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Tissue Distribution and Bioavailability of Trace Elements in Ergeria Radiata from the Upper Reaches of the Nun River, Niger Delta, Nigeria

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Abstract
Fresh samples of the clam *Ergeria radiata* were collected at six stations spanning 50 km along the upper reaches of the Nun River, Niger Delta, Nigeria. The animals were dissected into the foot, gills, viscera and shell and digested separately according to standard methods. Selected trace elements (PZn, Cu, Cd, Pb, Cr, and Ni) were analysed in each category to delineate the portioning of the elements in the organism.

Introduction

The discharge of industrial, domestic and institutional effluents into the environment bearing heavy elements and other contaminants has raised concerns about their potential human health impact over the years. Despite these concerns which have been scientifically documented, households, institutions, industries and oil and gas organizations still discharge effluents into the environment with little or no treatment especially in less developed countries like Nigeria. The aquatic environment is usually the most hit in terms of effluent discharges which enters it by direct piping or indirectly by storm water runoffs and through wastewater treatment discharges. For several years it was thought that the aquatic environment has infinite ability to dilute contaminants, it has however been reported that its carrying capacity has been jettison since the industrial evolution due to discharges of unprecedented quality and quantity of waste into the aquatic environment resulting to pollution (Babatunde et al., 2013).

Aquatic organisms thus exposed to such pollution have been known to concentrate trace elements and other contaminants in their tissues passing it along the food chain where humans occupy the apex. The bioaccumulation of elements in aquatic organisms is influenced by several factors ranging from the ecology of the species to speciation of the elements, uptake and elimination kinetics, assimilation rates and cellular distribution in the body of the organisms (McGeer *et al.,* 2004; Chindah *et al.,* 2009). These factors which can be categorised into 3 as follows:
- Physiological parameters (uptake, filtration rates, assimilation efficiency, excretion rate)
- Biological factors (sex, species, health status, and reproduction cycles)
- Environmental parameters (concentrations and speciation, food quantity and quality)

are responsible for why animals and humans are not acutely poisoned after consuming seafoods from contaminated environment. However, I is strongly warned that continual exposure to sub-acute levels of trace elements above stipulated limits have been reported to cause serious health challenges including damage to the kidney and liver, leukaemia and other forms of cancer (Katz and Salem, 1993; ATSDR 200; Stift *et al.,* 2000; WHO 2011; (Martin and Griswold, 2009; WHO 2011).

The process of bioavailability of trace elements begins with partitioning of the elements in various parts of the organism. Bioavailability of the elements thus depends on if that part consumed by humans is where the organism concentrated the trace elements. The concentrated elements in the eaten part of the organisms undergo digestion, assimilation and elimination in similar manner as any other food eaten by the humans Figure 1. Depending on the consumption rate and concentration of the elements as well as other metabolic actions, the cells and tissues may receive trace metal doses above stipulated levels which accounts for health challenges experienced by the individual.

