Studies on Microbiological, Proximate Mineral and Heavy Metal Composition of Freshwater Snails from Niger Delta Creek in Nigeria

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Abstract

Microbial quality of freshwater snail samples from Itu creek, Niger Delta Nigeria was studied. The bacteria isolates were Proteus sp., Sreptococcus pyrogens, Shigella flexneri, Staphylococcus aureus, E. coli, Klebsiella aerogenes, Citrobacter, Bacillus subtilis, Bacillus cereus, Aeromonas sp., Micrococcus liteus, Streptococcus salivanus, Salmonella typhi, Vibrio parahaemolyticus, Vibrio sp. and Vibrio cholera. Proteus sp, Aeromonas sp. and Micrococcus liteus had the highest frequency of occurrence (10.25%). The fungi isolates were Aspergillus terreus, Cladosporium sp, Fusarium oxysporum, Cryptococcus sp, Aspergillus flavus, Aspergillus glaucus and Aspergillus niger in which A. niger, A. terreus and F. oxysporum had the highest occurrence (16.67%). The total heterotrophic count of the samples ranged from 4.0 x10^7-1.42 x 10^8 cfu/g. The coliform levels were generally high and it ranged from 2.2-6.4 x 10^7 cfu/g in which the highest was recorded from P. canaliculata (A_1). The Salmonella/Shigella counts ranged from 1.1-5.2 x 10^7 cfu/g in which the highest count was recorded from L. libycus (C_1). The total Staphylococci count ranged from 1.7-3.5 x10^7 cfu/g in which A. fulica (D_2) had the highest. The total vibrio counts ranged from 1.6-3.2 x 10^7 cfu/g. The fungi count ranged from 1.7-3.5 x 10^7 cfu/g. The total microbial counts obtained from this work were found to be higher than the specified standard limits (1x10^5 cfu/g) for bacteria and fungi and 1.x10^2 cfu/g for coliforms) by ICMSF (1982) and USFDA (1991). The crude protein content of the samples ranged from 28.87^{d} – $33.41^{a\%}$ in which the highest was obtained from A. fulica (D_3). The Crude Fat, crude fiber and carbohydrate content ranged from 1.8-4.25% and 00.10-0.18%, respectively. The mineral elements such Na, Ca, K and P were detected in all the freshwater snail samples. The concentration of metal ranges of Zn, Fe, Mn, Mg, Pb, As and Cu in the samples were 78.6^{j}-96.3^{a} mg/kg, 15.7^{j}-28.6^{a} mg/kg, 58.6^{j}-77.5^{a} mg/kg, 0.258^{j}-0.297^{a} mg/kg, 0.03^{i}-0.25^{a} mg/kg, 0.04^{i}-0.37^{a} mg/kg and 12.6^{j}-16.3^{a} mg/kg, respectively. It was observed that different metals were present in the samples at different levels but majority were found to be within the standard limits prescribed by EU and FAO. Freshwater snails from the creek in Niger Delta was found to be nutritionally richer but harbor pathogenic microorganism and heavy metals which can pose serious health hazard to consumers and they are not totally safe for human consumption.

Keywords: *Freshwater snail, microbiological, proximate, mineral and metal concentration.*

Introduction

The freshwater Apple snails (*Pomacea* sp. and *Lanistes libycus*) are popular, seasonal freshwater food widely distributed in Nigeria

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especially in the Niger Delta and Upper Cross River basins (Arene *et al*. 1999; Obureke *et al*. 1987). It serves as a major source of protein as well as generating income to the people (Ezeama 2000).

Apple snails belong to the phylum Mollusca, class Gastropoda (snails), subclass Prosobranchia, order Caenogastropoda (Mesogastropoda in older literature), super family *Ampullarioidae* and family *Ampullariidae* (Apple snails). Apple snails are tropical and sub-tropical freshwater snails from the family Ampullariidae (sometimes referred to as Pilidae) which are divided in several genera; genera *Asolene*, *Felipponea, Marisa*, and *Pomacea* are the New World genera (South America, Central America, the West Indies and the Southern USA), while the genera *Afropomus, Lanistes, and Saulea* are found in Africa. The genus *Pila* is native in both Africa and Asia (Ghesquiere 2011).

