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Bioaccumulation of heavy metals in some fauna and flora

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ABSTRACT: Bioaccumulation of heavy metals in fauna and flora in the Ijaw area of the Niger Delta of Nigeria were investigated. The fauna-molluscs and crustacea, and flora Hibiscus esculentus and vernonia amygdalina were selected for this study. Results show that Molluscs and crustacea accumulate considerable amounts of heavy metals. Higher levels of all the metals determined Hg, Pb, Cr, Cu, Ni, and Zn were observed in the shells than in the fleshly tissues. Hibiscus esculentus and vernonia amygdalina also accumulated these heavy metals. The mercury levels in the roots of Hibiscus esculentus and vernonia amygdalina is 0.010 μg/g ± 0.00, while the stem 0.17 ± 0.03 μg/g and leaves 0.25 ± 0.02μg/g was recorded for the v. amygdalina. The H. esculenta fruit has lead levels of 0.22 ± 0.03 μg/g. The levels of Cu, Ni, and Zn are generally higher than those of Hg, Pb and Cr in all the samples analyzed. There is a growing concern about the physiological and behavioral effects of environmental trace metals in human population. The toxicity of lead at high levels of exposure is well known but of a major concern is the possibility that continual exposure to relatively low levels of these heavy metals through the consumption of these fauna and flora may entail adverse health effects.

Key words: *Heavy metals, industrial effluents, molluscs, crustacea, Hibisucs esculentus, vernonia amygdalina, toxicity, bioaccumulation*

INTRODUCTION

Heavy metals are natural components of the earth's crust and they can enter the water and food cycles through a variety of chemical and geochemical processes. (Tinsley 1979; NDES 1999).

Advancement in technology as well as increase in population have led to environmental concerns relating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste water, and crude oil spills replete with most common heavy metals in our environment (Wills, 2000). SCEP (1971) reported that various activities by man in recent years have increased the quality and distribution of heavy metals in the atmosphere, land and water bodies. Pollution of streams and rivers could occur due to run-offs flowing through agricultural areas where pesticides and fungicides, etc., may have been applied and industrial districts where there may have been waste deposits (Gustav, 1974). Water sediments and the biota are generally metal reservoirs in aquatic environments (Warren, 1981). The concentrations of heavy metals in water may vary considerably depending on annual and seasonal fluctuations (SCEP, 1971). Bower (1979) noted that the extent of accumulation in biota is dependent on the chemical effects of the metal, its tendency to bind to particular materials and on the lipid content and composition of the biological tissue. Various activities by man in recent years have increased the quantity and distribution of heavy metals in the atmosphere, land and water bodies. The extent of this wide spread but diffused contamination has raised concern about their hazards on plants, animals and humans.

The fate of heavy metals introduced by human activities into aquatic ecosystems have recently become the subject of wide spread concern, since beyond the tolerable limits they become toxic (WHO 1971, Pocock, *et al.*, 1994, Koller, *et al.*, 2004). Determination of harmful and toxic substances in water sediments and biota, gives direct information on the significance of pollution in the aquatic environment (Hugget, *et al.*, 1973). We therefore thought it necessary to investigate the concentrations of Ag, Pb, Cr, Cu, Ni, and Zn in molluscs and crustacea, okro and bitterleaf found in the Amasoma District of Bayelsa state of Nigeria. This area is in the Niger Delta and one of the

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coastal areas of Nigeria where there is massive oil exploitation and exploration. This research has been done in Amasoma city in Bayelsa State of Nigeria onFebruary, 25th, 2006.

MATERIALS AND METHODS

Samples collection

Molluscs and crustacea were selected for the fauna, while H. esculentus (okro) and V. amygdalina (bitter leaf) were selected for the flora. The molluscs being harmless were hand picked from sources, but the crustacea were caught by trapping or netting device. (Hugget, *et al.*, 1973). The giant land snail, a terrestrial molluscs and the periwinkle, an aquatic molluscs were studied. While crustacea studied were the fiddler crab (Uca Pugilator) a terrestrial crustacea and the shrimp, (Crago nigricanda) an aquatic crustacea. The mollusc and crustacea have hard shells which house the soft tissue. The shells are often greater in quantity than the tissue. Upon collection, the samples were washed with tap water and rinsed thoroughly with distilled water, and transported in cellophane bags to the laboratory.

