International Journal of Bioassays 6.11 (2017) pp. 5543-5473

Open Access
CODEN: IJBNHY
ISSN: 2278-778X
International Journal of Bioassays

Research Article

Effects of standard permissible levels of Lead (Pb) for potable waters on fish innate immune response and health compared with Pb levels found in natural waterbodies

Halley Grayson Orville
Wuxi Fisheries College of Nanjing Agricultural University, Nanjing, China.

Received: 9/30/2017; **Revised**: 10/17/2017; **Accepted**: 10/23/2017
Available online: 1st November 2017

Abstract: Among the list of pollutants, the heavy metals group is attracting the focus of Aquaculturist, Environmentalist and Fisherfolks alike, since they not only affect aquatic organisms but also have the potential to ultimately affect human beings. The deleterious effects of heavy metals such as lead (Pb) on aquatic ecosystems necessitate continuous monitoring of its accumulation in key species since it affords an indication of its impact on organism's health. In natural waters, the total Pb concentrations range between 0.05 and 10.0 mg/l (Galvin 1996). While the standard recommended a limit for water for consumption range between 0.01 and 0.05 mg/L Pb. In the current study, we exposed juvenile tilapias (*Oreochromis niloticus)* to waterborne Pb in five concentrations 0 (control), 0.01, 0.05, 0.25 and 1.25 mg/L Pb, over six weeks period. The present study focused on the bioaccumulation of low to moderate concentrations of Pb while simultaneously assessing the effects of waterborne Pb on *O. niloticus* feed intake, growth performances, blood plasma Lysozyme, Immunoglobulin M (IgM), Complement 3 (C3) and Cortisol levels. Results from the present study showed that among the Pb exposed groups a trend emerged, which indicated a high bioaccumulation rate in fish exposed to the lower (0.01, 0.05 and 0.25 mg/L) Pb concentrations. Fish exposed to 0.01 mg/l Pb showed highest accumulation rates, after 2 weeks of exposure, they were able to accumulate muscle Pb level that was equal to the concentration of Pb to which they were exposed. Meanwhile, data showed that fish exposed to 0.05 and 0.25 mg/L waterborne Pb recorded mean muscle Pb levels equal to or above the concentration of Pb to which they were exposed in the 4th week of exposure time. Furthermore, the mean muscle Pb recorded in fish after the 6th week of exposure to 0.01 mg/L waterborne Pb was 6 times above the level of the water concentration, while fish exposed to 0.05 mg/L, 0.25 mg/L and 1.25 mg/L waterborne Pb recorded mean muscle Pb after the 6th week that was 5, 3 and 2 times above the concentration of Pb in their respective water environment. Compared with the control group no significant difference in C3 activities were observed in fish exposed to 0.01 mg/L, 0.05 mg/L, and 0.25 mg/L Pb; however, as the dose of Pb increased to 1.25 mg/L significant decrease in C3 activity was observed after 4 weeks of exposure when compared with the control group. Additionally, no significant difference in IgM activities was observed in the 0.01 mg/L, 0.05 mg/L and 0.25 mg/L Pb treated groups of fish when compared with the control respectively. However, as the dose of Pb increased to 1.25 mg/L significant decrease in IgM activity was observed after 2 weeks of exposure compared with the control group. Similarly, as the dose of Pb was increased to 1.25 mg/L significant decrease in lysozyme activity was observed after 2 weeks of exposure compared to the control group. While Pb dose 0.25 mg/L after 4 weeks of exposure showed a significant decrease in lysozyme activity compared with the control. On the other hand, results on cortisol showed no significant sustained variations in activity when the respective Pb treated groups were compared with the control. The present study also showed that 1.25 mg/L waterborne Pb significantly depressed *O. niloticus* feed intake, weight gain, and absolute growth rate. While the mortality records showed that 75% of deaths occurred during the first two weeks of Pb exposure and the highest mortality was recorded in the group that was exposed to 1.25 mg/L Pb. The present study concluded that at low waterborne Pb concentrations fish bioaccumulate Pb faster. Also, tilapias may have become tolerant to the low Pb exposure levels over time by producing metalloproteinase and or their organs may have coped by reaching a state of homeostasis; however, further research will be needed to verify these hypotheses.

Keywords: Heavy Metals; Immunity; Bioaccumulation; Hepatosomatic Index; Fulton's Condition Factor; *Oreochromis niloticus*

Introduction

The immune system of fish operates at the crossroads between innate and adaptive responses and is habituated to the environment and the poikilothermic nature of fish (Tort *et al.,* 2003). The cells supporting the fish immune system share functional and morphological similarities with mammalians (Zelikoff, 1998). Moreover, the immune response entails phagocytosis and inflammatory processes (Corbel, 1975), ably assisted by non-specific immune cells such as monocytes/ macrophages, neutrophils and non-specific cytotoxic cells and enzymes. The immune system of fish as in higher vertebrates is usually divided into two integral components: (1) the innate, natural or non-specific defense system formed by a series of cellular and humoral components and (2) the adaptive response through the production of antibodies and by the cellular immune response mediated by T-lymphocytes, capable of reacting specifically with antigens (Gomez and Balcazar, 2008). However, there is evidence from both mammalian and fish

***Corresponding Author**:
Mr. Grayson Orville Halley,
Wuxi Fisheries College of Nanjing Agricultural University, Nanjing, China.
E-mail: ace0779@yahoo.com

immunology, that these are combinational systems (Magnadottir, 2006). Whereby, innate immunity generally will precede specific immunity by activating and determining the nature of the adaptive response and co-operating in homeostasis maintenance (Magnadittir, 2006). Naturally, fish can protect themselves with the help of a complex innate defense mechanism that may be constitutive (already present) or responsive (inductive), (Ellis, 2001). The innate immune response of teleosts is highly developed. Recently, Blood biochemical test has been adopted in aquaculture as an important tool to assess the health status of fish. Biochemical parameters such as plasma proteins (C3 and IgM), lysozyme and cortisol are some of the elements that play a crucial role in fish innate immune response; these are key constitutive and inducible immune components and may enhance immune recognition, especially during stressful conditions such as environmental stressors example Pb.

Pb is one out of four metals that have the most damaging effects on health and can enter the fish body through uptake of food and water. Pb occurs naturally in the environment. However, Pb pollution caused by Pb overload that is found in the environment is a result of human activities. Mostly due to the application of Pb in gasoline an unnatural Pb-cycle has evolved. In car engines, Pb is burned, so that Pb salts (chlorines, bromines, and oxides) will originate. These Pb salts enter the environment through the exhausts of cars through which the larger particles will drop to the ground immediately and pollute soils or surface waters, while the smaller particles will travel long distances through air, part of which will fall back on earth when it rains. This Pb-cycle caused by human is much more extended than the natural Pb-cycle. It has caused Pb pollution to be a global issue. Other human activities such as industrial processes and solid waste combustion also contribute to Pb polluting waters. Although Pb in petrol has dramatically decreased over the last decades, thereby reducing environmental exposure, the persistence of this metal in the soil and natural water systems remains a point of great concern. The concentration of Pb in natural waters range between 0.05 to 10 mg/L while the dissolved Pb seldom exceed 0.010 mg/L (Statumm and Morgan, 1980; WHO, 1986; Standard Method, 1989; Morell and Hering, 1993).

WHO (1985) and USEPA (1986) set standard limits for Pb in water at 0.01 and 0.005 mg/L respectively; while FAO & WHO (1989), FEPA (2003), set limits for Pb in fish and fish products for human consumption at 0.05 mg/kg; EU Regulation 1881/2006 and UNEP (1985) at 0.03 mg Pb/kg. There are other doc for EU standards for estuary and harbor basin water set at 0.50 mg Pb/L. The objectives of the study were to assess the effects of standard permissible levels of Pb (0.01 mg/l and 0.05 mg/l) on tilapia (*Oreochromis niloticus*) innate immune response and health compared with Pb levels found in natural water bodies (0.25 and 1.25 mg/L); by measuring blood plasma lysozyme, immunoglobulin M (IgM), complement 3 (C3) levels, together with bioaccumulation and growth performance of exposed *O. niloticus,* over a six weeks period.

Justification

Kumar and Halley (2015) investigated the presence of selected toxic heavy metals in two commercial fish species (*Oreochromis niloticus* and *Hoplosternum littorale*) in Guyana, South America. Their research analyzed samples taken from the muscles tissues of these two commercial fish species found in Municipal Markets, to assess human exposure to heavy metals through their diets. Table1 below highlight their findings; it can be seen that Pb is above the standard limit for both species Tilapia (*Oreochromis niloticus)* and Hassar (*Hoplosternum littorale*) respectively. Given the fact that these fish were harvested from natural waterbodies, R. Kumar and G. Halley (2015) postulated that, it is possible that some of the breeding grounds of these fish species pass through human inhabited settlements and as a result sewage from households, chemicals from agricultural activities and chemical from other human activities has possibly gotten into their water habitats and may have cause the accumulation of the heavy metals.

The information presented in tables 1 and 2 below, corroborate our knowledge that heavy metals persist in soils and waters. Because of the voracious feeding habit of Tilapia and the fact that the species is commonly found in most natural water systems in lesser developed countries and serve as an important dietary input for the peoples of these countries, the author is of the belief that it is vitally important that further research be carried out on this species ability to bioaccumulate this toxic heavy metals Pb and examine its effects on fish health. Based on previous work done by several researchers, the content of heavy metals in natural waters and agricultural soils worldwide is listed in Table 2 and 3 respectively. This study is an attempt at providing new comprehensive information on tilapias (*O. niloticus*) ability to bioaccumulate low to moderate waterborne Pb concentrations in their muscle tissues and examine the effects of the various concentration of this toxic heavy metal on the fish stress and immune responses. The results from this study will also ascertain whether this species can be used as a bioindicator for this particular heavy metal (Pb).

