



Influence of Lead and Applied Singly and in Mixture with Chitosan on Tissue Accumulation of *Clarias gariepinus*

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Abstract: *Clarias gariepinus* was exposed to 1 ppm Pb singly and in mixture with 75 ppm chitosan (CT) and the levels of Pb in gill, liver, spleen, kidney and muscle tissues were determined after 1, 7 and 15 days. Lead accumulation increased in gill tissue compared to control when exposed to Pb alone and in mixture on 7th day. A significant decrease, however, was observed in gill level on exposure to Pb+CT mixture on day 15. Exposure to Pb alone increased the level in liver significantly after 15 days compared to control fish. Exposure to Pb alone and to mixture had no effect on kidney accumulation. Pb accumulation increased in spleen tissue compared to control when exposed to Pb alone on 1st and 7th days, it decreased when exposed to Pb+CT mixture at these exposure periods. Muscle Pb accumulation was higher in fish exposed to Pb only as compared to controls and in fish exposed to Pb+CT mixture on 1st day. The studied lead concentrations caused alterations in the metal accumulation especially gill and liver tissues. Chitosan seemed to decrease the harmful effects of Pb on long term exposures.

Keywords: Accumulation, *Clarias gariepinus*, chitosan, lead, mixture.

Kurşun ve Kurşun-Kitosan Karışımının *Clarias gariepinus*'da doku birikimi üzerine etkileri

Öz: Bu çalışmada 1 ppm kurşunun tek başına ve 75 ppm kitosan ile karışımlarının 1, 7 ve 15 günlük etki sürelerinde *C. gariepinus* 'un solungaç, karaciğer, böbrek, dalak ve kas dokularındaki metal birikiminin belirlenmesi amaçlanmıştır. Kurşunun tek başına ve karışımın 7 günlük etki süresinde solungaç dokusunda biriktiği belirlenmiştir. Karışımın 15 günlük etki süresinde ise solungaç dokusundaki kurşun birikimi azalmıştır. Kurşunun tek başına 15 günlük süreyle etkisinde karaciğerde birikim artış göstermiştir. Böbrek dokusunda kurşunun tek başına ve karışımın etkisinde birikim gözlenmemiştir. Dalak dokusunda kurşunun tek başına 1 ve 7 günlük etki süresindeki kurşun birikimi artış gösterirken, karışımın aynı sürelerle etkisindeki birikim düşüş göstermiştir. Kas dokusunda ise kurşunun tek başına ve karışımın etkisinde yalnızca 1 günlük etki süresinde birikim gözlenmiştir. İncelenen kurşun derişimleri özellikle solungaç ve karaciğer dokusunda birikme neden olmuş, kitosanın uzun süreli etkisi kurşun birikimini azaltmıştır.

Anahtar kelimeler: Birikim, *Clarias gariepinus*, karışım, kitosan, kurşun.

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INTRODUCTION

Lead (Pb) has physical and chemical properties that make it extremely useful in various industries such as lead battery production and colored inks and paint preparation. Discharge of Pb containing urban, agricultural and industrial waste waters to aquatic environments result in a number of physiological and biochemical disturbances to organisms living in these environments. (Shaw et. al., 2006; Kusemiju et al., 2012). Pb have no biological function and is toxic even at very low concentrations. It is a potentially toxic chemical for aquatic animals such as fish and shellfish (Cicik, 2003). Chitin is a polymer of glucosamine which is found in shells or walls of invertebrates, fungi and yeasts. Chitosan (CT), an amino polysaccharide, is prepared from shellfish chitin by treatment with alkali. (Muzzarelli, 1977). CT is able to form complexes with many of the transition metals. The heavy metal-polymer complexes are believed to form as a result of dative bonding with CT (Muzzarelli, 1973). Physiological and biochemical profiles in fish and other aquatic organisms under heavy metal stress serve as important bioindicators in aquatic environment monitoring (Abbas et al., 2007; Firidin, 2019, Tunçsoy, 2019). *Clarias gariepinus* was chosen as an experimental fish species since the species is commonly found in streams and drainage channels of Mediterranean region, its consumption as a protein source in the region, its wide tolerance against pollutants and its habitat being under the effect of agricultural and industrial activities.

