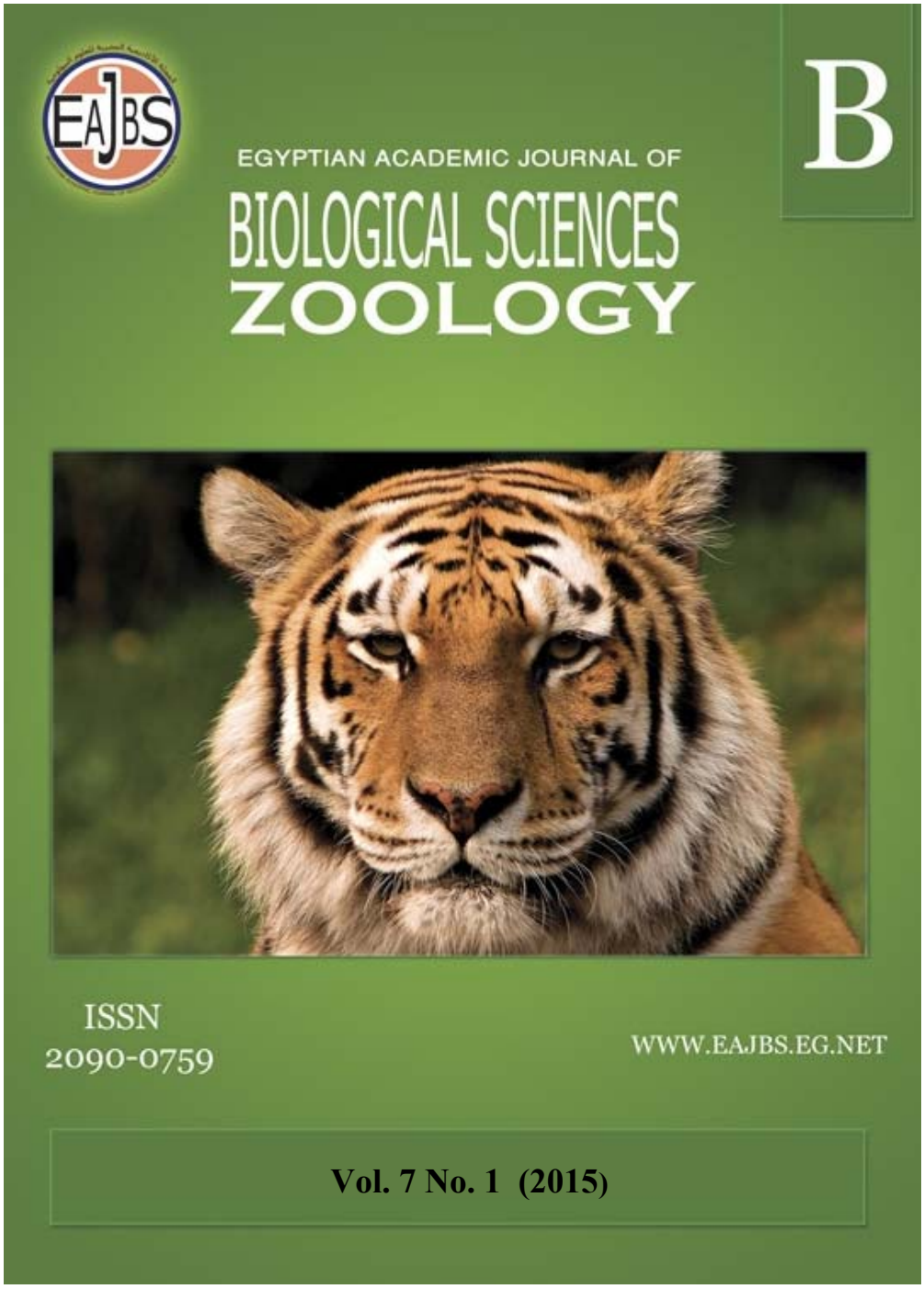

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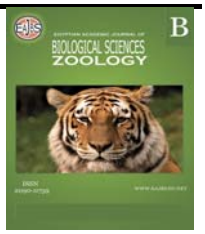