Shellfishes are highly nutritional balanced food and therefore are excellent sources of proteins, a good source of minerals like calcium, sodium, phosphorus, iron and some vitamins, and they are also low in fats and cholesterol (Ifon and Umoh 1987). Microbiological, proximate and heavy metal concentration in *Penaeus* sp. (shrimp) and *Calllinectes* sp. (crab) from creeks in Niger delta, Nigeria, has been reported by Adebayo-Tayo and Okpo (2010).

Survey on the microbiological quality of shellfishes has shown shellfishes to harbor pathogens which have been implicated in outbreaks of food-borne diseases in many parts of the world; there illnesses include typhoid fever, hepatitis and similar disorder of the digestive system and neurological disorder (Ukpong and Utuk 1992, Metcalf *et al*. 1973; Cutting and Spencer 1968). In major part of the world, about 10-19% of food-borne illness involved shellfishes as a vehicle and between 1993 and 1997, 6.8% of the food borne illnesses involved consumption of fish and shellfishes (Huss *et al*. 2003).

However, snails are also found in bodies of water contaminated with human and industrial wastes like metals, these pose a serious health hazard to the consumers as these substances tend to concentrate and accumulate in the shellfishes and thereby increasing their toxicity as the shellfishes are being consumed. Due to high level of consumption of these freshwater snails in Riverine area, there is need for continuous microbiological analysis and also to check the chemical contaminant in foods from the aquatic environment in order to understand their hazard levels and thus creating awareness to the public on health risks in consuming raw or undercooked and under processed shellfishes. The aims and objectives of these works were to enumerate the microbial quantity as well as proximate, mineral and heavy metal concentration in Apple snails from a creek in Niger Delta in order to evaluate their hazard level in relation to the maximum residual limit for human consumption.

Materials and Methods

Eighty six samples each of the freshwater snails (*Pomacea canaliculata*, *Pomacea bridgesii*, *Lanistes libycus and Achatina fulica)* used in this study were freshly harvested mechanically from Itu creek in Akwa Ibom state, a famous shellfish producing areas in Niger Delta, Nigeria. The sample was collected in triplicate. It was placed in the ice and brought to the laboratory.

Microbiological analysis

The fresh water snail samples were scrubbed, rinsed with water, surface sterilized (70% ethanol), and edible portion of the meat were aseptically extracted as described by American Public Health Association (APHA). The sample was homogenized and about 10g was taken for microbiological analysis. Standard pour plates were prepared from 10-fold dilutions into nutrient agar medium for total heterotrophic bacteria counts, MacConkey agar 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^oC for 24-48 hours, while fungal plates were incubated at room temperature (28 ± 2^oC) for 3-5 days. Colonies were selected randomly and were characterized using morphological and biochemical test such as gram stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP and 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) and Bergey's Manual of Determinative Bacteriology (Hott *et al*. 1994). Fungal isolates were identified based on their morphological and cultural characteristics as recommended by Sampson *et al*. (1984).

Proximate and mineral analysis

Proximate composition was determined according to the method of AOAC (1998). This includes determination of Ash content, crude protein, Dry matter, moisture content and crude fiber. The mineral contents were also determined using Jenway Digital flame photometer (PFP 7 Model).

Metal concentration analysis

The edible portions of the meat from the samples were removed; homogenized and about 2.5 g was taken for analysis. Ten milliliters of nitric acid - perchloric acid (10:4) mixture were added to the sample, covered and left overnight at room temperature. The samples were digested, allowed to cool to room temperature, filtered (glass wool) and made up to 50ml. The filtered samples were analyzed in triplicate, using Buck 2000 Atomic Absorption Spectrophotometer (AAS) as per standard conditions (Table 1). The blanks and calibration standard solution were also analyzed in the same way as for the samples. All the chemical analysis was carried out in duplicate.

