Assessment of Physicochemical Qualities, Heavy Metals Concentrations and Bacterial Pathogens in Creek Road / Bonny Estuary, Rivers State, Nigeria

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Abstract: ***An assessment of water bodies for pollution is very important, especially in tropical countries, such as Nigeria. Hence, this study analysed the levels of physicochemical parameters, concentrations of heavy metals and the presence of bacteria, mainly E. coli in the water body. A total of 72 water samples were used for the analysis of physicochemical parameters, 18 for Heavy metals and 120 for bacterial detection. The physicochemical parameters were analysed both periodically (morning and evening) and seasonally (start of rainy season (November), start of rainy season (April) and middle of rainy season (July). The parameters analysed are: Potential hydrogen (pH), Temperature, Salinity, Total dissolved solid (TDS), Dissolved oxygen (DO) and Biological oxygen demand (BOD). The heavy metals were analysed only seasonally. Heterotrophic Plate Count (HPC) technique was carried out both periodically and seasonally, while the Most Probable Number (MPN) technique and Eijkman test were performed for the detection of E. coli. Then, confirmed isolates of E. coli were subjected to antibiotic susceptibility testing by disc diffusion method. All the parameters, except Temperature fell within the recommended values. The levels of the Temperature were slightly higher than the permissible values. In the morning hours, the mean values ranged from 29.89±0.10 to 30.23±0.31, while in the evenings, the mean values ranged from 30.57±0.21 to 31.80±0.56. However, the highest mean value was obtained in the evening of July. The statistical analysis showed that there was a significant difference periodically, which was observed in April and July (p=0.011; t=-4.409 and p=0.004; t=-6.124), respectively. Throughout the study, the results of the heavy metals, such as Cadmium, Mercury, Lead, Arsenic, Chromium and Nickel did not fall within the recommended values. Results of the Heterotrophic Plate Count (HPC) showed that July had the highest HPC ($7.30x\ 10^6$) in the mornings and $7.47x10^6$ in the evenings. Total coliform and Total E. coli counts revealed that coliform and E. coli were found in the water body in high number. These results confirmed observations made during the sampling; dried and fresh human and animal excreta were seen along the shores of the water body. One hundred and fifty four isolates of E. coli were subjected to antibiotic sensitivity testing and according to the results; all the isolates were susceptible to Meropenem, Imipenem and Colistin, while only two isolates were resistant to Tigecycline. The Multiple Drug Resistance (MDR) isolates were 11 (7.14 %). One hundred and twenty two (122) showed Multiple Antibiotic Resistance Index (MARI) of 0.0; that is, susceptible to all antibiotics that were tested. However, 12 isolates (7.79 %) showed MARI of 0.1, while 9 isolates (5.84 %) showed MARI of 0.2.***

Keywords: *Escherichia coli*; ;Multiple Drug Resistance; Multiple Antibiotic Resistance Index; Antibiotics; Heavy metals; Physicochemical parameters; Pollution; Water bodies; Seasonal; Periodic; Antibiotics; Resistance

1. Introduction

There are different types of water bodies, however, among them all, the most polluted is the river; the reason is that it is often polluted by sewage disposal, human activities and industrial effluents. Although, river water is the most important resource for human beings, if it is polluted, the pollution will affect the microbial quality, heavy metals concentration and the physicochemical characteristics (Koshy & Nayar, 1999). Researchers have noted that the increase in the number and amount of agricultural, industrial and commercial chemicals discharged in to the water body have caused harmful effects on microbes found in the water environment (Hammer, 2004; Mohammed, 2009).

Some researchers have previously analysed the levels of physicochemical parameters and heavy metals in water bodies (Premlata, 2009; Patil et al., 2012; Hemant et al., 2012; Olorode et al., 2015; Onojake et al., 2017; Ollor et al., 2018). It has been revealed that heavy metals are also found in fish species; these fish species may have been exposed to heavy metals in their water environment. When these fishes are consumed by humans, the metals might be dangerous to their health (Abdullali *et al.*, 2003; Mazlin *et al.*, 2009; Baharom & Ishak, 2015).

