Turkish Journal of Agriculture - Food Science and Technology, 9(2): 277-280, 2021
DOI: https://doi.org/10.24925/turjaf.v9i2.277-280.3619

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

The Effects of Lead and NTA Mixtures on ALP levels in blood tissue of *Oreochromis niloticus*

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ARTICLE INFO

Research Article

Received : 23/06/2020
Accepted : 28/01/2021

Keywords:
Alkaline phosphate (ALP)
Blood
Oreochromis niloticus
Lead
NTA

ABSTRACT

In this study effects of Pb and Pb+NTA mixtures on the activity of alkaline phosphatase (ALP) in the blood tissues of *Oreochromis niloticus* were investigated. The fish were exposed Pb (0.1 mg/L Pb ve 1.0 mg/L Pb) and Pb+NTA ((0.1 mg/L Pb+0.3 mg/L mixtures NTA ve 1.0 mg/L Pb+3.0 mg/L NTA) for 7 and 21 days. ALP activity in the blood tissue was determined by spectrophotometric methods. In both exposure concentrations ALP activity increase with increasing exposure periods. activity in the blood tissues of fish exposed to Pb for 7 and 21 days was lower when compared to those exposed to Pb+NTA: As a result, it is thought that the toxic effect of lead on *O. niloticus* on biochemical parameters **is** prevented by NTA.

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Introduction

The increasing technology with the industrial revolution has led mankind to search for new raw materials. With the increase in the human population, the need for raw materials has accelerated, as a result, the amount of waste has increased. Heavy metals constitute a significant amount in the class, also called chemical waste, and threaten human, animal and environmental health.

Lead, which is the most important pollutant metal in terms of environmental health, is mixed into the aquatic environment as a result of mining activities (Viarengo, 1985). Lead inhibits the development of living things (Burden et al., 1998) and inhibits the enzyme d-aminolevulinic acid dehydratase, which is involved in the synthesis of erythrocytes in the blood (Campana et al., 2003). In addition, lead has been reported to have a toxic effect on many biochemical pathways (Ruparelia et al., 1989). In recent years, various methods have been developed for removing heavy metal accumulation in the water environment and for the continuity of natural resources. Among these methods; Neutralization, chemical precipitation, adsorption, sorption, ion exchange, reverse osmosis, membrane and evaporation methods are generally known. The most effective, convenient and low cost treatment process in the removal of heavy metals in the aquatic system is the adsorption method (Kablan, 2018). In recent years, scientifically the most synthetic chemical compounds such as (such as EDTA, NTA and DTPA) and zeolite have been benefited.

Synthetic chemical compounds are used to serve different purposes in all areas of the industry today, as they are agents in chelating metals (Çoğun and Şahin 2012). While EDTA is a very strong metal binder, NTA is now widely used in the production of large quantities for phosphate-free detergent formulations and is less necessary to form metal complexes with a 1: 1 mole ratio by weight.

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate monoesters in the organism and is a cell membrane-bound glycoprotein. The organism is thought to play a role in bone calcification and the transport of phosphate to the epithelial cells of the intestine.

Although *Oreochromis niloticus* is an important protein for the human food source, it is used by scientists as a bioindicator species due to its physiological responses to various toxic and non-toxic substances (Almeida et al., 2002).

The blood tissue parameters of the fish, provide information about the healthy status of the fish (Blaxhall ve Daisley 1973). Fort his purpose, it is aimed to determine the changes in ALP activity in the blood tissue of those niloticus fish, under the effect of 0.1 mg/L Pb, 1.0 mg/L Pb, 0.1 mg/L Pb+0.3 mg/L NTA and 1.0 mg/L Pb+3.0 mg/L NTA left for 7 and 21 days.

Material and Method

O. niloticus was taken from Ç. Ü. the Faculty of Fisheries the production pools, brought to the laboratory and adapted at 25 ± 1°C for two months. Experiments were conducted in five (5) groups to determine the blood ALP activity of *O. niloticus* for 7 and 21 days under the effect of lead and their mixtures with NTA. Fish in the first group 0.1 mg / L Pb, 1.0 mg / L Pb in the second group, 0.1 mg / L Pb + 0.3 mg / L NTA concentrations in the third group, 1.0 mg / L Pb + 3.0 mg / L NTA concentrations in the fourth group, and the last group is used for control. Experiments were carried out in three replicates, with two fish per repeat.

