Determination of Bacterial Composition, Heavy Metal Pollution, and Physicochemical Parameters of Andoni River, Rivers State, Nigeria

ABSTRACT

Water bodies can be polluted through different means, including microorganisms. This study was carried out to analyze physicochemical parameters, heavy metals concentrations, and bacterial pathogens in Andoni River, Rivers State, using 72, 18, and 120 water samples, respectively. Physical and chemical characteristics were examined on a periodic and seasonal basis. Only seasonally was the concentration of heavy metals examined. The heterotrophic plate count method was applied seasonally and periodically. Escherichia coli (E. coli) and other coliforms were found using the most probable number technique and the Eijkman test. Antibiotic profiling was done on verified E. coli isolates. Only temperature did not fall within the suggested standard values of the physicochemical parameters. In April, the temperature was at its maximum in the evenings. Cadmium, arsenic, chromium, and nickel concentrations were within the suggested limits, however mercury and lead concentrations were over the limits. Mercury concentrations were higher in April than in other months, while lead concentrations were higher in November. The highest count of heterotrophic bacteria observed was on the evening of July. Coliforms and *E. coli* were found in high numbers in both periods and seasons. Of the 139 isolates of *E. coli* subjected to antibiotic profiling, 102, 28, and 3 showed multiple antibiotic resistance indexes of 0.0, 0.1, and 0.2, respectively. This investigation discovered microorganisms, including E. coli, which is a sign of faecal pollution. In reality, chemical contaminants in the water body may have also contributed to E. coli's resistance to antibiotics.

Keywords: Water bodies; Pollution; *Escherichia coli*; Multiple antibiotic resistance index; Multiple drug resistance

INTRODUCTION

"Although oil pollution occurs when oil from roads and parking lots is carried in runoffs into water bodies, intentional and accidental oil spills are also sources of oil pollution. Oil pollution and other chemicals are sources of heavy metals, which could be carcinogenic and mutagenic. The latter is capable of causing antibiotic resistance in bacteria found in the river" (Fontham and Trapido, 2010; Sreenivas, 2021). "Oil spill contains toxic chemicals and can have serious short- or long-term health effects on workers at the oil rig, first responders to the area of the oil spill, those that will help with clean-up, marine life, such as fish, plants, turtles, otters and birds, residents of the area (land or water) of the oil spill, sailors and fishermen, as well as those who eat food from the spill-affected areas" (Fontham and Trapido, 2010; Sreenivas, 2021).

"The river is one of the water bodies that are the most important resource for humans. If the river is polluted, the pollution affects the microbial quality, heavy metals concentration, and physicochemical characteristics" (Koshy and Nayar, 1999). Water from streams, lakes, rivers, and other water bodies is contaminated with domestic, industrial, and agricultural wastes. "The water is likely to lead to adverse health effects, including gastrointestinal illness, neurological disorders, and reproductive problems" (CDC, 2014). There are four main sources of aquatic pollution, they are municipal wastes, industrial wastes, intentional and unintentional oil spillage, and agricultural runoffs (Walsh, 1980).

Pollutants such as microorganisms can also find their way into water bodies, even if it is not their natural habitat. For example, *Escherichia coli* (*E. coli*), which is one of the normal flora of the gut of endotherms (Aleru and Wachukwu, 2020; CDC, 2021). Most strains of *E. coli* are not pathogenic, however, some strains are pathogenic (Kaper *et al.*, 2004; CDC, 2021). "The pathogenic strains of *E. coli* are grouped into six and are Enterotoxigenic *E. coli*

(ETEC), Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC) or Enterohaemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC)" (Ali *et al.*, 2014).

"The analyses of the levels of physicochemical parameters and heavy metals in water bodies have been carried out in Rivers State, Nigeria". (Ollor *et al.*, 2018; Aleru *et al.*, 2019a; Aleru *et al.*, 2019b). According to findings, heavy metals are found in water bodies, as well as in fish species. These fish species may have been exposed to heavy metals in their water environment and the heavy metals might be dangerous to human health if they are consumed (Abdullali *et al.*, 2003; Mazlin *et al.*, 2009; Baharom and Ishak, 2015). Building toilets and defecating on water bodies could be the reason bacteria, especially, *E. coli* are found in water bodies. This is a practice that is common in some rural areas in Rivers State, Nigeria (Aleru *et al.*, 2019a; Aleru and Wachukwu, 2020), Andoni River is not an exception. For this reason, the assessment of physicochemical qualities, heavy metals concentration, and bacterial pathogens in Andoni River, Rivers State, Nigeria, was carried out.

