışmadan elde edilen verilere göre izlenen biyobelirteçlerin kentsel atık suyun biyokimyasal mekanizmalarını erken uyarı göstergesi olarak değerlendirmede faydalı ve etkili olduğu sonucuna varılabilir.

Anahtar kelimeler: Antioksidant, biyokimyasal mekanizma, biyoişaretçi.

INTRODUCTION

Varied types of waste water have varied physical and chemical properties. Wastewater naturally contains a wealth of salts, heavy metals, and organic and inorganic elements. According to Rajasulochana and Preethy (2016), these qualities harm the water quality and the delicate balance of life in the areas where they are released. Particularly, complex wastewater discharged from industrial and urban wastewater treatment plants to receiving environments contains a high concentration and variety of pollutants, including polycyclic aromatic hydrocarbons (PAHs), solvents, heavy metals, and drugs, which pose a serious threat to living ecosystems. The immunological responses, oxidative stress, and estrogenic effects of living things are activated when this type of effluent is exposed to fish collected in fish farms or contaminated water bodies (Oakes et al., 2004).

Accoding to Van der Oost et al (2003), the most noticeable impact of wastewater is the formation of reactive oxygen species (ROS) related to oxidative stress. Despite the fact that ROS is typically formed during metabolism and that it has the potential to harm, including lipid peroxidation (LPO), it is also conceivable to discuss its benefits when occurring naturally Droge et al. (2002). Oxidative stress also has the ability to activate antioxidant enzymes like catalase and glutathione S-transferases. Different strategies can be used by living cells with antioxidant defense mechanisms to repair broken macromolecules and reduce oxidative stress. Enzymatic and non-enzymatic antioxidants that eliminate ROS and free radicals make up the body's main defense system (Elshamy et al., 2019). Catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD), non-enzymatic glutathione (GSH), and glutathione-Stransferase (GST) are examples of these antioxidant enzymes. Vitamin E (gamma-tocopherol) and Vitamin C (ascorbic acid) are examples of these (Gusti et al., 2021). One of the most crucial enzymes that shields cells from ROS-induced oxidative damage is catalase (E.C.1.11.1.6). Hydrogen peroxide is converted by catalase into oxygen and water (Krishnamurthy and Wadhwani, 2012). In addition to mending cells, superoxide dismutase (E.C.1.15.1.1) is a crucial antioxidant defense enzyme that lessens the harm done to cells by radicals (Orozco et al, 1998).

Utilizing bioindicator organisms, which allow for the long-term monitoring of biological and biochemical characteristics, allows for the evaluation of contaminants affecting aquatic ecosystems (Dalzochio et al., 2016). An essential model organism for in situ monitoring and ecotoxicological tests of various contaminants seeping into aquatic habitats is *D. polymorpha*. The utilization of these organisms has become widespread due to their biological structure's high rate of filtration and their presence in water reservoirs throughout Europe (Pastorino et al., 2021). By filtering and redistributing nutrients in the water, zebra mussels also aid in the reduction of eutrophication in some lakes (Karatayev et al., 2002; Goedkoop et al., 2011). These creatures are now referred to as "ecosystem engineers" as a result of these characteristics. While the aforementioned *D. polymorpha* species are also thought to be invasive, Bowman and Bailey (1998) note that they are preferred due to their ability to withstand high temperatures (up to 28 °C), moderate salinity (6 to 10%), a wide pH range (6.5 to 9.3), and a high tolerance for physico-chemical conditions and also chemical contaminations.

Up to this point, the majority of studies have concentrated on the antioxidant response in various wastewaters. The examination of oxidative stress indicators, particularly in *D. polymorpha*, under urban wastewater conditions was not included in any of these investigations. In order to investigate the oxidative harm and anti-oxidative responses of *D. polymorpha* in synthetic residential wastewater at various concentrations, this research was carried out.

