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Histopathological Changes in Periwinkle (*Tympanotonus fuscatus* var. *radula)* Exposed to Graded Doses of Nickel

A.A. Enuneku[a*], L.I. Ezemonye[b], C.U. Ajuzie[c] and P. Orobor[d]

[a]Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Nigeria.
[b]Ecotoxicology and Environmental Forensics Unit, National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, Nigeria;
[c]Department of Microbiology, Faculty of Life Sciences, University of Benin
[d]Department of Environmental Science, University of Benin

ABSTRACT: The periwinkle *Tympanotonusfuscatus* var. *radula* was exposed to sublethal concentration (0.5, l, 2, 4 and 8mg/L) of nickel in the laboratory for 30 days. The test was conducted using the Organization for Economic Cooperation and Development (OECD) protocol #218 in a sediment medium. There were three replicates per treatment and 6 periwinkles per tank of nickel concentration including control groups. There was high bioaccumulation of nickel in the test organism. It was observed that bioaccumulation of nickel in *T. fuscatus*var. *radula* increased with increase in concentration of the heavy metal. Histopathological alterations were studied in the Kidney and Muscular foot of the *T. fuscatus var. radula*. Periwinkle exposed to lower concentrations of toxicant (0.5mg/L) showed hyperchromatic nuclei while fatty changes occurred in the 1, 2 4 and 8 mg/L exposures in kidney. Necrosis was observed in the highest concentrations (4mg/L and 8mg/L). For the muscular foot, it was found that periwinkle exposed to lower concentrations of toxicant (0.5mg/L and 1mg/L) showed fatty changes while the periwinkle exposed to higher concentrations (2mg/L, 4mg/L and 8mg/L) showed excess fatty changes and hyperchromatic nuclei in the muscular foot. Brown deposits were observed around the fringes of the foot. This result shows that nickel can cause histopathological changes in*T. fuscatus* var. *radula*. The discharge of effluents containing heavy metals especially nickel should be discouraged as this could affect the health of the organism and exacerbate the problem of global biodiversity loss.

Introduction

Aquatic systems may be contaminated with heavy metals released from industrial and agricultural activities. When exposed to higher concentrations, organs of aquatic animals may accumulate heavy metals (1, 2).Nickel (Ni) is the 24th most abundant element in the Earth's crust. Nickel is a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when too little or too much Ni is taken up. Although Ni is ubiquitous and vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms (3).Nickel and nickel compounds have many industrial and commercial uses. Nickel is used for the production of stainless steel and other nickel alloys with highcorrosion and temperature resistance. Nickel metal and its alloys are used widely in the metallurgical, chemical and food processing industries, especially as catalysts and pigments. The nickel salts of greatest commercial importance are nickel chloride, sulphate, nitrate, carbonate, hydroxide, acetate and oxide (4).

Aquatic organisms accumulate metals to concentrations many times higher than present in water or sediment and can take up metals concentrated at different levels in their different body organs (5).

In Nigeria, most studies on heavy metal pollution have concentrated on the levels of occurrence and distribution of these pollutants in sediment and surface water of aquatic resources (6) without relating the observed occurrence to biological effects on resident biota such as periwinkle such as acute toxicity and sublethal chronic action including bioaccumulation in resident biota such as periwinkles.

Periwinkle (*Tympanotonus fuscatus)* is very abundant in mangrove mud flats exposed during low tide (7) in the Niger Delta. These invertebrates are an important group in estuarine ecosystems, which spend part of their life cycle in the water column where they comprise a temporal community. When periwinkles are exposed to elevated levels of metals in a polluted aquatic ecosystem, they tend to take these metals up from their environment (8).

Histological biomarkers are the indicators of pollutants in the overall health of the entire population in the ecosystem (9). The exposure of aquatic organisms to sub lethal concentrations of chemical contaminants in their environment may result in various bio-chemical, physiological and histological alterations in vital tissues. *Tympanotonus* species provide a relatively cheap source of animal protein and its shell can be used as a source of calcium in animal feeds and for construction purposes. They are collected from the wild and their marketing form an important industry in the Niger Delta area of Nigeria.