Escherichia coli, which is a Gram negative rod bacterium was only found in the gut of animals and humans, but the bacterium has been reported in fishes and seafood; this then makes it important to the public health. This is a serious problem as people who live in the area might rely on the water body for uses, such as bathing, drinking and so on. As a result, the microorganisms that might have acquired mutation would find their way into fishes and seafood. As local residents swim in the water body, they could contract gastroenteritis (Kennedy et al., 2008; Odonkor & Ampolo, 2013).

In recent years, another strain of *E. Coli* was reported on BBC News London, which was broadcasted by James Gallagher in December, 2015.According to the reports, the gene found in the microorganism was an MCR gene, which was said to have resulted from antibiotics given to animals and poultry as growth factors, therapeutic and prophylactic purposes. In addition, in 2005, Aerestrup reported that the newest strain of *E. Coli* was found in animals and poultry (Aerestrup, 2005). If that is the case, it means that the strain of *E. Coli* might be found in water bodies, as toilets are built on water bodies in some parts of Rivers State, Nigeria. Besides, untreated sewages are disposed in the water bodies in Nigeria very often; even though the sewages are treated, they may not be treated properly. Again, as it rains, especially during the rainy season, human and animal excreta might be carried by erosion and emptied in the water bodies. Hence the assessment of physicochemical qualities, heavy metals concentration and bacterial pathogens in Creek Road/Bonny Estuary, Rivers State, Nigeria.

2. Materials and Methods

Study area

This study was carried out at Creek Road/Bonny Estuary, which is one of the Rivers in Rivers State located at the Rivers West Senatorial District, Rivers State, Nigeria. Oil and gas industries are located in Rivers State and at present, the State is the only oil and gas-producing region in Nigeria. Oil and Gas Free Zone, Fertilizer Company, Petroleum Refining Companies, Petrochemical Company, Harbours are located in Rivers State. As a result of this, the State is exposed to activities of Oil Multinational Companies, which have been linked to the degradation of the natural environment, pollution and low agricultural productivity. Additionally, because of many numerous industrial activities and increase in population in the area, industrial and domestic wastes generated have increased. Creek Road/Bonny Estuary is located at the coastal area in Rivers State, consequently when it rains, all the waste including domestic and industrial wastes are washed into the Water bodies. Finally, unplanned development of the area has resulted in the destruction of forests; eventually, the State is exposed to effects of radiation (Abutudu et al., 2007, Ibeanu et al., 2007).

Study design

This study carried out the physicochemical parameters both periodically and seasonally in November (end of rainy season), April (start of rainy season) and July (middle of rainy season). The analysis of the concentrations of heavy metals were carried out only seasonally, while the detection of bacteria was carried out periodically (morning and evening) and seasonally.

Sample size

A total number of two hundred and ten (210) water samples were collected for the analysis of physicochemical parameters, heavy metals and bacterial detection (72, 18 and 120, respectively). This was determined according to Daniel's formula (Daniel, 1999).

Collection of water samples for physicochemical parameters

At Creek Road/Bonny Estuary in the months of November, April and July, water samples were collected in 72 separate containers (4 oz. (118.3 ml) sterile specimen containers) for the measurement of physicochemical parameters. The 72 water samples were collected for both seasonal and periodic variations. In a day, three water samples were collected in the mornings and three were collected in the evenings, so that in two days 12 water samples were collected. Then, in the three seasons a total of 36 water samples were collected from November 2016 to July 2017. Therefore, 72 water samples were collected from November 2016 to July 2018 (November 2016, April 2017, July 2017, November 2017, April 2018 and July 2018). Additionally, all, but Biological oxygen demand (BOD) were measured *in situ* using Horiba Water Checker (Model U-10) and mercury thermometer (APHA, 1998).

