

## MICROBIOLOGICAL ANALYSIS OF THE FRESHWATER CLAM (*Galatea paradoxa*, BORN 1778) CAUGHT FROM CROSS RIVER, NIGERIA

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### ABSTRACT

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Microbiological analysis of the freshwater clam, *Galatea paradoxa*, from Cross River, Nigeria, was carried out using standard microbiological techniques. The total heterotrophic bacterial count (THBC) varied from  $2.80 \times 10^4$  cfu g<sup>-1</sup> to  $4.80 \times 10^4$  cfu g<sup>-1</sup>. The total coliform count (TCC) ranged  $2.72 \times 10^2$  cfu g<sup>-1</sup>-  $6.36 \times 10^2$  cfu g<sup>-1</sup>. The total faecal coliform count (TFCC) was from  $1.81 \times 10^2$  cfu g<sup>-1</sup> to  $4.54 \times 10^2$  cfu g<sup>-1</sup> and the total fungal count (TFC) fluctuated between  $3.36 \times 10^3$  cfu g<sup>-1</sup> and  $9.64 \times 10^3$  cfu g<sup>-1</sup>. The bacteria and fungi species isolated and their frequencies of occurrences were: *Staphylococcus aureus* (16.7%), *Streptococcus* sp (8.3%), *Vibrio cholerae* (8.3%), *Escherichia coli* (4.2%), *Enterobacter* sp (4.2%), *Salmonella* sp (8.3%), *Candida albicans* (8.3%), *Aspergillus niger* (16.7%), *Penicillium chrysogenum* (16.7%) and *Rhizopus stolonifera* (8.3%). The different anatomical sites of the clam sampled and their isolation rates were: guts (37.5%), inner shell (29.2%), and the homogenized flesh (33.33%). The isolates were mostly susceptible to Gentamycin and Ciprofloxacin and resistant to Rocephin and Zinnacef. The least susceptible isolate, *Staphylococcus aureus*, was susceptible to only Gentamycin, Ciprofloxacin and Streptomycin out of ten antibiotics used. Isolation of *E. coli* indicated faecal contamination of the clam sample, therefore depuration of clam sample is necessary to prevent food borne disease outbreaks.

**Keywords:** Bacterial isolates, *Galatea paradoxa*, *Aspergillus niger*, depuration

### INTRODUCTION

Molluscs are an extremely diverse group of animal with more living species than birds, mammals, reptiles and fishes combined (Lydeard and Linderberg, 2003). Molluscan shellfish are characterized by the number of shell valve. Bivalve shellfish (oysters, mussels and clams) have two shell valves hinged by an elastic ligament. Molluscan bivalves such as oysters and clams are seafood that are widely consumed; they are excellent source of protein but are highly perishable as fresh seafood, their short life thus poses serious practical problems of their distribution and storage (Frazier and Westhoff, 2000). The freshwater clam *Galatea paradoxa* (Born 1778) formerly *Egeria radiata* (Lamarck 1804) is a filter-feeding bivalve mollusc belonging to the order Veneroidea, superfamily Tellinoidea and family Donacidae (Purchon 1963) within this group the highest diversity is found in West African sub-region with a range that extends from the Gulf of Guinea to the Congo (Moses, 1990) and restricted to the lower reaches of a few large rivers in West Africa such as the Volta (Ghana), Cross and Nun (Nigeria), and Sanaga (Cameroon), (Etim and Brey, 1994).

These clams live in many types of habitats ranging from small ponds, ditches to lakes, canals, estuaries and rivers where it is partly buried in gravels and coarse sands, generally in waters at depth between 0.5-2.0 m, sometimes at greater depth where they are widely caught and farmed for human consumption (Obirikorang et al., 2013). The flesh of clam has vitamins that are essential for healthy skin, bones and teeth, they have an exceptional nutritional value making them ideal for the human diet, their flesh is rich in calcium, iron, magnesium and vitamins A, B, B<sub>12</sub>, B<sub>6</sub> and C and as such are mostly harvested for food (Serfor-Armah et al., 2010). Unfortunately, the freshwater ecosystem is constantly polluted and as a result there is bioaccumulation of bacteria and other pathogenic organisms (Hatha et al., 2005). According to Gram et al. (2002), clams that inhabit the freshwater ecosystem are also susceptible to becoming host and carriers of these bacteria. Being a filter feeder, *Galatea paradoxa* can accumulate human pathogenic bacteria, fungi and viruses when grown or sampled from sewage contaminated waters. For instance, Adjei-Boateng et al. (2009), studied the bacteriological contamination of *G. paradoxa* from the Volta estuary, Ghana, and reported that due to its water activity and nutritional content, such shellfish represents a public health risks especially when not properly depurated.

