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METALS ASSESSMENT AND MICROBIOLOGICAL SAFETY OF DIFFERENT SPECIES OF PERIWINKLE FROM DIFFERENT CREEKS IN CROSS RIVER STATE, CALABAR.

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Abstract

Periwinkle are common source of aquatic protein in Africa. The level of consumption increases daily due to its availability and health benefits. Evaluating the microbiological quality of the different species of periwinkle in and associated metals for public safety became our outmost importance and was carried out using Standard Bacteriological techniques. Bacterial cell count for P. *byronensis, P. aurita* and T. *fuscatus*under 3 processing conditions (washed, boiled and blended) were 2.4, 2.4 and 1.4 x 10^{7} CFU/mL, 1.6, 1.1 and 1.2 x 10^{7}CFU/mL and 9.9, 9.8 and 8.5 x 10^{7}CFU/g respectively. Prominent microorganisms isolated were; *S. aureus, Enterococcus Spp, Acinetobacter Spp, Serratia Spp, E. coli Spp, Pseudomonas Spp, Salmonella Spp, Vibrio Spp, S. epidermidis, Shigella Spp* with sum total % occurrence of 79.8, 53.8, 23.8, 11.9, 42.3, 33.5, 43.1, 34.5, 17.0 and 17.6 % for each isolates in all periwinkle species.*S. aureus* was the most abundance (79.8 %) followed by *Enterococcus* (53.8 %), *E. coli* (42.3 %), *and Salmonella* (43.1 %) species. There was no significance difference at P = 0.005in the level of contamination of the 3 periwinkle species. The level of contamination based on creeks varied. The mean concentration of mineral elements in different periwinkles species were; *T. fuscatus> P. aurita>P. byronensis* for Ca and Cu. *P. aurita>T. fuscatus>P. byronensis* for Zn and Pb while *P. aurita>P. byronensis>T. fuscatus* for Fe all in Jebs creek. In Ikang creek Cu; *P. byronensis > T. fuscatus >P. aurita*, Zn; *P. aurita > P. byronensis > T. fuscatus*, Fe; *T. fuscatus >P. byronensis> P. aurita*, Ca; *T. fuscatus > P. aurita> P. byronensis*, Pb; *P. aurita > T. fuscatus > P. byronensis*. The index findings reveals that all species of periwinkles despite location of harvest may serve as potential source of pathogenic organisms highly implicated in food borne infections. The presence of Zinc, Iron, copper and Calcium are known to provides healthy functions to humans although over consumption may be detrimental. Lead (PB) is highly toxic, thus, it presence in food that is frequently consumed is a threat to public health. Ways in which toxic substances are introduced into water bodies should be prohibited to minimize high accumulation of such into aquatic lives.

Keywords*: Periwinkle (P.aurita, Byronensis and T. fuscatus), Microorganism, Mineral elements Cu, Zn, Fe, Ca and Pb.*

1. Introduction

Periwinkles are protein-rich univalve invertebrates belonging to the phylum *Mollusca*, class *Gastropoda* and sub-class *Prosobranch Ia* [1]. They are turreted granular and spiny shell with a tapering end [2]. They arer epresented by two genera: *Tympanotonus* and *Pachymelania*. *Tympanotonus*is made of two species (*T. fuscatusvar fuscatu s*and *T.*

fuscatusvarradula). Pachymelania comprised of *P aurita* and *P byronensis***.** Their harvesting is usually through handpicking. The genus *Pachymelania*is the commonest and most dominant in the South-Western estuaries, and mangrove swamps lagoon of Nigeria [3]. It is a native of West Africa and is usually harvested by natives of coastal towns and villages in Nigeria as a staple source of protein [4].

They represent one of the cheapest sources of proteins, iron, magnesium, Omega 3 fatty acids, selenium and calcium and mostly used within Cross River State, Oron, Akwa-Ibom State, and Yenogoa, in Bayelsa State. Methods of preparations differs among different ethnic groups. Some remove the shell, washed the meat thoroughly before making it part of delicacy, others cut-off the pointed end of the tail, wash the shell before used. Stabilizing blood pressure, development of the brain and bone, good sight, prevent heart related diseases, healthy foetus are some of the noticeable health benefits of periwinkle. Shell fishes including Periwinkles thrive better in water bodies contaminated with domestic, agricultural and industrial waste effluent. Overtimes, accumulation and concentration of toxic substances including heavy metals in the univalve (shell) occurs [5]. Such conditions favors the proliferation of pathogenic organisms which also forms part of the periwinkle contaminant [6]. Different publications reports isolations of food borne pathogens in periwinkle from several localities, however, no public documentation has been made for Jebs and Ikang creeks that are common periwinkle creeks within our locality (Cross River State). Thus, the study aimed at examining microorganisms, mineral elements as well as heavy metals associated with different species of periwinkles t common in Ikang and Jebs Creek respectively.

