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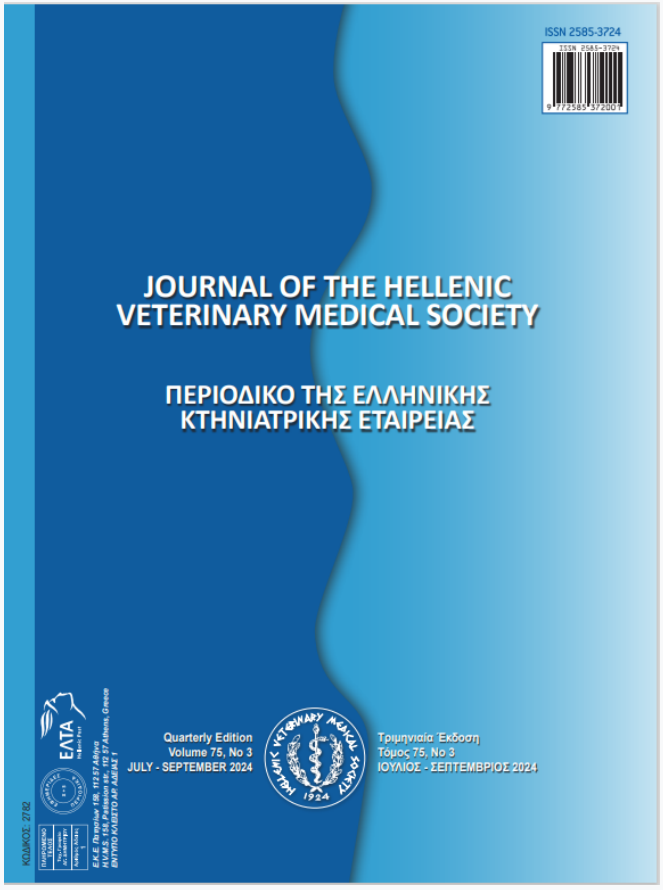

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Research article
Ερευνητικό άρθρο

The α-Al_2O_3 microparticles drive decreasing effects on the sperm quality of rainbow trout

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ABSTRACT: This work examined the effects of alpha-alumina microparticles (α-Al_2O_3-MPs) on the reproductive efficiency of rainbow trout, *Oncorhynchus mykiss* sperm cells. The α-Al_2O_3-MPs (~100 nm) were characterized by Fourier Transform Infrared Spectrometer, X-ray spectrum and Scanning Electron Microscope. After exposure with α-Al_2O_3-MPs (0.125, 0.25, 0.5, 1.0, and 5.0 g/L 24 h, *in vitro*) on sperm cells, it was determined to the motility parameters and total glutathione (tGSH) and superoxide dismutase (SOD) as biochemical parameters of sperm cells. The velocities ofsperm cells decreased after exposure to a 0.125 g/L dose of α-Al_2O_3-MPs. However, while the tGSH levels increased in a dose-dependent manner α-Al_2O_3-MPs, the SOD activities decreased. All these changes were significant ($p < 0.05$) in 1 g/L dose of α-Al_2O_3-MPs when compared with the control group. Finally, we provide a basis for understanding of α-Al2O3-MPs toxicity in this study. So, it may be enabled the assessment of risks associated with α-Al2O3-MPs pollution in aquatic environment, and allow better predictions of the hazardous properties of similar materials in the future.

***Keywords*:** Alpha-Alumina microparticles; *Oncorhynchus mykiss*; Reproduction toxicity.

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INTRODUCTION

Metal oxide structures have been used widely in the industry(Jeng and Swanson 2006; Kumar et al. 2013). Among these metal oxide structures, α-Al_2O_3 has an important place in the field of diffuse industrial technology(Stankic et al. 2016). AlO_x used in batteries, grinding, fire protection, paints, optical polishing, cosmetics, and clothing is produced over 100 t/year(Piccinno et al. 2012). However, the Economic Cooperation and Development (OECD) reported that the Al_2O_3 particles should be placed on the priority list of engineered materials requiring tests the save the environment(Bhuvaneshwari et al. 2016). Depending on the widespread use of Al_2O_3, it can accumulate in natural and natural resources as waste and play a role as a pollutant. Because, it has been known that the metal oxide microparticles have highly toxic potential in both human health and other organisms because they have inhibition survival and molecular degeneration of cells(Xiong et al. 2013). However, due to natural processes, the sizes of microparticles can continuously degrade, and thus their toxicity increases. Other hands, the microparticles can have several colloidal properties and toxicity due to different morphologies(Zhao et al. 2018).

The microparticles have been classified according to their their aerodynamic equivalent diameter into particles > 10 μm, or< 10 μm (PM10), or< 2.5 μm (PM2.5), and ultrafine particles < 0.1 μm. The size category of α-Al_2O_3-MPsin this study is PM2.5 and these microparticles may even pass through the alveoli. It was also reported these size particles are stronger toxic than larger particles(Wieland et al. 2022).

According to recent studies about Al_2O_3-MPs; African green monkey kidney cells (*Vero fibroblasts*) (Keçeli and Alanyali 2004), some plant species such as *Allium cepa* L*., and Zea mays*(Asztemborska et al. 2015), green algae (*Porphyridium aerugineum Geitler*) (Karunakaran et al. 2015), freshwater algae (*Chlorella pyrenoidesa*)(Zhao et al. 2018). However, there were some studies about Al_2O_3-NPs, for example; *Ceriodaphnia dubia*(Pakrashi et al. 2013), *Artemia salina* (Ates et al. 2015), gram +/- bacteria (Bhuvaneshwari et al. 2016), *Carassius auratus*(Benavides et al. 2016), *Scenedesmus obliquus*(Li et al. 2016), *Daphnia manga*(Rostami et al. 2017)*, Oreochromis mossambicus*(Murali et al. 2018), male rats (M'rad et al. 2018; Hamza et al. 2018). According to searching of literature, there has not been found any study about the effects of α-Al_2O_3-MPson the reproduction toxicity or especially sperm quality in fish. Therefore, in this study, the effects *in vitro* of α-Al_2O_3-MPs (0.125, 0.25, 0.50, 1 and 5 g/L) for 1 day on rainbow trout, *Oncorhynchus mykiss* sperm cells were investigated in detail as a metal oxide structure widely used in today's technology.