Results and Discussion

The microbial load in the freshwater snail samples from Itu creeks in Akwa Ibom state were shown in Table 1. The total heterotrophic count varied from 4.0 x10^7-1.42 x 10^8 cfu/g for the freshwater snail in which the highest growth was recorded from *P. bridgesii* (B_2).

Coliform density ranged from 2.2 – 6.4 x 10^7 cfu/g in which the highest was recorded from *P. canaliculata* (A_1).

The *Salmonella/Shigella* counts varied from 1.1-5.2 x 10^7 cfu/g in which the highest count was recorded from *L. libycus* (C_1). The total *Staphylococci* count ranged from 1.7-3.5 x10^7 cfu/g in which *A. fulica* (D_2) had the highest. The total *vibrio* counts ranged from 1.6-3.2 x 10^7 cfu/g.

The fungal count ranged from 1.7-5.6 x 10^7 cfu/g in which *P. bridgesii* (B_1) had the highest. The total microbial counts obtained from this work were found to be higher than the specified standard limits (1x10^5 cfu/g) for bacteria and fungi and 1x10^2 cfu/g for coliforms) by ICMSF (1982) and USFDA (1991). This high level of microbial loads could be as a result of human activities done in the rivers where the freshwater snails are harvested. These activities include; bathing, washing of clothes or other materials, disposal of faecal matters and sewage discharge.

Table 1. Total microbial groups in freshwater snail samples from Itu creek, Niger Delta.

Freshwater snail samples	Sample code	Total bacterial count x10^7	Total Coliform count x 10^7	Total Salmonella/ Shigella count x 10^7	Total Staphylococci count x 10^7	Total Vibrio count x 10^7	Total fungi count x 10^7
P. canaliculata	A_1	8.7	5.3	2.7	2.0	2.7	2.1
	A_2	1.04 x10^8	4.0	1.6	3.3	1.8	3.2
	A_3	1.42 x10^8	6.4	2.5	2.7	3.2	5.6
P. bridgesii	B_1	5.0	2.2	3.4	4.8	2.5	1.9
	B_2	5.8	3.2	2.2	3.5	2.8	2.7
L. libycus	C_1	1.20 x10^8	4.6	1.1	2.5	3.2	3.2
	C_2	4.0	4.0	3.2	1.8	1.6	2.9
Achatina fulica	D_1	7.6	6.2	2.9	2.3	2.7	3.5
	D_2	8.2	2.4	5.2	1.7	2.5	2.2
	D_3	5.0	3.6	4.8	2.4	2.8	1.7

Table 2 shows the probable identity of the microorganism isolated from the freshwater snail samples. The bacteria isolates include:

Proteus sp., *Sreptococcus pyrogens, Shigella flexneri, Staphylococcus aureus, E. coli, Klebsiella aerogenes, Citrobacter, Bacillus subtilis, Bacillus cereus, Aeromonas* sp., *Micrococcus liteus, Streptococcus salivanus, Salmonella typhi, Vibrio parahaemolyticus, Vibrio* sp. *and Vibrio cholera*. *Proteus sp., Aeromonas sp. and Micrococcus liteus* had the highest frequency of occurrence (10.25%) (see Fig. 1) and the fungi isolates were; *Aspergillus terreus, Cladosporium sp, Fusarium oxysporum, Cryptococcus sp, Aspergillus flavus,* Aspergillus *glaucus* and *Aspergillus niger* in which *A. niger, A. terreus* and *F. oxysporum* had the highest occurrence (16.66%) as shown in Fig. 2. This result is in agreement with the report of Adebayo-Tayo and Ogunjobi (2008) who isolated *E. coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Bacillus cereus* from freshly harvested *Trypanotonous* spp. (Periwinkle) and *Crassostrea* spp. (Oyster).