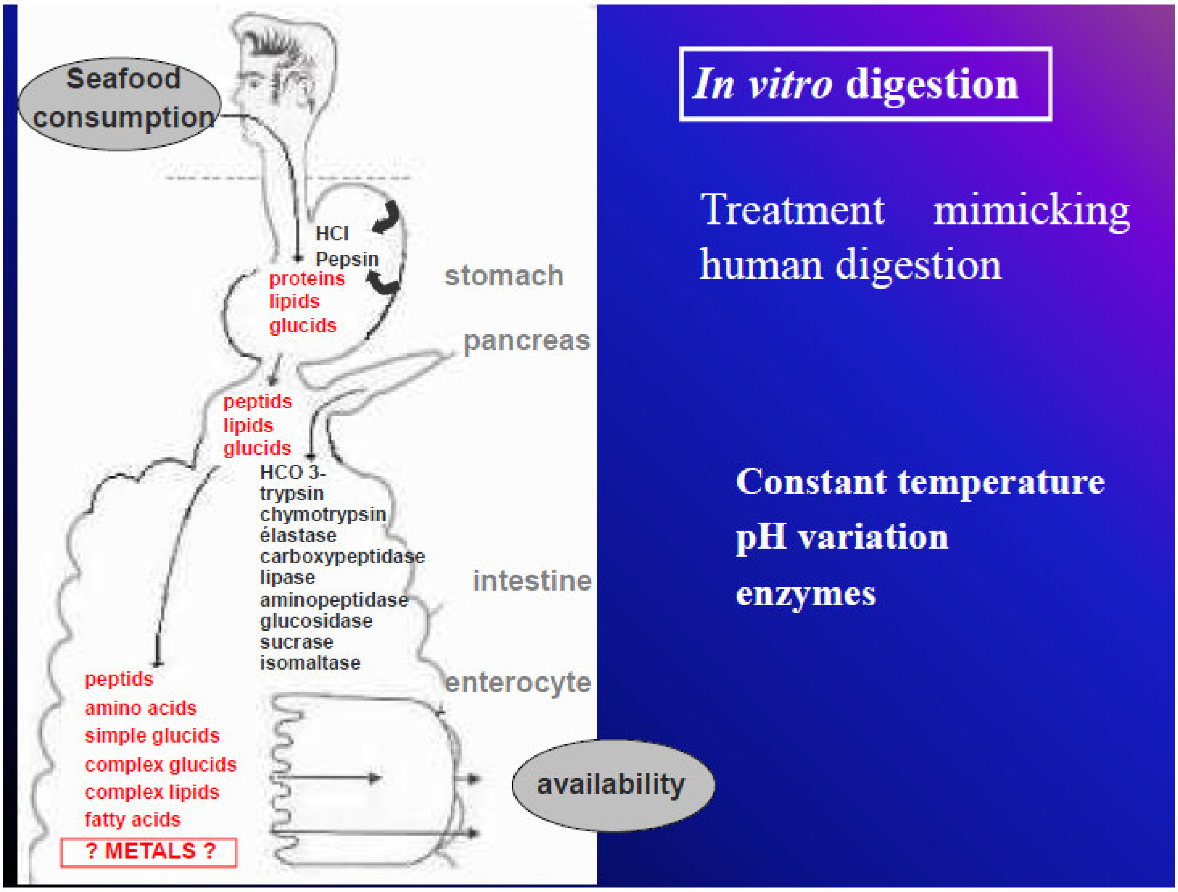

Fig. 1: Digestive Process of elements in seafood showing bioavailability to body cells.

The freshwater clam studied in the present research, *E. radiata,* also called River oyster or Volta clam, is endemic to the Volta River in Ghana and some southern Nigerian rivers, particularly common Itu River in Cross River State (Edmunds, 1978; Yoloye, 1988). The clam is also exploited in artisanal fisheries for food in the Nun River basin in the Niger Delta. Shellfish, especially clams like *E. radiata,* are used largely as a condiment in most meals eaten in the Niger Delta and its environs (Gomna and Rana, 2007; Babatunde et al., 2015) and may accumulate elements at levels which can become deleterious to human consumers. *Ergeria radiata* as a bivalve has been known to bioaccumulate heavy elements (Ekpo et al, 2015) and thus a useful bioindicator of metal contamination in aquatic environment. For example, Nwanbueze, (2011) reported elevated concentrations of heavy elements in tissues of *E. radiata* from some creeks in Delta State, Nigeria above concentrations in the environment and particularly Pb, Mn and Cd as higher than FAO/WHO acceptable limits of heavy metal contamination in fishes and shell fish. Similarly, Etim, (1990) reported elevated heavy metal contamination in tissues of *E.radiata* from Calabar River, Cross River, Nigeria above the environmental concentrations indicating the animal bioaccumulated the elements. Indeed, numerous studies around the world have demonstrated their ability to concentrate trace elements, even in areas far from anthropogenic sources such as the Antarctic Ocean (e.g., Mauri et al., 1990; Berkman and Nigro, 1992; Viarengo et al., 1993), with seasonal variations in the concentrations at various stages of their lives (Bryan, 1973) and in the Bay of La Rochelle in France (Bustamante and Miramand, 2005).

