Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 8 (3): 281-286
1st April, 2008

Bioconcentration of Lead in the Tissues of Feral and Laboratory Exposed *Clarias gariepinus*

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This study focuses on Pb bioaccumulation in the different tissues of the African catfish (*Clarias gariepinus*). Ten *Clarias gariepinus* were randomly allotted to each of experimental groups A-E and exposed to sublethal concentrations (0.0, 0.05, 0.1, 0.5 and 1 mg L^{-1}, respectively) of lead nitrate in triplicates. Bioconcentration of lead was between 9-600 and 20.2-800 mg kg^{-1} at 4 and 8 weeks, respectively in different tissues. Rate of bioconcentration of lead in tissues decreased with increase in lead concentration in culture water. The heart generally had the highest metal concentrations. The liver, in its role as a storage and detoxification organ, also accumulated high levels of lead. Muscles and skin accumulated much less lead concentrations. The lowest lead concentration (10.28 mg kg^{-1}) accumulated in the muscle of fish sampled from commercial fish ponds. This study has shown that lead pollution is of hazardous proportions in Ibadan and environs. This has severe consequences on aquatic fauna and humans who consume such contaminated fish and shellfish. It is therefore recommended that human and animal health surveillance and environmental monitoring of lead be more intensified. Skin and muscles should also be included in biomonitoring programmes, because they are consumed by the general public.

Key words: Bioconcentration, lead, *Clarias gariepinus*, Nigeria, water pollution

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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INTRODUCTION

There is an increasing concern regarding the roles and fates of trace metals in Nigerian environment. Much of this concern arises from the low level of available information on the concentration of these metals within the environment. Bioavailable heavy metals represent that portion of the total environmental metal load that is of direct ecotoxicological relevance since they can produce alterations of biochemical and physiological processes in the organisms (El-Shaikh *et al*., 2005). Aquatic organisms accumulate metals to concentrations many times higher than is present in water. Advancement in technology as well as increase in population have led to environmental concerns relating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste water and crude oil spills replete with most common heavy metals in our environment. The fate of heavy metals introduced by human activities into aquatic ecosystems have recently become the subject of wide spread concern, since beyond the tolerable limits they become toxic (Koller *et al*., 2004).

Metals therefore, tend to accumulate in the aquatic environment and thence in fish, either directly from the surrounding water or by ingestion of food (Antón *et al*., 2000). In addition, Heath (1991) indicated that when metals reach sufficiently high concentrations in body cells they can alter the physiological functioning of the fish. Lead has been shown to bioconcentrate in aquatic organisms such as plants, bacteria, invertebrates and fish. Determination of harmful and toxic substances in water sediments and biota, gives direct information on the significance of pollution in the aquatic environment. Freshwater fish are an important food source in both the developed and less developed countries. Fish remain the major source of protein and processed fishmeal plays a prominent although indirect role in human nutrition as an important component in the production of meat (Olaifa *et al*., 2004). As a result, fish have become an indispensable model system for the evaluation and/or measurement of the extent of aquatic pollution. Fish are used as biomarkers of not only acute toxic effects but also of the consequences of long-term exposure to low concentrations of pollutants (Nussey *et al*., 2000). This study was conducted to assess the accumulation of Pb in the tissues and organs of *Clarias gariepinus* from feral sources and those exposed to lead under laboratory conditions. It is expected that this study will give an insight into the Pb accumulation potentials of *Clarias gariepinus* and hence the public health implications of the findings.

MATERIALS AND METHODS

Study area: This was conducted in July 2005 in Ibadan, Nigeria. Ibadan (Oyo State, Nigeria) is the largest city in West Africa and the second largest in Africa, with land size covering an area of 240 km^2. The city is located on geographic grid reference longitude 3°5 E, latitude 7°20 N.