MATERIAL AND METHOD

Preparation of sythetic wastewater: In the study, 5 liters of synthetic domestic wastewater was prepared. For this purpose, 800 mg peptone and 550 mg malt extract medium, 140 mg urea (CH_4N_2O), 35 mg sodium chloride (NaCl), 20 mg calcium chloride, according to the methods specified in Krismastuti and Hamim (2019) were put into a sterile 5-liter plastic bottle. Dehydrate ($CaCl_22H_2O$), 10 mg magnesium sulfate heptahydrate ($MgSO_47H_2O$) was added and mixed. This synthetic wastewater obtained consists of 106 g/L of (OC) organic carbon (approximately), 46 mg/L nitrogen (N) and 5 mg/L phosphorus (P).

Animals and experimental procedure: Individuals of *D. polymorpha* were found in the Euphrates River (38º 48' 25" N, 38º 43' 51" E). Before being employed in tests, the organisms were swiftly transported to the lab in plastic bottles and fed with plankton on a 12-hour light: dark cycle in a 20 L vented aquarium for 15 days. For the duration of the experiment, organisms at comparable developmental stages were chosen for the study, and they were not fed. The experimental aquarium was checked every 24 hours, and any dead organisms were counted and destroyed. The death criterion was allowed to be inactivity.

Each tank holds 20 L of pure water, and each experiment consists of three replications with ten participants. The creatures in the experiments received no nutrition. Every 24 hours, the organisms were examined. The two groups used for the experiments were synthetic wastewater xxposure froup (B) and Reference water (A) from Munzur River. Two groups of *D. polymorpha* (n = 10) were exposed to wastewater samples for 24 and 96 hours as part of the research. The animals that underwent tests for 24 and 96 hours were housed in the same tank.

Multi-biomarker analysis: The samples were first weighed at this point in the study, and then PBS buffer (phosphate buffered saline solution) was added at a rate of 1/5 w/v to an ice homogenizer to homogenize the samples. These homogenized materials were then spun in a chilled centrifuge for 15 minutes at 17.00 rpm. These supernatants were quickly preserved until measurements could be done at -70 ºC in a deep freezer. The ELISA ASSAY kit (catalog numbers SOD: 706002, CAT: 707002, GPX: 703102 GSH: 703002 TBARS: 10009055) obtained from Cayman Chemical was used to measure the levels of SOD, CAT, GPX, TBARS, and GSH in tissues. All biochemical parameters werer measured by Thermo multiscan FC microplate reader according to Assay Kit procedure handbook.

Statistical analysis: In the study, one-way ANOVA and independent t-test were preferred for statistical analysis to reveal the importance of differences in oxidative stress biomarkers.

RESULTS

The level of TBARS in *D. polymorpha* varies with exposure time. It was observed that the amount of TBARS increased in *D. polymorpha* exposed to synthetic wastewater (SW) for 24 and 96 compared to the control group, but the increase in TBARS level was statistically insignificant (p>0.05) (Figure 1)

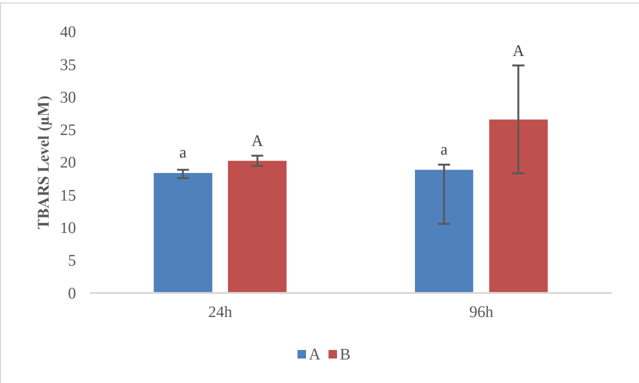

Figure 1. TBARS (µM) levels of *D. polymorpha* exposed to sythetic wastewater.

GSH levels and GSH – Px activities decreased in *D. polymorpha* exposed to SW compared to the reference water during 24 h and 96 h (p <0.05) are dempnstrated in Figure 2 and 3 respectively.

