Downloaded from jifro.ir on 2025-10-29

Acute toxicity and effects of titanium dioxide nanoparticles (TiO₂ NPs₎ on some metabolic enzymes and hematological indices of the endangered Caspian trout juveniles (Salmo trutta caspius Kessler, 1877)

Kaviani F.E.¹; Naeemi A.S.^{1,2}*; Salehzadeh A.³

Received: November 2016 Accepted: November 2018

Abstract

Titanium dioxide nanoparticles (TiO₂ NPs) have been incorporated into a large range of materials for different usages and they are very likely to come in wastewater and sewage, finally reaching the aquatic ecosystems. Therefore, valuating the impact of TiO₂ NPs on aquatic environment is a major concern. The aim of this work was to study the effects of TiO2 NPs on metabolic enzymes activity and haematological indices of the Caspian trout juveniles. After determining 96h-LC₅₀, juveniles have been exposed to 0.1 LC_{50-96h} TiO₂ NPs in three replicates for 28 days. The blood samples were collected from fish after acute (24, 48, 72, 96 hours) and sub chronic (7, 14, 21 and 28 days) exposure to the TiO₂ NPs. The analysis showed that the red blood cells count (RBC), haemoglobin (Hb), haematocrit (Hct), white blood cells count (WBC) and lymphocytes have been increased after acute and sub chronic exposure to TiO₂ NPs. Levels of neutrophils and monocytes were increased mostly in acute treatments. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) showed no significant differences. According to analysis of metabolic enzymes activities, levels of Alkaline Phosphatase (ALP) and Aspartate Amino Transferase (AST) after acute and sub chronic exposure as compared to control group were increased/decreased, respectively. Alanine Aminotransferase (ALT) levels showed significant decrease (p<0.05) after 28 days. Lactate Dehydrogenase (LDH) enzyme level increased mostly after acute exposure. The obtained results indicated that the presence of very low amount of TiO₂ NPs could affect most haematological and metabolic enzymes of Caspian trout juvenile.

Keywords: Metabolic enzyme, Haematological indices, Titanium dioxide nanoparticles, Caspian trout

¹⁻Department of Biology, Faculty of sciences, University of Guilan, Rasht, Iran.

²⁻ Department of Marine Science, Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran

³⁻Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran.

^{*}Corresponding author's Email: akramnaeemi@yahoo.com

Introduction

Nanotechnology is the ability of producing materials, devices and new systems production at the molecular and nuclear level (1 to 100 nm) which takes advantage of the properties of that scale (Amelia et al., 2012; Yan et al., 2012). Aquatic nano toxicology is facing the challenge of evaluating the impact of nanoparticles on diverse biological endpoints (Purushothaman et al., 2014). The nanoparticles have widespread usages in many industries especially in paints, materials and cosmetics, which make them possible for them to be released in environment. Therefore, it is necessary to determine their potential risk for living organisms in terrestrial and aquatic environments (Nicolas et al., 2015).

Metal oxide nanoparticles are widely used in many fields of science like food, materials, chemicals and biological sciences (Aitken et al., 2006). Titanium dioxide nanoparticles (TiO2-NPs), as one of the metal oxide nanoparticles, have been widely used in recent years. It has many applications in paint and coating textile industry. This nanoparticle has many usages due to the extraordinary and unique properties like photocatalytic properties. The extensive use of TiO2 NPs can lead to release the significant amounts of these substances with potentially dangerous consequences to the environment (Hall et al., 2009) which raise the concern of the risk to both ecosystems and humans (Ali, 2015). It is reported that TiO₂ NPs toxic effects, including cause inflammation and cell toxicity in mammalian, plants and microorganisms (Warheit et al., 2007; Wang et al., 2007, 2009).

Salmonids are the most valuable aquatic species, which are distributed widely in the most parts of the world. Salmo trutta caspius, is an anadromous fish that is living in the western and southern parts of the Caspian Sea. It is considered as critically endangered species according to the IUCN criteria (Coad, 2000). Most of the rivers of the Caspian Sea have lost their ecological values because of losing their sand and gravel, making dams and barriers development in the path of migrating fish, and overfishing, especially the emission of pollutants and toxins, as a result, these fishes are endangered (Coad, 2000). Therefore, studying the effects of pollutants to protect this species is necessary.

Many reports have been published on the ecotoxicity effects of nanoparticles on fish (Boyle *et al.*, 2013; Abdel-Khalek *et al.*, 2015; Louei Monfared *et al.*, 2015), but findings about the consequences of TiO₂ NPs on blood indices of salmonid fishes are scare (Federici *et al.*, 2007) and as far as it is known no investigation has been conducted on the effects of TiO₂ NPs on haematological indices and metabolic enzymes of Caspian trout.