Collection of water samples for the measurement of heavy metal concentrations

In November (2016/2017), April (2017/2018) and July (2017/2018), water samples were collected in 18 separate containers (4 oz. (118.3 ml) sterile specimen containers) for the measurement of heavy metal concentrations. The 18 water samples were collected for just seasonal variations. In each month, three water samples were collected from each location, so that in three months, 9 water samples were collected and throughout the sampling period, 18 water samples were collected. In order to preserve the samples for the measurement of heavy metal concentrations, 5 drops (0.1 ml) of concentrated nitric acid (HNO_3) were added.

Collection of water samples for the detection of bacteria

A total of 120 water samples were collected aseptically in sterile bottles (4 oz. (118.3 ml) capacity) for bacteriological analysis from November 2016 to July 2018. After the collection, the water samples were put in a cooler filled with ice packs, transported immediately to the laboratory and processed within two hours. The sampling was carried out periodically (in the morning and evening hours) and seasonally (Greenberg, 1985).

Sample analysis for the detection of microorganisms

Before samples were analysed, all media and reagents were prepared in the laboratory according to manufacturers' instructions and directions.

Cultivation of microorganisms

Media such as Nutrient agar, MacConkey agar, MacConkey broth and CHROMagar for *Escherichia coli* and other coliforms (CHROMagar ECC) were prepared and used following manufacturers' instructions and directions. After the preparation of the media, the media plates (petri dishes) were labelled clearly and stored appropriately in the refrigerator at 4-6 ^{0}C.

For the cultivation of microorganisms, data were acquired from HPC, TCC and *Total E. coli* Count (TE); the TCC and TEC were carried out using the Most Probable Number (MPN) technique, which was statistically determined by the use of MacCrady table.

Analysis of water samples for physicochemical parameters

All the physicochemical parameters (pH, Temperature, Salinity, Total dissolved solids and Dissolved oxygen) except Biological oxygen demand were analysed in situ using Horiba Water Checker (Model U-10) and the Lovibond CM-21 Tintometer; mercury thermometer was used for the analysis of Biological oxygen demand.

Analysis of water samples for heavy metals
The presence of Heavy metals was determined by using the Atomic Emission Spectrophotometer (Agilent Technologies 4210, MP-AES, USA); the water samples were treated with 0.1 ml of concentrated nitric acid (APHA, 2005).

Antibiotic sensitivity testing (AST)
Susceptibility of *E. coli* isolates to Meropenem (10µg), Imipenem (10µg), Tigecycline (15µg), Cefotaxime (30µg), Gentamicin (30µg), Cefoxitin (30µg), Amoxicillin / Clavulanic acid (30µg), Amikacin (30µg), Ciprofloxacin (5µg) and Colistin(10 µg) (all from Oxoid Co. UK)was determined on Nutrient agar using the Kirby-Bauer-CLSI (Clinical and Laboratory Standards Institutes) modified disc agar diffusion (DAD) method as described by Isenberg (1998) (Table 8). Results were interpreted in accordance with the Clinical and Laboratory Standards Institutes (CLSI) guidelines (CLSI, 2008).

3. Results

The results of the periodic variations in November, morning and evening hours are presented in Table 1. The values obtained for Potential hydrogen (pH), Temperature, Salinity, Total dissolved solid (TDS), Dissolved oxygen (DO) and Biological oxygen demand (BOD) are clearly shown in the Table. Statistically, there was a significant difference in TDS (p=<0.001; t=-11.363). However, there was no significant difference in other parameters.

Also, morning and evening values of Physicochemical parameters in April were compared and the following results were obtained: pH, 6.83 ± 0.02 and 6.92 ± 0.03; Temperature, 29.70±0.26 and 30.57 ± 0.21; Salinity, 7.27 ± 0.03and 6.85 ± 0.03; TDS, 9.14 ± 0.04and 7.45 ± 0.05; DO, 2.57 ± 0.03 and 2.57 ± 0.04; BOD, 3.18 ± 0.45 and 2.67 ± 0.10, respectively. According to the statistical analysis, the following were significant: pH (p=0.012; t=-4.380), Temperature (p=0.012; t=-4.409), Salinity (p=<0.001; t=18.379) and TDS (p=<0.001; t=45.347). The ones that were none significant are DO (p=0.907; t=0.125) and BOD (p=0.123; t=1.951) (Table 2).