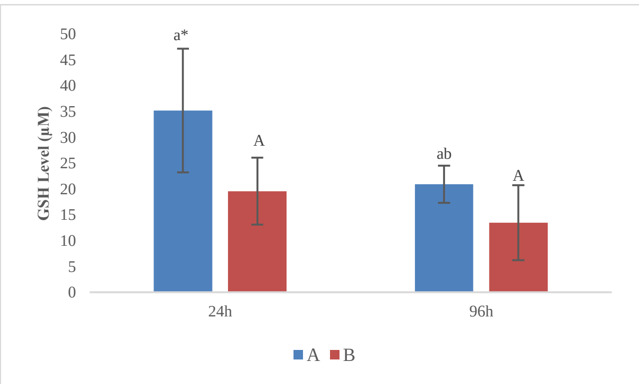

Figure 2. GSH (µM) levels of *D. polymorpha* exposed to sythetic wastewater.

SOD activities were in SW group were found lower than the control group in *D. polymorpha* during 24 and 96 h (p <0 .05) **(**Figure 4 and 5)

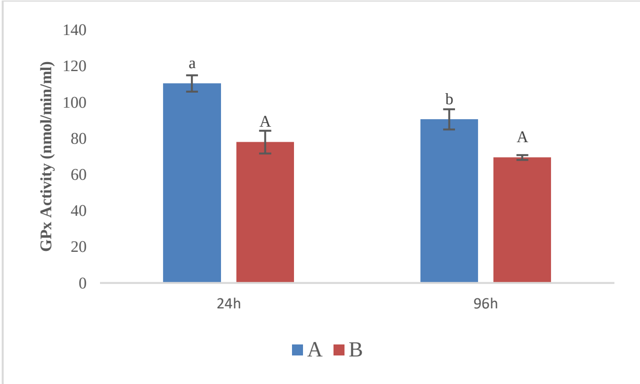

Figure 3. GPx (nmol/min/mL) levels of *D. polymorpha* exposed to sythetic wastewater.

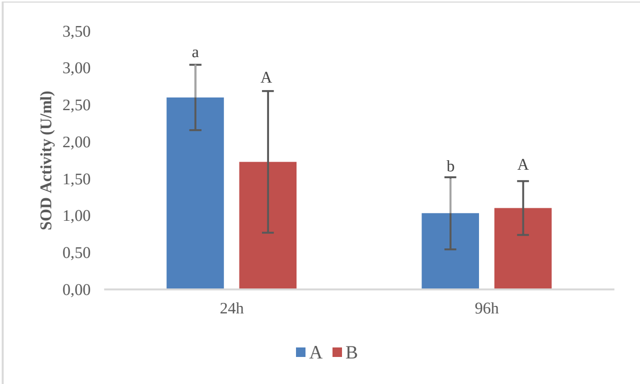

Figure 4. SOD (U/mL) levels of *D. polymorpha* exposed to sythetic wastewater.

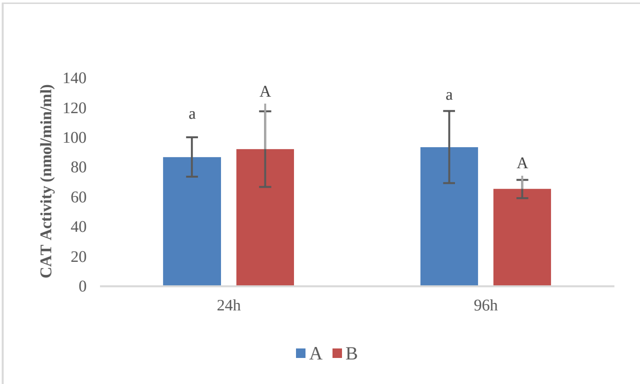

Figure 5. CAT (nmol/min/mL) levels of *D. polymorpha* exposed to sythetic wastewater.

