

1-1-2022

Effects of in vivo exposures to nanoparticles (Al₂O₃, CuO, TiO₂) on the activities of ATPases in the gill and muscle of freshwater mussel (*Unio tigridis*)

ESİN GÜLNAZ CANLI

MUSTAFA CANLI

Follow this and additional works at: <https://journals.tubitak.gov.tr/zoology>



Part of the [Zoology Commons](#)

Recommended Citation

CANLI, ESİN GÜLNAZ and CANLI, MUSTAFA (2022) "Effects of in vivo exposures to nanoparticles (Al₂O₃, CuO, TiO₂) on the activities of ATPases in the gill and muscle of freshwater mussel (*Unio tigridis*)," *Turkish Journal of Zoology*. Vol. 46: No. 4, Article 5. <https://doi.org/10.55730/1300-0179.3089>
Available at: <https://journals.tubitak.gov.tr/zoology/vol46/iss4/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Zoology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Effects of in vivo exposures to nanoparticles (Al_2O_3 , CuO, TiO_2) on the activities of ATPases in the gill and muscle of freshwater mussel (*Unio tigridis*)

Esin Gülnaz CANLI^{1*}, Mustafa CANLI²

¹Department of Biology, Faculty of Sciences and Arts, University of Niğde Ömer Halisdemir, Niğde, Turkey

²Department of Biology, Faculty of Sciences and Arts, University of Çukurova, Adana, Turkey

Received: 18.01.2022 • Accepted/Published Online: 22.06.2022 • Final Version: 06.07.2022

Abstract: Mussels are effective bioindicator organisms for aquatic environments. Therefore, they were often used to determine the effects of various xenobiotics in the aquatic systems. There is no study to our knowledge on the in vivo effects of nanoparticles (NPs) on the activities of ATPases in freshwater mussels (*Unio tigridis*). This work demonstrates the effects of Al_2O_3 , CuO, and TiO_2 NPs on Na-ATPase, Ca-ATPase, and Mg-ATPase activities in the gill and muscle of mussels following 14-day exposures to different concentrations of NPs (0, 1, 3, 9 mg/L). Mussels were fed with laboratory cultured algae (*Chlorella vulgaris*) during the exposures. There was no ouabain-sensitive ATPase activity in the tissues of *U. tigridis*. The activities of Na-ATPase and Ca-ATPase in the gill decreased significantly ($p < 0.05$), while Mg-ATPase activity increased. However, the activities of all ATPases decreased significantly after NP exposures in the muscle. There were more significant alterations in the gill compared to the alterations in the muscle. The order of NP effects was determined as $TiO_2 > CuO > Al_2O_3$. There was no significant alteration in algae consumption between control and NP-exposed mussels. The present study reporting the first data on the effects of NPs in *U. tigridis* demonstrated the sensitivity of mussel physiology towards NP exposures, suggesting further studies to understand better the physiological response of mussels.

Key words: Mussel, nanoparticle, ATPase, toxicity, biomarker

1. Introduction

Metal-oxide nanoparticles have recently emerged following the developments in nanotechnology. These matters have size ranges between 1–100 nm and superior physical and chemical characteristics (e.g., high surface to volume ratio, high reactivity, special surface structure, and unique electronic properties) that make them desirable metal complexes in different areas (Chavali and Nikolova, 2019). It is postulated that these matters might be the dominant contaminants in the future considering environmental pollution, especially the aquatic ones. Although there is no considerable data coming from the field studies, laboratory studies demonstrated that NPs are not innocent compounds as they can accumulate and cause toxic effects on different systems in animal metabolisms (Tiple et al., 2020). The environmental quality standard levels for metals were determined to set the maximum allowed metal levels in food and water (McLusky, 1989), though these standards have not been set NPs.

ATPases are a group of enzymes that play an important role in the functional regulation of the cell, and they control metabolic events that require high energy, such as osmotic

pressure, ion movements, and membrane permeability in tissues. For example, Na,K-ATPases are responsible for cell resting potential, active transport, and regulation of cellular volume. In many animal cells, 20% of the total ATP consumed in the cell is used by ATPases, while this situation increases up to 66% in neurons (Kaplan, 2002; Howarth et al., 2012). Moreover, ATPase group enzymes show high electronegative properties and it has been stated that they are highly sensitive to transition metals due to these properties (Kaplan, 2002). Since ATPases are sensitive to environmental pollutants, they were suggested to be used as the early warning systems for the aquatic animals before the osmoregulatory damage occurred due to xenobiotics, making them an important biomarker (Canli and Stagg, 1996; Ay et al., 1999). Although the effects of metals on ATPase group enzymes are well documented, studies on the effects of NPs have been published in the last decades (Khan et al., 2016; Canli et al., 2018; Carmo et al., 2018; Canli and Canli, 2020; Qian et al. 2020). These studies generally demonstrated that NPs alter the activities of ATPases in animals, depending on exposure conditions and species in concern.