Heavy Metals (Pb, Fe and Zn) in Fish Due to Water Toxicity

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ABSTRACT

Water Toxicity is a worldwide problem, heavy metals belonging to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. The fish were divided into four groups, control group and three groups exposed to 5, 10 and 15 mg/l of lead acetate, 10, 20 and 30 mg L^{-1} of ferric chloride and 10, 20 and 300 mg L^{-1} of zinc sulfate respectively, the experimental fish were exposed to treatment for periods of 15 days, 30 days and 45 days. The Nile tilapia was exposed to water pollution with lead, iron and zinc. The Nile tilapia 80 - 100 gm body weight was obtained from Abbassa farm. The fish were reared in large tanks contained water from their farm. The fish were acclimatized and fed on 30 % protein diet. Result of the present study revealed that lead concentrations in the tissues of the fish were elevated and highest contamination were increased in the gill and liver than that in the muscles. Also, there was vary significant increase in the concentrations of lead in the gill, liver and muscles tissues of *Oreochromis niloticus* exposed to different concentrations of lead acetate than that control fish tissues. Iron concentrations in the tissues of the fish were elevated in the gill and liver than that in the muscles. Also, there was vary significant increase in the concentrations of iron in the gill, liver and muscle tissues of *O. niloticus* exposed to different concentrations of ferric chloride at different period than that control fish tissues. Zinc concentrations in the gill and liver were higher than that in the muscles. Also, there was significant increase in the concentrations of zinc in the gill, liver and muscle tissues of *O. niloticus* exposed to different concentrations of zinc sulfate at different period than that control fish tissues. Results generally showed that metal concentrations were always lowest in the muscles and highest in the gill and liver.

INTRODUCTION

The freshwater fish has been affected and accumulated the heavy metals in their tissues as reported by Shokr (2007) who indicated that heavy metals accumulated in their tissues due to exposed to heavy metales. Some of the marine environment world-wide has been contaminated by heavy metals and as a result of (Mance, 1987; Langston, 1990; Merian, 1991; Bryan and Langston, 1992) this animals living in contaminated waters showed high metal concentrations in their tissues.

Over a few decades there has been growing interest to determine heavy metal levels in the marine environments and attention was drawn to find out contamination level of public food supplies particularly fish. Therefore, the marine environments are occasionally monitored for heavy metal contamination in water, sediment and animals. It is well known that heavy metals accumulate in tissues of aquatic animals and therefore heavy metals measured in tissues of aquatic animals can reflect the past exposures. Tissue concentrations of heavy metals can also be a reasonable measurement for public health standards and for animals' health point of view. Human population growth and industrial development have been the major causes of coastal contamination around the world during recent years (Caussy *et al.,* 2003). The subsequent accumulation of xenobiotic chemicals in sediments, seawater, or prey organisms has been shown to be directly linked to adverse health effects in both humans and fish (Boening, 2000, Riba *et al.,* 2004 and Ashraf, 2005). Contaminant levels in biotic and abiotic components of an ecosystem can easily be measured using accessible chemical analysis techniques, few reliable diagnostic tools are currently available to fish health professionals to evaluate disease in fish. According to Hrubec *et al.,* (2000), many of the clinical tools used to assess mammalian health are not developed for use in fish. Yet various workers have shown that hematological parameters can provide satisfactory information on the physiological response of fish to environmental stressors for two major reasons, namely, the close association of the circulatory system with the external environment (Houston, 1997, Lohner *et al.,* 2001 and Cazenave *et al.,* 2005). Similarly, histopathological investigations have long been recognized to be reliable biomarkers of stress in fish for several reasons described previously (Teh *et al.,* 1997 and van der Oost *et al.,* 2003). Gill lesions as indicators of exposure to contaminants have previously been used in numerous laboratory and field studies around the world (Dalzell and Macfarlane, 1999; and Oliveira Ribeiro *et al.,* 2002) including Mauritius (Bhagwant and Elahee, 2002). Unlike the enucleated mammalian red blood cells, fish erythrocytes possess very large nuclei, which may occupy about 29% of the cell volume (Wilhelm-Filho *et al.,* 1992 and Shokr, 2011).

The aim of this study is to determine heavy metal (Pb, Zn and Fe) concentrations in the muscles, gills and livers of freshwater fish *(Oreochromis niloticus)* exposed to lead acetate ferric chloride and zinc sulfate. This fish was very important source of food to human being.

MATERIALS AND METHODS

The *O. niloticus* 80 - 100 gm body weight was obtained from Abbassa farm. The fish were reared in large tanks contained water from their farm. The fish were acclimatized and fed on 30 % protein diet. The fish were divided into four groups, control group and three groups exposed to 5, 10 and 15 mg/l of lead acetate, 10, 20 and 30 mg L^{-1} of ferric chloride and 10, 20 and 300 mg L^{-1} of zinc sulfate respectively, the experimental fish were exposed to treatment for periods of 15 days, 30 days and 45 days. Each group consists of two-glass aquaria 30 x 60 x 40 cm^3. Each glass aquarium was contained 10 fish. In the laboratory, they were immediately dissected with clean equipment. Total size and weight of the animals were measured. Since size may be an important factor in heavy metal accumulation of aquatic animals (Canli and Furness, 1993a and b). six fish were obtained from each group after 15, 30 and 45 days. The gill, liver and muscle tissues of the animals were dissected using clean equipment and put in petri dishes. Tissues of the each group were pooled to make 5 subsamples and pooled tissues were transferred to an oven set at 120 °C to

