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MICROBIOLOGICAL QUALITY ASSESSMENT AND PHYSICOCHEMICAL ANALYSIS OF SELECTED MAJOR STREAMS AND RIVERS IN CALABAR MUNICIPALITY

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ABSTRACT

The study was aimed at assessing the microbiological quality and physicochemical properties of selected major streams and rivers in Calabar Municipality, Cross River State. Water samples were collected from streams and river (Great Kwa, Idundu, Atimbo, Ekabo, Marina and Ikot Effang Mkpa) of selected communities within Calabar Municipal Local Government Area of Cross River State. The collected water samples were analyzed using standard microbiological techniques. Results obtained from the study showed that the total heterotrophic bacterial count in the water samples ranged from 2.1 x 10^3cful/ml (Marina river) to 5.8 x 10^4cfu/ml (Ikot Effanga Mkpa River), while the total coliform count ranged from 13MPN/100ml (Idunda river) to 29MPN/100ml (Ikot Effanga Mkpa river), and faecal coliform ranged from 1MPN/100ml (Marina to 8MPN/100ml (Ikot effanga Mkpa river). However, from the result, it was observed that the total heterotrophic bacterial counts, total coliform and faecal coliform count of the analyzed water samples exceeded the accepted limits as stipulated by WHO and USEPA (total heterotrophic bacterial count (1.0 x 10^2cfu/ml), total coliform and faecal coliform count (OMPN/100ml). Similarly, the *Salmonella* (1.2 x 10^0 to 2.8 x 10^2cfu/ml), *Shigella* (1.1 x 10^2 to 2.4 x 10^2cfu/ml) and *Vibrio* (1.3 x 10^2 to 2.6 x 10^2cfu/ml) counts of the analyzed water samples also exceeded the WHO and USEPA accepted limits (1.0 x 10^2cfu/mkl) *Bacterial spp* identified from the water samples were *Escherichia coli* (20%), *Bacillus* (14.28%), *Streptococcus* (5.71%), *Klebsiella* (7.14%), *Salmonella* (8.57%), *Shigella* (5.71%), *Staphylococcus aureus* (8.57%), *Proteus* (4.29%), *Pseudomonas* (11.43%), *Micrococcus* (4.29%), and *Vibrio* (5.71%). Among the identified isolates, *Escherichia coli, Salmonella, Pseudomonas, Vibrio* and *Shigella* were present in all the water samples analyzed. The result of the physiochemical analysis showed that the pH, total dissolved solids (TDS), BOD, calcium, magnesium, nitrate, nitrite and zinc content of the collected water samples were lower than the WHO and USEPA accepted limits, while the temperature, turbidity and iron content of the collected water samples were higher than the WHO and USEPA accepted limits. Nevertheless, this study has shown that water samples from Idundu river, Great Kwa river, Marina, Ekabo, Atimbo and Ikot Effanga Mkpa are highly polluted and thereby needs a serious effort in limiting the number of microorganisms released into the water systems. The higher microbial load observed in the water samples render them unfit for ether human consumption or domestic uses. It is therefore, recommended that an effective hygienic and sanitary practices be implemented along the water bodies.

KEYWORDS: Microbiological, Physicochemical, Calabar Municipality, Implemented.

INTRODUCTION

Water is the most known and most abundant of all known chemical substance which occur naturally on the surface of the earth (Hemant *et al.*, 2012). It is fundamentally important to all plants, animals and man, it is a prime solvent and its properties determine many natural processes (Ngido, 2011). Water could be found in three studies, solid as ice, liquid as water and gas vapour and it can be obtained from a number of sources such as rivers, streams etc. Countries throughout the world are concerned with the effects of unclean drinking water because water-borne diseases are a major cause of morbidity and mortality (Nwabor *et al.*, 2016; Asuquo *et al.*, 2017). Clean drinking water is important for overall health and plays a substantial role in infant and child health and survival. Igbinosa and Okoh (2009) estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters and seafood. Persons with compromised immune systems, such as those with AIDs, are especially vulnerable to water borne infections, including those infections that are self-limiting and typically not threatening to healthy individuals (Chigor *et al*, 2010).

Throughout the less developed part of the world, the proportion of households that use unclean drinking water source has declined, and it is extremely unlikely that all households will have a clean drinking water source in the foreseeable future (Chigor *et al*, 2010)..

UNICEF (2010) reports that 884 million people in the world use unimproved drinking water source, and estimates that in 2015, 672 million people will still use an unimproved drinking water source. In another report, UNDESA (2010) put the worldwide estimate for people without access to safe water at nearly 900 million. According to UNICEF (2010) about 2.6 billion, almost half the population of the developing world do not have access to adequate sanitation. Over 80 percent of people with unimproved drinking water and 70 percent of people without improved sanitation live in rural area (Mint *et al.*, 2011). In Nigeria, a vast majority of people living along the course of water bodies still source and drink from rivers, streams and other water bodies irrespective of the state of these water bodies without any form of treatment. These natural waters contain a myriad of microbial species, many of which have not been cultured, much less identified. The number of organisms present varies considerably between different water types, and it is generally accepted that sewage-polluted surface waters contain greater number of bacteria than unpolluted waters (Vidya-sagar, 2007). Polluted surface waters can contain a large variety of pathogenic microorganisms including viruses, bacteria and protozoa (Fewtrell *et al.*, 2015). These pathogens are often of faecal municipal wastewater treatment plants (Fewtrell *et al.*, 2015) and drainage from areas where livestock are handled or from non-point sources such as domestic and wild animals defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff (Okoh *et al.*, 2007). Fecal contamination of water is globally recognized as one of the leading causes of waterborne diseases. The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development. The outbreak of cryptosporidiosis of 1993 in Milwaukae, Wisconsin, in the United States provides a good example (Nwabor *et al.*, 2016). It was estimated that about 400,000 individuals suffered from gastrointestinal symptoms due to *Cryptosporidium* (Okoh *et al.*, 2007). Although subsequent reports suggest that this may be a significant over estimation (Nwabor *et al.*, 2016). More recent outbreaks involving *Escherichia coli* O157:H7, the most serious of which occurred in Walkerton, Ontario Canada in the spring of 2000, resulted in six deaths and over 2,300 cases. The number of outbreaks reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. In Nigeria, cases of water related diseases abound, agents of these diseases have been found to cut across various classes of organisms. However, most of these cases are not documented since majority of the affected individual subscribes to self-medication rather than seek professional medical attention. The most common waterborne diseases in Nigeria include cholera. dracunculiasis, hepatitis, and typhoid (Chigor *et al.*, 2010). Cases of water borne diseases linked to contaminations of drinking water with pathogens have also been reported in several towns (Edberg *et al.*, 2010). Waterborne outbreaks of enteric disease occurs either when public drinking water supplies were not adequately treated after contamination with surface water or when surface waters contaminated with enteric pathogens were been used for recreational and domestic purposes (Hughes and Thompson, 2014). Instances of disease outbreak due to contaminated drinking water with microbes were also reported with the drinking waters sampled from Shuni and Tambuwal towns in Sokoto State. The study revealed that *E. coli*, *Salmonella*, *Shigella* and *Vibrio* species in the water samples were far above the WHO (2003) allowable limit. The role of water as a vehicle for the transmission of all manner of water related illnesses is no longer a subject for debate, even ancient histories and books contain extracts indicative of this fact (Adeyinka *et al*., 2014; Raji *et al*., 2010).