To determine the health of an organism, blood is utilized as a useful indicator, and the haematological parameters are very important in diagnosis of functional status of animals, which are exposed to the toxic materials (Joshi *et al.*, 2002).

Nanoparticles are very reactive and they have the ability to pass through cell membranes of organisms. Furthermore, their interactions with biological systems are relatively unknown (Khabbazi et al., 2014). Therefore, the aim of this study was to assess the TiO2 NPs acute and subacute effects on metabolic enzymes activities and haematological parameters in S. trutta caspius. The results of the present study can be useful in aquatic toxicity management and environmental health.

Materials and methods

Test organisms

Juveniles of Caspian trout (Salmo trutta caspius) with average length of 20 ± 4 cm and average weight of 25±5 g were obtained from Breeding Centre of Salmonids (BCBCS) in Kelardasht, Mazandaran. Iran. Prior experiments, the fish were adapted to the laboratory conditions in 1000 L fiberglass tanks prior to the experiments for 10 days. Fish were fed daily with a commercial trout pellets and the total volume of water was renewed every 24 h. During the period of adaptation and experiment, fish were maintained in 12 h light/dark cycle of photoperiod with average temperature of $(14 \pm 1 \, ^{\circ}\text{C})$ and pH (7.5 ± 0.2) . The water hardness or the concentration of CaCO3 (230 mg L⁻¹) and dissolved oxygen (8 mg L⁻¹) were controlled every day.

*TiO*₂ –*NP characterization*

TiO₂ NPs with purity of 99% were purchased from Iranian Nanomaterials Pioneers Company. TiO2-NPs were mixed with certain amount of distilled water to obtain a uniform suspension, then sonicated for 15 minutes via ultrasound device (Company: QSonica, model: S3000) at room temperature. The morphology of the nanoparticles was studied by analysis of SEM (Company: Tescan, model: MIRA3) at Razi Metallogy Research Center (Fig. 1a). density and crystalline The structure of the purchased nanoparticles were studied by analysis of X-ray (Company: Philips, model: Xpert MPD) in the X-ray laboratory of Tarbiat Modarres University (Fig. 1b). The properties of the utilized TiO2 NPs are presented in Table 1.

Table1: Properties of used TiO2. NPs.

Bulk density	Specific surface area (SSA)	Color	Purity
0.64 g cm ⁻³	10-45 m ² g ⁻¹	white	99%

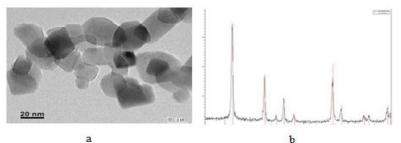


Figure 1: Characterization of TiO₂ nanoparticles: A: SEM image of TiO₂ –NPs, B: X-ray image of TiO₂ –NPs.

Acute lethal assay

For determination of the acute toxicity Caspian trout juveniles, experiment was planned and executed in 50-liter tanks containing 20 litres of aerated water (Static method) according to the procedure, which was described in Organization of **Economic** Cooperation Development and guideline (OECD guideline No. 203, 1992). The feeding process was stopped 24 h prior to beginning of the experiment. An initial test was done to determine the lethal range of TiO₂-NP. To obtain the results of the preliminary test, the fish have been exposed to TiO₂ NPs at concentrations of 50, 100, 125, 150, 175, 200, 225, 250, 275 and 300 mg L⁻¹ of TiO₂ NPs for 96 hours. In this study, 18 tanks were prepared, which were consisted of three tanks as control group and fifteen ones as treatment groups with different concentrations of TiO₂-NPs (five groups in replicates of each group). The number of fish in each tank was 10 (total number=180). Every 24 h the dead fish collected and recorded and the probit analysis has been used to determine the median lethal concentration of TiO2-NPs for Caspian trout (Finney, 1978).

Experimental exposure

After acclimatization, juveniles of Caspian trout (n=90) were transferred to six tanks. The tanks containing 16.054 mg L^{-1} ($10\% \text{ of the LC}_{50-96h}$) of TiO_2 NPs and the control thanks with no TiO_2 nanoparticle (in three replicates). The fish has been exposed to TiO_2 - NPs for 28 days. Sampling was done at 24, 48, 72 and 96 hours,

and 7, 14, 21 and 28 days after exposure to the nanoparticles.

Blood samples collection

At each sampling time, 3 fish were randomly sampled from the six tanks and anesthetized with powdered clove (150 mg L⁻¹) (Adel *et al.*, 2015). Blood samples were taken from the tail vein by heparinized insulin syringe and they were transferred to micro-tubes 0.5 ml containing 0.01 ml heparin. Half of the blood samples for the enzymes analysis were centrifuged for 10 min at 4500 g (Centurion Scientific, UK). The plasma were separated, and stored at -70 °C until analysis (Adel *et al.*, 2015; Abdel-Khalek *et al.*, 2016).