MATERIALS AND METHODS

Study area

This study was carried out at Andoni River. Andoni River is one of the rivers in Rivers State that is located in the Rivers South-East Senatorial District, Rivers State, Nigeria. Rivers State is the main oil and gas-producing region in Nigeria. The following are located in Rivers State: Fertilizer Company, Petrochemical Company, Petroleum Refining Companies, Oil and Gas Free Zone, and Harbours. "The State is exposed to activities of oil multinational companies; these have been linked to the degradation of the natural environment, low agricultural productivity, and pollution. Further, as a result of many numerous industrial activities and an increase in population in the area, industrial and domestic wastes generated, have increased. Andoni River is located in the coastal area of Rivers State and because of that, when it rains, all the wastes (industrial and domestic wastes) are washed into the water body" (Abutudu *et al*., 2007, Ibeanu *et al*., 2007).

Study design

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

Sample size

Two hundred and ten (210) water samples were collected for the analysis of physicochemical parameters (72), heavy metals concentrations (18), and bacterial detection (120). The sample size was determined according to Daniel's formula (Daniel, 1999).

Collection of water samples for physicochemical parameter

In the months of November, April, and July, water samples were collected in 72 separate sterile specimen containers (4 oz. (118.3 ml) for the measurement of physicochemical parameters. In addition, 72 water samples were collected for both seasonal and periodic analysis. On each day, three water samples were collected in the mornings and three in the evenings, so that in two days 12 water samples were collected. In the three seasons (November, April, and July) a total of 36 water samples were collected. Hence, 72 water samples were collected from November 2016 to July 2018 (November 2016, April 2017, July 2017, November 2017, April 2018, and July 2018). Moreover, all physicochemical parameters, but biological oxygen demand (BOD) were measured *in situ* (Horiba Water

Checker (Model U-10) (APHA, 1998). The acceptable limits of all the physiochemical parameters are found in Table 1.

Collection of water samples for the measurement of heavy metal concentrations

In the months of November (2016/2017), April (2017/2018), and July (2017/2018), water samples were collected in 18 separate sterile specimen containers (4 oz. (118.3 ml) for the measurement of heavy metal concentrations. The 18 water samples were collected for just seasonal variations. In each month, three (3) water samples were collected. Nine (9) water samples were collected in three months and in total, 18 water samples were collected, throughout the sampling period. Five (5) drops (0.1 ml) of concentrated nitric acid (HNO3) were added, in order to preserve the samples for the measurement of heavy metal concentrations.

Collection of water samples for the detection of bacteria

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

Sample analysis for the detection of microorganisms

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

Cultivation of microorganisms

The following media were used: MacConkey agar, MacConkey broth, Nutrient agar, and CHROMagar for *Escherichia coli* and other coliforms (CHROMagar ECC). They were prepared and used following manufacturers' instructions and directions. The media plates (Petri dishes) were labeled clearly and stored appropriately in the refrigerator at 4-6 ^{0}C after the preparation of the media.

Data were acquired from heterotrophic plate count (HPC), total coliform count (TCC), and total *E. coli* count (TE). The TCC and TEC were performed using the most probable number (MPN) technique. This was statistically determined by the use of the MacCrady table.

Analysis of water samples for physicochemical parameters

All the physicochemical parameters, such as the pH, temperature, salinity, total dissolved solids (TDS), and dissolved oxygen (DO), except BOD were analyzed *in situ* using Horiba Water Checker (Model U-10) and the Lovibond CM-21 Tintometer. Finally, a mercury thermometer was used for the analysis of biological oxygen demand.

Analysis of water samples for heavy metals

"Heavy metal concentrations were 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 the isolates of *E. coli* 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 Mueller-Hinton agar plates using the Kirby-Bauer-CLSI (Clinical and Laboratory Standards Institutes) modified disc agar diffusion (DAD) method as described by Isenberg (1998) (Table 8). McFarland standards were used as a reference to produce solutions that contained approximately similar numbers of bacteria for use in antibiotic susceptibility testing. Results were interpreted in accordance with the Clinical and Laboratory Standards Institutes (CLSI) guidelines (CLSI, 2008).

RESULTS

Comparison of periodic variations of physicochemical parameters in November

In November, the periodic values of pH, temperature, salinity, TDS, DO and BOD were 6.51±0.25 (morning) and 6.89±0.07 (evening); 29.10±0.36 (morning) and 29.97±0.50 (evening); 7.81±0.80 (morning) and 8.07±0.46 (evening); 8.82±0.38 (morning) and 6.77±0.59 (evening); 3.56±0.37 (morning) and 3.84±0.78 (evening), 4.100±0.01 (morning) and 5.46±1.29 (evening), respectively. The result showed that statistically, TDS and DO were significant when compared in the morning and evening ($p<0.05$) (Table 2).

