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COMPARATIVE ANALYSIS OF MICROORGANISMS AND HEAVY METAL CONTAMINATION OF PERIWINKLES (*Tympanotonus fuscatus and Pachymalania aurita*) IN AKWA IBOM STATE, NIGERIA

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ABSTRACT

Periwinkles are a common and affordable source of dietary protein in many coastal communities, yet their filter-feeding nature makes them susceptible to accumulating environmental contaminants from polluted waters; therefore, this study assessed the safety of periwinkle (*Tympanotonus fuscatus* and *Pachymelania aurita*) harvested from three aquatic ecosystems in Akwa Ibom State by determining microbiological and heavy metal concentrations using standard techniques, with results showing total heterotrophic bacterial counts ranging from $3.4 \times 10^7 \pm 0.07$ to $4.1 \times 10^7 \pm 0.29$ CFU/g, total coliforms from $2.8 \times 10^5 \pm 0.33$ to $4.1 \times 10^5 \pm 0.38$ CFU/g, total fungi from $1.4 \times 10^5 \pm 0.44$ to $2.2 \times 10^5 \pm 0.25$ CFU/g, total *Vibrio* from $9.2 \times 10^4 \pm 2.18$ to $1.0 \times 10^5 \pm 2.64$ CFU/g, faecal coliforms from $1.6 \times 10^5 \pm 1.93$ to $2.4 \times 10^5 \pm 0.14$ CFU/g, and total *Salmonella-Shigella* from $1.9 \times 10^5 \pm 2.13$ to $2.5 \times 10^5 \pm 0.22$ CFU/g, while eleven bacterial genera (*Bacillus, Salmonella, Escherichia, Enterobacter, Pseudomonas, Shigella, Staphylococcus, Vibrio, Serratia, Proteus*, and *Streptococcus*) and fungi (*Aspergillus, Mucor, Penicillium, Rhizopus*, and *Candida*) were isolated, with bacterial isolates showing high resistance to amoxicillin-clavulanate and cefuroxime but high sensitivity to gentamicin among *E. coli, Staphylococcus* spp., and *Pseudomonas* spp., and heavy metal concentrations (in mg/kg) for lead (6.076 ± 0.004 to 9.158 ± 0.050), copper (6.621 ± 0.006 to 103.850 ± 0.099), cadmium (0.641 ± 0.980 to 1.054 ± 1.441), chromium (0.050 ± 1.681 to 12.615 ± 2.051), and mercury (0.000 to 1.291 ± 0.185) were measured, revealing a significant correlation ($p < 0.05$) between microbial loads and heavy metal concentrations, and because all values exceeded permissible limits set by the International Commission on Microbiological Specifications for Foods (ICMSF) and the Federal Environmental Protection Agency (FEPA), proper processing of periwinkle before consumption is critical to prevent adverse health outcomes for consumers.

KEYWORDS*:* Periwinkle, heavy metal contamination, microbial load, antibiotic resistance, food safety

INTRODUCTION

Periwinkles (*Tympanotonus fuscatus* and *Pachymelania aurita*) are common and dominant molluscs of high economic value in and around the brackish water of the riverine areas of the Niger-Delta region of Nigeria. They are deposit feeders and bioindicators of heavy metals and hydrocarbon pollution in the marine environment (Davies *et al.*, 2006). They are mass consumer products and are a very cheap source of protein in Akwa Ibom State (Ekpo and Uzegbu, 2004). They are also transported to many non-riverine towns and cities where they are used to prepare various palatable dishes in hotels and restaurants across the country, Nigeria. The method of processing periwinkles before consumption differs among the populace. Some believe that the shell should be removed and the meat washed thoroughly before cooking, others believe that the periwinkle should be thoroughly washed; its pointed end cut off, and then cooked with its shell because of its perceived medical and nutritive value (Odu *et al.* 2010). Heavy metal pollution is a global issue, although its severity and the levels of pollution differ from place to place. At least twenty metals are classified as toxic, with half of them emitted into the environment in concentrations that pose great risks to human health (Deb and Fukushima, 1999). Contamination of the environment with toxic heavy metals has become one of the major causes of concern for humankind. Heavy metals are intrinsic, non-biodegradable and major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (Asaolu *et al*., 1997; Hussain *et al*., 2011). They are generally present in small amounts in natural aquatic environments as they generally enter the aquatic environment through natural (atmospheric deposition, erosion of geological matrix) or anthropogenic activities (caused by industrial effluent, domestic sewage, mining and agricultural wastes (Vautukuru, 2005). In recent times, industrial activities have raised natural concentrations, causing serious environmental problems. The biota that inhabits contaminated sites is generally exposed to very high concentrations of these pollutants (Woo, Sin and Wong, 1993; Farombi *et al*., 2007; Ekpo and Ettah, 2022). The study of toxic heavy metals is very important in comparison with other pollutants due to their non-biodegradable nature, accumulative properties and long biological half-lives. It is difficult to remove them completely from the environment once they enter it. With the increased use of a wide variety of metals and petrochemicals in industries, coupled with the African lifestyle of indiscriminate dumping of wastes, there is now a greater awareness of toxic metal pollution of the environment (Ukpebor *et al*., 2005).