Table 1: Standard critical limit for heavy metals in fish and detected levels in 2 fish species

Organization	METALS						References
	Cu	Pb	Mn	Zn	Cd	Cr	
FAO/WHO/FEPA Standards	**30.0**	**0.05**	**1.0**	**40.0**	**0.5**	**1.0**	**FAO/WHO (1989); FEPA (2003)**
Results from study:							
Tilapia Mean Metal Concentrations	0.65 ± 0.07	7.06 ± 1.53	1.20 ± 0.24hrs	4.62 ± 0.45	0.79 ± 0.20	0.25 ± 0.10	Below Standard Limit
Hassar Mean Metal concentrations	0.74 ± 0.16	5.60 ± 1.24hrs	0.60 ± 0.17	4.73 ± 0.30	0.60 ± 0.11	0.17 ± 0.06	Above Standard Limit

Note: The critical/standard limit of each heavy metal that is found in fish is set by organizations such as the FAO- Food & Agriculture Organization, WHO- World Health Organization and FEPA- Federal Environmental Protection Agency. The standard for Pb is also by the Australia New Zealand Food Standards Code.
*Metal Concentration expressed as *Mean* ± *SEM.* *Maximum and standard levels in (mg/kg) of metals in fish. WHO & USEPA recommended limits for water are, 0.01 and 0.005 mg/l respectively.

Table 2: The content of heavy metals in natural waters and sediments worldwide

Country	Amount Pb in Water (Rivers, etc.)	Amount Pb in Sediments	References	Location
Missouri, USA	30 to 54 µg/L (1982)	Average: 72 and 400 µg/g (dry weight basis). Max: 10550 and 124hrs00µg/g (dry weight basis)	Nord. L. Gale; Craig D. Adams, *et al.,* (2002)	Big River and Flat River Creek in Missouri
Nigeria	Between 1.10mg/L and 9.00mg/L		A.Uzairu, O.J.Okunola, R.J.Wakawa, and S.G.Adewusi (2014)	River Challawa
France	1.144 ± 0.003 (between 1960 and 2000) 1.166 ± 0.003 (2003) 1.166 ± 0.004 (2013)	1.138 ± 0.003 to 1.165 ± 0.002	S. Ayrault, P. Le Pape, (2013)	Seine River and Orge River
Egypt	(0.05 to 0.31 µg/L)		Shafei H. Mel (2015)	Manzala Lake (Damietta branch of Nile)
England UK	(2011) Calder- 0.28 to 1.75 (µg /L) Douglas- 0.48 to 3.81 Ribble- 0.35 to 1.88 Wyre- 0.42 to 1.37		Phil Rowland, Colin Neal, *et al.,* (2011)	Rivers of North West England
England UK	(period 1995 to 2001) 30–345 µg L			Rivers of North West England

Table 3: The content of heavy metals in agricultural soils worldwide (mg/kg)

City/Country	Cr	Cu	Pb	Zn	Ni	Cd	Hg	As	Reference
Beijing	75.74	28.05	18.48	81.10	-	0.18	-	-	Liu *et al.,* 2005
Guangzhou	64.65	24hrs.00	58.00	162.60	-	0.28	0.73	10.90	Li *et al.,* 2009
Yangzhou	77.20	33.90	35.70	98.10	38.50	0.30	0.20	10.20	Huang *et al.,* 2007
Wuxi	58.60	40.40	46.70	112.90	-	0.14	0.16	14.30	Zhao *et al.,* 2007
Chengdu	59.50	42.52	77.27	227.00	-	0.36	0.31	11.27	Liu *et al.,* 2006
Xuzhou	-	35.28	56.20	149.68	-	2.57	-	-	Liu *et al.,* 2006
Kunshan	87.73	34.27	30.48	105.93	31.08	0.20	0.20	8.15	Chen and Pu, 2007
Spain	63.48	107.65	213.93	427.80	34.75	1.42	-	-	Zimakowska- Gnoinska *et al.,* 2000
America	-	95.00	23.00	-	57.00	0.78	-	-	Han *et al.,* 2002
Korea	-	2.98	5.25	4.78	-	0.12	0.05	0.78	Kim and Kim, 1999
Changde	-	-	-	-	-	-	-	92.7	PSTV, 2014
Slovakia	-	65.00	139.00	140.00	29.00	-	-	-	Wilcke, 2005
USA	48.5	48	55	88.5	29	13.5	-	-	Jean-Philippe etal., 2012
India	2.19	1.20	0.95	28.24hrs	4.34	0.82	-	-	Raju *et al.,* 2013
India	1.23	2.62	2.82	4.65	0.14	0.05	-	-	Prajapati and Meravi, 2014
Iran	10.36	9.62	5.17	11.56	11.28	0.34	-	-	Sayyed and Sayadi, 2011
Iran	11.15	-	-	-	-	-	-	-	Zojaji *et al.,* 2014
Range	1.23~ 87.73	1.20~ 107.65	0.95~ 213.93	4.65~ 427.8	0.14~ 57.00	0.05~ 13.50	0.05~ 0.73	0.78~ 92.7	
Average	46.69	38.08	51.19	117.35	26.12	1.50	0.28	21.19	
Background	61	22.6	26	74.2	26.9	0.097	0.065	11.2	CEPA, 1995
Environ. Capacity	200	100	300	250	50	0.3	0.3	30	Zheng *et al.,* 2008

Materials and Methods

Experimental fish and management

This experiment was carried out over a 6 week period using tilapia (*Oreochromis niloticus)* fingerlings. Five (5) experimental groups with different concentrations of Pb were applied: control 0; 0.01; 0.05; 0.25 and 1.25 mg/l Pb, respectively. Water quality was kept within the optimum range for the species and mortality was recorded. The fingerlings were purchased from a local fish farm and transported to the laboratory in oxygenated waterproof plastic bags and handled properly to minimize injury and stress. Fish were acclimated to the laboratory conditions for 3 weeks before the initiation of the experiment. Fingerlings (average weight 30 ±2.85 grams) were randomly allocated to 15 partially closed 200 liters capacity aquarium supplied with 150 liters oxygenated freshwater and aerated using air pumps and individual air stone diffusers were provided for good aeration and to maintain constant dissolved oxygen (6.0 ± 0.5 mg/L), temperature (28 ± 0.2 °C); and pH (7.2 ± 0.2) and water hardness (140 ± 5 mg/L) were measured using YSI MDS multi-probe system. Fish were divided into five groups each containing 60 fish (3 replicates at 20 fish each). Group I was held in tap water as a control, and other groups were exposed to 0.01, 0.05, 0.25 and 1.25 mg/L Pb [Pb (NO3)2] respectively, for 6 weeks. The concentration of each toxicant was selected as nominal sub-lethal concentration and based on available literature data some of which are summarized in Tables 1, 2 and 3. Fish were maintained in static renewal conditions, where water and metals were completely replaced every 24hrs, transferring fish to freshly prepared toxicants solutions (Dutta and Arends, 2003; US EPA, 2002) and photoperiod 12L: 12D was maintained. During acclimation and throughout the experiment, fishes were fed with commercial fish diet (No.5271, 35% crude protein, Ningbo Tech-Bank co. ltd, Yuyao city, China) *Ad libitum* until apparent satiation and consumption were recorded. A total daily ration (feed) of 5% of initial Body Weights (BW) of fish in each replicate was weighted and place in plastic bags. Which was then fed *Ad libitum* trice daily during 30 minutes feeding sessions (between 7:30-8:00am, 12:0-12:30pm and 17:00-17:30pm). Feed remaining at the culmination of the last feeding session was weighted and recorded by replicate. All additional feed consumed above the estimated 5% BW allotment was also weighted and recorded. The feed was fed in small sprinkles to avoid uneaten food and feces were removed before each feeding session. The fish were visibly free of any deformities, lesions or disease. The experiment was carried out under completely randomized design with three replications. A stocking density of twenty (20) acclimatized *Oreochromis niloticus* fingerlings were randomly selected and allocated to each aquarium/container (in 150 L water) with three replicates totaling 60 fish being tested at each Pb concentration and a control group (no metal added). The replacement of the test solution was carried out every 24hrs (all stock solutions were made immediately before use). The water was changed daily during acclimation and throughout the experiment to reduce the build-up of metabolic wastes and to keep concentrations of Pb close to the nominal levels during the experiment.

The Pb solutions used in the experiment were prepared by dissolving Pb nitrate Pb (NO3)2]): as Pb nitrate salt laboratory grade, with 99.0% purity from Merck Co., Germany) was used in distilled water at nominal concentrations of 0.01, 0.05, 0.25,1.25 mg/L of Pb and a control. It is mainly soluble in soft and slightly acidic water (Moore and Rainbow, 1987). The stock solution was prepared using appropriate calibrated analytical pipettes and graduated cylinders. Pb metal was prepared by adding a calculated volume from the stock solution into test containers considering an equivalent to respective desired Pb concentrations.