MATERIALS AND METHODS

The measurement of α-Al_2O_3-MPs

In this study, Al_2O_3 powder and ethanol were supplied with high purity from Sigma-Aldrich. Other chemical reagents used were analytical grade.

Characterization

The chemical structure of α-Al_2O_3-MPs particles was determined by Fourier Transform Infrared Spectrometer (FTIR). It was recorded in the range of 400-4000 cm^{-1} by Perkin Elmer spectrum two FTIR spectrometer for the infrared spectrum of α-Al_2O_3-MPs. To examine its crystal structure, the powder X-Ray diffraction (XRD, Rigaku Rad B-Dmax II) was used. The 2θ values were taken by Cu Kα radiation (λ value of 2.2897 Å) from 5° to 80° with a step size of 0.04°. The morphological structure of α-Al_2O_3-MPs was observed by Scanning electron microscope (SEM; LEO EVO-40xVP). The chemical composition of α-Al_2O_3-MPs was investigated by EDX; a Rönteck Xflash detector analyzer attached to an LEO EVO-40xVP scanning electron microscope. The hydrodynamic diameter was measured by using a Malvern Zetasizer Nano ZS (Malvern Instruments, Southborough, Massachusetts).

The motility parameters of sperm cells

The semen samples of *Oncorhynchus mykiss* (1120±30 g) were collected on January 2021 in Malatya, Turkey. The semen samples for the semen pool were performed by gentle massage on the fish abdomen without anesthesia. The semen pool (6 individual fish) was diluted with inactivation solution (IS) (103 mmol/L NaCl; 40 mmol/L KCl; 1 mmol/L $CaCl_2$; 0.8 mmol/L $MgSO_4$; 20 mmol/L Hepes; pH 7.8; ratio 1:100, Sperm:IS) (Özgür et al 2019). The sperm density was calculated at about 11.4×10^8 cells/mL. The sub-semen samples were exposed with nominal doses such as 0.125, 0.25, 0.5, 1.0, and 5.0 g/L of α-Al_2O_3-MPsat 4 °C for 24 hours incubation. These nominal doses were selected by following the approaches from Asztemborska et al (2015). After incubation, the sperm cells were activated with activation solution (AS) (NaHCO3, 60 mmol/L; Tris, 50 mmol/L; pH: 9; at ratio 1:20 (Sperm:AS)(Özgür et al 2019). All semen samples were kept on the ice and

examined under an Olympus BX53 microscope with a 200x magnification lens and Sony CCD camera with 30 fbs video record capacity. The motility parameters of sperm cells were analyzed by the computer assisted sperm analysis system (Model:BASA-Sperm Aqua, made in Turkey). The values of motility parameters such as the straight line velocity (VSL, μm/s), the curvilinear velocity (VCL, μm/s), the angular path velocity (VAP, μm/s), linearity (LIN, %), the beat cross frequency (BCF, Hz) and the amplitude of lateral displacement of the sperm cell head (ALH, μm)(Özgür et.al 2019) are examined in the study.

Biochemical parameters of sperm cells

For the biochemical parameters, the semen samples were taken into plastic tubes with 500 μL of PBS buffer (pH: 7.4) and then they were homogenized 8 times in cold, 15s by sonicator (Bandelin Sonopuls HD 2070). The resulting homogenate was centrifuged at 10000 rpm for 15 min, and the supernatant was separated and stored at -20°C until the analysis period. The protein content was analyzed using bovine serum albumin (BSA) as the standard (Bradford 1976).

SOD activity was determined using the xanthine oxidase/cytochrome C method(Esrefoglu et al. 2016). Results were expressed in U/mg protein.

Evaluation of tGSH level was carried out as described according to Akerboom and Sies (1981). First, samples were added to 200 μL of phosphate buffer (pH 7.5) followed by the reaction mixture containing NADPH (4 mg/ml), 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) (1.5 mg/ml), and Glutathione reductase (6 unit/ml). In the method, the formation of 5-thio-2-nitrobenzoate (TNB) was measured spectrophotometrically at 412 nm with a microplate reader(Akerboom and Sies 1981). The results were given as nmol/mg protein.

Statistics analysis

The differences between groups were done by Variance Analysis (one-way ANOVA) with the Duncan test after the Test of Homogeneity of Variance in each group. Normality test and descriptive analysis (Means±SE, $p< 0.05$) were performed between the data. The SPSS 15 program for the statistics and Graph Pad Prism 5 for drawing graphics were used.

RESULTS

Characterization of α-Al_2O_3-MPs

The chemical characterization of the α-Al_2O_3-MPs structure was carried out with the FTIR spectrum and X-Ray spectrum. Figure 1a shows the FTIR spectrum of α-Al_2O_3-MPs. In this spectrum, strong bands were caused by the Al-O stretching vibrations, at 543 cm^{-1} 603 cm^{-1}, and 420 cm^{-1}. This spectrum shows the chemical structure of pure α-Al_2O_3-MPs. The X-ray spectrum of the α-Al_2O_3 particles was shown in Figure 1b. In this spectrum, peaks are seen in the values of 25.7, 35.24, 43.44, 52.67, 57.65, 61.27, 66.60, 68.32, 70.51 2 Theta (°). These peaks show the peaks of (012), (104), (113), (024), (116), (122), (214), (300), and (119), respectively. These peaks prove the chemical structure of pure α-Al_2O_3.