Sampling of fish from the wild, fish ponds and experimental fish population: Live specimens of adult *Clarias gariepinus* of both sexes were purchased from fishermen at Eleyele River and another batch was purchased from local fishermen along the Ogunpa water course. The fish specimens weighed between 153 and 165 g, (mean weight, 160.7 g and had total length of 25-31 cm, (mean total length, 27 cm). Live specimens of *Clarias gariepinus* weighing between 67 and 180 g with total length ranging between 28 and 35 cm were also purchased from some of the fish ponds sampled.

Fish exposure to lead under laboratory conditions: One hundred and sixty *C. gariepinus* weighing between 165.3 and 177.5 g (mean weight, 169.6 g and total length of between 26.5 and 33.5 cm, (mean total length of 29.5 cm) were purchased from Zartech farms in Ibadan. The fish specimens were acclimated to experimental conditions in the fish laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for 14 days after which the experiment commenced; the mortality recorded during the acclimation period was <10%. Fish were randomly allotted to five treatment groups (A-E) in static renewal plastic aquaria at ten per group and the experiment was set up in triplicate. Stock solution of lead nitrate {(Pb $(NO_3)_2$} was prepared by dissolving nalar reagent grade chemical (Merck, BDH, Poole, UK) in deionised water at a concentration of 100 mg L^{-1}. The fish in each group were exposed to different previously determined (96 h LC_{50} was 72.09 mg L^{-1}) sub-lethal concentrations of Lead Nitrate as follows: A (0.0 mg L^{-1}), B (0.05 mg L^{-1}), C (0.1 mg L^{-1}), D (0.5 mg L^{-1}) and E (1 mg L^{-1}). Experimental fish were fed commercially prepared fish pellets twice and once daily at 4 mg kg^{-1} body weight throughout the acclimation and experimental period respectively. The fish were observed several times daily and dead fish were promptly removed. Feces and food debris were siphoned out every 48 h. The experiment was allowed to run for 8 weeks.

Collection of samples for bioaccumulation study: Samples of selected tissues and organs (liver, spleen, gonads gill tissue, skin, muscle, heart and brain) from individual fish were pooled according to treatment for bioaccumulation studies.

Laboratory procedures

Analysis of samples for lead accumulation: Tissue samples were thawed and rinsed in distilled water; approximately 5 g of each sample was dried in an oven at 60°C for a period of 48 h. One gram of dried tissue was then accurately weighed into 100 mL Erlenmeyer flasks where after 5 mL perchloric acid (70%) and 10 mL nitric acid (55%) were added. Digestions were performed on a hotplate, at 200 to 250°C, for at least 4 h or until solutions were clear (Van Loon, 1980). After digestion each sample was filtered using an acid-resistant 0.45 μm filter paper and a vacuum pump. After filtration, the filtering system was rinsed with distilled water to remove all traces of metals, where after the samples were made up to 50 mL with distilled water. The Pb concentration in the tissue samples of the fish was determined using a Varian Atomic Absorption Spectrophotometer (Spectra AA-10).

Bioconcentration Factor (BCF): Bioconcentration factors between the fish tissues and the water (BF_w) were calculated, using the mean metal concentration in each tissue and the corresponding metal concentration in culture water.

C_{wf} is the concentration of lead in a fish's tissue or organ due to uptake of lead from the water, in which it is being cultured and C_w is the concentration of lead in the water, then the bioconcentration factor, BCF is calculated as follows:

$$BCF = \frac{C_{wf}}{C_w}$$

where, C_{wf} and C_w are in the same units (mg kg^{-1} and mg L^{-1}, respectively) as such BCF will be a simple number without units.

Statistical analysis: Samples were pooled and as such could not be statistically compared. The Spearman-R test was used to determine the relationship between the lead concentrations in the Gills relative to the other tissues and organs.

RESULTS

Lead was not detected in the tissues and organs of control fish population (group A), while BCF values of the control group (group A) was below detection limit.