Sampling and analysis

Feeding was withheld 24hrs before sampling. Fish were immediately anesthetized upon capture in bath treatment with the highly potent MS222 (also known as tricaine methanesulfonate or Ethyl 3-aminobenzoate methane sulfonate salt). The anesthesia was prepared by dissolving 98% pure MS-222 salt 500 mg/L, (Sigma Aldrich) in water and buffered by adding an equal weight of sodium bicarbonate. Concentrations higher than 250 mg/L MS-222 are lethal for fish and are used for euthanasia (AVMA 2007). The induction time – i.e. elapsed from the immersion of the fish in the bath until they stopped moving was between 20 to 40 seconds. After which, blood samples were taken from the caudal vein within 1 minute of their capture, using sterile syringes. The period from capture-anesthesia-blood extraction was kept strictly within a 1 minute time frame achieved by assigning each anesthetized fish to a highly skilled technician in blood extraction, aided by a team of assistants. Followed by decapitation with a sharp blade. The blood was collected in anticoagulant-free centrifuge tubes, and plasma was obtained by centrifugation of blood at 3000 rpm for 15 minutes. Plasma samples were then stored at -40^{o}C until analyzed. The plasma was used for stress and immune parameters analyses. The following parameters were analyzed**:**

Complement component 3 (C3):- Component 3 C3) ELISA kit was used. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of fish C3 in samples. Add C3 to monoclonal antibody enzyme well which is pre-coated with fish C3 monoclonal antibody, incubation; then, add C3 antibodies

labeled with biotin, and combined with Streptavidin-HRP to form an immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen solution A, B, the color of the liquid changed into blue, and then the color finally became yellow at the effect of acid. The Chroma of color and the concentration of the fish C3 for each sample were then positively correlated.

Immunoglobulin M (IgM):- Fish immunoglobulin M (IgM) ELISA kit was used. This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with the goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for IgM and Horseradish Peroxidase (HRP) conjugated IgM. The competitive inhibition reaction is launched between with HRP labeled IgM and unlabeled IgM with the antibody. A substrate solution is added to the wells, and the color develops in opposite to the amount of IgM in the sample. The color development is stopped and the intensity of the color is measured.

Lysozyme:- Lysozyme (LZM) ELISA kit was used. This kit is used to assay the lysozyme in the sample of fish's plasma. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of fish lysozyme in samples. Add lysozyme to monoclonal antibody enzyme well which is pre-coated with fish lysozyme monoclonal antibody, incubation; then, add lysozyme antibodies labeled with biotin, and combined with Streptavidin-HRP to form an immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen solution A, B, the color of the liquid changes into blue and at the effect of acid, the color finally becomes yellow. The Chroma of color and the concentration of the fish substance lysozyme (LZM) of the sample were positively correlated.

Cortisol analysis:- cortisol ELISA kit was used. This test kit applies competition method to detect the content of cortisol, represented in Figure 1. Samples were added to enzyme well which was pre-coated with antibodies, then add recognition antigen labeled with horse radish peroxidase (HRP); after being incubated for 1 hour at 37 ℃ both compete with solid phase antigen and formed an immune complex. The formed complex then undergo washing by PBST, the combined HRP catalyzes TMB (Tetramethyl benzidine) into blue, which subsequently turned into yellow by the action of an acid; the test has absorption peak under 450nm wavelength, and its absorbance is negatively correlated with antigen density of the sample.

Figure 1: Representation of a competitive assay

Growth performance analysis:-
Fish growth was assessed regarding Weight Gain (WG), Absolute Growth Rate (AGR), Fulton Condition Factor (FCF) and Hepatosomatic Index (HSI).

$WG\ (g) = (W_2 - W_1)$

$AGR\ (gday^{-1}) = (W_2 - W_1)/\ t$

$FCF\ (g\ cm^{-3}) = (W/\ L^3) \times 100$

$HSI\ (\%) = (\text{liver weight [g]}\ /W) \times 100$

Feed utilization analysis: - Feed Intake (FI), Feed Conversion Ratio (FCR) and Feed Efficiency Ratio (FER).

$FI\ (g/\text{fish}) = \text{dry feed intake} / \text{number of fish}$

$FCR = FI\ (g) / WG\ (g)$

$FER = WG\ (g) / FI\ (g)$

Where W is body weight of fish; W_1 is initial body weight of fish (g); W_2 is final body weight of fish (g); t is the feeding trial period (days); L is total length of fish (cm).

Hepatosomatic index (HSI) analysis:- Whole livers were carefully removed and weighted to allow for calculation of the Hepatosomatic Index.

Mortality: - Additionally, mortality was calculated using the following formula:

$\text{Mortality}\ (\%) = (\text{number of deaths} / \text{initial number of fish}) \times 100$

Tissue sampling
Fish tissue sampling was done on day zero and weekly after that from all treatments for 6 weeks. Two fish from each tank (6 fish/treatment) were sacrificed at each sampling period, and muscle tissues were removed, wrapped in Teflon grade polythene bags and kept at –40° C until analyzed.

Tissue Pb testing
Fish muscle tissue samples were dried and pulverized in liquid nitrogen with glass mortar and a pestle (precleaned with 10% HNO3) and allowed to air-dry overnight at room temperature to a constant weight (5 gram). The samples were digested by adding 6 ml nitric acid (65 %) and 1ml H2O2 (35%). A ramped temperature control program was applied at 150ºC during 15 minutes followed by 15 minutes at 150ºC and 10 minutes cooling down in the microwave until they reached room temperature. The residues were then dissolved and diluted to 50 ml for muscle in deionized water then the samples were filtered using Whatman filter paper (0.45 µm). The concentration of heavy metals in fish samples were determined by ICP–OES (Perkin Elmer AA Analyst). All glassware were soaked in nitric acid for 3 days and rinsed with deionized water before being used (Csuros and Csuros, 2002). The instrument was calibrated with chemicals standard solution prepared from commercially available

chemicals. Standard stock solutions of Pb were prepared from titrasol (1000 mg/L) to make the calibration, all reagents used were of analytical reagent grade (Merck, Germany). The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions. In order to check the Aldoghachi *et al.,* J. Anim. Plant Sci., 26 (2) 2016 509 validity of the measurements for accuracy and precision, certified reference materials (Dogfish muscle: DORM-2, National Research Council, Canada) were analyzed. The detection limit is defined as the concentration corresponding to 3 times the standard deviation of 10 blanks.

Transfer Factor (TF) analysis

The Transfer Factor (TF) in fish tissues from the aquatic water was calculated according to Kalfakakour and Akrida-Demertzi (2000) and Rashed (2001) as follows:

TF= Mtissue/ Msediment or water

Where, Mtissue is the metal concentration in fish tissue; Msediment, metal concentration in sediment or water.

Statistical analysis

All data were recorded in Microsoft excel. Then transferred into the format necessary for statistical packages (SPSS20). Data were expressed as mean (M) ± standard error (M± SE). One-way analysis of variance ANOVA with Pb inclusion levels as a factor was computed. Duncan's multiple range tests at a significance level of 95% were used to determine significant differences between treatments. Mean value of P<0.05 were considered significant.

Results

Plasma complement C3

Changes in C3 activity are recorded in Table 4. Compared with the control group no significant difference in C3 activities was observed in the 0.01 mg/L, 0.05 mg/L and 0.25 mg/L Pb treated groups; however, as the dose of Pb was increased to 1.25 mg/L significant (P < 0.05) decrease in C3 activity was observed after 4 weeks of exposure compared to the control group. Weekly plasma C3 levels of fish exposed to 1.25 mg/l Pb consistently showed significantly lower C3 levels when compared with the other Pb treated groups. Empirical results also showed that after 6 weeks of exposure, there were no significant differences in mean C3 among fish exposed to 0.01 mg/L, 0.05 mg/L and 0.25 mg/L waterborne Pb when compared to the control group respectively. However, there were significantly (P < 0.05) lower plasma C3 in fish exposed to 1.25 mg/L waterborne Pb compared to groups exposed to 0.01 mg/L, 0.05 mg/L, 0.25 mg/L waterborne Pb and the control respectively.

Table 4. Changes in plasma C3 in tilapia exposed to different waterborne Pb concentration (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Time (weeks)	C3 Concentration (µg/ml)				
	Control	0.01	0.05	0.25	1.25
1	0.749± 0.16^{a}	0.790± 0.17^{a}	0.941± 0.12^{a}	1.038± 0.41^{a}	0.427± 0.13^{a}
2	1.141± 0.03^{a}	0.749± 0. 70^{a}	0.946± 0. 06^{a}	1.289± 0. 28^{a}	0.600± 0. 17 ^{a}
3	1.072±0. 23^{a}	1.004±0. 17^{a}	0.983±0.43^{a}	0.793±0.26^{a}	0.793±0.14^{a}
4	1.012±0.05^{a}	1.101±0.13^{a}	0.904±0.07^{a}	0.807±0.03^{a}	0.390±0.23^{c}
5	0.829±0.10^{a}	1.005±0.46^{a}	0.931±0.10^{a}	0.902±0.10^{a}	0.500±0.17^{a}
6	0.793±0.08^{a}	0.872±0.05^{a}	0.790±0.11^{a}	0.956±0.19^{a}	0.653±0.25^{a}

Note: Data are expressed as a mean ± standard error (m± SE). Different lower-case letters indicate significant difference from the control and with P < 0.05 being considered significant.

Plasma IgM

Changes in IgM activity are recorded in Table 5. Compared with the control, no significant difference in IgM activities was observed in the 0.01 mg/L, 0.05 mg/L and 0.25 mg/L Pb treated groups; however, as the dose of Pb was increased to 1.25 mg/L significant (P < 0.05) decrease in IgM activity was observed after 2 weeks of exposure compared to the control group. Weekly plasma IgM levels of fish exposed to 1.25 mg/l Pb consistently showed significantly (P < 0.05) lower levels when compared with the other Pb treated groups.