Studies on the microbiological quality of shellfish have shown that they harbour many pathogenic microorganisms (Ukpong and Efuk, 1992; Ekpo *et al.*, 2010). Most times, the accumulation and concentration of pathogenic microorganisms and other toxic materials are usually from untreated human waste and industrial effluents that find their way into the water bodies that are inhabited by these shellfish (Montgomery and Needselman, 1997). The

continuous release of these pollutants has resulted in an enriched microbial community. These microbes often find surfaces or organs of aquatic organisms for colonisation (Mahalarmi *et al.*, 2013). Bacterial species acknowledged to be potential food pathogens of enteric origin, namely, *Bacillus spp. E. coli, Shigella spp. Staphylococcus aureus and Listeria monocytogenes* can be isolated from coastal water, as a result of human activities, such as improper waste disposal, which includes human, domestic and industrial wastes (Odu *et al.*, 2010). Pathogenic bacteria in marine waters are most abundant in the sediments; they can also be found on the surface film, as well as in the water column (Bassey *et al.*, 2014). However, a proper understanding of the transfer of microorganisms through the food web is essential to predict the exposure of consumers of this seafood to possible health consequences associated with their consumption. This research was therefore carried out to determine the microorganisms and the heavy metal concentration of periwinkles and their effect on consumers.

MATERIALS AND METHODS

Collection of Samples

Periwinkles (freshly harvested) sample (*Tympanotonus fuscatus* and *Pachymelania aurita*) used in this study were harvested from Itu, Oron and Ibeno, which are famous aquatic environments in Akwa Ibom State, Nigeria. The samples were preserved with the water and sediment from the same environment and taken to the laboratory in an ice-cold chest, where they were analysed.

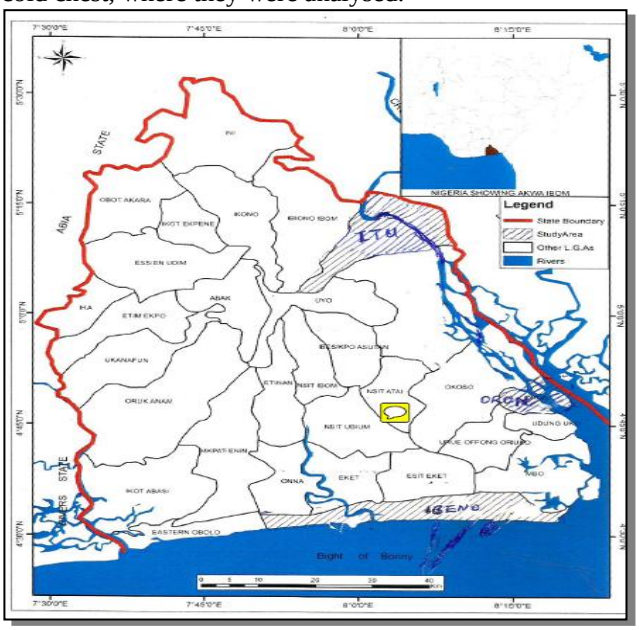

Fig. 1. Sampling location on the map of Akwa Ibom State

Microbiological Analyses:

