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INVESTIGATION OF THE PHYSICOCHEMICAL AND BACTERIOLOGICAL QUALITIES OF OKOKPON RIVER, EDO STATE, NIGERIA FOR ITS PORTABILITY STATUS

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ABSTRACT

Water occupies about 75% of the earth surface, yet access to portable water remains the most daunting task for everyone due to its frequent contamination. The physicochemical and microbiological characteristics of water samples from Okokpon River was determined on a monthly basis between October, 2011 and May, 2012. Routine laboratory procedures were employed in water analyses. The physicochemical parameters recorded were all lower than SON and WHO limit except turbidity (1.73±0.33 to 7.42±1.51NTU), iron (0.76±0.15 to 1.35±0.27mg/l) and lead (0.76±0.15 to 1.35±0.27). Electrical conductivity, total dissolved solid, alkalinity, potassium and total hardness showed significant difference ($p < 0.05$) at the downstream. The heterotrophic bacterial and fungal counts ranged from 2.16 to 5.89 $\times 10^5$cfu/ml and 3.01 to 3.69$\times 10^5$cfu/ml respectively with the highest at downstream which also had a significant difference for the heterotrophic bacterial counts ($P<0.05$). The coliform and *Escherichia coli* counts ranged from 35 to 180 MPN/100ml and 6 to 50 MPN/100ml respectively with the highest at the midstream. *Enterobacter aerogenes* and *Aspergillus niger* had the highest frequency of occurrence, 5.56% and 7.82% for bacterial and fungal isolates at midstream and upstream respectively. Plasmids were detected in *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella*, *Acinetobacter* and *Streptococcus* species which ranged from 2027bp to 23130bp. The water had higher turbidity, iron, lead and microbial values than the SON and WHO limits. Therefore, it is not suitable for drinking except treatment by at least boiling. Members of the community are advised to halt indiscriminate pollution of the water body to prevent further contamination.

Keywords: Coliform, Okokpon River, Plasmid, Set limits, Turbidity, Water Regulatory Agencies

INTRODUCTION

Water being the second most important life sustainer after oxygen is the medium in which all biochemical reactions of the body take place and constitutes about 65-70% of the body' weight. Therefore, the availability of water through surface and groundwater resources has become more critical on a daily basis (Aluyi *et al*., 2006). Only 1% is available on land for domestic, industrial and agricultural purposes (Ranee and Vasantha, 2010). Water bodies are constantly used as receptacles for untreated waste-water or poorly treated effluents accrued from domestic and industrial activities. However, when the absorptive capacity is exceeded, eutrophication results which consumes the available dissolved oxygen leading to loss of aquatic lives and contamination (Enerijiofi *et al*., 2018).

According to World Health Organisation, (WHO) (2008), more than 3.4 million people die yearly from water borne diseases, most of whom are young children and 80% of ill health especially in developing countries arise due to the lack of portable water supply. This makes infections contracted from contaminated water supply a leading cause of illness and death worldwide and also reveal why the provision of portable water for drinking is such a high priority for governments, allied agencies and well-meaning individuals (Enerijiofi *et al*., 2018). The provision of portable water for the world's estimated 1.3 billion deprived population has become one of the recent and topmost priorities of many governments. Water quality problems have intensified through the ages in response to the increased population size and urbanization (Lehloesa and Muyima, 2000). The discharges from sewage works and runoff from informal settlements have been identified to be the main factors compromising the microbiological quality of water (Zamxaka *et al*., 2004). Diarrhoea, fever and other secondary complications are outcomes of high total and faecal coliform counts in water (Fatoki *et al*., 2001). Nigeria, the most populous country in Africa is endowed with generous water resources which include its use in fishery, transportation, irrigation, recreational and domestic uses. In majority of the rural areas, the populace find it difficult to access portable water making them to depend on raw water supplies from wells, streams and rivers (Adekunle *et al*., 2007).

The Okokpon river is a small stream in the Okokpon community located in Ovia South West Local Government Area in Edo State. The river is used by the community and the surrounding ones for recreational and domestic purposes. The aim of this study was to examine the portability status of Okokpon river using the physicochemical and microbiological indices as well as plasmid profiling analysis of some bacterial isolates identified from the water samples.

MATERIALS AND METHODS

Study Area: The studied river is Okokpon in Ovia South West Local Government Area of Edo State. The water from the river is used by some neigbouring communities. These are settlements like Urhobo Camp, Sakpoba and Okponha. The river flows from Urhobo camp into Okokpon community and out splitting into three smaller tributaries.

Collection of surface water samples: Water samples were collected from three sampling points along Okokpon River (Fig.1). The samples were collected once per month throughout the sampling period (October, 2011 – May, 2012). Samples for physicochemical analysis, were collected with clean 2 litres plastic containers, while 100 ml sterile bottles with stopper attached were used in collecting samples for microbiological analysis and were labelled appropriately. The samples were immediately transported to the laboratory for analysis and those that could not be analyzed immediately were stored at 4^{o}C in a refrigerator for subsequent analyses. Sampling points designated as 1, 2 and 3 were upstream, midstream and downstream respectively.

