

Assessment of Heavy Metals in African Giant Snail (*Archachatina marginata*) and its Parasites Collected from three communities in Edo State, Nigeria

¹Awharitoma, A. O., ^{*2}Ewere, E. E., ³Alari, P. O., ⁴Idowu, D. O. and ⁵Osowe, K. A.

^{1,2,3,4,5}Department of Animal and Environmental Biology, faculty of Life Sciences,
University of Benin, P.M.B 1154, Benin City, Nigeria

*Corresponding author: endurance.ewere@uniben.edu

Abstract—*Archachatina marginata*, a mollusk, is highly prized as food in Africa and Asia, and is a vector of parasites and defoliators. The environments where the snails thrive are highly contaminated with heavy metals through various anthropogenic activities. Levels of heavy metals (Fe, Ni, Mn, Cu, Pb, Cd and Co) in *A. marginata* (parasite infected and uninfected) and its parasites were assessed from three communities (Ugbogui, Ugo and Okogbo) in Edo State to check the pollution status. Samples of snail were collected, cracked and parasites were isolated from the samples using standard methods. The isolated parasites and the snail samples were analyzed for heavy metals using Flame Atomic Absorption Spectrophotometry (FAAS). The parasite isolated from the infected snail was identified as nematode (*Rhabditis axei*). The mean concentration of Fe, Ni, Mn, Cu, Pb, Cd and Co ranged 38.61 – 70.49mg/kg, 9.09 – 16.58mg/kg, 5.09 – 8.90mg/kg, 4.10 – 7.48mg/kg, 0.39 – 0.71mg/kg, 0.19 – 0.35mg/kg and 0.04 – 0.07mg/kg respectively in infected snail; 14.94 – 28.45mg/kg, 3.26 – 5.96mg/kg, 2.85 – 4.79mg/kg, 1.47 – 2.69mg/kg, 0.14 – 0.26mg/kg, 0.07 – 0.13mg/kg and 0.03 – 0.05mg/kg respectively in uninfected snail tissues; 11.34 – 27.61mg/kg, 1.17 – 1.92mg/kg, 1.73 – 4.89mg/kg, 0.51 – 0.87mg/kg, 0.05 – 0.08mg/kg, 0.03 – 0.04mg/kg and 0.04 – 0.05mg/kg respectively in parasite. The result from this study showed that the accumulation of heavy metals was parasites <Uninfected snail tissues<Infected snail tissues. The levels of the heavy metals (Cu, Pb, Cd and Co) in the samples of *A. marginata* collected from the communities exceeded the maximum permissible limits and consumption of these contaminated snails could pose potential health risks to humans. It is therefore necessary to continuously monitor heavy metal content in snails to check the pollution status and the possible health risk associated with consumption.

Index Terms— Assessment, Giant Snail, Heavy metals, Nigeria, Parasites

1 INTRODUCTION

There are many heavy metals (HMs) occurring in our environment both naturally and from pollution. These heavy metal pollutants can be bioconcentrated in organisms and their parasites [22]. When chemicals bioconcentrated are toxic, bioconcentration becomes an environmental threat to the organism concerned. Hence toxicity occurs along the food chain when the contaminated species is consumed by another organism on the higher trophic level [12]. The Giant Africa land snail (*Archachatina marginata*) is an important source of animal protein especially in West African and the snails are usually collected in forests and transported to markets for sales. The link between heavy metals in the soil and land snails is through cutaneous contact with the upper soil horizon and feeding activities in the soil. They have been extensively used as bioindicator for heavy metal pollution, and have been shown to accumulate and concentrate high amounts of heavy metals in their body, especially in the hepatopancreas [18], [8]. It has been reported that most ingested metals are metabolically regulated in the snail body either by cellular

compartmentalization or by complexation to specific metallo-thioneins [4], [7]. Such processes of bioaccumulation in organisms may be associated with significant interactions between these HMs and metallic macroelements (K, Ca, Na, Mg), which can be explained by intensive and prevailing access of toxic metals in toxic reactions [16]. It has been shown that Ca is essential to snail reproduction, shell development, and other physiological needs [13], whereas both Na and K are key players for normal cell functioning [9].