Four weeks post exposure of experimental fish to lead nitrate: At 4 weeks post exposure Experimental fish in group B accumulated lead in the order of heart>liver>brain>skin>testis>gill>muscle>spleen>ovary with BCF values of 10400, 3000, 2400, 1162, 433.4, 368, 300, 276 and 180, respectively. In Group C, the trend was heart>liver>brain>skin>testis>spleen>gill> muscle>ovary (5500, 2000, 1700, 601.1, 249.8, 232, 201, 165 and 121, respectively). While in fish in group D, the bioconcentration trend was heart>liver>brain>skin> gill>testis>spleen>ovary>muscle (1160, 477.7, 360, 118.4, 70, 52.2, 52, 37.5 and 37.4, respectively). Group E accumulated lead in heart>liver>brain>spleen>skin> gill>testis>ovary>muscle (600, 241.7, 230.3, 65.5, 62.3, 57.1 43.5 and 21.9, respectively) (Table 1, 3).

Eight weeks post exposure of experimental fish to lead nitrate: At 8 weeks post-exposure, group B bioconcentrated lead in the order of heart>liver> brain>skin>testis>gill>ovary>spleen> muscle with BCF values of 10000, 4500, 3000, 2500, 2000, 640, 600, 427.4 and 404, respectively; group C's trend was Heart>Liver>

Table 1: Comparative assessment of lead concentration in tissues and organs of fish exposed to different concentrations of lead nitrate at 4 weeks post exposure to lead nitrate

Experimental fish	Lead concentration in different tissue at 4 weeks post exposure (mg kg^{-1})								
	Skin	Gill	Brain	Liver	Ovary	Testis	Spleen	Heart	Muscle
A (0.0 0 mg L^{-1} Lead)	0.01	0.001	0.00	0.002	0.003	0.00	0.02	0.00	0.02
B (0.0 5 mg L^{-1} Lead)	58.10	18.400	120.00	150.000	9.000	21.67	13.80	520.00	15.00
C (0.1 mg L^{-1} Lead)	60.11	20.100	170.00	200.000	12.100	24.98	23.21	550.00	16.50
D (0.5 mg L^{-1} Lead)	59.20	35.000	180.00	238.840	18.750	26.09	26.00	580.00	18.70
E (1.0 mg L^{-1} Lead)	62.30	57.140	230.30	241.670	21.860	43.51	65.50	600.00	20.00

*Samples were pooled, yet represent average values of 3 replicates

Table 2: Comparative assessment of lead concentration in tissues and organs of fish exposed to different concentrations of lead nitrate at 8 weeks post exposure to lead nitrate

Experimental fish	Lead concentration in different tissue at 8 weeks post exposure (mg kg^{-1})								
	Skin	Gill	Brain	Liver	Ovary	Testis	Spleen	Heart	Muscle
A (0.00 mg L^{-1} Lead)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B (0.05 mg L^{-1} Lead)	125.00	32.00	150.00	225.00	30.00	27.70	21.87	700.00	20.20
C (0.1 mg L^{-1} Lead)	128.00	40.50	340.00	500.00	39.77	34.90	32.65	700.00	21.25
D (0.5 mg L^{-1} Lead)	130.89	60.00	450.00	756.00	50.00	48.77	55.89	750.00	39.10
E (1.0 mg L^{-1} Lead)	137.50	89.29	637.50	800.00	60.00	62.50	121.90	750.00	42.17

Table 3: Comparative assessment of the Bioconcentration factors (BCFs) of tissues and organs of *Clarias gariepinus* exposed to different concentration of lead at 4 weeks post exposure

Experimental fish	Bioconcentration factors at 4 weeks post exposure								
	Skin	Gill	Brain	Liver	Ovary	Testis	Spleen	Heart	Muscle
A (0.00 mg L^{-1} Lead)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
B (0.05 mg L^{-1} Lead)	1162.0	368.0	2400.0	3000.0	180.0	433.4	276.0	10400	300.0
C (0.1 mg L^{-1} Lead)	601.1	201.0	1700.0	2000.0	121.0	249.8	232.1	5500	165.0
D (0.5 mg L^{-1} Lead)	118.4	70.0	360.0	477.7	37.5	52.2	52.0	1160	37.4
E (1.0 mg L^{-1} Lead)	62.3	57.1	230.3	241.7	21.9	43.5	65.5	600	20.0

