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Heavy Metal Analysis of *Oreochromis Mossambicus* using Atomic Adsorption Spectroscopy

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Abstract

The present study to evaluate the bioaccumulation, of *Oreochromis mossambicus* reared with heavy metals concentration viz; Ni, Pb, and Cd fed with different protein diets D1 (40%), D2 (45%), D3 (50%) and D4 (55%) for 14 days. The fish was exposed to Ni, Pb and Cd at different sub lethal concentration of 6.2, 7.2 and 8.3 mg/l twice a day after a feed was given. The heavy metals of Ni, Pb and Cd were assayed after 14 days by using Atomic Adsorption Spectrophotometry and the results were given. The overall heavy metal bio-accumulation of *O. mossambicus* was reported. Certain tissue morphological difference was observed due to metal exposure. In the gill region high accumulation of (3103.47) Cd were observed in (high conc.) of 9.3 mg/l fed with 55% diet, whereas low accumulation of 543.43 were observed in Ni (low conc.) of 6.2 mg/l fed with 45% diet. Likewise muscle, ovaries low and high accumulation were observed. The order of heavy metal accumulation in the region of Gills was Cd > Pb, and Ni and followed by Muscle Cd, > Pb and Ni and Ovary Pb, > Ni and Cd than followed by control diet of 40% protein diets. The results were statistically significant at $p<0.05$.The accumulation of Nickel, Lead and Cadmium was significantly high where compared with control diet (40%) of fish tissues were reported.

Keywords: Heavy metals, different diets, bio-accumulation of gills, muscle, ovary

Introduction

In the last decades, contamination of aquatic systems by heavy metals has become a global problem. Heavy metals may enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic waste water, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, *et al.*, 2009).

In aquatic environment, larger animals such as fish have been exposed to heavy metals as a direct consequence of bio-magnifications (Ekwanyanwu *et al*., 2011; Javed and Usmani, 2011). The metals were enter and can be deposited in aquatic organisms through the effects of bio-concentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level (Huang, 2003). The danger is that heavy metals even at low concentrations in fish and water have a particular significance in ecotoxicology and their toxic effects have been widely published for a number of water bodies (Agatha, 2010; Abdel-Baki *et al*., 2011). Fish play an important role in human nutrition and therefore need to be carefully and routinely screened to ensure that there are no high levels of heavy metals being transferred to man through consumption (Muiruri *et al.*, 2013).

Lead has been of particular concern due to its toxicity and ability to bio-accumulation of aquatic ecosystems, as well as persistence in the natural environment (Miller *et al*., 2002; Anim *et al*., 2010). Lead is known to accumulate in fish tissues such as bones, gills, liver, kidneys and scales, while gaseous exchange across the gills to the blood stream is reported to be the major uptake mechanism (Oguzie, 2003; Tawari-Fufeyin and Ekaye, 2007). Exposure to high Pb levels can severely damage the brain and kidneys, cause miscarriage in pregnant women, damage the organs responsible for sperm production in men and it may ultimately cause death (ATSDR, 2002).

It can enter into the fresh water by disposal of industrial and household waste. Fertilizers often contain some cadmium. Reproduction rate of aquatic organisms may also be affected due to Exposure to heavy metals and can lead to a gradual extinction of their generations in polluted waters Sridhara *et al*., (2008). Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in the blood stream to either a storage point (i.e bone) or to the liver for transformation and/or storage (Obasohan, *et al.*, 2008).

Risk Assessment of Heavy Metals in Human

Like in other organisms, heavy metals are not destroyed by humans Castro-(Gonzeza, *et al.*, 2008). Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver; muscles and bone threaten the health of humans. Therefore, the heavy metals are among most of the pollutants, which received attention in various countries and considered the most dangerous category of pollutants in the sea (Hassaan, *et al.,* 2007). The real importance of fish in human diet is not only in its content of high-quality protein, but also to the two kinds of omega-3 polyunsaturated fatty acids: icosapentenoic acid (EPA) and docosahexenoic acid (DHA). Omega-3 (n-3) fatty acids are very important for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Burger, *et al.,* 2005; Al bader, *et al.,* 2008).