dry. Drying continued until all the wet tissues reached to a constant weight, Dry tissue samples were put into digestion flasks and 4 ml perchloric acid and 8 ml nitric acid (Merck) were added. The digestion flasks were then put on a hot plate set at 120 °C (gradually increased) until all the materials were dissolved. After digestion the digested samples were diluted with distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals (Merck). Metal concentrations in the samples were measured using a Perkin Elmer AS 3100 flame atomic absorption spectrophotometer. Metal concentration in a tissue was presented as μg metal/gm dry weight.

Statistical Analysis:

Data were recorded as means ± S. E. (n) significant differences ($p<0.05$) within each group were tested with Student's two-tailed t – test and one-way ANOVA by SPSS for windows XP2 2002, comparisons between groups were tested by Student's two-tailed t – test unpaired design, ($p<0.05$.

RESULTS

Results of Table (1) revealed that the level of lead in gills increased significantly ($P< 0.05$) with each increase in its concentration in water, however concentrations in the liver and muscle showed in significant increase with its increase in water to 5 mg/l compared to the control group and significantly with its increasing level to 10 and 15 mg/l compared to control group and the group that exposed to 5 mg/l of lead acetate with each exposure period.

Table 1: the concentrations of lead (μg/gm d. w.) in the tissues of *Oreochromis niloticus* exposed to 5, 10 and 15 mg/l of lead acetate at different period

Organs	Doses /Periods	15 days	30 days	45 days
Gills	Control	3.65±0.095	3.10±0.065	3.32±0.054
	5 mg/l of lead acetate	7.85±0.042*	8.83±0.065*	9.25±0.042*
	10 mg/l of lead acetate	9.24±0.47**	10.25±0.089**	12.24±0.036**
	15 mg/l of lead acetate	12.05±0.048***	12.25±0.069***	14.02±0.95***
Liver	Control	1.33±0.084	1.24±0.18	1.81±0.21
	5 mg/l of lead acetate	4.20±0.32	5.21±0.64	6.02±0.46
	10 mg/l of lead acetate	8.11±0.27**	9.41±0.24**	9.22±0.51**
	15 mg/l of lead acetate	10.02±0.35***	10.71±0.24***	12.28±0.44***
Muscles	Control	1.24±0.22	1.27±0.68	1.24±0.66
	5 mg/l of lead acetate	2.95±0.58	3.83±0.14	5.82±0.57
	10 mg/l of lead acetate	5.35±0.54**	6.23±0.26**	8.23±0.24**
	15 mg/l of lead acetate	7.21±0.97***	8.69±0.84***	9.94±0.64***

In the gills level of iron increased significantly ($P< 0.05$) with each increase in its concentration in water, however concentrations in the liver and muscle showed in significant increase with its increase in water to 10 mg/l compared to the control group and significantly with its increasing level to 20 and 30 mg/l compared to control group and the group that exposed to 10 mg/l of ferric chloride with each exposure period as shown in Table (2).

Zinc concentrations in the gills, liver and muscles of *Oreochromis niloticus* exposed to different concentrations of zinc sulfate at different periods are shown in Table (3). level of Zinc in gills increased significantly ($P< 0.05$) with each increase in its concentration in water, however concentrations in the liver and muscle showed in significant increase with its increase in water to 10 mg/l compared to the control group and significantly with its increasing level to 20 and 30 mg/l compared to control group and the group that exposed to 10 mg/l of zinc sulfate with each exposure period.

Table 2: The concentrations of iron (µg/gm d. w.) in the tissues of *Oreochromis niloticus* exposed to 10, 20 and 30 mg L^{-1} of ferric chloride at different period