The surface and particle morphology of the α-Al_2O_3-MPs structure used in this study were determined by SEM technique. The SEM images of the α- Al_2O_3-MPs structure are given in Figure 2 in different magnifications. Smooth surface morphology of α- Al_2O_3-MPs structure is observed according to SEM images of different sizes with 500X, 2500X, 5000X, 20.000X, and 40.000X.

Finally, the hydrolytic size distribution was examined in the solution used. There is a 204 nm hydrolytically sized dimension. This result proves that the pure structure is less than about 100 nm and has a homolytic distribution (Figure 3).

The α-Al_2O_3-MPs effects on the motility parameters of sperm cells

The motility parameters of rainbow trout sperm cells exposed α-Al_2O_3-MPs (*in vitro*, 24 h) at different doses as 0.125, 0.25, 0.5, 1.0, and 5.0 g/L were determined (Table 1 and Figure 4). The VSL value changing started with 0.125 g/L dose and it was significantly ($p < 0.05$) decreased after 1 g/L of α-Al_2O_3-MPs with the compared control group. The decrease in the VCL due to α-Al_2O_3-MPs exposure was significant ($p < 0.05$) in 0.125 g/L of α-Al_2O_3-MPs with the compared control group. Besides, the lowest VCL value was observed at the doses of 1 and 5 g/L doses. While the VAP value tended to increase after exposure to 0.125 g/L dose of α-Al_2O_3-MPs, it decreased after 1 g/L dose. These increases and decreases were significant ($p < 0.05$) with the compared control group.

The LIN value significantly ($p < 0.05$) increased in dependence on doses of α-Al_2O_3-MPs. The highest LIN value was observed at 5 g/L of α-Al_2O_3-MPs when compared with all groups. The BCF value decreased as statistically insignificant ($p > 0.05$) with up

to α-Al_2O_3-MPs doses and the lowest BCF value was determined at 5 g/L dose. The ALH value showed a decrease with increasing doses of α-Al_2O_3-MPs (Table 1, Figure 4).

The α-Al_2O_3-MPs effects on biochemical parameters of sperm cells

The biochemical parameters as oxidative stress biomarkers such as tGSH and SOD were measured after sperm cells were exposed to α-Al_2O_3-MPs. At the end of this study, while there was a significant ($p< 0.05$) increase in the tGSH levels in 5 g/L dose of α-Al_2O_3-MPs, the SOD activity decreased compared to the control value. However, this decrease in the SOD activity was significant ($p< 0.05$) (Figure 5).

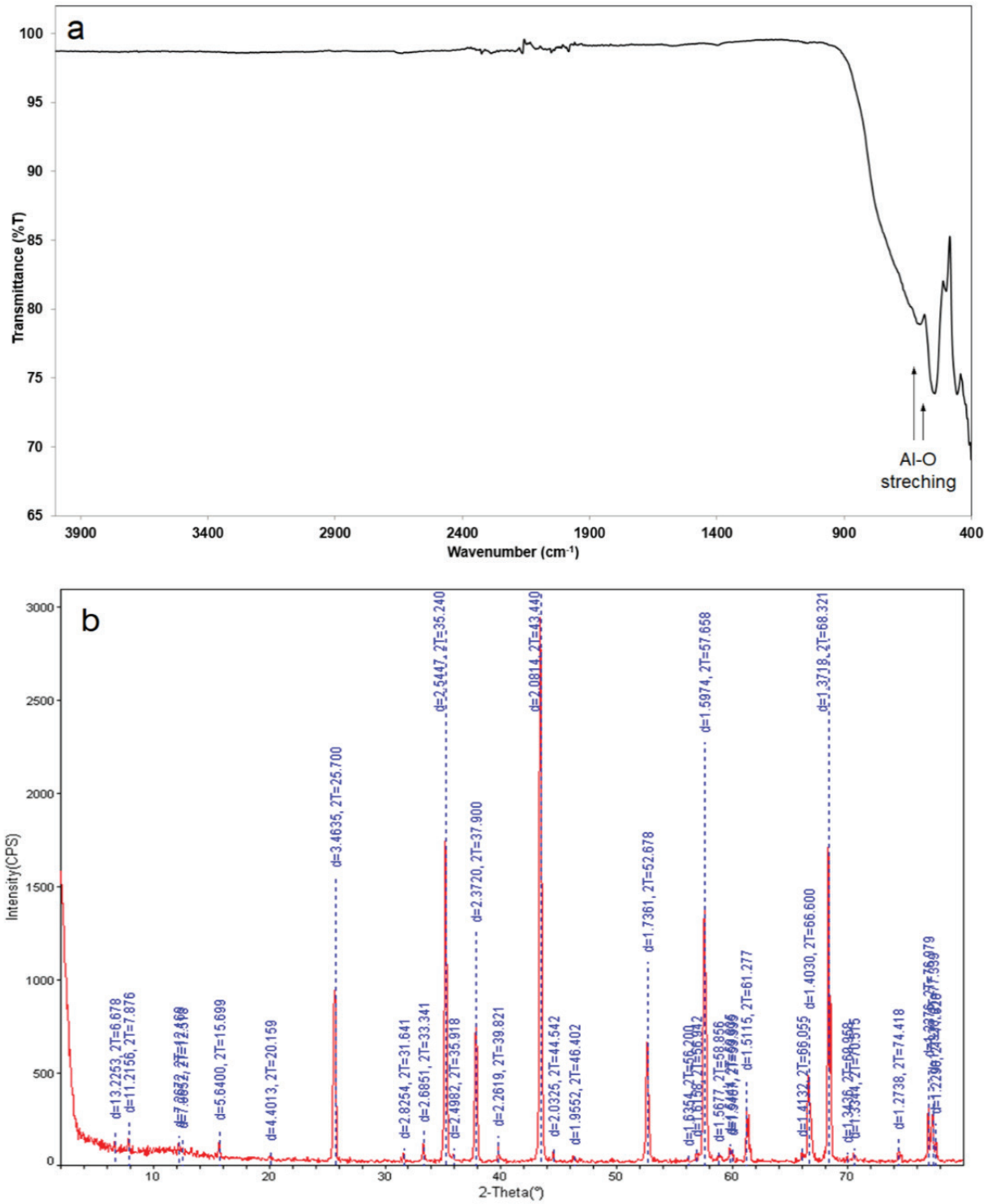

Figure 1. The FTIR spectrum (a) and the XRD pattern (b) of α-Al_2O_3-MPs.