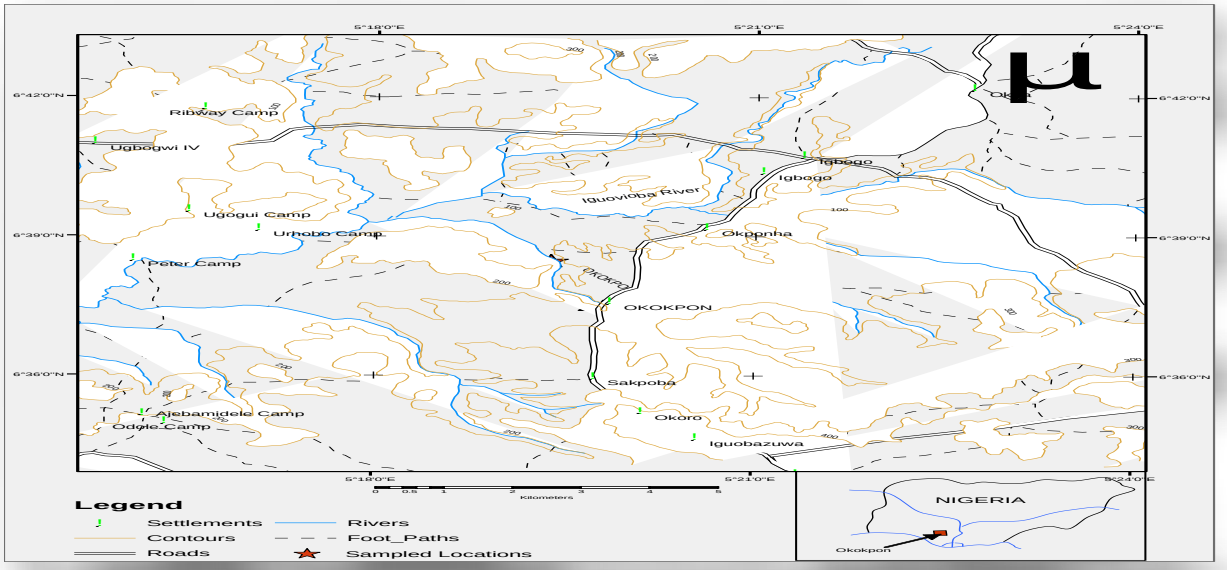

Fig. 1: Map code: 1 = Upstream, 2 = Midstream, 3 = Downstream

Determination of physicochemical parameters: The pH and temperature were measured using a HACH digital pH/temperature metre. Alkalinity was determined by titrimeteric method using standardized hydrogen tetraoxosulphate VI acid, phenolphthalein and methyl orange indicator, electrical conductivity by portable conductivity metre. Total hardness and Total Suspended Solid (TSS) was determined with oven to a constant weight, Total Dissolved Solids (TDS) and total solids were measured with a portable TDS metre. The total solid was derived from the addition of the TSS and TDS values. Chloride, nitrate, Ammonia-Nitrogen (NH_4-N_2), sulphate, phosphate and turbidity were determination by spectrophotometric method. Calcium, magnesium and dissolved oxygen were determined by titration. Sodium and potassium were determined with the aid of flame photometer (Jenway, PF-PF. USA) while oil and grease, Biological oxygen demand, Dissolved oxygen were detected using wrinkler's solution (APHA, 2011and Onyeonwu, 2000).

Heavy Metals Determination: The concentration of the respective heavy metals (iron, chromium, cadmium, nickel, copper, zinc, lead and manganese) present in the water samples were determined with the aid of an atomic absorption spectrophotometer (Buck Scientific model 210 VGP USA). Appropriate standards of known concentrations of the respective metals were prepared and used to calibrate and auto

zero the electrode. The water samples were dispensed into sterile plastic bottles, electrode was inserted and read at specific wavelengths (Ademoroti, 1996).

Determination of total heterotrophic bacterial and fungal counts: The total heterotrophic bacterial and fungal counts of the respective water samples were evaluated using both serial dilution and pour plate methods. Serial dilution was carried out on the stock culture by transferring 1ml from stock solution to 10^6 dilution while each test tube initially contained 9ml of sterile peptone water as diluent. The total heterotrophic bacterial count for each sample was determined using nutrient agar. One (1) ml aliquot of the serially diluted water sample (10^{-2} and 10^{-5}) was transferred onto sterile labeled plates before the addition of 15 ml of sterile nutrient agar maintained at 45°C under aseptic conditions and allowed to solidify. Plating was done in triplicate and incubated at 30^0C for 48 hours in an incubator. The mean bacterial counts were enumerated and recorded. Gram stain reaction was used to classify bacteria as either gram positive or negative. For the determination of fungi, One (1) ml aliquot of the serially diluted water sample (10^{-2} and 10^{-5}) was pipetted into each plate before dispensing 15ml sterile Potato Dextrose Agar (PDA) followed by the addition of 1 ml an antibiotic solution (500µg of chloramphenicol dissolved in 20 ml of distilled water) and maintained at 45°C. The PDA plates in triplicate were incubated at ambient temperatures (28^0C ± 2^0 C) for 5 days, after which the mean counts of the fungal colonies were also recorded. Fungi were characterised macroscopically as well as microscopically using lactophenol cotton blue stain (Cheesbrough, 2006).

