Full Length Research

The Effects of Lead (Pb) on *Clarias gariepinus* (B.) Juveniles in Captivity

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Anhydrous Lead Chloride (PbCl) was used to prepare a stock solution of 1000 mg/l with sterile water. Three concentrations were made in three replicates at 80.52 mg/l, 161.07 mg/l and 241.50 mg/l solvent and each in 30 litres of water respectively. The fourth treatment was without Lead solution and served as control. The effects of the varied concentrations were investigated on groups of *Clarias gariepinus* juveniles in glass tanks each measuring 60 × 60 × 30 cm³ for 96 hours. Aeration of water was done with electric air pumps, rubber holes and air stones mounted closed to the tanks throughout the period of trials. Growth, survival and other physical parameters were monitored in fish to ascertain the toxicity effects of lead. Proximate composition of fish carcasses obtained before and after the exposures revealed no significant difference (P<0.05). Fish mortality and abnormal behaviour were more in treatments with higher concentrations of lead. The LD50 value was determined at 161.07 mg/l concentration. The paper furthered reviewed the implications of heavy metals, especially ‘lead’ in fish and man; ‘the ultimate consumer of fish’ and further recommended industrial effluents, wastes, batteries etc. be discarded appropriately and that automobiles, electrical and related workshops be located or relocated in confined places to prevent lead and other heavy metals migration into water bodies.

Key words: *Clarias gariepinus*, Juveniles, Growth, Heavy metals, Lead, Survival, Toxicity, Water bodies.

INTRODUCTION

Heavy metals are elements with specific gravity greater than 5.0. They are metals which can be toxic even in small concentrations. Heavy metals are introduced into the environment through many sources such as weathering of rocks and soils; volcanic eruptions and various forms of other human activities involving mining, processing or use of metals and other substances containing contaminants (Lincoln et al., 2007; Ewuim and Akime, 2012). According to Biney et al. (2005), heavy metals are partitioned between water sediments, suspended solids and aquatic biota in water bodies and tend to accumulate more in sediments than in aquatic organisms and water as such sediments acts as sinks and supply of heavy metals to overlying water columns (Brooks et al., 2010, Gbem et al., 2001). When heavy metals enter water bodies, they change water quality, bind to sediments and accumulate in aquatic biota causing anemia, disturbance of physiological functions and mortalities of fish (Baeyans et al., 2001; Eichler et al., 2006). Specifically, aquatic organisms experience histological and morphological changes in circulatory systems, biochemistry, physical behaviour and organism’s reproduction (Kedebe and Wondimu, 2004; Olaifa et al., 2003).

The introductions of toxicants into an environment where fishes are found stun them and or acts as a stressor on the fish and other organisms found in that environment (Capar and Yess, 1996, Kakulu et al., 1987). The toxicants in water bodies may dissolve the oxygen concentration which eventually impairs aquatic organism...
respiration thus leading to asphyxiation. Reproduction is also impaired during bioaccumulation of toxicants (Mayer, 2001). As water temperature increases, rate of biological processes increases and may result in increase in metal uptake by fish and gram (g) hydrogen ion concentration (pH). As pH decreases, more dissolved metals ions are produced. Ionic forms of heavy metals induce acute poisoning in aquatic animals leading to immediate fish kills while complex forms lead to chronic poisoning and bioaccumulation in fish tissues over a longer period (Ellis et al., 1989; Baeyens et al., 2005).

Toxicology testing is conducted to determine the degree to which a substance can damage a living or nonliving organisms by analyzing the actual chemical in the samples or the used laboratory animals in studies. It has been in use for a long time in detecting potential hazards effects chemicals can have on living organisms via bioaccumulation of level of toxicants. The measure of a chemical’s toxicity is its Median Lethal Dosage (LD50) value which is the concentration that can cause average kills of 50 percent of a test population of animals on trial. This is usually reported in milligrams of the chemical per kilogram of a test animal’s life weight. The smaller the values, the more toxic the chemical referred. Chemicals with LD50 values greater than 500mg/kg in rats or mice are generally considered safe in agriculture (Mayer, 2001; Cunningham et al., 2011; Hadioui et al., 2005).

Heavy metal pollution of water has become a major environmental problem since the advent of agricultural and industrial revolution and today most water bodies are contaminated with heavy metals released from domestic, industrial and other man-made activities. In modern times, one of the main threats to the health of ecosystems is the exposure to a myriad of toxic substances and compounds such as mercury, cadmium, lead, copper, arsenic, air pollutants, pesticides, plastics, cigarette smoke, diesel fumes and nano-particles found in products like perfumes and sunscreens. Their introduction into aquatic environment is caused by direct or indirect agricultural and industrial discharges (Zhang et al., 2009; Von Linden et al., 2003).