Again, at Creek Road/Bonny Estuary in July, morning and evening values were compared and the values obtained for pH, Temperature, Salinity, TDS, DO and BOD are shown in Table 3. There was a significant difference in the values of the pH (p=0.040; t=-3.004), Temperature (p=0.003; t=-6.472), TDS (p=0.018; t=3.895) and DO (p=<0.001; t=-28.128). There was no significant difference among the rest of the obtained values between the morning and evening periods.

Table 4 shows the values of the Heavy metal from all the Seasons. The variance in the mean were compared statistically using the ANOVA and according to the result, there was a significant difference in half of the values of all the Heavy metals (p<0.05). In order to see which group is the variation significant, Tukey Multiple Comparison Test was performed and the result showed that there was a significant variation between A and B, as well as A and C. However, betweenB and C, there was no significant difference in all, except Chromium.
Moreover, heterotrophic plate count (HPC) was carried out in November, April and July. According to the results, seasonally, the highest HPC was obtained in July (middle of rainy season) and periodically, the highest HPC was obtained in the evening (Table 5).

The total coliform count and total *E. coli* count were also carried out. In accordance with the results, the two groups of microorganisms were isolated and the highest number of coliform was obtained in the morning of day 1 (48 MPN/100 ml) in the April, while the lowest was observed in the morning of day 3 (7 MPN/100 ml) in the July (Table 6 and Table 7).Additionally, the results of the total *E. coli* count are presented in Table 6 and 7.

The antibiotics susceptibility testing is shown on Tables 9 to 11. Among the 10 antibiotics selected from 7 Classes (Carbapenems, Glycylcycline, Cephalosporin, Aminoglycoside, Beta-lactamase, Fluoroquinolone and Polymyxins), resistance among Beta-lactamase and Cephalosporins were found to be most frequent than Carbapenem, Glycylcycline, Fluoroquinolone, Aminoglycoside and Polymyxins. However, 25 of the isolates were resistant to Cefoxitin, representing Cefoxitin resistance of 16.23 % (n=154).

Table 1: Comparison of Periodic Variationsof Physicochemical Parameters in November

	pH	Temperature (^{0}C)	Salinity (%)	Total Dissolved Solid(mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.82±0.32	30.23±0.31	8.09±0.37	9.11±0.13	2.65±0.18	3.37±0.49
Evening	6.92±0.61	30.77±0.15	8.31±0.55	8.03±0.10	2.70±0.21	3.42±0.37
p-value	0.66	0.54	0.59	< 0.001	0.74	0.90
t-value	-2.509	-2.704	-0.589	11.363	-0.358	-0.131
Remarks	NS	NS	NS	S	NS	NS

Periodic:
Morning
Evening
***(This was done in replicate)**

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Table 2: Comparison of Periodic Variations of Physicochemical Parameters in April

	pH	Temperature (^{0}C)	Salinity (%)	Total Dissolved Solid(mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.83 ± 0.02	29.70 ± 0.26	7.27 ± 0.03	9.14 ± 0.04	2.57 ± 0.03	3.18 ± 0.45
Evening	6.92 ± 0.03	30.57 ± 0.21	6.85 ± 0.03	7.45 ± 0.05	2.57 ± 0.04	2.67 ± 0.10
p-value	0.012	0.011	< 0.001	< 0.001	0.907	0.123
t-value	-4.380	-4.409	18.379	45.347	0.125	1.951
Remarks	S	S	S	S	NS	NS

