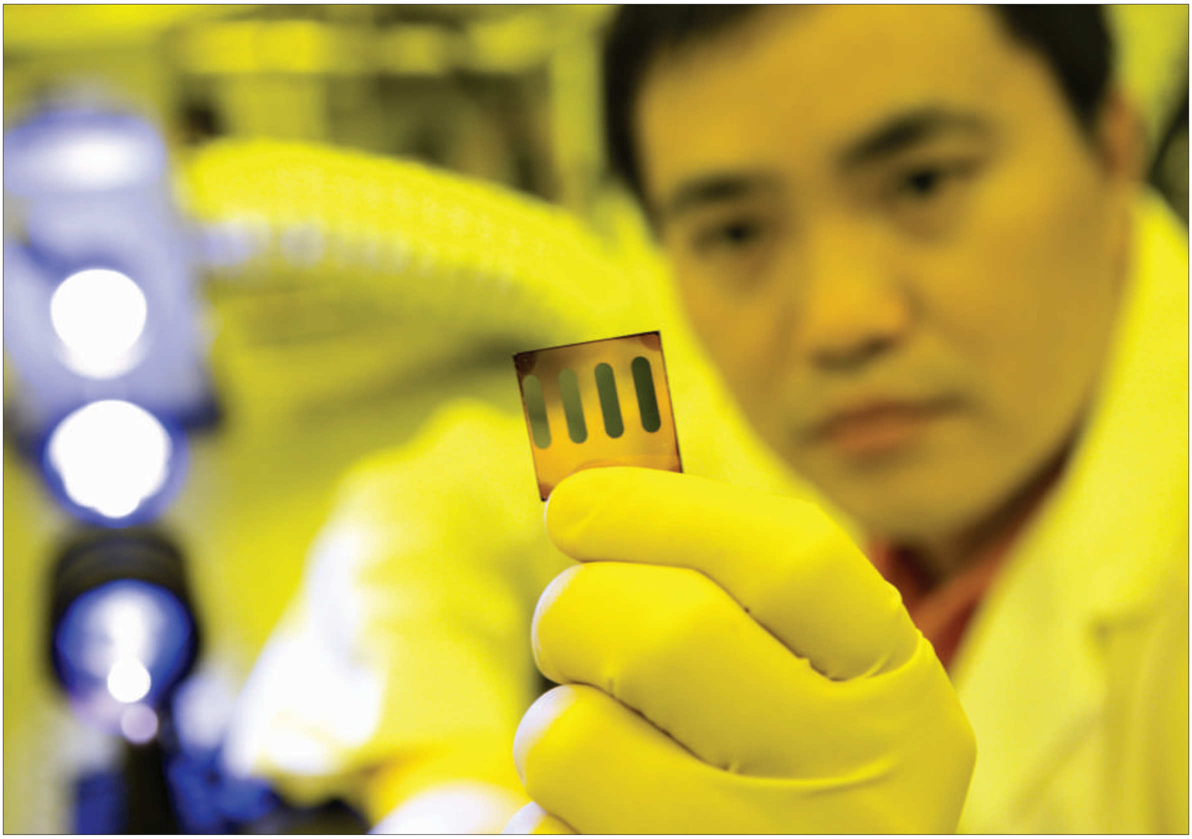

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Research Article
Proximate Composition, Essential and Toxic Elements Content of Bivalve Shellfish from Bonny Estuary, Nigeria

[1]Sunday Peter Ukwo, [2]Ofonime Ime Obot and [1]Ubanozie Chioma Sandra

[1]Department of Food Science and Technology, University of Uyo, Akwa Ibom State, Nigeria
[2]Department of Fishery and Aquatic Environmental Management, University of Uyo, Akwa Ibom State, Nigeria

