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BLOOD LEAD LEVEL AS BIOMARKER OF ENVIRONMENTAL LEAD POLLUTION IN FERAL AND CULTURED AFRICAN CATFISH (*CLARIAS GARIEPINUS)*

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SUMMARY

Research has demonstrated a positive link between the presence of lead in freshwater, sediments or food organisms and the onset of sub-lethal effects characterized by neurological defects, kidney dysfunction, endocrine disruption, reproductive/developmental defects, behavioural abnormalities and anemia in demersal aquatic fauna. Considering the significance of haematological parameters as indicators of fish health, this present work assessed the blood lead level (BLL) and haematological parameters of feral and cultured African catfish (*Clarias gariepinus*) in Ibadan, a metropolitan city. BLL and haematological parameters of fifty *Clarias gariepinus* were determined. Each set of ten fish were randomly collected from three different fish ponds and two rivers. BLL was relatively higher in feral fishes than those from fish ponds; while all the haematological parameters (PCV, HB, RBC, MCH, MCV and MCHC) were non-significantly ($p<0.05$) higher in fishes sourced from cultured environment relative to those from surface water and vice versa for immunological parameters (WBC and differential counts). The BLL observed in this study (0.85 ± 0.38 mg/dl and 0.90 ± 0.6 mg/dl) from cultured and feral fish respectively is indicative of lead pollution of the culture water and environment. The ideal blood lead level is now considered to be zero. Lead pollution of the study area has serious consequences on aquatic fauna and humans who consume such contaminated fish. It is therefore recommended that human and animal health surveillance and environmental monitoring of lead should be initiated.

Keywords: Lead, Pollution, Haematology, African Catfish, *Clarias gariepinus*

INTRODUCTION

Blood tissue reflects physical and chemical changes occurring in organisms, therefore detailed information can be obtained on general metabolism and physiological status of fish in different grouping of age and habitat (Kocabatmazve and Ekingen, 1978). Blood parameters have been commonly used to observe and follow fish health, since variations in blood tissue of fish are caused by environmental stress (Shah and Altindag, 2005), malnutrition (Casillas and Smith, 1977), gender (Collazos *et al.*, 1998), seasonal differences and breeding (Cech and Wohlschlag, 1981).

During recent years considerable attention has been focused on the fates of metals and their derivatives in the aquatic environment. Human activities and increased use of metal containing fertilizers in agriculture could lead to a continued rise in the concentration of metal pollutants in fresh water reservoirs as a result of water run off, thereby representing the greatest hazard to human

consumers of fish (Gutenmann *et al.*, 1988).

Various workers have shown that the use of haematological parameters as indicators of metal toxicity can provide information on the physiological response of fish due to the close association of the circulatory system with the external environment (Casillas and Smith, 1977, Wepener *et al.*, 1992). Haematology also helps to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals and hypoxia.

In recent years, the effects of sub-lethal concentrations of toxic substances have been recognized to be of biological importance (Zelikoff *et al.*, 1991). These have driven the suite of toxicological assessments away from death to sub-lethal indicators; such as impairment of physiology, reproduction, and performance, which are more subtle measures, but would ultimately, represent impaired fitness (Hinton, 1993).

Immunotoxicology specifically evaluates the impact of toxicant exposure on an organism's mechanism to ward off pathogens and disease. However, no single end point change (haematological, immunological and biochemical) in fish can currently be said to be pathognomonic. The use of immune system parameters to assess alterations in fish experiencing heavy metal exposure and interest in defense mechanisms stem from the need to develop health management tools to support a rapidly growing aquaculture industry (Jones, 2001). This study therefore assesses the blood lead level, haematological and immunological parameters of feral and cultured African catfish *Clarias gariepinus*, sourced from Ibadan, a metropolitan city in Nigeria.