Haematological indices

The total number of red and white blood cells was manually counted by haemocytometer. The haemoglobin (Hb) level of blood was performed by the cyamethaemoglobin method. Cyanomethemoglobin was measured spectrophotometrically at 540 nm wavelength (Abdel-Khalek al.. 2016). The haematocrit (Hct) was determined by micro centrifuge technique, using standards heparinized microhematocrit capillary tubes (75 mm at 7000 g for 10 min) (Adel et al., 2015). Thin blood smear slides were prepared and stained with Wright measuring differential Giemsa for leukocyte cells. To calculate percentages of leukocyte types, at least 100 leucocytes were counted under a light microscope (Blaxhall and Daisley, 2015). The 1973; Adel *et al.*, haematological indices of the mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were computed using the total RBC count, Hb concentration and Hct (Lee *et al.*, 1999).

Biochemical analysis

The activity levels of AST, ALT, LDH and ALP were estimated using commercial kits (Pars Azmoon, Tehran, Iran) and a biochemical auto analyser instrument (Eurolyser, Belgium) (Shahsavani *et al.*, 2010).

Statistical analysis

All of the data were reported as means±standard deviation (SD). A one-way analysis of variance (ANOVA) with Tukey's multiple comparisons was performed to see if there was any

significant difference among the groups. It was found that a difference was statistically significant when p<0.05.

Results

Acute lethal assay

The result of 96 h median lethal concentration values for Caspian trout calculated iuveniles were and summarized in Table 2. By increasing the concentration of TiO2-NPs in the Caspian trout, the acute toxicity increased. No mortality has been observed in the control tanks. By using probit analysis, it was found that the mortality of the 50% of the fish was observed in 160.54 mg L^{-1} . Then, the fish were exposed to 0.1 LC_{50-96h} (i.e. 16.054).

Table 2: Acute toxicity of Caspian trout juveniles (Salmo trutta caspius) exposed to different concentrations of TiO₂ NPs for 96 hours.

Concentration TiO ₂ NPs (mg L ⁻¹)	No. of fish exposed	Mortality (%)	LC50 (mg L ⁻¹)
50	10	0	
100	10	10	
125	10	20	$160.54 \text{ (mg L}^{-1}\text{)}$
150	10	50	
175	10	60	
200	10	70	
225	10	80	
250	10	90	
275	10	90	
300	10	100	

 TiO_2 . NPs acute effects on blood indices

In the present study, red blood cells, haemoglobin and haematocrit were significantly increased when compared to control group at all treatments except 48 h group (Fig. 2 A, B, C). The MCV, MCH and MCHC indices of the samples did not have significant differences with the control groups

(Fig. 3 A, B, C). After 48 hours of exposure to TiO_2 , the number of white blood cells significantly increased in comparison with control group (Fig. 4A). The amount of the neutrophil at all times except 96 h group have been showed significant increase compared to control group (p<0.05) (Fig. 4B). The amount of lymphocyte at 48h group showed significant increase compared to control group (p<0.05)

(Fig. 4C). In the case of monocytes only at 24 and 48 h groups compared to the control group had a significant increase (p<0.05) (Fig. 4 D).

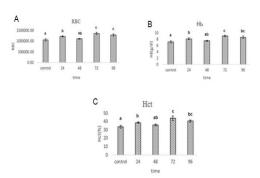


Figure 2: This figure indicates the relation of A: Red Blood Cell (RBC), B: Hemoglobin (Hb), C: Hematocrite (Hct) with time. Different letters in columns indicate a significant difference (p<0.05).

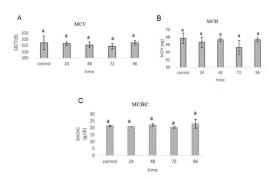


Figure 3: This figure indicates the relation of A: Mean cell volume (MCV), B: Mean cell haemoglobin (MCH), C: Mean cell haemoglobin concentration (MCHC) with time. Different letters in columns indicate a significant difference (p<0.05).

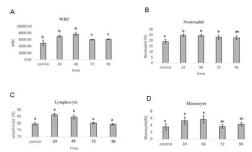


Figure 4: This figure indicates the relation of A: White Blood Cell (WBC), B: Neutrophils, C: Lymphocytes and D: Monocytes with time. Different letters in different columns indicate a significant difference (*p*<0.05).