Abstract
Background and Objective: Bivalve shellfish is a nutritious food item in the Niger Delta but the accumulation of toxic elements tends to undermine the nutritional benefits derived from bivalve consumption. This study investigated the proximate composition, minerals content and toxic elements in Bloody cockle (*Anadara senilis*), Donax clam (*Donax rugosus*), Knife clam (*Tagelus adansonaii*) and Mangrove oyster (*Crassosstra gasar*) from Bonny estuary. Percentage Recommended Daily Allowance (RDA) was estimated to determine if bivalve consumption was meeting the nutritional needs of consumers. **Materials and Methods:** Standard methods of analysis were employed to assess proximate composition, minerals and toxic elements while RDA (%) for minerals were determined according to the method of National academic of Science. **Results:** Results indicated variations among protein and mineral content in most cases were species-specific. Donax clam had the highest protein content while mangrove oyster had the least. Calcium and sodium were higher in concentration when compared to other minerals investigated. Iron was highest in Bloody cockle while zinc was highest in Mangrove oyster. Nutrient burden and RDAs for trace elements indicated that bivalve can provide adequate amount of required nutrients for an adult male of 65 kg b.wt. Lead, cadmium, mercury and arsenic concentrations in some species were higher than acceptable standards for shellfish as stipulated by FAO which portend health risks to consumers. **Conclusion:** Bivalves can be used to remedy problems of hidden hunger that is prevalent in the society, but the consumption of bivalve posits conflict between food benefits and risks.

Key words: Bivalve shellfish, niger delta, minerals, consumption, toxic element

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Corresponding Author: Sunday Peter Ukwo, Department of Food Science and Technology University of Uyo, Akwa Ibom state, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish and seafood products especially bivalve mollusk shellfish are currently the cheapest source of animal protein consumed by the average Nigerian and it accounts for about 50% of the total protein intake[1]. Bivalve mollusk shellfish are economically and nutritionally very important for human consumption and particularly playing a central role in the Niger Delta gastronomy. In Nigeria, mollusk and other shellfish constitute a greater part diet of the people of the Niger Delta. According to Akankali and Nwafili[2], fisheries resources constitute both traditional and primary source of enterprise and livelihood to most communities within the Niger Delta region of Nigeria. Bivalve mollusks are highly exploited by adult female and youth of the coastal communities. Mangrove oyster (*Crassosstrea gasar*) occurs abundantly in coastal swamps and estuaries of the Niger Delta where it is exploited at subsistence level. Other bivalve species harvested include clams, bloody cockle (*Anadara senilis*), knife clam (*Tagelus adansonaii*) among others[3]. At the subsistent level of their production, bivalves are usually sold or marketed either fresh or dried in local markets. Buyers and consumers include individual consumers who buy the products for home consumption, street food vendors, restaurant operators and exporters.

In several developing countries of Africa, there is a strong economic incentive derived from a sustained demand for bivalve mollusk shellfish as an animal protein source and this is particularly so in Nigeria, Ghana and Cameroon. They are believed to provide an inexpensive source of protein with high biological value, essential minerals such as selenium, Calcium, Iron, Phosphorus as well as vitamins[4]. In the Niger Delta, harvesting of bivalves has little or no regulatory mechanisms in place and this is further exacerbated by high degree of environmental degradation and aquatic perturbation posed by petroleum exploration activities in this region as well as poor sanitary facilities, which requires extra attention to curtail the incidence of shellfish borne diseases[5,6]. Consequent to inherent health hazards associated with consumption of bivalves, several developed nations have enacted laws based on microbiological assessment of water and/or bivalve flesh. Most of these regulations use coliform counts as an indication of faecal pollution.