All the organisms isolated from the freshwater snail samples have health implications on man. The presence of *E. coli* in the freshwater snail samples is an indication of secondary contamination as *E. coli* are known to be associated with gastrointestinal tracts of warm-blooded animals and are known to be present in the environment as a natural flora. This secondary contamination may be as a result of sewage contamination of freshwater snails harvesting areas. *E. coli* is the causative agent of diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infection, septicemia, pneumonia and meningitis (Kumar *et al*. 2005).

Table 2. Microorganisms isolated from freshwater snail samples from Itu creek.

Isolates	Freshwater Snail Samples			
	Pomacea canaliculata	*Pomacea bridgesii*	*Lanister libycus*	*Achatina fulica*
Proteus sp.	+	+	+	+
Sreptococcus pyrogens.	+	+	-	+
Shigella flexneri	-	-	+	+
Staphylococcus aureus	-	+	+	+
E. coli	-	+	+	+
Klebsiella aerogenes	+	-	+	-
Citrobacter freundii	+	-	-	-
Bacillus subtilis	-	+	-	+
Bacillus cereus	+	-	-	+
Aeromonas sp.	+	+	+	+
Micrococcus liteus	+	+	+	+
Streptococcus salivanus	-	+	-	+
Salmonella typhi	-	+	-	+
Vibrio parahaemolyticus	-	-	+	-
Vibrio sp.	-	-	+	+
Vibrio cholera	-	-	+	+
Aspergillus niger	+	+	+	-
Cryptococcus sp.	-	+	-	+
Pichia membrannaefacien	+	+	+	+
Aspergillus glaucus	-	+	-	+
Aspergillus terreus	-	+	+	+
Fusarium oxysporum	-	+	+	+
Cladosporium sp.	-	-	-	+

Note: + - present and - - absent.

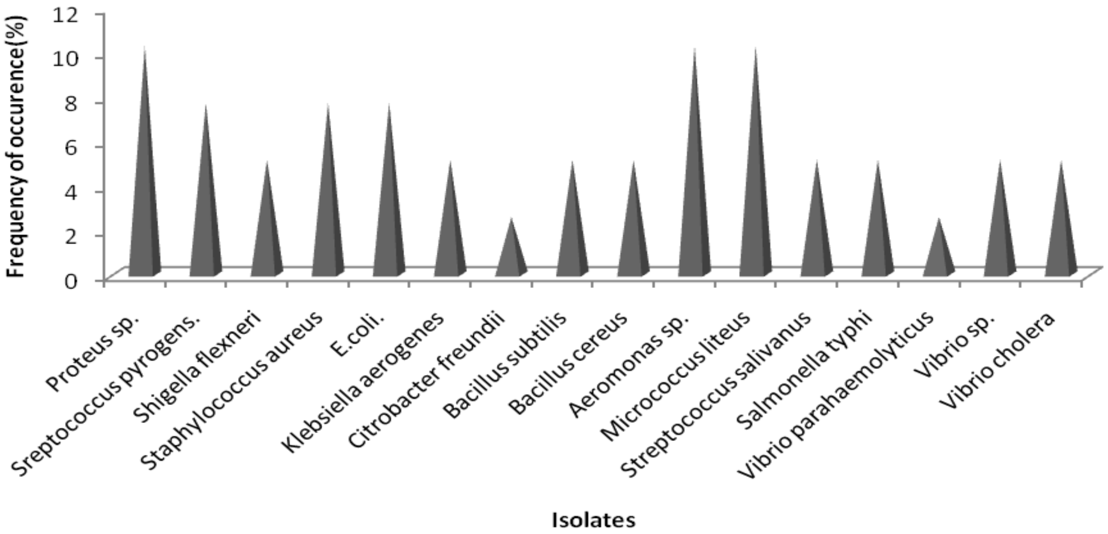

Fig. 1. Frequency of occurrence (%) of bacteria isolated from the freshwater-snail samples.