Heavy metals contamination could be detrimental to ecological balance of the recipient environment and to a diversity of aquatic organisms. Lead is a heavy metal which can seriously harm the human central nervous system, especially in children. It damages the kidneys and the immune system very easily. Exposure to lead causes premature births, diminished mental ability, mental retardation in infants and children making learning difficult and reduced rates of growth (Hu, 2000). Lead has been identified in at least 1,272 of the 1,684 hazardous waste sites that have been proposed for inclusion on the Environmental Protective Agency (EPA) National Priorities List (NPL) (Schmitt and Brumbaugh, 1990; Herbert, 2004; WHO, 2006). The majority of cases of lead poisoning are due to oral ingestion and absorption through the gut. Lead poisoning in adults occurs more frequently during exposure in the workplace and primarily involves the central nervous system. Symptoms of hemopoietic system involvement include hypochromic anaemia with basophilic stippling of the erythrocytes, hyperactivity, anorexia, decreased play activity; low intelligence quotient and poor school performance have been observed in children with high lead levels (USEPA, 1998, USEPA, 2002). Lead crosses the placenta during pregnancy and has been associated with intrauterine death, prematurity and low birth weight (Leighton et al., 2003; Kris-Ether ton et al., 2003; Nriagu et al., 1996).

Since fish is an animal that is particularly affected by these pollutants, different species have been widely used to evaluate the health of aquatic ecosystems (Hu, 2000; Igbal et al., 2008; Laidlaw et al., 2005; Mohammed et al., 2011). Fish is of paramount importance in many nations’ economics and food proteins. In a few countries in the world, fish consumption contributes up to 180 kcal per capita per day, but reaches such high levels only where there is a lack of alternative protein foods grown locally or where there is a strong preference for fish. Nigeria current national fish demand is around 1.6 million metric tons and it is supplied from imports about 700,000 MT per year while domestic production is 640,000MT from both marine and freshwater (FAO, 2006, 2007). Fish proteins are essential in the diet of some densely populated countries where the total protein intake level is low, and are very important in the diets of many other countries in the world. Fish is also a valuable source of essential fatty acids and its protein is highly and easily digestible to man. Even in small quantities, fish will have a significant positive impact on improving the quality of dietary protein intake by complementing the essential amino acids that are often present in low quantities in the rice and vegetable diets which are typical of nay developing states food. Fish is a rich source of lysine and other essential acids that are often deficient in carbohydrate diets and other plant protein sources (Lovell, 1988).

Fish meal is essential feedstuff rich in the essential proteins and essential amino acids nutrients needed by cultured fishes. In fact, livestock and fish feed without fish meal cannot meet the required nutrients for good performance of any of their culture animal (Lovell, 1988; Falayi, 2009).

Recent research shows that fish is much more than just an alternative source of animal protein. Fish oils are the richest source of a type of fat that is vital for brain development in unborn babies and infants and cardiovascular therapy (Kris-Etherton et al., 2003). This makes all fish and especially fatty fish, such as tuna, mackerel and sardine, particularly good components of the diet of pregnant and lactating women. It is therefore apparent that fish makes a valuable contribution to the nutritional quality of the diets of the populations of many developing countries in Asia and the Pacific region (FAO, 2006, 2007). Claris gariepinus is a hardy fish and highly
valued in Nigeria. Catfishes of the family Claridae comprise the most commonly cultivated fishes in Nigeria (Adesulu, 2007). The growth of aquaculture in Nigeria now is largely being boosted by a steady rise in catfish culture. It is therefore very important to guide our environment jealously from been polluted by the uncontrolled use of contaminated inland waters for aquaculture purposes as well as our territorial waters from oil spillages from petroleum exploration. The objective of the studies therefore is to ascertain the quantity of lead that can be accumulated within fish tissues and the implication in fish. This would then give an indication of how lead is indirectly consumed by man, the ultimate fish consumer.

