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Acute and Chonic Effects of Silver Nanoparticles (AgNPs) on *Unio delicatus*

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Abstract
The widespread use of silver nanoparticles (AgNPs) including water filters, paints, cosmetics, deodorants, clothing, textiles, food packaging, electrical appliances and medical devices inevitably leads to their release into the natural environment, bioaccumulation in organisms and persistent accumulation in natural aquatic systems. The aim of this study is to investigate the acute and chronic effects of silver nanoparticles, which can contaminate aquatic ecosystems, in freshwater mussels, one of the aquatic invertebrate organisms. The model organism of the study, *Unio delicatus*, was obtained from Gölbaşı Lake (Hatay). After that acclimation was performed in the laboratory for two weeks. The mussels were then exposed to 1 and 10 mg/L AgNPs for 7 and 21 days. At the end of the exposure period, hemolymph and tissue samples of the mussels were taken. Total hemocyte count from hemolymph samples, lipid peroxidation and glutathione levels from tissue samples (digestive gland and gill) were investigated. Acute exposure resulted in an increase in the total hemocyte counts, while chronic exposure resulted in a significant decrease ($P<0.05$). Changes in lipid peroxidation and glutathione levels in gill and digestive gland tissues of mussels also occurred but not significantly. In conclusion, AgNPs caused changes in physiological and biochemical parameters of freshwater mussels.

1. Introduction

Of all commercial goods using nanomaterials, silver nanoparticles (AgNPs) account for 24% of the total. The AgNPs are most frequently found in water filters, cosmetics, textiles, food packaging, electrical appliances, and medical devices [1]. The common use of AgNPs inevitably results in their discharge into the environment, bioaccumulates in the organisms and persistent accumulation in natural aquatic systems [2]. Once in the aquatic environment, AgNPs can go through various alteration processes (oxidation, dispersion, solubilization, adsorption with soluble and particulate organic matter, agglomeration /aggregation) [3]. AgNPs that accumulate in sediment can be transferred to aquatic organisms through environmental exposure. AgNPs taken up by aquatic organisms through the aquatic food chain pose a risk to human health [2].

Mussels have advantages such as the ability to make comparisons during the evaluation of information obtained from the aquatic environment,

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to collect information about pollutants in the same area for a long time and to be widely used to quickly assess the current status of many pollutants [4]. For this reason, mussels are one of the leading biological indicators used in the measurement and evaluation of pollution in water. A mussel with an average size of 7-8 cm has the ability to filter 10-15 liters of water per hour. They filter and feed on all kinds of organic and inorganic particles [5]. In addition, freshwater mussels take part in the natural purification function of the water in their environment due to their filter feeding and thus play a decisive role on the ecological balance of the environment [6]. Mussels are particularly at risk of environmental contamination by nanoparticles because they are immobile and feed on suspended matter. Particles trapped in the gills during feeding and respiration are lowered down the digestive tube and accumulate in the "stomach" (digestive gland) where they are broken down. Therefore, nanoparticles can easily accumulate in mussels with aggregation and cause negative effects [7]. The freshwater mussel *Unio delicatus* Lea, 1863 is one of the most common mussel species in large river basins in southwest-eastern Anatolia. Due to its distribution areas, it is an indicator species in biomonitoring, ecotoxicological and phylogenetic studies [8], [9].

Nanoparticles have the potential to affect the physiology and behavior of aquatic organisms, including mussels. Several studies have shown that exposure to nanoparticles can lead to various physiological changes in marine mussels, including changes in the immune system, metabolism, and oxidative stress response [1], [10], [11]. Conversely, freshwater mussels have been used in few studies to investigate the effects of nanoparticles. A study with freshwater mussels showed that acute exposure to nanoparticles did not change the filtering capacity of mussels but had an effect on biomolecules [12]. In the literature review, no chronic exposure to silver nanoparticles at freshwater mussels were found. The aim of this study is investigate the acute and chronic effects of silver nanoparticles on freshwater mussels *Unio delicatus* in terms of total hemocyte count (THC), lipid peroxidation and glutathione.