Aquatic organisms uptake the metals from water and sediment mainly via their gills and digestive track and accumulate them in various tissues. CT is well known as an excellent biosorbent for metal cation removal in near-neutral solutions because the large number of NH_2 groups (Seyedi, 2013). Hence the aim of the present study was to determine metal levels in gill, liver, kidney, spleen and muscle tissues of *C. gariepinus* after exposing the animals to 1ppm Pb and 1ppm Pb+75ppm CT over 1, 7 and 15 days.

MATERIAL AND METHODS

C. gariepinus was obtained from a private fish farm in Silifke-Mersin-Turkey. The mean length and weight of the animals were 21.9 ± 2.5 cm and 73 ± 3.11 g respectively. Fish were adapted to laboratory conditions for one month in glass aquaria 40x120x40 cm in height. The same sized five aquaria were used in the experiments. The first two aquaria were filled with 120 L of 1.0 ppm Pb and 1.0 ppm Pb + 75 ppm CT, respectively while the third one was filled with the same amount of lead-free tap water and used as control. Lead nitrate ($\text{Pb}(\text{NO}_3)_2$) was used in the preparation of experimental solution and trisodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 5\text{H}_2\text{O}$) was used to prevent precipitation and

adsorption of the metal. Experimental solutions were replaced daily, by serial dilution of freshly prepared 1000 ppm stock solution of the metal. 1 % acetic acid was used to prepare CT stock solution (Aldrich, GR, Deacetylation C75 %). Some physical and chemical properties of experimental water were as follows;

Temperature: $21.5 \pm 1^\circ\text{C}$,

Total alkalinity: 305 ± 0.5 mg CaCO_3/L

Dissolved Oxygen: 7.01 ± 0.6 mg/L,

pH: 8.2 ± 0.5 .

Experiments were run in triplicate being 3 fish in each replicate, hence 9 fish were placed in each aquarium totaling to 27 fish. Fish were fed once a day with readymade fish feed (Pınar, Pellet No: 2) at amounts of 2% of total biomass. Fish were anesthetized with MS222. They were then washed with tap water and dried with Whatman filter papers.

Three fish were removed from each aquarium at the end of 1, 7, and 15 days of exposure periods, they were washed dried and dissected for their gill, liver, spleen, kidney and muscle tissues. Tissues were placed in petri dishes after being wet weighted and were placed in a drying oven set at 150°C for 48 hours. Dried tissues were then transferred to experimental tubes and digested in nitric acid (Merck, 65%) / perchloric acid (Merck, 60%) mixture (2/1; v/v) at 120°C for three hours (Muramoto, 1983). Digested tissues were transferred to polyethylene tubes and their volumes were made up to 10 ml with distilled water. Metal levels in tissues were determined using atomic absorption spectrophotometric techniques.

Statistical analysis of the data was carried out by Analysis of Variance and Student Newman's Procedure (SNK) using SPSS-21 statistical package program (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

There was no mortality of fish during the experiments which was probably due to low metal concentration tested. The gills are considered as the main site of entry for the dissolved metal salts. They are sensitive to any change in water components hence serve as a good indicator of in water quality since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water (Gaber, 2007).

Metals entering from gills are firstly transferred to liver by the circulatory system, and when the carrying capacity of liver is exceeded, they are transferred and stored in metabolically active tissues, especially in kidney. Liver is a metabolically active tissue since it plays role in

