Microbiological safety and toxic element contaminants in bivalve shellfish from intertidal mudflats of IKO estuary, Niger delta, Nigeria

Sunday Peter Ukwo*[1], Chidi F. Ezeama[2] and Ofonmbuk Ime Obot[3]

[1]Department of Food Science and Technology University of Uyo, Akwa Ibom state, Nigeria
[2]Department of Food Science and Technology Michael Okpara University of Agriculture Umudike, Abia State, Nigeria
[3]Department of Fishery and Aquatic Environmental Management, University of Uyo, Akwa Ibom State, Nigeria
*E-mail: sonipeter75@gmail.com

Abstract

Bivalve shellfish is considered as delicious and healthy food items in several dietary regimes in the Niger Delta but the accumulation of hazardous substances tend to undermine the nutritional and health benefits derived from their consumption. Four species of bivalve shellfish: Bloody cockle (*Anadara senilis*), Donax clam (*Donax rugosus*), Knife clam (*Tagelus adansonaii*) and Mangrove oyster (*Crassosstra gasar*) harvested from the brackish water of Iko estuary were assessed of their safety for human consumption. Standard methods of analysis were employed to assess microbiological hazards and toxic element contaminants. Results of microbiological analysis revealed a total viable counts (TVC) lower than the recommended limit (5.7 log cfu/g) for fresh bivalve molluscs while *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella spp*, *Shigella spp*, *Listeria monocytogenes* and *E-coli* were far above the stipulated FDA standard for molluscian shellfish. Analysis for toxic elements clearly showed differential selectivity for the ranged of toxic elements. Lead concentration was within the acceptable limit (1.5mg/kg). Cadmium concentration in bloody cockle, knife clam and mangrove oyster was higher than the acceptable standard recommended by regulatory agencies for shellfish. The accumulation pattern and tissue burden of bivalve shellfish samples indicated that microbiological hazards and toxic element contaminants were higher in bloody cockle and knife clam when compared to donax clam and mangrove oyster. Therefore, bivalves shellfish harvested from the study location is not considered safe for human consumption without any further treatment and proper processing to reduce the levels of hazards which may pose health risk to the consumers.

Keywords: Bivalve, Hazards, Contaminants, Brackish water, Niger Delta

Introduction

There is growing scientific concern on the quality and safety of seafood harvested from the estuaries or brackish water ecosystems of the Niger delta region of Nigeria. The intertidal mudflat of brackish waters of the Niger delta is exceptional breeding ground for a vast variety of fish and shellfish. It provides an excellent habitat for diversity of bivalve molluscs shellfish such as Bloody cockle (*Anadara senilis*), Mangrove oyster (*Crassosstrea gasar*), Razor or Knife clam (*Tagelus andansonaii*), Donax clam (*Donax rugosus*) among others. But the prevailing pollution occasioned by exploration and production of petroleum have impacted negatively on the quality and safety of shellfish from this area (Yakubu, 2017). Many factors influencing seafood safety and quality include microbiological composition, environmental contaminants, water and product handling. Also many critical and lifelong diseases, from diarrheal disease to various forms of cancer have also been connected to unsafe seafood. Hazards

associated with bivalve shellfish may arise from raw, fresh, minimally processed, packaged, prepared, and stored items. The clinical symptoms of these hazards are dependent on the dosage and the health status of the consumer, ranging from mild to life-threatening and chronic adverse reactions (Huss *et al*., 2003).