* Correspondence: egcanli@gmail.com

The feeding behavior of mussels made them desirable bioindicator organisms to monitor the aquatic systems. As it is well known, mussels can accumulate xenobiotics in high amounts, levels reaching many folds comparing the levels in the water. Our previous work demonstrated that *U. tigridis* accumulated Al-NPs, Cu-NPs Ti-NPs in the gill and digestive gland (Canli and Canli, 2021b). Literature data indicated that metal compounds may have adverse effects on the growth, filtration, behavior, and enzyme activity of mussels that may cause a decline in the population densities and species diversities (Naimo, 1995; Khan et al., 2016). Our previous data demonstrated that *U. tigridis* does not have any ouabain-sensitive ATPase activity. Therefore, we characterized Na-ATPase activity which is dependent on Mg^{2+} and stimulated by Na^+ (Canli and Canli, 2021a). Likewise, Mg-dependent and Ca^{2+} stimulated Ca-ATPase activity was also characterized in the same paper. Data also showed that NPs (Al_2O_3 , CuO,) did alter significantly their activities in vitro, though ionic forms of aluminium and copper significantly inhibited the activities. The present study was planned to investigate in vivo effects of Al-NPs, Cu-NPs and Ti-NPs on the activities of Na-ATPase, Mg-ATPase, and Ca-ATPase in the gill of mussels to enlighten better the effects of NPs following a 14-day exposure protocol.

2. Materials and methods

2.1. Experimental protocols

Mussels were collected from Gölbaşı Lake (Hatay) by professional divers from 1–6 m depths ($36^{\circ}30'17.6''N$ $36^{\circ}29'10.8''E$). The mussels belonging to the Unionidae family from Hatay region were shown to contain very low metal levels in their tissues (Turkmen and Ciminli, 2007). Following the collection of mussels, they were immediately transported to the laboratory where the experiments were carried out and placed in glass aquariums ($36 \times 24 \times 15$ cm) containing tap water. The mussels were allowed to adapt to the laboratory conditions (21.9 ± 0.56 °C) for at least 2 weeks and then they were taken to the experimental mediums. The physical and chemical measurements of the experimental mediums were done regularly with multimeter probes (Orion 5-Star), though some measurements such as hardness and alkalinity were made using the standard titration methods. The results of those measurements (mean \pm se) were as follow; pH (7.52 ± 0.61), oxygen (6.70 ± 0.19 mg/L), total hardness (301 ± 18.4 mg Ca_2CO_3/L), alkalinity (198 ± 10.3 mg Ca_2CO_3/L) and salinity (0.15 ± 0.07 ppt). The mean values and associated standard errors for the length (mm), height (mm), and weight (g) of mussels used in the experiments were 61 ± 0.9 , 24.5 ± 0.4 , and 33 ± 1.5 respectively.

After the adaptation period, mussels were exposed to NPs (Al_2O_3 , CuO, TiO_2) at different concentrations (0, 1,

3, and 9 mg NP/L) for 14 days. A total of 100 mussels were divided among 3 NP exposure groups and control, each containing 10 mussels. The exposure waters of all media were refreshed every two days to keep NP concentrations close to nominal values and also to clean the exposure medium. After 14 days of in vivo exposures, the mussels were taken out from their mediums, washed with isotonic water (0.9 % NaCl), dried and their sizes (length and weight) were measured. Mussels were killed by cutting the anterior and posterior adductor muscles and the gills and food muscles were carefully dissected and frozen at -80 °C (Esco UUS-480A) until analysis. If the shells of the mussels were tightly closed during the dissection, they were accepted as alive.

2.2. Algae culturing and mussel feeding

The cultures of microalga *Chlorella vulgaris* were grown in glass jars, each one containing an 8 L culture medium (3NBBM+V medium) using a batch culture system and stirring continuously by air (Bischoff and Bold, 1963). During the culturing process, algae cultures were illuminated (22 ± 2 °C) with fluorescent lamps (Philips TLM 40W/54RS) at an irradiance level of $80 \mu\text{mol}/\text{m}^2/\text{s}$, using a photoperiod of 16 h light and 8 h dark regime. The irradiance level was measured by a radiation sensor LICOR (LI-250) and algae concentration (optical density) was measured each day by a UV-vis spectrophotometer (Shimadzu UV-1800) at 680 nm for algae growing period of one week until their optic density reached to 1513 ± 30 . Then these algae were used in the feeding of mussels. The mussels were fed 5 h before the water change. For feeding, unicellular algae of *Chlorella vulgaris* species cultured in laboratory conditions were used. Mussels were kept in complete darkness so that they can feed on algae, receiving an approximate algae concentration of 500,000 algae/mL. Algae consumption of mussels was calculated between feeding time zero and after 5 h feeding period using a light microscope (Lecia DM500) and the Thoma glass.