2. Material and Method

2.1. Test Organisms and Acclimation to Laboratory

Freshwater mussels *Unio delicatus* (mean weight 36.3±4.8 g, mean length 5.2±0.1 cm, mean thickness 2.2±0.2 cm, mean height 1.5±0.1 cm; N=60) acquired from local fishermen in Gölbaşı Lake (Hatay) were used in this study. Freshwater mussels were transported in aerated water and placed in 15 L aquaria containing 10 L of pre-rested tap water without chlorine (1 mussel/L) in the laboratory. Mussels were acclimatized to laboratory conditions for 2 weeks. During this period, the aquarium water was altered by siphoning 50% every two days and the mussels were fed with Spirulina sp. The aquaria water was measured by YSI Professional Plus Multiparameter Instrument (USA) in terms of water quality during the acclimation period. The mean values (mean±SEM) of physicochemical parameters of water are as follows: temperature 21.9±0.25ºC; dissolved oxygen 6.2±0.56 mg/L; pH 7.5±0.2; conductivity: 201.04±0.4 mS/cm; salinity; 0.19±0.02. This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

2.2. Exposure to AgNPs

Metal based AgNPs were obtained from Nanografi (Turkey) with the particle size specified as: 18 nm and >99.995% purity. Shape of AgNPs were spherical and crystal structure was cubic. The application concentrations of AgNPs were determined as 1 and 10 mg/L by reviewing literature studies [13], [14]. After freshwater mussels were placed in aquaria (10 mussels/L), 500 mL of aquarium water was taken, AgNPs were added at the determined concentrations and sonicated. After sonication by Ultasons (J.P. Selecta, Spain), AgNPs were added to the aquaria. The mussels were exposed to 1 and 10 mg/L AgNPs for 7 and 21 days [15]. There was one control group (aquarium water and mussels) in the experiments. Aquarium water quality parameters were measured during the experimental period (temperature 22±0.3ºC; dissolved oxygen 6.4±0.2 mg/L; pH 7.8±0.5; conductivity: 200.05±0.6 mS/cm; salinity; 0.20±0.01). After each treatment period, 10 mussel samples were taken and hemolymph tissues were removed with a 2.5 mL syringe after measuring biological data. Then, the gill (G) and digestive gland (DG) tissues of mussels were removed. The tissues were stored at -80ºC (Wisd Simplified Freezing System, WiseCryo Daihan, South Korea) until biochemical analysis.

2.3. Total Hemocyte Counts (THCs)

Hemolymph tissue was collected from freshwater mussels according to the method of [16]. The THCs were counted using a light microscope.

2.4. Biochemical Analysis

The lipid peroxidation was measured based on malondialdehyde (MDA) production in gills and digestive glands, the method of [17] was applied and the MDA levels were determined by reading at 535 and 520 nm using spectrophotometer (Biochrom Libra S22, UK). Briefly, following homogenization of 100 mg of tissue with 1/5 of 1.15% KCl buffer, 500 µL of homogenate sample was taken. The homogenate mixed with 3 mL of 1% phosphoric acid and 1 mL of 0.6% thiobarbutic acid was kept in a hot water bath for 45 minutes. Then, 4 mL of butanol was added and centrifuged at 3000 rpm for 5 minutes. The supernatant portion was read by spectrophotometer. The measurement of the glutathione levels in gills and digestive glands, the method of [18] was applied and the glutathione levels were determined by reading at 410 and 420 nm using spectrophotometer (Biochrom Libra S22, UK).

2.5. Statistical Analysis

To evaluate the experimental results obtained; total hemocyte count, glutathione and MDA determination results were subjected to normal distribution according to Kolmogorov-Smirnov normality test and homogeneity of variances tests to determine the differences between the experimental groups. After all samples met these conditions, one-way ANOVA test was performed and significance levels were determined with GraphPad 5 statistical data analysis program.

3. Results and Discussion

The acute and chronic effects of AgNPs on freshwater mussels was evaluated in the present study. Within the scope of the study, total hemocyte counts, which are physiological parameters, and lipid peroxidation and glutathione levels, which are biochemical parameters, were examined in freshwater mussels exposed to 1 and 10 mg/L silver nanoparticles for 7 and 21 days. The alterations in total hemocyte counts of freshwater mussels exposed to AgNPs for 7 and 21 days are shown in Figure 1. There was an increase in the THCs in the mussels exposed to AgNPs for 7 days compared to the control group ($p>0.05$). At 21 days of exposure, a decrease in total hemocyte count was observed in the experimental groups compared to the control group. There was a 1.7-fold decrease between the control group and the 10 mg/L AgNPs exposed group ($p=0.035$).