Periodic:
Morning
Evening
***(This was done in replicate)**

Table 3: Comparison of Periodic Variations of Physicochemical Parameters in July

	pH	Temperature (^{0}C)	Salinity (%)	Total Dissolved Solids (mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.86 ± 0.02	29.80 ± 0.10	7.90 ± 0.03	9.46 ± 0.03	3.21 ± 0.08	4.34 ± 0.34
Evening	6.92 ± 0.03	31.80 ± 0.56	7.31 ± 0.58	10.44 ± 0.07	3.78 ± 0.09	5.21 ± 0.10
p-value	0.040	0.004	0.083	< 0.001	<0.001	0.050
t-value	-3.004	-6.124	2.298	-20.964	-28.128	2.771
Remarks	S	S	NS	S	S	NS

Periodic:
Morning
Evening
***(This was done in replicate)**

Table 4: Seasonal Comparison of Heavy Metals among the Seasons
*(This was done in replicate)

	Cadmium (Cd) mg/l	Mercury (Hg)	Lead (Pb) mg/l	Arsenic (As) mg/l	Chromium (Cr) mg/l	Nickel (Ni) mg/l
November (A)	0.042 ± 0.003	0.490 ±0.026	0.042 ± 0.006	0.299 ± 0.046	0.314 ± 0.026	0.118 ± 0.020
April (B)	0.038 ± 0.005	0.378 ± 0.023	0.033 ± 0.013	0.799 ± 0.259	0.234 ± 0.031	0.108± 0.029
July (C)	-0.033 ± 0.003	0.331 ± 0.033	0.031 ± 0.012	0.714 ± 0.158	0.227 ± 0.024	0.104 ±0.029
p-value	0.065	0.001	0.453	0.014	0.013	0.805
F-value	4.460	26.797	0.906	9.561	9.813	0.225
Remarks	NS	S	NS	S	S	NS
Post hoc (Tukey) A and B		0.006 (S)		0.031 (S)	0.025 (S)	
A and C		0.001 (S)		0.016(S)	0.017 (S)	
B and C		0.169 (NS)		0.829 (NS)	0.935 (S)	

Table 5: Periodic Comparisons of Heterotrophic Plate Count (HPC) (CFU/ml)

	November 10^6	April 10^6	July 10^6
Morning	6.85 ± 0.73	7.18 ± 0.74	7.30 ± 0.61
Evening	7.18 ± 0.77	7.24 ± 0.75	7.47 ± 0.58

***(This was done in replicate)**

Table 6: Periodic Variations of Total Coliform Count (TCC) and Total *E.coli* in the Morning Hours

Days	November		April		July	
	TCC MPN/100 ml	TCC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml
1	28	7 (25.0 %)	48	7 (14.6 %)	15	3 (20.0 %)
2	20	7 (35.0 %)	11	3 (27.3 %)	20	3 (15.0 %)
3	11	3 (27.3 %)	21	3 (14.3 %)	7	4 (57.1 %)
4	15	3 (20.0 %)	11	3 (27.3 %)	15	3 (20.0 %)
5	21	4 (19.0 %	15	7 (46.7 %)	11	3 (27.3 %)

***(This was done in replicate)**

Table 7: Periodic Variations of Total Coliform Count (TCC) and Total *E. coli* in the Evening Hours

Days	November		April		July	
	TCC MPN/100 ml	TCC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml
1	15	3 (20.0 %)	28	7 (25.0 %)	14	3 (21.4 %)
2	28	7 (25.0 %)	11	3 (27.3 %)	11	7 (63.6 %)
3	21	3 (14.3 %)	23	4 (17.4 %)	21	3 (14.3 %)
4	20	3 (15.0 %)	11	3 (27.3 %)	15	7 (46.7 %)
5	15	7 (46.6 %)	20	3 (15.0 %)	15	3 (20.0 %)

*(**This was done in replicate)**

Table 8: Antibiotics, Strength, Zone of Inhibition and Classes of all the Antibiotics Used

