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-RESEARCH ARTICLE-

Effects of Clinoptilolite on Copper Accumulation of *Oreochromis niloticus*

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Abstract

The copper accumulation in liver and gill tissues of *Oreochromis niloticus*, exposed to 2 ppm Cu and 1g/L clinoptilolite singly and to the same concentrations of their mixture over 24, 48, 72 and 96 hours was studied. ICP-AES spectrophotometer techniques were applied in determining tissue copper levels. Statistical evaluation of the experimental data was carried out by Student Newman Keul's procedure. No mortality was observed during the experiments. Copper accumulation was lower in metal-clinoptilolite mixture group than metal singly group in gill tissue while no accumulation in both experimental groups in liver tissue ($P<0.05$). In addition, the copper level in the liver was lower in all experimental groups than in the control ($P<0.05$). Low Cu accumulation in gill tissue exposed in mixture groups can be explained by copper adsorption with chelating agent. The decrease of Cu reserves in the liver can be expressed by increase of copper-containing enzyme and protein synthesis.

Keywords:

O. niloticus, Copper, Clinoptilolite, Tissue, Accumulation

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Introduction

All domestic, industrial and agricultural wastes caused by anthropogenic activities are conveyed to the aquatic ecosystem that main receiver in the earth. The wastes including organic and inorganic pollutants change biotic and abiotic environment and damage to the ecological balance. The energy transfer in nature is occurred by food chain, it is inevitable to transport toxic substances to upper tropic levels. An important part of inorganic pollutants are heavy metals.

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Some of which (Fe, Se, Cu, Zn, Cr) have biological functions in animals at low concentration while others (Cd, Pb, Hg) have no function. Both groups are toxic to fish over a certain concentration. (Shukla et al., 2007; Kumar et al., 2011).

Copper is a trace element that acts as a prosthetic group in about 30 enzymes such as cytochrome oxidase, superoxide dismutase, tyrosinase, ceruloplasmin in aquatic animals. It produces energy via cytochrome c oxidase, provides iron homeostasis via hemoglobin and detoxifies free oxygen radicals via antioxidant enzymes such as superoxide dismutase and catalase (Grosell, 2012). The main source of copper in aquatic ecosystems are natural factors such as volcanic activities, floods and erosion, as well as human activities such as mining, leather industry, paint, plastic, pesticide and fertilizer production, especially electric industry (Patterson et al., 1998).

Heavy metals are absorbed from the whole body surface or nutrition by aquatic organisms and distributed to metabolically active tissues through the circulatory system and cause accumulation. If metal excretion in the tissue does not counterbalance the accumulation and storage capacity they are distributed to other tissues and organs then causing mortality. Gills are the main target organ for toxic chemicals in aquatic organisms as they are respiratory organs, and also function in osmoregulation and interact directly with the external environment (Fromm & Schiffman, 1958). Liver is a metabolically active tissue that converts the nutritional components absorbed from the intestines to each other when needed, synthesis of bile salts which function in the digestion of the fats and detoxifies of toxic chemicals (Heath, 1995). Besides, the major synthesis sites of the metal binding protein, such as metallothionein, and glutathione, a tripeptide (Langston et al., 2002).

Various engineering methods have been developed, such as adsorption, sorption, ion exchange, chemical precipitation, reverse osmosis, membrane and evaporation methods, to remove pollution caused by heavy metals in aquatic ecosystems, to protect aquatic organisms and sustainability of water resources. It has been reported that adsorption is the most efficient, cost-effective and low-cost treatment process. There are various adsorbents such as clay, activated carbon, gel, resin and zeolite used for the realization of the adsorption method (Patterson, 1985; Marani et al., 1995; Smith, 1996; James & Sampath, 1999; Papini et al., 1999). Zeolite is an eco-friendly and economical raw material that has wide natural reserves in our country and does not use any toxic chemicals in its processing. The strong adsorbing property of zeolite is due to the fact that the molecule has a lattice-like structure and thus a high internal and external surface area, and ion exchange ability. Clinoptilolite is a natural zeolite, that reduce the accumulation of heavy metals in aquatic organisms and avoid to the metabolic and physiologic dysfunctions has been determined (Patterson, 1985; Marani et al., 1995; Smith, 1996; James & Sampath, 1999; Papini et al., 1999).