Achachatina marginata has been shown to accumulate high amounts of heavy metals in their tissues [18]. The main route of metal uptake is through consumption of litter, fungi, soil, dead plants and animals and also via epithelium because land snails spend their entire lives on the upper soil horizon and therefore the snail tegument comes frequently in direct contact with polluted substrates [6]. However in natural environment, metal uptake is a cumulative process that occurs via mixed air, soil and food exposures in field exposure [10]. Also, parasites of organisms such as

snails are able to accumulate heavy metals at much higher or lower concentration than their host tissues or their environment [18]. This accumulation suggests accumulation by host and it is well accepted that parasitism and pollution may affect the physiological homeostasis of some terrestrial host like land snails. The effects of pollution on parasitism are variable and pollution may increase parasitism if the pollutant mainly affects the host rather than the parasites or it may exercise a negative effect over certain parasites, which are more susceptible to the particular pollutants than their hosts. The interaction between parasites and pollution can even be more complex, in fact, some parasites may even have a positive influence on their host when exposed to environmental pollution or on the contrary they may have synergetic effects. Several authors have reported hazardous concentration of HMs in organisms [20], [21], [11], [14]. To the authors' knowledge, there is no reported work on HMs in snails and its parasites. This work therefore assesses HMs in snail and its parasites in three communities of Edo State, Nigeria.

2. MATERIALS AND METHOD

2.1 Description of study area

The study was carried out in three different communities (Ugbogui community latitude 6° 40' N and longitude 5° 15' E; Okogbo community latitude 6° 12'0"N and longitude 5° 52'30"E and Ugo community latitude 6° 5'0"N and longitude 6° 0'0"E) in Edo state, Nigeria. The vegetation type in the region is naturally rain forest. The soil type is generally red yellow kind of ferral-sols, variation consist of shallow/stony reddish clay at the feet of inselbergs in the higher sections, lateritic clay and fine grained to sandy soils in the upper slope/lateritic tablelands and ferruginous soils on the crystalline acid rocks of the basement complex. The climate is characterized by two distinct conditions of wet and dry seasons with April – October as wet season with a brief break in August and the dry season is November – March. The annual rainfall averages 250cm. Temperature is usually high with an annual mean of 28°C. Topography can be described as generally low. Agricultural produce includes; cassava, maize, yam, plantain, fruits, palm produce, meat products. Mineral and natural resources includes; limestone, clay, bitumen, kaolin, marble and timbers.

2.2 Apparatus and Reagents

Petri dishes, Forceps, Dissecting scissors, Dissecting probe, Dissecting needle, Light microscope, Sample bottles, Saline, 70% Alcohol, Mechanical shaker, Extraction, Whatman filter paper No:42, Polythene funnels, Unicam 929 spectrometer, Extracting solution (9ml of Conc. HCl, 3ml of HNO₃ and 2ml of perchloric acid and make up to a final volume of 1litres with deionized water and 1000mg/l Stock Standard solution of Cu, Ni, Cd, Fe, Cd, Mg and Co were used in concentration 0, 0.2, 0.4, 0.6, 0.8, and 1mg/l for each analyte analysed.

2.3 Snail (*Archachatina marginata*)

A. marginata has a brown shell with conspicuous zigzag streaks with a narrow apex. The foot is grey in colour. The species is believed to be native to West Africa, within 160 to 300 kilometers of the coasts of Sierra Leone, Liberia, Ivory Coast, Togo, Benin, Ghana and Nigeria. The shells of these snails often grow to a length of 18cm (7.1in) with a diameter of 9cm (3.5in).



Plate 1: Showing the frontal view of *Archachatina marginata*.

2.4 Collection of snails and isolation of parasites

Snails were purchased monthly from traders in Ugbogui market in Ugbogui community, Edo state, Nigeria in the months of April, May and June 2015. In the laboratory the snail shell was washed with distilled water and cracked. The head region, reproductive system and digestive system were teased separately in petri dishes containing normal saline (0.72%NaCl). The specimens were viewed under the light microscope; some of the parasites found were transferred into specimen bottles for heavy metal analysis, some parasites were preserved in 70% alcohol in a separate specimen bottle for identification. The other parasites isolated together with the tissues of both the infected and uninfected snails were taken to the laboratory for heavy metals analysis. Three uninfected snail tissues and three infected snail tissues and their respective parasites from each of the communities were analysed monthly for heavy metals.