Bivalve shellfish are an excellent source of some essential elements however, bivalve shellfish is an interesting organism for various ecological studies, This is because of their sedentary nature or immobility, filter feeding pattern, low metabolic rate, contact with sediments, suitable size for bio-chemical analysis, wide distribution in marine, estuarine and freshwater environments, practicality in collection, ability to bio-accumulate and bio-concentrate pollutants and high tolerance to chemical exposure due to a remarkably active immune system[7]. Given all these morphological and biological characteristics associated to their habitat and the feeding pattern of bivalves, some environmental and chemical contaminants especially toxic metals may be accumulated in their tissues. Toxic elements are of interest to bivalve consumers due to their harmful effects on organisms and ability to bio-accumulate and bio-magnified in aquatic ecosystems and higher trophic level of food chain[8]. The incorporation rate of contaminants in bivalves depends on biotic factors such as species, age, sex, soft body weight and physiological status and abiotic factors such as availability of contaminants in the environment, filtration rate, temperature, salinity, pH, chemical and species[9]. Several species of bivalve shellfish inhabit estuaries that are exposed to several anthropogenic activities, thus being vulnerable to high levels of toxic elements contamination[7]. The routes of transmission of toxic elements from the environment to humans include the consumption of raw or lightly/extensively cooked shellfish, representing a significant human health hazard to consumers where they cause physiological impairment and other deleterious health effects[10].

Among seafood species, bivalve shellfish represent one of the most important groups captured globally. Although, numerous studies on the elemental composition of bivalve species exist, their objectives are mostly related to environmental contamination and its use as a biological monitoring agent[7]. Most of these studies focus on amounts present in several organs such as; digestive glands, liver and other tissues[7]. It is hoped that the study will contribute to a better knowledge of nutritional benefits and chemical hazards associated with bivalve shellfish from Bonny estuary and Niger Delta at a large. In this context, the aim of this study was to determine the proximate composition, essential elements and estimate their Recommended Daily Allowance (RDA). Toxic elements contamination associated to these bivalve species would also be investigated.

MATERIALS AND METHODS

Sample collection: The study was conducted between the months of February-June, 2019 at the Bonny River estuary in the Niger Delta region of Nigeria. The study location lies along Atlantic Coastline in the Niger Delta region of Nigeria at 4°23'-4°25' and 7°05'-7°15'. The Niger Delta region host the Africa's biggest and the world's third mangrove forest, containing not only Nigeria's most abundant petroleum resources, but also an ecosystems with and several aquatic and terrestrial organisms. The coastal waters of the Niger Delta provide an excellent habitat for diversity of fish, shellfish and other seafood products. The location was chosen because of availability of the four species of bivalve mollusks which served as an important delicacy and food for indigenous people. This location is essentially estuarine in nature with brackish water characterized by fine sandy beaches surrounded by mangrove swamp and intertidal mudflat in which Nypa vegetation dominate. The hydrology of this locations presents a cyclic pattern with large amount of precipitation (rainfall) and tidal interplay with annual rainfall level of about 2500 mm, an average humidity level of over 85% and the temperature range of 18-30°C. This coastal environment suffers from environmental degradation occasioned by exploration and production of petroleum, liquefied natural gas production and spillage of petroleum products.

Sample preparation and treatments: The traditional method of preparing bivalve for consumption in the Niger Delta was used for the study. At the laboratory, the bivalves were promptly cleaned of incrustations, washed in distilled water to remove all dirt, put into a stainless pot and blanched for 5 min at 100°C. After blanching, the samples were poured into a perforated basket to drain and allowed to cool at room temperature (28±2°C). Samples were then shucked with sterile scalpel to extract the flesh and intravalvular fluid into sterile container. The extracted tissue was homogenized for 60 sec in a stomacher (Seward Laboratory Stomacher 400, England) and stored at -20°C in a scan frost deep freezer for various experimental assays.

Proximate composition: Proximate composition of extracted bivalve samples was determined according to methods outlined by AOAC[11]. Moisture content was determined using the procedure of vacuum oven method, crude fiber was obtained using trichloroacetic acid method, the micro-Kjeldahl method was used for protein content determination, total ash content was determined by the method of incineration of dried sample obtained in muffle furnace at 550°C for 24 h. Also, crude fat content was analyzed by the Soxhlet extraction method, while carbohydrate content of bivalve samples were obtained by difference.