In the Niger Delta, Nigeria, oil exploration and exploitation of oil is associated with several operational and accidental spills, use of drilling chemicals, flaring of gases, and burning of fuel that release heavy elements into coastal waters. Pollution studies have revealed elevated levels of Pb, Cr, Ni, V, and Zn in surface water, sediments and some species of fauna, suggesting inputs from petroleum exploration and exploitation (Kakulu & Osibanjo, 1992; Horsfall and Spiff, 2002; Howard *et al.,* 2008; Davies et al., 2004; Chindah et al., 2004; Babatunde et al., 2013; Onojake et al., 2015). Consequently, the concentration of these elements in Nigerian coastal waters and sediment are of great concern, warranting the need for periodic sampling and analyses of both water and water resources in order to monitor the pollution and productivity status of the marine ecosystem and compare the data with international standards (Ajao *et al.,* 1996; Nubi *et al.,* 2008).

The present study analysed and evaluated the tissue distribution of trace elements in *E. radiata* caught in the upper reaches of the Nun River, Bayelsa State, Nigeria. This study is expected to elucidate and provide scientific knowledge on the parts of the organism favoured by the trace elements analysed. Such information would be useful to the consumers to know what part of the organism to consume in order to reduce exposure to trace elements concentrated by the organism.

Materials and Methods

Study Area

The Nun River is one of the major arm of the River Niger drainage system that empties directly into the Gulf of Guinea Atlantic Ocean Fig. 2. Six stations along the Upper reaches of the Nun River within Southern Ijaw Local Government Area of Bayelsa State, Nigeria were mapped out by geo-referenced coordinates (Fig.2) where samples of *Ergeria radiata* were collected bimonthly between August 2013 and February 2014. Within the area, the river drains and receives effluents from the activities of oil companies drilling for oil and commercial boat drivers. The river also serves as sewage/rubbish disposal medium for the surrounding communities, in which they also do most of their laundry along the riverbanks. The river is also, in recent times, "home" to the activities of illegal artisanal refineries and also a receptacle of runoffs from the surrounding agricultural fields. The climate of the area is typically tropical with dry (November-March) and wet (April-October) seasons. Rainfall is bimodal, peaking usually in July and again in September with a brief drop in August. Minimal rainfall is in January and February, followed by the onset of heavy rainfall in April. Annual temperature ranges from 22 - 32^{oC}, while annual humidity is between 69 and 96% (NIMET, 2010).

The sampling stations with their coordinates are: Ogbonogbene (Station 1) N04^0 53' 17.8" E 006^0 53' 58.3", Umbugbene (Station 2) N04^0 48' 06.9" E 006^0 01'04.2", Ondewari (Station 3) N04^0 46' 14.0" E 006^0 00' 24.2", Korokorosei (Station 4) N04^0 45' 19.8" E 006^0 00' 54.4", Ogbainbiri (Station 5) N04^0 50' 00.8" E005^0 58'40.0", and Ogbainbiri (Station 6) N04^0 49'17.7" E 005^0 57'51.4" (Figure 1). There are oil wells being currently drilled for oil at stations 5 and 6.

Sample Collection

Collection of *Ergeria radiata* samples

Matured *E. radiata* specimens were collected directly from the river at each sampling station with the help of fishermen who harvested the shellfish by diving. They were then put in plastic containers with ice, labelled and taken to the laboratory the same day and refridgerated for further analysis. An average of five animals were taken per station on each sampling date.

Each animal was dissected fresh and the various parts (foot, gills, viscera and shell) separated before drying in hot air oven according to ASTM (D 4638). Sample were homogenised into fine powder using Agatha mortar and pestle.