2.5 Samples processing and heavy metal analysis

Samples were placed in glass Petri dishes and dried in the oven at 105°C. After 24 hours of drying, lumps present were broken up with a clean glass rod in order to expose the inside for drying. After the samples were dried, it was left in the oven for further 24 hours before grinding. Grinding was done using a mortar and pestle to breakdown the pieces. 1g of the dried snail and parasite sample were transferred into an acid –wash 250ml extraction bottle, 9ml of HCl, 3ml of HNO₃ and 2ml of perchloric acid were added. The samples were digested for 3-4 hrs on mechanical shaker hot plate. The suspension was filtered through a Whatman No 42 filter paper and made up to 100ml. the filtrate was analyzed for, Cu, Ni, Cd, Fe, Co, Mn and Pb using Spectrometer PG 550. Control procedure was carried out to ensure the reliability of the results. In all the metal determinations, analytical blanks were prepared in a similar manner without using the snail or parasite sample. Single elemental standards were prepared by dilution of 1000mg/L stock solutions of the individual elements (Cu, Ni, Cd, Fe, Co, Mn and Pb). External calibration was done by running deionised water and a suite of calibration standards for each element. The calibration curve was then generated for each metal. In order to check the reliability of the instrument blanks and extracted solution were the run on the AA to obtain the absorbance values. Concentrations of the metals in digested samples were then calculated from the equation of the calibration curve by the equipment.

2.6 Statistical analysis

All data obtained was subjected to statistical analysis using the statistical package (SPSS). The result comparison was tested

using Duncan multiple range test.

3. RESULTS

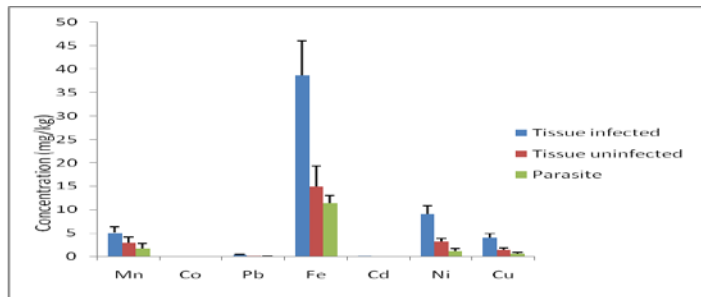


Figure 1: Concentration of Heavy Metals in uninfected snail tissues, infected snail tissues and parasite at Okogbo

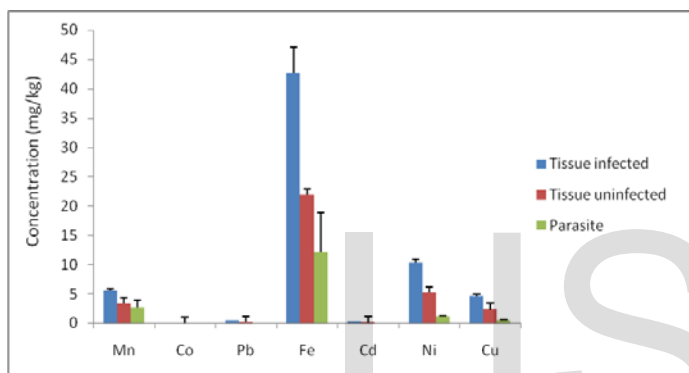


Figure 2: Concentration of Heavy Metals in uninfected snail tissues, infected snail tissues and parasite at Ugbogui

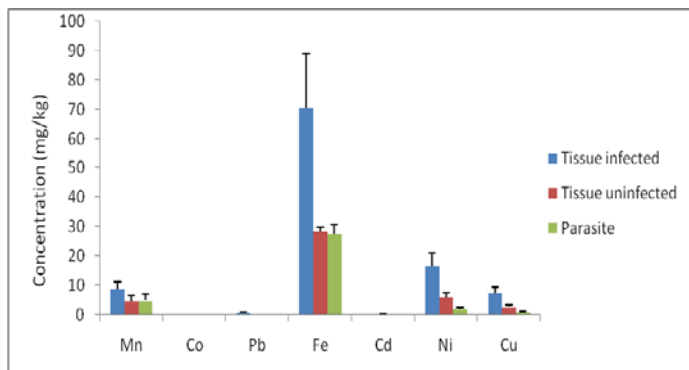


Figure 3: Concentration of Heavy Metals in uninfected snail tissues, infected snail tissues and parasite at Ugo

4. DISCUSSION

4.1 Parasites in Snails

Molluscs are significant as hosts for only two major groups of nematodes: as intermediate hosts for metastrongyloids and as definitive hosts for a number of rhabditids. Although most rhabditid nematodes have been reported not to kill their mollusc hosts prior to their reproduction, some species are pathogenic. Achatinids have been found to also act as reservoir host for the rat lung worms (*Angiostrongylus cantonensis* and *A. costricensis*), which cause eosinophilic meningoencephalitis in humans [5].