I. Materials and Methods

a. Study Area

Akpabuyo Local Government Area are autonomous since 1991, created from the former Odukpani Local Government Area. It became the 14th and 589th Local Government Area in Cross River State and Nigeria respectively. It has 10 council wards with a population of 360,000 people and is located in the Southern Senatorial District with headquarters at Ikot Nakanda.

Calabar South is a Local Government Area of Cross River State with its headquarters in Anantigha town. It has 11 council wards with an area of 264 km² and a population of 191,630 at the 2006 census. The postal code of the area is 540.

Ikang Creek is located in Akpabuyo while Jebs Creek is situated in Calabar South.

b. Collection and Preparation of Samples

A 60 g weight each of *P. byronensis, aurita*and *Tympanotonus fuscatus species* were purchased from the above-mentioned creeks and wrapped in sterile aluminum foil paper into a clean flask and transported immediately to Microbiology laboratory UNICROSS for analysis. Each 60g were divided into 3 segments for wash, boil and blended analysis respectively. The length of each sample ranged were within 2.5-4.9 cm weighing 2.0-3.8g.

A 20g weigh of the first portion was thoroughly washed using distil water, the second portion was boiled in 70 mL of distilled water at 100 ^{oC}, the shell of the third portion was broken then the viscera removed using a sterile needle. This was then blended using a sterile laboratory mortar and pestle. Thereafter1mL of both diluent and 1g of the homogenate (blended portion) were used to perform a ten-fold serial dilution down to 10^{-10}.

Bacterial cell count was determined using pour plating method. A1mL amount of 10^{-7} dilution of the sample suspension was placed in a clean sterile Petri dish; 20 mL of molten Nutrient (India), MacConkey (Diego), *Salmonella-Shigella agar* (Diego) and Thiosulfate Citrate Bile Salt Agar (Diego) of about $45^{oC}$was poured into the Petri dish. The Petri dishes were gently swirled until the contents mix thoroughly. The agar plates were allowed to set before incubation at 37°C for 24hrs in a humidified incubator. After 24hrs of incubation, the plates were examined for growth, morphological appearance of colonies were described, counted and recorded. Thereafter, discrete colonies were isolated, purified after three successive sub-culturing and re-isolations on Nutrient agar. Isolates were characterized by standard bacteriological techniques as described by Cheesbrough.

***C (i). Preparation of Samples for Metal Analysis* (Step one)**

Samples were dried in a hot air oven at 100 ^{oC} for 24 hrs. before digestion. Samples tissues were freeze dried to preserve the needed elements. Thereafter, homogenized to fine powder by ball-milling. A 0.20-0.25g of powdered tissue was weigh into a conical flask with addition of 3mL of HN03, mix and content heated in a 130^{oC} oven until digestion was completed. Contents were then diluted with distil water to a final volume of 20mL and stored in bottles for further analysis with UV-visible spectrophotometer.

***(ii). Metal analysis using UV-visible spectrophotometer (*Hach D.r 5000) (step two).**

(Copper (Cu), Zinc (Zn), Iron (Fe), Calcium (Ca) and Lead (Pb)) were analyzed in this study. A multi-cell Adapter with 1-inch square cell holder was used for the analysis. A clean square cell was fill with 10mL of already prepared samples in Step1 above. One Ferro Ver iron reagent pillow powder was added to the cell and swirl to mix. The appearance of an orange color indicates the presence of an iron. Five minutes reaction time was allowed to eliminate contaminant like rust iron and others. Results are display in different mg/L of the analyzed metals. This methodology was used for other metals varying the reagent depending on the type of metal with color formation serving as a guide for different metal detections.

d. Data Analysis

The statistical analysis was done using SPSS version 20 for descriptive statistics. The student Unpaired T-test compared the significance difference at P = 0.005 in isolated organisms from different periwinkle species and different creeks.