The aim of the study was to assess the bioaccumulation of nickel and histopathological alterations in the periwinkle, *T. fuscatus var. radula* exposed to nickel.

*Corresponding Author; e-mail: alex.enuneku@uniben.edu

Materials and Methods

Test animals, acclimation and test chemical

*Tympanntonus fuscatus*var. *Radula* (Periwinkle) (Mollusca; Gastropoda; Mesogastropoda, Potamididae) from Warri River in the Niger Delta Region of Nigeria was used for the experiments. Similar sizes (shell length of 39mm- 52mm) were collected by hand-picking into a plastic container from the Warri River at low tide.

The periwinkles were taken to the laboratory and kept in holding tanks (35cm x 24cm x 26cm). Also collected was mud from the same site and placed in the holding tank as substrate for periwinkles as they were being transported to the laboratory. The test animals were acclimatized to by diluting the water every 24hours using dechlorinated tap water for three days. Then, the animal was placed in dechlorinated tap water. Nickel chloride ($NiCl_2$) was obtained as metallic salt for the test.

Sub-lethal toxicity bioassay

The test was conducted using the Organization for Economic Cooperation and Development (OECD) protocol #218 (10) in a sediment medium. Nickel as Nickel (11) Chloride was used to prepare the stock solution. Treatments were then prepared by serial dilutions; the treatment concentrations were 0.5mg/L, 1mg/L, 2mg/L, 4mg/L and 8mg/L. Periwinkles were then exposed to the treatment nickel concentration in plastic tanks measuring (27cm x 18cm x 20cm) for 30days. There were three replicates tanks per treatment and 6 periwinkles per tank including control groups. Periwinkles numbering 108 of similar sizes were used. They were fed once in two days with fish feed. On the 30th day, periwinkle tissues were carefully removed from their shells cleaned in distilled water and put in labelled plastic containers for digestion and analysis for nickel content.

Bioaccumulation and histopathology

For the metal analysis oven-dried samples were homogenised to powder by mechanical methods. Samples (0.5g) were digested in 10mls of concentrated H_2S0_4 at 100^{o}C until samples dissolved. They were allowed to cool and each digest was diluted to 100ml with distilled water. The nickel in the sample was estimated by atomic absorption spectrophotometer (AAS).

In carrying out the histopathological analysis, Sections of organs were fixed in 10% saline following removal from periwinkle shell. They were fixed for 24hours following which, tissues were processed in an automatic tissue processor machine (*Lieca 2000*) as previously described (11). Tissues were dehydrated by taking them through 70% alcohol for 1hour, 90% for 1hour, Absolute alcohol I for 1hour, Absolute alcohol II for 2hours, Absolute alcohol IV for 2hours. For clearing, the dehydrated tissues were taken through 3 baths of toluene 2 hours in each. The cleared tissues were each impregnated in 3 thermostatically controlled baths of molten paraffin wax (1 hour in bath 1, $1\frac{1}{2}$hours in bath 2 and $1\frac{1}{2}$hours in bath 3). The tissues were then brought out from the processing machine and then embedded using aluminium casts. The embedded tissues were then trimmed on a rotary microtome at 0 micrometer gauge. After this, tissue blocks were arranged on ice for 5 minutes in other to enhance section or slicing of the tissue. The sections were cut at 3 micrometre gauge and allowed to dry on a hot plate for 15 minutes. The dry slide/sections were then stained with haematoxylin and eosin staining technique. For the staining technique, Sections were dewaxed in 2 changes of xylene two minutes in each. Sections were taken through absolute alcohol, 90% alcohol, 70% alcohol and then water (H_2O) two minutes in each. They were then stained in Cole's haematoxylin for fifteen minutes. Sections were rinsed to remove excess dye solution and differentiated in 1% acid alcohol briefly. They were again rinsed in water and blued in running tap water for 10minutes. Counter staining was done in 1% aqueous eosin for three minutes. Sections were rinsed in water and dehydrated through 70%, 90% and absolute alcohol. The sections were transferred into 2 baths of xylene and then mounted in Canada balsam. The stained sections were examined using a light microscope. Magnification was (x 100).