Sample Preparation

Sample Digestion

The method described by Allen *et al.,* (1974) and APHA, (1998) were used. Few drops of water was added to 10 g portion of the biota pulverised samples to wet it in a 100 ml beaker. Thereafter, 2 ml perchloric acid, 4 ml nitric acid, and 1 ml sulphuric acid were added and the sample was slowly digested for one hour. More acids was added until a clear solution was obtained. The completely digested samples were allowed to cool at room temperature, diluted using 1.5 M HCL acid and filtered through a 0.45 μm membrane filter and the volume made up to 50 ml in volumetric flasks with double distilled water. All digested samples were analyzed three times for the elements using AAS (Varian Spectre AA 200 Fast Sequential Flame Atomic Absorption Spectrometry). The instrument was calibrated with standard solutions prepared from Merck. The analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. The accuracy and precision of our results were checked by analysing standard reference material (SRM, IAEA-360 Mediterranean sediment). All metal concentrations were quoted as mg kg-1 dry weight unless otherwise stated. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. Doubled distilled water was used throughout the study. All glassware and other containers were thoroughly cleaned with 10% (w/v) nitric acid solution and finally rinsed with double distilled water several times and air dried prior to use. One-way analysis of variance (ANOVA) and Duncan's test (p=0.05) were used in order to access whether heavy metal concentrations varied significantly between sites and species. The probabilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

Results and Discussion

Results of trace elements analysed in the river clam, *E. radiata* are presented in Figures 3 and 4 for wet and dry seasons respectively. In the wet season, (Figure 3), Pb concentrations were 0.04, 0.17, 0.34 and 0.04 mg/kg in the gills, foot, shell and viscera of the clam, respectively. Cr concentration was 0.12, 0.18, 1.05 and 0.23 mg/kg for gills, foot, shell and viscera, respectively. Cu had values of 0.43, 0.63, 0.30, and 0.88 mg/kg in gills, foot, shell and viscera, respectively. Zn levels were 3.63, 3.62, 2.32 and 3.53 mg/kg in the gills, foot, shell and viscera of the clam, respectively, while Ni and Cd were not detected in any of the parts of the clam.

In dry season, Pb was not detected in any part of the clam, Cr concentration was 0.9, 0.35, 2.73 and 0.89 mg/kg in the gills, foot, shell and viscera of the clam, respectively. Cu had values of 0.56, 0.70, 0.29 and 0.77 mg/kg in the gills, foot, shell and viscera, respectively. Zn concentrations were 2.86, 2.43, 1.32, and 3.05 mg/kg in the gills, foot, shell and viscera of the clam, respectively, while, Ni was not detected in the shell but had concentrations of 1.05, 0.11 and 1.18 mg/kg in the gills, foot and viscera, respectively. Cd was only detected in the gills (0.03 mg/kg).

