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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Comparison to Toxic Effects of Copper Oxide Nanoparticles and Copper Sulphate on Some Serum Parameters and Enzyme Activities of *Oreochromis niloticus*

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Abstract: Today, human effects due to industrial development and population increase caused water ecosystems to be polluted by various pollutant such as heavy metals. Serum biochemical parameters are widely used in blood analysis and these parameters are suitable indicators for monitoring physiological changes in fish. Hence, the aim of present study to the effects of waterborne copper on serum glucose, total protein, albumin and triglyceride levels and serum cholinesterase (ChE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalane phosphatase (ALP) and lactate dehydrogenase (LDH) activities of *O. niloticus* were determined after exposing the fish to 10, 50 and 100 µg/L copper applied as CuO nanoparticles (CuO NPs) and $CuSO_4$ over 1, 7 and 15 days.

No mortality was observed during the experiments. Serum glucose and albumin levels increased while serum total protein, cholesterol and triglyceride levels decreased compared to control at the end of the 15th day. There was also a decrease in serum ChE activity whereas serum AST, ALT, ALP and LDH activities increased. Overall, $CuSO_4$ and CuO NPs had similar effects in serum parameters of *O. niloticus*.

Keywords: *O. niloticus*, Copper oxide nanoparticles, Copper sulphate, Serum parameters.

Bakır oksit nanopartikülleri ve Bakır sülfatın *Oreochromis niloticus*'da Serum Parametreleri ve Serum Enzim Aktiviteleri Üzerine Toksik Etkilerinin Karşılaştırılması

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Öz: Günümüzde endüstriyel gelişmeler ve nüfus artışına bağlı insan etkileri su ekosistemlerinin ağır metaller gibi çeşitli kirleticiler tarafından kirletilmesine neden olmuştur. Serum biyokimyasal parametreleri kan analizinde yaygın olarak kullanılmaktadır ve bu parametreler balıklarda fizyolojik değişikliklerin izlenmesi için uygun belirteçlerdir. Bu çalışmada 10, 50 ve 100 µg/L CuO nanopartikülleri (CuO NP) ve bakır sülfatın ($CuSO_4$) 1, 7 ve 15 günlük sürelerle etkisinde *O. niloticus*' da serum glikoz, total protein, albumin, kolesterol ve trigliserit düzeyleri ile serum Kolinesteraz (ChE), Aspartat aminotransferaz (AST), Alanin aminotransferaz (ALT), Alkalen fosfataz (ALP) ve Laktat dehidrojenaz (LDH) enzim aktivitelerinin belirlenmesi amaçlanmıştır.

Deney süresince tüm derişimlerde herhangi bir mortalite gözlenmemiştir. 15 günlük deney süresi sonunda her iki ugulamada da serum glikoz ve albümin düzeyleri artış gösterirken, serum total protein, kolesterol ve trigliserit düzeylerinde azalış gösterdiği belirlenmiştir. Serum enzim aktivitelerinde ise ChE aktivitesi azalma gösterirken, serum AST, ALT, ALP ve LDH aktiviteleri deney süresi sonunda artış göstermiştir. Sonuç olarak *O. niloticus* serum parametreleri üzerine toksik etkileri karşılaştırıldığında her iki uygulamanın da benzer etkiler gösterdiği belirlenmiştir.

Anahtar kelimeler: *O. niloticus*, Bakır oksit nanopartikülleri, Bakır sülfat, Serum parametreleri.

INTRODUCTION

Small amounts of heavy metals enter aquatic environments by volcanic activities, floods and erosion. These natural gradual entries do not impose any danger to aquatic biota due to various adaptive mechanisms. Severe increases observed in the amount of heavy metals entering these habitats mainly due to anthropogenic activities, however, result in serious environmental and human health problems (De et al., 2010; Jorgensen, 2010). In recent years,

the rapid development in industry and agriculture and population growth in the industry cause the aquatic environment to be polluted by various water pollutants especially heavy metals (Wang et al., 2013; Ji et al., 2015).