After 7 and 21 days, the fish were removed from the aquarium, after anesthesia with MS-222, the water droplets on it were removed with blotter paper and the tail section was cut and their blood was taken. Blood was centrifuged and measured by spectrophotometric methods. Statistical analysis of the obtained data was done by using SNK test and t-test using SPSS 20 package program.

Results

In the study, there was no death in the concentrations of lead and the effects of lead + NTA mixtures in fish at the end of the 21st day. Fish blood tissue ALP levels are given in tables 1, 2 and Figure 1.

Table 1. Blood ALP activity in *O. niloticus* left to the effect of 0.1 Pb and 0.1 mg / L Pb + 0.3 mg / L NTA

Time	Control	0.1 mg/L Pb	0.1 mg/L Pb+0.3 mg/L NTA
7	32.7 ± 2.9^{ax}	30.0 ± 4.5^{ax}	27.3 ± 4.1^{ax}
21	26.7 ± 1.5^{ay}	37.7 ± 1.5^{by}	24.7 ± 3.7^{ax}

Statistical difference between the letters a and b concentrations; the letters x and y are used to determine statistical difference between time ($P<0.05$).

Table 2. Blood ALP activity in *O. niloticus* left to the effect of 1.0 Pb and 1.0 mg / L Pb + 3.0 mg / L NTA

Time	Control	1.0 mg/L Pb	1.0 mg/L Pb+3.0 mg/L NTA
7	32.7 ± 2.9^{ax}	25.3 ± 1.8^{bx}	25.7 ± 2.7^{bx}
21	26.7 ± 1.5^{ay}	42.0 ± 3.2^{by}	27.0 ± 0.6^{ax}

Statistical difference between the letters a and b concentrations; the letters x and y are used to determine statistical difference between time ($P<0.05$).

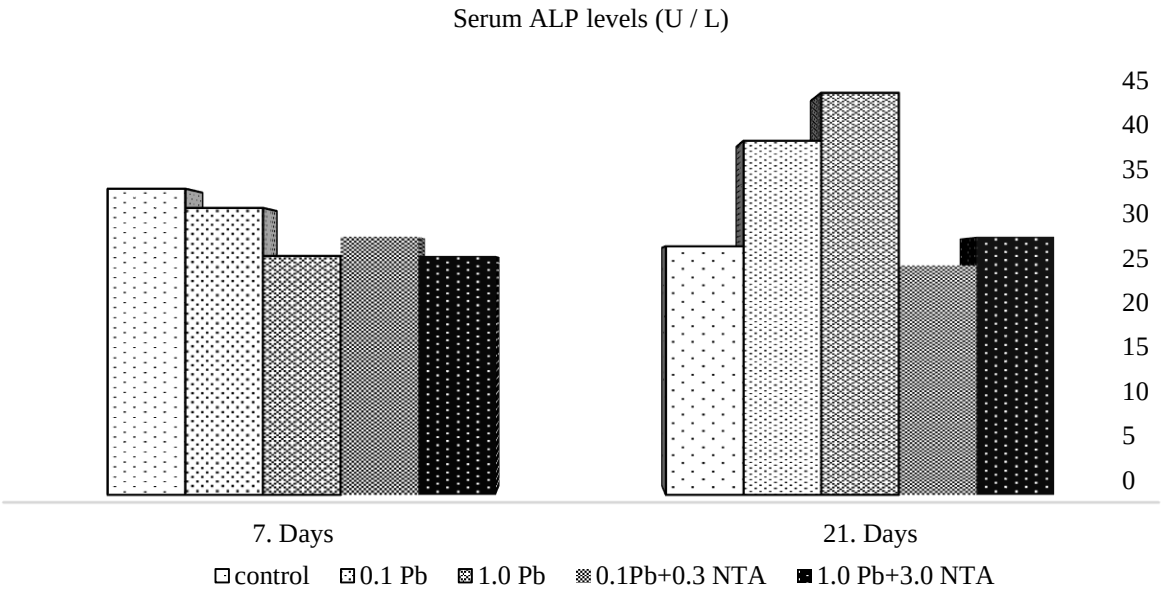

Figure 1. The effect of time on blood ALP activity in O. niloticus, which is left to the effect of Pb and Pb + NTA.