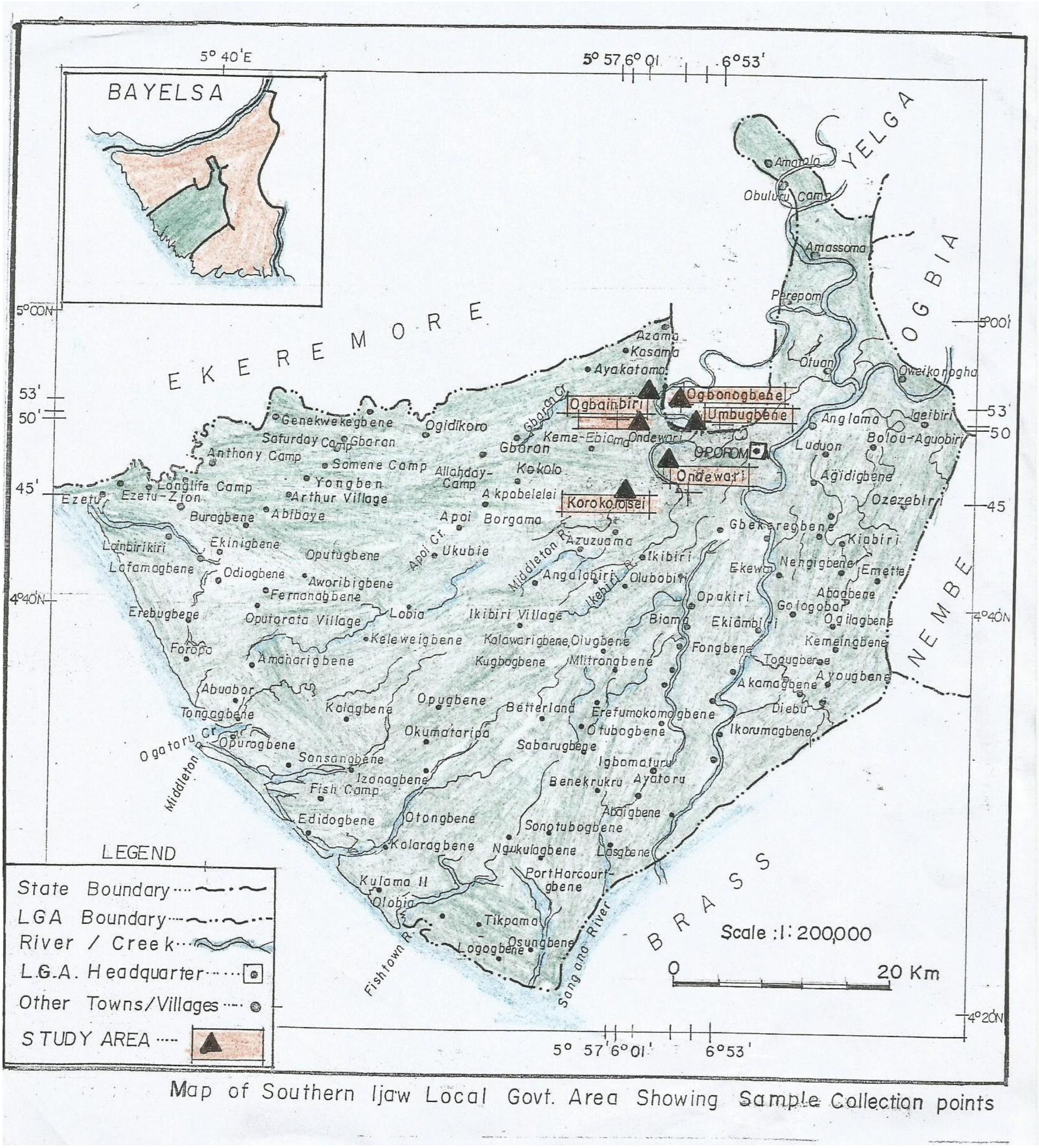

Fig.2: Map of Southern Ijaw LGA in Bayelsa showing sample locations with black triangles

Heavy metal concentrations in the clam showed irregular distribution pattern in the tissue compartments between the seasons. This may be as a result of seasonal ecological changes, which may influence feeding habit of the clam. Life cycle attributes reflecting availability of food may also influence the different patterns of compartmentalization of elements in the organism in the different seasons.

In the wet season, Zn recorded the highest concentration in all the organs but its highest value was in the foot and gills in wet season followed by the viscera and the shell recorded the least concentration of Zn in the wet season. While in the dry season, Zn was favoured by the viscera followed by gills, foot and again, the shell recorded the least concentration of Zn. Cr was retained more in the shell in wet season followed by viscera, foot and gills respectively. In dry season, Cr recorded higher values in all the organs than in wet season but the shell still retained more of the element followed by the gills, viscera and the foot recorded the least concentration of Cr in dry season. Pb was also more concentrated in the shell of the clam followed by the gills, foot and viscera concentrated the least Pb in wet season. In dry season, Pb was not detected in any of the clam's compartments. Although the dynamics of the partitioning of metals in organisms is complicated, undetected Pd in dry season may be as a result of less runoffs carrying industrial discharges from upland due to no rain in the dry season, this may result to less uptake and depuration or loss of accumulated elements into the environment.

Cu was consistent in its partitioning in both seasons. It recorded higher values in the organs in dry season and recorded the highest and lowest concentrations in the viscera and shell respectively in both seasons. The foot and gills recorded the second and third highest concentrations of Cu in both seasons as well. Ni and Cd were not detected in any of the organs in the wet season.