MATERIAL AND METHODS

Study area

The research work was carried out in streams and rivers (Great Kwa river, Idundu, river Atimbo river, Ekabo river, Marina river and Ikot Effanga Mkpa river) of selected communities within Calabar Municipal Local Government Area of Cross River State. Atimbo river takes its source from Oban hill and joins the Calabar river. It is a source of water for Atimbo residents and its environs. Marina river takes its source from Calabar river, which flows from the north pass the city of Calabar. It forms a natural habour deep enough for vessels and it serves as a major water source for Duke town residents and its environs. Ikot Effanga Mkpa river serves as a major water source for Lemna residents and its environs. Idundu river serves as a major water source for both Atimbo and Akpabuyo residents and its environs. Ekabo river serves as a major water source for residents in Ekabo and Nassarawa communities. Great Kwa river takes it soruce from the Oban hills and flows southwards to the Cross River estuary. Its lower reaches are tidal with broad mud flats and drain the eastern coast of the city of Calabar. It serves as a major water source to different communities within Calabar Metropolis.

Materials

Glass ware

Glass ware used for this study were; petridishes, test tubes, pipette, burette, measuring cylinder, beaker, conical flask, L-shaped glass spreader, slides and sample bottles.

Equipments

They include, autochlave, incubator, microscope, weighing balance, wireloop, spatula, pressure pot, bursen burner, membrane filteration machine,

spectrophotometer, hardness total test kit, turbidity meter and pH meter.

Miscellaneous (other materials used)
Disposable hand gloves, nose mask, cotton wool, foil paper, whatman filter paper, syringes (2mls and 10mls), hand towel, detergent, methylated spirit, masking tape, matches and test tube racks.

Reagents
Ethanol, crystal violet, safranin, 70% alcohol/acetone and hydrogen peroxide.

Media
The media used for this study were MacConkey Broth (oxoid), Brilliant Green Lactose bile Broth (oxoid), Eosine Methylene Blue (EMB) agar, *Salmonella/Shigella* agar (SSA), Thiosulphate Citrate Bile Salt (TCBS) agar, and Mueller-Hinton agar.

Chemicals and reagents
Chemicals used for this study were of analytical grade. They include; absolute alcohol, methanol (sigma, USA) indicator, urea (Titan biotech, India). Reagents used were oxidase strips, indole. Kovacs and were products of hardy diagnostics, USA.

Sample collection
Water samples were collected from Great Kwa River, Marina, Idundu river, Ikot Effanga Mkpa river, Atimbo river and Ekabo river, using sterile glass sample bottles (500ml). The water samples were collected at different points at a considerable distant apart to ensure homogeneity and proper representation of the water. All the samples were collected in duplicates, and were covered with sterilized closures and transported to the Microbiology Laboratory of University of Calabar, in anicebox at 4^0 for analyzes (WHO, 2003).

Bacteriological analysis
Enumeration of total heterotrophic bacteria count
The total heterotrophic bacteria in the water samples were obtained using the spread plate method. Dilutions of 10^{-1} to 10^{-4} of the samples were prepared in 0.1% buffered peptone water (oxoid) and 0.1ml aliquots of each dilution was inoculated onto the surface of dried nutrient agar plate in triplicates and incubated at 37^0C for 24 hours. Petri-dishes from dilutions containing between 30 and 300 discrete colonies were counted and the result expressed as colony forming unit per milliliter.

Examination of total and faecal coliform
Presumptive test
Total coliform and faecal coliform were enumerated by multiple tube fermentation tests as described by APHA (2005). Coliform count was obtained using three tube assay of the most probable number (MPN) technique. Presumptive coliform test was carried out using MacConkey broth (oxoid). The first set of the five tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength both. Durham tube was place in all the tubes before sterilization. The three sets of the tubes received 10ml, 1ml and 0.1ml of the water samples using sterile pipettes. They were carefully labeled and incubated at 37^0C for 24-48 hours for estimation of total coliforms and at 44^0C for faecal coliforms for 24-48hours and examined for acid and gas production, and production was determined by colour change in broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the durham tube. The MPN was then determined from the MPN table for the three set of tube.

Confirmed test
The confirmed test was carried out by transferring a loopful culture from a positive tube from presumptive test into a tube of Brilliant Green lactose bile (GBLB) broth (oxoid) with durham tubes. The tubes were incubated at 37^0C for 24-48 hours for total coliform and 44.5^0C for faecal coliforms and observed for gas production.

Completed test
Using Eosine methylene blue (EMB) agar plate for pure colonies. The plates were incubated at 37^0C for 24-48 hours. Colonies developing on EMB agar were further identified as faecal coliform (*Escherichia coli*). Colones with green metallic sheen were confirmed to be faecal coliform bacteria with rod shape.