TiO₂ NPs sub-chronic effects on blood indices

In the present study, the number of red haemoglobin blood cells, haematocrit after 1 and 3 weeks of exposure were significantly increased in comparison with control group (Fig. 5 A, B, C). The MCV and MCH indices showed no significant differences. **MCHC** index only increased significantly compared to control group after 4weeks (Fig. 6 A, B, C). The number of white blood cells was significantly increased in comparison to the control group after 1 and 3 weeks of exposure (Fig. 7A). The amounts of neutrophil and monocyte did not show significant difference with the control group but after 4 weeks significant decrease has been observed comparison to the first and third weeks of exposure (Fig. 7 B, D). Lymphocytes levels increased significantly compared to control at the end of 2, 3 and 4 weeks (Fig. 7C).

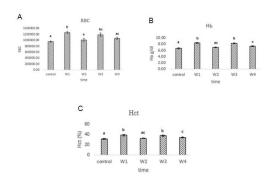


Figure 5: This Fig indicates the relation of A: Red Blood Cell (RBC), B: Haemoglobin (Hb), C: Haematocrit (Hct) with time. Different letters in different columns indicate a significant difference (p<0.05).

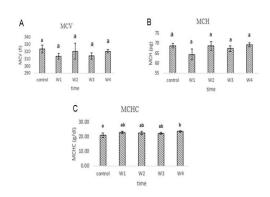


Figure 6: This Fig indicates the relation of A: Mean cell volume (MCV), B: Mean cell haemoglobin (MCH), C: Mean cell haemoglobin concentration (MCHC) with time. Different letters in different columns indicate a significant difference (p<0.05).

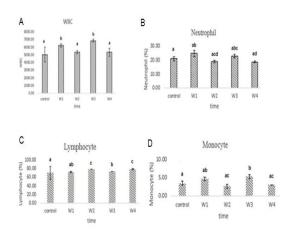


Figure 7: This Fig indicates the relation of A: White Blood Cell (WBC), B: Neutrophils, C: Lymphocytes and D: Monocytes with time. Different letters in columns indicate a significant difference (p<0.05).

TiO₂ NPs acute effects on blood enzymes

In the biochemical analysis of blood, ALP level was significantly increased compared to control group after 72 and 96 hours (Fig. 8A). AST amount in all of the acute treatments showed significant decrease compared to control (Fig. 8B). There were no significant differences in ALT amount (Fig. 8C). LDH showed significant increase in comparison with control enzyme after 72 and 96 hours of exposure (Fig. 8D).

TiO₂ NPs sub-chronic effects on blood enzymes

The levels of ALP were significantly increased in the biochemical analysis of blood, compared to control group after 1, 2 and 3 weeks of experiment (Fig. 9A). The amount of AST showed significant differences and were decreased compared to control group in all treatments except for week 1 treatment group (Fig. 9B). The ALT enzyme only showed significant decrease compared to control after 4 weeks (Fig. 9C). The LDH enzyme showed significant difference after 1 and 2 weeks of exposure in comparison to the control group. The highest level of LDH was observed after 1 week (Fig. 9D).

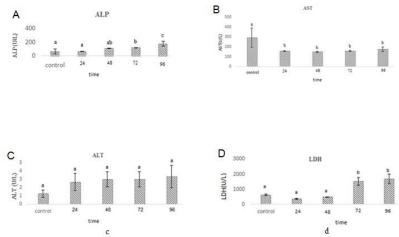


Figure 8: This Fig indicates the relation of A: Alkaline phosphatase (ALP), B: Aspartate aminotransferase (AST), C: Alanine aminotransferase (ALT) and d: Lactate dehydrogenase (LDH) with time. Different letters in columns indicate a significant difference (p<0.05).

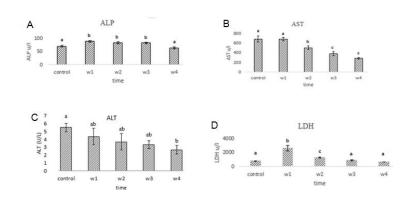


Figure 9: This Fig shows the relation of A: Alkaline phosphatase (ALP), B: Aspartate aminotransferase (AST), C: Alanine aminotransferase (ALT) and D: Lactate dehydrogenase (LDH) with time. Different letters in columns indicate a significant difference (p<0.05).

Discussion

According to the results of acute toxicity during the experimental period, 50 percent of the mortality (LC_{50-96h}) occurred at 160.54 mg L⁻¹. The obtained data of the acute toxicity of TiO₂ NPs on the tested fish species indicated low acute toxicity of TiO₂ NPs within the exposure period of studied: the 48 h LC₅₀ for *Pimephales promelas* >500 mg L⁻¹, the 96 h LC₅₀ for *Oncorhynchus mykiss* > 100 mg L⁻¹

and no lethal effects on *Danio rerio* have been observed below 500 mg L⁻¹ (Menard *et al.*, 2011). In another study, 14 days of exposure to 1 or 10 mg L⁻¹ concentrations of TiO₂ NPs were not lethal to *Oreochromis niloticus* (Nile tilapia) (Perera and Pathiratne, 2012).