In recent years, the production of nanomaterials has been increasing in industrial areas, in addition to this, there has been an increase in the widespread use of various uses, especially in the fields of biomedical and biotechnology (Gomes et al., 2013). Considering their chemical composition, the most important of the nanoparticles are carbon based - and metal oxides (Klaine et al., 2008; Bhatt & Tripathi, 2011). As a result of the increasing use of metal oxide nanoparticles, these particles reach aquatic environments via after disposal of NP-containing products and the effluent of wastewater treatment plants and application of biosolids to soil, or leachates from landfills (Tolaymat et al., 2017; Bundschuh et al., 2018). In recent years, toxicological research has focused on metal oxide nanoparticles from these type of nanoparticles. (Ringwood et al., 2010; Buffet et al., 2011).

Hematological and biochemical parameters are widely used in blood analysis and these parameters are suitable indicators for monitoring physiological changes in fish. Serum biochemical analysis provides information about internal organs, electrolytes, proteins, nutritional and metabolic parameters (Newman et al., 1997).

O. niloticus, which is used as a material in the research, is a tropical fish, and it is widely cultivated in culture conditions since it is consumed as a protein source. The reason why their cultivation in culture conditions is widespread is that they are highly resistant to temperature and salinity changes in tropics and subtropics, have a short food chain, adapt in a short time to intensive stocking, and are suitable for maintenance and nutrition under culture conditions. (Tunçsoy & Erdem, 2018). It is also important to know whether different forms of metal have different effects on biological functions of fish. Hence the present study was undertaken to compare the toxic effects of CuO nanoparticles and $CuSO_4$ on some serum parameters and serum enzymes of *O. niloticus*

MATERIAL AND METHOD

Commercially available CuO nanopowder (Sigma-Aldrich; particle size <50nm) and $CuSO_4$ (Sigma Aldrich; $CuSO_4$:$5H_2O$) were used in the experiment. Solutions were replaced daily by serial dilutions of freshly prepared 100 ppm stock solutions of the metal. Before each renewal CuO solution was sonicated for 30 min (Ultrasonic bath 230V, 200 W, 45kHz frequency) before each renewal to break down the size of aggregates. Results of characterization of CuO nanoparticles are given Tunçsoy and Erdem, (2018).

O. niloticus was obtained from the rearing pools of Fisheries Faculty, Çukurova University, Adana, Turkey. The mean length and weight of fish were 20.50±1.50 cm and 170.95±5.21 g respectively. Fish were adapted to laboratory conditions for one month in glass aquaria 40x120x40 cm in height. Experiments were run in triplicate being one fish in each replicate, hence nine fish were placed into each aquarium totaling to 63 fish (n = 63). The same sized seven aquaria were used in the experiments. The first six aquarium was filled with 120 L of 10, 50, 100 µg Cu/L as a Cu NPs and 10, 50, 100 µg Cu/L as a $CuSO_4$ solution whereas the seventh one was filled with the same amount of copper free tap water and used as control. Some physical and chemical properties of experimental water are given Tunçsoy and Erdem, (2018).

Fish were fed once a day with readymade fish feed (Pınar, Pellet No: 2) at amounts of 2% of total biomass and 12h/12h light/dark illumination regime was adopted. Three fish were removed from each aquarium at the end of exposure period and were anesthetized with MS222. They were then washed with tap water and dried with Whatman filter papers. Blood samples to be used for determining sera parameters were obtained by cutting caudal peduncle vertically. They were transferred to anticoagulant free centrifuge tubes and centrifuged at 4000 rpm for 10 minutes (Nuve NF400). Sera samples were then transferred to sera tubes and analyzed using a Beckman Coulter LH 750 auto-analyzer.