Table 4: Comparative assessment of the Bioconcentration factors (BCFs) of tissues and organs of *Clarias gariepinus* exposed to different concentration of lead at 8 weeks post exposure

Experimental fish	Bioconcentration factors at 8 weeks post exposure								
	Skin	Gill	Brain	Liver	Ovary	Testis	Spleen	Heart	Muscle
A (0.00 mg L^{-1} Lead)	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0.0
B (0.05 mg L^{-1} Lead)	2500.0	640.0	3000.0	4500	600.0	2000.0	427.4	10000	404.0
C (0.1 mg L^{-1} Lead)	1280.0	405.0	3400.0	5000	397.7	349.0	326.5	7000	212.5
D (0.5 mg L^{-1} Lead)	261.8	120.0	900.0	1512	100.0	111.8	97.5	1500	78.2
E (1.0 mg L^{-1} Lead)	137.5	89.3	637.5	800	60.0	62.5	121.9	750	42.2

Table 5: Bioconcentration profile of lead in tissues and organs of fish sampled from the wild and commercial fish ponds

Fish population	Lead bioconcentration (mg kg^{-1})								
	Skin	Gill	Brain	Liver	Ovary	Testis	Spleen	Heart	Muscle
Wild fish	52.35	36.00	100.50	53.80	14.87	25.73	52.00	580.00	26.80
Cultured fish	15.22	17.85	30.11	36.62	10.28	20.77	15.87	14.37	21.91

Brain>Skin>Gill>Ovary>Testis>Spleen>Muscle (7000, 5000, 3400, 1280, 405, 397.7, 349, 326.5 and 212.5, respectively); while that of fish in group D was liver> heart>brain>skin>gill>spleen>ovary>testis>spleen> Muscle and BCF was 1512, 1500, 900, 261.8, 120, 111.8, 100, 97.5 and 78.2, respectively. Group E accumulated lead in the order of liver> heart>brain>skin> spleen>gill>testis>ovary>Muscle with BCF values of 800, 750, 637.5, 137.5, 121.9, 89.3, 62.5, 60 and 42.2, respectively (Table 2, 4).

Bioconcentration profile of lead in the wild fish and cultured fish: An evaluation of the bioconcentration profile of lead in the wild fish and cultured fish (Table 5) revealed that in the wild fish population, the highest proportion (580) of lead accumulated in the heart while the lowest lead level was observed in the ovaries (14.87). In the cultured fish sampled, the highest lead level was observed in the liver (36.62) and the lowest also in the ovaries (10.28).

Relationship between lead bioconcentration profile in the gills relative to other tissues and organs: At 4 and at 8 weeks post exposure, very strong significantly ($p<0.01$) positive correlations were observed between the lead accumulated in the gills relative to the other tissues and organs sampled. The relationship between all other organs was also significantly ($p<0.01$) positively correlated.

DISCUSSION

In natural water the total Pb concentrations generally range between 0.05 and 10.0 mg L^{-1}, whilst the dissolved Pb concentration normally does not exceed 0.01 mg L^{-1} (Gbaruko and Friday, 2007). When fish are exposed to elevated metal levels in an aquatic environment, they can absorb the bioavailable metals directly from the environment via the gills and skin or through the ingestion of contaminated water and food. Metals in the fish are then transported by the bloodstream which brings it into contact with the various organs and tissues (Rodriguez Moreno *et al*., 2003). Fish can regulate metal concentrations to a certain extent; where after bioconcentration will occur (Heath, 1991). Therefore, the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal (Rodriguez Moreno *et al*., 2003). During the present study, at four weeks post exposure, lead bioconcentration was predominantly found to be highest in the heart tissue, followed by the other tissues (liver, brain, spleen, skin, gill, testis, ovary and muscle). The uptake of aqueous Pb across the gill into the bloodstream is the primary mode of uptake in freshwater fish (Fialkowski *et al*., 2003). At eight weeks post exposure, the bioconcentration was highest in the liver followed by other tissues in the order of heart>brain>skin>spleen>