Determination of the total coliform and feacal coliform counts: The total coliform and feacal coliform (*Escherichia coli*) counts of the water samples were evaluated. Both tests were conducted in three stages namely: Presumptive, confirmatory and completed (Cheesebrough, 2006).

Presumptive stage: Fifty (50) ml of each sample was dispensed in a sterile labeled 100 ml conical flask containing 50 ml of sterile MacConkey broth and an inverted Durham tube. Also, five 10 ml sterilized tubes containing both 10 ml of MacConkey broth and inverted Durham tubes were prepared and 10 ml of the respective water samples were added under aseptic conditions. Five 9 ml sterilized tubes containing 1 ml of MacConkey broth and inverted Durham tubes were also arranged and 1 ml of the corresponding water samples were added under sterile conditions. These tubes and conical flasks were incubated at 30^0 C for 48 hours. The procedure was repeated for the faecal coliform count but the inoculated test tubes and conical flasks were incubated at 44^0 C for 24 hours. At the end of the respective incubation periods, the test tubes and conical flasks were examined for both acid and gas production and reference was made to most probable number (MPN) statistical table to ascertain the most probable number of both the total coliform and faecal coliform count in 100 ml of the water samples.

Confirmatory stage: About 0.1 ml of the test tubes that had displayed positive result (acid and gas production) in the presumptive test was pipetted onto test tubes containing 10 ml of sterile MacConkey broth and inverted Durham tubes to detect gas production. The tubes were incubated at 30^0C for 48 hours for total coliform count and 44^0 C for 24 hours in respect of faecal coliform count.

Completed stage: The contents of the positive confirmatory test tubes were streaked on freshly prepared Eosin methylene blue agar plates with the aid of a sterile inoculating loop, under aseptic conditions. For the completed stage of the faecal coliform test, indole test was carried out to differentiate between streaked colonies of *Enterobacter aerogenes* and *Escherichia coli*.

Identification and characterization of microbial isolates: Pure cultures of the heterotrophic, coliform and feacal coliform counts of the bacterial isolates were identified and characterized on the basis of their cultural, morphological and biochemical characteristics (Cullimore, 2000). The fungal isolates were identified through macroscopic observation of their sub cultured colonies, microscopic examination of their respective spores and hyphal appendages using lactophenol cotton blue stain (Barnett and Hunter, 1972).

Plasmid Extraction: Confluent lawn of the bacterial culture were collected with a glass rod and resuspended in 100 µl of PEB 1 (50 mM glucose- 10 mM Ethylene dinitrilo tetra-acetic acid (EDTA) at 0 ^{0}C in a 1.5 ml Eppendorf tube. After 10 minutes, 200 µl of PEB II (0.2 N Sodium hydroxide- 1% Sodium Dodecyl Sulphate) at room temperature was added and mixed gently by inverting the tube several times. After 5 minutes of incubation at 0^0 C, 150 µl of PEB III (3 M Potassium acetate- 1.8 M Formic acid) at room temperature was added, mixed gently several times, and incubated for 15 minutes at 0^0C. About 1.5 ml of the culture broth was spun for 1 minute in a micro-centrifuge to pellet cells. The supernatant was gently decanted leaving 50 – 100 ul together with cell pellet and vortexed at high speed to resuspend cells completely. Three hundred (300) ul of TENS (Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%) was added and mixed by inverting the tubes 3 – 5 times until the mixture became sticky. About 150ul of 3.0M sodium acetate (pH 5.2) was added and vortexed to mix completely. The solution was spun for 5 minutes in the micro-centrifuge to pellet cell debris and chromosomal DNA and the supernatant transferred into a fresh tube, mixed with 900 ul of ice cold absolute ethanol. It was spun to pellet plasmid DNA (white pellet was observed). The supernatant were discarded and the pellet rinsed twice with 1 ml of 70% ethanol and dried. The pellets were re-suspended in 20 – 40 ul of distilled water and used in agarose gel electrophoresis (Kraft *et al*., 1988).