RESULTS AND DISCUSSION

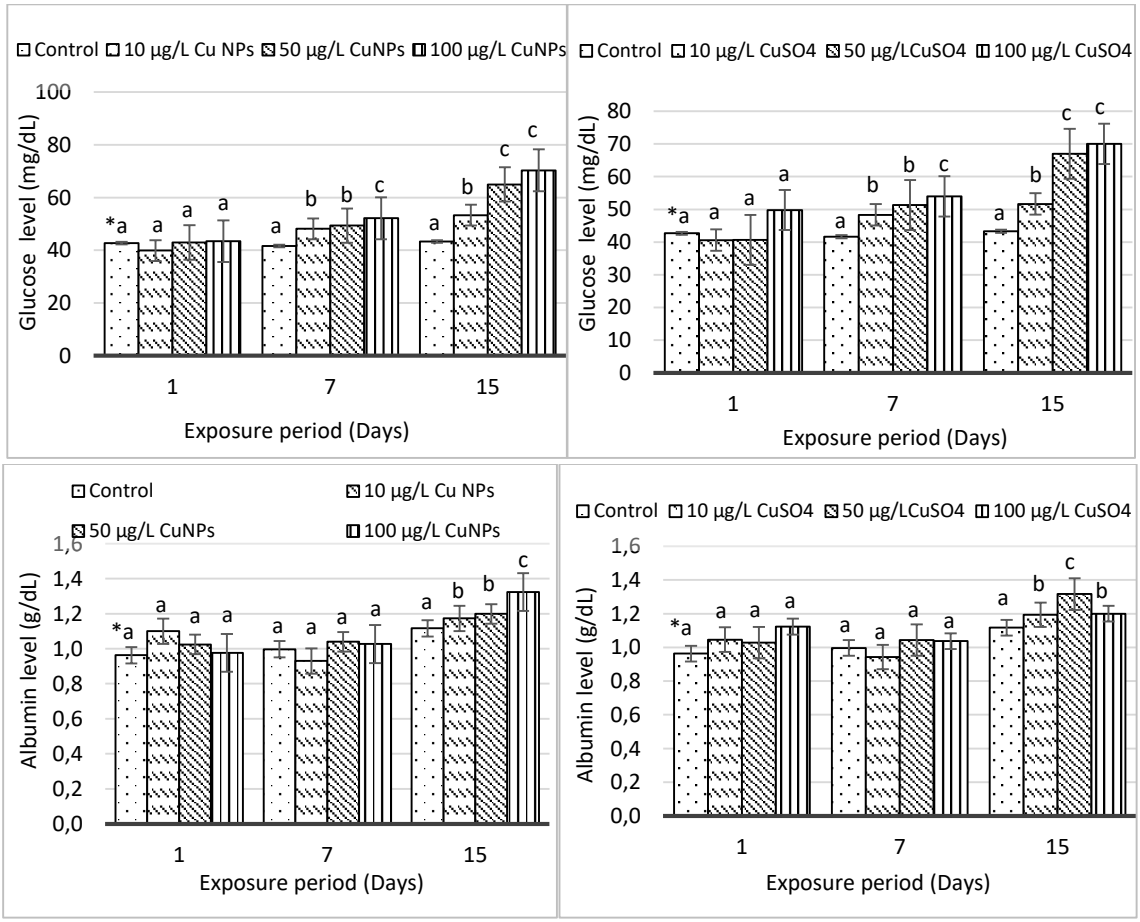
No mortality was observed in experiment. Stress factors such as metal effects cause changes in carbohydrate metabolism by increasing the release of hormones such as cortisol, epinephrine and catecholamine (Vosyliene, 1999). It is stated that the heavy metal effect causes stress in fish, the energy requirement increases under stress conditions and as a result, it stimulates the release of these hormones and causes glycogenesis and mobilization of muscle and liver glycogen (Brown, 1993; Tuncsoy et al., 2016). Many studies have shown that serum glucose levels increase in the case of stress caused by metal effects. (Iwama et al., 1999; Cicik & Engin, 2005; Ramesh, 2007). Determination of serum total protein level is used as an indicator of liver damage and is a parameter related to the nutritional status of fish and is used as a marker of xenobiotic-induced stress. Total protein and cholesterol are markers of nutritional status in the organism (Yang & Chen, 2003). The main function of triglycerides is to store and supply cellular energy. It also shows the feeding status of fish.

Table 1. Effects of various NPs and Cu on blood serum parameters in different fish species.

Tablo 1. Farklı nanopartiküllerin ve bakırın çeşitli balık türlerinde serum parametreleri üzerine etkileri.