2.2. Measurements of ATPase activity

Samples of gill and muscle tissues were homogenized using a homogenizer (Janke and Kunkel Ultra Turrax T25) in a buffer (1/10) containing 20 mM Tris-HCl, 0.25 M sucrose, and 1 mM EDTA (pH 7.7). Homogenizations were carried out on the ice for 2 min, setting the speed of the homogenizer to 9500 rpm. Then, the homogenates were centrifuged for 20 min using a centrifuge (Hettich Universal 30 RF) at 13,000 g (4 °C). The supernatants were obtained and transferred into Eppendorf tubes (2 mL) for subsequent analysis.

The methods described in our previous paper were used in the measurement of ATPase activities (Canli and Canli, 2021a). This work demonstrated that there was no ouabain-sensitive ATPase activity in *U. tigridis* up to 10 mM ouabain concentrations. Therefore, the activities of

Na-ATPase, Ca-ATPase, and Mg-ATPase were measured in the tissues. Na-ATPase activity was measured from the differences between buffer A (contains 100 mM Na⁺, 3 mM Mg²⁺, 2 mM ATP, 40 mM Tris, pH 7.5) and buffer B (3 mM Mg²⁺, 2 mM ATP, 40 mM Tris, pH 7.5) and Ca-ATPase activity was measured from the differences between buffer C (5 mM Ca²⁺, 4 mM Mg²⁺, 4 mM ATP, 40 mM Tris, 2 mM EGTA, pH 7.5) and buffer D (4 mM Mg²⁺, 4 mM ATP, 40 mM Tris, 2 mM EGTA, pH 7.5). The residual activities were accepted as Mg-ATPase activity. Reaction tubes containing buffers and supernatants (50 μ L) were preincubated at 37 °C for 5 min and then ATP was added to the tubes to start the reaction. All reactions were continued at 37 °C in a water bath (Wise Bath WSB-30) shaking continuously. After 30 min, 0.5 mL of ice-cold distilled water was added to the tubes to stop the reactions. The method of Atkinson et al. (1973) was used to measure ATPase activity. The method relies on determination of Pi liberated from ATP, reading the absorbance at 390 nm (Schimadzu UV-1800 spectrophotometer). Phosphate (KH₂PO₄) standards between 50–600 μ M were prepared to estimate the phosphate concentrations in the reaction mediums. Protein levels were measured using the method of Lowry et al. (1951).

2.3. Nanoparticle characterization

Characterization of metal oxide nanoparticles (Al₂O₃, CuO, TiO₂) was detailed in our previous papers (Canli and Canli, 2019). According to data, Al₂O₃ NPs have an approximate size of ~40 nm and >30 m²/g surface area, whereas CuO NPs have an approximate size of ~40 nm and >20 m²/g surface area and TiO₂ NP have an approximate size of ~21 nm and >30 m²/g surface area. X-ray diffraction analysis (XRD) analysis showed that gamma Al₂O₃ NP was polycrystalline structure and had a cubic phase, whereas Cu NP was a polycrystalline structure and monoclinic phase and anatase TiO₂ NP was a polycrystalline structure and tetragonal phase (Figure 1). Energy-dispersive X-ray

(EDX) analysis was done using a field-emission scanning electron microscope (Zeiss/Supra 55 VP) to determine the contents of NPs. Metal contents of Al₂O₃, CuO, and TiO₂ were 51.1%, 79.1%, and 60.5%, respectively, the remaining atoms being oxygen.

2.4. Statistical analysis

A statistical package program (SPSS 20) was used to perform the statistical test on the data. Data were checked for the homogeneity of variance. First, the one-way ANOVA was applied to the data to estimate significant differences ($p < 0.05$) among controls and exposure groups. Significant results were reanalyzed by post-hoc tests (Tukey) to estimate groups differing significantly from controls. Nonparametric tests (Kruskal-Wallis one-Way ANOVA and Mann-Whitney U-test) were used for data with nonnormal distributions. Mean values and associated standard errors of data were presented as figures, indicating the results of statistical tests.

3. Results

After 14 days of experimental duration, no mussel death was noted in both controls and NP-exposed mussels. ATPase activities were affected from 14-day NP exposures in all tissues (Table). Statistical analysis showed that the activities of Na-ATPase and Ca-ATPase decreased significantly ($p < 0.05$) in the gill of mussels after Cu-NP and Ti-NP exposures, though Al-NP exposure did not cause any significant alteration ($p > 0.05$). Oppositely, Mg-ATPase activity in the gill of mussels increased significantly ($p < 0.05$) after exposure to all NPs (Figures 2–4). However, both Mg-ATPase and Ca-ATPase activities in the muscle of mussels decreased significantly ($p < 0.05$) after all NP exposures (Figures 5–6). During the feeding of mussels, algae numbers ($\times 1000$ algae /mL) in the exposure aquariums ranged between 610–490 and 590–480 at the time 0 (algae addition) and after 5 h feeding period, respectively. Statistical analysis showed that algae