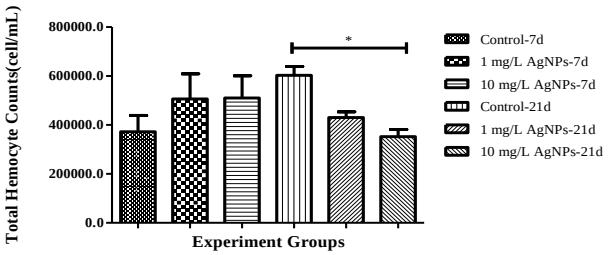

Figure 1. Changes in total hemocyte counts of freshwater mussels after exposure to silver nanoparticles.

The effect of silver nanoparticles on lipid peroxidation levels in gill and digestive gland tissues is shown in Figure 2. As a result of exposure of mussels to 1 and 10 mg/L silver nanoparticles for 7 days, gill and digestive gland MDA levels decreased compared to the control group ($p>0.05$). In 21-day exposure, an increase in MDA levels occurred in the digestive gland tissues exposed to both 1 mg/L AgNPs and 10 mg/L AgNPs ($p>0.05$).

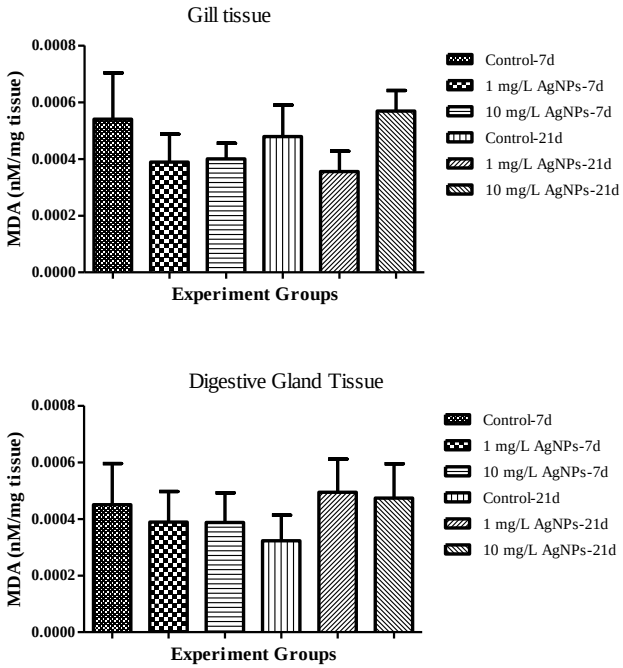

Figure 2. Changes in MDA levels in gill and digestive gland tissues of freshwater mussels after exposure to silver nanoparticles.

The effect of silver nanoparticles on glutathione levels in gill and digestive gland tissues of freshwater mussels is shown in Figure 3. As a result of exposure of mussels to 1 and 10 mg/L AgNPs for 7 days, gill glutathione levels increased 3.3 and 4.1 times compared to the control group. In 21-day exposure, a decrease was observed in the 1 mg/L group compared to the control group and an increase was observed in the 10 mg/L group. The glutathione

levels of digestive gland tissues were decreased in 7-day exposure and increased in 21-day exposure.

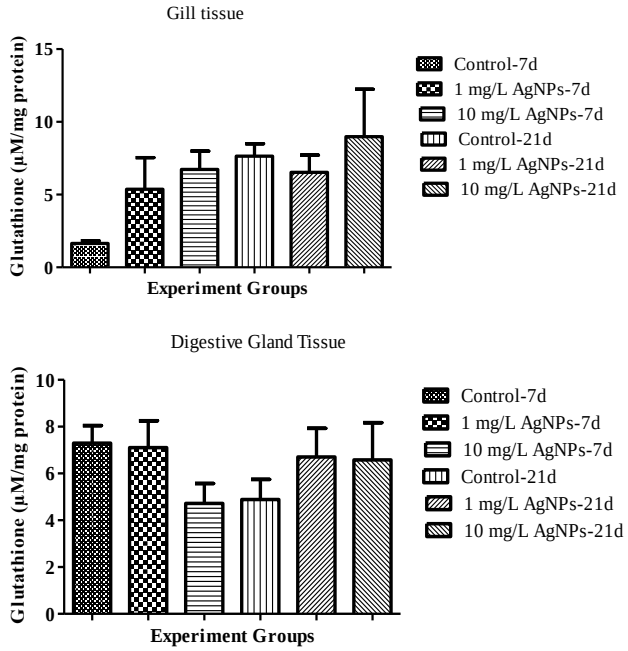

Figure 3. Changes in glutathione levels in gill and digestive gland tissues of freshwater mussels after exposure to silver nanoparticles.