II. Results and Discussion

Bacterial cell count for P. *byronensis*, *P. aurita* and T. *fuscatus* under 3 processing conditions (washed, boiled and blended) were 2.4, 2.4 and 1.4 x 10^{7} CFU/mL, 1.6, 1.1 and 1.2 x 10^{7} CFU/mL and 9.9, 9.8 and 8.5 x 10^{7} CFU/g respectively. (Table 1). The colony count reported here is higher than that reported by Nwiyi and Okonkwo, (2013) [7] who documented on pathogenic Microorganisms isolated from periwinkle in South-South creeks of Nigeria.

Isolated organisms were; *S. aureus, Enterococcus Spp, Acinetobacter Spp, Serratia Spp, E. coli Spp, Pseudomonas Spp, Salmonella Spp, Vibrio Spp, S. epidermidis, Shigella Spp* with sum total % occurrence of 79.8, 53.8, 23.8, 11.9, 42.3, 33.5, 43.1, 34.5, 17.0 and 17.6 % for each isolate in all periwinkle species. *S. aureus* was the most abundance (79.8 %) followed by *Enterococcus (*53.8 %), *E. coli* (42.3 %), *and Salmonella* (43.1 %) species (Table 2). 100, 71.4 and 99.9 % were the sum total of all isolates for *P. byronensis*, *T. fuscatus* and *P. aurita* (Figure 1). The report of this study is in close agreement with the study of Bukola *et al*, 2006 [8] who states similar organisms from periwinkle harvested from Ishiet and Oron creeks in Akwa Ibom State.

S. aureus was the most abundance (79.8 %) followed by *Enterococcus (*53.8 %), *E. coli* (42.3 %), *and Salmonella* (43.1 %) species.

Variations in the occurrence of isolated organisms were observed in all periwinkle species as well as harvested creeks. All isolated organisms except *Salmonella Spp* was not isolated from *Pbyronensis* from Jebs creek (Figure 2). *S. aureus, S. epidermidis* and *Enterococcus Spp* were absent in T *fuscatus* fromJebs while *Salmonella* was not detected from same species from Ikang (Figure 3). *Vibrio* was not found in *P. aurita* from Ikang as Pseudomonas *Spp* not detected in Jebs creek (Figure 4). The reason for variations in the occurrence of isolated organisms may be based on the different human activity that is allow in a particular creek.

S. aureus from periwinkle samples should be noted for its ability to produce enterotoxin that may result to toxic shock syndrome, food poisoning, occasional death after consumption. The Presence of *E. coli* Spp is a clear indication of fecal contamination. This organism is highly implicated in newborn meningitis and infantile diarrhea. *Salmonella Spp* are reasons for paratyphoid fever in humans, who are the only reservoir of this organism.

Other organisms such as *Enterococcus, Acinetobacter, Serratia, Pseudomonas* species occurred sparingly. According Cheesbrough, [9], there is potency in any pathogen recover in 10^{-5} of any diluents.

Five Cu, Zn, Fe, Ca and Pb were the mineral elements and heavy metals analyzed in this study. Result shows varying concentrations of these elements in different periwinkle species harvested from different creeks (Table 3-10). The mean concentration of elements in different periwinkle species were;*T. fuscatus*> *P. aurita*>*P. byronensis* for Ca and Cu.*P. aurita*>*T. fuscatus*>*P. byronensis* for Zn and Pb while *P. aurita*>*P. byronensis*>*T. fuscatus* for Fe all in Jebs creek. In Ikang creek Cu; *P. byronensis > T. fuscatus >P. aurita,* Zn; *P. aurita > P. byronensis > T. fuscatus,* Fe; *T. fuscatus >P. byronensis> P. aurita,* Ca; *T. fuscatus > P. aurita> P. byronensis,* Pb; *P. aurita > T. fuscatus > P. byronensis.*

Unlike essentials elements and nutrients, too much or too little nutritional ingestion of these elements can result in a corresponding condition of these elements.

Lead is heavy metal characterized as hazardous, non-essential with no known role in living organisms. Exhibits extreme toxicity even at very low exposure levels. There is no known safe exposure concentration for lead. Health effects include interference with the development of a child's brain and central nervous system, reduction of IQ, behavioral problems and reduced cognitive development. WHO and Food and Agricultural Administration (FAA) suggest d minimum intake of copper to be 12mg and 10mg/day for adult males and females.