Bivalve shellfish-associated hazards can be broadly classified into environmental, intrinsic, and process-related, however the environmental hazards are the major class of hazards of concerned. They are caused by exposure of bivalves to microbial pathogens, parasites, toxic metals, and other chemical pollutants from their habitats (Venugopal and Gopakumar, 2017). The number and type of pathogenic bacteria present in marine or estuarine waters depend on seasonal, climatic and anthropogenic factors (Vernocchi *et al.*, 2007). The bacteria found in bivalves can be indigenous to marine or estuarine environments, non-indigenous/enteric bacteria that occur due to faecal contamination and bacteria from cross-contamination during food preparation and processing (Lee *et al.,* 2008). The retention of bacteria by bivalve depends on several factors, such as its form and dimension that influence bacteria adsorption and capture (Silva and Batista, 2008). The feeding physiology of bivalves also determines the accumulation of pathogenic bacteria filtered from the overlying water (Burkhardt and Calci, 2000). According to Lees (2010), hazards posed by the routes of transmission from the environment to humans include the consumption of raw, uncooked or lightly cooked bivalve shellfish, representing a significant health risk to the consumers. Also the bioaccumulations of harmful micro organisms in shellfish are compounded by the traditional consumption of certain bivalve shellfish in raw or only mildly cooked dishes. The most notable examples include *Vibrio* species, which account for 20% of all outbreaks of disease (Potasman *et al*., 2002). The presence and concentration of toxic elements in the aquatic environment, including biota, specifically in bivalve molluscs shellfish can be attributed to natural and anthropogenic sources. Toxic elements particularly mercury, cadmium, lead and arsenic are cumulative in nature and can be assimilated, stored and concentrated by living organisms through the food chain, causing sometimes serious physiological effects.

In several developing countries in Africa, there is a strong economic incentive derived from a sustained demand for bivalve mollusc 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 (Astorga-Espana *et al.,* 2007). 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 require extra attention to curtail the incidence of shellfish-borne diseases (Wala *et al*., 2016; Zabbey and Babantunde, 2015). Due to the health hazards inherent with consumption of bivalves, many developed countries have enacted regulations based on microbiological analysis of water and/ or bivalve flesh. Most of these regulations use coliform counts as an indication of faecal contamination. The objective of this study is to assess microbiological hazards and toxic element contaminants of fresh bivalve species harvested from the brackish water of the Niger delta. This study will assess their suitability for human consumption and provide baseline information on the quality and safety of fresh bivalve species produced from this area.

Materials and Methods

Sample Collection: The study area lies along Atlantic Coastline in the Niger Delta region of Nigeria at ($4^o20 – 4^o35'$ and $7^o40' - 7^o50'$). The Niger Delta sustains Africa's largest, and the world's third mangrove forest, bearing not only Nigeria's most abundant petroleum resources, but also a diversified ecosystem, and numerous aquatic and terrestrial organisms (Okonkwo *et al*., 2015). 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 (4) species of bivalve molluscs 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 dominates. The hydrology of this locations presents a cyclic pattern with large amount of precipitation (rainfall) and tidal interplay with annual rainfall level of about 2500mm, 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: At the laboratory, the bivalves were promptly cleaned of incrustations, washed in distilled water to remove all dirt. Samples were shucked with sterile scapel to extract the flesh and intravalvular fluid into sterile container. The extracted tissues were homogenized for 60s in a stomarcher (Sewad Laboratory Stomacher 400, England) and a portion taken for microbiological analysis while the other portion were stored at -20°C in a scanfrost deep freezer for various toxic element analysis

Microbiological Analysis: Microbiological analysis includes enumeration and isolation of pathogenic microorganism in fresh bivalve tissues. For the purpose of this study, microorganisms indigenous to marine or estuarine environment and those from faecal contamination or enteric origin were assessed. The total viable count or aerobic plate count were carried out using the Pour Plate Method as outlined by Hatha *et al*., (2005). The method of Food and Drug Administration (FDA) as outlined by Feng *et al*., (2002) was used for enumeration of *E coli*. The isolation and enumeration of *salmonella* spp and *shigella* spp were carried out using a selective and differential medium, salmonella shigella agar. *Salmonella* do not ferment lactose but produce hydrogen sulphide resulting in colonies appearing colourless but black in centres while *shigella* spps do not ferment lactose or produce hydrogen sulphide hence the colour appears colourless on the media. Analysis for *Vibrio parahaemolyticus* and *Vibrio cholerae* were performed according to the ISO/TS 21872-1 method (ISO, 2007). The green characteristic colonies were presumptively selected as *Vibrio parahaemolyticus* while yellow characteristics colonies were selected as *V. cholerae*. Enumeration of *Listeria monocytogenes* was

done according to the method of ISO11290-1 (ISO, 2004). Morphological, physiological, biochemical and other confirmatory tests were carried out on the isolates to ascertain the organisms.