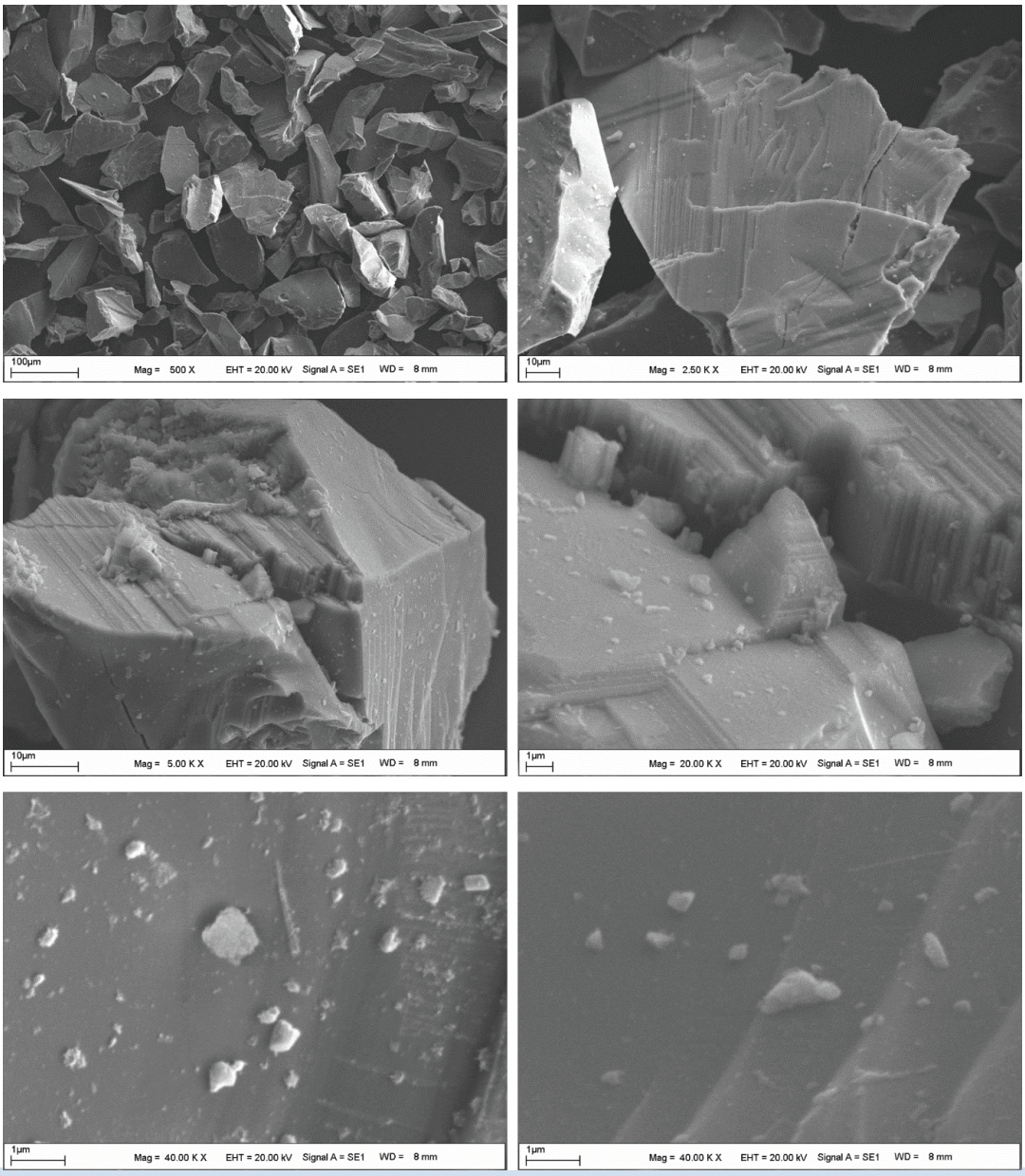

Figure 2. The SEM images of α-Al_2O_3-MPs.

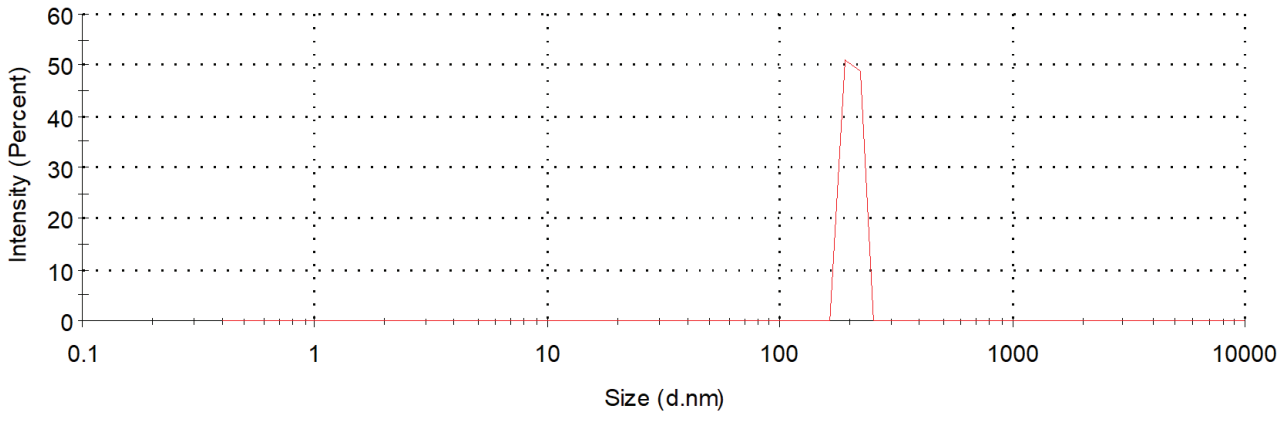

Figure 3. The particle size distribution of α-Al_2O_3-MPs.