THCs are commonly used physiological/immunological parameter to assess the health status of mussels and indicator of the mussels to stressors such as pollutants, disease, and changes in environmental conditions [8], [9], [16], [19], [20]. In the current study, significant decreases in the THCs occurred with increasing exposure time to nanoparticles. Similar decreases in the THCs were observed in bivalves *Mytilus coruscus* under TiO NPs [21], [22]. These results demonstrate the immunological effects induced by mussels and nanoparticles.

MDA is a reactive compound that is commonly used as a marker of lipid peroxidation and are often used as an indicator of the extent of lipid peroxidation in tissues exposed to oxidative stress [23]. In the context of mussels, measuring MDA levels can provide valuable information on the health of these organisms and the potential impacts of environmental stressors, such as pollution or exposure to nanoparticles, on their cellular and physiological functions [24]. The gill and digestive gland tissues are particularly vulnerable to the damaging effects of oxidative stress caused by pollutants including nanoparticles [25], [26]. In this study, it was observed that there was a change in MDA levels in the gill and digestive gland tissues from the groups treated with AgNPs. Studies have shown that exposure to nanoparticles indicates similar increase or decrease on lipid peroxidation in gill and digestive gland tissues of mussels, which may lead to cellular damage and dysfunction [24], [26], [27], [28]. A significant increase in MDA levels were observed at 10 µg/L AgNPs in *Mytilus galloprovincialis* [1].

One potential mechanism by which NPs can induce toxicity is through the modulation of glutathione (GSH) levels. GSH is an important antioxidant and detoxification molecule in the organisms, and changes in its levels can have significant impacts on cellular functions [29], [30]. Researches shown that exposure to NPs caused the changes in GSH levels in the mussel tissues including gills and digestive gland [29], [31], [32].

4. Conclusion and Suggestions

In this study, the first response to exposure to AgNPs was alterations in physiological parameter of the mussels. Acute exposure to AgNPs caused increase in the THCs, while chronic exposure to AgNPs caused a significant decrease. Changes in MDA and GSH levels are not significant in the different tissues of AgNPs exposed mussels. As a result of the study, AgNPs caused alterations in the physiological and biochemical parameters of freshwater mussels.

Contributions of the authors

Plan and design of the study: İlker Şimşek, Pınar Arslan ; Materials, methods and data collection: Pınar Arslan, Aysel Çağlan Günal ; Data analysis and interpretation: Özgür Kuzukıran, Ayhan Filazi ; Writing and critical evaluation: İlker Şimşek, Pınar Arslan, Aysel Çağlan Günal, Özgür Kuzukıran, Ayhan Filazi.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

References

[1] A. Ale, G. Liberatori, M. L. Vannuccini, E. Bergami, S. Ancora, G. Mariotti, N. Bianchi, J. M.Galdopórpora, M. F. Desimone, J. Cazenave, and I. Corsi, ‘‘Exposure to a nanosilver-enabled consumer product results in similar accumulation and toxicity of silver nanoparticles in the marine mussel *Mytilus galloprovincialis*,’’ *Aquatic Toxicology*, vol. 211, pp. 46–56, March 2019.

[2] J. Zhao, X. Wang, S. A. Hoang, N. S. Bolan, M. B. Kirkham, J. Liu, X. Xia, and Y. Li, ‘‘Silver nanoparticles in aquatic sediments: Occurrence, chemical transformations, toxicity, and analytical methods,’’ *Journal of Hazardous Materials*, vol. 418, p. 126368, September 2021.

[3] M. Auguste, C. Ciacci, T. Balbi, A. Brunelli, V. Caratto, A. Marcomini, R. Cuppini, and L. Canesi, ‘‘Effects of nanosilver on *Mytilus galloprovincialis* hemocytes and early embryo development,’’ *Aquatic Toxicology*, vol. 203, pp. 107–116, August 2018.

[4] A. G. Şahin, and M. Karatepe, 2022: ‘‘Vitamins A, E, C, β -carotene contents and MDA level of freshwater mussel, (*Unio elongatulus eucirrus* Bourguignat 1860) in the Karakaya Dam Lake,’’ *Ege Journal of Fisheries and Aquatic Sciences*, vol. 39, no. 2, pp. 120-124, June 2022.