The haematological studies are valuable to assess to the environmental impacts of contaminants on fish (Stentiford *et al.*, 2003). In this study, acute exposure to TiO₂ NPs led to an

increase in number of red blood cells, haematocrit and haemoglobin. haematocrit and haemoglobin levels in the rainbow trout blood, which were exposed to 1 mg L⁻¹ of TiO₂-NPs for 14 days, increased (Boyle et al., 2013). In the other study, significant increases were found in erythrocytes count and haemoglobin levels of the peripheral blood of Nile tilapia, which were exposed to 1 and 10 mg L⁻¹ of TiO₂ for 14 days (Perera and Pathiratne, 2012). The increased levels of haematocrit and haemoglobin in the fish blood, exposed to TiO₂-NPs, credited as a recompense for respiratory hypoxia related to the gill damages (Boyle et al., 2013).

In the present study, sub-chronic (3 and 4 weeks) exposure to TiO₂ NPs did not show significant increase in RBC, haematocrit and haemoglobin levels. In addition, MCV, MCH and MCHC indices did not show significant differences at all treatments (except MCHC on 4th week). In some studies, chronic and sub-chronic exposure to TiO₂ NPs did not show any significant changes in erythrocyte quantities of the blood (Federici *et al.*, 2007; Ramsden *et al.*, 2013; Ramsden *et al.*, 2009).

In this study, the WBC, neutrophils, lymphocytes and monocytes levels were significantly increased at acute TiO₂-NPs exposure. In sub-chronic treatments, neutrophil and monocyte levels did not show any significant change but white blood cells were increased significantly after 3 weeks and lymphocytes after 2, 3 and 4 weeks of exposure to NP. Some studies showed that the acute exposures of TiO₂-NPs to rainbow trout did not

cause noticeable changes in the blood neutrophils count (Federici et al., 2007; Ramsden et al., 2009). However, more studies that are recent have described important effects of TiO2 NPs on leucocytic factors of rainbow trout, zebra fish and fathead minnow after sub chronic exposure (Perera 2012). The Pathiratne. increasing amount of leucocytes count in the peripheral blood of the Nile tilapia was related to the 14 days of exposure to 1 and 10 mg L⁻¹ TiO₂ NPs (Perera and Pathiratne, 2012). The changes in leucocytes counts of the blood has been described in rainbow trout exposed to 1 mg L-1 TiO2 NPs for 14 days (Boyle et al., 2013) and subsequent exposure to 0.1 and 1 mg L⁻¹ TiO₂ NPs for 14 days in zebra fish (Ramsden et al., 2013). Since lymphocytes, monocytes and neutrophils, as phagocytes, involved in the immune reactions, their high levels is indicative that the immune system responds to the nanoparticles as a foreign agent and the decrease in the number of WBC may be attributed to unhealthy functioning bloodforming tissues (spleen and kidneys) or it can be a response to several infectious diseases (Al-Bairuty et al., 2013).

The determination quantities of the fish blood biochemical parameters provide critical information for the diagnosis and management of contaminated and uncontaminated samples (Pincus, 1996). Analysis of data obtained from measurement of enzymes activities in the blood showed that, levels of ALP in most treatments and LDH (in 72, 96 h, W1 and W2)

were increased significantly when compared to control group (p < 0.05). The changes in the ALP activities could be resulted from functional physiological changes in fish exposed to pollutants (Jiraungkoorskul et al., 2003). Several studies have been reported increasing the levels of ALP after short or long terms exposure to nanoparticles. Abdel-Khalek et al., (2015) found that 96 hours exposure to CuO - NPs in O. niloticus significantly increase the levels of ALP enzyme when compared to the control groups. In addition, Louei Monfared et al. (2015) reported increasing of ALP activity in common carp exposed to Ag NPs after 8 weeks.

LDH is an enzyme found in almost all tissues, such as liver, kidney, heart, gill and erythrocytes. Measurement of LDH levels is currently used for tissue damage detection (Banaee et al., 2011). LDH activity may signal changes in carbohydrate and protein metabolism in fish under the stress of metals (Elia et al., 2017). The marked increase after treatment with TiO2 NPs indicates that metal exposure induced a hypoxic condition, shifting from aerobic to anaerobic metabolism (Elia et al., 2017). Increasing the levels of LDH concentration has been showed after exposure to ZnO NPs and Ag NPs in common carp (Lee et al., 2014; Louei Monfared et al., 2015). In addition, exposure to Fe NPs significantly increased the levels of LDH activity in O. niloticus (Ates et al., 2016).