Statistical analysis

Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Duncan's Multi sample Range post hoc test using SPSS 15 software (SPSS Inc. Chicago). Statistical significance was considered at $p<0.05$ level of significance

Results

The physicochemical parameters of the test media are shown in table 1. Values are expressed as mean ± standard error of mean (SEM).

Table 1: Physicochemical Parameters of test water

Parameter	Unit	Value
pH	–	6.81 ± 0.03
EC	µS/cm	97.00 ± 0.01
Temperature	^{o}C	24.30 ± 0.02
Dissolved Oxygen	mg/L	4.90 ± 0.01
Salinity	mg/L	31.33 ± 0.03
Total Dissolved Solids	mg/l	3.93 ± 0.02

Values are expressed as mean ±standard error of Mean (SEM).

Bioaccumulation results

Results showed that *T. fuscatus*var.*radula*bioaccumulated nickel in its tissues.

Bioaccumulation of nickel increased with increase in concentration of the metal (Fig 1) except in the 2mg/l exposure which was lower (0.136±0.01 mg/kg) than the 1.00mg/l exposure (0.163±0.00 mg/kg). Bioaccumulation of nickel in the periwinkle was significantly higher in all treatment concentrations than control groups. The highest and the lowest bioaccumulation value of Nickel in *T. fuscatus var. radula* were observed in the organisms placed in the test tank having 8mg/l and 0.001mg/l of nickel respectively