Agarose Gel Electrophoresis: Agarose powder was weighed (0.8g), mixed with 100 ml of 1x TBE buffer and dissolved by boiling using a magnetic stirrer. It was allowed to cool to about 60^0C then 10ul of ethidium bromide was added and mixed gently. Afterwards, poured into electrophoresis tank with the comb in place to obtain a gel thickness and to avoid bubbles then allowed to solidify for 20 minutes before the comb was removed. The tray was placed in the electrophoresis tank and 1X TBE buffer was poured into the tank ensuring that the buffer covered the surface of the gel. About 15ul of the plasmid sample was mixed with 2 ul of the loading dye and carefully loaded into the wells created by the comb, the electrodes were connected to the power pack in such a way that the negative terminal is at the end close to the sample loading well. Electrophoresis was run at 60 – 100 V until the loading dye migrated about three-quarters of the gel. The electrophoresis machine was turned off, the electrodes disconnected and thereafter observed on a UV-transilluminator (Zolgharnein *et al*., 2007).

Statistical Analysis: The analysis of variance of the mean physicochemical concentrations and microbial counts were conducted (p=0.05). Duncan Multiple Range (DMR) tests were conducted to locate the point of significant differences in the analyzed mean counts (Ogbeibu, 2005).

RESULTS

Table 1 recorded the mean values of the physiochemical parameters determined for the water samples collected from the respective sampling points. Turbidity (1.73±0.33 to 7.42±1.51NTU), Iron (0.76±0.15 to 1.35±0.27mg/l) and Lead (0.036±0.01 to 0.05±0.01) concentrations were higher than the Standard organization of Nigeria (SON) and WHO standard. The downstream had the highest value for all physicochemical parameters studied except copper, biological oxygen demand, dissolved oxygen and total suspended solids.

The mean bacterial counts ranged from 2.16×10^5 cfu/ml at the upstream to 5.89×10^5 cfu/ml at the downstream sampling. The observed differences in the mean bacterial counts was statistically significant ($P<0.05$). A range of values; 3.01×10^5 cfu/ml to 3.69×10^5 cfu/ml was recorded in respect of the total fungal counts of the respective samples with no statistically significant difference ($P>0.05$). (Table 2). The total coliform and *Escherichia coli* counts showed ranges of 35 MPN/100 ml to 180 MPN/100 ml and 6 MPN/105 ml to 50 MPN/105 ml as recorded in (Tables 3a) and (Tables 3b) respectively.

Seven bacterial and six fungal isolates were identified and characterized. Amongst the bacterial isolates, *Enterobacter aerogenes* had the highest frequency of occurrence (5.56%) at midstream while *Streptococcus* sp. had the least (0.24%) at the downstream. However, of the fungal isolates, *Aspergillus niger* displayed the highest frequency of occurrence (7.82%) at the upstream while *Fusarium* sp. had the least (0.68%) at the midstream (Table 4). Plasmids were detected in *Acinetobacter* sp., *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella* sp. and *Streptococcus* sp. with weights between 2,027 to 23,130bp (Figure 2).