[5] A. G. Şahin, M. R. Gül, and M. Küçükyılmaz, ‘‘Karakaya Baraj Gölü'ndeki Tatlı Su Midyesi (*Unio elongatulus eucirrus* Bourguignat 1860)’nin Ağır Metal Düzeylerinin Belirlenmesi,’’ *Fırat Üniversitesi Fen Bilimleri Dergis*i, vol. 28, no. 1, pp. 13-19, January 2016.

[6] M. Güler, D. Çoban, and B. Kırım, ‘‘*Unio terminalis* (Bivalvia: Unionidae)' in üreme biyolojisi üzerine gözlemler,’’ *Ege Journal of Fisheries and Aquatic Sciences*, vol. 34, no. 3, pp. 303-309, June 2017.

[7] F. Gagne, J. Auclair, P. Turcotte, and C. Gagnon, ‘‘Sublethal Effects of Silver Nanoparticles and Dissolved Silver in Freshwater Mussels,’’ *Journal of Toxicology and Environmental Health, Part A*, vol. 76, no. 8, pp. 479–490, February 2013.

[8] P. Arslan, ‘‘Determinations of the effects of cyfluthrin on the hemocytes parameters of freshwater mussel (*Unio delicatus*),’’ *Ege Journal of Fisheries and Aquatic Sciences*, vol. 39, no. 1, pp. 39-45, January 2022.

[9] P. Arslan, and A. Ç. Günal, ‘‘Does Fipronil Affect on Aquatic Organisms? Physiological, Biochemical, and Histopathological Alterations of Non-Target Freshwater Mussel Species,’’ *Water*, vol. 15, no. 2, p. 334, January 2023.

[10] T. Gomes, J. P. Pinheiro, I. Cancio, C. G. Pereira, C. Cardoso, and M. J. Bebianno, ‘‘Effects of copper nanoparticles exposure in the mussel *Mytilus galloprovincialis*,’’ *Environmental science & technology*, vol. 45, no. 21, pp. 9356-9362, October 2011.

[11] T. Gomes, O. Araújo, R. Pereira, A. C. Almeida, A. Crav, and M. J. Bebianno, ‘‘Genotoxicity of copper oxide and silver nanoparticles in the mussel *Mytilus galloprovincialis*,’’ *Marine environmental research*, vol. 84, pp. 51-59, March 2013.

[12] E. G. Canli, A. Baykose, L. H. Uslu, and M. Canli, ‘‘Changes in energy reserves and responses of some biomarkers in freshwater mussels exposed to metal-oxide nanoparticles,’’ *Environmental Toxicology and Pharmacology*, vol. 98, p.104077, March 2003.

[13] A. Katsumiti, D. Gilliland, I. Arostegui, and M. P. Cajaraville, ‘‘Mechanisms of toxicity of Ag nanoparticles in comparison to bulk and ionic Ag on mussel hemocytes and gill cells,’’ *PLoS One*, vol. 10, no. 6, p. e0129039, June 2015.

[14] M. Munari, J. Sturve, G. Frenzilli, M. B. Sanders, P. Christian, M. Nigro, and B. P. Lyons, ‘‘Genotoxic effects of Ag2S and CdS nanoparticles in blue mussel (*Mytilus edulis*) haemocytes,’’ *Chemistry and Ecology*, vol. 30, no. 8, pp. 719-725, March 2014.

[15] REACH, ‘‘Guidance on Information Requirements and Chemical Safety Assessment,’’ Chapter R.7b: Endpoint specific guidance Retrieved from https://echa.europa.eu/documents/10162/17224/information_requirements_r7b_en.pdf/1a551efc-bd6a-4d1f-b719-16e0d3a01919?t=1498476047712, 2017.

[16] H. Y. Yavuzcan, A. Ç. G. Benli, ‘‘Nitrite toxicity to crayfish, *Astacus leptodactylus*, the effects of sublethal nitrite exposure on hemolymph nitrite, total hemocyte counts, and hemolymph glucose,’’ *Ecotoxicology and Environmental Safety*, vol. 59, no. 3, pp. 370–375, November 2004.

[17] M. Mihara, and M. Uchiyama, ‘‘Determination of malonaldehyde precursor in tissues by thiobarbituric acid test,’’ *Analytical Biochemistry*, vol. 86, no. 1, pp. 271-278, May 1978.