	Periods/organs	10 days	20 days	30 days
Gills	control	61.94±0.58	60.32±0.14	60.61±0.52
	10 mg L-1 of ferric chloride	83.66±0.95***	86.81±0.57***	84.24±0.57***
	20 mg L-1 of ferric chloride	88.23±0.91***	89.25±0.41***	93.35±0.84***
	30 mg L-1 of ferric chloride	90.04±0.52***	93.35±0.42***	94.32±0.81***
Liver	control	48.23±0.51	49.51±0.81	49.21±0.62
	10 mg L-1 of ferric chloride	65.29±0.45	65.36±0.91*	65.56±0.56*
	20 mg L-1 of ferric chloride	80.26±0.41**	82.52±0.95**	83.35±0.47**
	30 mg L-1 of ferric chloride	82.32±0.83***	85.23±0.71***	88.89±0.61***
Muscle	control	50.3±0.51	50.5±0.83	50.3±0.47
	10 mg L-1 of ferric chloride	55.22±0.51	65.24±0.61*	65.89±0.52*
	20 mg L-1 of ferric chloride	63.55±0.71*	71.52±0.61**	75.88±0.91**
	30 mg L-1 of ferric chloride	69.52±0.54**	72.35±0.86***	85.56±0.76***

Table 3: The concentrations of zinc (µg / gm d. w.) in the tissues of *Oreochromis niloticus* exposed to 10, 20 and 30 mg L^{-1} of zinc sulfate at different period

	Periods/organs	10 days	20 days	30 days
Gills	control	14.3±0.84	14.3±0.51	14.2±0.57
	10 mg L-1 of zinc sulfate	17.11±0.24*	18..21±0.81*	20.25±0.62*
	20 mg L-1 of zinc sulfate	19.85±0.31***	20.98±0.61***	23.87±0.95***
	30 mg L-1of zinc sulfate	25.21±0.22***	26.57±0.71***	27.98±0.75***
Liver	control	13.2±0.42	13.9±0.71	13.6±0.84
	10 mg L-1 of zinc sulfate	15.88±0.87*	16.52±0.63*	20.3±0.85*
	20 mg L-1 of zinc sulfate	18.22±0.81***	21.35±0.64***	25.6±0.62***
	30 mg L-1of zinc sulfate	24.21±0.83***	26.11±0.76***	27.55±0.81***
Muscle	control	10.6±0.80	10.2±0.63	10.7±0.41
	10 mg L-1 of zinc sulfate	13.32±0.44*	14.36±0.44*	15.24±0.88*
	20 mg L-1 of zinc sulfate	16.31±0.83***	17.14±0.82***	18.25±0.77***
	30 mg L-1of zinc sulfate	21.22±0.51***	21.86±0.91***	23.15±0.69***

Significant at $p<0.05$, ** highly significant at $p< 0.01$, *** very highly significant at $p < 0.001$

Results generally showed that metal concentrations were always lowest in the muscles and highest in the gills and liver.

DISCUSSION

In the present study the fish that exposed to 5, 10 and 15 mg/l of lead acetate, 10, 20 and 30 mg L^{-1} of ferric chloride and 10, 20 and 300 mg L^{-1} of zinc sulfate respectively, showed that metal concentrations were always lowest in the muscles and highest in the gills and liver. This is probably due to their physiological roles in fish metabolism. It has been shown that target tissues of heavy metals are metabolically active ones, like the liver, kidney and gills. Therefore, metal accumulation in these tissues occurs at higher level compared to some other tissues, like the muscles, where metabolic activity is relatively low (Heath, 1987; Langston, 1990; Roesijadi and Robinson, 1994; Canli *et al.,* 1995). It is generally agreed that heavy metal uptake occurs mainly from water, food and sediment. However, effectiveness of metal uptake from these sources may differ in relation to ecological needs and metabolism of animals and also contamination gradients of water, food and sediment as well as some other factors such as salinity, temperature, interacting agents (Heath, 1987; Langston, 1990; Roesijadi and Robinson, 1994). Kilgour (1991) indicated that animals which have close relationship with sediment, show relatively high body concentrations of cadmium, although uptake from water was more important route than uptake from sediment for animals which do not burrow. Canli and Furness (1998) also showed that tissue distribution of metals in the Norway lobster *Nephrops norvegicus* differed

significantly following to an uptake protocol from food and from seawater. Ecological needs of fishes are also one of the most important factors in the accumulation of heavy metal. For example, in the eel *Anguilla anguilla,* whiting *Merlangius merlangius,* flounder *Platichthys flesus* and plaice *Pleuronectes platessa,* levels of Zn, Cu, Pb and Cd in the muscle ranged considerably (Wharfe and Van Der Broek, 1977).