Isolation of *Salmonella/Shigella species*
Salmonella and *Shigella species* were isolated using *Salmonella/Shigella* agar (SSA). The media was prepared following the manufacturer's directive and 0.1ml aliquot of each water sample was transferred onto the surface of a dried sterilized SSA plates. The plates were inoculated in triplicates and incubated at 37^0C for 24 to 48 hours. Pure cultures were obtained through sub-culturing and the colonies were identified using standard procedures (Cheesbrough, 2000).

Isolation of vibrio species
Thiosulphate citrate bile salt (TCBS) agar was used to screen for the presence of vibrio species. The media was prepared according to manufacturer's directive and was poured into sterilized petri-dishes and allowed to solidify. Then 0.1ml of each water sample was transferred onto the dried TCBS agar plates in triplicates using 1ml pipette and spread evenly with a hockey stick. The plates were incubated at 35^0C for 24 to 38hours. Thereafter, yellow colonies were counted and identified following standard procedures (Cheesbrough, 2000).

The cultural, morpholtical and biochemical (characteristics of the isolates in a pure culture were determined following the procedures of Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Physicochemical analysis of water samples
The conventional parameters used for assessing the quality of the water samples a were determined according to procedures outlined in the standard methods for examination of water and waste water (APHA, 1998).

pH
The pH meter was calibrated by inserting its probe in a standard pH solution at 7.0, then rinsed with distilled water and sintered into the water samples. The pH level was read off above the temperature level displayed on the screen.

Turbidity
The turbidity of the water sample was determined with the use of turbidity meter. The samples were placed in the turbidity bottle and the bottle was then wiped clean to erase any finger print that may affect the reading. The bottle was then placed on turbidity meter and reading was taken.

Electronic conductivity
Conductivity meter was used to determine the electrical conductivity of the water samples. The conductivity probe was rinsed and immersed into the sample and the reading was noted.

Total hardness
Total hardness was determined by the spectrophotometric procedure. The procedure involved the addition of 1ml of sample into a reaction cell and 1ml of total hardness reagent (H-1K) was then added with a pipette. Three minutes reaction was allowed before the total hardness was read out in the spectrophotometer at a wavelength of 450m.

Total suspended solid (TSS)
For the determination of total suspended solid (TSS) filter paper was weighed using an electronic digital balance and the initial reading was noted. 100mls of the water samples were then filtered through and the filter oven dried at 50^0C for 1hour. The filter paper was then re-weighed and the final weight noted. The difference between the initial and the final weight of the filter paper gives the value of total suspended solids (TSS).

Nitrate, phosphate, sulphale, manganese and alluminium
They were all determined using spectrophotometer (model spectroquant).

Copper
5ml of the water sample was placed in a WTW spectrophotometer reaction cell and 5 drops of copper reagent cu-1k, added into it and shaken. A reaction time of 5 minutes was allowed before reading was taken in the spectrophotometer at 420µm have length.

Nitrite
5ml of the water sample was placed in a test tube and 1 micro spoonful of nitrate reagent no 2 was added and shaken to dissolve. After 10 minutes, the concentration was determined using the spectrophotometer at a wavelength of 40µm.

Ammonium
5mls of the water sample was placed in a test tube and 0.60ml ammonium reagent nh4-1 was added using a syringe. 1 level microspoonful of ammonium reagent nh4-2 was also added, shaken and allowed to stand for 5 minutes. Ammonium concentration was then determined using the spectrophotometer at a wavelength of 520nm.

Manganese
5ml of the water sample was placed in test tube and 4 drops of manganese reagent mn-1 was added and shaken. 2 minutes thereafter, 2 drops each of manganese reagents mn-2 and mn-3 were added, shaken and allowed to stand for another 2 minutes before reading the manganese concentration from the spectrophotometer at a wavelength of 520nm.

Lead
5ml of the water sample was placed in a reaction cell and 5 drops of lead reagent pb-1k was added and mixed. The concentration of lead was determined in the spectrophotometer at a wavelength of 620nm.

Chromate
6 drops (0.6ml) of chromate reagent cr-3k was added to a reaction cell and shaken to mix. This was allowed to stand for 1minute and then 5ml of the water sample added and shaken also to mix. This was again allowed to stand for another 1 minute and the chromate concentration was read from the spectrophotometer at a wavelength of 560nm.

Iron
5ml of the water sample was placed in a test tube and 0.30ml of iron reagent Fe-1 was added, shaken and allowed to stand for 3 minutes. The iron concentration was then determined at a wavelength at 420nm in the spectrophotometer.

Zinc
0.5ml of zinc reagent Zn-1k was placed in a reaction cell and mixed and then 0.5ml of the water sample was added and shaken to mix. Finally, zinc reagent Zn-2k was added, shaken and allowed to stand for 15 minutes. The zinc concentration was determined at a wavelength of 720µm.

Statistical analysis
Data from the physicochemical parameters of the analyzed water samples were subjected to two-way anaylsis of variance (ANOVA).

RESULTS

Microbial load of the analyzed water samples

Table 1 present the result of total heterotrophic total coliform, faecal coliform, *Salmonella*, *Shigella* and *Vibrio* counts obtained from the analyzed water samples. It showed that Ikot Effanga Mkpa river had the highest total heterotrophic bacteria count (5.8 x 10^4cfu/ml), total coliform (29MPV/100ml), faecal coliform count (10MPN/100ml), *Salmonella* (2.8 x 10^2cfu/ml), *Shigella* (2.4 x 10^2cfu/ml), and *Vibrio* counts (2.6 x 10^2cfu/ml), compared to counts obtained from the other water samples analyzed. However from the result, it was also observed that the total heterotrophic bacteria counts, total coliform, faecal coliform count *Salmonella*, *Shigella* and *Vibrio* counts (cfu/ml) of the analyzed water samples exceeded the accepted limits as stipulated by WHO and USEPA (total heterotrophic bacterial count (1.0 x 10^2cfu/ml), total coliform counts (OMPN/100ml), faecal coliform count (OMPN/100ml), *Salmonella* (1.0x x 10^2cfu/ml), *Shigella* (1.0 x 10^2cfu/ml) and *vibrio* counts (1.0 x 10^2cfu/ml).