MATERIALS AND METHODS

Fish Procurement and Acclimatization

This study was conducted at the Department of Biological Sciences Laboratory of the College of Natural and Applied Sciences, Wesley University of Science and Technology, Ondo, Nigeria. Juveniles Clarias gariepinus fish were obtained from a reputable hatchery farm in Osogbo, Nigeria and transported in a plastic container to the laboratory. The fish were acclimatized for four weeks and fed Copen (imported) floating fish feed ad-lib daily in three (3) glass tanks each measuring 60 × 30 × 30 cm\(^3\) and holding 30 litters freshwater and stocked at the rate of 50 fingerlings per tank during the acclimatization period. The tanks were filled to ¾ of the holding capacity. Aeration was done via a giant electric aerator, rubber holes and air stones accessories mounted closed to the tanks. At the end of acclimatization, the fish were sorted and weighed into tanks.

Preparation of the Stock and Test solutions of Lead

Anhydrous lead chloride was used for the experiment because it is of low in toxicity compared to the other forms of leads (Odiete, 1999). A stock solution of 1000mg/l (l g/l) of the lead was added to 1 litre of distilled water to make the solution. The different concentrations required were calculated using the formula.

\[
\text{Weight of Lead (Pb) required } \times \frac{\text{Molecular weight of Lead (Pb)}}{\text{Atomic weight of Lead (Pb)}}
\]

For the four different treatments, the concentrations were as follows:

Treatment 1 = 80.52mg/l solvent was added in 30 litres of water
Treatment 2 = 161.07mg/l solvent was added into 30 litres of water
Treatment 3 = 241.50mg/l solvent was added in 30 litres of water
Treatment 4 = Control (No lead (Pb) was added to 30 litres of water

Experimental Tanks and Design

The system consists of twelve (12) rectangular glass tanks each measuring 60 × 60 × 30 cm\(^3\) covered with netting materials (to prevent fish from jumping out of the tanks). The tanks were filled with borehole water to \(\frac{3}{4}\) of holding capacity. Aeration was done by electric aerators and air stones as in the acclimatization process.

Three replicates juveniles fish samples mean weight ranged from 28.70 – 29.40g were put in each tank. The tanks were blocked and randomly treated to make a Completely Randomized Block Design (CRBD). The photographs of the fish were taken with a digital camera before and after the experiment to see if there are differences in colouration.

Dead Fish Observation and Preservation

The fish remained in the inoculated lead waters for a period of 96 hours (4 days). During those hours all fish samples were fed daily with Copen floating fish feed at 5% of fish biomass shared into two (2) and fed twice daily at 07.00 hours at sunrise and 18.00 hours at sunset. The weights of fish were recorded at the beginning and end of the experiment.

Other parameters such as fish physical appearance, fish behaviours or disposition in tanks were observed and recorded accordingly.

Dead fish were identified by an absolute lack of movement and such fingerlings were removed as soon as noticed, wrapped in pre-cleansed polyethene bags and labelled according to number, tanks and put in refrigerator. The toxicity of the test chemical was determined using logarithm methods of analysis (Litchfield and Wilcoxon, 1949). The numbers of dead fish were recorded; changes in colouration of fish in tanks were noted and recorded. Proximate analysis of fish and feed fed were determined following the methods of AOAC (1990). The fish carcasses were also examined for Lead toxicity following the bioassay techniques (Raish and Oshida, 1978).

Fish Digestion

The digestion of fish carcasses was done following the methods of FAO/SIDA, (1993). Ten (10) g flawed fish samples from each treatment were placed in a beaker and 15ml of freshly prepared Nitric acid and Hydrogen peroxide 1:1 was added. The beaker was covered with
watch glass and left for the reaction to subsidise. The beaker with its content was heated on hot plate at 160°C for two (2) hours until the content reduced to about 2-5mls and transferred into a 25mls volumetric flask and diluted with distilled water. The digest was transferred again into plastic bottles and analysed using the Atomic Absorption Spectrophotometer (AAS) apparatus.

The actual concentration of Lead (Pb) in fish tissue was determined as:

Actual concentration of Lead (Pb) in sample = PPMR x Dilution Factor

Where PPMR represents the AAS reading and dilution factor 50/1 = 50 and actual value of lead in tissue = AAS reading minus blank x, and x = actual value of lead in fish tissue

The physiochemical parameters of the experimental waters observed include: Temperature (0°C) of water using mercury in glass thermometer inserted into the water after standardization. Dissolved oxygen (D.O) (mg/l) was measured by Winkler’s methods. The hydrogen ion concentration (pH) was determined by pH meter following the methods of (APHA, 1990).

Percentage Fish survival was calculated from:

Number of Fish stocked – Mortality x 100

No. of fish stocked

As described in Falayi, (2009).