In both Pb concentrations, blood tissue alkaline phosphatase (ALP) activity increased with prolonged time. The alkaline phosphatase (ALP) activity in the blood tissues of fish exposed to Pb for 7 and 21 days is higher compared to those exposed to Pb + NTA

Discussion

Alkaline phosphatase (ALP), which was first studied by Suzuki et al. (1907) still has little information, plays a role in bone isoenzymes and skeletal mineralization as its physiological function. Evaluation of the activity of liver and blood alkaline phosphatases is used extensively in routine examinations. Generally, the normal serum range of alkaline phosphatase is between 20 and 140 U / L. Alkaline phosphatase an enzyme is an important serum parameter as a result of its high serum level; It is associated with the presence of bone, liver and other diseases (Epstein et al., 1986).

Generally high ALP levels in children and pregnant women, shows conditions such as vitamin D deficiency or liver damaged cells, as well as high ALP levels, may indicate obstruction of bile ducts. In addition, high ALP enzyme activity indicates that active bone a formation may occur because it is a by-product of osteoblast activity (Rodan and Rodan, 1984).

There are no more studies on the effects of environmental pollution with NTA on the blood levels of *O. niloticus* fish. In studies with NTA, there is mostly work on the removal of environmental pollution. In a study by Sprague (1968), biological research on trout also showed that NTA is effective against copper and zinc pollution as an anti-pollution agent. In the present study, the effects of lead and lead + NTA mixtures on blood alkaline phosphatase (ALP) of *O. niloticus* fish were investigated.

Blood biochemical parameters in fish provide important information about the physiological status of the fish, and also, the blood parameters are affected by the climatic and physiological factors (Munoz et al., 1991; Chen et al., 2003).

Significant increases were observed in fish serum ALP enzyme levels in metal (Yang and Chen, 2003; Chen et al., 2004) and pesticide effect (Agrahari et al., 2007). In a study, it has been reported that increases in ALP activity in plasma occur due to plasma membrane permeability (Rahman et al., 2000), and lead levels in the environment increases the stress level in animals.

Hilmy et al. (1985), in a study on the effect of Cd effect on serum ALP enzyme activities in *Mugil cephalus*, it was reported that it caused an increase in Cd blood serum ALP activities. Wang and Zhai (1988) found in their study that increases in the ALP level were due to the passage of the enzyme into the blood as a result of cell deaths in the liver. In our study, it is thought that the reason for the increase in ALP levels in the effect of lead media levels may be a result of liver tissue damage. In our study, the blood ALP activity of the fish under the effect of both NTA mixtures at the end of the 21st day compared to 7 days showed significant decreases compared to the fish under the effect of lead media concentrations. These reductions are thought to be caused by the chelating feature of NTA.

Conclusion

In conclusion our study, while ALP activity lead media levels increased at the end of the 21st day compared to 7 days, the lead + NTA mixtures showed significantly decreases. The reason for the increase in ALP levels may be a result of liver tissue damage, and in the mixture of NTA + Pb, it can be concluded that NTA reduces the effect of lead with the chelating effect.

Acknowledgment

This research was supported by Çukurova University Scientific Research Projects Coordination Unit (BAP) (FBA-2018-10696).

References

Almeida JA, Diniz YS, Marques SFG, Faine LA, Ribas BO, Burneiko RC, Novelli LB. 2002. The Use of Oxidative Stress Responses as Biomarkers in Nile Tilapia (*Oreochromis niloticus*) Exposed to *in vivo* Cadmium Contamination. Environ. Int., 27: 673-679. https://doi.org/10.1016/S0160-4120(01)00127-1

Blaxhall PC, Daisley KW. 1973. Routine Haematological Methods for use with Fish Blood. J. Fish Biol. 5: 771-781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Burden VM, Sandheinrich MB, Caldwell CA. 1998. Effects of lead on the growth and d-aminolevulinic acid dehydratase activity of juvenile rainbow trout, *Oncorhynchus mykiss*. Environ Pollut, 101: 285-289. https://doi.org/10.1016/S0269-7491(98)00029-3