Table 1: Profile of total heterotrophic (THB), total coliform and faecal coliform counts *Salmonella*, *Shigella* and *Vibrio* counts obtained from the analyzed water samples.

Sampling site	THB (cfu/ml)	TCC (MPN/100ml	FCC (MPN/100ml)	*Salmonella sp* (cfu/ml)	*Shigella sp* (cfu/ml)	Vibrio (ful/ml)
GKR	24 x 10^3	17	3	1.3x 10^2	1.2 x 10^2	1.5 x 10^2
IR	6.1x 10^3	24	6	1.8 x 10^2	1.5 x 10^2	1.9 x 10^2
ATM	2.7x 10^3	23	5	1.5x 10^2	1.4x 10^2	1.7 x 10^2
EK	3.1 x 10^4	27	8	2.2 x 10^2	1.9 x 10^2	2.4 x 10^2
RM	2.1 x 10^3	13	1	1.2 x 10^2	1.1x 10^2	1.3 x 10^2
ITEM	5.8 x 10^4	29	10	2.8x 10^2	2.4 x 10^2	2.6 x 10^2
WHO Standard	1.0x 10^2	0	0	1.0 x 10^2	1.0 x 10^2	1.0 x 10^2
USEPA standard	1.0 x 10^2	0	0	1.0 x 10^2	1.0 x 10^2	1.0 x 10^2

Legend: GKR = Great Kwa River, IR = Idundu River, ATM = Atimbo River, EK = Ekabo River, RM = Marina River, IEM = Ikot Effanga Mkpa River, WHO = World Health Organization, USEPA = United State Environmental Protection Agency

Distribution and frequency of occurrence of bacterial isolates from the analyzed water samples

Table 2 present the result of distribution of bacterial isolates from the analyzed water samples. It showed that *Escherichia coli*, *Salmonella spp*, *Pseudomonas spp*, *Vibrio spp* and *Shigella spp* had the highest distribution (they were present in all the water samples) compared to other bacterial counterparts.

Table 3 present the result of frequency of occurrence of bacterial isolates from the analyzed water samples. It showed that *Escherichia coli* had the highest occurrence (14) while *Proteus* and *Micrococcus* had the least (3 each respectively) occurrence compared to other bacterial counterparts.

Fig. 1 present the result of percentage occurrence of bacterial isolates from the analyzed water samples. It showed that *Escherichia coli* had the highest percentage occurrence (20%) while *Proteus spp* (4.29%) and *Micrococcus* (4.29%) had the least percentage occurrence compared to other bacteria isolates.

Physicochemical characteristics of the collected water samples

Table 4 present the result of the physiochemical parameters of the collected water samples. It showed that some of the measured physiochemical parameters of the water samples varied with that stipulated by WHO and USEPA. The temperature (^{o}C) of the collected water samples were significantly higher (p=0.0026) than the stipulated WHO and USEPA limits, while the pH of the water samples were significantly lower (p = 0.0026) than the WHO and USEPA stipulated limits (fig 1). Fig 2 present the result of turbidity (NTU), total hardness and BOD (mg/l).

Table 3: Distribution of bacterial isolates from the analyzed water samples.

Samples site	*Bacillus spp*	*Echerichia coli*	*Streptococcus spp*	*Klebsiella spp*	*Shigella spp*	*Salmonella spp*	*Staphylococcus*	*Proteus spp*	*Pseudomonas spp*	*Micrococcus sp*	*Vibrio spp*	*Shigella spp*
Great Kwa River	+	+	-	+	+	+	-	-	+	+	+	+
Marina River	-	+	-	+	+	+	-	+	+	+	+	+

Ikot Effanga Mkpa River	+	+	-	+	+	+	+	+	+	+	+	+
Atimbo River	+	+	+	-	-	+	-	-	+	-	+	+
Ekabo River	+	+	+	+	+	+	+	-	+	-	+	+
Idundu River	+	+	+	+	+	+	+	+	+	-	+	+

Legend: + = Present, - = Absent

Table 3: Frequency of occurrence of bacterial isolates from analyzed water samples.

Isolate	Occurrence (n=68)	Frequency of occurrence (%)
Escherichia coli	14	20
Bacillus spp	10	14.28
Streptococcus spp	4	5.71
Klebsiella spp	5	7.14
Salmonella spp	6	8.57
Shigella spp	4	5.71
Staphylococcus aureus	6	8.57
Proteus spp	3	4.29
Pseudomonas spp	8	11.43
Micrococcus spp	3	4.29
Vibrio spp	5	5.71
	68	100

Table 4: Physicochemical parameters of the collected water samples.

Parameters	Units	RI	IEM	RM	ATM	GKR	EK	WHO	USEPA
Temperature	^{o}C	26.20	26.51	26.40	27.43	26.10	26.30	<25	<25
pH		6.91	5.87	6.80	6.01	6.53	5.92	6.5-8.2	6.5-8.5
Conductivity	µs/cm	25.10	26.10	49.70	24.71	23.10	28.32	Unobjectional	Unobjectional
Turbidity	NTU	26.30	43.61	39.50	26.31	38.19	41.21	5	5
TDS	Mg/l	15.06	27.43	22.82	14.82	19.26	24.21	<600	No limit
Total hardness	Mg/l	17.10	21.24	19.10	24.43	20.10	22.33	100	-
BOD	Mg/l	7.72	11.13	8.40	9.78	9.15	10.48	14	-
Iron	Mg/l	0.38	0.47	0.46	0.48	0.41	0.45	0.30	0.30
Manganese	Mg/l	0.18	0.98	0.15	1.12	0.20	0.85	0.50	-
Calcium	Mg/l	10.80	12.11	12.10	11.41	12.40	10.48	50	-
Magnesium	Mg/l	6.90	6.42	5.14	8.04	4.70	6.83	30	-
Sulfide	Mg/l	0.87	1.88	1.14	1.85	1.64	1.72	-	-
Nitrate	Mg/l	4.21	4.61	3.93	4.40	4.10	3.82	50	-
Nitrite	Mg/l	0.65	0.84	0.55	0.91	0.61	0.82	50	-
Fluoride	Mg/l	0.94	1.02	0.61	0.83	0.67	0.68	-	-
Copper	Mg/l	2.10	2.14	2.81	2.08	1.82	1.98	2.0	-
Ammonia	Mg/l	1.08	1.34	0.88	1.06	0.84	0.87	0.5	-
Chromium	Mg/l	0.15	0.18	0.11	0.11	0.12	0.10	0.07	-
Aluminum	Mg/l	1.61	1.78	1.23	1.18	0.76	1.13	0.2	-
Cadmium	Mg/l	0.11	0.14	0.10	0.13	0.12	0.16	-	-
Zinc	Mg/l	0.45	0.52	0.43	0.52	051	0.48	3.0	-
Lead	Mg/l	0.10	0.12	0.08	0.14	0.11	0.13	0.01	-
Arsenic	Mg/l	0.12	0.13	0.10	0.14	0.09	0.13	-	-
Alkalinity	Mg/l	5.71	5.47	6.23	6.46	6.47	5.92	-	-