Analysis for Toxic element contaminants of bivalve: The levels of toxic element present in the bivalve samples were determined using a perkin – Elmer model 3030 Atomic Absorption spectrophotometer (AAS) at their respective lamp and wavelength. Standard stock solution of the element to be analysed were prepared, diluted to the corresponding working standard solution for recovery experiment according to the methods as outlined by Onwuka (2018). The wet ashing method as outlined by Onwuka (2018) was used to determine the concentration of toxic element in the bivalve samples. The method of preparation and digestion procedure as outlined by AOAC (2010) for biological sample was also employed.

Assessment of bioaccumulation factor: Bioaccumulation factor (BAF) is multipliers used to estimate concentrations of chemicals that can accumulate in tissues through any route of exposure (Benson *et al*., 2011). It can also be referred to as bio-concentration factor (BCF) for aquatic invertebrates. The BCF of the bivalve samples for specific toxic elements at the study location were determined for different bivalve samples as the ratio of the concentration of heavy metal in animal tissue to that of concentration of heavy metal in water sample.

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 and Discussion

Microbiological Hazards in bivalve shellfish

Microbiological hazards in surface water and bivalve mollusc shellfish from Iko brackish water (Table 1) indicates a total viable counts (TVC) ranging from 3.48-3.85 log cfu/g. There was no significant difference (P>0.05) between the TVC for bloody cockle and knife clam as well as between donax clam and mangrove oyster. However, significant difference (P<0.05) existed between TVC for

the surface water sample and bivalve mollusc samples. The highest value for *Vibrio cholerae* (3.30 log cfu/g) was isolated in bloody cockle followed by donax clam (3.10 logcfu/g) while the surface water sample had the least value of 2.10 log cfu/L. *Vibrio cholerae* was not detected in knife clam while *Vibrio parahaemolyticus* was not also isolated in knife clam and mangrove oyster. Salmonella spp. was isolated from the surface water and all the bivalve shellfish samples while Shigella spp was not isolated in mangrove oyster. The highest count for *Salmonella spp* and *Shigella spp* were noted in mangrove oyster (3.30 log cfu/g) and bloody cockle (3.11 log cfu/g) respectively. *Listeria monocytogenes* was not isolated in the brackish water, donax clam and mangrove oyster. *E coli* were isolated in all the samples analysed. The highest count was observed in brackish water samples. But values indicated no significant difference in the *E coli* counts between that of brackish water and all the bivalve shellfish samples under investigation. The cumulative microbiological hazards indicated that bloody cockle had the highest hazards loads followed by knife clam while donax clam accumulated the least microbiological hazard load. Bioaccumulation of harmful micro-organisms in bivalve molluscs have been attributed to various outbreaks of bivalve – associated infections globally. The threats posed by microbial hazards are compounded by traditional consumption of certain bivalve species in raw or mildly cooked dishes. The total viable count (TVC) gives a quantitative estimate of both viable and non-viable cells in bivalve tissues. As regard to current legislation, the TVC for bivalve molluscs harvested from the Niger Delta coastal waters were lower than the recommended limit (5.7 log cfu/g) for a good quality fresh bivalve molluscs as outlined by the centre for food safety and applied nutrition (CFSAN, 2003) of the US Food and Drug Administration. The TVC recorded in his present study was similar to values obtained by Anaclato *et al.,* (2013) in Portugal. *Vibrios* are primarily associated with estuarine and coastal marine environment. *Vibrio* cholerae and *vibrio parahaemolyticus* have been implicated in food poisoning associated with seafood consumption especially from countries with high ambient temperature and where shellfish are consumed raw (Lee *et al.,* 2008). The *Vibrios* are