Antibiotic	Strength	Resistant Zone Diameter	Class of Antibiotics
Meropenem	10µg	≤ 13 mm	Carbapenems
Imipenem	10µg	≤ 13 mm	Carbapenem
Tigecycline	15µg	≤ 12 mm	Glycylcycline
Cefotaxime	30µg	≤15 mm or≤17 mm	Cephalosporin
Gentamicin	30µg	≤ 12 mm	Aminoglycoside
Cefoxitin	30µg	≤ 14 mm	Cephalosporin
Amoxicillin/ Clavulanic acid	30µg	< 13 mm	Beta-lactamase
Amikacin	30µg	≤ 14 mm	Aminoglycoside
Ciprofloxacin	5µg	≤ 15 mm	Fluoroquinolone
Colistin sulphate	10µg	≤ 12 mm	Polymyxins

Table 9: Antibiotic Resistance of *E. coli*

Antibiotics	Creek Road/Bonny Estuary (154)
Meropenem	0 (0 %)
Imipenem	0 (0 %)
Tigecycline	2 (1.30 %)
Cefotaxime	20 (12.99 %)
Gentamicin	4 (2.60 %)
Cefoxitin	25 (16.23 %)
Amoxicillin/Clavulanic acid	21 (13.64 %)
Amikacin	8 (5.19 %)
Ciprofloxacin	3 (1.95 %)
Colistin	0 (0 %)

Table 10: Multidrug Resistance (MDR) Pattern of the Isolates of *E. coli*with MARI of >0.2 (n=11)

Multidrug Resistance (MDR) Pattern	Number of Isolate (n=11)	Percentage (%)
CTX, FOX, AMC	1	9.09
CN, FOX, AMC	1	9.09
CTX, FOX, AMC, AK	3	27.27
CTX, FOX, AMC, CIP	2	18.18
CTX, CN, FOX, AMC	1	9.09
CTX, CN, FOX, AK	1	9.09
TGC, CTX, FOX, AMC, AK	2	18.18
Total	**11**	**100**

Table 11: Antibiotic Resistance Index of *E. coli* Isolated from Creek Road/Bonny Estuary

MAR Index	Number of Isolates (n=154)	(%) MARI
0.0	122	79.22
0.1	12	7.79
0.2	9	5.84
0.3	2	1.30
0.4	7	4.55
0.5	2	1.30
Total	154	100

4. Discussion

The quality of water is not determined by only measuring the physicochemical parameters, heavy metals, or bacterial load. This is because it has been reported that physical, chemical and biological components are found in water bodies. These components affect water quality as their presence in water bodies denotes that the water environment is polluted (UNEP, 2000).

In November, the values of the physicochemical parameters obtained in the mornings and evenings are shown in Table 1. When compared with the Federal Environmental Protection Agency Nigeria (FEPA), United States Environmental Protection Agency (US EPA) and World Health Organization (WHO), all the parameters fell within the permissible values for water bodies. Although, the Temperature was higher; this was observed both in the morning and evening hours (World Bank, 1990; WHO, 1993; EPA, 2001). Statistical analysis of the results showed a significant difference in TDS (p=<0.001; t=-11.363), while other parameters were not significant when compared (Table 1).

The mean values of the physicochemical parameters in the morning and evening hours of April (start of rainy season) are presented in Table 2. A higher Temperature level was also observed in the month of April. According to the findings, all the values of the parameters were statistically significant (p<0.05), except DO (p=0.907; t=0.125) and BOD (p=0.123; t=1.951) when compared. Moreover, the values of physicochemical parameters obtained in the mornings and evenings of July (middle of rainy season) are shown in Table 3. The statistical analysis showed that there was a significant difference in all the parameters except, Salinity and BOD.

Generally speaking, although all the physicochemical parameters, except the Temperature fell within the permissible levels, the parameters were compared statistically. Periodically, the highest temperature was observed in the evenings (29.80±0.10 to 31.80±0.56 ^{0}C). Conversely, the highest mean Temperature value was recorded in the evening of July (31.80±0.56 ^{0}C). Considering the Student's T-test values for the periods, there was a significant variation (p=0.004; t=-6.124) observed as can be seen in Table 3.The Climate of Rivers State is dominated by rainy season (April to November) and dry season (November to March). In the present study, during the end of rainy season (November), mean temperature values ranged from 29.10±0.36 to 30.77±0.15 ^{0}C. Then, the start of rainy season (April), it ranged from 29.33±0.15 to 30.77±0.15^{0}C. However, in the middle of rainy season (July), it ranged from 29.33±0.15 to 31.80±0.56.