Accumulation Factor (Transfer Factor)

The accumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals in organs and/or tissues of organisms, such as fish (Obasohan, *et al.,* 2008). The accumulation factor consists of ratios of the concentration of a given contaminant in biota (a particular metal concentration in fish muscle) to that in an abiotic media (water, sediment and food). Having a good understanding of the accumulation factor is important in predicting the relative contributions of abiotic media as a source of heavy metals accumulation in fish and the accumulation efficiency for any particular pollutant in any fish organ.

The greater transfer factor for any environmental source indicates that metals transferred to fish tissues from that source more than other sources (Rashed, *et al.,* 2001). Toxic substances may knock down immune, reproductive, nervous and endocrine systems in animals and these effects can be at organ, tissue and cell level (Geeraerts and Belpaire, 2009). In our present study was conducted to investigate the tissue accumulation of Ni, Pb, and Cd in the edible part, of *O.mossambicus* under in lab condition fed with different diets, to determine the relationship between the current aquatic contamination with heavy metals and the health hazards to fish consumers from higher trophic levels.

Material and Methods

To study the sub lethal effects of heavy metals on the *O. mossambicus* was selected as an experimental animal. The body weights of the fishes are ranging between 14 to 16gm and total length 14 to 15cm were collected from the local dam Kullursandai at Virudhunagar district. The fishes were brought to the laboratory with care and acclimatized for the laboratory condition. After acclimatization, the fishes were transferred to plastic tub of 15 litre capacities of water, each tub containing 10 fishes along with control group at a room temperature of 28^0C. The fishes were treated with heavy metals water after the feed was given @ twice a day with different concentration to observe the LC_{50} and the biochemical analysis and morphological changes. Each group was exposed to gradually increased the concentration (i.e.) Ni-7.2 mg/l, Pb-6.2mg/l, Cd-8.2mg/l for a period of 14 days and triplicate were maintained. The biochemical parameter was analysed, before and after the end of the experiment.

The fishes were divided into two groups namely control group and experimental group. The first is a control group and second is an experimental group. The second group treated with heavy metals (Ni, Pb, Cd) at different a sub-lethal concentration. Whereas the control group fishes, were not treated with heavy metals. Both the group of fishes were fed with different percentage of protein diet 40%, 45%, 50%, 55% fish feed at a rate of 5gm/kg of fish/day. The faecal matter and left out food materials were removed every day. The tissues sample like gill, muscle, and ovary were carefully removed from both control and experimental group at end of the 14 days. The tissue was immediately washed in 0.9% NaOH remove the adherence of mucous and blood. It was kept on the blotting paper to drain the moisture. The dried material was first digested using Nitric-perchloric acid digestion (AOAC, 1990). The experimental tissues of control and metal treated fish tissues were analysed the AAS with the help of Research Department of Zoology, ANJA College, Sivakasi, Tamil nadu.

Statistical Analysis

The data obtained were subjected to analysis of variance using statistical Analysis (ANOVA) was performed to compare the means of the heavy metals accumulation in different fish tissues at P=0.05 level of significance.

Results

The concentration of Ni, Pb, and Cd in the Muscle, Gills, and Ovary of the *O. mossambicus* commercial fish species are presented in (Table1). The highest concentrations of heavy metal were for Pb (high conc. in ovary; 3839.74 ± 5.03 ppm) and the lowest were for Cd (low conc. in ovary; 105.33 ± 4.9ppm). These values were compared with the control the accumulation is very low (11.2 ± 0.02 ppm) in ovary of control fish. Calculation of the average concentrations of Ni, Pb, Cd in muscle, gills and ovary of the fish gave the following results in control gills (143.50 ± 2.76), muscle (12 ± 0.02), ovary (11.2 ± 0.02) were presented in (Table 1).