conversion of food, binding of harmful substances and in digestion of macromolecules especially lipids (Heath, 1995). When the metal binding capacity of liver is exceeded, the excess amount of metals is sent to kidneys for excretion (Hollis et al., 1999). Spleen is an important member of the body's immune and lymphatic system as well as it is a hematopoietic organ in which blood cells synthesized and stored (Tayel et al., 2008), also it acts as a filter and purifier of the blood (Garcia-Abiado et al., 2004). Exposure to Pb alone and mixture had no effect on kidney accumulation (Figure 1C; $P>0.05$). Pb accumulation increased in spleen tissue compared to control when exposed to Pb alone on 1st and 7th days whereas its accumulation decreased with increasing periods (Figure 1D; $P<0.05$). Muscle is not an effective tissue in binding metals, it is, however, important to know metal levels in this tissue as far as food chain and human health is concerned (Duran et al., 2015). Pb accumulation in gill tissue exposed to Pb alone increased with increasing exposure periods and in mixture increased on 7th day while decreased on 15th day. Exposure to Pb alone increased Pb accumulation of *C. gariepinus* on 7th day whereas its accumulation decreased with increasing periods (Figure 1A; $P<0.05$). Pb accumulation increased in gill tissue of *C. gariepinus* compared to control when exposed to Pb alone on 7th and 15th days while decreased in this tissue exposed to mixture on 15th day (Figure 1A; $P<0.05$). Pb accumulation increased in liver tissue compared to control when exposed to Pb alone on 15th day. Exposure to Pb alone increased Pb accumulation of *C. gariepinus* on 7th day whereas its accumulation decreased with increasing periods (Figure 1B; $P<0.05$). Pb accumulation increased in muscle tissue of *C. gariepinus* compared to control when exposed to Pb alone on 1st day while decreased in this tissue exposed to mixture at same exposure periods (Figure 1E; $P<0.05$).

Metals entering from gills are firstly transferred to liver by the circulatory system, and when the carrying capacity of liver is exceeded, they are transferred and stored in metabolically active tissues, especially in kidney. Liver is a metabolically active tissue since it plays role in conversion of food, binding of harmful substances and in digestion of macromolecules especially lipids (Heath, 1995). When the metal binding capacity of liver is exceeded, the excess amount of metals is sent to kidneys for excretion (Hollis et al., 1999). Spleen is an important member of the body's immune and lymphatic system as well as it is a hematopoietic organ in which blood cells synthesized and stored (Tayel et al., 2008), also it acts as a filter and purifier of the blood (Garcia-Abiado et al., 2004). Exposure to Pb alone and mixture had no effect on kidney accumulation (Figure 1C; $P>0.05$).