Table 5. Changes in plasma IgM in tilapia exposed to different waterborne Pb concentration (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Time (weeks)	plasma IgM (mg/ml)				
	Control	0.01	0.05	0.25	1.25
1	0.733 ± 0.04^{a}	0.823 ± 0.29^{a}	1.05 ± 0.07^{a}	0.98 ± 0.47^{a}	0.713 ± 0.15^{a}
2	1.05 ± 0.1^{a}	0.537 ± 0.45^{a}	1.043 ± 0.33^{a}	0.997 ± 0.32^{a}	0.437 ± 0.08 ^{c}
3	1.11 ±0.18^{a}	0.98 ±0.15^{a}	0.93 ±0.43^{a}	0.87 ±0.17^{a}	0.717 ±0.38^{a}
4	1.037 ±0.14^{a}	1.167 ±0.14^{a}	0.823 ±0.24^{a}	0.893 ±0.12^{a}	0.77 ±0.27^{a}
5	0.923 ±0.23^{a}	0.887 ±0.37^{a}	0.87 ±0.01^{a}	0.883 ±0.12^{a}	0.707 ±0.19^{a}
6	0.83 ±0.07^{a}	0.767 ±0.03^{a}	0.763 ±0.16^{a}	1.013 ±0.05^{a}	0.737 ±0.16^{a}

Note: Data are expressed as a mean ± standard error (m± SE). Different lower-case letters indicate significant difference from the control and with P < 0.05 being considered significant.

Results from the present study also showed that after 6 weeks exposure, no significant differences in mean Plasma IgM were observed among fish exposed to 0.01 mg/L, 0.05 mg/L and 0.25 mg/L waterborne Pb when compared to the control group. Table 5 also showed, there was significantly ($P < 0.05$) lower mean plasma IgM in fish exposed to 1.25 mg/L Pb compared to fish exposed to 0.05 mg/L, 0.025 mg/L Pb and the control respectively; however, fish exposed to 1.25 mg/L waterborne Pb showed no significant difference in mean plasma IgM when compared to fish exposed to 0.01 mg/L waterborne Pb, after the 6 weeks exposure period.

Plasma lysozyme

Changes in lysozyme activity are recorded in Table 6. Compared with the control no significant difference in lysozyme activities was observed in the 0.01 mg/L and 0.05 mg/L Pb treated groups; however, as the dose of Pb was increased to 1.25mg/L significant ($P < 0.05$) decrease in lysozyme activity was observed after 2 weeks of exposure compared to the control group. Additionally, at Pb dose 0.25 mg/L after 4 weeks of exposure significant ($P < 0.05$) decrease in lysozyme activity compared with the control group. Weekly plasma lysozyme levels of fish exposed to 1.25 mg/l Pb consistently showed significantly lower levels when compared with the other Pb treated groups. Empirical data revealed that there were no significant differences in mean plasma lysozyme among fish exposed to 0.01 mg/L, 0.05 mg/L and 0.25 mg/L waterborne Pb after 6 weeks of exposure when compared with the control group respectively. Moreover, table 6 showed there were significantly ($P < 0.05$) lower plasma lysozyme in fish exposed to 1.25 mg/L waterborne Pb after 6 weeks of exposure compared to groups exposed to 0.01 mg/L, 0.05 mg/L, 0.25 mg/L waterborne Pb and the control respectively.

Table 6. Changes in plasma lysozyme activity in tilapia exposed to different waterborne Pb concentration (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Time (weeks):	Plasma lysozyme activity (mg/L)				
	Control	0.01	0.05	0.25	1.25
1	0.773 ± 0.07[a]	0.927 ± 0.29[a]	0.960 ± 0.22[a]	1.027 ± 0.31[a]	0.670 ± 0.25[a]
2	1.000± 0.17[a]	0.580± 0. 48[a]	1.080 ± 0. 29[a]	1.067± 0. 21[a]	0.430± 0. 04 [c]
3	1. 983 ±0. 25[a]	0. 887 ±0. 17[a]	0. 913 ±0.43[a]	0. 873 ±0.25[a]	0. 557 ±0.55[a]
4	1.160±0.01[a]	1.260±0.12[a]	0.873±0.11[b]	0.867±0.10[c]	0.500±0.12[d]
5	0.947±0.17[a]	0.923±0.46[a]	0.867±0.12[a]	0.923 ±0.17[a]	0.577±0.10[a]
6	0.877 ±0.09[a]	0.770±0.05[a]	0.737±0.08[a]	0.940±0.18[a]	0.517±0.27[d]

Note: Data are expressed as a mean ± standard error (m± SE). Different lower-case letters indicate significant difference from the control and with $P < 0.05$ being considered significant.

Plasma cortisol

Changes in cortisol activity are recorded in Table 7. No significant differences in cortisol activities were observed between the 0.01 mg/L Pb treated group compared with the control. Though as the dose of Pb increased to 0.05 mg/L, 0.25 mg/L and 1.25 mg/L significant ($P < 0.05$) decrease in cortisol activities were observed at the 4th week of exposure when compared to the control group respectively. However, the decline did not persist into the succeeding weeks, which is also reflected in table 7 which showed that although there was a general decrease in cortisol in all Pb treated groups compared to the control group, statistical analysis of the data revealed that there was no significant difference in plasma cortisol between the control and the Pb treated groups after 6 weeks of exposure. Among the Pb-treated groups, there was no significant difference in mean plasma cortisol either.

Table 7. Changes in plasma cortisol in tilapia exposed to different waterborne Pb concentration (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Time (weeks):	Plasma cortisol (mg/L)				
	Control	0.01	0.05	0.25	1.25
1	0.829 ± 0.12[a]	0.810 ± 0.25[a]	0.935 ± 0.62[a]	0.955 ± 0.43[a]	0.827 ± 0.06[a]
2	1.034± 0.11[a]	0.511± 0. 39[a]	1.058 ± 0. 39[a]	1.018± 0. 26[a]	0.977± 0. 28 [a]
3	1.123 ±0. 19[a]	1.010 ±0. 17[a]	0.908 ±0.38[a]	0.882 ±0.24[a]	0.923 ±0.09[a]
4	1.067±0.12[a]	1.24hrs5±0.10[a]	0.803±0.13[c]	0.809±0.10[b]	0.700±0.10[d]
5	0.897±0.08[a]	1.004±0.45[a]	0.858±0.15[a]	0.968 ±0.11[a]	0.858±0.07[a]
6	0.818 ±0.01[a]	0.860±0.08[a]	0.750±0.05[a]	0.953±0.26[a]	0.813±0.06[a]

Note: Data are expressed as a mean ± standard error (m± SE). Different lower-case letters indicate significant difference from the control and with $P < 0.05$ being considered significant.

Feed utilization and growth performance

Feed Intake (FI), empirical data from the present study showed that there was no significant difference in FI among fish exposed to 0.01, 0.05, 0.25 mg/L Pb nor the control. However, fish exposed to 1.25 mg/L Pb recorded significant ($P <$ 0.05) differences in feed intake compared with fish exposed to 0.01, 0.05, 0.25 mg/L Pb and the control respectively. The highest mean FI was recorded in fish that were exposed to 0.05 mg/L Pb followed by the control group, 0.25 mg/L group, 0.01 mg/L group and 1.25 mg/L Pb group respectively.

Weight Gain (WG): fish that were exposed to 0.05 mg/L waterborne Pb recorded the highest mean WG, which proved to be significantly ($P < 0.05$) higher than the gains observed in fish exposed to 0.01, 0.25, 1.25 mg/L Pb and the control respectively. Although the lowest WG was observed in fish exposed to 1.25 mg/L waterborne Pb which proved to be significantly lower compared to the mean WG of fish exposed to 0.01, 0.05, 0.25 mg/L Pb and the control respectively. There were no significant differences between the mean WG of the control and the WG of fish exposed to 0.01 mg/L and 0.25 mg/L Pb respectively.

Absolute Growth Rate (AGR): fish that were exposed to 0.05 mg/L waterborne Pb recorded the highest mean AGR, which proved to be significantly higher than the rate observed in fish exposed to 0.01, 0.25, 1.25 mg/L Pb and the control respectively. Though the lowest AGR was observed in fish exposed to 1.25 mg/L waterborne Pb which proved to be significantly lower compared to the absolute growth rate of fish exposed to 0.01, 0.05, 0.25 mg/L Pb and the control respectively. There were no significant differences between the AGR of the control and the AGR of fish exposed to 0.01 mg/L and 0.25 mg/L Pb respectively.

Feed Conversion Ratio (FCR): data from the present study showed that there was no significant difference between feed conversion ratio of the control and FCR of fish exposed to 0.01, 0.05, 0.25 and 1.25 mg/L waterborne Pb respectively.

Feed Efficiency Ratio (FER): furthermore, data showed that the FER of fish exposed to 0.01, 0.05, 0.25 and 1.25 mg/L Pb revealed no significant difference when compared with the control respectively. Additionally, among the Pb-treated groups, there were no significant differences in FER.

Fulton Condition Factor (FCF): empirical data from the present study showed fish exposed to 0.05 mg/L waterborne Pb recorded the highest FCF, which proved to be significantly higher compared to fish exposed to 0.01, 0.25, 1.25 mg/L Pb and the control respectively while the lowest FCF was observed in fish exposed to 1.25 mg/L waterborne Pb which proved to be significantly ($P < 0.05$) lower compared to fish exposed to 0.01, 0.05, 0.25 mg/L Pb and the control respectively. There were no significant differences between FCF of the control and the FCF of fish exposed to 0.01 mg/L and 0.25 mg/L Pb respectively.

Hepatosomatic Index (HSI): data from the present study showed that there was no significant difference between HSI of the control compared to HSI of fish that were exposed to 0.01, 0.05, 0.25 and 1.25 mg/L waterborne Pb.

Mortality

No deaths were recorded in the control group and the group that was exposed to 0.01 mg/L Pb. Nevertheless, 1.7% mortality was recorded in the groups that were exposed to 0.05 and 0.25 mg/L waterborne Pb respectively whereas the highest (6.7%) mortality was recorded in the group that was exposed to 1.25 mg/L Pb (See Table 3-2 below).