In the present study *O. niloticus* exposed to 5, 10 and 15 mg/l of lead acetate, 10, 20 and 30 mg L^{-1} of ferric chloride and 10, 20 and 300 mg L^{-1} of zinc sulfate respectively, for a period of 20 days, 30 days and 45 days this different from ecological needs of the fishes and play an outstanding role as all the fish species regardless of their ecological needs showed high metal concentrations. This may indicate that the exposure of *O. niloticus* to 5, 10 and 15 mg/l of lead acetate, 10, 20 and 30 mg L^{-1} of ferric chloride and 10, 20 and 300 mg L^{-1} of zinc sulfate respectively, for a period of 20 days, 30 days and 45 days causes increase precipitation of heavy metals as lead, iron and zinc in the tissues of this fish as gills, liver and muscles and increase the precipitation in the gills and liver than that in the muscle. Data from the literature showed that metal concentrations in the tissues of fish varied widely depending on where the animals were caught and exposure to heavy metals toxicity. Doganoc (1995) found that fishes from Slovania contained (ppm w.w.) <0.05-0.34 Pb, <0.003-0.05 Cd, in the muscle between the period of 1982-1993. Serra et al. (1993) showed that in tissues of *Liza ramada* and *Leuciscus cephalus* fished from the northern Adriatic, zinc levels were higher in the chubb than the grey mullet. They also found that the concentrations of iron and copper in the red muscle of the grey mullets were remarkably high and they indicated that this was probably due to swimming activity of this species. Remarkably high lead, iron and zinc concentrations in the liver of the *O. niloticus* in the present study support the high lead, iron and zinc concentrations of this species. Medina *et al*. (1986) measured the concentrations of heavy metals in the muscle of *Mullus barbatus* and *Mullus surmelatus,* collected from Spanish coasts. They found that Cd, Pb and Cr levels ranged between 0.019-0.187, 0.03-0.803 and 0.023-0.638 μg/g d.w. These values are lower when compared to the present results. Iron and zinc concentrations also may vary between different fish. For example, iron and zinc concentrations in the muscles of various marine fishes from Bangladesh ranged between 8.50-22.2 ppm w.w. for iron and 7.40-22.5 ppm w.w. for zinc (Quazi *et al.* 1995). The present study also showed that iron and zinc concentrations also varied among the groups of fishes that exposed to acute toxicity of ferric and zinc. This is perhaps due to the fishes that exposed to acute toxicity of ferric and zinc. Romeo (1987) also found relatively low concentrations of Cu, Zn, Cd and Pb in the muscle of *Mugil cephalus* (mullet roe) from the northern coast of Mauritania in the Atlantic Ocean as 2.3, 142, <0.1, <0.5 ppm d.w. respectively. However, this is not surprising because oceans are less contaminated marine environments comparing to seas that generally face human impact more than oceans. This study also emphasized that some metal levels were higher than the acceptable values for human consumption set by various health organizations. For example, zinc, iron and Pb data given in the present study are considerably high compared to daily tolerable cadmium and lead intake from food according to WHO/FAO committee's proposal (Merian, 1991). These are approximately 1 μg Cd/kg body weight and 7 μg Pb/kg body weight. This study showed that heavy metal concentrations in the tissues of fishes, regardless of their exposure levels and periods, were considerably high at all groups compared to metal concentrations in the literature given above from other waters. So, there is a high risk

for consuming this fish to human being, and treatment of water is highly recommended before using it in aquaculture.

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ARABIC SUMMERY

المعادن الثقيله (الرصاص و الحديد و الزنك) فى انسجة الاسماك نتيجه لتلوث المياة

السيد احمد محمد شكر
قسم الفسيولوجى- كليه الطب- جامعة حائل

اوضحت النتائج ان تعرض اسماك البلطى النيلى الى تركيزات مختلفة و اوقات مختلفة من اسيتات الرصاص الى ارتفاع فى مستوى الرصاص فى انسجة البلطى النبلى المختلفة من الخياشيم و الكبد و العضلات.
وجد ارتفاع فى مستوى تركيز الرصاص فى الخياشيم والكبد اكثر من العضلات تحت تاثير الجرعات المختلفة من اسيتات الرصاص.
اظهرت النتائج ان تعرض اسماك البلطى النيلى الى تركيزات مختلفة و اوقات مختلفة من كلوريد الحديدالى ارتفاع فى مستوى الحديد فى انسجة البلطى النبلى المختلفة من الخياشيم و الكبد و العضلات.
وجد ارتفاع فى مستوى تركيز الحديد فى الخياشيم والكبد اكثر من العضلات تحت تاثير الجرعات المختلفة من كلوريد الحديد
اوضحت النتائج ان تعرض اسماك البلطى النيلى الى تركيزات مختلفة و اوقات مختلفة من كبريتات الزنك الى ارتفاع فى مستوى الزنك فى انسجة البلطى النبلى المختلفة من الخياشيم و الكبد و العضلات.
وجد ارتفاع فى مستوى تركيز الرصاص فى الخياشيم والكبد اكثر من العضلات تحت تاثير الجرعات المختلفة من كبريتات الزنك.