involved in the transmission and epidemiology of disease leading to outbreaks at endemic epidemic and pandemic levels (Goel *et al.,* 2010). Microbiological standard for shellfish as outlined by FDA (2009), indicated that *Vibrio choleae* should be absent in seafood for human consumption while the count of *Vibrio parahaemolyticus* should not exceed 1000 cfu/g. *Salmonella* and *Shigella* are non-indigenous/enteric bacteria that occur due to faecal contamination of the estuarine waters. These enteric pathogens from the polluted environment can enter the bivalve during feeding where sea water is being filtered into their gut. Consumption of bivalve with these bacteria usually results in infectious diseases such as typhoid fever and other serious salmonellosis in case of *Salmonella* Spp and bacillary dysentery or shigellosis in the case of *Shigella* Spp (Potasman *et al.,* 2002). From the public health point of view, the current legislation (FDA 2009) requires total absence of *Salmonella* and *Shigella* spps in 25g of bivalves so as to ensure safe product. *Listeria monocytogenes* is indigenous to marine environment and can be present in bivalve eaten raw or fermented. *L monocytogenes* have been implicated as a causative agent in invasive listeriosis, meningitis and febrile gastroenteritis (Lee *et al.,* 2008). *E. coli* is an enteric bacterium which is usually used as an indicator of food safety and sanitary conditions. The presence of this bacterium in food therefore serves as indicator of the present pathogenic micro organisms. *E. coli* was detected in all bivalve samples analysed indicating that contamination is associated with human activities as the major source of pollutants in this area. The result from this study was similar to the findings observed by Udoh *et al.,* (2017) on fresh water clam from Cross River, in Nigeria. *E. coli* have been implicated in acute diarrheas and gastroenteritis particularly serotypes such 0148, 0157 and 0124. According to FDA (2009), *E-coli* in bivalve should equal or less than 230MPN/100g and it was observed that the result from this study exceeded the standard. Therefore, in this study, bivalve molluscs harvested from the coastal waters of the Niger Delta are not considered to be safe for human consumption without any treatment or proper coking time. This is due to high accumulation of pathogenic bacteria which posed serious health risk to the consumers.

Table 1: Microbiological hazards (Log cfu/g) isolated from the surface water and bivalve species.

Microbiological isolates	SW	Bivalve species				Pr>sam -ples	Signif -icant
		BC	DC	KC	MO		
Total Viable count(TVC)	3.48±0.16[c]	3.85±0.12[a]	3.70±0.05[b]	3.80±0.01[a]	3.64±0.04[a]	<0.0001	Yes
Vibrio cholerae	1.10±0.02[d]	3.30±0.04[a]	1.26±0.17[c]	ND	2.64±0.04[b]	0.003	Yes
Vibrio parahaemolyticus	2.23±0.02[b]	3.02±0.18[a]	3.01±0.35[a]	ND	ND	<0.001	Yes
Salmonella spp	2.43±0.02[b]	2.10±0.02[c]	2.10±1.83[c]	3.23±0.15[a]	3.30±0.09[a]	<0.001	Yes
Shigella spp	1.15±0.01[d]	3.11±1.01[a]	1.80±0.05[c]	2.83±0.16[b]	ND	<0.001	Yes
Listeria monocytogenes	ND	1.02±0.02[b]	ND	3.02±0.01[a]	ND	<0.001	Yes
Echerichia coli	3.14±0.05[a]	3.13±0.16[a]	3.04±0.13[a]	2.97±0.13[a]	2.86±0.13[ab]	0.2321	No

*Values are Means ± SD of triplicate determination Means in the same row with different superscript are significantly different at (P <0.05).   SW=Surface water; BC=Bloody cockle; DC= Donax clam; KC= Knife clam; MO=Mangrove oyster, ND= Not Detected