The samples were washed, scrubbed and soaked in water for 24 hours. The meat was aseptically extracted from the shell and this makes the first group of 'raw extracted periwinkle' samples, the second group of 'processed periwinkle with shell' was aseptically scrubbed, washed and the pointed ends cut off, the third group of processed periwinkles without shell was thoroughly scrubbed, washed and the meat was aseptically extracted. The second and third groups (processed periwinkle samples) were boiled under laboratory conditions at 100^{o}C for 15min as described by APHA (2005). Standard pour plate technique as described by APHA (2005) was used for the microbiological analysis from 10-fold dilutions, nutrient agar medium (NA) was used for the enumeration of total heterotrophic bacteria counts, MacConkey agar was used for total coliform counts, Salmonella-shigella agar for total salmonella-shigella counts, Thiosulphate citrate bile salt sucrose agar for total vibrio counts and Sabouraud dextrose agar for total fungal counts. The bacterial plates were incubated at 37 °C for 24 hours, while fungal plates supplemented with 0.5mg/l streptomycin were incubated at room temperature (28 ± 2^{o}C) for 3-5 days. Colonies were selected randomly and were characterized using morphological and biochemical tests such as Gram stain, spore stain, motility, catalase, oxidase, coagulase, indole, MR-VP, Urease and sugar fermentation tests. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the Identification of Medical Bacteria (Cowan, 1985). Fungal isolates were identified based on their morphological and cultural characteristics as recommended by Sampson *et al.* (1984).

Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was determined using the disk diffusion technique (Cheesbrough, 2006), on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006). The antimicrobial drugs used were Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Amoxycillin/Clavulanates (30µg), Nitrofurantoin (300µg) and Ampicillin (10µg).

Metals Concentration Analyses

The concentrations of cadmium (Cd), chromium (Cr), Copper (Cu), lead (Pb) and mercury (Hg) in the periwinkle samples were determined using an atomic absorption spectrophotometer (Model: UNICAM 939) according to the recommended methods of the Association of Official Analytical Chemists AOAC} (2005).

Statistical Analysis

Analysis of variance (ANOVA) and Correlation matrix were used to compare means, and values were considered significant at $p<0.05$. Post-Hoc multiple comparisons for the ANOVA were done using Least Significant Difference (LSD).

Results

The mean microbial loads in periwinkle samples from the three study areas are shown in Figure 1. The total heterotrophic bacterial count ranged from $3.4 \times 10^{7} \pm 0.07$ to $4.1 \times 10^{7} \pm 0.29$ CFU/g**.** Total coliforms ranged from $2.8 \times 10^{5} \pm 0.33$ to $4.1 \times 10^{5} \pm 0.38$ CFU/g, while total fungi ranged from $1.4 \times 10^{5} \pm 0.44$ to $2.2 \times 10^{5} \pm 0.25$ CFU/g. The faecal coliform count varied from $1.6 \times 10^{5} \pm 1.93$ to $2.4 \times 10^{5} \pm 0.14$ CFU/g**,** and the total *Vibrio* count ranged from $9.2 \times 10^{4} \pm 2.18$ to $1.0 \times 10^{5} \pm 2.64$ CFU/g**.** The total *Salmonella-Shigella* count ranged from $1.9 \times 10^{5} \pm 2.13$ to $2.5 \times 10^{5} \pm$