MATERIALS AND METHOD

Study Area

Ten (10) each apparently normal adult *Clarias gariepinus* of both sexes weighing between 250-350gm (mean=338.4± 12g) and a total length of between 30-37cm (mean =36.9± 2.5cm) were collected from three fishponds and two surface water from Ibadan. The fishponds which were randomly sampled are Dominican, Basil and Peace farms, while those from surface water were collected from Ogunpa and Asejire Rivers. Basil and Peace farms were earthen ponds, while Dominican's pond was made with concrete.

Laboratory Experiment

Blood Collection and Processing

Blood was drawn from the posterior caudal vein using needle and syringes according to Schmitt *et al*. (1999) and 2ml was decanted in EDTA bottles for the assessment of haematological parameters, while another 2ml was decanted into heparinized bottles for the BLL determination. Whole blood (50µl) was stained for enumeration of red and white blood cells (Shaw, 1930). Blood smears were air-dried for five minutes, fixed in absolute methanol, and stained for 60 seconds in Giemsa stain.

Haematological Analyses

Packed cell volume (PCV), red blood cell count (RBC) and hemoglobin concentration (Hb) were conducted immediately. PCV was determined by spinning blood samples contained in heparinized capillary tubes in a microhematocrit centrifuge. The RBC count was carried out in a modified Neubauer chamber after saline dilution of the blood, while Hb was determined by the cyano-methaemoglobin method (Hesser, 1960). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from previously obtained RBC, PCV and Hb values.

Blood smears made and stained with Giemsa, were used to determine the white blood cell count (WBC), thrombocyte count and differential WBC counts (Stoskopf, 1993).

Determination of Blood lead level (BLL*)*

Lead in blood was determined by atomic absorption spectrometry (AAS) as described by the method of Hassel (1968). One mL of 5% triton x-100 was added to 5 mL well mixed EDTA blood to lyse the erythrocytes and release lead. Mixing the content of the tube in a vortex mixer enhanced rapid haemolysis. One mL of 2% ammonium pyrrolidine dithiocarbamate solution was added to chelate the lead. The solution was then extracted with 5 mL methyl-isobutyl ketone and the organic supernatant separated into screw-capped specimen containers and analysed in a Perkin-Elmer 703 Atomic Absorption Spectrophotometer at a wavelength of 283.3 nm.

Statistical Analysis

The data of BLL and haematological parameters are presented in figures as mean ± SD. Relationships between BLL and haematological parameters were also determined by Pearson correlation coefficient using environment (feral and culture) and different structure of ponds (surface water, earthen and concrete ponds) as indices. The confidence limit was 95% for all the tests conducted.

RESULTS

Blood lead level and Haematological parameters

Figure 1 demonstrate that the mean value of the BLL of fishes from surface water (0.9 µg/l) was not significantly higher ($p<0.05$) than that of the culture ponds (0.85 µg/l). All the haematological parameters (PCV, HB, RBC, MCH, MCV and MCHC) were generally higher (Figure 2) in fishes sourced from cultured environment relative to those from surface water. However, the difference in values were not statistically significant ($p>0.05$). Conversely, leucocytic parameters (WBC, lymphocyte, monocyte, platelet, Heterophil, and eosinophil counts) were generally higher (Figure 3) in fishes sourced from surface water relative to those from cultured environment.

Relationships between BLL and haematological parameters of fish in different culture environments (feral and culture)

As presented in Table I, non-significant ($p>0.05$) negative correlations were observed between the BLL and all haematological parameters of cultured fish [e.g. PCV ($r=-0.11$, $p=0.5645$), Lymphocyte counts ($r=0.02$, $p=0.9091$)], except for MCV ($r=0.32$, $p=0.0828$), MCH ($r=0.27$, $p=0.1489$) and heterophil counts ($r=0.05$, $p=0.776$) which presented non-significant positive correlations.