Table 1 Heavy metals concentration in various tissues of *Oreochromis mossambicus* fed with different level of protein

		Nickel sulphate			Lead nitrate			Cadmium chloride		
	Protein diet 40%	Protein diet 45%			Protein diet 50 %			Protein diet 55 %		
Organs	Control	Low(6.2mg/l)	Optimum(7.2mg/ll)	High(8.2mg/l)	Low(5.2mg/l)	Optimum(6.2mg/l)	High(7.2mg/l)	Low(7.3mg/l)	Optimum(8.3mg/l)	High(9.3mg/l)
Gills	143.50±2.7^{a}	543.43±19.94^{d}	893.72±19.65^{d}	1035.74±5.30^{b}	740.38±1.90^{a}	2176.28±5.90^{b}	3400.64±2.72^{a}	1524.22±23.99^{d}	2471.41±10.15^{c}	3103.47±2.97^{a}
Muscle	12±0.02^{a}	686.07±5.97^{b}	1004.81±3.95^{a}	1236.45±9.86^{c}	913.46±2.89^{d}	51.66±1.19^{a}	3759.61±8.96^{d}	1349.0±10.01^{d}	1356.79±5.94^{b}	3488.56±7.9^{c}
Ovary	11.2±0.02^{a}	769.38±8.85^{d}	1266.74±0.95^{a}	1176.49±5.85^{b}	2073.71±13.2^{d}	3471.1±10.01^{d}	3839.74±5.03^{b}	3494.54±4.16^{b}	626.24±6.28^{c}	105.33±4.9^{b}

The mean values having different superscripts in the same row are significantly different at < 0.05 % level

Table 2 Summary of ANOVA treatment of the effect of different heavy metals on the tissues of *Oreochromis mossambicus*

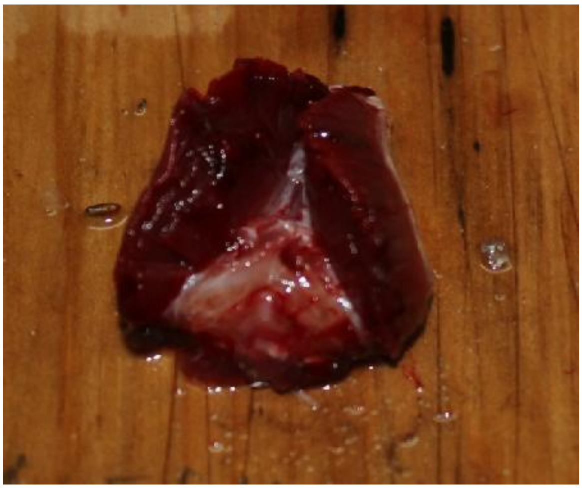
Parameters	Source of Variation	SS	Df	MS	F-value	significance
Gills	Between Groups	27078421	8	3384803	1.1734	0.05*
	Within Groups	2523.8224	18	140.2124		
	Total	27080944.82	26			
Muscle	Between Groups	37527063	8	4690883	7.0940	0.05*
	Within Groups	920.7701	18	51.15389		
	Total	37527984	26			
Ovary	Between Groups	39385330	8	5626476	0.041337	0.05*
	Within Groups	32061023	18	2003814		
	Total	71446353	26			

Plate I

Effect of Nickel Sulphate (Ni) treated with *Oreochromis mossambicus* after the experimental period of 14 days

a. Gills (6.2 mg/l) Low Conc.

b. Gills (7.2 mg/l) Optimum Conc.



c. Gills (8.2 mg/l) High Conc.

d. Gonad (6.2 mg/l) Low Conc.

e. Gonad (7.2 mg/l) Optimum Conc.