Table 1: Physicochemical parameters for the water samples for all sampled points

Physicochemical parameters	S. P 1	S.P 2	S.P 3	SON (PLS)	WHO(PLS)
pH	^{a}5.95±0.24	^{a}6.22±0.25	^{a}5.70±0.17	6.5-8.5	7.0 – 8.5
Turbidity(NTU)	^{a}1.88±0.32	^{a}1.73±0.33	^{b}7.42±1.51	1	5
Temperature (^{0}C)	^{a}28.76±0.15	^{a}28.595±0.16	^{a}29.18±0.49	Ambient	NS
Electrical Conductivity (µscm^{-1})	^{a}44.88±7.70	^{a}41.25±7.96	^{b}73.13±14.85	1000	NS
Total suspended solids (mg/l)	^{a}4.70±0.80	^{a}4.27±0.75	^{a}4.27±1.07	NS	NS
Total dissolved solids (mg/l)	^{a}22.44±3.85	^{a}20.63±3.98	^{b}36.56±7.42	500	NS
Total solids (mg/l)	^{a}8.42±2.29	^{a}7.69±1.98	^{b}14.26±3.91	NS	500
Total alkalinity (mg/l)	^{a}8.42±2.29	^{a}7.69±1.98	^{b}14.26±3.91	NS	100
Dissolved Oxygen (mg/l)	^{a}6.53±1.11	^{a}5.94±1.04	^{a}5.99±0.48	6.5 – 8.5	NS
Biochemical Oxygen Demand (mg/l)	^{a}4.70±0.80	^{a}4.27±0.75	^{a}4.27±1.07	6.5 – 8.5	NS
Chloride (mg/l)	^{a}13.01±2.23	^{a}11.96±2.31	^{b}21.21±4.31	NS	200
Phosphate (mg/l)	^{a}0.54±0.09	^{a}0.50±0.10	^{a}1.08±0.22	NS	NS
Manganese (mg/l)	^{a}0.02±0.00	^{a}0.02±0.00	^{a}0.04±0.01	0.2	0.5
Nitrate (mg/l)	^{a}0.45±0.08	^{a}0.41±0.08	^{a}0.59±0.12	50	50
Nickel (mg/l)	^{a}0.00±0.01	^{a}0.00±0.01	^{a}0.01±0.01	0.02	NS
Sulphate (mg/l)	^{a}0.72±0.12	^{a}0.66±0.13	^{a}1.54±0.31	100	200
Potassium (mg/l)	^{a}3.14±0.54	^{a}2.89±0.56	^{b}8.78±1.78	NS	1 - 2
Sodium (mg/l)	^{a}2.24±0.38	^{a}2.06±0.40	^{a}4.75±0.97	200	NS
Zinc (mg/l)	^{a}0.09±0.02	^{a}0.08±0.02	^{a}0.15±0.03	3	5.0
Magnesium (mg/l)	^{a}0.94±0.16	^{a}0.87±0.17	^{a}2.93±0.59	0.20	50
Oil and Grease (mg/l)	^{a}0.04±0.01	^{a}0.04±0.01	^{a}0.73±0.15	NS	NS
Calcium (mg/l)	^{a}1.39±0.24	^{a}1.28±0.25	^{a}3.51±0.71	NS	
Ammonium (mg/l)	^{a}0.22±0.04	^{a}0.21±0.04	^{a}0.44±0.09	NS	
Copper (mg/l)	^{a}0.02±0.05	^{a}0.01±0.01	^{a}0.01±0.01	1	
Total Hardness (mg//l $CaCO_3$)	^{a}2.33±0.40	^{a}2.15±0.41	^{b}6.44±1.31	150	
Iron (mg/l)	^{a}0.83±0.14	^{a}0.76±0.15	^{a}1.35±0.27	0.3	
Lead (mg/l)	^{a}0.03±0.01	^{a}0.03±0.01	^{a}0.05±0.01	0.01	
Chromium (mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.05	
Cadmium (mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.003	

KEY: mean± standard deviation of 8 samples. Means preceded with different superscript on the same row are significantly different ($P<0.05$) using Duncan multiple range Test.

S.P 1: Sampling point 1 (upstream), S.P 2: Sampling point 2 (midstream), S.P 3: Sampling point 3 (downstream),

SON (PLS): Standard Organisation of Nigeria (Permissible Limits), WHO (PLS): World Health Organisation (Permissible Limits), NS: Not Stated

Table 2: Heterotrophic bacterial and fungal counts (×10^{5}cfu/ml) from all sampled points

Sampling point	October	November	December	January	February	March	April	May	SON (PLS)	WHO (PLS)
			Bacterial counts							
1	^{a}2.32	^{a}2.79	^{a}2.16	^{a}2.45	^{a}2.51	^{a}2.56	^{a}2.69	^{a}2.57	NS	<100cfu/ml
2	^{a}2.79	^{a}2.57	^{a}2.62	^{a}2.73	^{a}2.77	^{a}2.82	^{a}2.57	^{a}2.53	NS	<100cfu/ml
3	^{b}5.32	^{b}5.14	^{b}5.53	^{b}5.74	^{b}5.81	^{b}5.89	^{b}5.75	2.85	NS	<100cfu/ml
			Fungal counts							
1	^{a}3.03	^{a}3.12	^{a}3.21	^{a}3.08	^{a}3.28	^{a}3.31	^{a}3.38	^{a}3.42	NS	<100cfu/ml
2	^{a}3.01	^{a}3.04	^{a}3.11	^{a}3.01	^{a}3.14	^{a}3.24	^{a}3.37	^{a}3.42	NS	<100cfu/ml
3	^{a}3.13	^{a}3.33	^{a}3.35	^{a}3.13	^{a}3.52	^{a}3.59	^{a}3.64	^{a}3.69	NS	<100cfu/ml

KEY: Means preceded with different superscript on the same column are significantly different (P<0.05) using Duncan multiple range Test.
1: upstream; 2: midstream; 3: downstream; SON: Standards Organization of Nigeria;
Pls: Permissible limits NS: Not stated

Table 3a: Coliform counts (MPN/100ml) from all sampled points

Sampling point	October	November	December	January	February	March	April	May	SON (PLS)	WHO (PLS)
1	50	40	50	90	50	40	35	40	Nil	0cfu/ml
2	160	180	180	180	160	90	50	35	Nil	0cfu/ml
3	50	160	90	180	160	50	90	50	Nil	0cfu/ml

KEY
Point 1: upstream; Point 2: midstream; Point 3: downstream; SON: Standards Organization of Nigeria; Pls: Permissible limits; WHO: World Health Organization

Table 3b: Total *E. coli* counts (MPN/100ml) for water samples from all sampled points