Table 8. Mortality rate of fish exposed to various waterborne Pb concentrations (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Mortality	Pb concentration (mg/L)				
	Control	0.01	0.05	0.25	1.25
Deaths (No)	0	0	1	1	4
Percentage (%)	0.0	0.0	1.7	1.7	6.7

Table 9. Growth performance and feed utilization of tilapia subjected to various waterborne Pb concentrations (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Parameters	Pb concentration (mg/L)				
	Control	0.01	0.05	0.25	1.25
WG (g)	56.61± 3.07^{a}	54.76 ± 3.34^{a}	63.98 ± 1.69^{b}	53.71± 2.85^{a}	28.41± 1.92^{c}
AGR (gday)	1.32 ± 0.07^{a}	1.27 ± 0.08^{a}	1.49 ± 0.03^{b}	1.25 ± 0.04^{a}	0.66 ± 0.07^{c}
HSI (%)	2.65 ± 0.49^{a}	2.66 ± 0.76^{a}	2.71 ± 0.72^{a}	2.80 ± 1.59^{a}	2.25 ± 0.70^{a}
FCF	1.96 ± 0.24^{a}	1.94 ± 0.27^{a}	2.05 ± 0.18^{aB}	1.92 ± 0.39^{a}	1.86 ± 0.21^{aC}
FI (g/fish)	60.06± 2.78^{a}	59.89 ± 3.44^{a}	65.16 ± 0.74^{a}	60.07± 2.48^{a}	34.18 ± 1.10^{b}
FCR (g/g)	1.06 ± 0.06^{a}	1.09 ± 0.03^{a}	1.02 ± 0.02^{a}	1.12 ± 0.10^{a}	1.21 ± 0.12^{a}
FER	0.94 ± 0.05^{a}	0.91 ± 0.03^{a}	0.98 ± 0.01^{a}	0.90 ± 0.08^{a}	0.83 ± 0.08^{a}

Note: Data are expressed as a mean ± standard error (m± SE). Values with the unlike subscript letter in the same row are significantly different ($P< 0.05$) and different upper-case letters indicate difference among exposed groups. Where, WG = Weight Gain, AGR = Absolute Growth Rate, FCF = Fulton Condition Factor, HSI = Hepatosomatic Index, FI = Feed Intake, FCR = Food Conversion Ratio and FER = Feed Efficiency Ratio.

Pb in fish muscle tissues (Bioaccumulation)

Among the Pb exposed groups, the mean weekly muscle Pb levels revealed interesting trends. One such trend indicated a high accumulation rate in fish within the group that was exposed to the lowest (0.01 mg/L) Pb concentration; after 2 weeks exposure, this group was able to accumulate muscle Pb level that is equal to the concentration of Pb in the water environment. Meanwhile, data showed that fish exposed to 0.05, 0.25 and 1.25 mg/L waterborne Pb did not record mean muscle Pb levels equal to or above the concentration of Pb to which they were exposed until the 4th week of

exposure time. Furthermore, the mean muscle Pb recorded in fish at the 6th week was exposed to 0.01 mg/L waterborne Pb was 6 times the concentration of the water environment, while those exposed to 0.05 mg/L, 0.25 mg/L and 1.25 mg/L waterborne Pb recorded mean muscle Pb at the 6th week that were 5, 3 and 2 times the concentration of Pb in their respective water environment. Analysis of the accumulated means of the 6 weeks exposure time and analysis of Pb Transfer Factor (TF) showed a similar trend. Among the Pb treated groups the highest mean accumulated muscle Pb at the end of six weeks exposure was observed in fish which were exposed to 1.25 mg/L waterborne Pb followed by groups exposed to 0.25 mg/L, 0.05 mg/L and 0.01 mg/L Pb respectively while the TF of fish exposed to 0.01 mg/L Pb showed Pb accumulation in muscle tissues at the end of six weeks of exposure was 2.44 times above the concentration of Pb to which they were exposed, followed by 1.64 times in fish exposed to 0.05 mg/L Pb, 1.19 times for fish exposed to 0.25 mg/L Pb and 1.02 times above in fish exposed to 1.25 mg/L Pb.

Table 10: Changes in muscle Pb levels in tilapia exposed to various waterborne Pb concentrations (Control; 0.01; 0.05; 0.25 and 1.25 mg/L Pb)

Time (weeks)	Pb levels in muscle (mg/kg)				
	Control	0.01	0.05	0.25	1.25
1	ND	ND	ND	ND	ND
2	ND	0.010 ± 0.001^{dD}	0.036 ± 0.005^{cD}	0.128 ± 0.004^{bD}	0.826 ± 0.031^{aD}
3	ND	0.012 ± 0.002^{dD}	0.037 ± 0.005^{cD}	0.131 ± 0.004^{bD}	0.864 ± 0.035^{aD}
4	ND	0.023 ± 0.002^{dC}	0.054 ± 0.005^{cC}	0.292 ± 0.009^{bC}	1.396 ± 0.057^{aC}
5	ND	0.041 ± 0.002^{dB}	0.120 ± 0.007^{cB}	0.491 ± 0.014^{bB}	2.074 ± 0.050^{aB}
6	ND	0.061 ± 0.002^{dA}	$0.24hrs6\pm0.007^{cA}$	0.741 ± 0.005^{bA}	$2.514\pm0.024hrs^{aA}$

Note: Data are expressed as a mean ± standard error (m± SE). Different lower-case letters indicate significant difference among concentrations at the same exposure period and different upper-case letters indicate difference among exposure time at the same Pb concentration by the Tukey test and with $P < 0.05$ being considered significant.

Discussion

Effect of Pb on non-specific immunity of *O. niloticus:*

Observations from this research indicate that concentration of 1.25 mg/L Pb in fish water environment has significant damaging effects on fish immune system activities. Results indicated that once Pb enters the aquatic environment, it subsequently enters the fish body through uptake of water and causes depression in immunological functions such as IgM, complement C3 and lysozyme responses, particularly at a moderately high concentration (1.25 mg/L Pb). Though not significant in all exposed groups, it was observed that in all Pb treated fish compliment C3 was lower than the non- exposed control group. However, the lowering of fish plasma C3 levels was only significant in fish exposed to 1.25 mg/L waterborne Pb. It can be assumed that the significant depression in fish plasma C3 which was observed in the present study at that level of Pb exposure can have wide-reaching effects since this particular compliment plays a vital role in the innate immunity of fish. Research work done by Holland and Lambris (2002), reported that this complement system which is predominant in fish has a primary role in their innate immunity by facilitating chemotaxic, opsonization and pathogen destruction. At the same time, it also has a direct link to the acquired immune system since complement activation enhances B cell proliferation (Morgan *et al.,* 2005). Additionally, the findings of the present research on fish C3 depression occasioned by the possible toxic effects of Pb is also supported by work done by Watts *et al.,* and Uribe *et al.,* who reported that C3 is among the first line of non-specific humoral defense in fish, and these defenses can be influenced by extrinsic factors such as toxins (Pb) present in the water.

Observation from the present study showed that other components of the fish innate immune system were also affected when fish were exposed to waterborne Pb, namely IgM and plasma lysozyme. In teleost, mainly IgM is present, and IgD has been recently described, but its function is yet unknown. However, different forms of fish IgM and its observed flexibility of structure may compensate for lack of Ig class diversity. The present study observed that fish exposed to 1.25 mg/L waterborne Pb showed significantly lower plasma IgM when compared to those exposed to 0.01, 0.025 mg/L Pb and the control respectively. The low expression of IgM in response to moderately high (1.25 mg/L) concentration Pb may have been directly related to possible damages to a particular cell or may involve more than one cells which regulate the proliferation and differentiation of other cells responsible for the normal function of the immune system. The finding infer that fish antibody synthesis is inhibited and or suppressed by Pb. This kind of immunotoxin effects of Pb on fish has also been documented in brown bullhead exposed for 183 days to 50 ppm Pb acetate which had their phagocytic activity in the peripheral circulation and lymphoid organs decreased and a reduced hematopoietic activity in spleen (Dawson, 1935). Additionally, Carp exposed for 40 days to 1 ppm Pb acetate presented a reduction in albumin and gamma-globulin levels in the electrophoretic patterns in sera (Fujiya, 1961) and Saltmo trutta

injected intraperitoneally with low concentrations of Pb (NO3)2 presented a decrease of antibody titer against MS2 bacteriophage (O'Neill, 1981a).

Furthermore, results from the present study showed that fish plasma lysozyme was significantly depressed by exposure to 1.25 mg/L waterborne Pb. Lysozyme is more important for fish as compared with mammals because it is an important enzyme in the blood of fish which forms an integral part of their innate immune system and is considered to be the first line of defense against a broad spectrum of pathogens and toxins. This researcher opine that lysozyme level or activity can be an important index for assessing innate immunity responses of fish exposed to Pb since it is ubiquitous in its distribution among fish species and because this enzyme is known to act as an Opsonin and activates the complement system as well as phagocytes (Magnadottir B., 2006). Moreover, the low expression of this enzyme in response to 1.25 mg/L Pb combined with the equally low compliment C3 and IgM that were obtained in the present research is clear indication of an overall depression of fish innate immunity.

