Acute Toxicity and Haematology of *Clarias gariepinus* Exposed to Selenium

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Abstract The acute toxicity and haematological effect of selenium was investigated on *Clarias gariepinus* juvenile. 180 healthy *C. gariepinus* juveniles with mean weight of 7.4 ± 0.64 g and length of 11.2 ± 0.88 cm were exposed to different concentrations (0, 2, 3, 4, 5 and 6 mg/l) of selenium under a static method of bioassay for 96 hours. The mortality rate of the experimental fish increased with increase in concentration of the selenium. The 24, 48, 72 and 96 hours LC₅₀ were estimated to be 8.49, 6.36, 4.80 and 3.39 mg/l respectively which was analysed using probit method. The dissolved oxygen of the culture media was significantly lower p < 0.05 in the treatments when compared to the control. The blood parameters: Pack Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Haemoglobin (Hb) showed decrease from lowest concentration (2 mg/l) to highest concentration (6 mg/l). There were variations in the derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). In conclusion, it was observed that selenium at high concentration is toxic and caused a successive population decline of *C. gariepinus*. Fish farmers should as much as possible locate farms from likely source of toxicants.

Keywords Selenium; *Clarias gariepinus*; LC₅₀; Haematology

1 Introduction

Water pollution has become a global problem and rapid industrialization is one of the main causes of aquatic pollution because most of their effluents are being discharged in the aquatic environment. Heavy metal pollution is one of the most important environmental problems today. Contamination of fresh water with a wide range of pollutants has become a matter of concern over last few decades (Vutukuru, 2005). Modern industries are to a large extent, responsible for contamination of the environment. Nriagu and Pacyna (1988) reported that industrial wastes contain various types of toxic metals. These include: Hg (mercury), Cr (chromium), Pb (lead) Se (selenium), Zn (zinc), Cu (copper), Ni (nickel), Cd (cadmium), As (arsenic), Sn (tin), etc. Heavy metals are high priority pollutants because of their relative high toxicity and persistent. Though, many metals play a vital role in the physiological processes of plants, animals and humans, yet excess concentration of metals is harmful (Ololade and Oginni, 2010). The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to pollution (Khalid, 2011). Selenium is an essential trace element which is required in animal diet, including fish for normal growth and physiological function of animal (Abbas, 2009) it is also required for maintenance of homeostatic functions at trace concentrations (Monterio et al., 2009a). However, selenium can bio accumulates and become toxic. It is used in a number of industrial and manufacturing processes including photoelectric cells, steel manufacture, anti-dandruff shampoos, fungicide, and glass manufacturing (Naggal, 2001). Selenides (-2) are usually present as organic compounds (Bowie et al., 1996), such as selenomethionine and selenocysteine (Combs and Combs, 1986), and are physically and chemically similar to sulphides (Banks, 1997). Major anthropogenic sources of Se include fossil fuel combustion, mining, and agricultural drainwater (Haygarth 1994; Lemly, 1999). Other anthropogenic sources that may increase selenium contamination are open pit phosphate mining, wetlands constructed to treat Se-laden wastewater, and feedlot waste (Lemly, 1999). Upon entering an aquatic ecosystem, Selenium may be absorbed or ingested by aquatic organisms, bind to particulate matter, or stay free in solution (Lemly and Smith, 1987). African catfish (*Clarias*
gariepinus) is appreciated by large population (Prusynski, 2003). It is an excellent species for aquaculture as it is omnivorous, grows fast, and tolerates relatively poor water quality (Rad et al., 2003). Several investigations have been carried out on various toxicants with Clarias sp. (Aguigwo, 1998; Maheswaran et al., 2008). Changes in several haematological variables are recognized as indicators of metal exposure (Cyrick et al., 1989). Blood parameters has been used as an indicator of stress in fish exposed to different toxicants such as heavy metals and industrial effluents. In fish, exposure to chemical pollutants can induce either increase or decrease in haematological levels (Mehjbeen and Nazura, 2012). The haematological effects of various metals such as Hg, Cu, Cd and Pb on Clarias sp have been reported in various studies. Oshode et al., (2008) reported that observation of haematological parameters allows the most rapid detection of changes in fish. Disrupted haematological patterns appear very quickly and precede changes in fish behaviour and visible lesions. The rapidity of toxic effects, exerted by heavy metals, is related to the blood’s transport function, with the blood distributing the metals to all the body parts. Literatures has reviewed that there are no much work on the haematological effects of selenium on C. gariepinus. Therefore this present study was to investigate the acute toxicity of selenium and its effect on blood parameters of C. gariepinus juvenile.