DISCUSSION

Wastewater-derived materials contain a variety of contaminants and put aquatic organisms in a difficult habitat (Deng et al., 2020). According to Tetreault et al. (2021), exposure to sewage caused antioxidant enzymes and ROS activity to be induced in fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*). Caged fish (*Prochilodus lineatus*) subjected to pollutants from wastewater showed an increase in oxidative stress (Cavenave et al., 2013). Parolini et al.

(2010) proposed that zebra mussel antioxidant responses serve as indicators of exposure to specific xenobiotics. Similarly, in the current study, it was observed that synthetic wastewater created oxidative stress in *D. polymorpha*.

An intermediate created during lipid peroxidation that directly evaluates cell damage and cellular toxicity is found in the thiobarbituric acid reactive species concentration (TBARS) (Belda et al., 2021). In cells cultured with a 100% concentration of domestic wastewater. Singh et al. (2022) discovered that the TBARS level in *C. vulgaris* and *S. vacuolatus* increased by 2.86 and 2.44 times, respectively. At 25%, 50%, and 75% concentrations of municipal wastewater, the amount of TBARS in *C. vulgaris* and *S. vacuolatus* increased 1.82, 2.03, 3.40 times, and 1.44, 1.85, 2.07 times, respectively. In a study by Tabrez and Ahmad (2009) discovered that oral wastewater delivery to rats significantly increased MDA levels in the kidney and liver by 2.5 and 5 times, respectively. According to Tatar et al. (2017), wastewater exposure enhanced the level of MDA in the leaves of *Lemna minor* and *Lemna gibba* compared to the control media. In parallel with these studies, in the current study, it was determined that synthetic wastewater increased TBARS levels in *D. polymorpha*.

The carp *Cyprinus carpio* exposed to hospital wastewater in Mexico showed signs of oxidative stress, according to a study by reference given by Cruz et al., (2015). Comparatively to the control group, there were noticeable increases in the gills of these animals at the 24th hour, in the brain at the 24 and 48th hours, and in the liver at the 72 and 96th hours. Tatar et al. (2018) also discovered that the MDA level in *G. pulex* may change based on the length of time it spends in contact with sewage. They discovered that after exposing *G. pulex* to wastewater for 24 hours, the amount of MDA dropped in comparison to raw water, but after 96 hours, MDA levels rose. Yildirim et al. (2020) asserted that exposure to treated municipal wastewater led to an increase in TBARS levels 24 and 96 hours later. This investigation also revealed that the amount of TBARS in *D. polymorpha* changes with exposure time. When compared to the control group, it was discovered that *D. polymorpha* exposed to synthetic wastewater for 24 and 96th hour exposure periods had higher TBARS levels, however this increase was not statistically significant ($p>0.05$). According to Upadhyay et al. (2016), the elevated TBARS level has been linked to increased lipid peroxidation and membrane degradation, which negatively affects zebra mussels' physiological reactions and cellular metabolism.

Catalase activity is not frequently utilized as a sensitive indicator of oxidative stress (Sarwar et al., 2014). A multifunctional collection of enzymes with broadly overlapping substrate specificities are housed in superoxide dismutase and are crucial in the detoxification of the superoxide radical (Sheng et al., 2014). As the SOD-CAT system serves as the first line of defense against oxidative stress brought on by ROS. Hassan and Scandalios (1990) claim that changes in CAT and SOD activity frequently happen in the presence of environmental contaminants. On the basis of catalase (CAT) as the biomarker produced under oxidative stress, which manifests itself in two sentinel mussel species (*Mytilus galloprovincialis* and *Perna perna*) represented by the three-dimensional class, Abdellah et al. (2020) evaluated the toxicological aspect of an industrial wastewater discharged into the BouIsmail bay area. In a 2009 study, Tabrez and Ahmad found that wastewater causes the kidney and liver tissues to suffer severely damaging damage. The inhibition of the antioxidant system by SOD, which is thought to be one of the liver toxicity biomarkers of the wastewater, causes this damage. Samanta et al. (2018) assessed the detrimental effects of wastewater pollution on three fish species found in the Eungcheon, Mihocheon, and Busocheon rivers: *Carassius auratus, Zacco platypus*, and *Zacco koreanus*. As a result, the effluent has considerably greater catalase activity, indicating the activation of antioxidant defense systems. Additionally, Cruz et al. (2015) discovered that *C. carpio* exposed to hospital wastewater had significantly less gills at 48 and 72th hours compared to 24 hours. Similar to these previous studies, similar changes in SOD and CAT activities were observed in the current study.