O. niloticus, an experimental material used in the research, is an economically important freshwater fish species that is widespread in the aquaculture of the world and consumed as a protein source by human. However, their natural habitats are under the influence of domestic, industrial and agricultural wastes, it was aimed to determine the effect of clinoptilolite on copper accumulation in gill and liver tissues of *O. niloticus* exposed to 2 ppm Cu and 1g/L clinoptilolite singly and to the same concentrations of their mixture during 24, 48, 72 and 96 hours.

Materials and Methods

O. niloticus 13.99 ± 0.64 cm in length and 43.84 ± 6.69 g in weight were used in the experiments. Fish were provided from the Aquaculture Unit located in Mersin University Water Products Faculty Implementation Unit, experiments were carried out in the Basic Science Research Laboratory with controlled environment conditions of the same unit (24 ± 1 ° C temperature applied 12 hours dark 12 hours dark photoperiod).

The fish were kept in 40x100x40cm size 4 glass aquariums for 30 days to adaptation, before the experiments. The ventilation was obtained from the central ventilation system, the fish were fed with readymade pellet feed (Pınar; Bream feed, Pellet No: 2) in quantity of 2% of total biomass once a day.

Experiments were run in triplicate being 3 fish in each replicate, hence 12 fish were placed in each aquarium, a total of 48 fish were used at the end of the experiments. The same size 4 glass aquaria were used in the experiment. Two aquariums were filled with 100 L 1 g/l clinoptilolite and 2 ppm Cu only, the third one filled with clinoptilolite and copper mixture at the same concentrations, the last one filled with metal and clinoptilolite free rested tap water and used as control. Experiments were conducted for 24, 48, 72 and 96 hours.

The water temperature (24 ± 1 °C), pH (8.41 ± 0.13), dissolved oxygen (6.07 ± 0.9 mgl^{-1}), total hardness (224 ± 0.39 mgl^{-1} $CaCO_3$) and alkalinity (329 ± 0.70 mgl^{-1} $CaCO_3$) in the experimental and control aquariums were determined.

$CuSO_4$. $5H_2O$ salt of copper was used to prepare the test solutions. Experimental medium was renewed every two days by dilution with fresh stock solution due to evaporation, precipitation and adsorption.

At the end of the defined periods, the fishes extracted from the experimental aquariums were stunned with anesthetic substance of ethylene glycol mono phenyl ether (= phenoxyethanol, $C_8H_{10}O_2$; Merck). After the washing on the body surface with tap water to remove metal residues, the fishes were dried with Whatman paper, and later on measuring their total length and weight, each fish was euthanized and gill and liver tissues were dissected.

ICP-AES spectrophotometer techniques were applied in determining tissue copper levels. Dissected tissues were dried to a constant weight at 105 ° C for 72 hours, and their dry weights were determined and then they were transferred to experimental tubes. There they were digested in nitric acid (65%, Baker) - perchloric acid (65%, Erba) (2/1; v / v) mixture at 120 ° C for four hours. Digested tissues were transferred to polyethylene tubes and the total volume was adjusted to 5 ml with distilled water and analyzed in ICP-MS.

Student's Newman Keul's (SNK) test was applied to statistical analysis of the experimental data using SPSS package program.

Results

No mortality was determined during the experiments. Metal accumulation in gill tissues of *O. niloticus* at the exposure periods and certain concentration of copper and clinoptilolite singly and with mixture in the same concentration during 24, 48, 72 and 96 hours are shown in Figure 1.