2 Materials and Methods

Juveniles of African catfish (C. gariepinus) with average weight of 7.4 ± 0.64 g were used for the study. 180 healthy fish which were purchased from a reputable fish farm were acclimatized (10 each) in eighty litres plastic container filled with forty litres of tap water each for a period of 4 weeks to the laboratory condition. During this period of acclimatization, the fish were fed with commercial pellets twice daily at 3% body weight and water was changed every other day. The fish were not fed 48 hours prior to experiment in order to minimise waste from fish.

Toxicant stock solution of the tested metal, a pure chemical: sodium bi-selenite was prepared by dissolving 5 g of reagent equivalent to 1 g of selenium in 1000 ml water at concentration of 1000 mg/l. From the stock solutions, different concentrations required were prepared after a range finding test using a screening procedure. The concentrations prepared for the experiment were: 2, 3, 4, 5 and 6 mg/l based on literature guidance (Burba 1999; Vinodhini and Narayanan, 2008). This was prepared 24 hours before the experiment in other for the chemical to properly dissolve.

Water quality monitoring was done every 24 hours throughout the period of the experiment. The pH, conductivity, dissolved oxygen, and temperature was done with the use of HI-769828 multi-parameter water analysis probe. Ammonia, nitrate and nitrite test was done with the use of NT LABS pond water multiparameter test kit.

Blood samples were collected from both the control and experimental fishes that survived the 96 h toxicant exposure period. The blood samples were taken by puncturing posterior caudal vein and collected into ethylenediaminetetraacetate (EDTA) bottle (Schmitt et al., 1999). Automated haematology analyser mindray Bc 300 plus was used to determine haematological parameter. The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1986): MCV was calculated in fentoliters = PCV/RBC x 10; MCH was calculated in picograms = Hb/RBC x 10; and MCHC = (Hb in 100 mg blood / Hct) x 100.

Data obtained were subjected to one-way analysis of variance (ANOVA) test and the means from the various treatments were compared for significant differences (P>0.05) using Duncan’s multiple range tests. Values were expressed as mean ± S.E. 96 hours LC50 was determined using probit analysis (Finney, 1971) and simple graphical method.
3 Results

3.1 Trends in mortality and physicochemical parameters
Time to death was shorter in the higher concentrations (Figure 1). At the first 12 hours, there were no noticeable effect on the test organisms even at higher concentrations. Few mortality were recorded at the first 24 hours of the toxicant introduction but at 72-96 hours, mortality rate increases speedily. The mortality rate of the experimental fish increased with increase in concentration of the toxicant. The 24, 48, 72 and 96 hours LC50 was estimated to be 8.49, 6.36, 4.80 and 3.39 mg/l respectively which was analysed using probit analysis.

![Figure 1 Trend in fish mortality with duration of exposure to selenium](image)

3.2 Haematological variables
Table 1 showed the mean value of the haematology. PCV of untreated group is significantly higher (48.33±0.58) than the treatment groups while that of the treatments decreases (22.67, 21.67, 19.00, 17.00 and 12.50) with increase in concentration of selenium except for treatment 1 which is slightly higher than treatment 2 but showed no statistical differences. The WBC of the treated groups were significantly lower than the control. No significant difference between treatments 1 and 2. Also, no significant difference between treatments 3, 4, and 5. The haemoglobin concentration was highest (25.71) in the unexposed fish while it was least (4.96) in the group exposed to highest concentration of selenium (6 mg/l). There was a slight decrease with increase in concentration of the treated groups while the unexposed fish had the highest RBC count. There were great variations in the values of MCV, MCH and MCHC. Mean corpuscular volume (MCV) of treatments 4 and 5 were significantly higher (p > 0.05) compared to control group. Treatment 5 had the highest mean corpuscular haemoglobin (MCH) value while mean corpuscular haemoglobin concentration (MCHC) was higher in the control than treatment groups.

3.3 Water parameters
In Table 2, dissolved oxygen decreased with increase in concentration of selenium. However, all other parameters showed no significant difference.

4 Discussions
The present study shows that C. gariepinus juvenile are susceptible to selenium toxicity; the LC50 reduces with increase in duration of exposure. Also, the higher the concentration of selenium, the higher the mortality rate (Figure 2). The 96 hours LC50 of selenium in this study (3.39 mg/l) has a close range with the 96 hours LC50 of
selenium (2.5 mg/l) exposed to Channa punctatus (Blouch) (Avinashe and Patil, 2011). 96 hours LC50 of 4.32mg/l was also recorded when Oreochromis species was exposed to selenium concentration (Hossam et al., 2009) The mortality recorded in the study might be a consequence of stress induced by selenium on the immune system of C. gariepinus which probably slow toxic progress and resulted in acute toxic response.