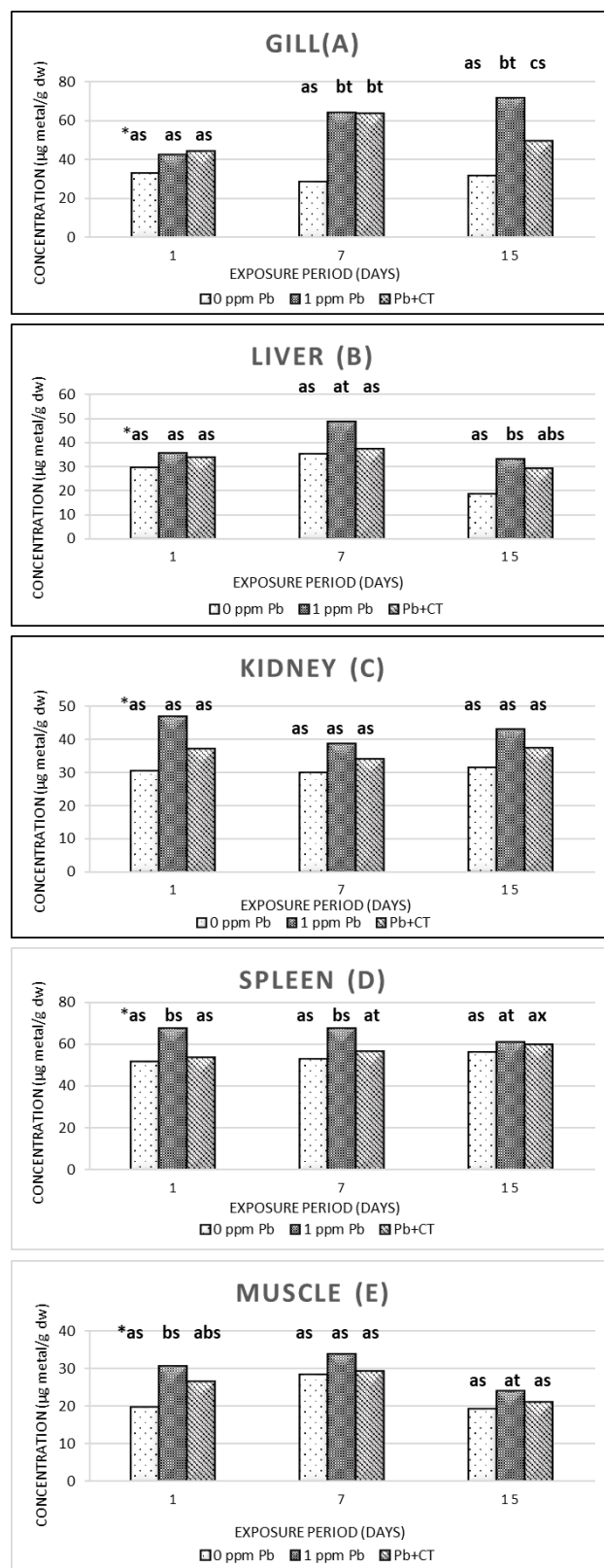


Figure 1. Lead accumulation in tissues of *Clarias gariepinus* exposed to Pb and Pb-CT mixture ($\mu\text{g metal/g.d.w.}$).

*=SNK; Letters a, b, c and s, t, x show differences among concentrations and exposure periods at a given tissue respectively. Data shown with different letters are significant at the $P<0.05$ level.

Pb accumulation increased in spleen tissue compared to control when exposed to Pb alone on 1st and 7th days whereas its accumulation decreased with increasing periods (Figure 1D; P<0.05). Muscle is not an effective tissue in binding metals, it is, however, important to know metal levels in this tissue as far as food chain and human health is concerned (Duran et al., 2015). Pb accumulation in gill tissue exposed to Pb alone increased with increasing exposure periods and in mixture increased on 7th day while decreased on 15th day. Exposure to Pb alone increased Pb accumulation of *C. gariepinus* on 7th day whereas its accumulation decreased with increasing periods (Figure 1A; P<0.05). Pb accumulation increased in gill tissue of *C. gariepinus* compared to control when exposed to Pb alone on 7th and 15th days while decreased in this tissue exposed to mixture on 15th day (Figure 1A; P<0.05). Pb accumulation increased in liver tissue compared to control when exposed to Pb alone on 15th day. Exposure to Pb alone increased Pb accumulation of *C. gariepinus* on 7th day whereas its accumulation decreased with increasing periods (Figure 1B; P<0.05). Pb accumulation increased in muscle tissue of *C. gariepinus* compared to control when exposed to Pb alone on 1st day while decreased in this tissue exposed to mixture at same exposure periods (Figure 1E; P<0.05).

Chitosan has recently been recognized as a biopolymer with significant potential for use as biosorbent for removal of metal ions from wastewater. Dynamic and static adsorption experiments with heavy metal ions indicated that chitosan can be effectively used to adsorb these metals by establishing their different interactions with its amino and hydroxyl groups (Vieira and Beppu, 2014). Several values have predicted the trend of adsorption behavior with respect to the stability of metal-bio-adsorbent complexes of Pb > Cu > Ni > Zn > Cd (Spotio, 1981). Pb ions are adsorbed to chitosan by coordinate and covalent linkages, ion exchange mechanisms, and electrostatic forces (Krishnamurthy and Harris, 1960). Copper-Chitosan complex increased metal accumulation in gill tissue while decreased in liver tissue of *C. gariepinus* (Tunçsoy et al., 2016).

Various biosorbents such as chitin and chitosan (CT) are known to form stable complexes with many metal ions. Chitin is widely distributed in nature, especially in the exoskeletons of marine invertebrates such as prawn, crab and lobster whereas its derivative CT has reactive amino groups which forms complexes between metal ions and the polymer chain. It was concluded that the studied lead concentrations caused alterations in the metal accumulation especially gill and liver tissues. Chitosan, which is a natural adsorbent, seemed to decrease the harmful effects of Pb on long term exposures.

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