Although various aspects of the normal flora of the freshwater clams have been studied in an attempt to define the bacterial group associated with the freshwater clams and those that came from its ecosystem so as to determine its microbiological quality for consumption, nevertheless, dearth of information still exists on the complete microbiological examination of *G. paradoxa* from freshwater at Itu, Akwa Ibom State, Nigeria. This research therefore seeks to estimate the microbial load, isolate and characterize the bacterial and fungal species associated with *G. paradoxa*, check the sensitivity of the bacterial isolates to different antibiotics and estimate the frequency of occurrences and isolation rate of microbial species from different parts of *G. paradoxa* from Cross River.

## MATERIALS AND METHODS

### Source and collection of samples

Fresh samples of *G. paradoxa* were obtained aseptically from catch landings by artisanal fishers at Itu Bridge monthly for six months (June-December 2016). The samples were collected in sterile ice-packed coolers and then transported to the Microbiology Laboratory University of Uyo, Uyo, Nigeria for analysis. Clams were washed with a brush and water to remove all materials adhering to the shells and allow to air dry. Subsequently, the clams were opened aseptically using a sterile scalpel. Care was taken to ensure that all the fresh clams sample analysed were healthy-looking in appearance and with no signs of diseases infections noted. Clam flesh weighing 10 g were homogenized in a blender with 90 ml of sterile distilled water, corresponding to a 10<sup>-1</sup> dilution (Hatha *et al.*, 2005). The guts of the fresh clams were obtained by dissection using a sterile surgical blade then mashed using a sterile stomacher. The clam muscle was also mashed separately and then all the specimens were used for analysis.

### Serial dilution of the homogenate

The homogenate was serially diluted up to 10<sup>-12</sup> using 9 ml of sterile dilution blanks. Overall 60 samples were collected. Using the pour plate method 1.0 ml of the dilutions were transferred to sterile petri dishes and plated in duplicate in standard plate count agar. The plates were incubated at 37°C for 24 hours. After incubation, the plates with 30 - 300 colonies were chosen for counting and the total plate count bacteria expressed as the number of colony forming units (cfu) per gram of shellfish. The organisms were sub-cultured for pure isolates. The purified isolates were tested biochemically and confirmed by microscopic examination prior to characterization and identification. These freshly sub-cultured organisms were stocked and preserved in a refrigerator for further tests.

### Enumeration of total heterotrophic bacteria or total viable count

This was carried out by picking up morphologically different colonies using a sterile inoculation needle and aseptically transferring them to a sterile nutrient slants for further characterization. The isolates were checked for their purity and characterized up to genera following a standard characterization key (Bodyfelt, 1979) based on Gram staining, catalase, motility, urease, Kovac's oxidase, oxidation/fermentation (O/F) test, citrate, indole, mannitol, glucose, sucrose and lactose tests.

### Enumeration of total and faecal coliforms

This was done by preparing a sample homogenate in the same way as described for the total plate count bacteria and 10<sup>-1</sup> to 10<sup>-12</sup> dilutions and using it for estimating the coliform bacteria. A standard 3 - tube dilution most-probable number (MPN) method (West, 1989) procedure was used to enumerate the coliform load in the clam samples. Using a sterile pipette, 1 ml sample each was inoculated into 10 ml sterile McConkey broth. After inoculation, the tubes were incubated at 37 °C for 24 h and checked for gas production. The tubes with gas production were recorded and referred to the MPN table to ascertain the MPN index for the coliforms.

### Microbiological analysis of clam samples for fungi

The clam samples were processed by weighing 1 g proportion of each samples (gut, flesh and shell) aseptically into 9 ml of (w/v) sterile peptone water in a beaker, and allowed to stand for 30 minutes stirring occasionally using a sterile wooden applicator stick. A drop of each specimen suspension was dispersed with a sterile pipette onto the center of the Petri dish containing Saboroud dextrose agar and spread with a sterile hockey stick. The plates were incubated at 24 °C for one week and observed each day for fungal growth. The fungi were identified by naked eyes and by use of a microscope.