Species	NPs Type	Effects	Reference
C. carpio	CuO NPs TiO_2 NPs	Both metals cause histopathological anomalies in the gill and liver tissue.	Mansouri et al., 2016
	Cu NPs	Serum triglyceride level increased under the effect of 25 ppm Cu NP on the 3rd days of exposure compared to the control. Serum cholesterol level increased on the 14th day under the effect of 0.25 ppm Cu NP and on the 14th day under the effect of 25 ppm Cu NP compared to the control.	Mazandarini & Hoseini 2015
	Ti NPs	Serum glucose level and AST, ALT ve ALP activities increased, cholesterol and total protein levels unchanged.	Tunçsoy & Duran, 2020
	Cu NPs	Serum AST and ALT activities increased.	Hoseini et al., 2016
Salmo trutta	$CuSO_4$	Serum glucose and cortisol levels increased	Nemcsok & Hughes, 1988
C. gariepinus	Ti NPs	Serum AST, ALT, ALP, LDH, glucose and albumin increased while serum Na^+,K^+ and Cl^-, total protein, cholesterol, ChE and triglyceride decreased.	Tunçsoy, 2021
Rhamdia quelen	$CuSO_4$	Serum glucose level increased, while total protein level decreased.	Pretto et al., 2014
O. mykiss	$CuCl_2$	Serum cholesterol levels decreased	Heydarnejad et al., 2013
O. niloticus	CuO NPs	Serum glucose level and serum AST, ALT and ALP activities increased, while serum total protein, total lipid, albumin and globulin levels decreased.	Abdel-Khalek et al., 2015
	$CuSO_4$	ALP activity increased.	Palandöken lier & Kargın, 2019
	Zn NPs	Serum triglyceride levels increased, whereas cholesterol levels decreased. There was no changed serum glucose and total protein levels.	Chen et al., 2004
	CuO NPs	Serum AST, ALT and ALP levels dose-dependent increased.	Abdel-Latif et al., 2021
	ZnO NPs	Serum AST, ALT and ALP activities increased, while serum total protein and albumin levels decreased compared to control.	Abdel-Daim et al., 2019
Salmo trutta caspius	CuO NPs	Although serum AST, ALP and LDH activities increased after 96 hours, ALT activity increased after 28 days of exposure.	Kaviani et al., 2019

In this study, serum glucose levels increased with increasing Cu concentrations and periods in *O. niloticus* exposed to both CuO nanoparticles and $CuSO_4$ (Fig 1; $P<0.05$). The increase in serum glucose can be explained by the increase in glycogenolysis due to the increased energy demand under the influence of metal stress. Copper increased serum albumin levels in *O. niloticus* after 15 days of exposure, both in metal form (Fig 1; $P<0.05$). This may be due to the increased synthesis of metal-binding proteins to provide more binding sites. In *O. niloticus*, serum total protein levels also decreased after 7 days of exposure to CuO NPs and after 15 days of exposure to $CuSO_4$ (Fig. 1; $P<0.05$), which may result from inhibition of serum protein synthesis due to metal-induced liver injury at prolonged exposures. Serum cholesterol and triglyceride levels of *O. niloticus* also decreased 15 days after exposure to CuO NPs and $CuSO_4$ (Fig 1; $P<0.05$). The reduction of total serum cholesterol under the influence of copper may be partly the result of liver damage, the use of cholesterol in the synthesis of corticosteroid hormones, or gluconeogenesis. The decrease in serum triglyceride was probably due to meeting energy needs from non-glycogen sources via gluconeogenesis.