Analysis for essential and toxic elements content of bivalve shellfish: The essential elements include the composition of macro mineral and trace present in the bivalve samples. Techniques of ultraviolet-visible spectrophotometry, atomic absorption spectrophotometry and flame photometry were used for analysis of both essential and toxic elements. Standard stock solution of the element to be analyzed was prepared, diluted to the corresponding working standard solution for recovery experiment according to the methods as outlined by Onwuka[12]. The wet ashing method as outlined by the author was used to determine the concentration of metallic element in the bivalve samples. The method of preparation and digestion procedure as outlined by AOAC[11], for biological sample was also employed. Determination of sodium and potassium content was done through the use of Jenway flame photometer at the wavelength of 589 nm and 767 nm for potassium and sodium, respectively, while analysis for other macro mineral, trace and toxic elements were carried out using a Perkin-Elmer model 3030 Atomic Absorption Spectrophotometer (AAS) using their respective lamp and wavelength. Analysis of phosphorus was done by using UV-visible spectrophotometer. The percentage recommended daily allowance for macro mineral and trace elements were also calculated according to the method of National Academic of Science[13].

Data analysis: All the analyses were carried out in triplicate and the experimental data generated were statistically analyzed using one way analysis of variance (ANOVA) using SPSS version 16.0. Duncan multiple range test was used to separate the means at p<0.05 significant differences.

RESULTS

Proximate composition of bivalve shellfish from bonny estuary: The proximate composition of bivalve shellfish harvested from Bonny estuarine waters is shown in Table 1. It indicated a moisture content ranged of 54.08-62.18% with Mangrove oyster having the highest moisture content followed by knife clam, while Donax clam with recorded the lowest moisture content. Knife clam with 7.95% had the highest ash content, Donax clam recorded the highest lipid and protein content of 5.23 and 22.78%, respectively. Mangrove oyster also recorded the highest carbohydrate content of 9.22% while Donax clam with 8.20% was the least.

Table 1: Proximate composition (%) of bivalve samples

	Bivalve species			
Parameters	BC	DC	KC	MO
Moisture	55.10±0.10[c]	54.08±0.11[d]	57.77±0.08[b]	62.18±0.39[a]
Ash	7.17±0.31[c]	7.24±0.36[b]	7.95±0.21[a]	5.03±0.22[d]
Lipid	3.75±0.07[b]	5.23±0.39[a]	3.04±0.17[c]	3.73±0.18[b]
Fibre	3.78±0.22[a]	2.41±1.13[b]	2.21±0.15[b]	1.17±0.09[c]
Protein	21.67±2.01[b]	22.78±2.05[a]	20.72±1.61[b]	18.66±0.81[c]
Carbohydrate	8.62±0.87[b]	8.20±0.51[d]	8.30±0.01[c]	9.22±0.89[a]

Values are Means±SD of triplicate determination, means in the same row with different superscript are significantly different at (p<0.05), BC: Bloody cockle, DC: Donax clam, KC: Knife clam, MO: Mangrove oyster

Table 2: Essential mineral content (mg/100 g) of bivalve samples

	Bivalve species			
Mineral element	BC	DC	KC	MO
Sodium (Na)	65.19±0.05[d]	75.31±0.19[c]	85.55±0.05[b]	104.63±2.12[a]
Potassium (K)	40.12±0.71[b]	26.63±1.53[d]	36.74±2.61[c]	46.78±1.45[a]
Magnesium (Mg)	53.13±2.21[a]	52.06±1.31[a]	40.16±0.42[b]	38.71±0.52[c]
Calcium (Ca)	109.75±2.88[a]	107.82±2.34[b]	85.47±1.33[c]	95.78±1.83[c]
Phosphorus (P)	15.73±0.41[a]	10.76±0.44[c]	13.39±0.36[b]	15.39±0.38[a]
Copper (Cu)	3.71±0.04[a]	1.72±0.03[d]	2.19±0.02[b]	1.74±0.10[c]
Zinc (Zn)	2.99±0.11[b]	1.56±0.04[d]	1.79±0.01[c]	5.69±0.04[a]
Manganese (Mn)	1.74±0.08[c]	1.37±0.01[d]	2.21±0.02[a]	1.88±0.01[b]
Iron (Fe)	5.78±1.03[a]	3.80±2.34[d]	4.94±0.03[b]	4.11±0.02[d]
Selenium (Se)	0.36±0.01[b]	0.15±0.01[d]	0.28±0.01[c]	0.44±0.02[a]