Macroscopic examination of fungi was based on the following characteristics: colony colour, nature of hyphae, type of soma, asexual spore, reproductive structure, conidia head and special vegetative structure. Microscopic examination of fungi was done by teasing small portion of the fungal pure culture and mounting in lactophenol cotton blue dye on a clean slide, covered with a clean cover slip and observed under the microscope (Fawole and Osho, 1995) with 10x objective lens and confirmed with 40x objective lens. Royal Horticultural Society (RHS) mini colour chart was used in this study as a guide for morphological identification.

### Experimental design

The design took the form of completely randomized design (CRD) with four treatments and three replicates. Total Heterotrophic Bacteria Count (THBC), Total coliform Count (TCC), Total Faecal Coliform Count (TFC) and Total Fungal Count (TFC) represented treatments 1, 2, 3 and 4 respectively. Each consecutive two months sampling represented a replicate and the six months sampling represented three replicates.

### Statistical analysis

All findings of MPN for Total Heterotrophic Bacteria Count (THBC) Total coliform Count (TCC) Total Faecal Coliform Count (TFC) and Total Fungal Count (TFC) were log transformed (log<sub>10</sub>) for the purpose of statistical analysis. The parameters were analysed using analysis of variance (ANOVA). Where significant difference existed, *post hoc* test was performed to separate the means using Duncan Multiple Range test.

## RESULTS

The cultural, morphological and biochemical characteristics of bacterial isolates from clams are presented in Table 1. The isolates were: *Salmonella* sp. *Vibrio cholera*, *Escherichia coli*, *Streptococcus* sp., *Staphylococcus aureus* and *Enterobacter* sp. Microbial load of the sample are shown in Table 2. Mean THBC of  $3.8 \times 10^4$  cfu g<sup>-1</sup> with the least seen in shell and the highest in the gut. Mean TCC was  $4.2 \times 10^2$  cfu g<sup>-1</sup> with the least clam flesh and the highest in gut. Mean TFCC of  $2.1 \times 10^2$  cfu g<sup>-1</sup> was recorded with no coliform in inner shell and highest in gut. Mean TFC was  $7.5 \times 10^3$  cfu g<sup>-1</sup> with least in gut and highest in clam homogenized flesh. Total heterotrophic Bacteria Counts (THBC) in clam gut was significantly higher ( $p < 0.05$ ) compared to flesh and shell. Total coliforms count (TCC) and Total Faecal Coliform portrayed a similar trend, being significantly higher ( $p < 0.05$ ) in the gut than flesh and shell. The reverse was the case of Total fungal count (TFC) which was higher in both flesh and shell than the gut. The frequency of occurrence of isolates from the clam sample is presented in Table 3. The *Staphylococcus aureus* (16.7%), *Penicillium chrysogenum* (16.7%) and *Aspergillus niger* (16.7%) had the highest frequency of occurrence while *Escherichia coli* (4.2%) and *Enterobacter* sp (4.2%) occurred the least. The isolation rates of all organisms from different anatomical sites are shown in Table 4. Microbial specimens in guts of the clams had the highest isolation rate 9(37.5%) followed by the homogenized clam flesh 8(33.3%) and lastly the inner shell 7(29.2%).

Table 1: Cultural, morphological and biochemical characteristics of bacterial isolates from clam sample

Bacteria shape	Gram reaction	Catalase	Motility	Urease	Oxidase	Citrate	indole	mannitol	Glucose	Sucrose	Lactose	Probable organism
Rod	-	+	+	-	-	-	+	+	+	+	+	<i>E. coli</i>
Cocci	+	+	-	-	-	+	-	+	+	+	+	<i>S. aureus</i>
Rod	-	+	+	-	-	-	+	+	+	+	-	<i>Salmonella</i> sp.
Rod	-	+	+	-	-	+	-	+	+	+	+	<i>Enterobacter</i> sp.
Comma	-	+	+	-	+	+	+	+	+	+	+	<i>Vibrio cholerae</i>
Cocci	+	-	-	-	-	+	-	+	+	+	+	<i>Streptococcus</i> sp.