According to results, ALT levels show significant decrease after 4 weeks exposure to TiO₂ NPs and the levels of

AST was significantly decreased in acute and sub-chronic (W2, W3 and W4) treatments (p<0.05). ALT and AST are non-functional enzymes of plasma, which are generally localized within the cells of numerous organs including liver. Furthermore, they are considered as significant indicators in evaluating kidney and liver status and tissue damage or organ dysfunction (Louei Monfared and Soltani, 2013). Therefore, reducing AST and ALT levels might be signs of liver cells insufficiency. Besides, ALT and AST enzymes play an important role in protein metabolism and catalyse the intermolecular transferal of the amino groups between amino acids and αketoacidosis. Decreasing the activity of **AST** show that glutamate oxaloacetate are not appropriate for this Krebs cycle via regular transamination (Lee et al., 2006). Some studies reported effects of pollutant on decreasing ALT and AST enzymes activity in fishes (Özdemir et al., 2016). According to Zhu et al. (2009), nanoparticle interaction with biological systems may lead to biochemical disorders or/and adaptive reactions. These reactions (biomarkers) can be used to evaluate the health condition of aquatic organisms (Wang et al., 2009). The results showed that the presence of low amount of TiO2-NPs could affect the blood indices of the Caspian trout iuvenile and subsequently, the processes of growth and fish physiology. It has been also showed that the most parameters of blood and metabolic enzymes have been affected even in the short term. The other observations in exposed fish were changes in the activity of the target enzymes in the fish blood parameters. The results showed that the blood parameters were very sensitive in monitoring the toxicity of TiO2-NPs, especially at sub lethal concentrations and the changes in blood parameters was a useful tool to measure the early exposure to TiO2-NPs. Thus, it can indicate the need for proper management of related organization to prevent the entering the contaminants such as nanoparticles to aquatic environments. Further studies including the effects of TiO2¬-NPs on histopathology, biochemical factors of different tissues and antioxidant gene expression in the Caspian trout need to be done to determine the effects of this nanoparticle.

Acknowledgement

This research was supported by the Caspian Sea Basin Research Centre at the University of Guilan (Project No.1343/ K M). Also we thank breeding centre of Salmonids in Kelardasht city, Mazandaran, Iran for fish supply.

References

Adel, M., AbedianAmiri, A., Zorriehzahra, J., Nematolahi, A. and Angeles Esteban, M., 2015. **Effects** peppermint of dietary (Mentha piperita) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (Rutilus frisii kutum). Fish and Shellfish Immunology, 45,841–847.

Abdel-Khalek, A., Kadry, M.A.M., Badran, S.R. and Marie, M.A.S., 2015. Comparative toxicity of copper oxide bulk and nano particles in Nile Tilapia; *Oreochromis niloticus*: Biochemical and oxidative stress. *The Journal of Basic and Applied Zoology*, 72, 43-57.

Abdel-Khalek, A.A., Badran, S.R. and Marie M.A., 2016. Toxicity evaluation of copper oxide bulk and nanoparticles in Nile tilapia, **Oreochromis** niloticus, using hematological, bioaccumulation and histological biomarkers. Fish Physiology and Biochemistry, 42(4), 1225-1236.

Agrahari, S., Pandey, K.C. and Gopal, K., 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). *Pesticide Biochemistry and Physiology*, 88 (3), 268-272.

Ates, M., Demir, V., Arslan, Z., Kaya, H., Yılmaz, S. and Camas, M., 2016. Chronic exposure of tilapia (Oreochromis niloticus) to oxide nanoparticles: **Effects** of particle morphology on accumulation. elimination. hematology and immune responses. Aquatic Toxicology, 177, 22–32.

Aitken, R.J., Chaudhry, M.Q., Boxall, A.B.A. and Hull, M., 2006. Manufacture and use of nanomaterials: Current status in the UK and global trends. *Occupational Medicine*. (Oxford), 56, 300–306.

- Al-Bairuty, G.A., Shaw, B.J., Handy, R.D. and Henry, T.B., 2013. Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology, 126, 104–115.
- Ali, D., 2015. Evaluation of environmental stress by comet assay on freshwater snail *Lymnea luteola* L. exposed to titanium dioxide nanoparticles. *Toxicological and Environmental Chemistry*, 96, 1185-1194.
- Amelia, M., Lincheneau, C., Silvi, S. and Credi, A., 2012.