Sampling point	October	November	December	January	February	March	April	May	SON (PLS)	WHO (PLS)
1	20	17	18	30	Nil	8	10	Nil	Nil	0cfu/ml
2	20	13	35	35	50	Nil	18	6	Nil	0cfu/ml
3	17	17	25	35	35	11	20	7	Nil	0cfu/ml

KEYS:
1: upstream; 2: midstream; 3: downstream; SON: Standards Organization of Nigeria
Pls: Permissible limits; WHO: World Health Organization

Table 4: Percentage frequency and occurrence of bacterial and fungal isolates from each sampled point

Bacterial Isolates	Sampling points	Sampling months: October n (%)	November n (%)	December n (%)	January n (%)	February n (%)	March n (%)	April n (%)	May n (%)	Total n (%)
Acinetobacter sp.	1	18 (23.68)	9 (11.84)	10 (13.16)	9 (11.84)	Nil	18 (23.68)	12 (15.79)	Nil	76 (1.03)
	2	22 (23.16)	11 (11.58)	11 (11.58)	6 (6.32)	Nil	22 (23.16)	17 (17.89)	6 (6.32)	95 (1.29)
	3	21 (39.62)	10 (18.87)	8 (15.80)	4 (7.55)	Nil	Nil	14 (26.42)	5 (9.43)	53 (0.72)
Klebsiella sp.	1	15 (10.14)	8 (5.41)	15 (10.14)	22 (14.86)	17 (11.49)	35 (23.65)	30 (20.27)	6 (4.05)	148 (2.00)
	2	17 (9.56)	11 (6.18)	22 (12.36)	28 (15.73)	17 (9.56)	33 (18.54)	39 (21.91)	11 (6.18)	178 (2.41)
	3	10 (11.63)	6 (6.98)	Nil	20 (23.26)	Nil	Nil	40 (46.51)	10 (11.63)	86 (1.16)
Escherichia coli	1	30 (15.79)	Nil	24 (12.63)	31 (16.32)	24 (12.63)	36 (18.95)	25 (13.16)	20 (10.53)	190 (2.57)
	2	39 (15.35)	33 (12.99)	27 (10.61)	33 (12.99)	28 (11.02)	39 (15.35)	33 (12.99)	22 (8.66)	254 (3.44)
	3	25 (13.59)	28 (15.22)	12 (6.52)	22 (11.96)	20 (10.87)	33 (17.73)	29 (15.76)	15 (8.15)	184 (2.49)
Staphylococcus aureus	1	15 (18.99)	20 (25.32)	Nil	Nil	10 (12.66)	18 (22.78)	12 (15.19)	4 (5.06)	79 (1.07)
	2	17 (17.17)	22 (22.22)	Nil	11 (11.11)	22 (22.22)	11 (11.11)	16 (16.16)	Nil	99 (1.34)
	3	12 (22.22)	16 (29.63)	Nil	10 (18.52)	6 (11.11)	Nil	10 (18.52)	Nil	54 (0.73)
Enterobacter aerogenes	1	34 (13.55)	45 (17.93)	30 (11.95)	42 (16.73)	Nil	40 (15.94)	60 (23.90)	Nil	251 (3.40)
	2	44 (10.71)	50 (12.17)	39 (9.49)	50 (12.17)	55 (13.38)	50 (12.17)	56 (13.63)	67 (16.30)	411 (5.56)
	3	20 (12.58)	15 (9.43)	20 (12.58)	23 (14.47)	20 (12.53)	20 (12.58)	33 (20.75)	8 (5.03)	159 (2.15)
Streptococcus sp.	1	Nil	2 (7.14)	4 (14.29)	Nil	Nil	18 (64.29)	2 (7.14)	2 (7.14)	28 (0.38)