The important role of iron is seen in hemoglobin formation and oxygen transport, although low iron intake and or bioavailability are the reasons for most anemia in industrialized countries [10]. The calcium contents of bones and teeth is over 99% of all calcium in the human body. It is an essential needed element for rebuilding of bones during aging, although, its concentration tends to decline with age. Thus, getting extra calcium from periwinkle is useful in rebuilding strong bones, strengthen the heart, nerves, and blood clothing system. Zinc support growth and boost immunity during childhood.

Chindah *et al.,* (2009) [11] reported the presence but varying mean range of Cu, Zn, and Pb in *T. fuscatus* from different ecological zones of Bonny River System, Niger Delta. Davies *et al.*, (2006) [12] found the concentration of Pb to be < 0.001mg/ kg among other metals like chromium (Cr) and Cadmium (Cd) in a comparative study of Bioaccumulation of heavy metals in water, sediment and periwinkle (*T. fuscatus*) from Elechi creek, Niger Delta. Udiba *et al.,* (2020)

[13] identified Pb, Cd, Cr, and Ni from *T. fuscatus* purchased from Watt and Marian market in Calabar with mean concentration of 0.011–0.056, 0.008–0.032, 0.014–0.157 and 0.053–0.261 respectively.

The mean range of Cu, Zn, Fe, Ca and Pb metals analyzed in this study are above the WHO recommended dosage. This is unhealthy and calls for periodic public health education on thorough washing, proper and prolong boiling of periwinkle before consumption. Creeks with high contamination should be monitored to discourage introduction of unhealthy substances into the water bodies. This will invariably minimize the level of contamination.

Table1: Bacterial Cell Count of Three Periwinkle Species from Jeb and Ikang Creeks

		P. byronensis	***T. fuscatus***	***P. aurita***
		JEB CREEK		
Media	**Sample code**	**CFU/mL(g)**		
N/A	JBL	9.8 x10^7 CFU/g	9.8 x10^7	
	JB	3.8 x10^7	1.4 x10^7	
MAC	JB	2.7 x10^7		1.2 x 10^7
	JBL	1.6 x10^7 CFU/g		8.9 x10^7 CFU/g
	JW	.8 x10^7	2.4 x 10^7	2.2 x 10^7
TCBS	JW		1.9 X 10^7	
	JB		1.2 x10^7	
	JBL		9.9 x10^7CFU/g	
SSA	JB			7.3 x 10^7
	JW			1.1 x10^7
	JBL			6.4 x10^7CFU/g
		IKANG CREEK		
N/A	IBL	3.2 x10^7 CFU/g		8.5 x10^7CFU/g
	IB	2.4 x10^7		2.4 x10^7
	IW			2.4 x x10^7
SSA	IB			2.4 x10^7
TCBS	IB		2.6 X10^7	
MAC	IBL	8.9 x10^7 CFU/g	9.8 x10^7CFU/g	
	IB	2.5 x10^7	5.2 x10^7	
	IW	5.0 x10^7	1.4 x10^7	

Key: N/A= Nutrient agar**, TCBS=** Thio Citrate Bile Salt **agar, SSA=** Salmonella Shigella agar**, MAC=** MacConkey agar**, JBL=**Jebs blended**, JB =** Jebs boil**, JW =** Jebs wash**, IBL=** Ikang blended, **IB=**Ikang boil**, IW=**Ikang wash**, CFU=**colony forming unit**.**

Table 2: Percentage Occurrence of Bacterial Isolates from three Species of Periwinkle

Bacterial Isolates	***P. byronensis* N=30**	***T. fuscatus* N=27**	***P. aurita* N=27**	**Total**
	% Occurrence			
S. aureus	26.7	21.4	18.5	79.8

Enterococcus Spp	23.3	7.1	14.8	53.8
Acinetobacter Spp	20			23.8
Serratia spp	10			11.9
E. coli Spp	10	10.7	14.8	42.3
Pseudomonas Spp	6.7	17.9	3.7	33.5
Salmonella Spp	3.3	10.7	22.2	43.1
Vibrio		17.9	11.1	34.5
S. epidermidis		14.3		17.0
Shigella Spp			14.8	17.6
Total	**100**	**71.4**	**99.9**	