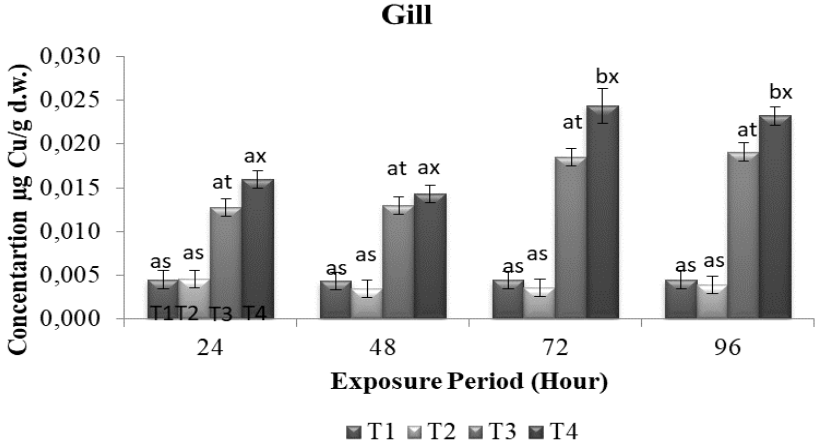

Figure 1. Copper Accumulation in gill tissues of *O. niloticus* exposed to Cu singly and with mixture of clinoptilolite during 24, 48, 72 and 96 hours. (µg Cu/g d.w.) (T1:Control, T2:1g/L Clinoptilolite, T3:1g/L Clinoptilolite-2ppmCu, T4:2ppmCu). Letters a and b are used to show differences between exposure period and s, t and x among concentrations (P<0.05).

Accumulation in gill tissue of fish exposed to Cu alone and Cu with mixture of clinoptilolite increased based on control and increasing exposure period. Copper accumulation in gill tissue in mixture group less than copper singly group was found. Decreasing of copper in the same tissue according to exposure period and control were not significant at clinoptilolite singly groups (P>0.05) (Figure 1).

Effect of accumulation in the liver tissue of copper and clinoptilolite singly and with mixture at the same amount during 24, 48, 72 and 96 hours are shown in Figure 2.

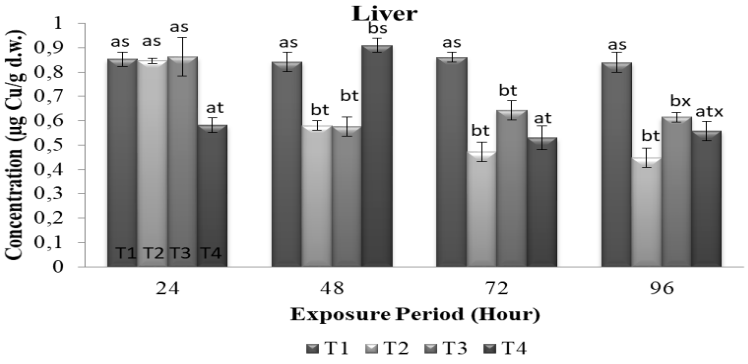

Figure 2. Copper Accumulation in liver tissues of *O. niloticus* exposed to Cu singly and with mixture of clinoptilolite during 24, 48, 72 and 96 hours. (µg Cu/g d.w.) (T1:Control, T2:1g/L Clinoptilolite, T3:1g/L Clinoptilolite-2ppmCu, T4:2ppmCu). Letters a and b are used to show differences between exposure period and s, t and x among concentrations (P<0.05).

There was no accumulation in liver tissue exposed to Cu alone and in mixture group. The Cu level in liver tissue exposed to all experimental groups was lower than control. Clinoptilolite decreased in Cu level in liver tissue at the end of the exposure time according to the 24 h exposure ($P<0.05$) (Figure 2).

Discussion

There was no mortality in *O. niloticus* exposed to 2 ppm copper and 1g/L clinoptilolite alone and with mixture groups at 24, 48, 72 and 96 hours. No mortality was reported to copper, cadmium alone and with mixture of zeolite and Ca in *O. niloticus* in previous study (Reyhan, 2014). The absence of mortality in fish can be result from either the inspected concentration are sub lethal for this species or the detoxification mechanisms in fish against to the toxic effects of metals are stimulated. In copper-clinoptilolite mixture group, chelator binds the metal and prevents metabolic and physiological changes that will cause toxic effects in fish is possible.