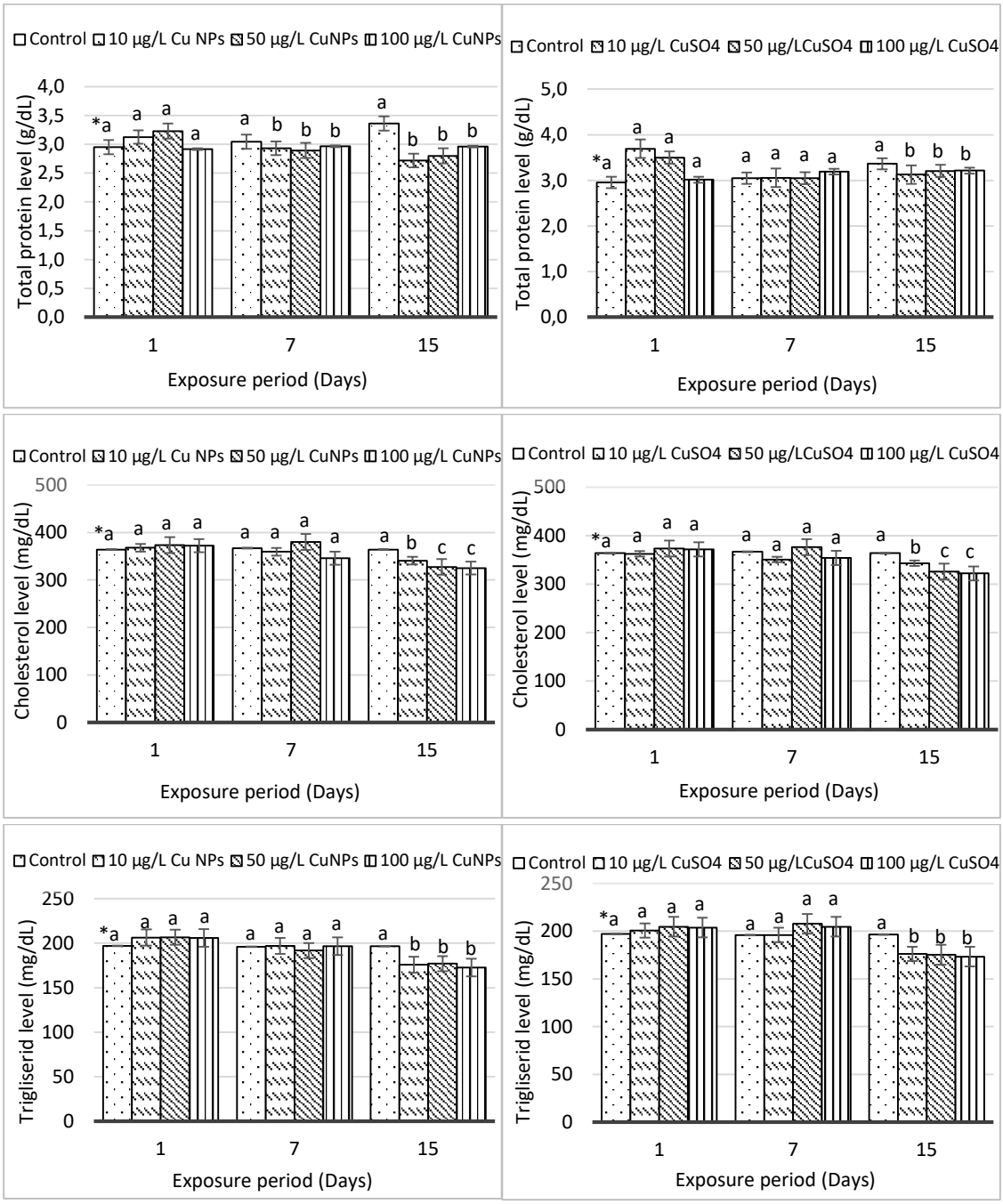
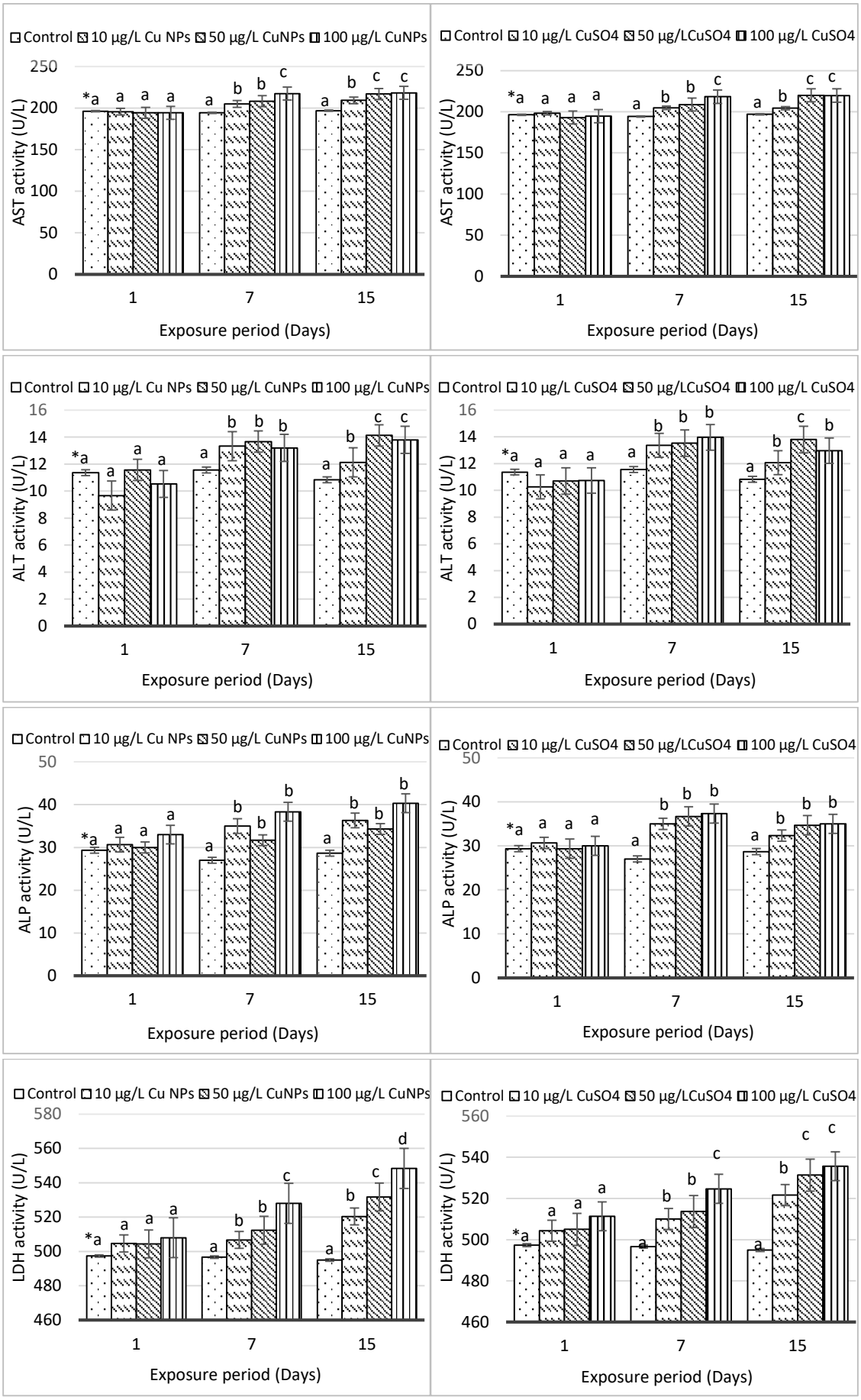