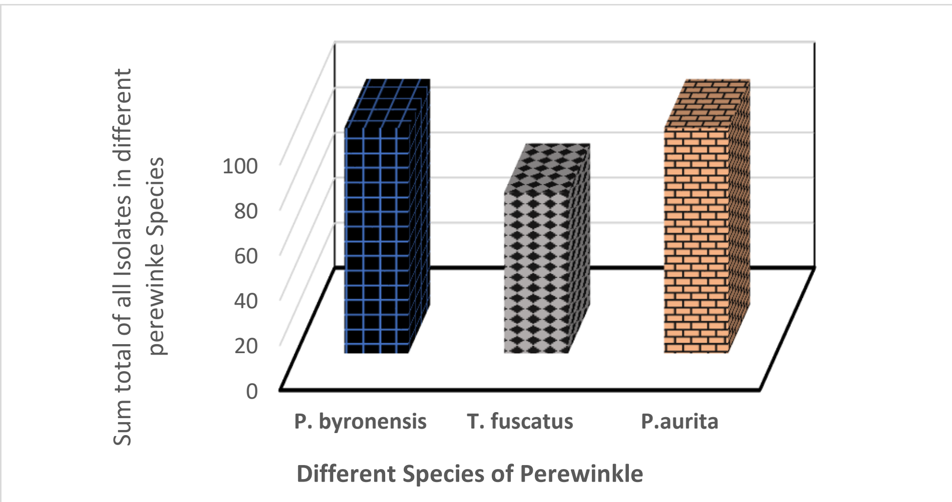

Figure 1: Sum total of all bacterial Isolates from different periwinkle species

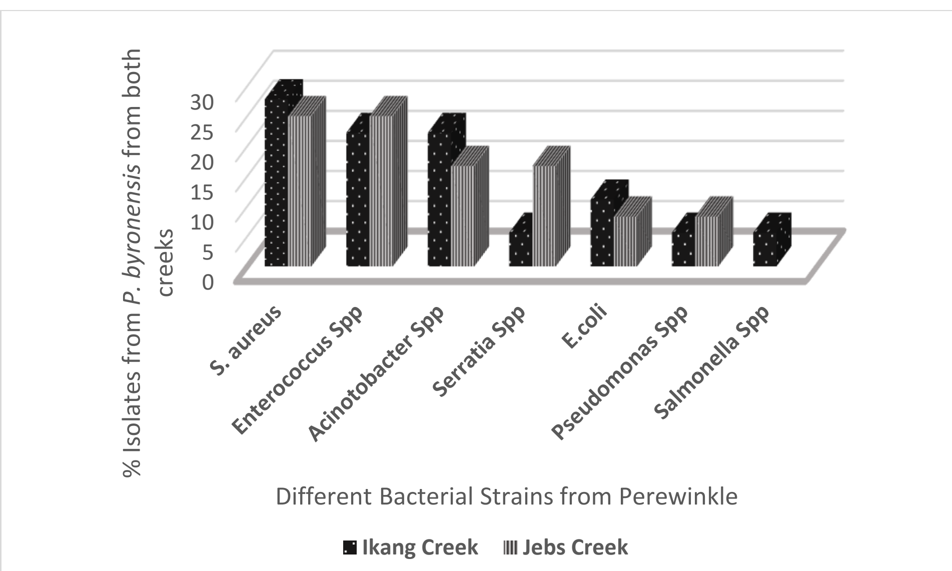

Figure 2: Percentage of bacterial Isolates from *P. byronensis* harvested from Ikang and Jebs Creeks.