Table 2: Microbial load (cfu g<sup>-1</sup>) of freshwater clam (*Galatea paradoxa*) samples caught from Itu freshwater

Anatomical sites	Total bacterial count	heterotrophic 10 <sup>4</sup> cfu g <sup>-1</sup>	Total coliform Count 10 <sup>2</sup> cfu g <sup>-1</sup>	Total faecal Coliform count 10 <sup>2</sup> cfu g <sup>-1</sup>	Total fungal count 10 <sup>3</sup> cfu g <sup>-1</sup>
Clam gut	4.8±2.1c		6.4±3.5d	4.5±2.6b	3.4±2.5a
Clam flesh	3.7±1.5c		2.7±3.6b	1.8±3.1a	9.6±5.4d
Clam shell	2.8±1.7a		3.6±4.0b	-	9.36±2.2c
MEAN	3.8±2.6		4.2±4.8	2.1±2.7	7.5±3.8

Mean±sd in the same column with different alphabets are significantly different ( $p < 0.05$ )

Table 3: Frequency of occurrence of isolates from different anatomical sites of clam sample

Isolates	Flesh	Gut	Inner shell	Total	%
<i>Escherichia coli</i>	-	1	-	1	4.2
<i>Enterobacter</i> sp.	-	1	-	1	4.2
<i>Staphylococcus aureus</i>	2	1	1	4	16.7
<i>Salmonella</i> sp.	-	2	-	2	8.3
<i>Vibrio cholera</i>	-	1	1	2	8.3
<i>Streptococcus</i> sp.	1	-	1	2	8.3
<i>Candida albicans</i>	-	1	1	2	8.3
<i>Rhizopus stolonifer</i>	1	-	1	2	8.3
<i>Penicillium chrysogenum</i>	2	1	1	4	16.7
<i>Aspergillus niger</i>	2	1	1	4	16.7
Total	8	9	7	24	100

Table 5 shows the antibiotic sensitivity test result for the bacterial isolates from the clams. The isolates were mostly susceptible to Gentamycin and Ciprofloxacin and resistant to Rocephin and Zinnacef. The *Staphylococcus aureus* was the least susceptible isolate and was susceptible to only three (Gentamycin, Ciprofloxacin and Streptomycin) out of the ten antibiotics.

## DISCUSSION

The results of this study showed that the freshwater clam, *Galatea paradoxa* in the Cross River harbours microorganisms including those that are pathogenic. The isolated microorganisms included six (6) bacterial

species and four (4) fungal species. Two groups of organisms which are of public health interest are, organisms naturally present in the freshwater ecosystem (e.g. *Vibrio* sp.) and Enterobacteriaceae which originates from contamination of the freshwater ecosystem with human faecal matter (e.g. *Salmonella* sp. and *E. coli*). In this study, we have identified these two groups in the body of *G. paradoxa* which implies that its consumption should be of public health importance. The detection of coliforms of faecal origin and *E. coli* gives relevant information regarding the food safety and sanitary conditions of clams and the freshwater respectively (Vierra et al., 2003). The presence of *E. coli* serves as an indicator for pathogenic organisms. The high load of microbiological contamination may be associated with the activities of humans and animals which are the major sources of pollutants in this area. This finding agrees with the work of Itah et al. (1996) that estuaries or rivers are constantly polluted with faecal matter from riverine dwellers.

Table 4: Isolation rate of microbial isolates from different anatomical sites of clam sample

Anatomical sites	Isolate number	Isolation rate (%)
Clam gut	9	37.5
Inner shell	7	29.2
Clam flesh	8	33.3
Total	24	100

Table 5: Antibiotic susceptibility test result of bacterial isolates from the clam samples

Test isolates	Zone of inhibition (mm)									
	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>E. coli</i>	12	12	8	0	10	0	9	11	10	0
<i>S. aureus</i>	0	4	0	0	0	0	16	5	0	0
<i>Enterobacter</i> sp.	8	16	4	0	7	0	11	11	0	7
<i>Salmonella</i> sp.	11	7	0	0	8	0	12	6	10	0
<i>Vibrio</i> sp.	0	16	10	10	0	5	17	0	6	16
<i>Streptococcus</i> sp	8	16	11	5	8	8	9	0	10	12

Legend: PEF:Perfloxacin; CN:Gentamycin; APX:Ampiclox; CPX:Ciproflaxacin; S:Streptomycin; SXT;Seprin Z:Zinnacef; AM:Amoxicillin; R:Rocephin; E:Erythromycin.