	2	Nil	6 (13.33)	11 (24.44)	Nil	Nil	22 (48.89)	6 (13.33)	Nil	45 (0.61)
	3	Nil	8 (44.44)	Nil	Nil	Nil	10 (55.56)	Nil	Nil	18 (0.24)
Pseudomonas aeruginosa	1	Nil	4 (4.94)	20 (24.69)	20 (24.69)	20 (24.69)	Nil	Nil	17 (20.99)	81 (1.10)
	2	6 (5.66)	Nil	33 (31.13)	22 (20.75)	28 (26.42)	Nil	Nil	17 (16.04)	106 (1.43)
	3	Nil	2 (3.64)	12 (21.82)	14 (25.45)	16 (29.09)	Nil	Nil	11 (20)	55 (0.74)
Fungal isolates										
Candida sp.	1	17 (7.26)	32 (14.10)	11 (4.70)	6 (2.56)	39 (16.67)	67 (28.63)	61 (26.07)	Nil	234 (3.17)
	2	15 (9.38)	Nil	8 (5.00)	2 (1.25)	21 (13.13)	60 (37.50)	54 (33.75)	Nil	160 (2.16)
	3	12 (11.21)	Nil	6 (5.61)	2 (1.87)	14 (13.08)	42 (39.25)	31 (28.97)	Nil	107 (1.45)
Fusarium sp.	1	11 (17.74)	44 (27.42)	Nil	Nil	6 (9.68)	11 (17.74)	Nil	17 (24.42)	62 (0.84)
	2	Nil	20 (27.78)	8 (11.11)	2 (2.78)	Nil	18 (25.00)	14 (19.44)	10 (13.89)	72 (0.97)
	3	Nil	14 (28.00)	4 (8.00)	2 (4.00)	4 (8.00)	14 (28.00	Nil	12 (24.00)	50 (0.68)
Aspergillus flavus	1	72 (20.28)	42 (12.39)	72 (20.28)	Nil	39 (10.99)	50 (14.08)	40 (16.60)	78 (21.97)	335 (4.53)
	2	60 (24.89)	8 (3.32)	80 (33.20)	Nil	30 (12.45)	23 (9.54)	23 (11.06)	Nil	241 (3.26)
	3	41 (19.71)	Nil	45 (21.63)	Nil	41 (19.71)	39 (18.75)	19 (9.13)	19 (9.13)	208 (2.81)
Penicillium sp.	1	Nil	50 (11.09)	67 (14.86)	72 (15.96)	67 (14.86)	56 (12.42)	50 (11.09)	50 (11.09)	451 (6.10)
	2	Nil	4 (1.33)	55 (18.33)	81 (27.00)	66 (22.00)	54 (18.00)	Nil	40 (13.33	300 (4.06)
	3	28 (12.96)	9 (4.17)	43 (19.91)	54 (25.00)	42 (19.44)	40 (18.52)	Nil	Nil	216 (2.92)
Aspergillus niger	1	72 (12.46)	78 (13.49)	72 (12.46)	89 (15.39)	50 (8.65)	67 (11.59)	61 (10.55)	89(15.39)	578 (7.82)
	2	60 (16.20)	Nil	90 (25.50)	Nil	56 (15.86)	88 (24.93)	59 (16.71)	Nil	353 (4.78)
	3	59 (20.56)	8 (2.79)	33 (11.50)	70 (24.39)	29 (10.10)	Nil	40 (13.94)	48 (16.72)	287 (3.88)
Mucor sp.	1	56 (10.16)	83 (15.06)	67 (9.07)	67 (12.16)	78 (14.16)	61 (11.07)	78 (14.16)	78 (14.16)	551 (7.45)
	2	50 (16.18)	18 (5.83)	50 (16.18)	Nil	60 (19.42)	59 (16.180	Nil	81 (26.21)	309 (4.18)
	3	39 (17.11)	Nil	33 (14.47)	51 (22.37)	Nil	42 (18.42)	Nil	63 (27.63)	228 (3.08)
Total										**7392**