The plant and animal samples were properly identified at the Departments of Plant Science and Biotechnology; and Animal and Environmental Biology, Abia State University, Uturu Nigeria respectively; and samples deposited at the University herbarium.

Sample preparation/chemical analysis

Molluscs and crustacea: Samples preparation

The molluscs were placed in clean water in a large laboratory trough. Soon their fleshly parts came out from the shell in order to move freely in water. They were held and pulled out and removed from their shells. Thus their fleshy parts were separated from the shells. Both parts were rinsed properly with distilled water. They were placed in clean watch glasses and dried in an oven at 105 °C until brittle enough for grinding. After grinding to fine powder the samples were further dried to constant weight at 105 °C; then put in labeled polythene bags and preserved in desiccator. The shells of the crustacea are more easily remove after drying. The shells and flesh were ground into fine powder, put in labeled polythene bags and preserved in a desiccator.

Chemical analysis

2 g of each of the samples were accurately weighed into digestion flasks. A mixture of concentrated trioxo-nitrate (V) acid (HNO_3) and tetra-oxo-chlorate (VII) acid ($HClO_4$) (2:1) was added and then digested. The resultant residue was dissolved in 10 cm^3(1:1) Hydrochloric acid (HCl); and then diluted to the 100cm^3 mark with distilled water. This solution was used for the determination of the heavy metals in triplicates on atomic absorption spectrophotometer (AAS) in accordance with Perkin-Elmer (1973) and standard methods APHA (1990).

H. esculentus and V. amygdalina: Sample preparation

Hibiscus esculentus and Vernonia amygdalina plants are cultivated as vegetables. The H. esculentus (okro) fruits and the tender leaves of V. amygdalina are used for the preparation of soup.

Young fruiting H. esculenta plants were uprooted, while the young, tender plants of V. amygdalina with edible leaves were uprooted. The plants collected were washed with tap water to remove sand, dirt, and then rinsed thoroughly with distilled water. Stainless steel knife was used to cut the plants into fruits, leaves, stems and roots. The separate plant parts were dried to constant weight in an oven at 105 °C. They were pulverized to fine powder using the laboratory mill. They were then put separately into labeled polythene bags preserved in a desiccator.

Chemical analysis

2 g of each sample was accurately weighed into clean platinum crucibles, ashed at 450-500 °C for 12 h, and then cooled to room temperature in a desiccator. The ash was dissolved in 5cm^3 20% hydrochloric acid (HCl), and the solution carefully transferred in into a 100 cm^3 volumetric flask. The crucible was well rinsed with distilled water and transferred to the volumetric flask, and was then made up to the mark with distilled water. The flask was thoroughly shaken to mix well. Analysis of the samples for heavy metals were also carried out in triplicate using the AAS in accordance with standard methods (APHA 1990) and Perkin Elmer (1973).

Statistical analysis

Data were analyzed using the one-way analysis of variance (ANOVA) and group means were compared using Duncans multiple range test. p values < 0.05 were considered significant.