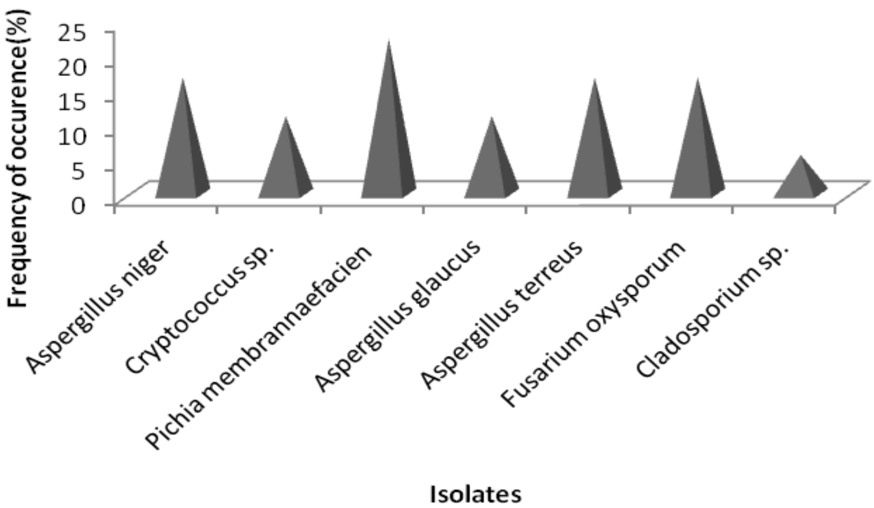

Fig. 2. Frequency of occurrence (%) of fungi isolated from the freshwater-snail samples.

Salmonella one of the most important food-borne pathogens are indication of sewage contamination and it is found to be associated with number of non-human hosts for example, reptiles (Winfield and Groisman 2003). It has been reported to survive and persist in the aquatic environment. *Salmonella* has been detected in periwinkles from different creeks (Adebayo-Tayo *et al*. 2006), in the gut of tilapia and crab (Iyer and Shrivastava 1989; Ogbondeminu 1993) and causes new born meningitis and infantile diarrhea.

The presence of *Streptoccus salivanus and Streptococcus pyrogens* is an indication of serious contamination. *Streptoccus sp*. has been implicated in human infections like pharyngities, scarlet fever and pneumonia. *Shigella* sp. and *Salmonella* sp. are causative agents of illnesses like shigellosis and salmonellosis in human who are the only reservoir of these organisms (Nester *et al*. 1995). *Vibrio* sp., a natural habitants of sea water, has been reported by Cash *et al*. (1974) that an oral dose of 10^4 to 10^8 *Vibrio cholerae* organisms can routinely induce cholera infection in humans.

A. niger, *A. flavus* and *A. terreus* have also been implicated in causing mycetoma in human (Cheesbrough 2000). *Aspergullus flavus* is involved in allergic aspergillosis (pulmonary aspergillosis) and also produces aflatoxin that is highly carcinogenic (Prescott *et al*. 2005).

The percentage proximate and mineral composition of the samples was shown in Table 3. The crude protein ranged from 28.88^{j}-$33.41^{a\%}$ in which the highest was obtained from *A. fulica* (D_3). The Crude Fat ranged from 3.18^{k}-4.25^{a}%, the Crude Fiber content ranged from 00.10^{d}-0.18^{a}%.

Proximate analysis has shown freshwater snail samples from Itu creek to be nutritionally rich. The fat, fiber and moisture contents in samples from the freshwater snails are constituents in shellfish which provide an energy source to the consumers. The mineral composition in the samples was shown in Table 4. The mineral elements such Na, K, Ca, and P were detected in all the freshwater snail samples. The sodium content ranged from 0.139^{e}-0.419^{a}%. The presence of mineral elements like sodium, calcium, potassium, magnesium and phosphorus found in freshwater snail are major constituents of protein, nucleic acid, co-factors and other cell components, when absorbed into the cells. Shellfishes have been reported to serve as a source of protein and mineral elements (Briggs *et al*. 1979), which helps in the repair of worn-out tissue and body building.