According to a study by Tatar et al. (2018), the CAT activities of *G. pulex* in the secondary wastewater group were lower for 24 and 96 hours than those of raw water. In another related investigation, Yildirim et al. (2020) discovered that SOD activity rose as well after the *G. pulex* samples were exposed to water from the municipal wastewater treatment facility during the 24 and 96th hours. In comparison to control groups, after 24 hours, CAT activities in groups exposed to treated municipal wastewater dropped from 25.29 to 14.12 nmol/min/ml. However, after 96 hours of exposure, this decline also got worse. For 24 and 96th hours, the synthetic wastewater group's *D. polymorpha'* s CAT and SOD activities were determined to be lower than those of the control components ($p<0.05$). According to Gwodziski et al. (2010), SOD inhibition explains this condition by realizing both O_2 buildup and overproduction of H_2O_2, which can also be created through spontaneous conversion of superoxide anion mediated by non-enzymatic mechanisms. Antioxidant enzymes that participate in the breakdown of hydrogen peroxide include catalase and glutathione peroxidase (GPx) (Modesto et al., 2010). It is clear that an increase in H_2O_2 and the accumulation of superoxide radicals brought on by SOD inhibition may lead to the formation of hydroxyl radicals via the Haber-Weiss reaction, which would then result in a significant rise in the levels of protein carbonylation and lipid peroxidation (Verlecar et al., 2008). In this study, antioxidant system activation may also cause oxidative stress resulting from multiple contamination of synthetically prepared urban wastewater. As a result of this, it may be evidence that exposure to wastewater at the end of the 96th hour causes a decrease in catalase activity, an imbalance in the oxidative state has been reached, or that zebra mussels have now undergone an adaptation process to synthetic wastewater exposure (Geba et al., 2021).

According to Zama et al. (2007), if antioxidants are not enzymatic, they prevent uncontrolled free radical formation as well as limit the reactions of biological components and free radicals. As a result, many free radicals responsible for the oxidation of endogenous antioxidants are also broken down. Periodic processes related to glutathione and GSH play a key role in antioxidant defense. Glutathione undertakes the task of maintaining the redox potential of cells and is responsible for ending the activities of oxyradicals. In previous literature studies, it has been reported that glutathione peroxidase activity is altered by some environmental pollutant parameters (Radi et al., 1985). Many studies in recent years provide evidence that pollutants inhibit GSH levels in living aquatic organisms. Tatar et al. (2018) determined a decrease in GSH in the aquatic organism *G. pulex*, which they exposed to municipal wastewater influx. Tatar et al. (2017) found that the GSH/GSSG ratio decreased in *L. minor* species exposed to the effluent of urban wastewater treatment plants compared to the control. In this study, GSH levels and GSH-PX activities in *D. polymorpha* exposed to synthetic wastewater were decreased for 24 and 96 hours compared to reference water ($p<0.05$). The increase in free radicals associated with the direct oxidation of glutathione peroxidases or the compounds containing it also causes the depletion of GSH (Serdar et al., 2021).

CONLUSION

This current study is the first report to examine the oxidative damage and anti-oxidative responses of *Dreissena polymorpha* to synthetic municipal wastewater. According to the results obtained, it has been determined that synthetic wastewater causes a significant oxidative stress by causing irregularity in the antioxidant defense system. Different pollutants from synthetic wastewater can induce different oxidative stress responses.

The results also reveal that the selected biomarkers are essential to having a knowledge about the biochemical mechanisms of municipal wastewater as early warning indicators.

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