Toxic Element contaminants in bivalve shellfish

The results obtained from instrumental analyses of surface water and bivalve shellfish samples for toxic elements indicated significant differences (p<0.05) in the concentrations of lead, cadmium, arsenic and mercury (table 2). Lead concentration was highest in donax clam (0.94 mg/kg) followed by knife clam. The concentration of lead was within the acceptable limits as outlined by EC, (2006) and FDA, (2009).   The highest concentration of cadmium (9.80 mg/kg) was noted in bloody cockle followed by knife clam with 5.53mg/kg. The concentration of cadmium in bloody cockle, knife clam and mangrove oyster were higher than the safety limits as shown in table 2. Arsenic and mercury were present in the bivalve and surface water samples but that of mercury was within the acceptable limit for molluscan shellfish. The accumulation pattern of toxic elements indicated that bloody cockle had the highest cumulative toxic element concentration of 10.18mg/kg followed by knife clam 6.24mg/kg while donax clam (2.71) mg/kg had the least concentrations of toxic element contaminants. The concentrations and levels of accumulation of toxic elements by bivalve samples in the study area clearly revealed that bivalve shellfish are differentially selective for a range of toxic element and these variations might be influenced by a number of intrinsic (e.g size, age, and sex) and extrinsic factors (e.g metal speciation, Temperature and salinity). Also,

the concentration of toxic elements in the tissue of marine invertebrates depends on the accumulation strategy adopted by each species for each element (Sarkar *et al*., 2008). 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 (Benson *et al.,* 2017). *Bivalve molluscs* have been extensively used as a model organisms in environmental studies of water quality and biomonitoring agent hence the results from this study reflects the level of contamination of the Niger Delta environment as well as the safety of bivalve molluscs consumed in this area. According to FAO (2003), lead concentration above acceptable limits 1.5mg/kg in bivalve tissue is unacceptable and can pose health risk to the consumers. Cadmium concentration above legal limit as well as the present of arsenic in the tissue of bivalve mollusc 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.,* (2015) at B/Dere in Ogoni land. The values are also lower when compared with the values reported by Sarkar *et al.,* (2008), in bivalve molluscs at Sunderban mangrove Wetland at Bay of Bengal in India. Toxic elements are assimilated, stored and concentrated by living organisms through food chain causing serious toxic effect to humans. 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 as well as combined with membranes, thereby altering their permeability and hindering other elements in their electrochemical functions in human system (Burger and Gochfield, 2005; FAO, 2003). The discharged effluents alongside the activities such as artisanal refining of petroleum product and pipeline vandalization has negatively affected the environment resulting in elevated toxic element concentrations in seafood from these locations which raised serious concern about their safety for human consumption.

Table 2; Toxic Element concentrations in the surface water (mg/L) and bivalve species(mg/kg).

Toxic elements	SW	Bivalve species				EC, 2006	FDA, 2009
		BC	DC	KC	MO		
Lead (Pb)	0.005 ± 0.02^e	0.18 ± 0.02^d	0.94 ± 0.03^a	0.51 ± 0.08^b	0.25 ± 0.04^c	1.5	1.7
Cadmium (Cd)	0.026 ± 0.03^e	9.80 ± 0.05^a	1.75 ± 0.01^d	5.53 ± 0.15^b	4.25 ± 0.04^c	1.0	3-4
Arsenic (As)	0.003 ± 0.02^e	0.05 ± 0.01^a	0.01 ± 0.01^b	0.07 ± 0.01^a	0.01 ± 0.01^b	-	-
Mercury (Hg)	0.001 ± 0.01^c	0.15 ± 0.01^a	0.01 ± 0.01^d	0.13 ± 0.10^b	0.07 ± 0.01^c	0.5	1.0