The moisture content was found to be higher in the freshwater snail samples which may be as a result of fresh water in Itu creek, the shellfish from this water trends to absorb water from the external environment into their cells which are of higher concentration in order to balance the osmotic pressure between the cell and the surrounding water.

The mineral elements analyzed in this work are essential minerals required by humans. The sodium and calcium are essential elements found in human bones phosphorus plays a major role in glucose metabolism and also essential element of the DNA molecule.

Table 3. Heavy metals concentration of freshwater snail samples from Itu creek.

Sample	Metals (mg/kg)						
	Fe	Zn	Cu	Mg	Mn	Pb	As
A_1	21.3^{d}	86.3^{g}	13.3^{i}	0.262^{i}	58.6^{j}	0.11^{f}	0.08^{h}
A_2	28.6^{a}	83.7^{h}	15.7^{c}	0.285^{c}	71.8^{d}	0.09^{g}	0.15^{f}
A_3	23.5^{b}	83.2^{i}	12.6^{j}	0.293^{b}	69.2^{f}	0.07^{h}	0.04i
B_1	19.4^{g}	89.7^{f}	15.2^{d}	0.274^{e}	61.3^{i}	0.17^{d}	0.21^{d}
B_2	19.6^{f}	96.3^{a}	16.3^{a}	0.264^{h}	77.5^{a}	0.24^{a}	0.31^{b}
C_1	19.8^{e}	94.6^{b}	13.8^{h}	0.297^{a}	71.9^{c}	0.13^{e}	0.18^{e}
C_2	17.6^{i}	78.6^{j}	14.3^{f}	0.266^{g}	73.5^{b}	0.19^{c}	0.26^{c}
D_1	18.3^{h}	92.5^{e}	15.9^{b}	0.258^{j}	68.3^{g}	0.03^{i}	0.00^{j}
D_2	15.7^{j}	94.2^{c}	14.1^{g}	0.269^{f}	66.5^{h}	0.21^{b}	0.11^{g}
D_3	21.9^{c}	92.8^{d}	14.6^{e}	0.279^{d}	69.5^{e}	0.25^{a}	0.37^{a}

Table 4. Proximate composition (%) and mineral contents (mg/kg) of freshwater snail samples from Itu creek.

Sample	Proximate (%)					Mineral(mg/kg)			
	Crude Protein	Crude Fat	Crude Fiber	Ash	DM	Na	K	Ca	P
A_1	31.38^{g}	3.26^{j}	0.11^{c}	11.38^{h}	86.75^{a}	0.152^{d}	0.412^{b}	0.196^{b}	0.314^{j}
A_2	31.49^{f}	3.44^{f}	00.10^{d}	11.57^{f}	86.29^{f}	0.157^{cd}	0.397^{e}	0.198^{a}	0.336^{h}
A_3	29.44^{i}	3.29^{i}	0.17^{a}	11.26^{i}	86.31^{e}	0.143^{e}	0.392^{g}	0.176^{j}	0.351^{d}
B_1	33.24^{b}	3.18^{k}	0.13^{b}	11.42^{g}	86.63^{b}	0.419^{a}	0.418^{a}	0.191^{d}	0.326^{i}
B_2	31.59^{e}	3.87^{d}	0.18^{a}	11.66^{e}	86.41^{c}	0.136^{e}	0.382^{h}	0.194^{c}	0.352^{c}
C_1	31.66^{d}	3.57^{e}	0.09^{e}	12.21^{b}	86.16^{i}	0.139^{e}	0.401^{d}	0.185^{f}	0.348^{e}
C_2	28.87^{j}	4.29^{a}	0.08^{e}	12.44^{a}	86.21^{h}	0.154^{d}	0.362^{j}	0.179^{i}	0.363^{b}
D_1	29.82^{h}	4.25^{b}	0.11^{c}	11.83^{d}	86.39^{c}	0.163^{c}	0.375^{i}	0.183^{g}	0.339^{g}
D_2	32.87^{c}	3.29^{i}	0.15^{b}	`2.36^{j}	86.35^{d}	0.316^{b}	0.406^{c}	0.187^{e}	0.342^{f}
D_3	33.41^{a}	4.13^{c}	0.15^{b}	12.17^{c}	86.24^{g}	0.146^{d}	0.394^{f}	0.181^{h}	0.369^{a}