n= no. of bacteria, % = percentage of total no. of bacteria

1: upstream; 2: midstream; 3: downstream

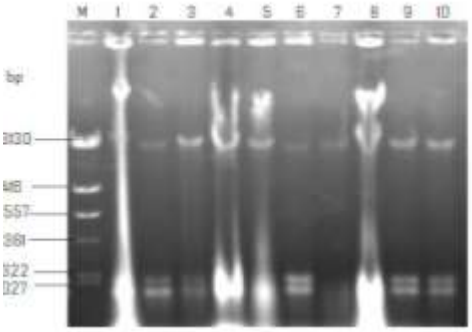

Fig 2: Agarose (0.8%) gel electrophoresis of the respective bacterial plasmid DNA. Lane M: *E. coli* marker plasmids of known molecular weights.
Lane 1 and 8; *Staphylococcus aureus*; Lane 3 and 9; *Acinetobacter* sp.; Lane 2; *Escherichia coli*,
Lane 4 and 5; *Klebsiella* sp.; Lane 6; *Streptococcus* sp.; Lane 7; *Pseudomonas aeruginosa*.

DISCUSSION

Most of the mean physicochemical attributes of the water samples collected from the Okokpon river fell within the permissible limits recommended by WHO. (2008) and SON. (2007) for potable water. However pH values were below the allowable limits stipulated by WHO. (2008) and SON. (2007) while the turbidity, iron, lead values were above the allowable limits stipulated by SON. (2007) for drinking water. Enerijiofi *et al.* (2018) reported pH values ranging from 5.43 ± 0.11 to 5.73 ± 0.14 from Ujiogba river, Edo State. Zamxaka *et al*. (2004) reported pH values ranging from 3 to 10.5 on domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. This range favours the growth of both indicator and pathogenic microorganisms. The concentrations of iron reported were higher than the standard by SON. (2007) meaning that the river is contaminated with iron. The mean temperature of the respective water samples ranged from 28.76^{0}C to 29.18^{0}C. These values were in agreement with report of Kulkarni. (1998) who observed a range of temperature values of 29.1^{0}C to 29.5^{0}C on water samples from suspected wells in Maharashtra of India. However, turbidity, electrical conductivity, total dissolved solid, total solid, total alkalinity, chlorine, potassium and total hardness showed significant difference ($p < 0.05$) at the downstream. This could be due to the effect of surface run offs and other human activities. The increasing levels of conductivity and cations could have resulted from decomposition and mineralization of organic materials (Dirisu and Olomukoro, 2015, Abida and Harikrishna, 2008). The low phosphate, sulphate, nitrate and lead values recorded in water samples from the respective sampling points was an indication of reduced contamination by organic matter. The concentrations of heavy metals were all below SON permissible except iron and lead. This could be due to the indiscriminate washing of cars and farm implements as well as release from exhaust of cars. However, this concentration could have resulted from low industrial activities around the river. The Biological Oxygen Demand (BOD) values reported ranged from 4.27±0.75 to 4.70± 0.80mg/l, which were higher than the SON. (2007) permissible limit, water samples from Okokpon river was not fit for consumption.

Generally, the heterotrophic bacterial and fungal counts recorded in the water samples from the river were high. However, the downstream had the highest with statistically significant in the heterotrophic bacterial counts ($p<0.05$). This high counts could have resulted directly from high anthropogenic activities such as indiscriminate laundry, passage of faeces, washing of cars and bathing as well as reduced dilution effect and surface run off at the downstream prior to sampling (Enerijiofi *et al.*, 2018). However, these counts disagreed with the report of Anyanwu and Okoli. (2012), who reported bacterial counts ranging from 0.78×10^4 cfu/ml to 1.06×10^4 cfu/ml in spring water samples collected from a location within Nsukka and lower fungal counts ranging from 7.0×10^3 cfu/ml to 8.0×10^3 cfu/ml for water samples sourced from Foma River, Ilorin (Agbabiaka and Oyeyiola, 2012). However, DEFRA. (2011) reported that surface waters contain greater amounts of organic matter which provide nutrients for bacterial growth. The bacterial isolates identified in this study corroborates with earlier findings of Anazoo and Ibe. (2005) and Enerijiofi *et al*. (2018) where they identified *Escherichia coli, Klebsiella, Salmonella, Pseudomonas, Shigella, Staphylococcus* and *Bacillus, Micrococcus, Enterobacter* sp. However, it was at variance with a report by Aluyi *et al*. (2006) who isolated *Proteus* sp., *Salmonella typhi*, *Clostridium perfringens*, *Enterococcus faecalis*, *Micrococcus* sp., *Acetobacter* sp. and *Pseudomonas* sp. in water samples from Udu River, Warri. Generally, the total and faecal coliform, *Escherichia coli* counts observed for the water samples rendered the water samples unfit for direct human consumption as the counts were higher than the permissible limits stipulated by the Standards Organization of Nigeria (SON, 2007) and World Health Organization (2008) for portable water. Also, the recovery of *E. coli* from the water samples sourced from the respective sampling points along the river could be indication of recent faecal contamination prior to

sample collection. *Enterobacter aerogenes* had the highest percentage frequency of occurrence amongst the bacterial isolates identified from the water samples. Agbabiaka and Oyeyiola, (2012) also reported the same trend amongst bacterial isolates identified from Foma river. *A. niger* was the most predominant isolate amongst the fungal isolates identified from Okokpon River. Also, DEFRA. (2011), similarly observed that *Aspergillus* sp. was one of the most abundant fungal genera present in both surface and ground water as reported in this study. The downstream which recorded the highest concentration of physicochemical parameters may have accounted for the reduced frequency of occurrence of bacterial and fungal isolates at the sampled point. This may be due to the adverse effect on bacterial and fungal growth.

Plasmids were detected in *Acinetobacter* sp., *Enterobacter aerogenes*, *E. coli*, *Klebsiella* sp. and *Streptococcus* sp. This observation is significant as these plasmids could confer several unique metabolic attributes such as degradation of organic or inorganic compounds and resistance to several classes of antimicrobials. Also, in the discharges of faecal materials from humans, animals and birds, drug resistant bacteria are distributed in the sewage and surface water where exchange of resistance plasmids can occur under certain physicochemical and biological conditions (Ash *et al.*, 2002). There is a possibility that drug resistant bacteria could be spread in the environment where man and animals acquire infection with bacteria carrying drug resistant plasmids. The implication is that infections arising from such resistant isolates becomes expensive and difficult to treat.

CONCLUSION AND RECOMMENDATION

The results obtained from this research revealed that water from Okokpon river is unsuitable for direct human consumption because they did not meet the set standard by WHO and SON. However, measures such as boiling, filtration and addition of flocculants would invariably enhance the portability of the water. It is recommended that enlightenment programmes be organized by relevant government agencies and non - governmental agencies to educate the people living within the catchment area on the need to stop disposing domestic waste and faeces into the water body.

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