Figure 1. Influence of Cu NPs and $CuSO_4$ on serum parameters of *O. niloticus.*
*=SNK; Letters a, b and c show differences among concentrations. Data shown with different letters are significant at the P<0.05 level.

Serum enzymes such as ChE, AST, ALT, ALP and LDH are used as sensitive biomarkers to determine the effects of contaminants for aquatic organisms (Levesque et al., 2002; Nel et al., 2009). ChE hydrolyze acetyl choline and other choline-esters. AST and ALT play roles in amino acid metabolism and their increased levels in serum indicate mainly liver, kidney and gill damages (Hoseini et al., 2012). Any damage to liver and kidney tissues increases the level of serum ALP (Karan et al., 1998; Jiraungkoorskul et al., 2003). LDH is an enzyme that catalysis reversible oxidation of lactate to pyruvate and LDH level increase at any tissue damage (Yousef et al., 2007) .

In the present study, AST, ALT, ALP and LDH activities increased compared to control after 7 and 15 days and ChE activity decreased after 15 days of exposure to both forms of metal in *O. niloticus.* These variations in enzyme activities became more pronounced with increasing concentrations of copper and exposure periods (Fig 3; P<0.05). Excess energy used under the effect of copper might be compensated from non-carbohydrate sources and the increase in serum enzyme levels might be attributed to damages in liver and kidney tissues.