Effect of Pb on Plasma Cortisol, Hepatosomatic Index, Growth Performance, Feed Utilization and Survival of Tilapia

Stress in fish has been widely studied, and cortisol is one of the most common stress indicators. The current study showed that cortisol was low in all the experimental groups inclusive of the control. And Pb at the tested concentrations did not have any significant long-term effect on cortisol compared to the control. The researcher is of the opinion that cortisol may not be the ideal indicator for measuring stress caused by waterborne Pb in fish because cortisol production and secretion can be influenced by a wide range of factors. Despite the extended use of this indicator and its acceptance, some inconsistencies have been reported in the results of several experimental studies, much of them associated to undefined and uncontrolled variables which may alter the response in the secretion of cortisol into the bloodstream. Some of the variables are related to metabolic changes in the organisms as an adaptation or acclimation mechanism while others are extrinsic to the fishes. Also, the researcher believes that because cortisol is known to have rapid release into the bloodstream, make capture and handling of fish during sample collection very likely to cause cortisol elevation in all tested fish. For this reason, the time from capture to blood extraction was curtailed to under 1 minute by using a very potent sedative and highly skilled team of technicians. Hontela, A., Rasmussen (1992), investigated cortisol stress response to capture in two species of fish (*Perca flavescens* and *Esox lucius*) from sites polluted by high levels of polycyclic aromatic hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), and mercury, and from reference sites in the St. Lawrence river system. Their results showed, fishes from the reference sites exhibited the normal elevation of plasma cortisol in response to the acute stress of capture and had large pituitary corticotropes. In contrast, fish from the most polluted sites were unable to increase their plasma cortisol in response to the acute stress of capture and their pituitary corticotropes were atrophied. These results suggest that chronic exposure to chemical pollutants may Pb to an exhaustion of the cortisol-producing endocrine system, possibly as a result of prolonged hyperactivity of the system. However, the level of cortisol recorded in the present research was consistently lower than those recorded by most other researchers for the tilapia species. This may have been attributed to the precautionary methods used to reduce stress during the process of sample collection. The method was aimed at reducing the time from capture to sample extraction to 1 minute or below to reduce the normal neuroendocrine cascade (alarm response) that usually occurs during capture and sampling. On the other hand, the hormone cortisol is not stored in the body of fish but is synthesized on demand (Sunmter, 1997). Thus, the elevation or lowering of circulating cortisol must be a function of recent stimulation and not solely attributed to the influence of Pb. Results from the present study is in direct contradiction with those of Fırat Ö, Cogun H.Y, Yüzereroğlu T.A, *et al.,* (2011), who exposed *O. niloticus* to 0.05 mg/L Pb for 4 and 21 days, and concluded that the alterations in plasma cortisol activities of metal-exposed fish showed increases in cortisol levels (Control 3.98 ± 1.03 and 8.12 ± 1.24hrs ng/dl for Pb treated group) at 4 days, followed by a return to control levels at the end of the exposure period(control 4.11 ± 1.49 and 4.11 ± 1.41 Pb treated group). However, the trend is similar to what was observed in the present study though the plasma cortisol levels in this study were significantly lower than those obtained by Fırat Ö, *et al.,* for the identical waterborne Pb concentration (0.05 mg/L). Though not significant, looking at the graphical representation of plasma cortisol in Table 7, one can appreciate the inconsistent nature of this biological indicator which also lend support to the discussion above. From a purely biological viewpoint, the following researchers Stegeman *et al.,* 1988, Galgani *et al.,* 1992, George 1994, Escart´ın & Porte 1996, Kirby *et al.,* 2000 opine that many substances can affect the physiological processes of living organisms through the induction or suppression of enzymatic reactions. While some researchers specifically alluded to Pb (Pb) causing disruptions of metabolic and endocrine functions; others suggested Pb disruption of cortisol secretion through a direct toxic effect on adrenocortical cells. However, the specifics of Pb exposure on fish plasma cortisol is yet to be proven in detail. Hence, few studies are available for definitive comparison. This has led to

varying interpretations of Pb effects, coupled with the fact that there is not any officially accepted normal physiological range for cortisol in fish (Tilapia). Additionally, the researcher believes that to meaningfully investigate cortisol as a bio-indicator for stress caused by Pb, an experimental design must be tailored to address the multiple variables that can influence this particular enzyme activity and secretion. Other researchers such as Hasan Kaya and Mehmet Akbulut (2015), tested waterborne Pb at various concentration (0.5, 2.5, and 5.0 mg/L) over a 14 days period and their findings suggest that these concentrations of Pb can disrupt the health of Mozambique Tilapia and cause oxidative stress and osmoregulatory damage.

Could it be that the cortisol levels recorded in the present study normal physiological range for the particular species (*Oreochromis niloticus*) and size of fish? Unfortunately, there is still little concrete information on the physiological response of fish to a persistent stressor and little is also known about how fish respond when exposed simultaneously to two or more different stressors, or to sequential stressors. This is to say that variables such as the environment (temperature, etc.) and other habitat factors (feed and feeding, etc.) also have a role to play in fish homeostasis and or stress, since it is known that the environment can set physiological performance in a variety of ways and set the bounds within which normal physiological operation and function can take place. Investigators need to be aware of the various 'non-stress' factors that can also influence cortisol activity. Thus, the question may only find an answer through further profound investigative research.

The present study concurrently demonstrated that tilapia *(Oreochromis niloticus*) growth performance and feed utilization were also hampered as a consequence of being exposed to 1.25 mg/L waterborne Pb. Correctly, the present study observed that WG, Absolute Growth Rate (AGR) and FI of fish significantly decreased owing to exposure to 1.25 mg/L waterborne Pb. This depression in performance may have been as a result of direct toxic and damaging effects Pb may have had on the organism which could be responsible for integrated biological effects related to essential physiological functions, like metabolism of nutrients, growth and development being compromised. Several cited literatures recorded similar depressive effects on fish growth performance owing to Pb exposure. Another possible reason for low growth performance may have been due to the well-documented premise that Pb tends to compete with other essential metals for the binding sites of metalloproteinase, resulting in the disruption of essential metabolic activities relating to growth and feed utilization. Mirroring the depression in growth was the low Fulton Condition Factor (FCF) which was recorded for fish exposed to 1.25 mg/L waterborne Pb. Since FCF is a simplified morphometric measurement for assessing fish growth and general health, results from the present study showed that 1.25 mg/L Pb hampered the wellbeing of fish. Little comparative data is available to assess the impact of Pb on fish growth performance, and the researcher was unable to find a single research aimed at assessing growth performance of fish exposed to the toxic metal Pb. In this regard, the present study is indeed the first to offer insights into the impact of Pb on fish growth performance and feed utilization. However, research works carried out by Spahn S. and Sherry T (1999), concluded that Pb (Pb) exposed little blue Heron chicks (Egretta caerulea) in South Louisiana wetlands had significantly slower growth rates than non-exposed chicks, and exposure to Pb was correlated with increased nestling mortality.

Additionally, several researchers alluded to Hepatosomatic Index (HSI) as being a useful biomarker in detecting and assessing the hazardous effects of environmental stressors. This morphometric measurement is another such indicator used for assessing the general health of fish and is considered a mirror that reflects exposure to a variety of anthropogenic pollutants. However, the present study observed no significant differences in HSI among the various Pb treated groups, nor did the HSI of the Pb treated groups significantly differ from the control. The researcher is of the opinion that inspection of the liver is pertinent and should be a part of future investigations since liver indeed plays an important role in the metabolism and excretion of xenobiotic compounds. In general, most cited articles on heavy metals researchers opined either increase or decrease hepatic enzyme activities leading to histopathological changes, depending on the metal type and concentration, fish species, length of exposure and other factors. Ologo E. A. A, Olurin K. B, *et al.,* (2005), exposed fingerlings of African catfish *Clarias gariepinus* to sub-lethal concentrations (0.006 mg/l and 0.008 mg/l) of Pb for three weeks. Results from histological examination concluded that the degree of distortion in liver was proportional to the exposure periods and concentration of the metals and was found to be dose and time-dependent. However, in order to have an accurate and effective assessment of the effects of the xenobiotic compound Pb in the field and experimental studies, a proper monetorization of histological changes in the fish liver is a more accurate way and is highly sensitive.

In the present research, mortality (6.7%) was recorded for the group of fish which were exposed to 1.25 mg/L waterborne Pb, which is significantly higher than the 0 % observed in the control group. This is interesting to note since globally Pb levels in natural water bodies such as rivers and lakes have

been recorded above the highest (1.25 mg/L) concentration that was utilized in the present study which led to an insinuation that Pb may play a role in issues of decreasing fish stocks being experienced in many open waters around the world. Also, during the present study, abnormal physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure to1.25 mg\L Pb after which it became occasional. At the same time, the data also provided information which suggested *O. niloticus* tolerance to chronic low Pb exposures; research works carried out by Pascoe and Beattie (1979) lend support to the possibility of organisms developing tolerance to heavy metals by the metal itself acting as a stimulus in the stimulation of metallothionein synthesis which in turn provide the organism with certain level of tolerance. Grocell M, *et al.,* (2006) reported tolerance to chronic Pb exposure in fathead minnow (*Pimephales promelas*) and concluded, recovery of Na+ and K+ levels and reversal of effects on Ca2+ homeostasis during continued exposure strongly suggest fathead minnow can acclimate to Pb. While at higher concentration (1.25 mg/L) Pb may have caused extensive damages to the fish body which may have in principle affected the processes associated with the accumulation of the Pb and general health of fish.

Bioaccumulation of Pb in the muscle of tilapia

The data from the present study provided evidence in support of fish ability to bioaccumulate Pb. From the results of bioaccumulation, the observed trend suggests that at lower levels exposures, Pb has faster bioaccumulation rates. The observed ability of *O niloticus* to assimilate Pb at very low concentrations draw attention to a high possibility of transfer of Pb to consumers particularly in peoples of lesser developed territories who depends on 'capture fisheries' from inland freshwater bodies. Given that Pb is particularly dangerous and has been shown to accumulate in fish among other organisms, gives credence to possible accumulation in entire food chains. The Transfer Factor (TF) also points to the inability of the tilapia species to excrete Pb efficiently which resulted in it accumulating in the muscles tissues of the fish in levels that were well above the exposure concentrations. Fish ability to efficiently bio accumulate waterborne Pb was also reported on by Abdel-Baki A. S, *et al.,* (2011), in their research on tilapia, concluded that the transfer factors of Pb in fish from water was greater than that from sediments and that fish bioaccumulation of Pb from aquatic ecosystems was mainly from water and a lesser extent sediments . The amount of Pb in muscle tissues that were recorded in fish at the end of the six weeks exposure to 0.05, 0.25 and 1.25 mg/L waterborne Pb were higher than the standard recommended permissible limit (0.05 mg/kg, EPA, WHO) set for fish products destined for human consumption.