Determination of LD₅₀ value

This was determined by plotting a graph to the concentration at which 50 percent fish kills was observed.

Statistical Analysis

T-test package was used to test the significant differences between the mean weights replicate samples of fish at 5 percent confidence following (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 shows the different concentrations of PbCl in 30 litres water treatment and Table 2 also revealed concentration of Lead in each treatment and the Control without lead (ppm). Table 3 further showed Mean numbers and weights of Clarias gariepinus juveniles in different concentrations of lead (Pb) at the beginning and end of 96 hours treatment.

Table 3 shows the initial mean weights and final mean weights of experimental fishes and the numbers stocked at the beginning of experiment and the number remained at end of experiment. Since the fish were kept for only 96 hours (4 days) in the medium, we do not expect much weight increase. The daily weight increase ranged from 0.74g to 1.97gram per day. This was lower than expected of juvenile growth rate under intensive culture (Shepherd and Bromage, 1992). ANOVA’S showed no significant (P>0.05) difference in replicate fish weights (Table 5), but there was significant (P<0.05) difference in mean weight gain of fish at end of 96 hours of culture with Tt3 having highest lead solution having the highest weight gain than Tt4 without lead. This is an evidence that, though there may be lead in fish muscle, that this may not prevent the fish from growing.

Figure 1 showed the LD₅₀ value was determined at 161.07 mg/l concentration. The measure of a chemical’s toxicity is its Median Lethal Dosage (LD₅₀) value which is the concentration that can cause average kills of 50 percent of a test population of animals on trial. This is usually reported in milligrams of the chemical per kilogram of a test animal’s life weight. The smaller the values, the more toxic the chemical referred.

Table 4 showed the proximate composition of feed fed and that of fish tissues before and after 96 hours trial in different Lead (Pb) Concentrations.

Figure 2 shows the bar chart on fish survival in all treatments including the control. The control without lead dose had the highest survival (80%), next was the least lead dose (Tt1) and in that order until the least survival (36.3%) in Tt3 with highest lead concentrations. We may therefore suggest the mortality trends could be as a result of the lead.

There were no significant (P>0.05) differences in fish proximate analysis before and after the experiment. There is the slight decrease in crude lipids and Ash content of final fish carcasses compared with the initial composition. There was slight increase in crude proteins of final fish carcass in all treatments compared with the initial. The increase in body proteins and lipids are evidence of gains in the nutrients embedded in the feed fed for those hours despite the hazardous effects of lead on the fish. The proximate composition of feed was significantly (P<0.05) lower than fish Carcass in crude protein (44.6%) and significantly higher (P<0.05) in crude lipid (19.20%) in feed fed to Fish. The general increase in the protein composition of the fish carcasses in just 96 hours culture shows that the feed was within the nutrients requirement of catfish Clarias gariepinus juveniles in captivity.

The mean weight of Fish before and after the experiment revealed no significant differences (P>0.05) in weights of Fish from all treatment including the control in the 96 hours trials (Table 4). The mean weight of Fish before and after the experiment, t-tests revealed no significant differences (P>0.05) in weight of Fish from all treatment including the control in the 96 hours trials.

The survival rate of juveniles’ catfish decreased with increase in lead concentrations. The control without lead
Table 1. Different concentrations of PbCl in 30 litres water treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pb (mg/l)</th>
<th>PbCl (mg/l)</th>
<th>PbCl in 30 litres</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2.684</td>
<td>80.52</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5.360</td>
<td>161.07</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>8.05</td>
<td>241.50</td>
</tr>
<tr>
<td>4</td>
<td>Neutral water</td>
<td>Neutral water</td>
<td>Neutral water</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>16.094</td>
<td>483.09</td>
</tr>
</tbody>
</table>

Table 2. Concentration of Lead in each treatment and Control without lead (ppm).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of in water (mg/l)</th>
<th>AAS reading</th>
<th>Actual concentration of Lead in fish (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80.52</td>
<td>0.4085</td>
<td>0.00002</td>
</tr>
<tr>
<td>2</td>
<td>161.07</td>
<td>0.8175</td>
<td>0.00004</td>
</tr>
<tr>
<td>3</td>
<td>241.5</td>
<td>1.1780</td>
<td>0.00006</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0.2369</td>
<td>0.000009</td>
</tr>
</tbody>
</table>