RESULTS

Table 1 shows the concentration of heavy metals in the flesh and shell of mollusks and crustacean. The shells contain high concentrations of heavy metals compared to the flesh. The crustacea Uca pugilator (fiddler crab) shows higher concentration of the heavy metals investigated compared to the flesh of Arathchatina maginata; litorina littorea and crago nigricando; showing concentrations of 0.04±0.01, 1.07±0.02, 0.57±0.03, 2.28±0.04, 1.97±0.03 and 7.04±0.04 for Hg, Pb, Cr, Cu, Ni and Zn respectively. The molluscs – Arathchatina maginata showed very low concentration of the heavy metals, 0.1±0.01, 0.37±0.03, 0.17±0.02, 0.89±0.04, 0.33 ±0.03, and 2.58±0.05 for Hg, Pb, Cr, Cu, Ni and Zn respectively. The mollusc - litorina littorea showed higher heavy metal accumulation- 1.81 ±0.04,5.81 ±0.06, 4.68±0.06, 7.08±0.04, 3.48±0.31and 30.00±0.05 for Hg, Pb, Cr, Cu, Ni and Zn respectively, while the crago nigricando had lower heavy metal content- 0.16±0.03, 2.01±0.01, 1.10±0.03, 1.18±0.02, 1.08±0.04 and 5.58±0.03 for Hg, Pb, Cr, Cu, Ni and Zn respectively. Table 2 shows the concentration of heavy metals in the plants analysed. The edible parts of the plants is the fruit for H.esculenta, and the leaf for V amygdalina.The H.esculenta has lower concentration of the heavy metals- 0.01± 0.01, 0.22± 0.03, 0.04± 0.01, 0.12±0.03, 0.08±0.02 and 0.65 ± 0.03 for Hg, Pb, Cr, Cu, Ni, and Zn respectively, compared to V.amygdalina leaf – 0.25 ± 0.02,0.41 ±0.02, 0.06 ±0.02, 0.15±0.02, 0.13 ± 0.03 and 1.03±0.03 for Hg, Pb, Cr, Cu, Ni and Zn respectively. The stems of plants accumulate higher concentration of these metals. H.esculenta (0.05 ± 0.01, 0.41 ± 0.02, 0.06 ± 0.03, 0.16 ± 0.03, 0.14 ± 0.01,1.10 ± 0.00) and V. amygdalina (0.17 ± 0.03, 0.47 ± 0.02, 0.060 ± 0.02, 0.13 ± 0.01, 0.17 ± 0.01, 1.48 ± 0.01) for Hg, Pb, Cr, Cu, Ni and Zn respectively.While the roots show much higher concentration than the stem.

DISCUSSION AND CONCLUSION

Table 1 shows heavy metal concentrations in molluscs and crustacea. Generally our results show that the shells seem to contain higher concentrations of the heavy metals than the flesh. The giant land snail has a concentration of 0.53 ± 0.01mg/g Hg in its shell and 0.10 ± 0.01 mg/g in the flesh; while Pb, Cr, Cu, Ni, and Zn were 1.52 ±0.01; 1.46 ± 0.04, 2.10 ± 0.03, 2.13 ± 0.03 and 12.81 ± 0.04mg/g respectively for the shell, while in the flesh the concentrations of these metals were 0.37 ± 0.03; 0.17 ± 0.02, 0.89 ± 0.04; 0.33 ± 0.03 and 2.58 ± 0.05mg/g respectively.

Table 1: Heavy metal concentration in molluscs and crustacea (μg/g) dry weight determination was in triplicate

Biological name	Common name /part	Hg	Pb	Cr	Cu	Ni	Zn
H. esculenta	Okoro						
	Root	0.01 ± 0.00	0.50 ± 0.03	0.06 ± 0.01	0.20 ± 0.03	0.16 ± 0.04	1.83 ± 0.05
	Stem	0.05 ± 0.01	0.41 ± 0.02	0.06 ± 0.03	0.16 ± 0.03	0.14 ± 0.01	1.10 ± 0.00
	Leaf	0.02 ± 0.03	0.37 ± 0.01	0.05 ± 0.01	0.15 ± 0.01	0.13 ± 0.02	0.88 ± 0.02
	Fruit	0.01 ± 0.01	0.22 ± 0.03	0.04 ± 0.01	0.12 ± 0.03	0.08 ± 0.02	0.65 ± 0.03
V. amygdalina	Bitter Leaf						
	Root	0.01 ± 0.01	0.48 ± 0.03	0.06 ± 0.02	0.20 ± 0.00	0.19 ± 0.02	1.85 ± 0.02
	Stem	0.17 ± 0.03	0.47 ± 0.02	0.06 ± 0.02	0.13 ± 0.01	0.17 ± 0.01	1.48 ± 0.01
	Leaf	0.25 ± 0.02	0.41± 0.02	0.06 ± 0.02	0.15 ± 0.02	0.13 ± 0.03	1.03 ± 0.03

Table 2: Heavy metal concentration in hibiscus esculenta and vernonia amygdalina (μg/g) dry weight determination was in triplicate