There were non significant negative correlations between the BLL and PCV, HB, RBC, MCHC, lymphocyte ($r=-0.13$, $p=0.5833$) and monocyte ($r=-0.12$, $p=0.6158$) counts of feral fish. While non-significant positive correlations was observed between BLL and eosinophil ($r=-0.02$, $p= 0.9353$), MCV ($r=-0.13$, $p=0.5736$), MCH ($r=-0.06$, $p=0.7943$), WBC ($r=0.23$, $p=0.332$) and heterophil count ($r=0.17$, $p=0.4788$) of feral fish. Only the platelet counts of feral fish showed a significant ($P\leq0.05$) positive correlation ($r=0.50$, $P=0.0264$) with BLL

Table I: Relationships between BLL and Blood (haematological and immunological) parameters of cultured and feral fish

	Cultured Fish		Feral fish	
Variable	Correlation	Probability (at 95% confidence limit)	Correlation	Probability (at 95% confidence limit)
PCV (%)	-0.11	0.5645	-0.41	0.071
HB (g/100ml)	-0.17	0.3631	-0.46	0.0423
RBC (10^6/mm^3)	-0.37	0.046	-0.38	0.0966
MCV (fl)	0.32	0.0828	0.13	0.5736
MCH (pg)	0.27	0.1489	0.06	0.7943
MCHC (%)	-0.27	0.1532	-0.18	0.441
WBC ($X10^4$)	-0.17	0.3833	0.23	0.3332
PLATELET (X 10^5)	-0.34	0.0679	0.50	0.0264
LYMPHOCYTE (%)	-0.02	0.9091	-0.13	0.5833
HETEROPHIL (%)	0.05	0.776	0.17	0.4788
MONOCYTE (%)	-0.16	0.3984	-0.12	0.6158
EOSINOPHIL (%)	0.09	0.6436	0.02	0.9353

Table II: Relationships between BLL and Blood (haematological and immunological) parameters of fish sourced from surface water, earthen and cemented ponds

	Concrete pond		Earthen pond		Surface Water	
Variable	Correlation	Probability (at 95% confidence limit)	Correlation	Probability (at 95% confidence limit)	Correlation	Probability (at 95% confidence limit)
PCV (%)	0.06	0.8622	-0.56	0.0109	-0.41	0.071
HB (g/100ml)	0.13	0.7144	-0.59	0.0065	-0.46	0.0423
RBC (10^6/mm^3)	0.46	0.1797	-0.51	0.0219	-0.38	0.0966
MCV (fl)	-0.52	0.1221	-0.11	0.6508	0.13	0.5736
MCH (pg)	-0.49	0.1505	-0.10	0.6635	0.06	0.7943
MCHC (%)	0.51	0.1365	-0.05	0.8461	-0.18	0.441
WBC ($X10^4$)	-0.04	0.906	0.15	0.5342	0.23	0.3332
PLATELET (X 10^5)	-0.15	0.6728	-0.37	0.1091	0.50	0.0264
LYMPHOCYTE (%)	0.16	0.6676	-0.23	0.3256	-0.13	0.5833
HETEROPHIL (%)	-0.09	0.8094	0.16	0.4968	0.17	0.4788
MONOCYTE (%)	0.10	0.7765	-0.23	0.3283	-0.12	0.6158
EOSINOPHIL (%)	-0.45	0.196	0.36	0.1154	0.02	0.9353