Values are Means±SD of triplicate determination, means in the same row with different superscript are significantly different at (p<0.05), BC: Bloody cockle, DC: Donax clam, KC: Knife clam, MO: Mangrove oyster

Table 3: Recommended Daily Allowance (RDA) (%) for essential minerals as provided by 100 g/portion

		*RDA/100 g molluscs (%)			
Mineral element	RDA	BC	DC	KC	MO
Sodium (Na)	500 (mg)	13.19	15.06	17.72	20.93
Potassium (K)	2000 (mg)	2.01	1.33	1.84	2.34
Magnesium (Mg)	400 (mg)	9.68	13.02	10.04	13.28
Calcium (Ca)	1000 (mg)	9.58	10.78	8.55	10.91
Phosphorous (P)	700 (mg)	2.25	1.54	1.91	2.20
Copper (Cu)	900 (μg)	41.22	19.11	24.33	19.33
Zinc (Zn)	11 (mg)	27.18	14.18	16.27	51.73
Manganese (Mn)	2.3 (mg)	75.65	59.57	96.07	81.74
Iron (Fe)	8.0 (mg)	75.25	47.50	61.75	51.38
Selenium (Se)	55 (μg)	65.45	27.27	50.91	80.00

*Source: National Academy of Sciences[13], BC: Bloody cockle, DC: Donax clam, KC: Knife clam, MO: Mangrove oyster

Table 4: Toxic element content (mg kg^{-1}) of bivalve samples

	Bivalve species			
Mineral elements	BC	DC	KC	MO
Lead (Pb)	1.54±0.02[a]	1.03±0.01[d]	1.50±0.02[b]	1.08±0.03[c]
Cadmium (Cd)	2.48±0.01[a]	1.57±0.04[c]	2.36±0.01[b]	1.50±0.02[d]
Arsenic (As)	1.41±0.02[b]	1.06±0.03[d]	1.65±0.02[a]	1.26±0.01[c]
Mercury (Hg)	0.57±0.03[a]	0.28±0.01[c]	0.53±0.01[a]	0.34±0.02[b]

Values are Means±SD of triplicate determination, means in the same row with different superscript are significantly different at (p<0.05), BC: Bloody cockle, DC: Donax clam, KC: Knife clam, MO: Mangrove oyster

Essential element content of bivalve shellfish from Bonny Estuary: The essential elements assessed in bivalve shellfish include the macro minerals and trace elements as shown in Table 2. Mangrove oyster had the highest concentrations of Sodium, Potassium while Bloody cockle had the highest concentration of Magnesium, Calcium and Phosphorus. Also the result of trace elements content indicated the highest concentrations of copper and Iron content in Bloody cockle while Zinc and Selenium concentrations were highest in mangrove oyster.

Recommended daily allowance for essential elements: The percentage recommended daily allowance for macro minerals and trace elements are presented in Table 3. The results indicated that consumption of 100 g of bivalve samples can provide 13.19-20.93% of Sodium, 1.33-2.34% of Potassium 9.68-13.28% of Magnesium, 8.55-10.91% of Calcium and 1.54-2.25% of Phosphorus. Mangrove oyster provided the highest percentage of Sodium, Potassium while Bloody cockle supplied the highest percentage of Magnesium, Calcium and Phosphorus. The estimated percentage recommended allowance for trace elements indicated that values for copper ranged from 19.33-41.22%, Zinc ranged from 14.18-51.73%, manganese ranged from 59.57-96.07%, iron ranged from 47.50-72.25% and selenium 27.27-80.00%.