0.22 CFU/g. For processed periwinkle (Figure 2), no viable microbial growth was recorded in shelled samples. In contrast, the processed unshelled periwinkle showed a total heterotrophic bacterial count of 5.4 × 10^5 CFU/g. Morphological and biochemical characteristic of the bacteria and fungi isolated from the samples at the different locations are shown in Tables 1 and 2. A total of eleven bacteria species belonging to the following genera, *Bacillus*, *Salmonella*, *Escherichia*, *Enterobacter*, *Pseudomonas*, *Shigella*, *Staphylococcus*, *Vibrio*, *Serratia*, *Proteus*, and *Streptococcus* and a total of five fungal species were isolated from the samples. These include *Aspergillus niger*, *Mucor*, *Penicillum notatum*, *Rhizopus*, and *Candida* species.The results of the antimicrobial sensitivity screening of antibiotics against the test isolates ar e presented in Table 3. The results revealed that the bacterial species exhibited different sensitivity and resistivity pattern to the antibiotics used. Most of the isolates showed an intermediate or resistant pattern towards Amoxycillin/Clavulanic acid and Cefuroxime. All *E. coli*, *Staphylococcus* and *Pseudomonas* species were sensitive to Gentamicin but resistant to Cefuroxime. The levels of heavy metals (Pb, Cu, Hg, Cr, and Cd) in the periwinkle samples from the three studied locations are presented in Figure II. Means concentrations of heavy metals in periwinkle samples ranged between 6.076 mg/kg (Oron) and 9.158 mg/kg (Ibeno) for Lead, 6.621mg/kg (Ibeno) and 103.850mg/kg (Itu) for Cu, 0.050mg/kg (Oron) and 12.615mg/kg (Ibeno) for Cr, 0.641 mg/kg (Itu) and 1.054 mg/kg (Ibeno) for Cd, and 0.00 mg/kg (Itu) and 1.291 mg/kg (Ibeno) for Hg
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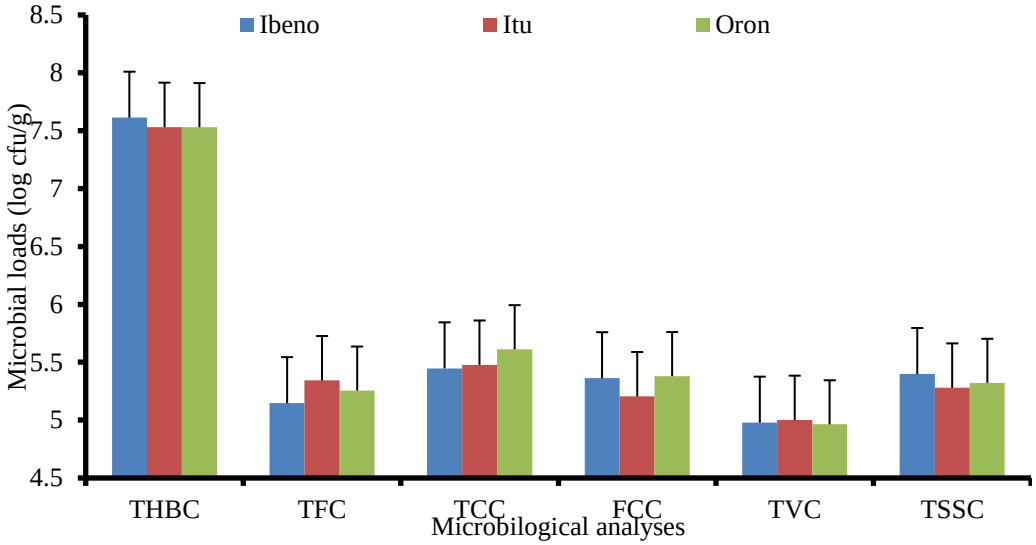
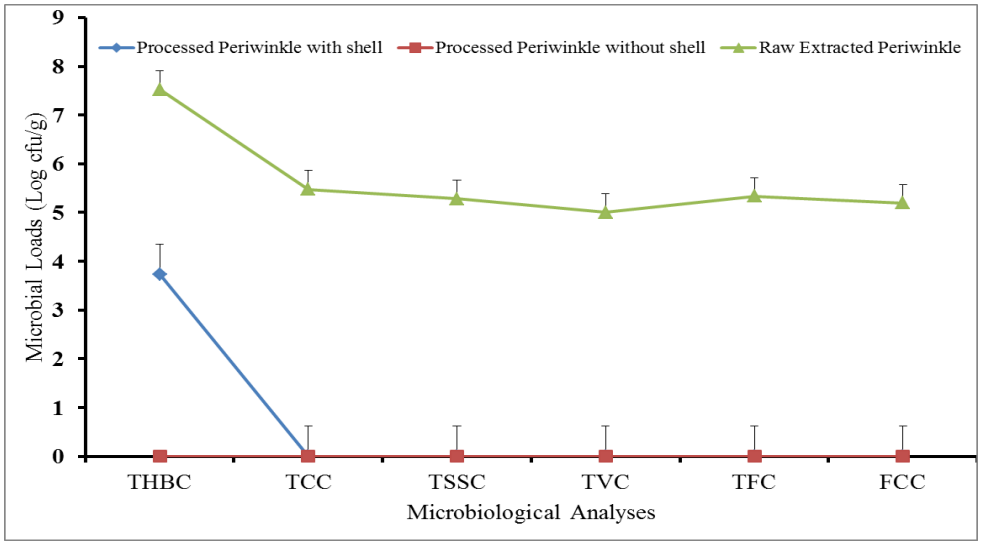


Key: THBC= Total heterotrophic bacteria counts, TFC= Total fungal counts, TCC= Total coliform counts, FCC= Faecal coliforms counts, TVC= Total vibrio counts, TSSC= Total *Salmonella-Shigella* Counts