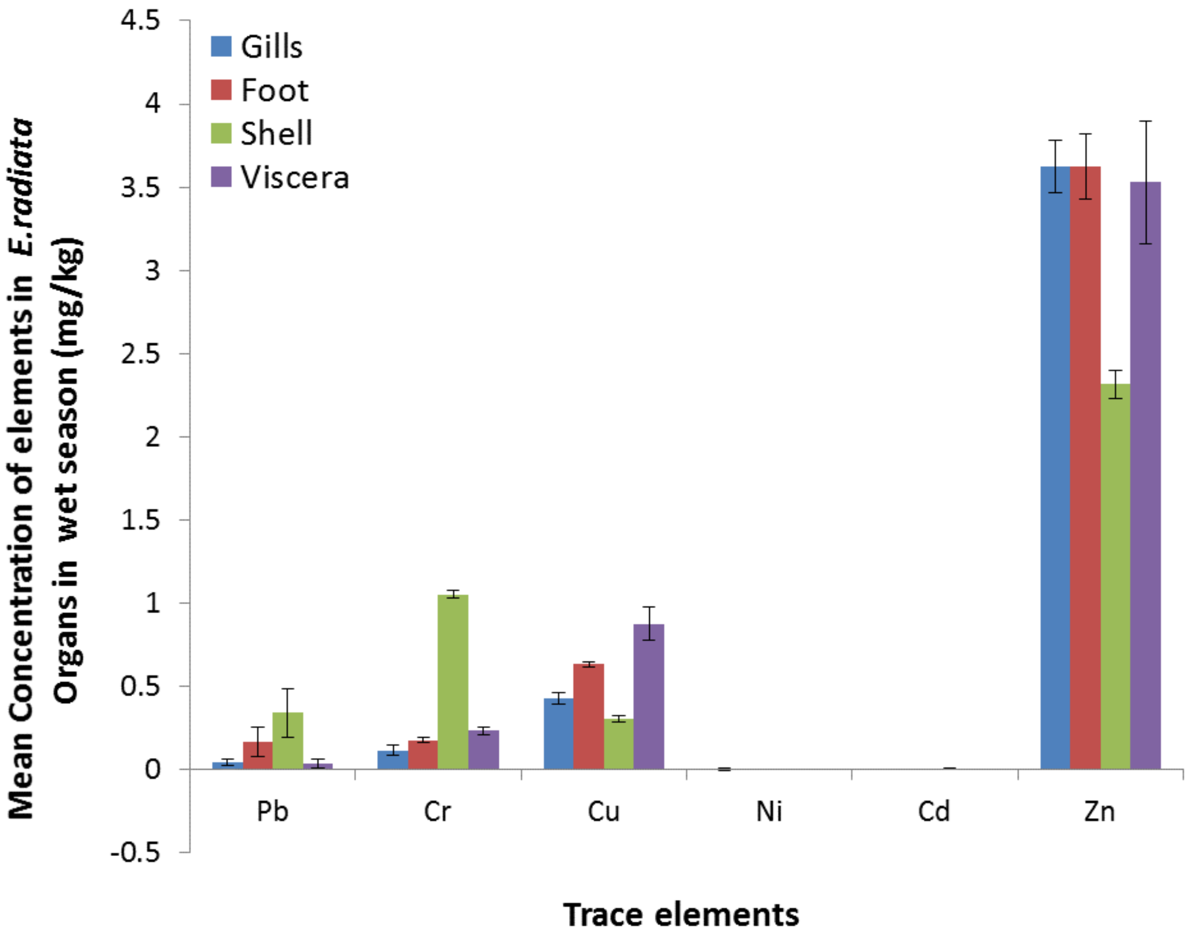

Fig. 3: Wet season concentration of heavy elements in various parts of *Ergeria radiata.*

Ni was below detection limit in the shell in dry season but recorded the highest value in the viscera followed the gills and foot respectively. In dry season, Cd was only detected in the gills.