The level of mean THBC reported in this study is lower than the range reported for *G. paradoxa* from Volta Lake while TCC fell within the range (Adjei-Boateng et al., 2009). Total heterotrophic bacteria and coliform may be directly influenced by many anthropogenic activities and rainfall. Run-off from rain might carry raw sewage from the surrounding villages and leachate from waste sites in the catchment area into the freshwater; the clams being filter feeders are able to accumulate the isolates in their tissues to level twice that in the surrounding waters (Hatha et al., 2005). This agrees with the work of Antai (1998) who reported that the high microbial load in the sample is a clear indication that the freshwater clam *G. paradoxa* serves as a medium through which microbes multiplied rapidly. This happens as a result of their feeding habit. This work also concurred with the findings of Ekanem and Adegoke (1995) that Cross river waters were generally of an unacceptable quality for growing market shellfish. These authors also posited that *Egeria radiata* samples collected from the waters were found to be highly contaminated by bacteria.

*Galatea paradoxa* is a protein rich food and therefore serves as a suitable substrate in supporting growth of different types of bacteria and fungi; the microbial growth in these fresh sea foods will encourage food spoilage and seafood poisoning. The presence of *Salmonella* sp. which indicates possible contamination with *Salmonella* laden faeces can expose the clam consumer to poisoning such as Salmonellosis and human diseases such as typhoid fever. For instance, Udoh (1994) reported the prevalence of *Salmonella* in Cross River estuaries explaining that *Salmonella* is largely an environmental pathogen being associated with infection from fishery products and contacts with natural aquatic habitat. The *Staphylococcus aureus* which is the most occurring bacterial isolate (16.7%) is also known to cause food poisoning in man. The presence of *Streptococcus* sp. in a low prevalence (8.3%) indicates a former contamination. However, this might also potentiate serious consequences to man if clam is consumed raw (Ayers et al., 2008). Among the fungal isolates were species of *Aspergillus* which have also been associated with outbreaks of seafood diseases. There is a great possibility that some of the isolates are micro flora of the clam itself but might also be a function of the micro flora of the freshwater environment as indicated by the similarities between the isolates and the typical freshwater organisms as reported by Itah (1996). Some of the isolates may have been derived from external sources during handling and as such, the clams become transient carriers of such microbes (e. g. *S. aureus*). However, in the study most isolates from the clam gut and homogenized flesh may be accounted for mainly by the filter feeding effect of the clam (Spanggard et al., 1993).

The economic and health problems caused by this shellfish contamination are further compounded by the development of antibiotic resistant among some of the isolates such as *S. aureus*. According to the work of Malik

and Ahmad (1994), high levels of bacterial resistance to antibiotics are indications of abuse and misuse of antibiotics without medical advice and at such bacterial groups cohabiting common environments may express a similar antibiotic pattern. The result of this study also suggests that the freshwater clam, *G. paradoxa*, could serve as a substrate for growth of microorganisms (e.g. *Penicillium chrysogenum*) of laboratory and industrial importance.

From the foregoing, it is important to highlight the fact that the freshwater clam, *G. paradoxa* from Cross River in Nigeria has in them much pathogen that could cause disease outbreaks. Therefore, microbial growth must be controlled through proper handling and / or postharvest best practices, in order to encourage desired products and to discourage growth of spoilage organisms and pathogens in the interest of public health. According to Grayzna and Bonnie (2010) contaminants tend to grow faster in fresh sea food, this is consistent with the present findings from the isolation rate and prevalence data. In conclusion, considering the nutritional value and important protein source of the freshwater clam, *G. paradoxa*, to the riparian human communities where it occurs (King, 2000), the aquatic foods should not be eaten raw without adequate handling and / or postharvest best practices to avoid disease outbreaks /health challenges.

#### ACKNOWLEDGEMENT

The authors profoundly acknowledge the contributions of all staff of Microbiology Laboratory and Fisheries Laboratory, University of Uyo, Nigeria.

