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Impact of heavy metal bioaccumulation on antioxidant activities and DNA profile in two earthworm species and freshwater prawn from Ogun River

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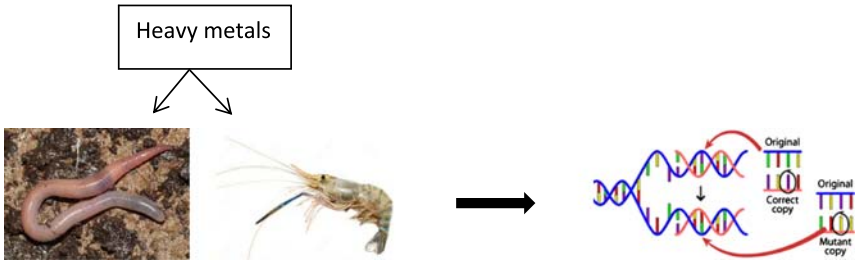
HIGHLIGHTS

- Test organisms accumulated varying concentration of heavy metals in their tissues.
- Accumulated metals resulted to the low activities of most antioxidants in the test organisms.
- Earthworm indicated mutation due to heavy metals. However, freshwater prawns showed no variations.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of freshwater invertebrates as bioindicator of heavy metal pollution is an important tool for environmental biomonitoring. This study investigated antioxidant activities and DNA profile in two limicolous earthworms (*Alma millsoni* and *Libyodrilus violaceus*) and freshwater prawns (*Macrobrachium vollenhovenii*) at selected points of Ogun River, Abeokuta. Heavy metal concentrations and DNA profile in the earthworms and prawn tissues were measured using standard procedures. Zn concentration was higher than other heavy metals in *A. millsoni* (685.83 ± 114.42 mg/kg), *L. violaceus* (1913.3 ± 1098.7 mg/kg) and *M. vollenhovenii* (134.7 ± 13.61 mg/kg). Superoxide dismutase activity ranged from 62.44 ± 7.16–79.82 ± 11.18 U/g tissues, 60.26 ± 11.18–71.07 ± 7.54 U/g tissues and 74.07 ± 16.69–87.79 ± 8.50 U/g tissues in *A. millsoni*, *L. violaceus* and *M. vollenhovenii* respectively. RAPD-PCR revealed varying DNA profile among the earthworms samples; the UPGMA dendrogram formed two distinct clusters at genetic similarity coefficient of 0.15–0.2 with one cluster consisting of *Alma millsoni* and *Libyodrilus violaceus* from Sokori, Enugada and Iberekodo sampling points and the second cluster forming two distinct sub-clusters comprising Arakanga and Ago-ika's *L. violaceus* in one and *A. millsoni* in the other. High genetic variability was recorded among the earthworm species while the freshwater prawn showed no variability. Antioxidant activities and genetic variability in earthworms could serve as biomarkers of heavy metal pollution in freshwater environment.

1. Introduction

Heavy metals coming majorly from human activities (urbanization and industrialization) are considered major contaminants of freshwater (Ubwa et al., 2013). Heavy metals such as Cu, Fe and Zn are regarded as

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essential metals, because of their biological role in respiration, cell growth, protection and production of enzymes but when accumulated at higher concentrations, they can be toxic. On the other hand, heavy metals such as Cd, Hg and As are not needed even in a minute quantity.

Metal accumulation in living system is often associated with various deleterious physiological effects such as low cast production by earthworms (Dittbrenner et al., 2010), impaired metabolic rate and energy depletion (Fisker et al., 2011), histopathological damage (Tarasub et al., 2011), ineffective burrowing activity (Pelosi et al., 2013), abnormal behavioural activities (Mariappan and Karuppasamy, 2014) and gonad maturation (Peranandam et al., 2014). The often ionic nature of these metals enables them to penetrate body surfaces and phospholipids of cell membranes, hence causing harm to the cell by producing radicals cumulatively called reactive oxygen species (ROS) such as perhydroxl radical (HO_2), hydroxyl radical (•OH) (Pratviel, 2012). These radicals results to imbalance between pro-oxidants and oxidants otherwise known as oxidative stress (Metcalfe and Alfonso-Alvarez, 2010). Also, influx of metals can result to the degradation of macromolecules such as lipids, protein and DNA (Joseph and Kafilat, 2012) thus leading to deleterious conditions such as genotoxicity and lipid peroxidation (Li et al., 2011).

Many freshwater macro-invertebrates respond to heavy metals pollution either morphologically, behaviorally or physiologically (Pablo et al., 2013), hence lowering the risk of the resulting negative effects. The responses by these groups of animals to heavy metal pollution could be exploited as early warning signals and could serve as powerful tools for assessing anthropogenic influence on freshwater environment. The antioxidant defense system readily eliminates any form of toxic effect or repair any form of damage caused by the ROS. Antioxidants such as superoxide dismutase (SOD), peroxidase, glutathione-*S*-transferase and glutathione are produced to reduce the destructive effects of ROS in organisms (Azqueta et al., 2009). Superoxide dismutase is one of the major antioxidants produced to counter superoxide anion by dismutation, GST detoxify ROS by conjugating with these substances, hence bringing to a halt the damage of ROS (Otitoloju and Olagoke, 2011).