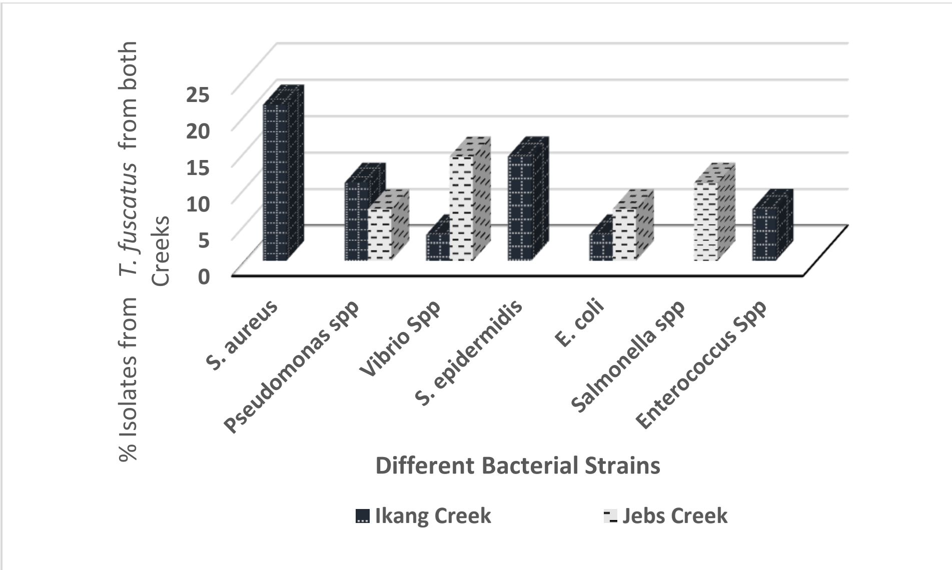

Figure 3: Percentage of bacterial Isolates from *T. fuscatus* harvested from Ikang and Jebs Creeks.

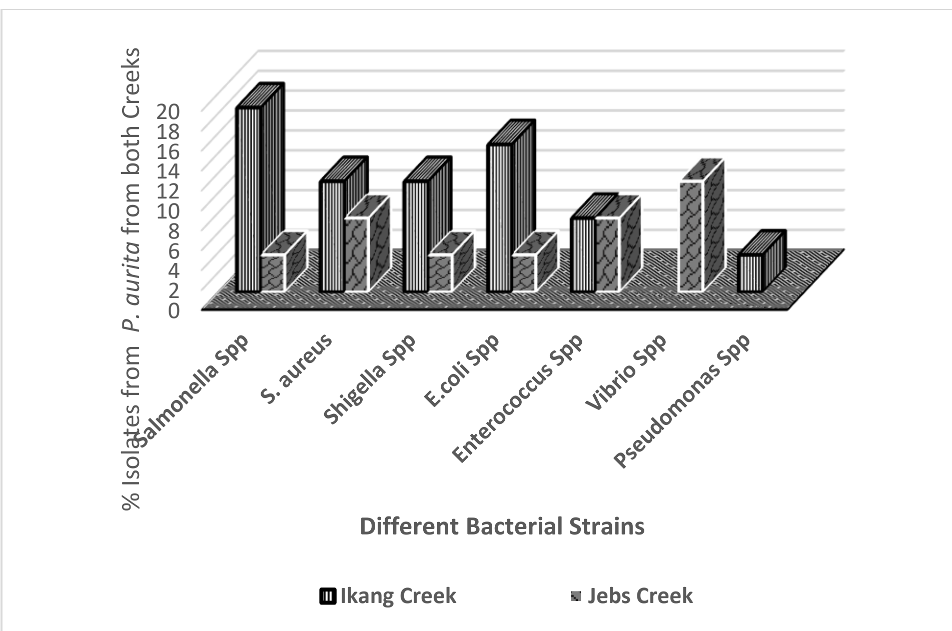

Figure 4: Percentage of bacterial Isolates from *P. aurita* harvested from Ikang and Jebs Creeks.

Table 3: Heavy Metal Analysis of *P. byronensis* from Jebs Creek using UV-Spectrophotometer

Samples (n=3)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.11	00.27	4.93	0.79	0.028
2	1.12	0.30	5.05	0.78	0.033
3	0.96	0.26	4.96	0.79	0.030
Mean	1.0633	0.2767	4.98	0.7867	0.0303
Standard Deviation	0.0896	0.0208	0.0624	0.0058	0.0025

F-statistical value = 4974.00983
P-value = 0

Table 4: Heavy Metal Analysis of *P. aurita* from Jebs Creek using UV-Spectrophotometer

Samples (n=3)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.48	0.66	5.29	0.82	0.048
2	1.46	0.66	5.33	0.81	0.048
3	1.47	0.68	5.27	0.82	0.047
Mean	1.47	0.6667	5.2967	0.8167	0.0477
Standard Deviation	0.01	0.0115	0.0306	0.0058	0.0006

F-statistical value = 54752.77831
P-value = 0

Table 5: Heavy Metal Analysis of *T. fuscatus* from Jebs Creek using UV-Spectrophotometer

Samples (n)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.73	0.46	3.29	1.11	0.042
2	1.73	0.45	3.31	1.09	0.045
3	1.75	0.46	3.32	1.10	0.043
Mean	1.7367	0.4567	3.3067	1.1	0.0433
Standard Deviation	0.0115	0.0058	0.0153	0.01	0.0015

F-statistical value = 48861.24521
P-value = 0

Table 6: Heavy Metal Analysis of *P. byronensis* from Ikang Creek using UV-Spectrophotometer