The partitioning of the trace elements showed that Zn was the most concentrated in all organs in both seasons. Apart from Zn, the other elements Cr, Cu and Pb were more abundant in the muscle tissues than gills in wet season and that disagrees with the report of Ebenezer and Eremasi, (2012) and Eneji et al., (2011). They noted that elements favoured the gills more than other organs of *T . zilli* from Kolo Creek in Bayelsa. In dry season, the reverse was the case, more elements were concentrated in the gills than foot as suggested in the report of Ebenezer and Eremasi, (2012).

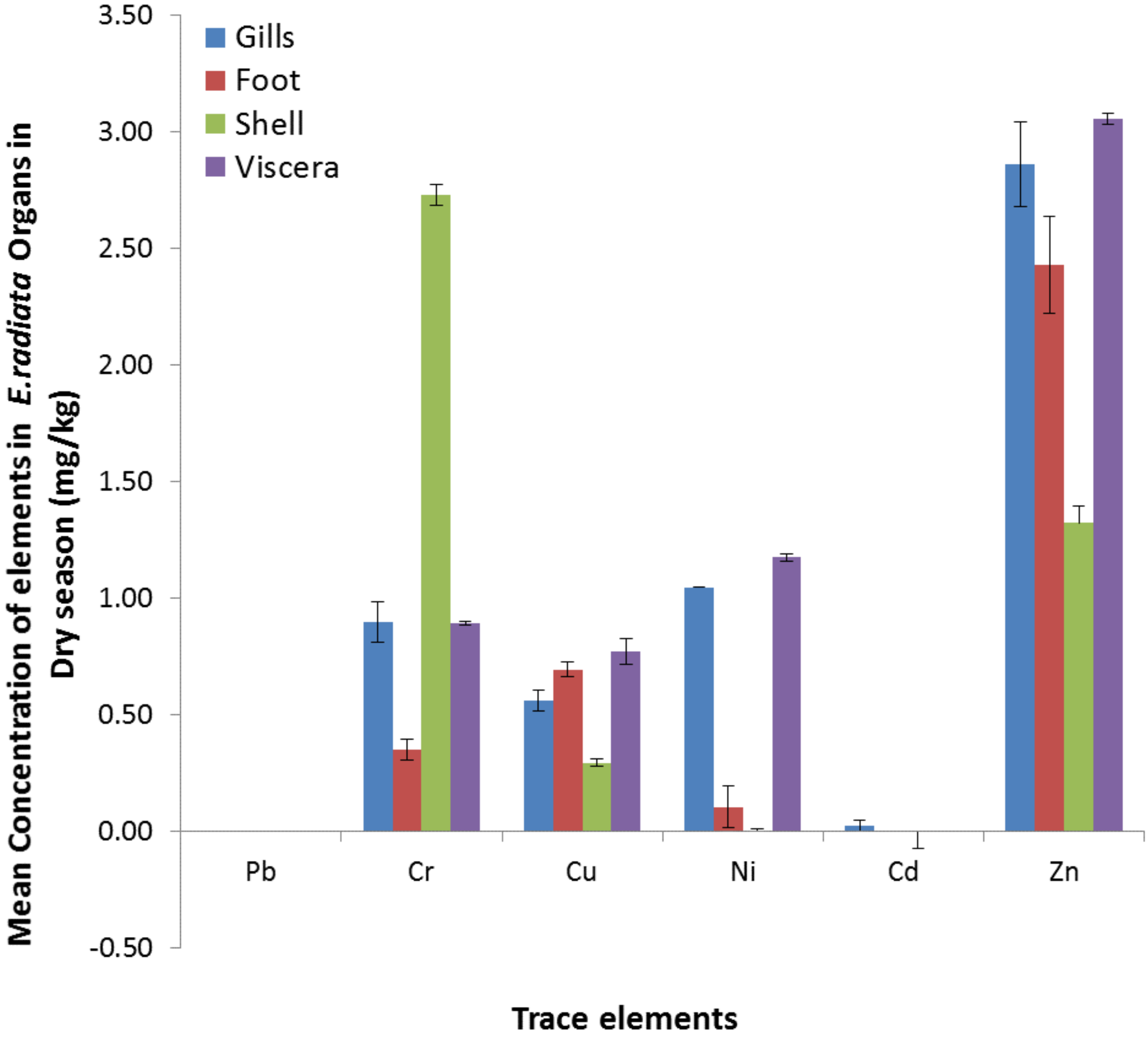

Fig. 4: Dry season concentration of heavy elements in various parts of *Ergeria radiata.*