Fig. 1: Mean Viable Microbial Counts of Periwinkle's Samples



Key: THBC= Total heterotrophic bacteria counts, TCC= Total coliform counts, TSSC= Total Salmonella-Shigella Counts, TVC= Total vibrio counts, Total fungal counts, FCC= Faecal coliform counts

Fig. 2: Viable Microbial Counts of Processed and Raw Extracted Periwinkle Samples

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Table 1: Morphological and biochemical characteristics of Bacteria Isolates

Isolates	Cell Shape	Gram's Reaction	Catalase	Coagulase	Motility	Citrate	Urease	Indole	Oxidase	Vogesproskaurer	Methyl/Red	Glucose	Lactose	Maltose	Mannitol	Dextrose	Sucrose	Probable Organism
1.	Rod	+	+	-	+	+			-	+	-	A	A	A	A	A	A	*Bacillus* sp.
2.	Rod	-	+	-	+	+	-	+	+	+	-	A	AG	A	AG	A		*Salmonella* sp.
3.	Rod	-	+	-	+	+	-	+	-	-	+	A	AG	A	AG	AG	A	*Escherichia coli*
4.	Rod	-	+	-	+	+			-	+	-	A	A	AG	AG	AG		*Enterobacter* sp.
5.	Rod	-	+	-	+	+			+	-	+	AG	A	A	AG	AG		*Pseudomonas*
6.	Rod	-	+	-	-	-	-	-	-	-	+	A	A	A	A	A	A	*Shigella* sp.
7.	Cocci	+	+	-	-	-	+	-	+	+	-	AG	A	AG	AG	A		*Staphylococcus aurous*
8.	Rod	-	+	-	+	+	-	+	-	+	-	A	A	A	A	A	A	*Vibrio* sp.
9.	Rod	-	+	-	+	+			-	+	-	A	A	A	AG	A	A	*Serratia marcessens*
10.	Rod	-	+	-	-	-	+	-	-	-	+	A	A	A	AG	A		*Proteus* sp.
11.	Cocci	+	-	-	+	+	-	+	-	-	+	A	AG	A	AG	A	A	*Streptococcus* sp.

A =Acid production, G = Gas production

Table 2: Morphological characteristics of Fungi Isolates

Isolates	Colour Hyphae	Colour Colony	Nature of Hyphae	Type of Spore	Special Structure	Shape of Sporagiophore or Conidiophores	Spore Character	Probable Organism
1.	Black	Black To grey Black with edge	Septate	Globose	Smooth walled erect conidiophores	Dome gradually enlarging	Compact	*Aspergillus niger*
2.	White	White and pale grey	Unseptated	Oval spores	Sporangiophores	Erect sporangiophore	Globose	*Mucor* sp.
3.	Blue to green colony	Green	Septate multinucleated, branched	Chain of green round globose	Absent	Erect and branched Condiophore	Broom like straginata with conidia	*Penicillium notatum*
4.	White To grayish brown	White	Coenocytic		Rhizoids Ovoid sporangiophores	Filamentous Stolon		*Rhizopus* sp.
5.	Moist milky colony		Unseptated	Blastoconidia				*Candida* sp.

Table 3: Antibiotic Sensitivity Pattern of Bacterial Isolates

Bacterial Isolates	CAZ 30 µg	CRX 30 µg	GEN 10 µg	CPR 5 µg	OFL 5 µg	AUG 30 µg	NIT 300 µg	AMP 10 µg
Streptococcus sp.	I	I	S	I	S	R	I	R
Staphylococcus sp.	S	R	S	S	S	R	R	I
Enterobacter sp.	R	R	S	S	R	R	S	R
Vibrio sp.	R	I	I	R	S	I	R	R
Serratia marcescens	R	R	S	S	S	R	I	R
Shigella sp.	R	I	S	S	S	R	I	R
Escherichia coli	R	I	R	R	S	R	R	I
Salmonella sp.	R	R	S	R	R	R	S	I
Proteus sp.	R	R	S	S	S	R	S	R
Bacillus sp.	I	I	R	R	R	R	R	I
Pseudomonas sp.	I	R	S	S	S	I	S	R

Resistant = R(≤15), Intermediate = I(16-20), Sensitive = S(≥21), CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamicin, CPR = Ciprofloxacin, OFR = Ofloxacin, AUG = Amoxycillin/Clavulanates, NIT = Nitrofurantoin, AMP = Ampicillin