The nematode parasite *Rhabditis axei* recorded in this study in *A. marginata* has been reported previously in *A. marginata* [1], [3].

4.2 Heavy Metals in Snails and Parasites

Iron has the highest concentration both in the snail samples and their parasites in the three communities sampled. The mean concentrations of iron in this study were within the safe limit since the permissible limit of iron is (30-150mg/kg) [24]. The concentration of this heavy metal in the snail maybe due to the fact that iron-proteins are found in all living organisms and it is a necessary trace element found in nearly all living organisms, also the presence of heavy metals in the soil of the various communities could be due to anthropogenic activities occurring in the areas. Humans experience iron toxicity above 20 milligrams of iron for every kilogram of mass, and 60 milligrams per kilogram is considered a lethal dose. Excess iron encourages the formation of cancer-causing free radicals. Of course, the body needs a certain amount of iron for healthy blood cells, but beyond this rather small amount, iron becomes a dangerous substance, acting as a catalyst for the formation of free radicals.

The mean concentration of Nickel was highest in the infected snails. Nickel is not only favorable as an essential element, it can be dangerous when the maximum tolerable amounts is exceeded, thereby causing respiratory problems and cancer. This can cause various kinds of cancer on the different sites within the bodies of animals. Mean concentration of nickel in this study were lower than the upper tolerable nickel intake (40mg/day) [2]. Concentrations of nickel in this present study are similar to the values for nickel in *A. marginata* from Southern Nigeria [23].

The mean concentration of Manganese in the infected and uninfected snail was below the maximum permissible limits of 11mg/kg. Manganese is important for human health, it is necessary for development, metabolism and the antioxidant system. However excessive intake may lead to manganism, a neurodegenerative disorder. From the results, the concentration of manganese was higher in parasites infected snail than uninfected snail tissues in the three communities studied.

The mean concentration of Copper in the infected snails was above the maximum permissible limit given by the World Health Organization (approximately 1.3mg of copper per day). Copper stimulates the Immune system to fight infections, to repair injured tissues, and to promote healing. Despite the benefits accrued to copper, excess copper intake cause headache, dizziness, abdominal pain, kidney failure, respiratory difficulty and cancer.

The mean concentration of Lead in the snail tissue exceeded the maximum permissible limit of lead in food (0.1mg/kg) [15]. Lead is only weakly mutagenic, but in vitro, it inhibits DNA repair and acts synergistically with other mutagens [16]. There is weak evidence associating lead with cancer and the most likely candidates are lung cancer, stomach cancer and gliomas.

The mean concentration of Cadmium was above the maximum permissible limit of 0.15mg/kg in food. Nawrot *et al.* [17] showed that cadmium (Cd) is carcinogenic and associated with lung cancer. In this study levels of cadmium were much lower than those reported previously [23].

The mean concentration of Cobalt (Co) in the snail (Infected and uninfected) exceeds the maximum permissible limit of

0.02mg/kg at the various communities sampled. The levels of cobalt reported in this study were within the concentration ranges of 0.02–0.85mg kg⁻¹ reported for mollusc tissues [19].

5. CONCLUSION

The results of this study showed that snails infected with parasites accumulated higher concentration of heavy metals relative to uninfected snails. The results of this study showed that parasitism makes the snail more susceptible to intake/poor elimination of heavy metals and subsequently accumulating/concentrating more of the metals. The levels of the heavy metals (Cu, Pb, Cd and Co) in the samples of *A. marginata* collected from Ugbogui, Ugo and Okogbo communities exceeded the maximum permissible limits. Consequently, consumption of the contaminated snails could pose a potential health risk to consumers. It is therefore imperative to continuously monitor heavy metals content in snails to check the pollution status and the risk associated with the consumption.

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