Samples (n)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.48	0.65	4.99	1.09	0.019
2	1.46	0.64	4.98	1.11	0.021
3	1.46	0.64	5.06	1.09	0.017
Mean	1.4667	0.6433	5.01	1.0967	0.019
Standard Deviation	0.0115	0.0058	0.0436	0.0115	0.002

F-statistical value = 26047.79306
P-value = 0

Table 7: Heavy Metal Analysis of *P. aurita* from Ikang Creek using UV-Spectrophotometer

Samples (n)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.22	0.89	4.72	1.31	0.038
2	1.22	0.90	4.74	1.31	0.038
3	1.23	0.89	4.72	1.33	0.037
Mean	1.2233	0.8933	4.7267	1.3167	0.0377
Standard Deviation	0.0058	0.0058	0.0115	0.0115	0.0006

F-statistical value = 145994.2019

P-value = 0

Table 8: Heavy Metal Analysis of *T. fuscatus* from Ikang Creek using UV-Spectrophotometer

Samples (n)	Cu (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Pb (mg/g)
1	1.35	0.54	5.31	1.69	0.026
2	1.36	0.56	5.34	168	0.027
3	1.35	0.54	5.32	1.70	0.026
Mean	1.3533	0.5467	5.3233	1.69	0.0263
Standard Deviation	0.0058	0.0115	0.0153	0.01	0.0006

F-statistical value = 129971.56979

P-value = 0.

Table 9: Distribution of Total Heavy Metals in Jebs Creeks

Metals	Cu	Zn	Fe	Ca	Pb
Range	0.96 – 1.75	0.26 – 0.68	3.29 – 5.33	0.78 – 1.11	0.028 - 0.048
Mean	1.4233	0.4667	4.5278	0.9011	0.0404
S.D	0.2972	0.1695	0.9267	0.1499	0.008

Table 10: Distribution of Total Heavy Metals in Ikang Creeks

Metals	Cu	Zn	Fe	Ca	Pb
Range	1.22 – 1.48	0.54 – 0.90	4.72 – 5.34	1.09 – 1.70	0.017 – 0.038
Mean	1.3478	0.6944	5.02	1.3678	0.0277
S.D	0.1057	0.1551	0.2596	0.2599	0.0082

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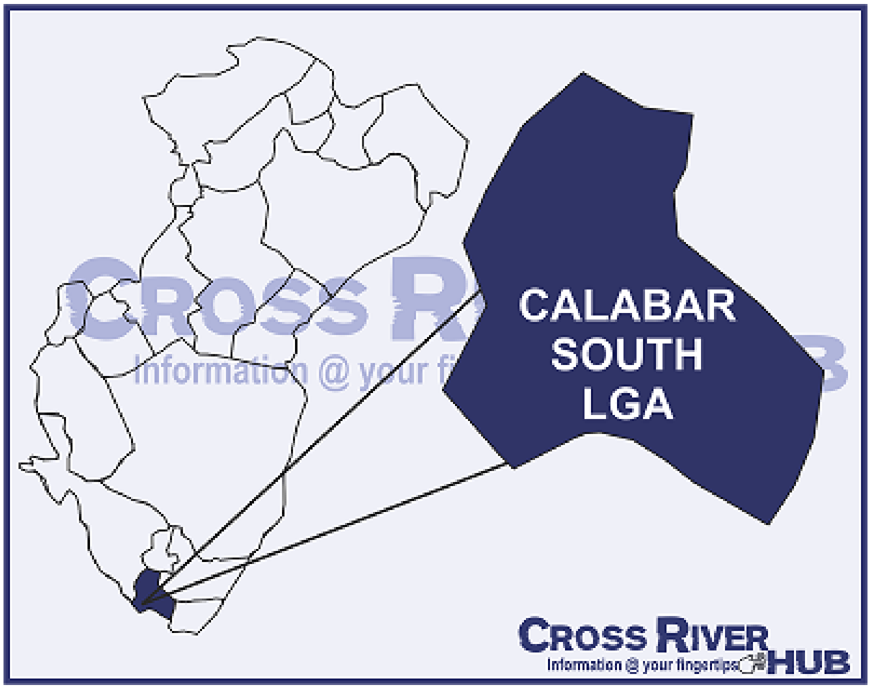
Appendix



APP 1: Map of Calabar South LGA of Cross River State

App 2: Map showing Akpabuyo LGA of Cross River State