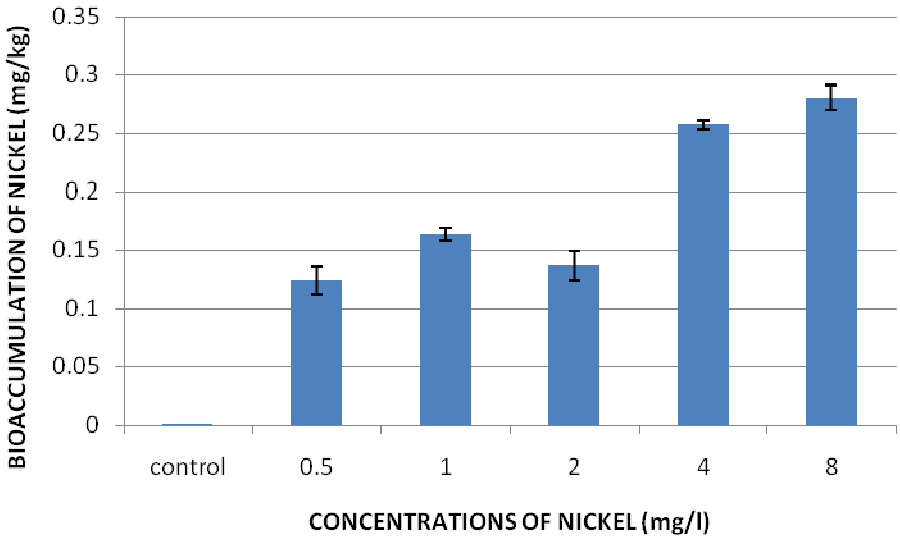


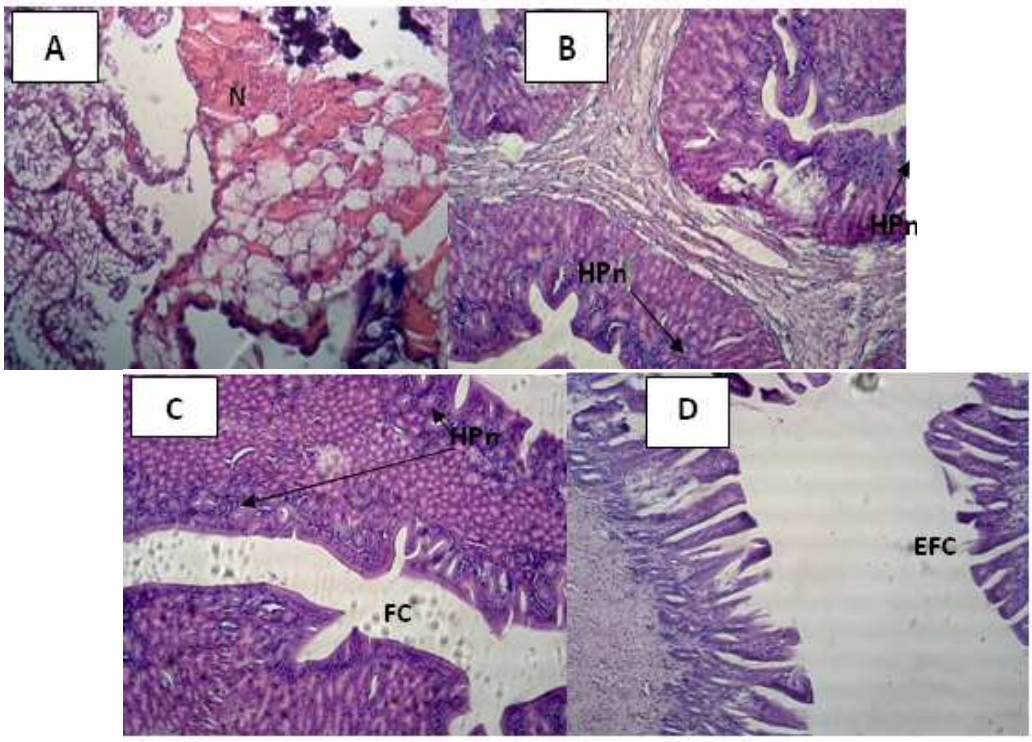
Fig 1: The bioaccumulation of Nickel after 30 days exposure.

The regressive statistics showed that there was a significant relationship between the concentration of nickel in the various test concentrations and the tissue bioaccumulation. The r^2 value is 0.714, this implies that the concentration of nickel in the various compartment contributed about 71.4% of the bioaccumulation of nickel by the *Tympanntonus fuscatus.*

Histopathology

Periwinkles in control groups showed normal histological structure of kidney and foot. Periwinkle exposed to lower concentrations of toxicant (0.5mg/L) showed hyperchromatic nuclei while fatty changes occurred in the 1, 2 4 and 8 mg/l exposures in kidney (Plate 1). Necrosis was observed in the highest concentrations (4mg/L and 8mg/L).

In the muscular foot (Plate 2), periwinkle exposed to lower concentrations of toxicant (0.5mg/L and 1mg/L) showed fatty changes while the periwinkle exposed to higher concentrations (2mg/L, 4mg/L and 8mg/L) showed excess fatty changes and hyperchromatic nuclei in the muscular foot. Brown deposits were observed around the fringes of the foot