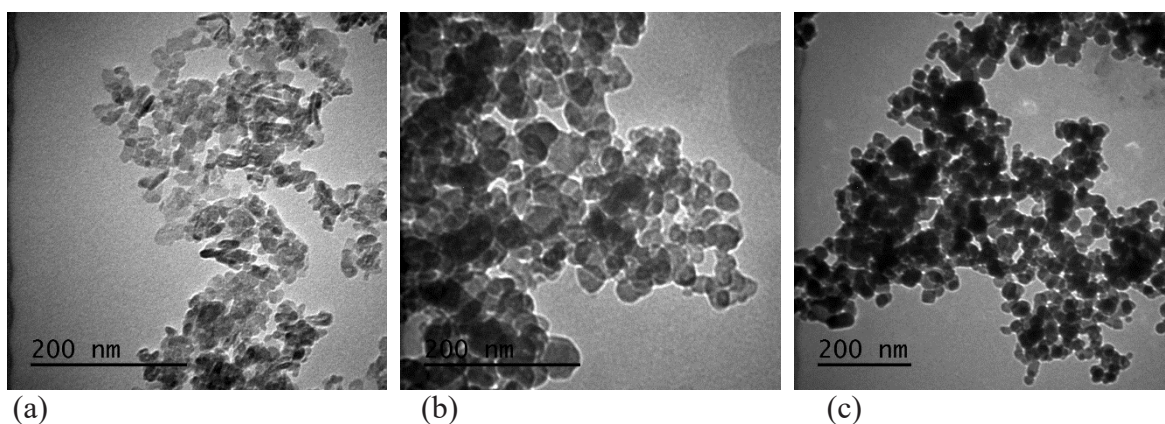


Figure 1. TEM images of Al₂O₃ (a), CuO (b), and TiO₂ (c) nanoparticles in stock solutions (Canli and Canli, 2020).

Table 1. A summary of significant alterations in ATPase activities in the gill and muscle of mussels after 14-day exposure to different concentrations (1, 3, and 9 mg/L) of Al₂O₃, CuO, and TiO₂ nanoparticles. Upward arrows indicate increases and downward arrows indicate decreases. Only significant ($p < 0.05$) alterations were indicated.

TISSUE	ENZYME	Al ₂ O ₃ NP			CuO NP			TiO ₂ NP		
		1	3	9	1	3	9	1	3	9
Gill	Na-ATPase						↓	↓	↓	↓
	Mg-ATPase		↑	↑	↑	↑	↑	↑	↑	↑
	Ca-ATPase				↓	↓	↓		↓	↓
Muscle	Mg-ATPase		↓	↓					↓	↓
	Ca-ATPase			↓		↓	↓		↓	↓

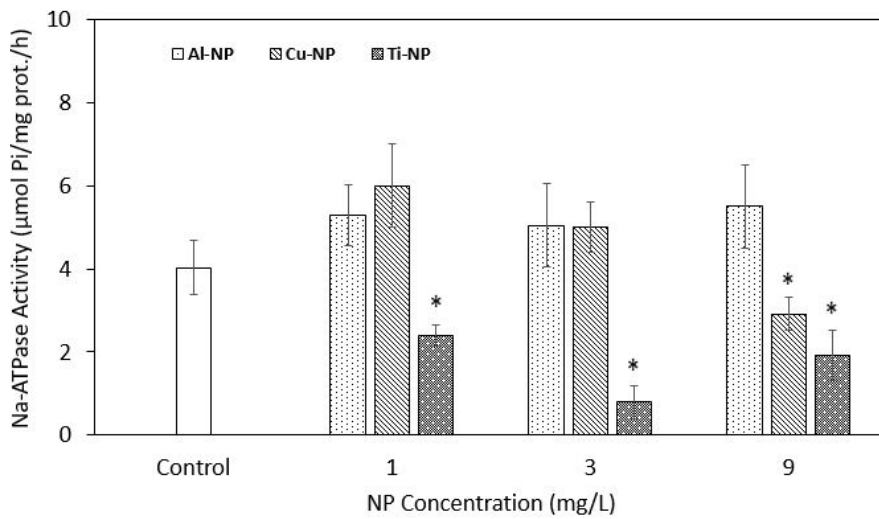


Figure 2. Effects of NPs on Na-ATPase activity in the gill of mussels after 14 days. * indicates significant ($p < 0.05$) differences compared to control.