Toxic elements content in bivalve shellfish from bonny estuary: The results obtained from instrumental analyses bivalve shellfish samples for toxic elements indicated significant differences (p<0.05) in the concentrations of lead, Cadmium, Arsenic and Mercury (Table 4). Lead concentration was highest in Bloody (1.54 mg kg^{-1}) followed by knife clam (1.48 mg kg^{-1}) while the least concentration of 1.03 mg kg^{-1} was obtained in Donax clam. Also both Bloody cockle and Knife clam also accumulated the highest concentrations of all the toxic elements assessed when compared to Donax clam and Mangrove oyster.

DISCUSSION

Bivalves shellfish is veritable source of nutrients as indicated in the results obtained in various analysis carried out. Bivalve can provide an rich source of protein with high biological value, essential minerals such as Selenium, Calcium, Iron and Phosphorus among others to the population of the Niger Delta who depend on them as their main delicacies[14]. As indicated in the results obtained, the nutritional characteristics of bivalves vary among species and between individuals of same species. These variations may be attributed to the effects of environment and species as well as other intrinsic factors[15]. Values for moisture content obtained in this study was higher than values reported by Gopalsamy *et al.*[16] on *Donax cuneatus* and Eswar *et al.*[17] on marine clam (*Gafrarnim divaricatum*) from the cuddalore and Mumbai both in India. However, values for moisture from in this study was lower than values reported by Kiin-Kabari *et al.*[18] on selected shellfish (mangrove oyster and clam) from River State, Nigeria. Moisture plays a key role as solvent for organic and inorganic solutes. Change in the amount of water present in the muscle has a profound effects on their internal organs, nutritional value and their sensory properties[19]. Bivalve and other shellfish contain higher protein when compared to finfish[20]. Protein plays a very role in growth and maintenance of vital bodily functions[19]. Lipid content of bivalves ranged from 3.04-5.23% with high variability. Despite considerable variations between bivalve species, research has shown that fatty acids profile of bivalve mollusk is particularly interesting, as the n-3 long chain Polyunsaturated Fatty Acids (PUFA) which are essential nutrients are generally predominant[21,22]. There is a strong evidence to suggest protective effect of n-3 PUFA on the risk of cardiovascular disease and stroke[23,24]. Macro minerals such as Na, K, Mg and P were detected in significant level in this study which is in agreement with reports from several authors that the edible portions of bivalve shellfish are rich in macro minerals[16,18,20,25]. Sodium and Potassium are important in osmo-regulation, balance and membrane potential of cells as well as transport across membranes. Sodium and potassium are also needed to activate amylase, an enzyme which is important in glucose metabolism in human body[21]. Calcium is a major component in bones and constitutes over 95% of bivalve shell in the form Calcium carbonate. Calcium is involved in structure of muscle system and controls essential processes like muscle contraction, blood clothing and actively involves in brain cells and their growth[21]. Magnesium just as calcium formed a greater part of human bones as well as prosthetic group in enzyme that hydrolysis and transfer phosphate groups, consequently, Mg is essential in energy requiring biological functions such as membrane transport, generation and transmission of impulses, contraction of muscles and oxidative phosphorylation[14]. Phosphorus, the main structural element found in shellfish is usually found equal amount with sulphur. Bivalve shellfish was found to be rich in micro mineral such as Zinc, Copper, Manganese Iron and Selenium. Trace elements play significant role of functional elements in several metalloenzymes, which possess catalytic functions in living organisms. As observed, bivalve mollusc shellfish in this study were rich sources of trace element when consumed. Variations in trace element content within species could be attributed to factors such as habitat of the organisms, dietary pattern, other ecological interactions and overall body size of the bivalve species[26]. Copper is a component of a number of enzymes involve in glucose metabolism, synthesis of haemoglobin, connective tissue and phospholipid. Several authors have reported higher concentration of Copper in shellfish from Niger Delta[26,27]. Zinc is an essential element for human and its presence in bivalve species analyzed is in agreement that Zinc is always presence in shellfish and that