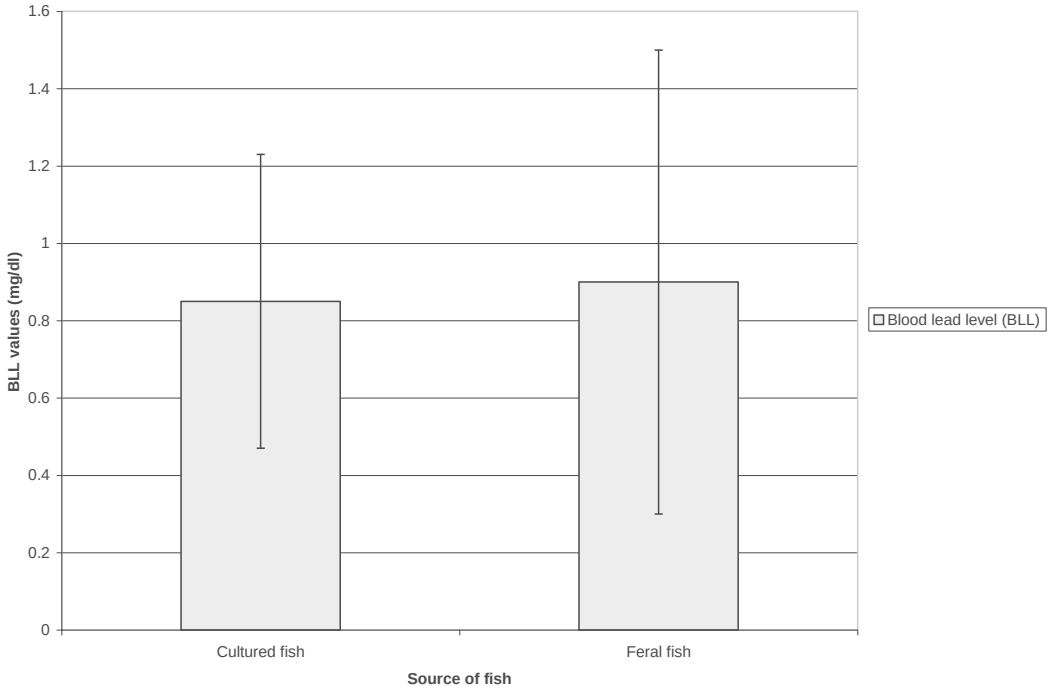

Figure 1: Blood lead level (BLL) of Cultured and Feral Fish

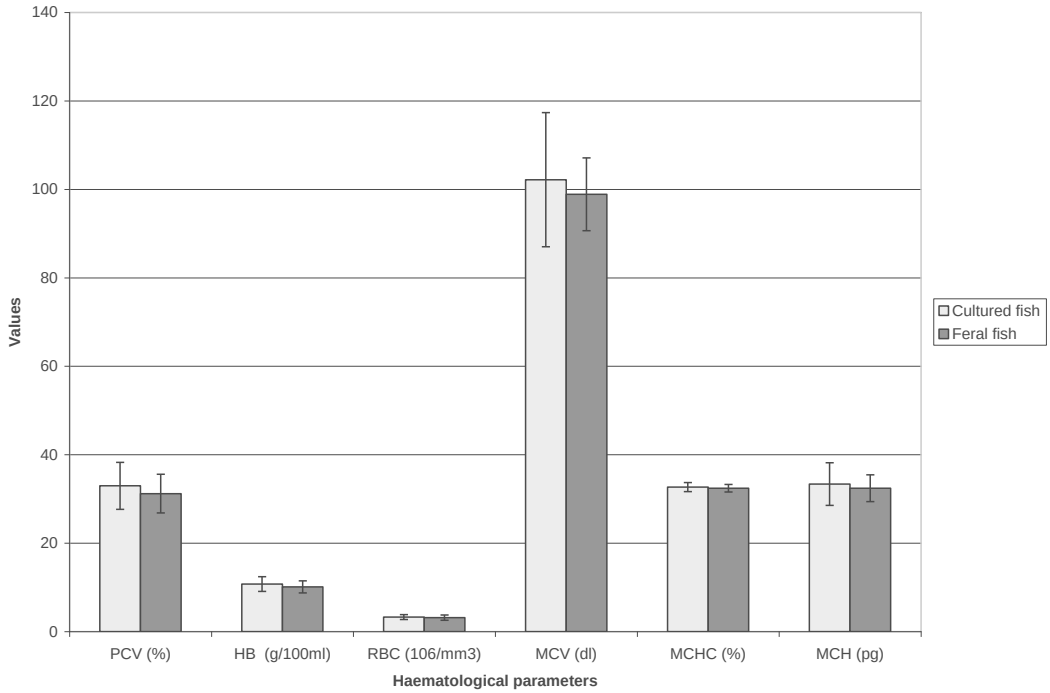

Figure 2: Haematological Parameters of Cultured and Feral Fish

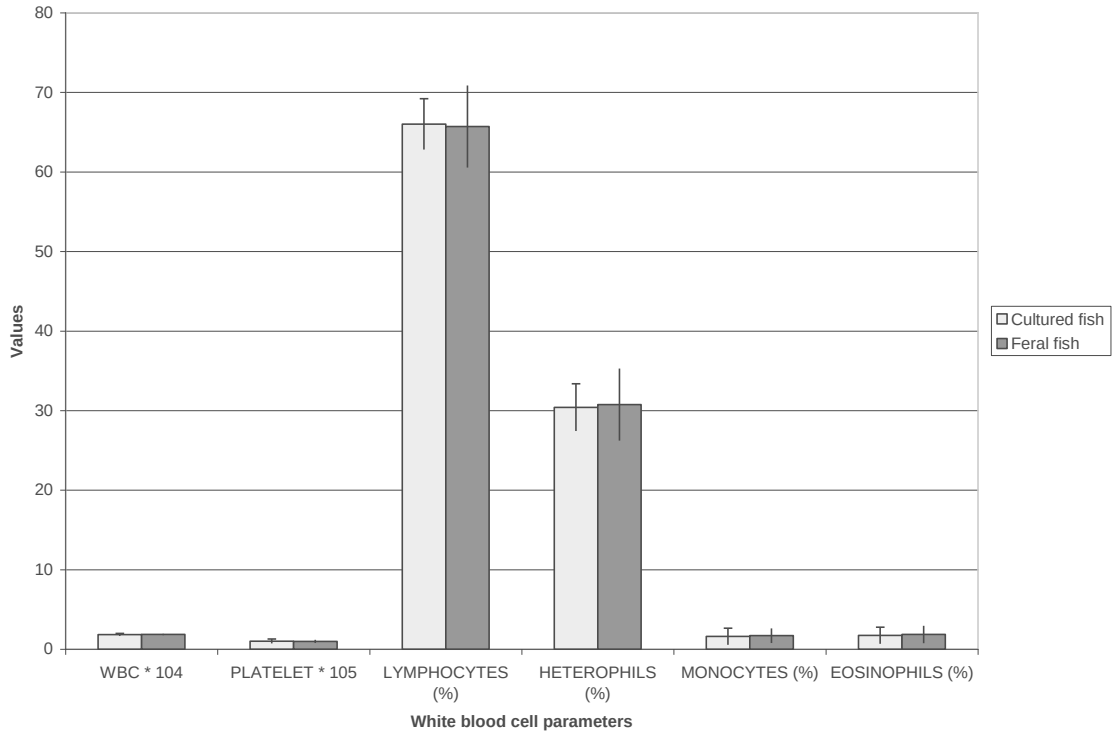

Figure 3: White blood Cell and differential Parameters of Cultured and Feral Fish

Relationships between BLL and haematological parameters of fish grown in different culture structures (surface water, earthen and concrete ponds)