Sample	Biological name	Common name	Hg	Pb	Cr	Cu	Ni	Zn
Flesh moldiness	Arathchatina maginata	Giant land snail	0.1 0± 0.01	0.37 ± 0.03	0.17 ± 0.02	0.89 ± 0.04	0.33 ± 0.03	2.58 ± 0.05
	Litorina littorea	Periwinkle	0.22 ± 0.02	1.77 ± 0.03	0.56 ± 0.02	2.50 ± 0.03	1.17 ± 0.04	10.86± 0.06
Shell molluscs	Arathchatina maginata	Giant land snail	0.53 ± 0.03	1.52 ± 0.01	1.46 ± 0.04	2.10 ± 0.03	2.13 ± 0.03	12.81± 0.04
	Litorina littorea	Periwinkle	1.81 ± 0.04	5.81 ± 0.06	4.68 ± 0.06	7.08 ± 0.04	3.48 ± 0.31	30.00± 0.05
Flesh crustacea	Uca pugrlator	Fiddler crab	0.04 ± 0.01	1.07 ± 0.02	0.57 ± 0.03	2.28 ± 0.04	1.97 ± 0.03	7.040± 0.04
	Crago nigricando	Shrimp	0.15 ± 0.02	0.46 ± 0.03	0.23 ± 0.01	0.68 ± 0.03	0.92 ± 0.01	3.700± 0.01
Shell crustacea	Uca pugilator	Fiddler crab	1.51 ± 0.02	6.03 ± 0.01	4.40 ± 0.04	5.72 ± 0.04	4.27 ± 0.04	26.41± 0.03
	Crago nigricaudo	Shrimp	0.16 ± 0.03	2.01 ± 0.01	1.10 ± 0.03	1.18 ± 0.02	1.08 ± 0.04	5.580± 0.03

The periwinkle also has higher concentrations of these metals in its shell than in the flesh – 0.22 ± 0.02, 1.77 ± 0.03, 0.56 ± 0.02, 2.50 ± 0.03, 1.17 ± 0.04 and 10.86 ± 0.06mg/g respectively for the flesh, compared to those of the shell – 1.81 ± 0.04, 5.81 ± 0.06, 4.68 ± 0.06, 7.08 ± 0.04, 3.48 ± 0.31 and 30.0 ± 0.05mg/g respectively. The periwinkle flesh seems to have accumulated higher concentrations of the heavy metals compared to the flesh of the giant land snail – 0.22 ± 0.02, 1.77 ± 0.03, 0.56 ± 0.02, 2.50 ± 0.03, 1.17 ± 0.04 and 10.86 ± 0.06mg/g for Hg, Pb, Cr, Cu, Ni, and Zn respectively, compared to 0.10 ± 0.01, 0.37 ± 0.03, 0.17 ± 0.02, 0.89 ± 0.04, 0.33 ± 0.03 and 2.58 ± 0.05mg/g for the flesh of the giant land snail respectively for the same metals. There is a significant difference at ($p < 0.05$) level of significance for all the metals in the analyzed sample of periwinkle and the giant land snail.

A similar trend was observed with the crustacea. The shells of the fiddler crab and shrimp seem to have higher concentrations of the heavy metals compared to the flesh. For the fiddler crab, the shell contains 1.51 ± 0.02, 6.03 ± 0.01, 4.40 ± 0.04, 5.72 ± 0.04, 4.27 ± 0.04 and 26.41 ± 0.03mg/g respectively for the metals; compared to the flesh with 0.04 ± 0.01, 1.07 ± 0.12, 0.57 ± 0.03, 2.28 ± 0.01, 1.97 ± 0.03 and 7.04 ± 0.04 mg/g respectively. The shrimp also had higher concentrations of these metals in its shell compared to the flesh; 0.16 ± 0.03, 2.01 ± 0.01, 1.10 ± 0.03, 1.18 ± 0.02, 1.08 ± 0.04 and 5.58 ± 0.03mg/g respectively compared to 0.15 ± 0.02, 0.46 ± 0.03, 0.23 ± 0.01, 0.68 ± 0.02, 0.92 0.01 and 3.70 ± 0.01mg/g respectively for the flesh. The fiddler crab flesh seems to contain higher concentrations of the heavy metals compared to the flesh of the shrimp – 0.04 ± 0.01, 1.07 ± 0.02, 0.51 ± 0.03, 2.28 ± 0.04, 1.97± 0.03 and 7.04 ± 0.04mg/g respectively; while the shrimp flesh had 0.15 ± 0.02, 0.46 ± 0.03, 0.23 ± 0.01, 0.68 ± 0.03, 0.92 ± 0.01 and 3.70 ± 0.01mg/g respectively. Except for Hg, the difference in concentration for each of the heavy metals for the shrimp and fiddler crab flesh is quite significance at ($p < 0.05$) level of significance.