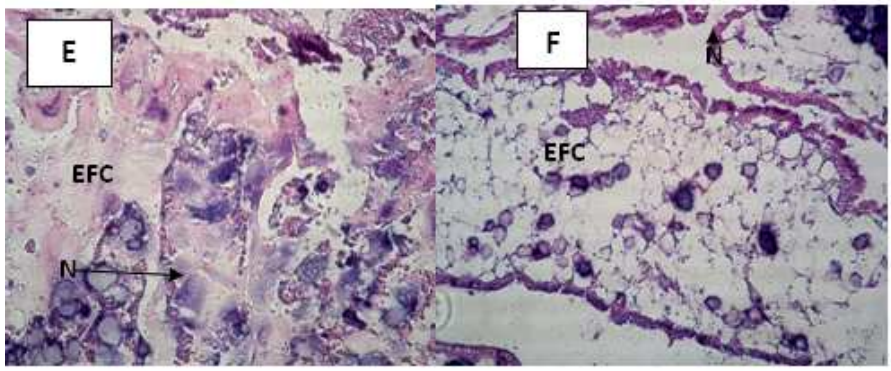


Plate 1: Histology of normal kidney and histopathological alterations of nickel exposed kidney.
(A) Normal kidney tissue with nuclei cells (n), (B) Showed hyperchromatic nuclei(HPn) with 0.5mg/l conc.(C) Hyperchromatic nuclei in tissue(HPn), fatty changes(FC) with 1mg/l(D) excess fatty changes(EFC) formed in 2mg/l. (E) Necrosis(N), excess fatty changes(EFC) in 4mg/l conc. (F) Necrosis(N), excess fatty change(EFC) and refractile bodies in 8mg/l conc.