Characteristics of the analyzed water samples. It showed that the turbidity (NTU) of the water samples were significantly higher ($p = 0.1078$) than the stipulated limits approved by WHO and USEPA, while the total hardness and BOD of the water samples were significantly lower ($p = 0.1078$) than the accepted WHO and USEPA limits.

Fig 3 present the result of elemental characteristics of the analyzed water samples. It showed that manganese was significantly higher ($p<0.001$) in Ikot Effanga Mkpa and Ekabo river than the accepted limits, while calcium, magnesium, nitrite in the water samples were significantly lower ($p<0.001$) than the WHO and USEPA limits. The result also showed that the ammonia component of the water samples was significantly higher ($p<0.001$) than WHO and USEPA accepted limits. Fig 4a

present the result of heavy metal iron, manganese and copper) characteristics of the analyzed water samples. It showed that the iron concentration of the water samples was significantly higher ($p<0.0001$) than the accepted WHO and USEPA limits. The result also revealed that the manganese concentration in Ikot Effanga Mkpa and Ekabo river was significantly higher ($p<0.0001$) than the accepted WHO and USEPA limits. The result also revealed that the manganese concentration in Ikot Effanga Mkpa and Ekabo River was significantly higher ($p<0.0001$) than the accepted WHO and USEPA limits. Furthermore the result showed that the copper contents in Indundu river, Ikot Effanga Mkpa, Marina and Atimbo River were significantly higher ($p<0.0001$) than the accepted WHO and USEPA limits. Fig 4b present the result of the heavy metal (chromium, Aluminum and cadmium) characteristics of the analyzed water samples. It showed that the alumumium content of the water samples were significantly higher ($p<0.0001$) than the accepted WHO and USEPA limits.

Fig 4c present the result of heavy metal (zinc, lead and arsenic) characteristics of the analyzed water samples. It showed that the zinc content of the water samples were significantly higher ($p = 0.010$) than the accepted WHO and USEPA limits, while the lead content of the water samples were significantly higher ($p=0.0154$) than the accepted limits.

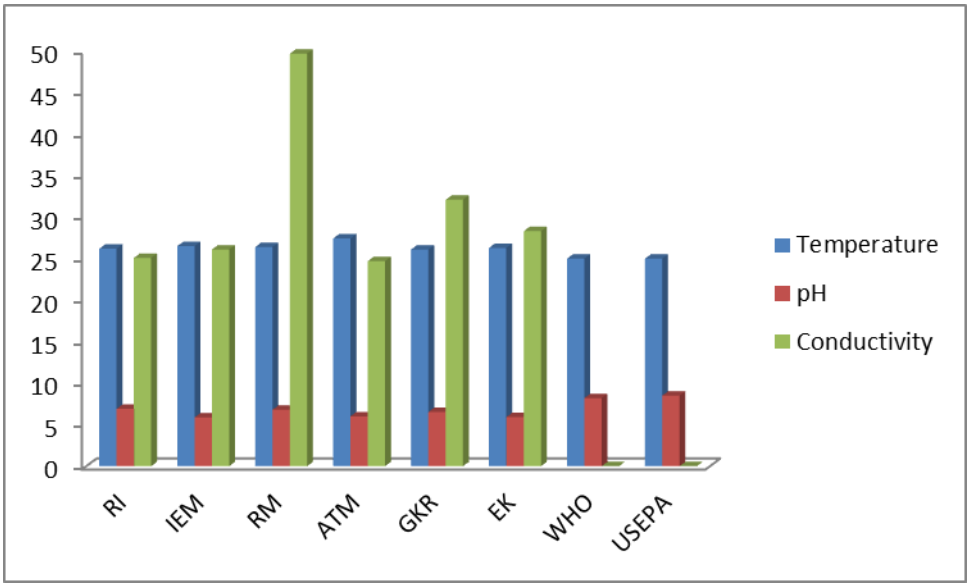

Fig. 1: Temperature, pH and conductivity characteristics of the analysed water samples.

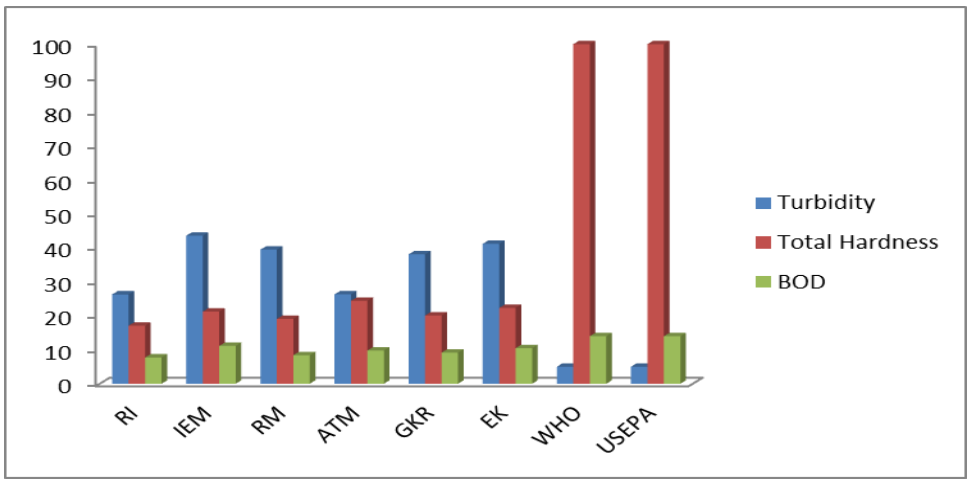

Fig. 2: Turbidity, Total hardness and BOD characteristics of the analysed water samples.