There are indicated studies that the potential role of chelators in preventing or reducing the toxic effects of heavy metals depending on the origin of the chelators (natural or synthetic), metals, concentrations, exposure periods, test materials and environmental factors such as hardness, pH (Batchelder et al., 1980; Hung, 1982; Hansten et al., 1996; Gopal et al., 2009; Palaniappan et al., 2009; Sivakumar et al., 2012). It has been reported that humic acid decreases Cd and Cu toxicity while increases Zn toxicity, EDTA and Fe decrease in toxicity of all three metals, Mn increases Zn toxicity but without any effect on Cd and Cu toxicity in *Anodonta anatina* (Hansten et al. 1996). Lead accumulation in *O. niloticus* decreased with Pb-Se mixture and AChE (Acetylcholinesterase) enzyme activity increased in brain while decreased in the liver, kidney and gill tissues were determined (Maruldali, 2010). Ca reduced Cd toxicity in liver and gill tissues of *Phoxinus phoxinus* has been reported (Wicklund & Runn, 1988). EDTA prevented Cu accumulation and improved the changes in hematological parameters on *O. niloticus* (James et al., 1998). Changes in the biochemical parameters of Pb exposed *Heteropneustes fossilis* were found to decrease with zeolite (Jain, 1999). Cd changed on hematological parameters of *Oreochromis mossambicus* while zeolite with mixture to Cd prevented these changes (James, 2000). It has been determined that 4gZ / L decreased Cd toxicity based on increasing DNA / RNA ratio while there was any adverse effect on fish at 8gZ / L in *O. mossambicus* (James & Sampath, 1999). The optimum concentration of zeolite to prevent from Cu toxicity is 1 g Z dm^3 (James et al., 2004). The chronic effect of sub lethal Pb concentration caused by accumulation of gill, liver, kidney, spleen and muscle tissues of *O. niloticus* depending on exposure periods while metal clinoptilolite mixture decreased in accumulation compared to the metal alone (Çoğun & Şahin, 2012).

In this study, the accumulation of copper in gill tissue of *O. niloticus* exposed to copper singly and in mixture group was determined. The accumulation level of metal singly group was higher than in mixture group. Increasing concentration of copper in gill tissue of metal singly group is due to the direct interaction of the gill with the contaminant medium is possible. There was no accumulation in the liver tissue of *O. niloticus* in spite of 2 ppm Cu exposure during 24, 48, 72 and 96 hours. The Cu level of the liver tissue exposed to metal and clinoptilolite alone and in mixture group reduced based on control and increasing exposure period. Decreasing in copper level in the liver may result from either the using of Cu increasing in enzyme and protein synthesis or removal of the existing Cu from the medium by feedback mechanism. Cu

accumulation of fishes exposed to metal clinoptilolite mixture group was lower in gill while was higher in liver tissues according to metal singly group. Decreasing level in gill tissue may be due to the adsorbtion of Cu by the chelator when increasing level in liver tissues except to 24 h effect may be due to the slowing of the stimulation of the detoxification mechanism. The effect of clinoptilolite alone decreased the amount of copper reserves in both tissues except 24 h effect. This may be due to the fact that Cu is adsorbed by clinoptilolite from medium and inhibited to metal uptake by fishes so homeostatic mechanism is stimulated. As a result, it was determined that 1g Z / L concentration of clinoptilolite decreased Cu toxicity in *O. niloticus*.

Consequently, the use of chelators to prevent the toxic effects of heavy metals is of great importance for the environment and human health. However, it is necessary to determine the most suitable concentration of chelator to be applied for the ecosystem and aquatic organisms. Thus, using enough chelator, the ecosystem will not be polluted, the health of aquatic organisms will be preserved and the economy will be ensured.

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