The rise in the temperature reported in this study can be as a result of heat that is generated from the sunlight that is brighter in the day than in the mornings. The results of this study are in agreement with a work carried out in Nigeria, in 2005 (Fafioye *et al.*, 2005). These researchers reported temperature at 31.5 ^{0}C and stated that temperature increased between 1.00 pm and 3 pm throughout their experiment, which they assumed may be time of high insolation. Additionally, Obunwo and his co-researchers reported temperature of 29 ^{0}C at Elechi Creek in 2011. In 2015, researchers reported temperature levels of (27 ^{0}C and above) (Olorode et al., 2015). Notwithstanding, the highest temperature observed in July (middle of rainy season) did not conform to the reports of other researchers, who observed a lower temperature in July (Silas et al., 2018).

In this study, the analysis of heavy metals was carried out seasonally, in November (end of rainy season), April (start of rainy season) and July, 2019 (middle of rainy season) at Creek Road/Bonny Estuary. In general, according to the results, the levels of the heavy metals obtained did not fall within the recommended levels. This work agrees with the findings of Ukpaka and Chuku who reported high levels of Pb, Cd, Zn, Ni, Cr and As in different researches they carried in 2012. The seasonal comparisons of the heavy metals in the three seasons are presented in Table 4. In accordance with the results, the mean values of all, but Arsenic were highest in November (start of rainy season); followed by April (start of rainy season). Additionally, considering the Tukey Multiple Comparison test, there was a significant variation observed in Mercury, Arsenic and Chromium as can be seen in Table 4. The high concentrations of heavy metals observed at the end of rainy season (November) could be as a result of reduced rainfall during this season. Conversely, during the start of rainy season, especially, during the middle of rainy season, increased rainfall allows the dilution of the contents of water bodies. The findings from this work is in line with previous work carried out in 2013, where the concentrations of heavy metals were more in the dry season than in the rainy season (Bouka *et al.*, 2019).

The present investigation observed a high number of heterotrophic bacteria at Creek Road/Bonny Estuary. According to periodic variations, the highest number of bacteria was observed in the evenings (7.18±0.77 to 7.47±0.58 (10^6). The activities of local residents may have contributed to the high heterotrophic bacteria in the evenings. In accordance with the seasonal variations, the highest number of bacteria was recorded in July (7.30±0.61 to 7.47±0.56), followed by April (7.18±0.74 to 7.24±0.75). This is in conformity with the work by Nwabueze (2011) who reported a higher microbial load in the rainy season than the dry season. As far back as 1987, Christensen noted that during the rainy season, more water percolate through the waste, which promotes and assists the process of decomposition by bacteria. In addition, a report in 2004 also recorded a higher bacterial density during the rainy season, which the researchers claimed might be due to the higher bacterial inputs from soil particles with runoffs (Isobe *et al.*, 2004).

Periodically, the presence of Coliforms was revealed at Creek Road/Bonny Estuary; this is shown in Table 6 and 7. In the mornings, the highest total coliform count (TCC) was observed in the morning of April, day 1 (48 MPN/100ml). However, the lowest was observed in the morning of July, day 3 (7 MPN/100ml). Additionally, *E. coli* was isolated from all the water samples and the results are in Table 6 and 7. According to FEPA, WHO and EPA, no detection of faecal coliform (*E. coli*) is desirable. Results from this study showed that all the water samples harboured *E. coli* in them, which does not agree with the recommended standard of zero. As stated by WHO, the most frequent adverse health outcome associated with exposure to faecally contaminated or polluted water is enteric illness (WHO, 2006). The presence of coliforms and *E. coli* revealed in the present study is in conformity with the findings of other researchers, who reported their presence in Water bodies (El-Jakee *et al.*, 2009; Pal & Gupta, 2011; Edun *et al.*, 2016; Ollor *et al.*, 2018; Amosu *et al.*, 2018). The presence of *E. coli* in all the water samples could be related to the building of toilets on the water body, nearby waste dumps and the discharge of sewage into the water body.