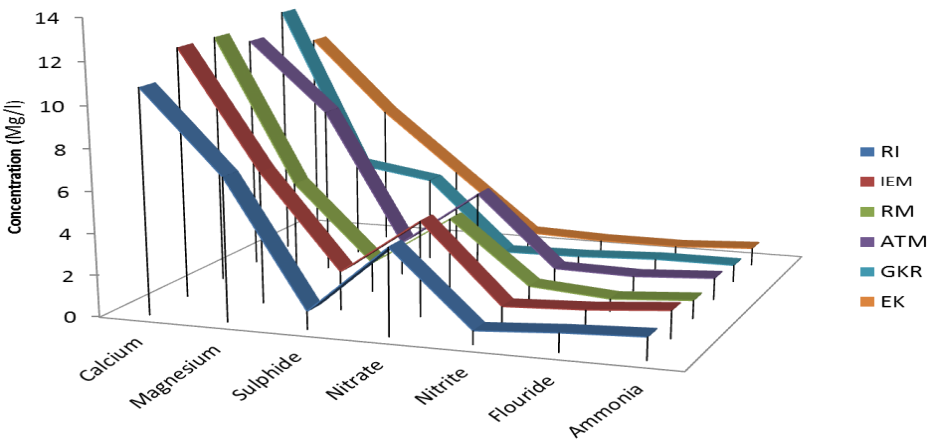

Figure 3: Elemental characteristics of the analysed water samples

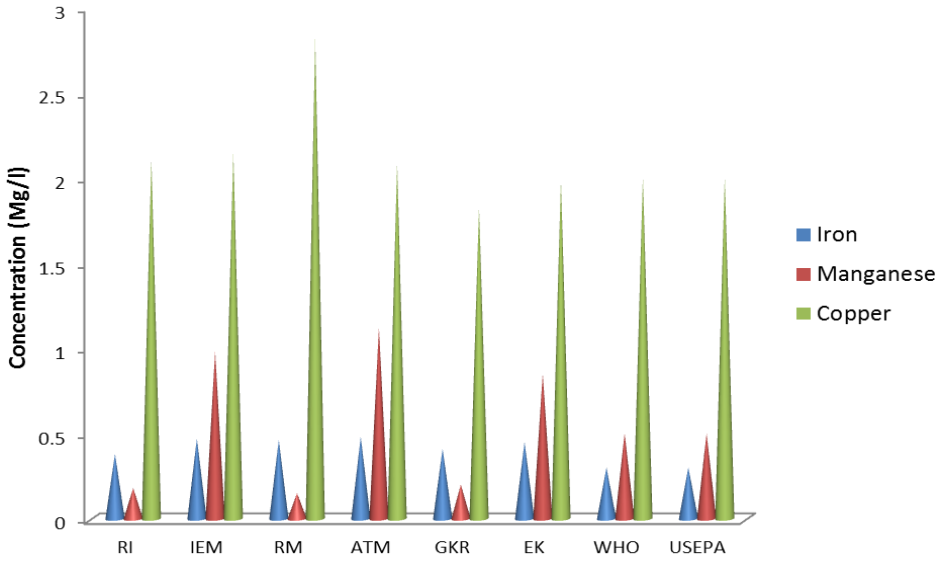

Fig. 4a: Heavy metal (Iron, Manganese and Copper) Characteristics of the analysed water samples.

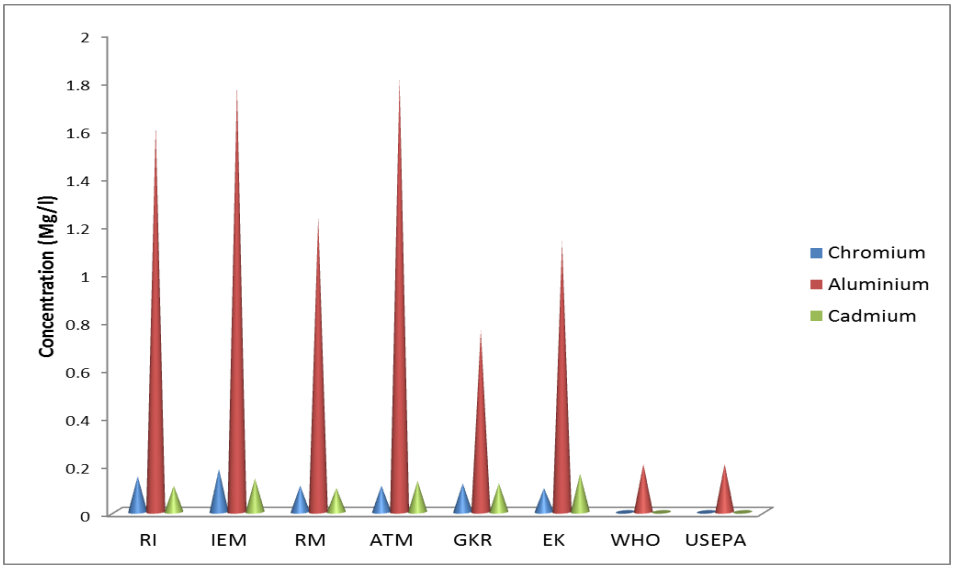

Fig. 4b: Heavy metal (Chromium, Aluminium and Cadmium) characteristics of the analysed water samples.