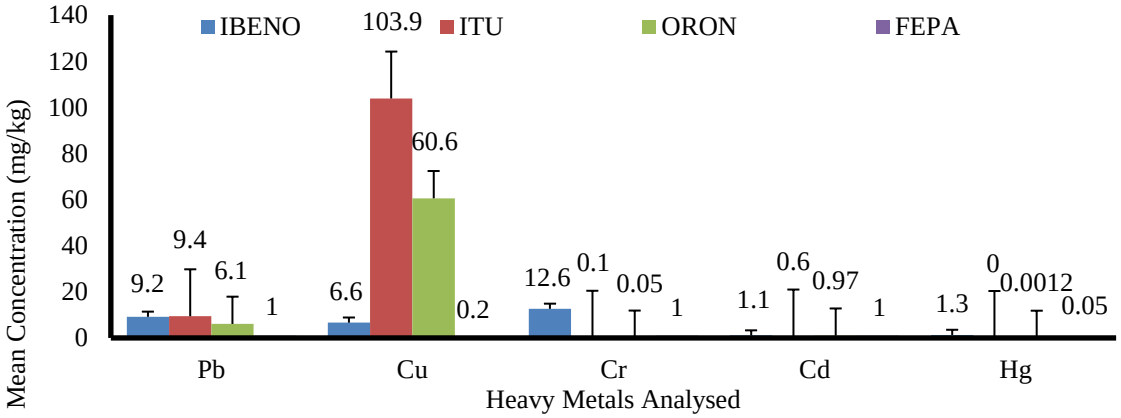

Fig. 3: Concentrations of Heavy Metals in the Periwinkle Samples

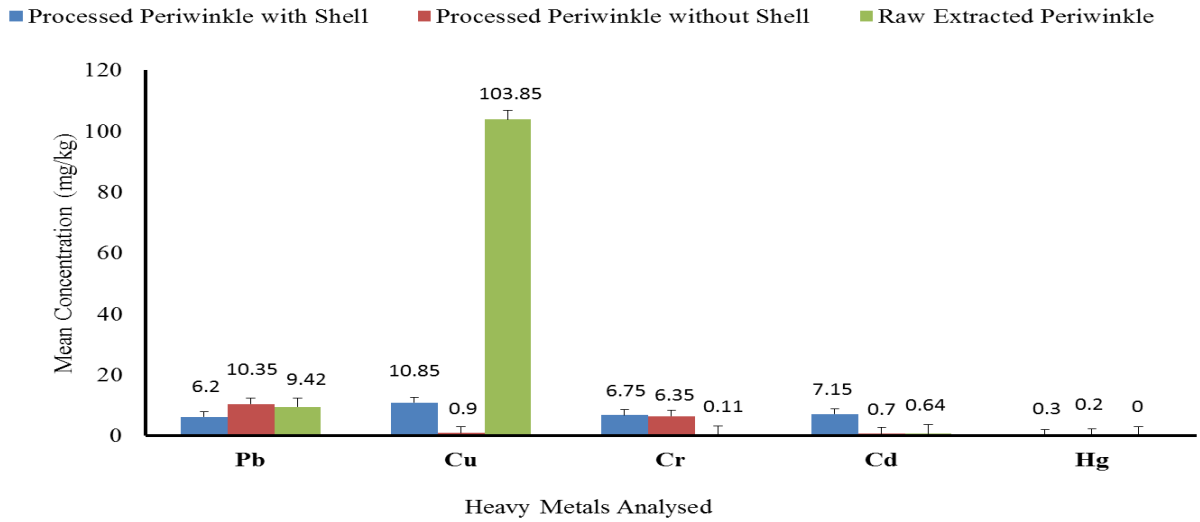

Fig. 4: Concentrations of Heavy Metals in Processed and Raw Extracted Periwinkle Samples

DISCUSSION

The results of this study have revealed a high incidence of microbial contamination of the mollusc. The total microbial counts obtained were higher than permissible limits (1 x 10^5 CFU/g) for bacteria and fungi, and (1 x 10^2 CFU/g) for coliforms. It is also higher than the number of E. *coli* in 25g of mollusc meat by the International Commission of Microbiological Specification for Food (ICMSF) (1982) and United States Food and Drug Agency (USFDA) (2005). The anthropogenic activities (i.e., bathing, washing of cloths or other materials, deposition of faecal matters and sewage discharge by municipal authorities and independent outfits) and other contaminants (Singh *et al.*, 2017) in the water bodies where the periwinkle is harvested contributed to the

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high microbial profile of the samples analyzed. The isolation of *Bacillus* and *Aspergillus* spp. is a source of serious concern since they have been implicated in the spread of diseases such as anthrax, food poisoning, gastroenteritis and aspergillosis (Frazier and Westhoff, 2004). *Bacillus* sp. causes a toxin-mediated disease rather than infections such as diarrhoea and emetic illness characterized by nausea and vomiting (Adebayo-Tayo *et al.,* 2012).