*Values are Means ± SD of triplicate determination Means in the same row with different superscript are significantly different at (P <0.05). SW=Surface water; BC=Bloody cockle; DC= Donax clam; KC= Knife clam; MO=Mangrove oyster

Trace metal accumulation based on bioconcentration factor

The bioconcentration factor (BCF), computed for bivalve shellfish is presented in Table 3. The results show a more significant increase in trace metal levels in bivalve shellfish samples than in the surface or brackish water samples. BCF values for trace metals in bivalve shellfish increases as the concentration of the toxic elements increased in the bivalve samples. Relatively high concentration factors were observed for Cd; bloody cockle (376.9), Knife clam (212.7), mangrove oyster (163.5) Enhanced bioconcentration factors for these toxic elements in tissues of bivalve shellfish show that the organisms may serve as good bioindicators for monitoring metals in polluted aquatic ecosystems. However, BCF values found for Pb and Cd were comparatively higher than those of As and Hg. This may suggest that bivalve shellfish retains more Pb and Cd than other toxic elements in the aquatic ecosystem. According to Benson *et al*., (2017), Oysters and mussels, which have been reported to accumulate Cd in their tissues at upto 100,000 times levels observed in water environments where they live. Such toxic element accumulation levels in bivalve shellfish found in the present study complement the reports that the concentration of toxic elements in the tissue of marine invertebrates depends on the accumulation strategy adopted by each species for each element (Sarkar *et al*., 2008). Therefore, our results indicate that bivalve shellfish serves as a sentinel organism for the biomonitoring of toxic elements in fresh water ecosystems.

Table 3: Toxic Element accumulation levels in bivalve tissue based on bioconcentration factor

Toxic elements	Bivalve species			
	BC	DC	KC	MO
Lead (Pb)	36.0	188.0	102.0	50.0
Cadmium (Cd)	376.9	67.3	212.7	163.5
Arsenic (As)	16.7	3.3	23.3	3.3
Mercury (Hg)	150.0	10.0	130.0	70.0

BC=Bloody cockle; DC= Donax clam; KC= Knife clam; MO=Mangrove oyster

Conclusion

The consumption of bivalve molluscs from the Niger Delta coastal waters posit or exemplify the conflict between food benefits and risks. This is because of the accumulated hazardous substances due to anthropogenic activities which tend to undermine the nutritional and health benefits derived from bivalve consumption. Bivalve shellfish

samples harvested from the brackish water if Iko Town were loaded with both indigenous marine bacteria vibrios (*Vibrio cholera* and *Vibrio parahaemolyticus*) *Listeria monocytogenes* and enteric bacteria *Salmonella spps, Shigella spp* and *E- coli*. The bacteria count of each bivalve samples were higher than the limit stipulated by FDA for shellfish quality. Therefore, the bivalves samples harvested from the locations are not considered safe for human consumption without any further treatment or proper processing. This will help reduce the levels of pathogenic bacteria which may pose health risk to the consumers. The concentrations and level of accumulation of toxic elements by bivalve samples revealed that bivalve molluscs are differentially selective for a range of toxic element and their concentration depends on the accumulation strategy adopted by each species for a particular element. Lead concentration was within the acceptable FAO limit of 1.5mg/kg while cadmium, and the present of arsenic were above their acceptable limit in food which are likely to pose health risk to bivalve consumers. This may be attributed to large effluent discharge from petrochemical companies, artisanal refining of petroleum product as well as vandalization and spillage of petroleum product in the Niger Delta. The accumulation pattern and tissue burden of bivalve shellfish samples indicated that microbiological hazards and toxic element contaminants were higher in bloody cockle and knife clam when compared to donax clam and mangrove oyster. Also, BCF values for trace metals in bivalve shellfish increases as the concentration of the toxic elements increased in the bivalve samples. The bioconcentration factor for cadmium was higher when compare to other elements while bloody cockle and knife clam were better bioindicators for environmental monitoring. From these findings it is unlikely that bivalve - related infection in the Niger Delta is lacking but it is rather underreported.

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