Zn seems to have the highest concentration in the flesh of all the samples analyzed. Giant land snail has 2.58 ± 0.05, periwinkle 10.86 ± 0.06, fiddler crab 7.04 ± 0.04 and shrimp 3.70 ± 0.01. Zinc is followed by copper, then chromium, nickel, lead and mercury. In terms of nutritional implications, it seems the giant land snail and the shrimp should be recommended due to their lower heavy metal content.

Table 2, shows the result on the plants. The roots of the plants seem to accumulate higher concentrations of the heavy metals. The okro fruit seems to accumulate lower concentrations of heavy metals compared to the root, stem and leaves. The difference in concentration of the heavy metals in the okro leaves and fruits is quite significant at ($p < 0.05$) level of significance. The leaf contains 0.02 ± 0.03, 0.37 ± 0.01, 0.05 ± 0.01, 0.15 ± 0.01, 0.13 0.02 and 0.86 ± 0.02μg/g of Hg, Pb, Cr, Cu, Ni, and Zn respectively, while the fruit contains 0.01 ± 0.01, 0.22 ± 0.03, 0.04 ± 0.04, 0.12 ± 0.03, 0.08 ± 0.012 and 0.65 ± 0.03μg/g respectively. For the bitter leaf, the concentration of the heavy metals is slightly lower in leaves compared to those of the root and stem, which is not statistically significant at ($p < 0.05$) level of significance. For both plants, it is the leaves of V. amygdalina and fruits of H. esculentus that is used for the preparation of soup, while the flesh of the molluscs and crustacea is consumed by man as protein supplement. Cr, Cu, Ni and Zn are beneficial to man at lower or standard levels, since they are integral parts of important physiologic compound such as Zn, Cr, Ni and Cu in certain enzymes, where it is essential for their activity (Stansell and Deutsch, 1965). Cr, Ni, and Zn have been suggested as essential trace elements in nutrition, whose functions included regulation of apoptosis, activation of depressed immune system, and as cofactors for metalloenzymes. Ni is invloved in fat metabolism and aid in fat deposition (Hardy, 1962, Goyer, 1995). The levels of Cu, Ni and Zn in both fauna and flora investigated (Tables 1 and 2) are generally higher than those of Hg, Pb, and Cr. Cu, Ni, and Zn and are of no toxicological significance, since for example hypercupremia occurs but has no diagnostic significance (Harper 1975). From our results, it seems all the samples analyzed have very high concentration of the heavy metals investigated. The nutritional implication is that consumers of these food materials may be exposed to heavy metal toxicity if bioaccumulation results due to regular consumption (WHO, 1971, Goyer, 1995, Ross and Morison 2002).

The high levels of Hg, Pb and Cr in the flesh of the fauna, fruit of okro and leaves of bitter leaf (Tables 1 and 2) are of public health concern. The levels are far beyond the tolerable level of 0.001 μg/g set by WHO.

(WHO, 1971). Though these food materials are processed (heating, cooking) before consumption, the effect of processing could be minimal, since the heavy metals are non-degradable. Mercury toxicity can occur after microbial degradation of Hg to dimethyl mercury. Human exposure to dimethyl mercury occurs through consumption of contaminated marine or aquatic foods. Hg affects the central nervous system and brain due to its ability to cross the blood brain barriers (Clark, *et al.*, 1997). There is growing concern about the physiological, biochemical and behavioural effects of environmental trace metal in human population. The toxicity of Pb at high level of exposure is well known, but the concern of today is the possibility that continual exposure by the use of the analyzed samples as source of protein and sauce making may result to gradual accumulation of these metals in the human system and may lead to adverse health effects (Bergback, *et al.*, 1992, Koller, *et al.*, 2004).

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