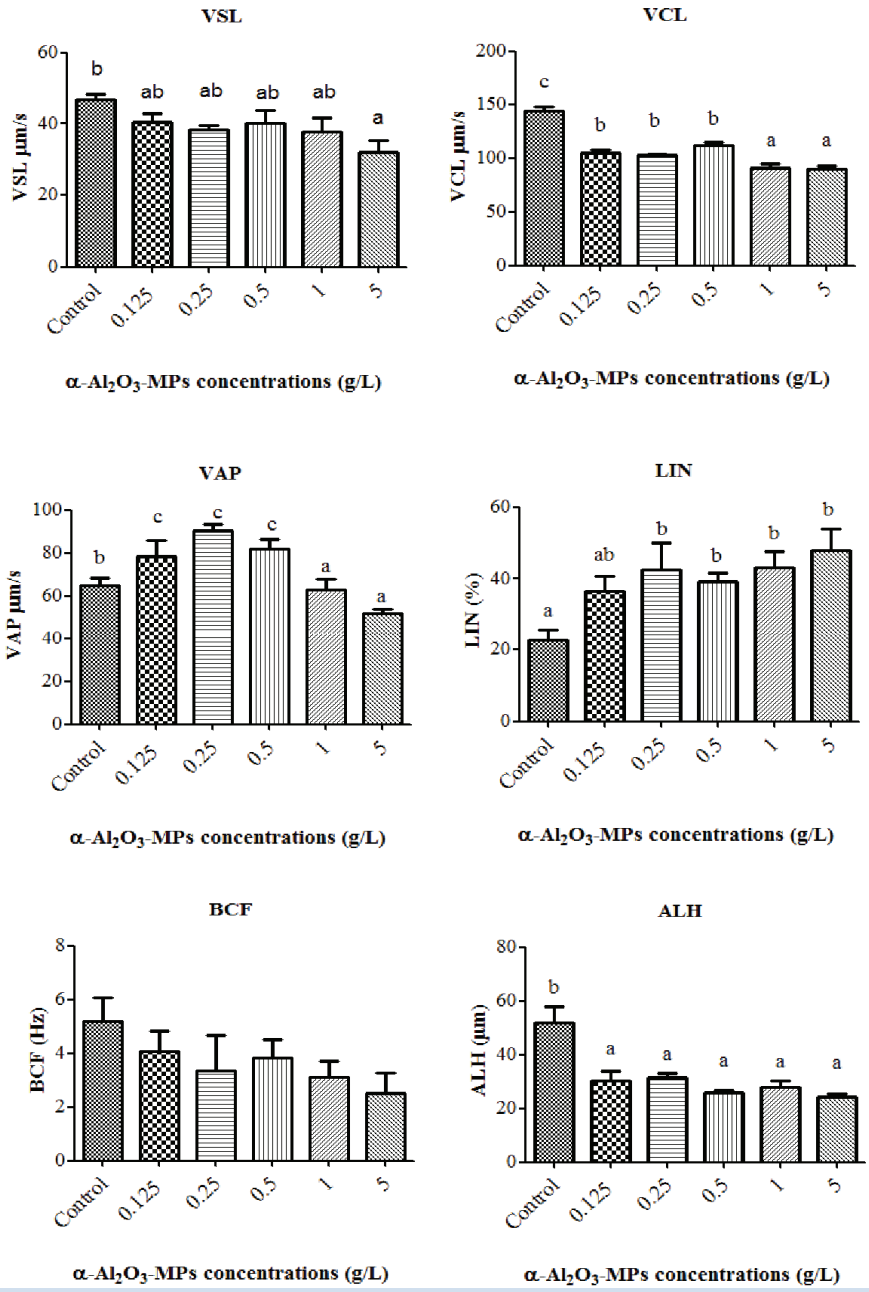

Figure 4. The changes of motility parameters in rainbow trout sperm cells after different α-Al_2O_3-MPs doses (Mean±SE; $p < 0.05$).

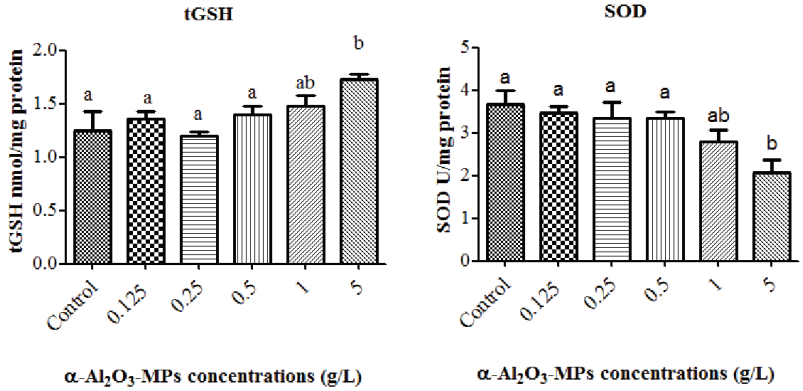

Figure 5. The changes of tGSH level and SOD activity in rainbow trout sperm cells after different α-Al_2O_3-MPs doses (Mean±SE; $p < 0.05$).