Faecal coliform includes several pathogenic bacteria species, including *Salmonella* sp., *Shigella* sp., *E. coli*, *Klebsiella* and *Aeromonas hydrophila* (APHA, 2005) which are all species that have been causes of shellfish-borne diseases in humans. *E. coli* is the causative agent of diarrhoea, dysentery, haemolytic uremic syndrome, bladder and kidney infection, septicemia, pneumonia and meningitis (Kumar *et al.,* 2005). *Enterobacter aerogenes* causes septicemia and neonatal meningitis, *Salmonella paratyphi* is the causative agent of paratyphoid fever in humans, who are the only reservoir of this organism (Adebayo-Tayo *et al.,* 2012). *Salmonella*, one of the most important food-borne pathogens, is an indication of sewage contamination and appears to be associated with several non-human hosts like reptiles, and has been reported to survive and persist in the aquatic environment (Winfield and Groisman, 2003). The presence of *Streptococcus* sp. is also implicated in human infections like pharyngitis, scarlet fever and pneumonia. *Shigella* sp. is the causative agent of shigellosis in human who are the only reservoir of the organism (Adebayo-Tayo *et al.,* 2012). *Pseudomonas aeruginosa* is prevalent among patients with wounds, burns, cystic fibrosis and some bloodstream infections (Llopis *et al.,* 2004). These organisms are likely to have been introduced into the environment by swimmers and infected individuals who use these creeks for recreational purposes.

The presence of *Serratia marcescens*, an enterobacterium in the samples, is a major source of concern as it has been implicated in human pulmonary and urinary infections (Cheesbrough, 2006). The findings show that the raw extracted periwinkle is unacceptable for consumption. *Vibrio* species were also isolated from the aquatic samples. Some researchers have opined that the presence of pathogenic *Vibrios* in shellfish, sediment and overlying waters is most pronounced in the waters of tropical and sub-tropical ecosystems (Zimmerman *et al.,* 2007). In addition, *Staphylococcus aureus* was isolated in the samples. *Staphylococcus aureus* may be expected to exist at least in low numbers in any or all food product of animal origin or in those that are handled directly by humans unless heat processing steps are applied to aid their destruction. The presence of *Staphylococcus aureus* indicates possible cross contamination or post-process contamination through unhygienic handling, processing, packaging and storage as well as the filthy environment in the market place. Staphylococcal food poisoning usually develops within 4 hours of the ingestion of contaminated food. The periwinkles are sedentary and will accumulate pathogenic organisms in the body of water and sediment. It is also possible that some other organisms may have been introduced by the shellfish harvesters as well as other human activities. The findings in this study in respect of the periwinkles, water and sediment are in agreement with the observations of Omenwa *et al.* (2011); Adebayo-Tayo and Ogunijobi (2008) and Odu *et al.* (2010), who isolated similar organisms from freshly harvested *Tympanotonus* sp. (Periwinkle) and *Crassostrea* sp. (Oyster). The efficacy of the depuration and pasteurisation process was shown in the results of the processed periwinkle samples. The shelled and unshelled periwinkles were hygienically processed hence, the average total heterotrophic bacterial count (THBC) observed in the processed unshelled periwinkle was 10^3 cfu/g, which is lower than the acceptable limits of 10^5 cfu/g stipulated by International Commission on Microbiological Specifications for Food (ICMSF, 1982) standards. Previous studies have shown that heating in water at 100 °C for 5 minutes can reduce the bacterial load by 75% (Odu *et al.,* 2010). This further indicates that micro-organisms associated with periwinkle from the harvesting grounds are mostly eliminated during pasteurisation, with the exception of the spore-forming rods, *Bacillus*, that get resuscitated and germinate. The heat treatment is also responsible for the absence of coliforms, fungi, Vibrio, *Escherichia coli*, *Salmonella* and *Shigella* in the processed periwinkle. This indicates no extraneous introduction of organisms of faecal origin. This meets the ICMSF standard of no *E. coli* in 25g of mollusk meat (ICMSF, 1982). It also revealed that there was no post-process contamination of this sample category and the result obtained is in agreement with the findings of Odu *et al.* (2010).