Table 1 Haematological parameters of C. gariepinus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TRT 1</th>
<th>TRT 2</th>
<th>TRT 3</th>
<th>TRT 4</th>
<th>TRT 5</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%V)</td>
<td>22.67±1.15a</td>
<td>21.67±0.58a</td>
<td>19.00±1.00a</td>
<td>17.00±0.50a</td>
<td>12.50±0.55a</td>
<td>48.33±0.58c</td>
</tr>
<tr>
<td>WBC(x109/mm³)</td>
<td>1816.67±76.38b</td>
<td>1703.33±28.92b</td>
<td>1440.00±69.28a</td>
<td>1536.67±124.23a</td>
<td>1410.00±75.50a</td>
<td>2470.00±26.46c</td>
</tr>
<tr>
<td>HB (x10³/g/dl)</td>
<td>7.93±0.47c</td>
<td>7.37±0.06a</td>
<td>6.21±0.11b</td>
<td>5.00±0.46a</td>
<td>4.96±0.83a</td>
<td>25.71±0.34d</td>
</tr>
<tr>
<td>RBC (x10³/µl)</td>
<td>2.95±0.16d</td>
<td>2.81±0.06d</td>
<td>2.47±0.13c</td>
<td>1.31±0.02b</td>
<td>1.02±0.01a</td>
<td>6.29±0.07c</td>
</tr>
<tr>
<td>MCV (x10⁷-Fl)</td>
<td>76.53±0.47a</td>
<td>76.88±0.46a</td>
<td>76.92±0.00a</td>
<td>128.08±3.02a</td>
<td>122.52±3.70b</td>
<td>76.80±0.00a</td>
</tr>
<tr>
<td>MCH (x10⁷-pg)</td>
<td>27.72±1.04a</td>
<td>26.16±0.67a</td>
<td>25.15±0.89a</td>
<td>37.97±3.50a</td>
<td>46.68±8.33c</td>
<td>40.84±0.76b</td>
</tr>
<tr>
<td>MCHC (pg)</td>
<td>36.07±1.29bc</td>
<td>34.02±1.07bc</td>
<td>32.71±1.15bc</td>
<td>29.64±2.16a</td>
<td>39.81±7.26c</td>
<td>53.23±1.06d</td>
</tr>
</tbody>
</table>

Note: Mean with different superscripts are significantly different

Table 2 Water quality parameters of culture system of C. gariepinus observed over period of 96 hours

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (0 mg/l)</th>
<th>TRT 1 (2 mg/l)</th>
<th>TRT 2 (3 mg/l)</th>
<th>TRT 3 (4 mg/l)</th>
<th>TRT 4 (5 mg/l)</th>
<th>TRT 5 (6 mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMP(OC)</td>
<td>25.53±0.13ab</td>
<td>25.53±0.09ab</td>
<td>25.48±0.16a</td>
<td>25.68±0.68b</td>
<td>25.48±0.10a</td>
<td>25.50±0.18a</td>
</tr>
<tr>
<td>pH</td>
<td>6.58±0.16a</td>
<td>6.48±0.15a</td>
<td>6.44±0.12a</td>
<td>6.40±0.07a</td>
<td>6.44±0.10a</td>
<td>6.47±0.10a</td>
</tr>
<tr>
<td>D.O (ppm)</td>
<td>4.11±0.22a</td>
<td>3.08±0.17a</td>
<td>2.99±0.18a</td>
<td>2.75±0.28a</td>
<td>2.27±0.20a</td>
<td>1.52±0.34a</td>
</tr>
<tr>
<td>SAL (ppm)</td>
<td>0.08±0.00a</td>
<td>0.08±0.00a</td>
<td>0.08±0.00a</td>
<td>0.08±0.00a</td>
<td>0.08±0.00a</td>
<td>0.08±0.00a</td>
</tr>
<tr>
<td>COND(mm-cm)</td>
<td>0.0042±0.00a</td>
<td>0.0043±0.00a</td>
<td>0.0042±0.00a</td>
<td>0.0043±0.00a</td>
<td>0.0043±0.00a</td>
<td>0.0042±0.00a</td>
</tr>
<tr>
<td>T.D.S (ppm)</td>
<td>95.83±1.10a</td>
<td>96.00±1.75a</td>
<td>95.67±2.31a</td>
<td>95.17±1.80a</td>
<td>95.17±1.11a</td>
<td>95.08±1.93a</td>
</tr>
<tr>
<td>Ammonia(ppm)</td>
<td>0.4±0.16a</td>
<td>0.34±0.15a</td>
<td>0.33±0.15a</td>
<td>0.35±0.17a</td>
<td>0.40±0.15a</td>
<td>0.33±0.15a</td>
</tr>
<tr>
<td>Nitrate(ppm)</td>
<td>7.08±2.57a</td>
<td>7.50±2.61a</td>
<td>6.67±2.46a</td>
<td>7.50±2.46a</td>
<td>6.67±2.46a</td>
<td>7.08±2.57a</td>
</tr>
<tr>
<td>Nitrite(ppm)</td>
<td>1.42±0.51a</td>
<td>1.33±0.49a</td>
<td>1.42±0.51a</td>
<td>1.33±0.49a</td>
<td>1.42±0.51a</td>
<td>1.41±0.51a</td>
</tr>
</tbody>
</table>