Table 1. The motility parameters of rainbow trout sperm cells after exposed α-Al_2O_3-MPs.

N=6			95% Confidence Interval for Mean	
Parameters	**Doses (g/L)**	**Mean± SE**	**Lower Bound**	**Upper Bound**
VSL (µm/s)	**Control**	46.80±1.31^{b}	43.43	50.16
	0.125	40.36±2.52^{ab}	33.87	46.85
	0.25	38.40±1.18^{ab}	35.36	41.44
	0.5	40.13±3.57^{ab}	30.94	49.31
	1.0	37.75±3.93^{ab}	27.65	47.85
	5.0	32.12±3.23^{a}	23.83	40.41
VCL (µm/s)	**Control**	143.52±4.13^{c}	132.90	154.15
	0.125	104.24±3.54^{b}	95.14	113.35
	0.25	103.12±0.80^{b}	101.07	105.17
	0.5	111.32±3.57^{b}	102.15	120.49
	1.0	90.49±4.48^{a}	78.99	102.00
	5.0	89.64±3.48^{a}	80.70	98.57
VAP (µm/s)	**Control**	64.90±3.54^{b}	55.79	74.02
	0.125	78.30±7.47^{c}	59.09	97.51
	0.25	90.39±2.91^{c}	82.90	97.88
	0.5	81.65±4.81^{c}	69.28	94.01
	1.0	41.21±3.01^{a}	33.46	48.95
	5.0	43.70±0.92^{a}	41.33	46.08
LIN (%)	**Control**	22.84±2.85^{a}	15.51	30.16
	0.125	36.48±4.12^{ab}	25.88	47.07
	0.25	42.63±7.25^{b}	23.98	61.27
	0.5	39.09±2.41^{b}	32.90	45.27
	1.0	42.99±4.55^{b}	31.29	54.69
	5.0	47.82±6.12^{b}	32.10	63.55
BCF (Hz)	**Control**	5.17±0.89	2.89	7.45
	0.125	4.08±0.75	2.14	6.01
	0.25	3.33±1.36	0.16	6.81
	0.5	3.84±0.65	2.16	5.53
	1.0	3.12±0.59	1.61	4.63
	5.0	2.52±0.73	.64	4.40
ALH (µm)	**Control**	51.78±6.18^{b}	35.89	67.67
	0.125	30.16±3.82^{a}	20.33	39.98
	0.25	31.55±1.57^{a}	27.53	35.58
	0.5	26.02±0.75^{a}	24.09	27.95
	1.0	27.80±2.53^{a}	21.30	34.31
	5.0	24.41±1.06^{a}	21.70	27.12

Data are presented Mean±SE of values. Different letters as ^{a,b,c} show differences between groups ($p < 0.05$)

Table 2. Biochemical parameters of rainbow trout sperm cells after exposed α-Al_2O_3-MPs.

N=6 Parameters	Doses (g/L)	Mean±SE	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
tGSH (nmol/mg protein)	Control	1.25±0.18[a]	0.78	1.72
	0.125	1.36±0.07[a]	1.18	1.54
	0.25	1.19±0.05[a]	1.07	1.31
	0.5	1.40±0.08[a]	1.20	1.60
	1.0	1.48±0.10[ab]	1.23	1.73
	5.0	1.72±0.05[b]	1.59	1.86
SOD (U/mg protein)	Control	3.67±0.31[a]	2.87	4.47
	0.125	3.46±0.15[a]	3.07	3.86
	0.25	3.34±0.38[a]	2.36	4.32
	0.5	3.34±0.15[a]	2.95	3.74
	1.0	2.78±0.29[ab]	2.05	3.51
	5.0	2.06±0.30[b]	1.28	2.85

Data are presented Mean±SE of values. Different letters as [a,b,c] show differences between groups ($p < 0.05$)

DISCUSSION

Recently, especially in fish reproduction systems, there are limited studies on the toxicity of micro sized and nanosized particles for risk assessment in ecotoxicity. The focus of the present study is to determine the toxic effects of different doses of α-Al_2O_3-MPs on sperm cells of rainbow trout, *Oncorhynchus mykiss*. According to the results in this study, the motility parameters of sperm cells began to be adversely affected after α-Al_2O_3-MPs exposure. While the sperm cell velocity, VCL value started to reduce after 0.125 g/L of α-Al_2O_3-MPs, the VSL value started after 1 g/L dose compared to the control group.

In vitro fish sperm toxicity tests, Linhartova et al (2015) studied the effects of different in vitro doses and over 2 h (0.5, 1.75, 2.5, 5, and 10 µg/L) of Tetrabrombisphenol A on Sterlet (*Acipenser ruthenus*) sperm cells. They determined that the velocity of the sperm cells decreased after exposure to about a 2.5 µg/L dose. SOD activity increased after a 0.5 µg/L dose (Linhartova et al. 2015). Shaliutina et al (2017) investigated the toxicity effects of nonylphenol, propranolol, and diethylstilbestrol on sperm cells of *Acipenser ruthenus*. The motility and speed of sperm cells decreased at all doses according to their results(Shaliutina et al. 2017). Özgür et al. (2019) studied the toxic effects of SiO_2-NPs on sperm cells of rainbow trout. They determined that the values of VSL, VCL, and VAP decreased in a dose-dependent manner of SiO_2-NPs(Özgür et al. 2019). According to these literatures (Linhartova et al. 2015; Shaliutina et al. 2017; Özgür et al. 2019), the decreasing the velocities of sperm cells in our results were supported.

SOD and tGSH are commonly used to measure oxidative stress, which are important cellular antioxidant defense mechanisms. Although the tGSH level increased, the SOD activity decreased with the compared control group at 5 g/L of α-Al_2O_3-MPs.