Table 3. Mean numbers and weights of *Clarias gariepinus* juveniles in different concentrations of lead (Pb) at the beginning and end of 96 hours treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tt.1</th>
<th>Tt.2</th>
<th>Tt.3</th>
<th>Tt.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of fish stocked</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Initial Mean weight</td>
<td>29.30</td>
<td>28.79</td>
<td>29.40</td>
<td>29.10</td>
</tr>
<tr>
<td>Total final weight (g)</td>
<td>32.26</td>
<td>33.59</td>
<td>37.28</td>
<td>36.36</td>
</tr>
<tr>
<td>Total no. of fish remained</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Mean weight gain</td>
<td>2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LD<sub>50</sub>

Figure 1. LD<sub>50</sub> value determined in the 96 hours trial at 161.07mg/l.

dose had the highest survival (80%), next to the control was the least lead dose (Tt1) and in that order until the least survival (36.3%) in Tt3 with highest lead concentrations. The results therefore suggest that the mortality
Table 4. Proximate composition of fish tissues before and after 96 hours trial in different Lead (Pb) Concentration.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Final Fish Proximate Analysis in Treatments</th>
<th>Initial Fish Proximate Analysis</th>
<th>Fish Feed Proximate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tt1</td>
<td>Tt2</td>
<td>Tt3</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>7.54</td>
<td>7.20</td>
<td>7.50</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>10.37</td>
<td>10.88</td>
<td>9.56</td>
</tr>
<tr>
<td>Crude Fibre (%)</td>
<td>0.62</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>13.50</td>
<td>13.80</td>
<td>12.90</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>65.00</td>
<td>64.00</td>
<td>64.50</td>
</tr>
</tbody>
</table>

Table 5. t-Test comparing the mean replicates of fish weights.

<table>
<thead>
<tr>
<th></th>
<th>Variable 1</th>
<th>Variable 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.72</td>
<td>4.425</td>
</tr>
<tr>
<td>Variance</td>
<td>0.047</td>
<td>0.393233333</td>
</tr>
<tr>
<td>Observations</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.023783534</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Df</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>-5.177573723</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.006992145</td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>2.353363435</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.01398429</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>3.182446305</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Fish survival Chart in 96 hour treatment in different Lead dosage and control without lead.

trends could be as a result of the lead toxicity as earlier mentioned by Brooks et al. (2010), FAO, (1983), Enomoto and Uchida (1973), Gbem et al. (2001) in their findings. There were only little differences in skin colourations. The larger the concentration, the darker the fish colour. These may have happened due to the effects
of lead exposition on direct fish skins since catfish possess no scales as coverage.

Aquatic Bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals. Bioassay is necessary to determine the concentration of a toxicant, which may be allowed in receiving waters without adverse effects on the living resources (Ward and Parrish, 1982; Reish and Oshida, 1987, Ouyemi and Olabanji, 2011).

As seen in the study, the accumulation of lead in the fish tissue increased with increasing concentration of lead in the water. Health conscious people trying to get a healthy protein source are at risk of heavy metal accumulation if they depend on lead polluted water bodies.

The rate of survival reduced with increase in concentration of lead. Lead concentration gets to a point where it becomes lethal to the organisms around. The highest mortality occurred in T13 with the highest concentration of lead. This result agrees with Olaifa et al. (2003) who observed toxic stress of lead in fingerlings Clarias gariepinus and Kedebe and Wondimu, (2004) in distribution of trace elements in tilapia Oreochromis niloticus. There is a need for more work to set maximum permissible levels of metals for fish meant for human consumption.

Conclusions and Recommendations

In conclusion, lead is a heavy metal and should be prevented from entering into water bodies meant for agriculture. From the results obtained from the varied dosage of lead in 30 litres of water where the juveniles fish were raised for 96 hours, the growth of fish, the survival of fish and health of fish were affected negatively as the dose increases.

It is therefore recommended that all waters meant for aquaculture should be thoroughly screened for lead and other heavy metals composition to avoid bioaccumulation of these heavy metals harbour in the fish tissues. When in the tissues of fish, it can be transferred to man when such fish is eaten by man or when the fish is used as fish meal to poultry fish and other livestock feed, it will enter into man when such fish or livestock or poultry is eaten.

Our mechanisms, petrochemicals industries and electronics workshop and wastes should be properly discarded far from our water bodies to prevent the heavy metals from getting into our water bodies meant for drinking or for aquaculture purposes.

The water quality parameters of the experimental waters and the mean values include:

- Temperature 24.9°C, dissolved oxygen was 5.4mg/l, and the hydrogen ion concentration was 8.33. They are within the values recommended for Fish Culture in tropical countries (APHA, 1990).

REFERENCES