Confirmed isolates of *E. coli* recovered (157) from the water body were evaluated for their antibiogram profiling by the disc diffusion method (CLSI, 2008). The antibiotic susceptibility profile of the confirmed isolates is presented in Table 9 to 11. Among the 10 antibiotics selected from 7 Classes (Carbapenems, Glycylcycline, Cephalosporin, Aminoglycoside, Beta-lactamase, Fluoroquinolone and Polymyxins), resistance among Beta-lactamase and Cephalosporins were most frequent than the other classes. The resistance to these classes of antibiotics is sometimes encoded by plasmids, which may distribute resistance in susceptible bacteria via horizontal gene transfer (Hall & Barlow, 2004; Sayah *et al.*, 2004).

All the isolates of *E. coli* were susceptible to both Meropenem, Imipenem and Colistin. However, 25 of the isolates were resistant to Cefoxitinand 21 were resistant to Amoxicillin / Clavulanic, representing Cefoxitin and Amoxicillin / Clavulanic acid resistance of 16.23 % and 13.64 % (n=154), respectively. The susceptibility of all the isolates of *E. coli* to Meropenem, Imipenem and Colistin could be due to low abuse of these antibiotics. Colistin is regarded as a last-resort antibiotic and presently, it is used for the treatment of infections caused by carbapenemase-producing Gram-negative bacteria (Falagas & Kasiakou, 2005). In fact, the emergence of multidrug-resistant Gram-negative bacteria, especially those producing carbapenemases, has reintroduced colistin as a last-resort antibiotic for the treatment of severe infections (Lim et al., 2010).

The Multiple Drug Resistance (MDR) isolates were 11 (7.14 %). In addition, 122 isolates showed Multiple Antibiotic Resistance Index (MARI) of 0.0; that is, susceptible to all antibiotics that were tested. However, 12 isolates (7.79 %) showed MARI of 0.1, while 9 isolates (5.84 %) showed MARI of 0.2. When *E. coli* is isolated from sources of water, it shows health hazards, for example, diarrhoeal disease, which is rated high in morbidity and mortality in both adults and children (Park et al., 2018). MAR index greater than 0.2 indicates that the isolates originate from an environment where antibiotics are often used, which could make choices of therapy very difficult and limited. It is therefore; recommended that there should be restrictive policies on the use of antibiotics, building of toilets on water bodies, the disposal of human/animal sewages and bathing/washing in or close to water bodies.

5. Conclusion

Rapid population growth and urbanization in Rivers State have led to serious water pollution problems in the region. The high concentrations of Heavy metals in the water body could be as a result of non-point pollution. Increase in the population density of bacteria, including total heterotrophic bacteria, Coliform and *E. coli* counts observed might be due to the addition of materials from nearby waste dump sites and through land runoff carrying high bacterial population. Intensive monitoring, especially of faecal pollution arising from building of toilets on water bodies, the use of faeces as manure and industrial effluents is important.

The use of antibiotics including polymyxins (Colistin) in humans and food animals/poultry poses a serious public health risk. This is because the antibiotics could cause genetic changes in the microorganisms, which could result to mutation and probably cause antibiotic resistance in the microorganisms. Restraints in the use of antibiotics as prophylaxis, food supplements in animal feeds and the misuse of antibiotics by humans are advocated to reduce the emergence and spread of resistance in *E. coli*.

It is therefore recommended to coordinate different efforts at the level of the community dwellers and the government to rescue the residents of Rivers State and the water bodies in the State from the current hazard-posing environmental problems.

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