The study of heavy metal concentrations in aquatic organisms is important due to the nature, management and human consumption of the organisms. Bioaccumulation of metals in periwinkles can be considered an index of metal pollution in aquatic bodies (Davies *et al.,* 2006). Heavy metals, being trace components of the aquatic environment, have increased due to industrial wastes, agricultural and mining activities, which can only be recognised if the quantity is within detection limits (Faleye *et al.,* 2017). Different orders of heavy metals might be attributed to the different uptake, metabolism and detoxification of metals in periwinkle. Similar observation was recorded by Ibrahim and El-Naggar (2006). Mercury, lead and cadmium top the list of studied heavy metals because they are more damaging to organisms and the environment (Nwabueze *et al.,* 2011; Nkpaa *et al.,* 2010). Mercury occurs naturally in nature but can be released into the environment via industrial activities. As a metal it can accumulate in soils, sediments and surface water. It is present in seafood as methyl-mercury, where it has the potential to bioaccumulate in the food chain and cause damage to the kidney and central nervous system (Andem *et al.,* 2013). The developing brain of the foetus is even more sensitive (Gbaruko and Friday, 2007). The concentration in the samples from Ibeno was higher than the FEPA permissible limit of 0.05 mg/kg. The concentrations of mercury were highest in the Ibeno sample location and lowest in Itu sample location. Lead, like cephalosporin-resistant bacteria in this research, which of critical concern (Adegoke, *et al.,* 2020), has the potential to accumulate in

the body and also affect the nervous system, kidneys and reproductive system. It is also linked to reduced cognitive development in children (Zodape *et al.,* 2010). Lead was highest in the samples from Itu, the concentration of lead in the samples were higher than the permissible limits of 1.0mg/kg by FEPA and this is similar to the findings of Otitoju and Otitoju (2013). Cadmium mean concentrations in the samples were within the permissible limits of 1.0mg/kg by FEPA, except for Ibeno samples. All mean concentrations of Hg, Cd and Pb were lower and does not agree with obtained by Zodape *et al.* (2010). In another study, Hg was not detected in periwinkle from Itu and Abuloma Rivers but was 64.2mg/kg in Oron River sediment (Zodape *et al.*, 2010). In Warri, Hg and Cd were not detected in a similar study in *Tympanotonus fuscatus* (Nwabueze *et al.*, 2011). The low detectable levels of Hg, Cd and Pb in the samples could be explained by the absence of industries close by as opposed to other parts of the Niger Delta region and agrees with the findings of Otitoju and Otitoju (2013) in Niger Delta region of Nigeria. Copper is an important cellular component of organisms, but have been found to be toxic under certain conditions and at high concentrations. Their major inputs include natural and anthropogenic sources (Pfafflin and Ziegler, 2006; Valavanidis and Vlachogianni, 2014). Cu was detected in all samples and in varying concentrations. The mean concentration of copper in the samples was far above the FEPA permissible limit, with the periwinkle samples from Itu and Oron exhibiting higher concentrations of the heavy metal and the trends in the samples are similar to those observed by Otitoju and Otitoju (2013). Chromium levels were above the FEPA standard permissible limits of 1.0mg/kg in the samples, Cr plays an important role in glucose metabolism, and its bioaccumulation in shellfish has been reported to cause impaired respiratory and osmoregulatory functions through structural damage to the shellfish (Heath, 1991). The work is in line with the observation of Ekpo and Ukpong (2014) who observed a high concentration of chromium in periwinkle.

CONCLUSION

The comparative studies show that when periwinkles were processed under hygienic conditions, the microbial load was greatly reduced, making the periwinkle meat safe for consumption. The heavy metals analysed from this work provide information about the concentration of metals in periwinkles, which can be related to the industrial activity and other human activities taking place in these three areas (Ibeno, Itu and Oron). Although the tropical periwinkle is a cheap source of quality protein, the microbiological profile and heavy metal concentrations underscore the possible public health risk associated with inadequate cooking after pasteurisation. The appropriate agencies must ensure that processors and handlers maintain high sanitary standards.

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