Campana O, Sarasquete C, Blasco J. 2003. Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. Ecotoxic Environ Safe, 55: 116-125. https://doi.org/10.1016/S0147-6513(02)00093-3

Chen CY, Wooster GA, Bowser PR. 2004. Comparative Blood Chemistry and Histopathology of Tilapia Infected With *Vibro vulnificus* or *Streptococcus iniae* or Exposed to Carbon Tetrachloride, Gentamicin, or Copper Sulfate. Aquaculture, 239: 421-443. https://doi.org/10.1016/j.aquaculture.2004.05.033

Chen CY, Wooster GA, Getchell RG, Bowser PR, Timmons MB. 2003. Blood Chemistry of Healthy, Nephrocalcinosis Affected and Ozon-Treated Tilapia in a Recirculation System, with Application of Discriminant Analysis. Aquaculture, 218: 89–102. https://doi.org/10.1016/S0044-8486(02)00499-4

Çoğun HY, Şahin M. 2012. The effect of zeolite on reduction of lead toxicity in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). Kafkas Üniversitesi Veteriner Fakültesi Dergisi 18.1: 135-140.

Epstein E, Kiechle FL, Artiss JD, Zak B. 1986. The clinical use of alkaline phosphatase enzymes. Clin Lab Med.6: 491–505. https://doi.org/10.1016/S0272-2712(18)30795-9

Hilmy AM, Shabana MB, Daabees AY. 1985. Effects of Cadmium Toxicity upon the *In Vivo* and *In Vitro* Activity of Proteins and Five Enzymes in Blood Serum and Tissue Homogenates of *Mugil cephalus*. Comp. Biochem. and Physiol. C: Comp. Pharma., 81, 1: 145–153.

Kablan B. 2018. Klinoptilolitin *Cyprinus carpio* 'nun doku ve organlarndaki bakir birikimi üzerine etkisi Mersin Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 22.

Munoz MJ, Carbailo M and Tarazona JV. 1991. The Effects of Sublethal Levels of Copper and Cyanide some Biochemical Parameters of Rainbow Trout along Subacute Exposure. Comp. Biochem. Physiol., 100C: 577–582. https://doi.org/10.1016/0742-8413(91)90043-S

Rahman MF, Siddiqui MK and Jamil K. 2000. Acid and Alkaline Phosphatase Activities in a Novel Phosphorothionate (RPR-11) Treated Male and Female Rats. Evidence of Dose and Time-dependent Response. Drug. Chem. Toxicol., 23: 497-509. https://doi.org/10.1081/DCT-100100131

Rodan GA, Rodan SB. 1984. In: Peck WA, editor. Advances in bone and mineral research annual II. Amsterdam: Excerpta Medica; p. 244–85.

Ruparelia SG, Verma Y, Mehta NS, Salyed SR. 1989. Lead-induced bio-chemical changes in freshwater fish Oreochromis mossambicus. Bull Environ Contam Toxicol, 43, 310-314.

Sprague JB. 1968. Promising anti-pollutant: chelating agent NTA protects fish from copper and zinc. Nature, 220 (5174):1345-1346.

Suzuki U, Yoshimura K, Takashi M. 1907. Uber ein enzymephytasek das anhydro-oxy-methylen-diphosphorsaure spaltet. Bull Coll Agri Tokyo Imp Univ.7: 503–12.

Viarengo A: 1985. Biochemical effects of trace metals. Mar Pollut Bull, 16 (4): 153-158. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=9281021

Wang X and Zhai W. 1988. Cellular and Biochemical Factors in Bronchoalveolar Lavage Fluids of Rats Exposed to Fenvalereate. Zhongguo Yaolixue Yu Dulixue Zoghi., 2: 271-276.

Wepener V, Vanvuren JHJ, Du Preez HH. 2001. Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of a freshwater teleost, *Tilapia sparmanii*. Water SA 27(1): 99–108. DOI: 10.4314/wsa.v27i1.5016

Yang JL, Chen HC. 2003. Effects of Gallium on Common Carp (*Cyprinus carpio*): Acute Test, Serum Biochemistry, and Erythrocyte Morphology. Chemosphere, 53: 877–882. https://doi.org/10.1016/S0045-6535(03)00657-X