#### REFERENCES

- Adjei-Boateng, D., Amisah, S. and Quagraine, K. K. 2009. Bacteriological contamination of the freshwater clam (*Galatea paradoxa*) from the Volta estuary, Ghana. *African Journal of Microbiological Research*, 3(7):396-399.
- Antai, S. P. 1998. Study of the microbial flora of Nigeria mussel species. *International Journal of Food Microbiology*, 6:259-261.
- Ayers, T., Iwamoto, M., Swerdlow, D. L. and Williams, I. 2008. *Epidemiology of seafood associated outbreaks in the United States, 1973-2006, abstr. P3-70. Abstract of International. Association of Food Protection. and Annual Meeting, Columbus, OH.*
- Bodyfelt, F. W. 1979. Sensory, shelf-life, microbial and chemical evaluation of creamed cottage cheese treated with sorbates. *J. Food Protection*. In: Buchanan, R.E. and Gibbons, N.E., Editors, 1974. *Bergey's Manual of Determinative Bacteriology* (8th edn.), The Williams and Wilkins Co., Baltimore pp. 217-243.
- Ekanem, E. O. and Adegoke, G. O. 1995. Bacteriological study of West African clam (*Egeria radiata* Lamarch) and their overlying waters. *Food Microbiology*, 12: 381-385.
- Etim, L. and Brey, T. 1994. Growth, productivity, and significance for fishery of the bivalve *Egeriaradiata* (Donacidae) in the Cross River, Nigeria. *Archive of Fishery and Marine Research*, 42, 63-75.
- Fawole, M. and Osho, A. 1995. *Laboratory methods in microbiology*. Books Ltd; Nigeria. Pp 34-35.
- Frazier, W. C. and Westhoff, D. C. 2000. *Food Microbiology*. McGraw-Hill Inc, New York. pp. 17-48, 441-443.
- Gram, L., Ravn, L. and Rasch, M. 2002. Food spoilage interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78: 79-97.
- Grazyna, E. D. and Bonnie, S. P. 2010. *Environmental Effects on Seafood Availability, Safety, and Quality. Chemical and Functional Properties of Food Components*. CRC Press.
- Hatha, A. A. M., Christi, K. C., Singh, R. and Kumar, S. 2005. Bacteriology of the fresh water bivalve clam *Batissaviolacea* (Kai) sold in the Suva market. *The South pacific Journal of Natural Science*, 23: 48-50.
- Itah, A. Y., Etukudo, S. M., Akpan, E. J. 1996. Bacteriological and Chemical Analysis of Some Rural Water Supplies of Calabar, Nigeria. *West African Journal of Biological and Applied Chemistry*, 40:10-12.
- King, R. P. 2000. Population structure, growth performance and mortality rates of the freshwater clam *G. paradoxa* Born 1778, in Nun River, Nigeria. *Archive of Fisheries and Marine Research*, 48(1):21-30.
- Lydeard, C. and Lindberg, D. R. 2003. *Molecular Systematics and Phylogeography of Molluscs; Smithsonian Series in Comparative Evolutionary Biology* (ed. Lydeard C, Lindberg DR), Smithsonian Books Washington and London.
- Malik, A. and Ahmed, M. 1994. Incidence of drugs and metal resistance in *E. coli* strains from sewage water and soil. *Chemical and Environmental Research* 3:3-11.
- Moses, S. B. 1990. Growth, biomass, mortality, production and potential yield of the West African clam, *Egeria radiata* (Lamarck) (Lamellibranchia, Donacidae) in the Cross River system, Nigeria. *Hydrobiologia*, 196:1-15.
- Obirikorang, K. A., Amisah, S. and Adjei-Boateng, D. 2013. Habitat description of the threatened freshwater clam, *Galatea paradoxa* (Born, 1778) at the Volta estuary, Ghana. *Curriculum of World Environment* 8(3). doi: <http://dx.doi.org/10.12944/CWE.8.3.01>
- Purchon, R. D. 1963. A note on the biology of *Egeria radiata* L (Bivalvia: Donacidae). *Proceedings of the Malacotogical Society of London* 35:251-275.

- Serfor-Armah, Y., Amoah, C., Odamtten, G., Opata, N. and Akaho, E. 2010. Determination of elemental and nutrient composition of water, clam mantle and shell of the Volta clam (*Galatea paradoxa*) using neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry* 286(1) DOI: 10.1007/s10967-010-0664-8.
- Spanggard, B., Jorgenses, F. G. and Huss, H. H. 1993. Antibiotic resistance in bacteria isolated from three freshwater farms and an unpolluted stream in Denmark. *Aquaculture* 115:195-207.
- Udoh, S. M. 1994. Pollution Associated with Shrimps harvest from cross river estuary in Nigeria. *Journal of Educational Laboratory Sciences* 7(2):34-44.
- Vieira, R. F., Ménages, F. R., Fonteles-Filho, A. A., Torres, R. O. and Ernani, A. S. 2003. Bacteria of fecal origin in mangrove oysters (*Crassostrea rhizophorae*) in the Coco River estuary, Ceara State, Brazil. *Brazil Journal of Microbiology* 34: 126-130.
- West, P. A. 1989. Human pathogens and public health indicator organisms in shellfish. In: B. Austin and DA Austin (Ed). *Methods for the microbiological examination of fish and shellfish*. Ellis Harwood. Chichester, West Sussex, England pp. 273-308.