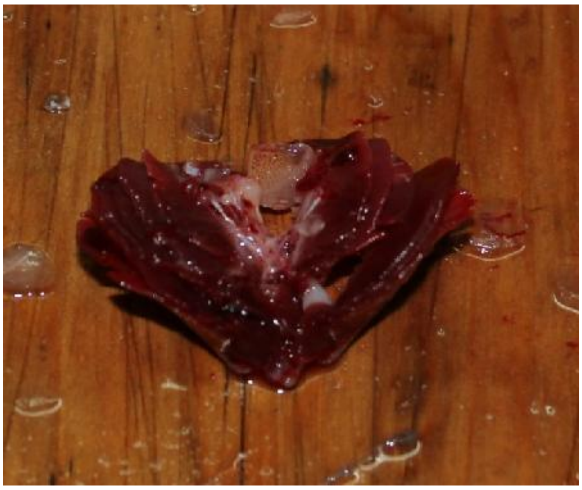
f. Gonad (8.2 mg/l) HighConc.

Plate II

Effect of Lead Nitrate (Pb) treatment on *Oreochromis mossambicus*

a. Gills (6.2 mg/l) Low Conc.

d. Gonad (5.2 mg/l)Low Conc.



b. Gills (6.2 mg/l) Optimum Conc.

e. Gonad (6.2 mg/l) Optimum Conc.

c. Gills (7.2 mg/l) High Conc.

f. Gonad (7.2 mg/l) High Conc.

Plate III

Effect of Cadmium Chloride (Cd) treatment on *Oreochromis mossambicus*

a. Gills (7.2 mg/l) Low Conc.

d. Gonad (7.2 mg/l) Low Conc.

b. Gills (8.2 mg/l) Optimum Conc.

e. Gonad (8.2 mg/l) Optimum Conc.

c. Gills (9.2 mg/l) High Conc.

f. Gonad (9.2 mg/l) High Conc.

Discussion

This study was undertaken to investigate the heavy metal concentrations in the edible parts (muscles, gills, and ovary) of commercially important fish species, and to detect, whether their levels are potentially harmful for human health if included in the diet. *O.mossambicus* fish were selected because they are the most commonly consumed fish. The levels of heavy metals were determined in the muscles of fish species because of its importance for human consumption**.**

In Egypt concentrations of Zn, Cu, Pb and Cd were determined in gills, skin and muscles of two fish species (*M. cephalus and Liza ramada*) from five locations in Lake Manzala Sodoumou, *et al.*, (2005). According to the National Health and Medical Research Council (NHMRC) limits were indicated that, the values of the metals detected in the fish muscles (the edible part) were within the permissible levels.

The present study revealed that concentration of the metals differs in water and the gills, muscles, ovary of the sampled fish. Bio-accumulations of the metals were in the order of Pb > Cd > Ni. The observed alterations in the muscle, gills, and ovary were mildly and moderately damaged respectively. This indicated that Pb, Cd, and Ni polluted the water. Authman *et al.*, (2007) revealed that the consumption of fishes in the lake could have the health damage to the local fish population .In the study to determine the accumulation and distribution of Cu and Zn in two fish species, of bio-accumulation factor showed the trend of accumulation of metals in fish organs was apparent in liver, gills and muscles, respectively. Moreover they reported that *T. zillii* seemed to be more contaminated with Zn and Cu than *M. Cephalus*. As they mentioned their result was in disagreement with many previous findings which pointed that *Mugil* species seemed to be more accumulated with heavy metals than Tilapia species.

In our investigation showed that the experimental fish contained different concentrations of metal (LC_{50} of Ni, Pb, Cd : 7.2, 6.2, 8.3 mg/l) in their muscle, gills, and ovary. Compared to these three tissues were more accumulation determined in the ovary (Pb: 3839 ppm) of the fish, were analysed using the Atomic Absorption Spectroscopy instrument. In Libya, Khalifa, *et al.*, (2010) determined concentrations of Co, Cd, Pb, Fe and Cu indifferent tissues of six samples of fishes of the Mediterranean sea. They found that the concentrations of Co, Cd and Pb in all examined tissues were more than the allowed literature values by WHO.