 Electrochemical properties of Cd Se and Cd Te quantum dots. *Chemical Society Reviews*, 41, 5728–5743.
- Banaee, M., Sureda, A., Mirvaghefi, A.R. and Ahmadi, K., 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). Pesticide Biochemistry and Physiology, 99 (1), 1-6.
- Blaxhall, P.C. and Daisley, W., 1973.
 Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.
- Boyle, D., Al-Bairuty, G.A., Ramsden, C.S., Slomanb, K.A., Henrya, T.B. and Handya. R.D., 2013. Subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles are associated with gill rather than brain injury. *Aquatic Toxicology*, 126, 116–127.
- Borges, A., Scotti, L.V., Siqueira, D.R., Zanini, R., Amaral, F.D.,

- Jurinitz, D.F. and Wassermann, 2007. G.F., Changes in hematological and serum values biochemical Jundia in Rhamdia quelen due to sub-lethal cypermethrin. toxicity of Chemosphere, 69 (6), 920-926.
- **Coad, B. W., 2000**. Criteria for assessing the conservation status of taxa (as applied to Iranian freshwater fishes). *Biologia*, 55, 537–555.
- Elia, A.C., Magara, G., Righetti, M., Dörr, A.J., Scanzio, T., Pacini, N., Abete, M.C. and Prearo, M., 2017. Oxidative stress and related biomarkers in cupric and cuprous chloride-treated rainbow trout. Environmental Science and Pollution Research, 24(11), 10205-10219.
- El-Sayed, Y.S. and Saad, T.T., 2008. Sub-acute intoxication of a deltamethrin-based preparation (Butox 5% EC) in monosex Nile tilapia, *Oreochromis niloticus* L. *Basic and Clinical Pharmacology and Toxicology*, 102 (3), 293–299.
- Federici, G., Shaw, B. and Handy, R., 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress and other physiological effects. *Aquatic Toxicology*, 84, 415–430.
- **Finney, D.J., 1978.** Statistical method in biological assay. Griffin, London, UK. 508 P.
- Hall, S., Bradley, T., Moore, J.T.,
 Kuykindall, T. and Minella, L.,
 2009. Acute and chronic toxicity of nano-scale TiO₂ particles to freshwater fish, cladocerans, and

- green algae, and effects of organic and inorganic substrate on TiO₂ toxicity. *Nanotoxicology*, 3, 91–97.
- **Heath, A.G., 1995.** Water pollution and fish physiology. 2nd ed. CRC Press, New York. 384 P.
- Jiraungkoorskul, W., Upatham, E.S. and Kruatrachue, M., 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environmental Toxicology*, 18, 260-267.
- Joshi, P.K., Bose, M. and Harish, D., 2002. Changes in certain haematological parameters in a siluroid catfish *Clarias batrachus* (Linn) exposed to cadmium chloride. *Pollution Resources*, 21(2), 129-131.
- Khabbazi, M., Harsij, M., Hedayati, S.A.A., Gholipoor, H., Gerami, M.H. and Ghafari Farsani, H., 2014. Effect of CuO nanoparticles on some hematological indices of rainbow trout (*Oncorhynchus mykiss*) and their potential toxicity. *Nanomedicine Journal*, 2, 67-73.
- Lee, R.G., Foerster, J., Lukens, J., Paraskevas, F., Greer, J.P. and Rodgers, G.M., 1999. Wintrobe's clinical hematology. 10th ed. Lippincott Williams and Wilkins, New York. 2763 p.
- Lee, J.W., Kim, J.E., Shin, Y.J., Ryu, J.S., Eom, I.C., Lee, J.S., Kim, Y., Kim, P.J., Choi, K.H. and Lee, B.C., 2014. Serum and ultrastructure responses of common carp (*Cyprinus carpio* L.) during long-term exposure to zinc oxide nanoparticles.

- Ecotoxicology and Environmental Safety, 104, 9–17.
- Louei Monfared, A. and Soltani, S., 2013. Effects of silver nanoparticles administration on the liver of rainbow trout (Oncorhynchus mykiss): Histological and biochemical studies. European Journal of Experimental Biology, 3(2), 285-289.
- Louei Monfared, A., Bahrami, A., Hosseini, E., Soltani, S. and Shadde, M., 2015. Effects of nanoparticles on histo-pathological changes of the fish. *Journal of Environmental Health Science and Engineering*, 13, 62.
- Menard, A., Drobne, D. and Jemec, A., 2011. Ecotoxicity of nanosized TiO₂. Review of in vivo data. Environmental Pollution, 159, 677-684.
- Nicolas, M., Severine, L.M., Anne, B.N. and Pascal, P., 2015. Effect of two TiO₂ nanoparticles on the growth of unicellular green algae using the OECD 201 test guideline: influence of the exposure system. *Toxicological and Environmental Chemistry*, 98, 860-876.
- **OECD, 1992.** OECD Guidelines for the Testing of Chemicals, Test No. 203: Fish, acute toxicity test. Paris. pp. 1-9.
- Özdemir, G., Çelik, E.S., Yılmaz, S., Gürkan, M. and Kaya, H., 2016. Histopathology and blood parameters of bogue fish (*Boops boops*, Linnaeus 1758) parasitized by *Ceratothoa oestroides* (Isopoda: Cymothoidae). *Turkish Journal of*