Freshwater macro-invertebrates such as prawns and limicolous earthworms, which thrive successfully in the freshwater environment (Marioghae, 1990; Owa et al., 2010), occupy an important position in the food chain feeding on detritus and still a good recipe for other higher organisms. Studies have documented heavy metal bioaccumulation in freshwater prawn (Omoigberale and Ikponmwosa-Eweka, 2010; Manosathiyadevan et al., 2013) and limicolous earthworm such as *Alma millsoni* and *Libyodrilus violaceus* (Ebenezer et al., 2013), hence they are good biomonitoring agents for heavy metal pollution.

DNA damage caused by heavy metal pollution has been measured by several authors using RAPD-PCR (Ilhan et al., 2016; Kumar et al., 2015 and El Assal et al., 2014). The method is less expensive, yet very sensitive to identify DNA damage. For the DNA damage, we hypothesized to detect genetic mutation in sampled population, which might have been caused by heavy metal bioaccumulation resulting from the exposure of the population to these pollutants.

The objective of this study is to measure the effects of heavy metal bioaccumulation on antioxidant activities and DNA profile in two limicolous earthworms (*Alma millsoni* and *Libyodrilus violaceus*) and freshwater prawns (*Macrobrachium vollenhovenii*) collected from Ogun River, Abeokuta.

2. Materials and methods

Natural occurring specimen of limicolous earthworms and freshwater prawns were collected at different five sampling sites (A-Arakanga, B-Iberekodo, C-Ago-Ika, D-Sokori, E-Enugada) along Ogun River shown in Fig. 1 with their respective co-ordinates. Adult limicolous earthworms with well-developed clitella were collected at a depth of 0–2.0 cm in soil particles containing high humus along the bank of the river using the procedure of Owa (1992). Freshwater prawn samples were baited and trapped at the various sites in fishing baskets. Prawns were transported in ice-chest to the laboratory for further analysis, while, earthworm samples were transferred to the laboratory in well labeled clean polyethene bags.

2.1. Identification of earthworm and prawn samples

The earthworms were identified and described by an Animal Taxonomist in the Department Pure and Applied Zoology of the Federal University of Agriculture, Abeokuta while the prawn identification was done using Marioghae (1990) as a guide.

2.2. Determination of heavy metal analysis in the water and tissue

Frozen prawns were thawed partially at room temperature before opening. Shell and soft tissues were carefully separated from each prawn sample. Both parts of the samples were dried in the oven at 105 °C for 24 h to obtain a constant dry weight (dw). Earthworm samples were separated based on its species in well labeled petri-dishes after which they were oven dried at 60 °C for 24 h. The dried samples were pulverized into powdery form. 1 g each of the dried powdered samples were digested by heating with 21 mL of concentrated nitric acid and 7 mL of concentrated hydrochloric acid according to the standard methods of the Association Of Analytical Chemist (AOAC, 2000). Digested sample were diluted with little amount of distilled deionized water. The solution was filtered using a Whatman No 1 filter paper. Heavy metal concentrations were determined using atomic absorption spectrophotometry.

2.3. Antioxidants assays

10% homogenate tissue sample was prepared in ice-cold homogenization buffer (125 mM sucrose, 125 mM mannitol, 1 mM EGTA and 5 mM HEPES) (pH 7.2 0.25 M) with 15 strokes in a Teflon pestle homogenizer centrifuged at 3000 rpm for 15 min at 4 °C. The supernatant was kept at −20 °C until analysis.

Glutathione-*S*-transferase activity determined according to the method of Habig et al. (1974). Two test tubes marked blank (B) and test (T) were arranged in a test tube rack. Potassium phosphate buffer (1.0 mL), 50 μL of glutathione and 50 μL of CDNB were pipeted into each of the tubes. Erythrocyte (50 μL) was added to the mixture in the tube marked T while 50 μL of buffer was added to the mixture in the tube marked B. They were mixed by inversion and the increase in absorbance was recorded for 5 min. The difference between the initial and final absorbance and average absorbance difference was calculated (ΔA 340/min). GST activity was calculated as

$$\text{Units/mL enzyme} = \frac{(\Delta A\ 340\text{nm}/\ \text{minTest} - \Delta A\ 340\text{nm}/\ \text{minBlank})\ (1.15)(\text{df})}{0.05 \times 9.6}$$

where 1.15 = Total volume (in milliliters) of assay

df = Dilution factor

9.6 = Millimolar extinction coefficient of Glutathione-1-Chloro-2,4-Dinitrobenzene conjugate at 340 nm

0.05 = Volume (in milliliter) of enzyme used

2.4. Determination of superoxide dismutase (SOD)

Superoxide dismutase activity was determined according to the method of Marklund and Marklund (1974). Two test tubes marked blank (B) and test (T) were arranged in a test tube rack. Potassium phosphate buffer (100 μL), 830 μL of distilled water and 50 μL of sample were pipetted into tube T while 150 μL of buffer and 830 μL of distilled water were pipetted into tube B. The contents of the tubes were incubated for