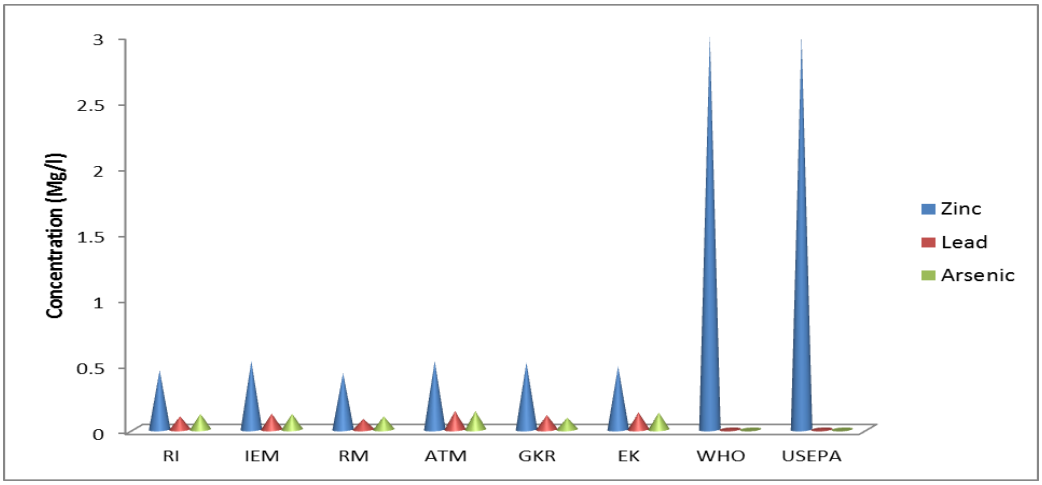

Fig. 4c: Heavy metal (Zinc, Lead and Arsenic) characteristics of the analysed water samples.

DISCUSSION

In rural and some urban areas, surface and underground water are generally supplied as drinking water either directly as in the previously mentioned areas or as borehole and individual or community wells in urban areas (Amanial, 2016). The total heterotrophic, coliform and faecal counts in any water body is used to estimate the total amount of bacteria in water and this indicates the overall microbial status of the water (Khalid *et al.*, 2017).

The analysis of THB count in the water samples revealed the presence of heterotrophic bacterial in all the water sources. The WHO standard for heterotrophic bacteria in potable water states that the total heterotrophic bacterial count should not be more than 100cfu/ml (WHO, 2003; WHO 2006). The presence of counts exceeding the WHO limits indicates that the water samples contain high concentration of bacteria that could make the water unsafe for drinking. Result obtained in this study showed that the THB count of the analyzed water samples ranged from 2.1 x 10^3cfu/ml in Marina river to 5.8 x 10^4cfu/ml in Ikot Effanga Mkpa river. These values exceeds WHO and USEPA permissible limit for drinking water. The result agrees with findings of Akubuenyi *et al.*, (2013) that there are high counts of total heterotrophic bacteria in major sources of water for domestic uses in Calabar Metropolis. Also, the result was consistent but higher than those reported by Ekpo *et al.*, (2013) who observed a THB count of 2.1 x 10^2cfu/ml to 3.810^{2}cfu/ml in borehole water samples collected from land fill and non-land fill areas in Calabar Municipality. Asikong *et al.*, (2017) reported a higher THB counts (8.2 x 10^4 to 9.0 x10^5cfu/ml) in water samples collected from major streams rivers in flood-prone area in Calabar South Local Government Area of Cross River State. This result also corroborates with the findings of Uzoigwa and Agwa (2012), who reported a higher counts of heterotrophic bacteria (2.5 x 10^3 to 2 x 10^4cfu/ml) in most river stream and borehole water samples in Port-Harcourt. Similar study by Sunday *et al.*, (2012) also reported a high THB count in major water bodies in Uturu, Abia State, Nigeria. The concentration of total coliform and faecal coliform obtained from the water samples exceeds WHO and USEPA standard of OMPN/100ml respectively. This result corroborates with the finding of Agbaiaka and Sule (2011) who reported that the MPN coliform index per 100ml of water samples from selected streams and boreholes in Ilorin metropolis ranged from 0 to 20MPN/100ml. This observation was also consistent with that of Aroh *et al.*, (2016), who reported a higher coliform count of 17 to 110MPN/100ml in selected upstream and downstream in Ebonyi, South-East Nigeria. Also, similar study by Asikong *et al.*, (2017) reported a higher coliform counts which were too numerous to count (TNTC) in water samples collected from Jebs, Anantigha and Idang communities in Calabar South Local Government Area of Cross River State. However, this observation was not surprising as the high THB, coliform and faecal counts observed in the water samples could have been as a result of human activities around the water bodies such as dumping of sewages and deposition of human excretas into the water bodies, run-off of fertilizers or manures from farmlands close to the shore of water bodies and excreta from grazing animals along the shores of the water bodies.

The bacteriological identification of bacterial isolates from the analyzed water samples revealed the presence of these genera; *Escherichia coli* (20%), *Bacillus spp* (14.28%), *Streptococcus* (5.71%), *Klebsiella* (7.14%), *Salmonella* (8.57%), *Shigella* (5.71%), *Staphylococcus aureus* (8.57%), *Proteus* (4.29%), *Pseudomonas* (11.43%), *Micrococcus* (4.29%) and *Vibrio* (5.71%). Result shows that *Escherichia coli, Salmonella, Pseudomonas, Vibrio* and *Shigella* were present in all the water samples analyzed. This observation corroborate with that reported by Ashang *et al.*, (2017) who reported to have identified *Salmonella, Shigella, Escherichia coli,* and *Bacillus* in selected water bodies in Calabar metropolis. Also study by Ikpeme *et al.*, (2011) reported to have identified *Campylobacter, Proteous, Vibrio, Bacillus Escherichia coli* and *Aeromonas* from dumpsite utisols and water sources in a rural community in Cross River State, Southern Nigeria. They further reported to

have observed *Escherichia coli* to have the highest percentage occurrence compared to other bacterial isolates identified in the water samples.