Strongly positive non-significant correlations (Table II) were observed between the BLL, PCV (r=0.06, p=0.8622), HB (r=0.13, p= 0.7144), lymphocyte (r=0.16, p=0.6676) and monocyte (r=0.10, p=0.7765) counts of fishes cultured in cemented fishponds. Non-significant strongly negative correlations were also observed between the BLL, WBC (r=-0.04, p=0.906), platelet (r=-0.15, p= 0.6728) and heterophil (r=-0.09, p=0.8094) counts of fishes cultured in cemented fishponds. For fish grown in earthen ponds, significantly ($p \leq 0.05$) negative correlations were observed between their BLL and PCV (r=-0.56, p=0.0109), Hb (r=-0.59, p=0.0065) and RBC (r=-0.51, p=0.0219). MCV, MCH MCHC, platelet, lymphocyte and monocyte had non-significant negative correlations, while WBC and heterophil counts had non-significant positive correlations with BLL. BLL of fish collected from surface water had significantly negative and positive correlations with Hb (r=-0.46, p=0.0423) and platelet counts (r=0.50, p=0.264) respectively. PCV, RBC, MCHC, lymphocyte, monocytes had non-significant negative correlations while all other parameters had non-significant positive correlations with BLL.

DISCUSSION

Fish can absorb the bio-available metal directly from the environment via the gills or the skin or through the ingestion of water and food. Metals in fish are then transported by the bloodstream to the various organs and tissues where it is accumulated. Blood lead levels (BLLs), which reportedly increase in proportion to waterborne lead concentrations, are easier to determine than whole body lead levels,

and are highly relevant to fish health (Hodson *et al.*, 1984). Advances in science and medicine indicate that lead levels hitherto considered innocuous are associated with significant adverse health outcomes. Lead has no threshold value below which there is no adverse effect. The ideal blood lead level is now considered to be zero (Landrigan, 1987). The BLL observed in this study (0.85 ± 0.38 mg/dl and 0.90 ± 0.6 mg/dl) from cultured and feral fish respectively is indicative of lead pollution of the culture water and environment. Ayodele *et al.*, (1996), reported adverse effects on aquatic biota at waterborne lead concentrations of 1.0 to 5.1 ug/l, which include reduced survival, impaired reproduction, reduced growth, and high bioconcentration from the medium. It has also been recognized that xenobiotics exposure can exacerbate disease state by lowering immune functions (Zelikoff *et al.*, 1991).

Lead levels as low as 0.05 ppm can cause neurological impairment in human beings, and may be causing neurological impairment in fish (Eisler, 1988; Canfield *et al*., 2003 and Fry, 2004). The World Health Organization lead threshold standard is >10 µg/dl for toxic effects. Mergler *et al.*, (1998) reported that about 80% of the total human lead burden is attributed to diet. (WHO, 1987, Goyer, 1988). Human consumption of contaminated fish with the potential for adverse health effects has been identified in the Great Lakes region. (Schwartz, et *al.*, 1983). A Michigan study (Hovinga *et al*., 1993) also reported that mean blood lead levels were significantly higher in fish eaters than among the control group.

Haematological parameters in this study were generally higher in cultured fish relative to feral fish; while a converse relationship was observed for immunological parameters. The haematological profile submitted is in agreement with the submission of Rogers *et al.* (2003), who concluded after their research that the mechanism of lead toxicity occurs by ion-regulatory disruptions with little or no marked changes in the haematological parameters. Shah and Altindag, (2005) reported significant increase in immunological parameters following lead exposure, which suggests that lead may weaken the immune system, resulting in increased susceptibility to infections. An increased WBC (leucocytes) count was also observed in *Anguilla anguilla* after lead exposure (Santos and Hall, 1990).

Gill and Pant, (1985) have reported that first the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage, but a prolonged or continuous stimulus may cause the suppression or exhaustion of this capacity, resulting in a decrease in lymphocytes and so in total WBC count. Non-significant ($p \leq 0.05$) negative correlations were observed between the BLL and most of the haematological parameters of cultured and feral fish. This is in agreement with previous reports on the inhibitive effects of lead on haematological parameters (Ayodele *et al*., 1996, Haffor and Al-Ayed, 2003 and Rogers *et al*., 2003). Assessment of BLL Correlations based on the source of surface water, earthen and concrete ponds revealed varying correlations of BLL relative to haematological and immunological parameters. This suggests that the culture environment affects the availability of lead to the fish in-situ.

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