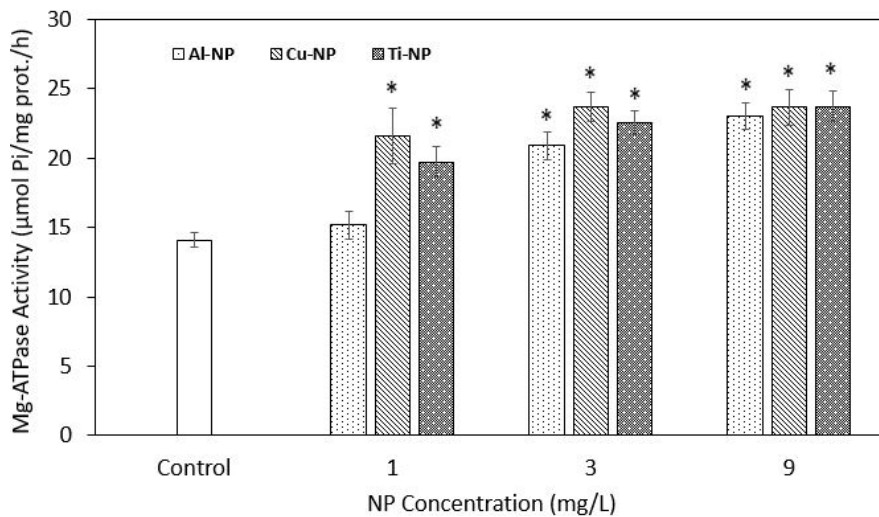


Figure 3. Effects of NPs on Mg-ATPase activity in the gill of mussels after 14 days. * indicates significant ($p < 0.05$) differences compared to control.

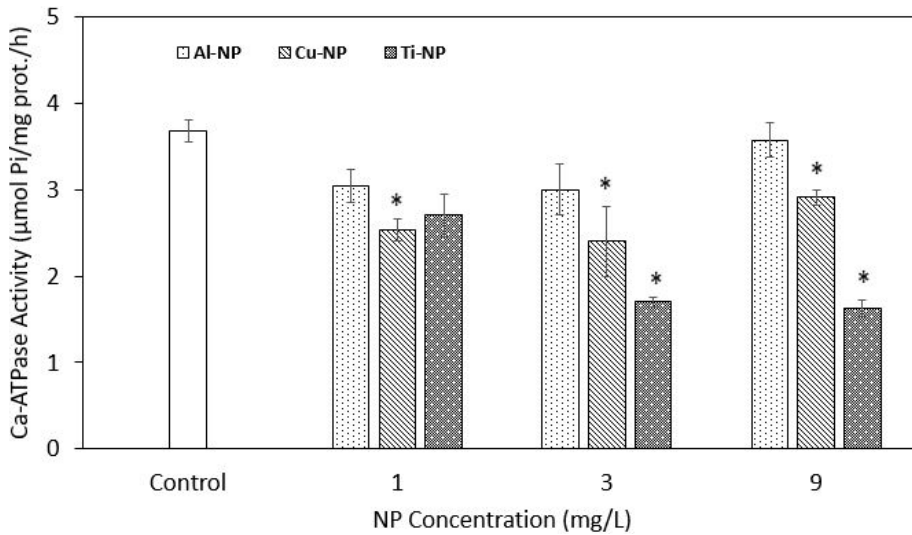


Figure 4. Effects of NPs on Ca-ATPase activity in the gill of mussels after 14 days. * indicates significant ($p < 0.05$) differences compared to control.

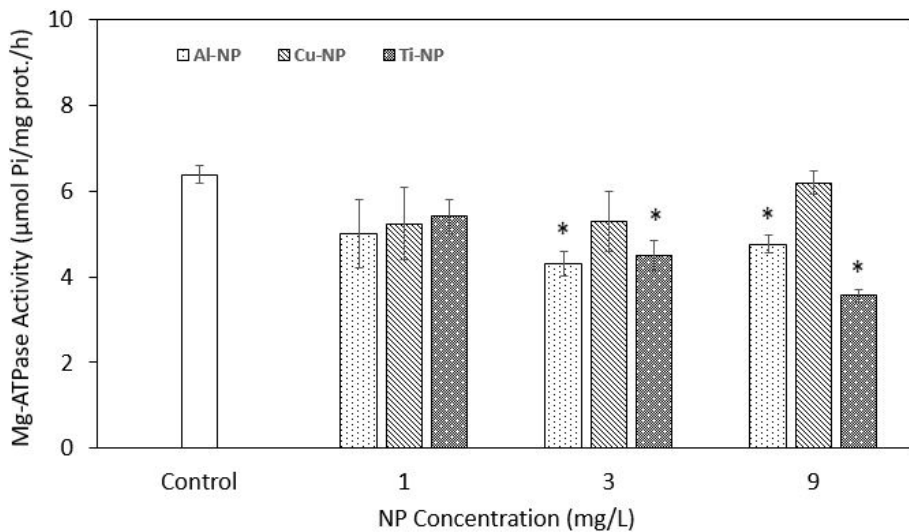


Figure 5. Effects of NPs on Mg-ATPase activity in the muscle of mussels after 14 days. * indicates significant ($p < 0.05$) differences compared to control.

consumption of mussels did not change significantly ($p > 0.05$) among controls and NP-exposed groups.