Several studies have been undertaken to investigate Al_2O_3-MPs. For example, Keçeli and Alanyali (2004) studied the cytotoxicity of Al_2O_3-MPs on African green monkey kidney cells (Vero fibroblasts) in a part of their study. They found that the cell numbers decreased 30% with Al_2O_3, while the cell morphology was not affected(Keçeli and Alanyali 2004). Asztemborska et al (2015) investigated the accumulation of aluminum by four plant species (*Allium cepa* L., *Zea mays, Lepidium sativum* and *Kalanchoe daigremontiana)* exposed to nano- and microsized particles of Al_2O_3. They determined that the accumulation of aluminum depended on the doses (1, 10, and 100 g/kg) of Al_2O_3 in the growth medium and the particle size. However, they found that no toxic effect was in all experiments(Asztemborska et al. 2015). Karunakaran et al (2015) studied the toxicity effects of micro and nanosized particles in SiO_2 and Al_2O_3 on the growth of green algae. They found that Al_2O_3-MPs are less toxic to *P. aerugineum Geitler*, whereas Al_2O_3-NPs nanoparticles are found to be highly toxic at 1 g/L. Additionally, Al_2O_3-NPs decreased the growth, chlorophyll, and protein content of *P. aerugineum Geitler*(Karunakaran et al. 2015). Zhao et al (2018) researched about toxicity effect on freshwater Algae (*Chlorella pyrenoidesa*) in Al_2O_3-MPs with different

morphologies in a part of their study. They tested different doses (300, 150, and 100 mg/L) of bulk Al_2O_3-MPs (100-300 nm) in their study. They found that the ROS levels increased in a dose-dependent manner at the end of the study(Zhao et al. 2018).

Pakrashi et al (2013) studied the acute toxicity of Al_2O_3-NPs (20, 40, 60, 80, 100 and 120 mg/L, 72 h) on *Ceriodaphnia dubia*, a bioindicator in freshwater environments. They determined that reactive oxygen species (ROS) increased with corresponding the increment in doses of Al_2O_3-NPs and exposure time. Another hand, the LC_{50} dose was 117.8 mg/L in 24 h (Pakrashi et al. 2013). Li et al (2016) investigated the effects of Al_2O_3-NPs (0-1 mg/L) on the toxicity and oxidative stress of copper (0.01, 0.05, 0.1, 0.2, and 0.5 mg/L) on freshwater green algae,*Scenedesmus obliquus.* Their results showed that SOD activity decreased either with or without Al_2O_3-NPs in a dose dependent on copper dose. While no significant difference in GSH was observed at 1.0 mg/L dose of Al_2O_3-NPs with the increase in copper dose, they increased gradually in copper and copper/ Al_2O_3-NPs systems. Malondialdehyde (MDA) level increased in both exposure systems(Li et al. 2016).

Benavides et al (2016) studied about single and combined effects of Al_2O_3-NPs (10 and 100 µg/L, 20 nm size) and ZnO-NPs (10 and 100 µg/L, 50 nm size) in the gills and liver of *Carassius auratus*. They found that catalase and superoxide dismutase activity in the gills and livers of fish decreased at both experiments. However, they determined an increase in glutathione S-transferase in gills for all experiments. Also, their histological results showed that there were alterations in liver and gills after NPs of exposure(Benavides et al. 2016). In another study, Murali et al (2018) investigated the toxic effects of Al_2O_3-NPs (120, 150, and 180 mg/L) on freshwater fish *Oreochromis mossambicus.* After the end of the study, they found that the histological alterations were started at a lower dose and the severity of lesions were formed in 180 mg/L of Al_2O_3-NPs in the brain, gill, intestine, kidney, and muscle tissues of fish(Murali et al. 2018).

In this study, while the tGSH level increased, the SOD activity decreased at 5 g/L of α-Al_2O_3-MPs. It can be explained that the capacity of SOD activity to scavenge O_2^- reduced at high α-Al_2O_3-MPs dose because the antioxidant defense system of sperm cells was restricted. Similarly, the negative effects of antioxidant system with Al_2O_3 micro and nanosized particles were reported and supported to our results by other studies(Keçeli and Alanyali 2004; Pakrashi et al. 2013; Karunakaran et al. 2015; Li et al. 2016; Benavides et al. 2016; Murali et al. 2018; Zhao et al. 2018; Özgür et al. 2019).

CONCLUSIONS

In this study, the toxicity of α-Al_2O_3-MPs on the reproduction system of rainbow trout (*Oncorhynchus mykiss*) was studied. While the velocities of sperm cells were affected negatively after 0.125 g/L dose, the antioxidant system started to affect after 1 g/L. Finally, the results investigated from rainbow trout sperm cells exposed to α-Al_2O_3-MPs are consistent with results obtained from other fish species. Thus,in vitro exposure of fish sperm cells could be used as a robust test model for studying the ecotoxicology of microsized particles on aquatic ecosystems. Additionally, we hope that our results can guide researchers to determine the toxic levels especially Al_2O_3-NPs and other microparticles in future studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Akerboom TPM, Sies H (1981) Assay of Glutathione, Glutathione Disulfide, and Glutathione Mixed Disulfides in Biological Samples. Methods Enzymol 77:373-382. doi: 10.1016/S0076-6879(81)77050-2

Asztemborska M, Steborowski R, Kowalska J, Bystrzejewska-Piotrowska G (2015) Accumulation of aluminium by plants exposed to nano-and microsized particles of Al2O3. Int J Environ Res

Ates M, Demir V, Arslan Z, et al (2015) Evaluation of alpha and gamma aluminum oxide nanoparticle accumulation, toxicity, and depuration in Artemia salina larvae. Environ Toxicol 30:109-118. doi: 10.1002/tox.21917