gill>testis>ovary>muscle. This pattern has also been reported other researchers Nussey *et al.* (2000) and Mouneyrac *et al.* (2001). However, this result is contrary to the experimental bioaccumulation studies in carp (*C. carpio*), which revealed that lead concentrations in the tissues was in the order of gill>liver>muscle as opposed to liver>gill>muscle observed in this study. This disparity may be attributed to differences among species and fish mass within populations (Olaifa *et al.*, 2004).

Nussey *et al.* (2000) reported that the lowest concentrations of lead detected in the tissue of *Labrax umbratus*, was in the muscle and skin. The lower levels of these metals might also indicate that the skin is an important excretory organ for these metals, presumably by means of mucus secretions (Heath, 1991). The skin and the gills are both characterized by a mucus layer on their outer surfaces, indicating that they are possible routes of excretion. This involves the sloughing off of metal-containing mucus from these surfaces (Antón *et al.*, 2000). In the present study, the concentrations of lead detected in the muscle and skin of *C. gariepinus* were also lower than most of the other tissues. The cultured fish sampled in this study shows that the lowest lead accumulated in the muscle of fish cultured in commercial fish ponds in Ibadan local government areas is 10.28 mg kg^{-1}, this is very important because the muscle is the edible part of the fish. Detection limit of lead in fish sample is 1 mg kg^{-1}, which shows that lead is being accumulated to a very dangerous proportion even in apparently safe commercially cultured fish. This results shows that humans who consume these fish may be subject to biomagnification lead and may suffer detrimental health effects as a result of this exposure (CIESM, 2002).

The calculated Bioconcentration Factor (BCF) values provide some indication of the bioavailability of lead to the fish from the water. A review of the ecotoxicological literature indicates that bioconcentration values of lead and certain lead compounds (lead salts) in aquatic plants and animals are often above a bioconcentration/ bioaccumulation factor of 1,000 and in some species at or greater than 5,000 (Fialkowski *et al.*, 2003).

In this study, at four and eight weeks post exposure, the range of the BCFs for all the groups and all tissues sampled is 21.9-10400 and 42.2-10000, respectively. This result is in agreement with studies on other fish species; USEPA (1984) reported that bioconcentration values for four freshwater invertebrate species ranged from 499-1,700. However, certain fish tissues have been observed to have much higher BCF values, e.g., the BCF value for the intestinal lipids in rainbow trout were as high as 17,300. Freshwater phytoplankton and both marine and freshwater algae have been known to accumulate or concentrate lead to very high levels (greater than 10,000x). These indicate that many of the BCF values and measured environmental concentration factors for lead are above 1,000 with several species having BCF or observed concentration factors at or above 5,000 (El-Shaikh *et al.*, 2005). This study also shows that BCFs decrease with lead concentration increase, thus suggesting accelerated saturation of uptake mechanisms. The bioconcentration factors also revealed that the fish exposed to the lower concentration of lead had higher BCF values, which indicates that lead is more available to African catfish at lower concentrations. This is because the Bioconcentration Factor (BCF) can be seen as a constant of proportionality between the concentration of the metal in the fish and the concentration in the water (BF_w), as such an increase in accumulation of metal in fish tissue will reflect as a decrease in the value of the BCF. The bioconcentration factor does not represent a hazard for the fish suffering the accumulation but for those populations which use these individuals as source for food (CSTEE, 2000). The findings from this study has shown that small concentrations of lead in the environment can find their way into fish in high enough dosages to bioconcentrate, thereby posing grievous health risk to the consumers. Regular biomonitoring of aquatic flora and fauna and health surveillance of the populace in Ibadan and environ is hereby recommended.

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