In our study showed that the accumulation of Pb is more (ovary: 3839 ppm, muscle: 3759 ppm, gills: 3400 ppm) in high concentration (Table 1) of treated fish tissues of ovary compared to other two concentration respectively., Marcovecchio, (2004) reported in the locations of Argentina the *M. furnieri* fish as a best good bio indicator of heavy metal pollution in ecosystem. A relationship between the metal contents of the fish species, their trophic and ecological habits was analysed. The results showed that the levels of Hg, Cd and Zn found in edible muscle tissue were lower than the standards for human consumption. Yilmaz, reported in (2009), to compare the concentrations of Cd, Cu, Mn, Pb,and Zn in tissues of three economically important fish (*Anguilla anguilla*, *M. Cephalus* and *O. niloticus*) the highest concentration of trace metals in the tissues of *M.cephalus* in lake was attributed to the trophic characteristics of this species, that *M.cephalus* reflects the metal concentrations in surface and suspended particulate matter, showing high metal concentrations.

Furthermore, Olowu, *et al.*,(2010) determined the concentrations of Zn, Ni and Fe in tissues of two fish species, Tilapia and Cat fish from two stations in Lagos, Nigeria. They concluded that both of the fish species may be considered safe for consumption, but the need for continuous monitoring to prevent bioaccumulation is necessary. In another study in Nigeria Christopher, *et al.,* (2009), studied the distribution of Pb, Zn, Cd, As and Hg in Bones, Gills, Livers and Muscles of Tilapia *(O. niloticus)* from Henshaw town beach market in Calabar. The results showed that the muscle of Tilapia contained the least concentrations of the heavy metals determined.

In the present study the levels of three heavy metals i.e Nickel, Lead, Cadmium has been determined from the fresh water fishes in the treated water medium. Heavy metals accumulate in fresh water and elevate through the food chain. So, in this study handled in laboratory condition using three different concentration heavy metals (Ni, Pb, Cd). The patterns of bioaccumulation of heavy metals are determined by the absorbance and excretion rates of fish. Different factors such as physical and chemical properties of water as well as the reason of significant accumulation of metals in different fish tissues.

In this study the order of heavy metal accumulation in the muscle, gill and ovary was Pb > Cd > Ni. Similarly Ni: high > optimum > low, Pb: high > optimum > low, Cd: high > optimum > low. To compare the accumulation of all (low, optimum, high) concentration of metals in fish tissue the more accumulation is present in high concentration of three metals. The lower value (gills, muscle, ovary: 143, 12, 11 ppm) obtained in control fish tissue because the control fish is untreated. In all heavy metals, the bio-accumulation of heavy metals Nickel, Lead, and Cadmium proportions was extensively augmented in the tissues of *Oreochromis mossambicus.*

A study on metal induced morphological alterations in the fish was therefore considered important. In the present study exposure of fish to sub-lethal concentration, low and high concentration of Nickel Sulphate (LC_{50} 7.2mg/l), Lead Nitrate (LC_{50} 6.2mg/l), and Cadmium chloride (LC_{50} 8.3mg/l) for a short term (two week) showed the changes in their behaviour of fish. Fish exposed to heavy metal pollutants can induce either percentage of increase or decrease in terms water quality parameters, growth performance and morphological changes of tissues were reported, when compared to control (Plate I, II, III).

In the present study focussed agreement with the previous studies that indicate that metal toxicity are mainly responsible for those morphological alterations (Muscles, gills, & ovary). Thus, it is concluded that physico-chemical analysis of the water sample, growth performance and morphological characters are the most sensitive parameters in monitoring the toxicity level of Ni, Pb, & Cd especially at sub-lethal concentration.

In over all our results were indicated that, in the tissue of *Oreochromis mossambicus* viz; ovary, gills, muscle were analysed using the AAS and the highest accumulation factor were observed in the ovary (Plate II), in the metal (lead) treated concentration of 7.2 mg/l (high conc) followed by other tissue viz; muscle (Plate II), gills respectively. The lowest metals observed in control fish tissues viz; gill, muscle, and ovary (143, 12, 11 mg/l). So that the human health studies are helpful to identify routes of pollutants entry and effect it is liable to cause.

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