Note: Mean with different superscripts are significantly different

Figure 2 96 hours LC50 of Clarias gariepinus juvenile exposed to selenium

Blood parameters have been used as sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as metals, biocides, pesticides and industrial effluents (Dharam et al., 2008). The study of
haematological picture are frequently utilised for the detection of pathological changes in different stress conditions such as exposure to heavy metals (Nussey et al., 1995). Jayapakash and Shettu (2013) reported after the exposure of *Channa punctatus* to deltamethrin that anaemia might have led to a fall in the red blood cell count, haemoglobin and pack cell volume. In the present study, PCV% value, Hb%, and RBC count of *C. gariepinus* exposed to selenium decreased significantly $P < 0.05$ when compared to the control group. This reduction could be an indication of anaemia condition caused by the exposure of experimental fish to selenium in water. The observed decrease in the haemoglobin and pack cell volume value in the fish could also be as a result of erythrocyte lysing. The decreased haemoglobin concentration also signifies that the fish ability to provide sufficient oxygen to the tissue is restricted considerably and this will result in decreased physical activity (Nussey et al., 1995). Several studies have been reported on the reduction of haematological parameters of fish exposed to heavy metals (Dhiaram et al., 2008; Ololade and Oginni, 2010; Khalid, 2011). A decrease in the haematological values of common carp fish when exposed to nickel, Khalid (2011). Ololade and Oginni (2010) also had similar report when African cat fish was exposed to nickel. Pamile et al. (1991) explained that reduction in haemoglobin content in fish exposed to toxicant could be due to the inhibiting effect of the toxic substance in the enzyme system responsible for synthesis of haemoglobin.

The reduction in WBC count of the treatment groups that was observed in this study agreed with the finding of Adeyemo et al. (2007) following the exposure of *C. gariepinus* to lead nitrate. This result is also similar to the findings of Olanike (2007) and Witeska (2003) that attributed this to the release of epinephrine during stress which causes a decrease in leucocyte count, and also shows the weakening of the immune system.

There were variations in the values of blood indices MCV, MCH and MCHC. Mean corpuscular volume (MCV) of treatment 4 and 5 were significantly higher ($p > 0.05$) compared to control group while mean corpuscular haemoglobin (MCH) also increased significantly in fish exposed to highest concentration of selenium. This result is similar to the finding of Adeyemo et al. (2007) that exposed *C. gariepinus* to lead nitrate. It also agreed with the work of Shah, (2006) following a short term exposure of tench (Tinca tinca) to lead. Mean corpuscular haemoglobin concentration (MCHC) was significantly higher in the control than treatment group. MCHC is a red blood cell morphological index reflecting the haemoglobin concentration, the observed decrease in this parameter may indicate that the haemoglobin concentration in the unexposed fish was higher than in the selenium exposed fish and this may further suggest impaired haemoglobin synthesis in the treated fish. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006).

Water parameters such as pH, turbidity, alkalinity, dissolved oxygen, temperature, and conductivity were influenced by the rate of pollutant entering aquatic environment (Fagbenro, 2002; Olufayo, 2009). The dissolved oxygen is significantly lower ($P < 0.05$) in the test medium of the treatment groups. This could be that the oxygen molecules is been degraded by selenium. This study agreed with the study carried out by Ololade and Oginni (2010) when *C. gariepinus* was exposed to nickel. No significant difference was recorded in pH. Selenium has been examined to have toxic effect on *C. gariepinus* juveniles. It is therefore recommended that industries should install waste treatment plant, with a view to properly treat waste water before discharging them into aquatic environment.

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