4. Discussion

The present study is the first one to our knowledge investigating the effects of in vivo NP impacts on ATPases of *U. tigridis*. Therefore, there is no comparable data to evaluate the present data with the literature data. Exposures of mussels to Al-NPs, Cu-NPs, and Ti-NPs for 14 days did not cause any mussel mortality, as the shells of all mussels were tightly closed. Likewise, filtration rates of algae by controls and NP-exposed mussels did not change significantly in the present study. However, there are a few

studies demonstrating the effects of NPs on the filtration rates of freshwater mussels. For example, Ray et al. (2020) found a decrease in the filtration rate of algae by freshwater mussel (*Lamellidens marginalis*) exposed to Cu-NPs for 14 days. Similarly, there are several data demonstrating the changes in food consumption of mussels after different xenobiotic exposures (Anandraj et al., 2002; Culbertson et al., 2008; Liu et al., 2014; Ray et al., 2020).

The present data demonstrated that ATPase activities in the gill and muscle of mussels decreased significantly, except Mg-ATPase activity in the gill. The strong and constant inhibition patterns of ATPases suggest that NPs harm the structures of the membranes of the cells and

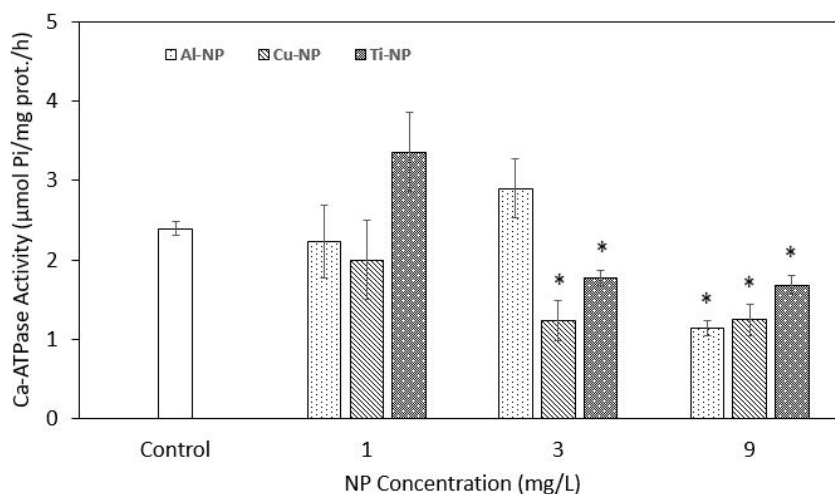


Figure 6. Effects of NPs on Ca-ATPase activity in the muscle of mussels after 14 days. * indicates significant ($p < 0.05$) differences compared to control.

inhibit their activities. Oppositely, increases in Mg-ATPase activity in the gill indicate that the cells need extra energy to cope with the harms caused by NPs, as mitochondrial Mg-ATPase involves in respiration processes. Our previous papers showed that there were significant amounts of Al-NPs, Cu-NPs, and Ti-NPs accumulation in the gill and muscle of *U. tigridis* following exposure to NPs (Canli and Canli, 2021b). Therefore, the alterations in ATPase activities in the present study were accepted as the results of NP accumulations in the gill and muscle of NP-exposed mussels.

The findings of the present work seem in accord with ATPase data obtained from other mollusk species exposed to different NPs. Katsumiti et al. (2015a and b) investigated the effects of Ag-NPs and Ti-NPs on the activity of Na,K-ATPase in the gill of marine mussels (*Mytilus galloprovincialis*). The authors showed that both NPs decreased Na-K-ATPase activity in gill tissue in vitro, suggesting that small-sized NPs are more effective than larger ones. They also emphasized that NP toxicity may vary with the size, mode of synthesis, crystalline structures, and other additives. Similar in vitro effects were also determined in mussels (*Mytilus galloprovincialis*) exposed to Cu NPs, as there was a decrease in Na-K-ATPase activity in gill tissues (Katsumiti et al. (2018). Qian et al. (2020) demonstrated that exposure to TiO₂ NPs altered the osmotic adjustment system in marine mussel *Perna viridis* by reducing Na,K-ATPase activity. They also found that that TiO₂ NPs could block the transformation and detoxification of arsenic in mussels, which would increase the risk of arsenic to marine animals and even humans via the food chain. Similarly, Völker et al. (2015) studied the effects of Ag NPs and found that the activity of Na,K-ATPase decreased up to 83% in the gill of freshwater clam *Sphaerium corneum*. There are more data concerning