Control 10 µg/L Cu NPs 50 µg/L CuNPs 100 µg/L CuNPs
AST activity (U/L)
Exposure period (Days)
Control 10 µg/L CuSO4 50 µg/LCuSO4 100 µg/L CuSO4
AST activity (U/L)
Exposure period (Days)
Control 10 µg/L Cu NPs 50 µg/L CuNPs 100 µg/L CuNPs
ALT activity (U/L)
Exposure period (Days)
Control 10 µg/L CuSO4 50 µg/LCuSO4 100 µg/L CuSO4
ALT activity (U/L)
Exposure period (Days)
Control 10 µg/L Cu NPs 50 µg/L CuNPs 100 µg/L CuNPs
ALP activity (U/L)
Exposure period (Days)
Control 10 µg/L CuSO4 50 µg/LCuSO4 100 µg/L CuSO4
ALP activity (U/L)
Exposure period (Days)
Control 10 µg/L Cu NPs 50 µg/L CuNPs 100 µg/L CuNPs
LDH activity (U/L)
Exposure period (Days)
Control 10 µg/L CuSO4 50 µg/LCuSO4 100 µg/L CuSO4
LDH activity (U/L)
Exposure period (Days)

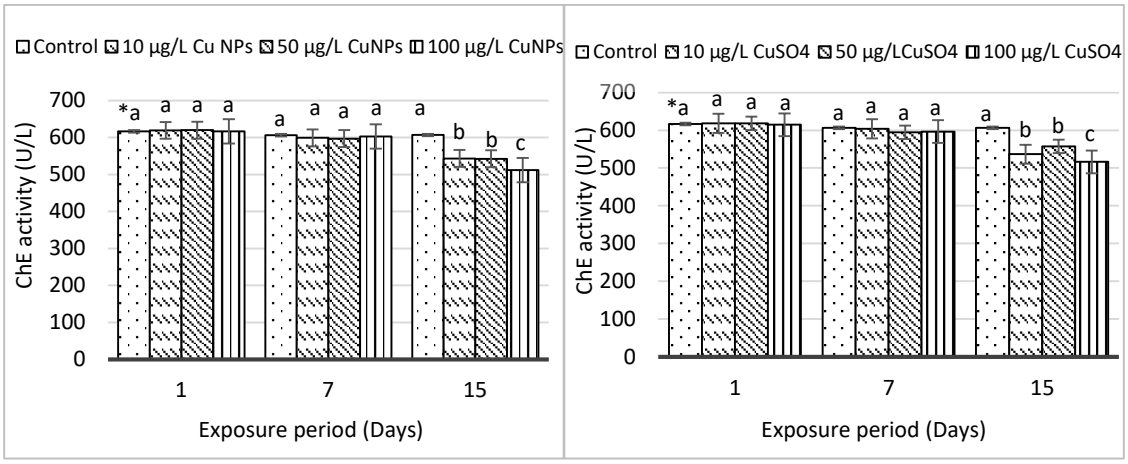


Figure 2. Influence of Cu NPs and $CuSO_4$ on serum enzyme activities in *O. niloticus*.
* See Fig. 1 for abbreviations

CONCLUSION

Studying sera biochemical parameters are not only the indicators of physiological stress responses but also can be used as indicators of heavy metal toxicity and these parameters also provide information concerning target organ toxicity. Our results indicate that exposure to the influence of Cu NPs and $CuSO_4$, serum glucose level increased at 7 and 15 days and serum albumin level increased compared to control at 15 days, while serum total protein, cholesterol and triglyceride levels decreased compared to control after 15 days of exposure. Also, influence of Cu NPs and $CuSO_4$, serum ChE activity decreased compared to the control at the end of the 15 days, while serum AST, ALT, ALP and LDH activities increased compared to the control. Overall, $CuSO_4$ and CuO NPs had similar effects in serum parameters of *O. niloticus*. Periodic determination of physiological and biochemical changes together with the metal concentrations that are consumed as food and accumulated in the tissues of an economically important species as a result of the increasing amounts of heavy metal pollution caused by the increase of technological developments and industrial wastes is of great importance in terms of the structural integrity of the ecosystem, both human health and economic is important. It was concluded that changes in serum parameters studied under the effect of two forms of copper in *O. niloticus* can be used as copper pollution indicators in freshwater habitats.

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