There are some similarities and dissimilarities between the present results and the report of Bustamante and Miramand, (2005) on 17 trace element concentrations in the tissues and organs of variegated scallops *C. varia* from the French coast of the Bay of Biscay. In their report, the digestive gland contained the highest concentrations of Ag, Al, Ce, Cr, La, Mo, Nd, Ti, and V. Second, kidneys exhibited the highest concentrations of As, Cd, Co, Cu, Mn, Ni, Pb, and Zn. The adductor muscle of *C. varia* always displayed the lowest trace element concentrations. In this tissue, concentrations were remarkably low for nonessential elements such as Ag, Cd, Pb, or rare earth elements and also for most of the essential ones, especially Co, Cu, and Mo. Compared to these low muscle concentrations, the gills and gonads of *C. varia* displayed generally relatively high concentrations of trace elements. Similarly, Metian et al., (2008) reported tissue distribution of trace elements a bivalve from two stations in the Bay of New Caledonia, France. The body distributions of the different elements among the tissues and organs of the scallops from both sites are compared showed that the digestive gland clearly exhibited the highest proportion of Ag, As, Cr, Fe and Zn in both areas. In the case of Cd and Ni, the digestive gland and kidneys contained most of the metal burden.

Seasonally, the present study showed that the trace elements were generally more concentrated in the organs in the dry season months. This may agree with the general reports on trace element concentrations in Nigeria aquatic environment which has been reported to be higher than the wet season due to concentrated solutions in the dry season due to low or no precipitation (Onojake and Frank, 2012; Babatunde et al., 2013).

Heavy elements have multiple effects on biological systems depending on the oxidation state, the formation of complexes and biotransformation of elemental species. Health effects of heavy elements such as Pb, Zn, Cr, Cd and Cu in humans have been demonstrated in acute toxicity, neurotoxicity and nephrotoxicity (Katz and Salem, 1993; ATSDR 200; Stift *et al.,* 2000; WHO 2001), while Pb is a confirmed carcinogen (WHO 2001; Lars, 2003; Martin and Griswold, 2009). Some observed effects of Ni in aquatic environments include tissue damage, genotoxicity and growth reduction in organisms. Molluscs and crustaceans are more sensitive than other aquatic fauna (Onojake and Frank, 2012). The inhabitants of the study area usually dissect the clam and remove the foot which constitute 90% of the soft tissue consumed. In the present study, trace elements such as Zn, Cu and Cd were within tolerable and permissible limits for biological systems. However, Cr, Pb and Ni when detected recorded concentrations in the edible foot 10-100 folds higher than permissible limits for safe consumption of seafood. It is therefore not unlikely that consumers of the clam at the study area may suffer cancer and other related neurological diseases as opined by (Martin and Griswold, 2009; WHO 2001).

Conclusion

The present project investigated the partitioning of trace elements in different tissues of a clam *E. radiata* from the Nun River, Niger Delta, Nigeria. Partitioning of trace elements in the body of bivalves or organisms generally is part of the dynamics that govern bioaccumulation and bioavailability of trace elements to biological systems leading health concerns. Zn exhibited the highest concentration in all the organs in both seasons but Zn, Cd and Cu were well within tolerable and permissible limits for the consumption of such seafood. Pb, Cr and Ni on the other hand when detected, were several folds higher than stipulated limits especially in the edible soft tissue (foot) generally relished by inhabitants of the study area. Further research and consistent monitoring is required to generate large amount of scientific data required for integrated catchment management of the aquatic resources available to people to guarantee seafood safety and forestall health issues arising from consuming elevated levels of trace elements in seafood.

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