- Fisheries and Aquatic Sciences, 16, 579-590.
- Palanivelu, V., Vijavavel, K.. Balasubramanian. S.E. and Balasubramanian, M.P.. 2005. Influence of insecticidal derivative (Cartap Hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish Oreochromis mossambicus. Journal of Environmental Biology, 26(2), 191-195.
- Perera, S.A.D.S. and Pathiratne, A., 2012. Haemato-Immunological and histological responses in Nile Tilapia, *Oreochromis niloticus* exposed to Titanium Dioxide Nanoparticles. *Sri Lanka Journal of Aquatic Sciencesi*, 17, 1-18.
- Pincus, M.R., 1996. Interpreting laboratory results: Reference values and decision making, In: Henry, J.B. (Ed.) Clinical Diagnosis and Management by Laboratory Methods. Nineteenth edition. W. B. Saunders, Philadelphia, PA, USA. pp. 74–91.
- Purushothamana, S., Raghunatha, A., Dhakshinamoorthya, V., Panneerselvam, L. and Perumal, E., 2014. Acute exposure to titanium dioxide (TiO₂) induces oxidative stress in zebrafish gill tissues. *Toxicological and Environmental Chemistry*, 96(6), 890-905.
- Ramsden, C.S., Smith, T.J., Shaw, B.J. and Handy, R.D., 2009. Dietary exposure to titanium dioxide nanoparticles in rainbow trout (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical

- disturbances in the brain. *Ecotoxicology*, 18, 939–951.
- Ramsden, C.S., Henry, T.B. and Handy, R.D., 2013. Sub-lethal effects of titanium dioxide nanoparticles on the physiology and reproduction of zebrafish. *Aquatic Toxicology*, 126, 404–413.
- Rao, J.V., 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish. Oreochromis mossambicus. Pesticide **Biochemistry** and Physiology, 86(2), 78-84.
- Saha, S. and Kaviraj, A., 2009. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. *Chemosphere*, 74(9), 1254-1259.
- Saravanan. M., Suganya, R.. Ramesh. M., Poopal, **R.K..** Gopalan, N. and Ponpandian, N., 2015. oxide Iron nanoparticles alterations induced in haematological, biochemical and ionoregulatory responses Indian major carp Labeo rohita. Journal of Nanoparticle Research, 17, 274.
- Shahsavani, D., Mohri, M. and Gholipour Kanani, H., 2010.

 Determination of normal values of some blood serum enzymes in Acipenser stellatus Pallas. Fish Physiology and Biochemistry, 36(1), 39-43.
- Stentiford, G.D., Longshaw, M., Lyons, B.P., Jonesa, G., Greena,

- M. Feist, S.W., 2003. and Histopathological biomarkers in estuarine fish species the assessment of biological effects of contaminants. Marine Environmental Research, 55, 137-159.
- Wang, J.J., Sanderson, B.J.S. and Wang, Н., 2007. Cytoand genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells. Mutation Research, 628, 99-106.
- Wang, Z., Zhao, J., Li, F., Gao, D. and Xing, B., 2009. Adsorption and inhibition of acetylcholinesterase by different nanoparticles. *Chemosphere*, 77, 67–73
- Warheit, D.B., Webb, T.R., Reed, K.L., Frerichs, S. and Sayes, Ch.M., 2007. Pulmonary toxicity study in rats with three forms of ultrafine-TiO₂ particles: differential responses related to surface properties. *Toxicology*, 230, 90–104.
- Yan, L., Zheng, Y.B., Zhao, F., Shoujian, L., Xingfa, G., Bingqian, X., Weiss, P.S. and Yuliang, Z., 2012. Chemistry and physics of a single atomic layer: Strategies and challenges for functionalization of graphene and graphene-based materials. *Chemical Society Reviews*, 41, 97–114.
- Zhu, H.J., Jia, Y.F., Wu, X. and Wang, H., 2009. Removal of arsenic from water by supported nano zero-valent iron on activated carbon. *Journal of Hazardous Materials*, 172, 1591–1596.