In general, the data showed that uptake rate and bioaccumulation ability decreased as Pb concentration increased. Similarly, muscle accumulation of Pb increased as a time of exposure increased. This was supported by M. S. Ahmed and S. Bibi (2010), who exposed freshwater cyprinid, catla catla to 1.0 µg/L, 2.5 µg/L, 5.0 µg/L, 7.5 µg/L and 10.0 µg/L Pb for 6 weeks and concluded that with the passage of time as the fish grew, metal accumulated in muscles and other organs increased steadily. Studies conducted by other researchers on Pb bioaccumulation in the other species (Falusi and Olanipekun, 2007; korai *et al.,* 2008; Ozturk *et al.,* 2009; Ganbi, 2010; Victor *et al.,* 2012; Mahdi Banaee *et al.,* 2013) also validated our findings.

Conclusion

The present study concluded that immunotoxicology effects of Pb in fish are severe at dose 1.25 mg/L in the aquatic environment. Furthermore, exposure to 1.25 mg/L Pb has been responsible for integrated biological effects related to the impairment of essential physiological functions, like metabolism of nutrients, immunological functions, growth, and development. Empirical data from the study also highlighted loopholes in documented methodologies used for collection of samples intended for cortisol analysis, since this particular hormone is very sensitive and can be activated by a large number of factors. Thus, in this study attention was paid on the formulation of a new methodology, one which considerably addressed the effects of extrinsic factors on the production and stimulation activities of cortisol during sampling. Further testing of the new method may prove its reliability and when this is achieved cortisol can be used as a reliable biomarker in the field of environmental biomonitoring of Pb pollution. Additionally, there was strong correlation between dose 1.25 mg/L Pb and low feed intake and weight gain. Strong correlation was also seen between exposure to 1.25 mg/L Pb and the low immunological (IgM, C3 and lysozyme) activities of fish. Though detailed study needs to be carried out to understand better the various pathophysiological and other changes undergone by the fish during these different concentrations of Pb exposure; and to assess the perceived immunological depression by further challenging the Pb fish with a pathogen (bacteria). Fish being an excellent model for assessing the immunotoxicology risk, relevant biological and dose-response and exposure data for xenobiotics such as Pb should continue to be analyzed in an attempt to establish qualitative and quantitative estimates of adverse outcomes. The alterations in plasma parameters may be a result of direct tissue (i.e., liver, gill, and kidney) damage and

dysfunction induced by Pb. Thus, plasma parameters such as IgM, C3 and lysozyme can be used as rapid and sensitive indicators for monitoring the impact of the toxicants Pb metal in fish and by extension other aquatic organisms and ultimately the whole of the ecosystem. From the data, most fish did not exceed the safe muscle Pb levels for human consumption, however, the constant presence of muscle Pb in concentrations near the limit considered safe for human consumption is a reason for concern, and populations who constantly consume fish from Pb polluted rivers should be cautious and warned about the regular consumption of fish, even when the fish consumed are caught in waters where Pb contamination levels are considered low.

Acknowledgment

The author is grateful for the financial support jointly provided by China Ministry of Commerce (MOFCOM) and the University of Guyana for the scholarship awarded to Grayson Orville Halley, which made this work possible. Many thanks to my supervisors Professors Xu Pao and Meng Shunlong for their guidance, constructive ideas, and professional advisory. I would also like to thank my wife Nalini Elizabeth Seepersaud Halley, my sons Jason, Jayson and Jaedyn, my mother Joylin Halley and my father Eon Jones and to the rest of my extended family especially John Christian and Lamont Crawford for their understanding and support. I am thankful to all my friends and lab mates at FFRC for the support and assistance given to me, 'One Love'. Lastly, I would also like to thank the Wuxi Fisheries College and staffs for their kindness and vital support afforded to me during my tenure at the University.

References

1. Abdel-Baki AS, Dkhil, MA, Al-Quraishy S. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arab [J]. African Journal of Biotechnology, 2011, 10: 2541-2547.

2. Abdullah A, Mehana EE, Meki A. Evaluation of Pb and cadmium levels in freshwater fish farms at Qassim region, KSA [J]. Journal of Agricultural and Veterinary Sciences, 2008, 1: 59-69.

3. Ahmed MS, Bibi S. Uptake and bioaccumulation of waterborne Pb (Pb) in the fingerlings of a freshwater cyprinid, *Catla catla* [J]. The Journal of Animal & Plant Sciences, 2010, 20: 201-207.

4. AVMA. Guidelines on Euthanasia: Formerly Report of the AVMA Panel on Euthanasia, 2007. http://www.avma.org/resources/euthanasia.pdf.

5. Berglind R, Dave G, Sjöbeck ML. The effects of Pb on delta-aminolevulinic acid dehydratase activity, growth, hemoglobin content, and reproduction in *Daphnia magna* [J]. Ecotoxicol Environ Saf, 1985, 9: 29-216.

6. Brown M, White R, Chaille J, Russell M, Oseto C. Evaluation of three anesthetic agents for crayfish (*Orconectes virilis*) [J]. Shellfish Res, 1996, 15: 433-435.

7. Burger J, Gochfeld M. Heavy metals in commercial fish in New Jersey. Environ Res, 2005, 99: 403-412.

8. Cavit K, Selim S. The Immune System Drugs in Fish: Immune Function, Immunoassay, Drugs, Recent Advances in Fish Farms, 2011, 978: 307-759.

9. Congleton JL, La Voie WJ. Comparison of blood chemistry values for samples collected from juvenile *Chinook salmon* by three methods [J]. Aquat Anim Health, 2001, 13: 168–172.

10. Csuros M, Csuros C. Environmental sampling and analyses for metals. Lewis Publishers, ACRC Press Company, 2002.

11. Dalmo RA, Bogwald J. β-glucans as conductors of immune symphonies [J]. Fish and Shellfish Immunology, 2008, 25: 384–396.

12. Drugs Act. United Kingdom Pb in Food Regulations. Food and HMSO, 1979. London.

13. Dunier M, Siwicki AK. Study of the effect of pollutants on fish defense mechanisms: In vitro influence of heavy metals on the spleen and head kidney lymphocytes and macrophages activity in carp (*Cyprinus carpio*). GCP Project, 1993.

14. Ellis AE. Innate host defense mechanisms of fish against viruses and bacteria. Dev Comp Immunol, 2001, 25: 827-839.

15. Ewa ŁT, Ewa DK, Paweł S, Magdalena S, Włodzimierz P. Effect of long-term dietary Pb exposure on some maturation and reproductive parameters of a female Prussian carp (*Carassius gibelio*) [J]. Environ Sci Poll, 2014.

16. Fábio PA, Lourenço AS, *et al.,* Bioaccumulation of mercury, cadmium, zinc, chromium, and Pb in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil [J]. Anais da Academia Brasileira de Ciências, 2016.

17. Fırat Ö, Cogun HY, Yüzereroğlu TA, *et al.,* Fish Physiol Biochem, 2011, 37: 657.

18. Galvin RM. Occurrence of metals in water. Water SA, 1996, 22: 7–18.

19. Goyer RA, Clarsksom WT. Toxic effects of metals (The Basic Science of Poisons) [J]. McGraw-Hill NY, 2001, 811–867.

20. Grocell M, Gerdes R, Brix KV. Influence of Ca, humic acid and pH on Pb accumulation and toxicity in the fathead minnow during prolonged waterborne Pb exposure [J]. Toxicology & Pharmacology, 2006.

21. Hajek G, Choczewski M, Dziaman R, Klyszejko B. Evaluation of immobilizing methods for the Chinese mitten crab (*Eriocheir sinensis*). Milne-Edwards, 2009, 12-18.

22. Hamouda EE. Pathological studies on fish experimentally intoxicated by certain heavy metals. Faculty of Veterinary Medicine, Alexandria University, Egypt, 1996.

23. Hasan K, Mehmet A. Effects of Waterborne Pb Exposure in *Mozambique* Tilapia: Oxidative Stress, Osmoregulatory Responses, and Tissue Accumulation [J]. Journal of Aquatic Animal Aealth, 2015.

24. Hontela A, Gagnon A, Jumarie C. Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol, 2006, 78: 59–65.

25. Hontela A, Rasmussen JB, Audet C, *et al.,* Arch. Environ. Contam Toxicol, 1992, 22: 278.

26. NIH. Guidelines for use of zebrafish.

27. http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf

28. Iwama GK, Ackerman PA. Biochemistry and molecular biology of fishes, analytical techniques for anaesthetics [J]. Elsevier Science, Amsterdam, 1994.

29. Jalali R, Ghafourian H, Asef Y, Davarpanah SJ, Sepehr S. Removal and recovery of Pb using nonliving biomass of marine algae [J]. Journal of Hazardous Materials, 2002, 92: 253-262.

30. Jerry MN. Bioaccumulation Mechanisms. Battelle Memorial Institute of Marine Environmental Sciences, 2002. https://www.researchgate.net/publication/279946385_Bioaccumulation_Mechanisms.

31. Koller LD, Kovacic S. Decreased antibody formation in mice exposed to Pb [J]. Nature, 1974, 250: 148-150.

32. Koller LD. Immunosuppression produced by Pb, cadmium and mercury. Am J Vet Res, 1973, 34: 1457-1458.

33. Lange S, Bambir SH, Dodds AW, Bowden T, Bricknell I, Espelid S, Magnadótti R. Complement component C3 transcription in Atlantic halibut (*Hippoglossus hippoglossus*) larvae [J]. Fish & Shellfish Immunology, 2006, 20: 285-294.