The concentration of different metals detected in the edible portion of the freshwater snail samples from the Itu creek was showed in Table 4. The heavy metal concentrations are found to be significantly higher the samples except for Pb and As. freshwater snail samples from the Itu creek had significantly higher concentration of metals.

Iron and Magnesium content in the samples ranged from 15.7^{j}-28.6^{a} mg/kg and 0.262^{j}-0.297^{a} mg/kg, respectively. Iron and Magnesium are essential trace elements; they play a major role in the metabolic processes that take place in human system and regulation of blood. Magnesium may function as co-factor to some enzymatic activities. Iron is a major component of the hemoglobin found in human blood.

The zinc content in the samples ranged from 78.6^{j}-96.3^{a} mg/kg in which *P. bridgesii* (B_2) had the highest. Since zinc is an essential element for both animals and humans, the recommended daily allowance is 10 mg/day in growing children and 15 mg/day for adults (FNB/CDA/CIRDA/NRC 1974). It has a protective effect against the toxication of both cadmium and lead (Calabrese *et al*. 1985; and Sanstead 1976). A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decrease fertility. Zinc toxicity is rare, but, at concentrations in water up to 40 mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (FNB/CDA/CIRDA/NRC 1974).

Lead was detected in all samples and it ranged from 0.03^{i}-0.24^{a} mg/kg. The highest was found in *A. fulica* (D_3) sample F. However, all the freshwater snail samples from the Itu creek contained lead below 0.5 mg/kg (The Commission of the European Communities 2001) and 1.0 mg/kg (Nauen 1983). Freshwater snail samples contained lead the concentration (0.03-0.24 mg/kg) that is below the standard limit of the Commission of the European Communities (2001) and that of Nauen (1983). Lead causes renal failure and liver damage in humans (Emmerson 1973; Luckey and Venugopal 1977).

The concentration of copper ranged from 12.6^{j}-16.3^{a} mg/kg in which the highest was found in *P. bridgesii* (B_2). The concentrations in the samples were above the limit of 10 mg/kg (Nauen 1983). Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin. Shellfish are the richest sources of copper especially oysters and Crustaceans (Underwood 1977). Underwood (1977) reported that deficiencies of Copper in infants can lead to anemia and hypo-proteinemia and no deficiency of copper in adult have been reported.

Manganese was also detected in all the samples and the concentration ranged from 58.6^{j}-77.5^{a} mg/kg, the highest being detected from *P. bridgesii* (B_2). Manganese deficiencies, can lead to severe skeletal and reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age. Total daily intake varies from 2.5 to 7.0 mg (SDWC/ACT/ALS/NRC 1977; SDWC/ BTEHH/ALS/NRC 1982). The Arsenic concentration ranges from 0.04^{i}-0.37^{a} mg/kg.

Conclusion

The present study revealed that freshwater snail samples from Itu creek in Niger Delta Area of Akwa Ibom state Nigeria contain unacceptable level of microorganisms (as laid down by ICMSF 1982 and USFDA 1991). Though nutritionally richer, the heavy metals analyzed from this work provide information about the concentration of metals in freshwater snails which can be related to the industrial activity and other human activities taking place in the area (Itu). Majority of the metal level remain within their permissible safe levels for human consumption as laid down recently by the Commission of the European Communities (2001) and Nauen (1983) except in few cases. The study on the whole evidenced the microbial, nutritional and metal status of freshwater snail samples from Niger Delta. However it did bring out the probable hazard associated with their consumption.

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