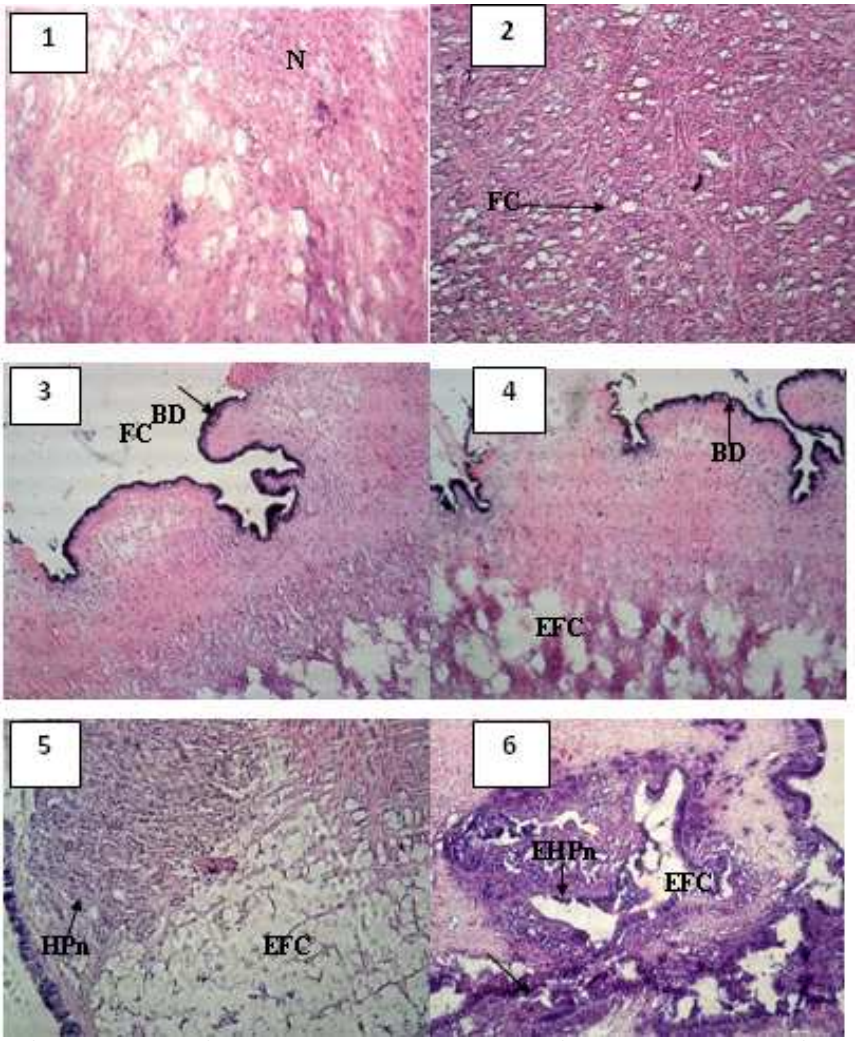


Plate 2: Histology of normal muscular foot and histopathological alterations of nickel exposed muscular foot.
(1) Normal muscular foot tissue with nuclei cells (n) (2) fatty bodies(FC) in 0.5mg/l conc. (3)Nuclei(n) and fatty changes(FC) in 1mg/l conc. (4) Nuclei (n) and excess fatty changes (FC) in 2mg/l conc. (5) Shows hyperchromatic nuclei(HPn) and excess fatty changes (EFC) in 4mg/l conc. (6) Excess hyperchromatic nuclei (EHPn) and excess fatty changes (EFC) in 8mg/l conc.

Discussion

Reports on bioaccumulation of Heavy metals by Gastropods are available (12, 13). The major observation made in this study indicated that the bioaccumulation of Ni increased as the concentration of the heavy metal increased. The increase was in line with the observation made by Otitoloju and Don- Pedro (14). The toxic functions of nickel probably result primarily from its ability to replace other metal ions in enzymes and proteins or to bind to cellular compounds containing O-, S-, and N-atoms, such as enzymes and nucleic acids, which are then inhibited.

The exposure of the periwinkle to sublethal concentrations of nickel caused varying degrees of histological alterations in the organs examined (kidney and muscular foot). The kidney and muscular tissue of *Tympanotonus fuscatus* from the control showed normal tissue and no visible gross lesions. For the exposed periwinkles, however, some pathological changes were observed in the tissues depending on ambient concentrations of the toxicant.

Periwinkle exposed to lower concentrations of toxicant (0.5mg/l) showed hyperchromatic nuclei while fatty changes occurred in the 1, 2 4 and 8 mg/l exposures in kidney. Necrosis was observed in the highest concentrations (4mg/l and 8mg/l). For the muscular foot, it was found that periwinkle exposed to lower concentrations of toxicant (0.5mg/l and 1mg) showed fatty changes while the periwinkle exposed to higher concentrations (2mg/l, 4mg/l and 8mg/l) showed excess fatty changes and hyperchromatic nuclei in the muscular foot. Brown deposits were observed around the fringes of the foot. This result shows that nickel could inflict histopathological changes on *T. fuscatus* var. *radula*

Hart and Ulonnam (15) determined the toxicity and histopathological effects of oil-based drilling mud, using the edible periwinkle, *Tympanotonus fuscatus*. Pronounced percentage mortality occurred in periwinkle exposed to as low as 2% concentration by volume of the drilling mud. Histopathological consequences of the tested mud on the periwinkles included irregular tissue shape, macrophage, inflammatory cells and basophilic spots. Uptake of barium, a carcinogenic component of the drilling mud by the periwinkle, the magnitude of barium uptake was relatively higher.

Otitoloju *et. al*., (16) reported the exposure of the snails to sublethal concentrations of heavy metals caused varying degrees of histological alterations in the organs examined (hepatopancreas and ovotestes. The exposure of the snails to sublethal concentrations of the metals resulted in a prevalence of hepatocellular foci of cellular alterations in thehepatopancreas of snails. Hepatocytes of test animals exposed to sublethal concentration of Cuwere also observed to be clogged together with peripheral thickening while exposure to lead caused inflammation of hepatictubules. Basophilic adenoma and ovotesticular fibrillar inclusions were also observed in the ovotestes of snails exposed to the test metals.

This ability of the periwinkle to adapt to nickel contamination and accumulate the metal confers on it the potential of being used in eco-toxicological monitoring as a sentinel of nickel pollution.

Conclusion

The observations made in this study which indicate that the concentration of nickel in the tissues of *Tympanotonus fuscatus* var. *radula* increased as the concentration of nickel in the test medium increased. This is an indication that this organism can serve as a good indicator of nickel pollution in the environment. Histopathological changes which could affect the health of the organism were observed. The discharge of effluents containing heavy metals especially nickel should be discouraged as this could affect the health of the organism and exacerbate the problems of global biodiversity loss.

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