34. Larsson A, Haux C, Sjöbeck ML. Fish physiology and metal pollution, results and experiences from laboratory and field studies [J]. Ecotoxicol Environ Saf, 1985, 9: 81-250.

35. Łuszczek-TE, Drąg-Kozak E, Popek W. Pb accumulation and elimination in tissues of Prussian carp (*Carassius gibelio*) after long-term dietary exposure, and depuration periods [J]. Environmental Science and Pollution Research, 2013, 20: 3.

36. Magnadóttir B, Guðmundsdóttir BK, Lange S, Bambir SH, Steinarsson A, Oddgeirsson M, Bowden T, Bricknell I, Dalmo R, Guðmundsdóttir S. Immunostimulation of cod (*Gadus morhua*) larvae and juveniles [J]. Journal of Fish Diseases, 2006, 26: 147-155.

37. Magnadottir B, Jonsdottir H, Helgason S, Bjornsson B, Jorgensen TO, Pilstrom L. Humoral immune [J]. Veterinarni Medicina, 1999, 56: 486–503.

38. Magnadottir B, Lange S, Gudmundsdottir S, Bogwald J, Dalmo RA. Ontogeny of humoral immune parameters in fish [J]. Fish and Shellfish Immunology, 2005, 19: 429–439.

39. Magnadottir B. Innate immunity of fish [J]. Fish and Shellfish Immunology, 2006, 20: 137–151.

40. Marcel MP, Luis RMC, Rogelio RE. Cortisol and Glucose, reliable indicators of fish stress? [J]. Pan-American Journal of Aquatic, 2009, 4: 158-178.

41. Marking LL, Meyer FP. Are better anesthetics needed in fisheries? [J]. Fisheries, 1985, 10: 2–5.

42. Mathan R, Manoharan S, Chokkalingam K. Hormonal responses of the fish (*Cyprinus carpio*) to environmental Pb exposure [J]. African Journal of Biotechnology, 2009, 4154-4158.

43. McCoy CP, Hara TM, Bennett LW, Boyle CR, Lynn BC. Liver and kidney concentrations of zinc, copper and cadmium in channel catfish (*Ictalurus punctatus*) [J]. Vet Hum Toxicol, 1995, 37: 11–15.

44. Moore PG, Rainbow PS. Copper and zinc in an ecological series of talitrodean *Amphipoda*. UK. Ecologia, 1987, 73: 120-126.

45. Muriel D. Water pollution and immunosuppression of freshwater fish [J]. Italian Journal of Zoology, 1996, 63: 303-309.

46. Nouri J, Mahvi AH, Jahed GR, Babaei AA. Regional distribution pattern of groundwater heavy metals resulting from agricultural activities [J]. Environmental Geology, 2008, 55: 1337–1343.

47. Omaima AS, Aboud A. Impact of pollution with Pb, mercury and cadmium on the immune response of *Oreochromis niloticus*. New York Science Journal, 2010, 3: 9.

48. Omaima AS, Aboud A. Impact of pollution with Pb, mercury and cadmium on the immune response of Oreochromis niloticus [J]. New York Science Journal, 2010, 3: 9.

49. O'Neill JG. Effects of intraperitoneal Pb and cadmium on the humoral immune response of *Salmo trutta* [J]. Environ Contamin Toxicol, 1981, 27: 42-48.

50. Ortuño J, Esteban MA, Meseguer J. Effect of four anaesthetics on the innate immune response of gilthead seabream (*Sparus aurata*) [J]. Fish Shellfish Immunology, 2002, 12: 49-59.

51. Passow H, Rothstein A, Clarkson TW. The general pharmacology of the heavy metals [J]. Pharmacological Reviews, 1961, 13: 185-224hrs.

52. Rashed MN. Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int, 2001, 27: 27-33.

53. Ronis MJ, Badger TM, Shema SJ, Roberson PK, Shaikh F. Reproductive toxicity and growth effects in rats exposed to Pb at different periods during development. University of Arkansas for Medical Sciences, Little Ro, 1996.

54. Ross LG, Geddes JA. Sedation of warm water fish species in aquaculture research [J]. Aquaculture, 1979, 16: 183 186.

55. Ross LG, Ross B. Anaesthetic and sedative techniques for fish. Institute of Aquaculture Handbook, University of Stirling, 1984, 45-52.

56. Ross LG. Restraint, anaesthesia and euthanasia, veterinary practice and procedures for ornamental fish. British Small Animal Veterinary Association, London, UK, 2001.

57. Schreck CB. Accumulation and long-term effects of stress. The Biology of Animal Stress, 2000, 7: 147-157.

58. Shalaka S, Pragna P. Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient [J]. World Journal of Zoology, 2013, 8: 110-118.

59. Sinah AK, Dasgupta P, Chakrabarty S, Bhat-Tacharya G, Bhatacharjee S. Bioaccumulation of heavy metals in different organs of some of the common edible fishes of Kharkai River, Jamshed pur Indian [J]. Journal of Environ Health, 2002, 46-102.

60. Spahn S, Sherry T. Arch Environ Contam Toxicol, 1999, 37: 377.

61. Strykowski JL, Schech JM. Effectiveness of recommended euthanasia methods in larval zebrafish (*Danio rerio*), 2015, 54: 81-84.

62. Tort L, Balasch JC, Tort L, Balasch JC, Mackenzi S. Fish immune system, a crossroads between innate and adaptive responses [J]. Inmunología, 2003, 277-286.

63. UNEP. Reference Methods for Marine Pollution Studies, Determination of total Hg in marine sediments and suspended solids by cold vapour AAS, 1985.

64. USEPA. Effects of exposure to heavy metals on selected freshwater fish, toxicity of copper, cadmium, chromium, and Pb to eggs and fry of seven fish species. Environmental Research Laboratory, Duluth, MN, 1976.

65. USEPA. Methods for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms, 2002.

66. Van-der-Oost RV, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment [J]. Environmental Toxicology and Pharmacology, 2003, 13: 57-149.

67. Velcheva E, Tomova D, Arnaudova D, Arnaudov A. Morphological investigation on gills and liver of freshwater fish from Dam Lake [J]. Bulgarian Journal of Agriculture Sciences, 2010, 16: 364-368.

68. Venkatramreddy V, Vutukuru SS, Tchounwou PB. Ecotoxicology of Hexavalent Chromium in Freshwater Fish. Reviews on Environmental Health, 2009, 24hrs: 129–145.

69. Vutukuru SS. Heavy metals [J]. International Journal of Environmental Research and Public Health, 2005, 2: 456-462.

70. Watts M, Munday B, Burke C. Immune responses of teleost fish [J]. Australian Veterinary Journal, 2001, 79: 570–574.

71. Weis JS, Weis P. Effects of environmental pollutants on early fish development. Rev Aquat Sci, 1989, 1: 45–73.

72. WHO. Directives for water quality, 1986. Geneva.

73. WHO. Guidelines for Drinking Water Quality, 1985. Geneva.

74. WHO. Guidelines for drinking water quality, 1993. Geneva.

75. WHO. Guidelines for drinking water, 2005. Geneva.

76. WHO. Trace elements in human nutrition and health, 1996. Geneva.

77. Wilson JM, Bunte RM, Carty AJ. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). JAALAS, 2009, 48: 785-789. http://www.ingentaconnect.com/content/aalas/jaalas/2009/00000048/00000006.

78. Windholz M. Encyclopedia of chemicals, drugs and biologicals. Merck and Company, Inc, 1983, 816-818.

79. Winemiller KO. Feeding and reproductive biology of the curito (*Hoplosternum littorale*) in the Venezuelan llanos with comments on the possible functions of the enlarged male pectoral spines [J]. Environmental Biology of Fishes, 1987, 20: 219-227.

80. Wong PT, Cha YK, Kramar O, Bengert GA. Accumulation and depuration of tetramethyl Pb by rainbow trout. Water Res, 1981, 15: 621.

81. Wren CD, McCrimmon HR, Loescher BR. "Examination of bioaccumulation and biomagnification of metals in a Precambrian shield lake [J]. Water, Air, and Soil Pollution, 1983, 19: 277-291.

82. Yang R, Yao T, Xu B, Jiang G, Xin X. Accumulation features of organochlorine pesticides and heavy metals in fish from high mountain lakes and Lhasa River in the Tibetan Plateau [J]. Environmental International, 2007, 33: 151-156.

83. Yilmaz E, Akyurt I, Mutlu E. Effects of energetic diets on growth, blood chemistry, and liver pathology of African catfish (*Clarias gariepinus*) [J]. Israeli Journal of Aquaculture, 2007, 58: 191-197.

84. Zare S, Afaghi A, Heidari R, Asadpoor Y, Shiri S. Effects of Pb nitrate (PbNO3) on the glucose and cortisol hormone levels in common carp (*Cyprinus carpio*) [J]. Pak J Biol Sci, 2007, 10: 90-2587.

85. Zelikoff JT, Raymond A, Carlson E, Li Y, Beaman JR, Anderson M. Biomarkers of immunotoxicity in fish, from the lab to the ocean. Toxicol Lett, 2000, 112: 325-331.

86. Zelikoff JT. Biomarkers of immunotoxicity in fish and other non-mammalian sentinel species, predictive value for mammals [J]. Toxicology, 1998, 129: 63-71.

87. Zhang J, Huang WW, Liu MG, Cui JZ. Eco-social impact and chemical regimes of large Chinese rivers [J]. Water Resources, 1994, 28: 609-617.

Cite this article as:

Halley Grayson Orville. Effects of standard permissible levels of lead (Pb) for potable waters on fish innate immune response and health compared with Pb levels found in natural waterbodies. *International Journal of Bioassays 6.11 (2017) pp. 5543-5557.*

DOI: http://dx.doi.org/10.21746/ijbio.2017.6.11.2

Source of support: Nil.

Conflict of interest: None Declared