[18] G. L. Ellman, ‘‘Tissue sulfhydryl groups,’’ *Archives of Biochemistry and Biophysics*, vol. 82, pp. 70-77, May 1959.

[19] H. E. Parry, and R. K. Pipe, ‘‘Interactive effects of temperature and copper on immunocompetence and disease susceptibility in mussels (*Mytilus edulis*),’’ *Aquatic toxicology*, vol. 69, no. 4, pp. 311-325, September 2004.

[20] Y. Sui, H. Kong, Y. Shang, X. Huang, F. Wu, M. Hu, and Y. Wang, ‘‘Effects of short-term hypoxia and seawater acidification on hemocyte responses of the mussel *Mytilus coruscus*,’’ *Marine pollution bulletin*, vol. 108, no. 1-2, pp. 46-52, July 2016.

[21] X. Huang, D. Lin, K. Ning, Y. Sui, M. Hu, W. Lu, and Y. Wang, ‘‘Hemocyte responses of the thick shell mussel *Mytilus coruscus* exposed to nano-TiO2 and seawater acidification,’’ *Aquatic Toxicology*, vol. 180, pp. 1-10, September 2016.

[22] T. Wang, X. Huang, X. Jiang, M. Hu, W. Huang, and Y. Wang, ‘‘Differential in vivo hemocyte responses to nano titanium dioxide in mussels: Effects of particle size,’’ *Aquatic Toxicology*, vol. 212, pp. 28-36, July 2019.

[23] D. Tsikas, ‘‘Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges,’’ *Analytical biochemistry*, vol. 524, pp. 13-30, October 2017.

[24] F. Gagne, J. Auclair, P. Turcotte, and C. Gagnon, ‘‘Sublethal Effects of Sılver Nanopartıcles and Dıssolved Silver in Freshwater Mussels,’’ *Journal of Toxicology and Environmental Health, Part A*, vol. 76, pp.479–490, February 2013.

[25] E.A. Almeida, A. C. D. Bainy, A. L. Dafre, O. F. Gomes, M. H. Medeiros, and P. Di Mascio, ‘‘Oxidative stress in digestive gland and gill of the brown mussel (*Perna perna*) exposed to air and re-submersed,’’ *Journal of Experimental Marine Biology and Ecology*, vol. 318, no.1, pp. 21-30, May 2005.

[26] S. Tedesco, H. Doyle, J. Blasco, G. Redmond, and D. Sheehan, ‘‘Oxidative stress and toxicity of gold nanoparticles in *Mytilus edulis*,’’ *Aquatic toxicology*, vol. 100, no. 2, pp. 178-186, March 2010.

[27] M. Dellali, A. Khallouli, A. H. Harrath, F. Falodah, S. Alwasel, H. Beyrem, and F. Boufahja, ‘‘Effects of Au/TiO 2 metallic nanoparticles on *Unio ravoisieri*: Assessment through an oxidative stress and toxicity biomarkers,’’ *Environmental Science and Pollution Research*, vol. 28, pp. 18176-18185, January 2021.

[28] S. E. Gürkan, ‘‘Impact of nickel oxide nanoparticles (NiO) on oxidative stress biomarkers and hemocyte counts of *Mytilus galloprovincialis*,’’ *Biological Trace Element Research*, vol. 200, no. 7, pp. 3429-3441, March 2022.

[29] L. Canesi, A. Viarengo, C. Leonzio, M. Filippelli, and G. Gallo, ‘‘Heavy metals and glutathione metabolism in mussel tissues,’’ *Aquatic Toxicology*, vol. 46, no. 1, pp. 67-76, June 1999.

[30] F. Regoli, and G. Principato, ‘‘Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers,’’ *Aquatic toxicology*, vol. 31, no. 2, pp. 143-164, February 1995.

[31] H. Falfushynska, L. Gnatyshyna, I. Yurchak, I. Sokolova, and O. Stoliar, ‘‘The effects of zinc nanooxide on cellular stress responses of the freshwater mussels *Unio tumidus* are modulated by elevated temperature and organic pollutants,’’ *Aquatic Toxicology*, vol. 162, pp. 82-93, May 2015.

[32] X. Huang, Z. Liu, Z. Xie, S. Dupont, W. Huang, F. Wu, and Y. Wang, ‘‘Oxidative stress induced by titanium dioxide nanoparticles increases under seawater acidification in the thick shell mussel *Mytilus coruscus*,’’ *Marine environmental research*, vol. 137, pp. 49-59, June 2018.