the concentration presents in bivalve is usually higher[28]. The important function of zinc is based on its role as an integral part of a number of metalloenzymes and as a catalyst in regulating the activity of specific Zinc-dependent enzyme. Among the bivalve species analyzed, mangrove oyster recorded the highest concentration of Zinc followed by bloody cockle. The higher concentration of Zinc in mangrove oyster have been attributed to the reason why therapists recommend eating of oyster for men with sexual disorder since the high content of Zinc can be helpful in raising libido in men[14]. Iron is present in cells of living organism and plays a vital role in several biochemical reactions. Most of iron is present in the haemoglobin (blood) and myoglobin (muscle tissue), pigments, cytochromes and other proteins participating in transport, storage and utilization of oxygen[12]. Bloody cockle as the name implies is the only bivalve among the species analyzed that contain haemoglobin in the internal fluid[14]. Selenium is recognized both as an essential trace elements and a toxic agent. It is an integral component of enzyme in human and animal tissue, which together with vitamin E and catalase enzymes acts as an antioxidant, thereby protecting cells against oxidative damage. It is also involved in thyroid metabolism and proper functioning of immune system[29]. Results from various RDA determinations indicated that consumption of bivalve shellfish can provide significant daily requirement for the trace elements particularly for Zinc, Manganese Iron and Selenium. The concentrations and levels of accumulation of toxic elements by bivalve samples in the study area clearly indicated that bivalve shellfish are differentially selective for a range of toxic element and these differences might be influenced by a number of intrinsic and extrinsic factors[30]. This strategy results from net differences between rate of absorption and excretion of elements, the permeability of the body surface and the nature of the food and the efficiency of the osmo regulatory system present[31]. According to FAO[32], lead concentration above acceptable limits 1.5 mg kg^{-1} in bivalve tissue is unacceptable and can pose health risk to the consumers. Cadmium concentration above legal limit of 3-4 mg kg^{-1}, mercury above 1.0 mg kg^{-1} as well as the present of arsenic in the tissue of bivalve mollusk is dangerous to the health of the consumers. The concentration of toxic elements in the tissue of bivalve molluscs from this study were higher when compared to values reported by Edoghotu *et al.*[27] at B/Dere in Ogoni land. The values are also lower when compared with the values reported by Sarkar *et al.*[30], in bivalve mollusks at Sunderban Mangrove Wetland at Bay of Bengal in India. The toxicity of those elements are due to their ability to replace other metals in the active sites of enzymes, form complexes and precipitates with enzyme metals or other groups involve in metabolism, catalyzes the breakdown of essential metabolites[32,33]. The anthropogenic activities in this area has negatively affected the environment resulting in elevated toxic element concentrations in bivalve shellfish thereby raising serious concern about their safety for human consumption.

CONCLUSION

The findings from this study revealed that bivalve shellfish from Bonny estuarine water are rich in protein and minerals. High protein values were noted in all the bivalve samples analyzed. Donax clam had the highest protein content closely followed by Bloody cockle. Bivalve mollusk shellfish are veritable sources of essential minerals which can be optimized to remedy the problem of hidden hunger that is endemic in the developing countries like Nigeria. The elevated concentration of toxic elements in bivalve shellfish in Bonny exemplify the conflict between food benefits and risks. This is because the accumulated hazardous toxic elements tend to undermine the nutritional and health benefits derived from bivalve consumption.

SIGNIFICANCE STATEMENT

This study revealed the proximate and minerals content of bivalve shellfish consumed in Bonny Island in the Niger Delta, Nigeria. The RDAs for minerals were also estimated to determine if their consumption were meeting the nutritional needs of the consumers. The study also exposed the levels of toxic elements accumulated by bivalve species vis-a-vis their safety. It is hoped that researchers will explore methods of reducing the level of pollution in the coastal waters of this area in order to enhance the safety, quality and nutritional benefits associated with bivalve shellfish consumption.

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