Benavides M, Fernández-Lodeiro J, Coelho P, et al (2016) Single and combined effects of aluminum (Al2O3) and zinc (ZnO) oxide nanoparticles in a freshwater fish, Carassius auratus. Environ Sci Pollut Res 23:24578-24591. doi: 10.1007/s11356-016-7915-3

Bhuvaneshwari M, Bairoliya S, Parashar A, et al (2016) Differential toxicity of Al2O3 particles on Gram-positive and Gram-negative sediment bacterial isolates from freshwater. Environ Sci Pollut Res 23:12095-12106. doi: 10.1007/s11356-016-6407-9

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254. doi: 10.1016/0003-2697(76)90527-3

Esrefoglu M, Akinci A, Taslidere E, et al (2016) Ascorbic acid and beta-carotene reduce stress-induced oxidative organ damage in rats. Bio-

tech Histochem 91:455-464. doi: 10.1080/10520295.2016.1220019

Hamza RZ, Al-Juaid NS, Althubaiti EH (2018) Antioxidant Effect of Carnosine on Aluminum Oxide Nanoparticles (Al2O3-NPs)-induced Hepatotoxicity and Testicular Structure Alterations in Male Rats. Int J Pharmacol 14:740-750. doi: 10.3923/ijp.2018.740.750

Jeng HA, Swanson J (2006) Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng. doi: 10.1080/10934520600966177

Karunakaran G, Suriyaprabha R, Rajendran V, Kannan N (2015) Toxicity evaluation based on particle size, contact angle and zeta potential of SiO_2 and Al_2O_3 on the growth of green algae. Adv nano Res 3:243-255. doi: 10.12989/anr.2015.3.4.243

Keçeli SA, Alanyali H (2004) A study on the evaluation of the cytotoxicity of Al2O3, Nb2O5, Ta2O5, TiO2 and ZrO2. Turkish J Eng Environ Sci 28:49-54

Kumar KY, Muralidhara HB, Nayaka YA, et al (2013) Low-cost synthesis of metal oxide nanoparticles and their application in adsorption of commercial dye and heavy metal ion in aqueous solution. Powder Technol. doi: 10.1016/j.powtec.2013.05.017

Li X, Zhou S, Fan W (2016) Effect of Nano-Al2O3 on the Toxicity and Oxidative Stress of Copper towards Scenedesmus obliquus. Int J Environ Res Public Health 13:575. doi: 10.3390/ijerph13060575

Linhartova P, Gazo I, Shaliutina-Kolesova A, et al (2015) Effects of tetrabrombisphenol A on DNA integrity, oxidative stress, and sterlet (Acipenser ruthenus) spermatozoa quality variables. Environ Toxicol 30:735-745. doi: 10.1002/tox.21953

M'rad I, Jeljeli M, Rihane N, et al (2018) Aluminium oxide nanoparticles compromise spatial learning and memory performance in rats. EXCLI J 17:200-210. doi: 10.17179/excli2017-1050

Murali M, Athif P, Suganthi P, et al (2018) Toxicological effect of Al2O3nanoparticles on histoarchitecture of the freshwater fish Oreochromis mossambicus. Environ Toxicol Pharmacol. doi: 10.1016/j.etap.2018.03.004

Özgür, Mustafa Erkan; Okumuş F. K (2019) A Novel Computer Assisted Sperm Analyzer for Assessment of Spermatozoa Motility in Fish; BASA-Sperm Aqua. El-Cezeri Fen ve Mühendislik Derg. doi: 10.31202/ecjse.486342

Özgür ME, Ulu A, Özcan İ, et al (2019) Investigation of toxic effects of amorphous SiO2 nanoparticles on motility and oxidative stress markers in rainbow trout sperm cells. Environ Sci Pollut Res 26:15641-15652. doi: 10.1007/s11356-019-04941-5

Pakrashi S, Dalai S, Humayun A, et al (2013) Ceriodaphnia dubia as a Potential Bio-Indicator for Assessing Acute Aluminum Oxide Nanoparticle Toxicity in Fresh Water Environment. PLoS One 8:e74003. doi: 10.1371/journal.pone.0074003

Piccinno F, Gottschalk F, Seeger S, Nowack B (2012) Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanoparticle Res 14:1109. doi: 10.1007/s11051-012-1109-9

Rostami I, Mahvi AH, Dehghani MH, et al (2017) Application of nano aluminum oxide and multi-walled carbon nanotube in fluoride removal. Desalin Water Treat 72:368-373. doi: 10.5004/dwt.2017.20699

Shaliutina O, Shaliutina-Kolešová A, Lebeda I, et al (2017) The in vitro effect of nonylphenol, propranolol, and diethylstilbestrol on quality parameters and oxidative stress in sterlet (Acipenser ruthenus) spermatozoa. Toxicol Vitr 43:9-15. doi: 10.1016/j.tiv.2017.05.006

Stankic S, Suman S, Haque F, Vidic J (2016) Pure and multi metal oxide nanoparticles: Synthesis, antibacterial and cytotoxic properties. J. Nanobiotechnology

Wieland S, Balmes A, Bender J, et al (2022) From properties to toxicity: Comparing microplastics to other airborne microparticles. J Hazard Mater 428:128151. doi: 10.1016/j.jhazmat.2021.128151

Xiong S, Tang Y, Ng HS, et al (2013) Specific surface area of titanium dioxide (TiO2) particles influences cyto- and photo-toxicity. Toxicology 304:132-140. doi: 10.1016/j.tox.2012.12.015

Zhao J, Dai Y, Wang Z, et al (2018) Toxicity of GO to Freshwater Algae in the Presence of Al 2 O 3 Particles with Different Morphologies: Importance of Heteroaggregation. Environ Sci Technol 52:13448-13456. doi: 10.1021/acs.est.8b00815