the effects of NPs on the response of ATPases of freshwater fish compared to freshwater mussels, emphasizing the hazardous effects of NPs on the osmoregulation system (Federici et al., 2007; Griffith et al., 2011; Remya et al., 2015; Carmo et al., 2018), though there were increases in ATPase activity in fish exposed to different NPs (Wang et al., 2014; Bessemer et al., 2015). Effects of NPs on Ca-ATPase activity in freshwater fish were less investigated and remain to be studied thoroughly. Nevertheless, our previous work demonstrated that Ca-ATPase in the gill, brain, and muscle of freshwater fish (*Oreochromis niloticus*) generally decreased following exposure to Al-NP, Cu-NP, and Ti-NP (Canli and Canli, 2019; 2020). Studies have shown that ATPases are inhibited or stimulated, in the aquatic animals exposed to NPs, depending on the sizes, metal contents, exposure conditions, and species in concern. There is a distinct difference between the in vivo and in vitro methods applied to measure the activities of ATPases. That is, if a generalization is made, in animal tissue homogenates under the influence of metal in vitro, Na, K-ATPase is inhibited to a certain degree depending on the dose, but in vivo metal exposures cause fluctuations in ATPase activity, depending on metal type and experimental conditions. However, in vitro and in vivo effects of NPs on ATPases are still to be investigated thoroughly in aquatic animals, especially in mollusks.

In conclusion, the present study demonstrated that all NPs were able to alter the activities of Na-ATPase, Mg-ATPase, and Ca-ATPase following the 14-day exposure protocol, suggesting the toxic effects of NPs. Thus, this work emphasizes that NPs can cause serious toxic effects in mussel physiology and some measures should be taken to limit the discharge of these matter into the environment, as they seem likely to be the dominant pollutants of the environment.

Acknowledgments

This study received financial support from the Scientific and Technological Research Council of Turkey (project no:120Y016).

References

- Anandraj A, Marshall DJ, Gregory MA, McClurg TP (2002). Metal accumulation, filtration and O₂ uptake rates in the mussel *Perna perna* (Mollusca: Bivalvia) exposed to Hg²⁺, Cu²⁺ and Zn²⁺. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 132 (3): 355-363.
- Atkinson A, Gatemby AO, Lowe AG (1973) The Determination of inorganic orthophosphate in biological systems. *Biochimica Et Biophysica Acta* 320: 195-204.
- Ay O, Kalay M, Tamer L, Canli M (1999) Copper and lead accumulation in tissues of a freshwater fish *Tilapia zillii* and its effects on the branchial Na, K-ATPase activity. *Bull Environ Contam Toxicol* 62 (2): 160-168.
- Besemer RA, Butler KMA, Tunnah L, Callaghan NI, Rundle A et al. (2015) Cardiorespiratory toxicity of environmentally relevant zinc oxide nanoparticles in the freshwater fish *Catostomus commersonii*. *Nanotoxicology* 9 (7): 861-870.
- Bischoff HW, Bold HC (1963) Phycological studies. some soil algae from enchanted rock and related algal species. *Univ. Texas. N.* 6318, pp. 32-36.
- Canli M, Stagg RM (1996) The effects of in vivo exposure to cadmium, copper and zinc on the activities of gill ATPases in the Norway lobster, *Nephrops norvegicus*. *Arch Environ Contam Toxicol* 31 (4): 494-501.
- Canli, E.G., Dogan, A. & Canli, M. (2018). Serum biomarker levels alter following nanoparticle (Al₂O₃, CuO, TiO₂) exposures in freshwater fish (*Oreochromis niloticus*). *Environmental toxicology and pharmacology* 62, 181-187.
- Canli EG, Canli M (2019) Nanoparticles (Al₂O₃, CuO, TiO₂) decrease ATPase activity in the osmoregulatory organs of freshwater fish (*Oreochromis niloticus*); histopathological investigations of tissues by transmission electron microscope. *EC Pharmacology and Toxicology* 7: 909-924.
- Canli EG, Canli M (2020) Investigations of the nervous system biomarkers in the brain and muscle of freshwater fish (*Oreochromis niloticus*) following accumulation of nanoparticles in the tissues. *Turkish Journal of Zoology* 44: 90-103.
- Canli EG, Canli M (2021a). Characterization of ATPases in the gill of freshwater mussel (*Unio tigridis*) and effects of ionic and nanoparticle forms of aluminium and copper. *Comp Biochem Physiol Part C: Toxicol Pharma* 24: 109059.
- Canli EG, Canli M (2021b). Accumulation and distribution of nanoparticles (Al₂O₃, CuO, TiO₂) in tissues of freshwater mussel (*Unio tigridis*). *Bull Environ Contam Toxicol* 108: 702-707.
- Carmo TLL, Azevedo VC, de Siqueira PR, Galvão TD, Dos Santos FA et al. (2018). Reactive oxygen species and other biochemical and morphological biomarkers in the gills and kidneys of the Neotropical freshwater fish, *Prochilodus lineatus*, exposed to titanium dioxide (TiO₂) nanoparticles. *Environ Sci Pollut Res* 25 (23): 22963-22976.
- Chavali MS, Nikolova MP (2019). Metal oxide nanoparticles and their applications in nanotechnology. *SN Applied Sciences* 1: 607-637.
- Culbertson JB, Valiela I, Olsen YS, Reddy CM (2008). Effect of field exposure to 38-year-old residual petroleum hydrocarbons on growth, condition index, and filtration rate of the ribbed mussel, *Geukensia demissa*. *Environmental Pollution*, 154 (2): 312-319.
- Federici G, Shaw BJ, Handy RD (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. *Aquat Toxicol* 84(4): 415-430
- Griffitt RJ, Feswick A, Weil R, Hyndman K, Carpinone P et al. (2011). Investigation of acute nanoparticulate aluminum toxicity in zebrafish. *Environ Toxicol* 26(5): 541-551.
- Howarth C, Gleeson P, Attwell D (2012). Updated energy budgets for neural computation in the neocortex and cerebellum. *Journal of Cerebral Blood Flow and Metabolism* 32 (7): 1222-1232.
- Kaplan JH (2002) Biochemistry of Na,K-ATPase. *Annual Review of Biochemistry* 71 (1): 511- 535.
- Katsumiti A, Berhanu D, Howard KT, Arostegui I, Oron M et al. (2015a). Cytotoxicity of TiO₂ nanoparticles to mussel hemocytes and gill cells in vitro: Influence of synthesis method, crystalline structure, size and additive. *Nanotoxicology* 9: 543-553.
- Katsumiti A, Gilliland D, Arostegui I, Cajaraville MP (2015b). Mechanisms of toxicity of Ag nanoparticles in comparison to bulk and ionic Ag on mussel hemocytes and gill cells. *PLoS one* 10(6): e0129039.
- Katsumiti A, Thorley AJ, Arostegui I, Reip P, Valsami-Jones E, Tetley TD, Cajaraville MP (2018) Cytotoxicity and cellular mechanisms of toxicity of CuO NPs in mussel cells in vitro and comparative sensitivity with human cells. *Toxicology in Vitro* 48: 146-158.
- Khan KU, Zuberi A, Nazir S, Fernandes JBK, Jamil Z et al. (2016). Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile *Tor putitora*. *Turkish Journal of Zoology* 40: (5): 704-712.