These organisms are important human pathogens associated with a variety of infectious diseases (Oluwayemisi *et al.,* 2015; Antai *et al.,* 2016) and their presence raises serious public health concern because they are known as causative agents of many water borne diseases and this indicates that these water sources are not potable. Also, the presence of these identified pathogenic organisms in the analyzed water samples could present a health risk factor to people who ingest them either directly or indirectly. *Staphylococcus aureus* could cause gastroenteriritis in individuals who ingest them directly or indirectly (Amr *et al.,* 2013). The presence of *Klebsiella, Escherichia coli, Salmonella, Shigella* and *Vibrio* in the water samples is a clear indication of faecal contamination of the water bodies and when ingested, they can cause diarrhea, cramps, nausea, headaches and other symptoms (USEPA, 2006). Also the presence of *Pseudomonas* in the water sample was worrisome as they have been implicated with diseases such endocarditis, Urinary tract infections, respiratory tract infections, bactermia, gastrointestinal tract infection, skin infections among others (Uzoigwe and Agwa, 2012). Their entry into the water sources could be attributed to seepages from nearby septic tanks as opined by Nguendo-Tongsi (2011) or through deliberate and indiscriminate deposition of animal waste and human faeces into streams as commonly observed in some riverine areas. Amir *et al.,* (2013) opined that the presence of *Escherichia coli* which is the most common indicator of feacal pollution in a water sample is an indication of the presence of other enteric pathogen.

The examination of the physicochemical parameters showed that the pH of all the samples collected from the water sources were below WHO and USEPA permissible limit of 6.5-8.5 (WHO 2003, WHO 2006; USEPA, 2006). Result shows that the pH of the water samples ranged from 5.87 to 6.91, indicating that the water sources were slightly acidic and this increased acidity could be attributed to the presence of acidic metabolites. The low pH values of the water samples may be an indication of low CO_2 content of the water bodies (Edema *et al.*,2010). The temperature of the analyzed water samples exceeded the WHO and USEPA accepted limits. This is believed to have been influenced by the intensity of sunlight as the water bodies sampled are open and exposed to sunlight.

The overall chemical richness of any water is a reflection of its conductivity values (Muduli and Panda, 2015). The conductivity values of the analyzed water in samples obtained in this study ranged from 25.10 to 49.70µs/cm. This may be attributed to high concentrations of chloride, sulphate and total dissolved solids in the water bodies. The higher conductivity values recorded may also probably be due to increased decomposition of organic matter or evaporation resulting in the concentration of nutrient (Hemant *et al.,* 2012). Turbidity relatively measures the physical or visual observable dirtiness of a water resource and are indicators of water pollution. The high values (26.30 to 41.21NTU) obtained in the analyzed water samples exceeded the WHO and USEPA permissible limit of 5.0NTU. This could be attributed to the direct empting of waste materials into the sample water sources, as this is a phenomenon that is common in Nigeria and Africa at large. Also, the high turbidity values obtained in this study may have to do more with the nutrient loads of the pharmaceutical and other industrial effluents that are being discharged into the river bodies. It could be appreciated that rivers receives a lot of human activities like bathing and washing clothes than others and these activities causes, the agitation of the particulate organic matter settled at the bottom of the river bed and may result in increased turbidity (Sunday *et al.,* 2012).

Total dissolved solids (TDS) refer to the non-filterable or dissolved substances in water that have formed an aqueous, non-colloidal mixture (Atiribom and Kolndadacha, 2014). They are mostly inorganic salts in unsaturated solutions and may impact inferior palatability, colour and tastes to receiving aquatic systems (Atiribom and Kolndadacha, 2014). The total dissolved solids values obtained in this study were lower than WHO and USEPA accepted limits. This observation may be related to the geo-chemistry of the sampled water bodies and their allochthonous input.

Total hardness is a function of the geology of the area with which the water is associated. It may affect the taste of water as well as influence its lathering ability when used for washing. Results obtained in the study shows that the values of total hardness, calcium and magnesium of the water samples were within WHO permissible limit. Calcium, which is essential for nervous system and for the formation of bones is commonly present in all water bodies where it usually comes from the leaching of rocks (Okonko *et al.,* 2013). On the other hand, magnesium is usually less abundant in water than calcium perhaps due to the fact that magnesium is found in the earth's crust in much lower amount s as compared to calcium (Michael *et al.,* 2015). High concentration of magnesium in drinking water gives unpleasant taste to the water (WHO, 2003). Manganese values of the water sources was within WHO guideline values except for those of Ikot Effanga Mkpa and Ekabo River. Manganese is a naturally occurring element in rocks. Like other heavy or trace metals, it is essential to sustenance of life. They are needed at low levels as catalyst for enzyme activities. However, drinking water containing high levels of these essential metal may be hazardous to human health (Hemnant *et al.,* 2012). Its contact with water sources could be through weathering of rocks and leaching. Excess manganese may inhibit the use of iron in the regeneration of blood heamoglobin. A high dose of manganese causes apathy, headaches,

insomnia and weakness of legs. Under extreme cases, neurological disorders such as Parkinson's disease may develop (Hemnant *et al.*, 2012). The value of iron in the water sources were above WHO and USEPA guideline of 0.30mg/L. This increase could also be attributed to weathering of rocks, as well as the presence of corrosive materials in the water bodies. Iron when presence in high detectable amounts can affect the colour of water, promote the growth of iron bacteria in water and also make water distasteful (Yagoub and Ahmed, 2009). The level of lead was higher in the water sources compared to the permissible limits and such water when consumed could be a possible source of cardiovascular dysfunction (Ashang et al., 2017).

CONCLUSION

This study has shown that water samples from Idundu River, Great Kwa River, Marina river Ekabo river, Atimbo river, and are Ikot Effanga, Mkpa river are highly polluted and thereby needs a serious effort in limiting the number of microorganisms released into the water systems. The high microbial load observed in the water samples render them unfit for either human consumption or domestic uses. However, the water quality of this water sources should be controlled inorder to minimize the acute problem of water-related disease which is endemic to the health of human. Therefore, it is recommended that an effective hygienic and sanitary practices be implemented along the water bodies. Also, there is need for intensive surveillance of isolates throughout water processing and treatment activities.

RECOMMENDATIONS

From this research work, the following recommendation were made

(i) The government should enact punishable law that will prohibit the dumping of refuse and open defecation into water bodies and also create awareness in the rural and urban areas concerning the hazardous effects of this habit.

(ii) It is also recommended that an effective hygienic and sanitary practice should be implemented along this water bodies in order to reduce contamination.

(iii) The government should provide treatment plants for these environments, so as to provide the residence with safe drinking water.

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