- Liu GX, Shu MA, Chai XL, Shao YQ, Wu HX et al. (2014). Effect of chronic sublethal exposure of major heavy metals on filtration rate, sex ratio, and gonad development of a bivalve species. *Bulletin of Environmental Contamination and Toxicology*, 92 (1): 71-74.
- Lowry OH, Rosebrough NJ, Farrar NJ, Randall RJ (1951). Protein measurements with the Folin Phenol Reagent. *Journal of Biological Chemistry* 193: 265-275.
- McLusky DS (1989). *The estuarine ecosystem*. 2nd edition, 215 pp. Blackie, Glasgow.
- Naimo TJ (1995). A review of the effects of heavy metals on freshwater mussels. *Ecotox* 4 (6): 341-362.
- Naime, T.J., 1995. A review of the effects of heavy metals on freshwater mussels. *Ecotoxicology*, 4 (6), 341-362.
- Santini O, Chahbane N, Vasseu, P, Frank H (2011). Effects of low-level copper exposure on Ca²⁺-ATPase and carbonic anhydrase in the freshwater bivalve *Anodonta anatina*. *Toxicol Environ Chem* 93(9): 1826-1837.
- Qian W, Chen CC, Zhou S, Huang Y, Zhu X et al. (2020). TiO₂ nanoparticles in the marine environment: Enhancing bioconcentration, while limiting biotransformation of arsenic in the mussel *Perna viridis*. *Environ Sci Tech* 54 (19): 12254-12261.
- Ray A, Gautam A, Das S, Pal K, Das S et al. (2020). Effects of copper oxide nanoparticle on gill filtration rate, respiration rate, hemocyte associated immune parameters and oxidative status of an Indian freshwater mussel. *Comp Biochem Physiol Part C: Toxicol Pharm* 237: 108855.
- Remya AS, Ramesh M, Saravanan M, Poopal RK, Bharathi S et al. (2015) Iron oxide nanoparticles to an Indian major carp, *Labeo rohita*: Impacts on hematology, iono regulation and gill Na⁺/K⁺ ATPase activity. *Journal of King Saud University-Science* 27 (2): 151-160.
- Tiple AD, Badwaik VJ, Padwad SV, Chaudhary RG, Singh NB (2020). A review on Nanotoxicology: Aquatic environment and biological system. *Materials Today: Proceedings* 29: 1246-1250.
- Turkmen M, Ciminli C (2007). Determination of metals in fish and mussel species by inductively coupled plasma-atomic emission spectrometry. *Food Chem* 103 (2): 670-675.
- Völker C, Kämpken I, Boedicker C, Oehlmann J, Oetken M (2015). Toxicity of silver nanoparticles and ionic silver: comparison of adverse effects and potential toxicity mechanisms in the freshwater clam *Sphaerium corneum*. *Nanotoxicol* 9 (6): 677-685.
- Wang T, Long X, Cheng Y, Liu Z, Yan S (2014). The potential toxicity of copper nanoparticles and copper sulphate on juvenile *Epinephelus coioides*. *Aquat Toxicol* 152: 96-104.