Comparison of periodic variations of physicochemical parameters in April

At Andoni River in April, morning and evening values were compared (Table 3). There was a significant difference in the values of the temperature ($p=0.003$; $t=-6.472$) and TDS ($p=0.018$; $t=3.895$). The rest of the parameters showed no appreciable difference between the morning and evening times.

Periodic variations of physicochemical parameters in July

At Andoni River in July, morning and evening values were compared and the values obtained for pH, temperature, salinity, TDS, DO, and BOD are clearly shown in Table 4. There was a significant difference in the values of salinity ($p=0.023$; $t=3.584$). Between the morning and evening time periods, there was no discernible variation in the other acquired values.

Seasonal comparison of heavy metals among the seasons

Table 5 shows the values of the heavy metal from all the Seasons at Andoni River. The variance in the mean was compared statistically using ANOVA and according to the result, there was no significant ($p>0.05$) difference in all the values, except Mercury ($p<0.05$). To see which group the variation significant, Tukey Multiple Comparison Test was performed and the result shows that there was significant variation between the three seasons ($p=0.837$).

Periodic comparisons of heterotrophic plate count (HPC) (CFU/ml)

The heterotrophic bacterial count was highest in the month of July (both in the mornings and evenings). Although, the count was highest in the evening (Table 6).

Periodic variations of total coliform count (TCC) and total *E. coli* in the morning hours

Additionally, the total counts of E. coli and coliforms were performed. 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 5 (150 MPN/100 ml) in July, while the lowest was observed in the evening of day 3 and day 5 (7 MPN/100 ml) in November and April, respectively (Table 7 and Table 8).

Antibiotic resistance of *E. coli* and antibiotic resistance index of *E. coli* isolated from Andoni River

Tables 10 to 12 display the results of the tests for antibiotic susceptibility. Among the 10 antibiotics selected from 7 Classes (Carbapenems, Glycylcycline, Cephalosporin, Aminoglycoside, Beta-lactamase, Fluoroquinolone, and Polymyxins), resistance among Amoxicillin/ Clavulanic acid, Cefotaxime, and Cefoxitin were found to be most frequent than the other antibiotics. Out of the 139 isolates of *E. coli* subjected to antibiotic profiling, 102,

28, and 3 showed multiple antibiotic resistance indexes of 0.0, 0.1, and 0.2, respectively.

Table 1: Safe Limits of FEPA, EPA and WHO for Determining Surface Water (World Bank, 1990; WHO, 1993; EPA, 2001)

Physicochemical Parameters	**FEPA**	**EPA**	**WHO**
pH	6-9	5.5-8.5	6.5-8.5
Temperature (^{0}C)	<40 ^{o}C (within 15 ^{o}C)	25	25
Salinity (%)	-	35	-
Total Dissolved Solid (mg/l)	2000	<60 %	1000
Dissolved Oxygen (mg/l)	-	-	6-8
BOD (mg/l)	30	5	6
Heavy Metals	**FEPA**	**EPA**	**WHO**
Cadmium (Cd) mg/l	<1	0.005	0.003
Mercury (Hg)	0.05	0.002	0.006
Lead (Pb) mg/l	<1	0.015	0.01
Arsenic (As) mg/l	0.1	0.010	0.01
Chromium (Cr) mg/l	<1	0.1	0.05
Nickel (Ni) mg/l	<1	-	0.02
Bacteria (MPN/100 ml)	**FEPA**	**EPA**	**WHO**
Total Coliform Count	Must not be detected	Must not be detected	Must not be detected
Total *E. coli* Count	Must not be detected	Must not be detected	Must not be detected

Table 2: Comparison of periodic variations of physicochemical parameters in November

	pH	Temperature (^{0}C)	Salinity (%)	Total Dissolved Solid (mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.51±0.25	29.10±0.36	7.81±0.80	8.82±0.38	3.56±0.37	4.10±0.01
Evening	6.89±0.07	29.97±0.50	8.07±0.46	6.77±0.59	3.84±0.78	5.46±1.29
p-value	0.07	0.07	0.64	0.007	0.178	0.02
t-value	-2.446	-2.425	-0.499	5.100	-1.631	-3.745
Remarks	NS	NS	NS	S	NS	S

***this was done in replicate**

Table 3: 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	6.50 ± 0.36	29.67 ± 0.25	7.46 ± 0.44	8.76 ± 0.58	3.20 ± 0.75	4.29 ± 0.27
Evening	6.97 ± 0.04	30.77 ± 0.15	6.84 ± 0.06	7.31 ± 0.28	3.83 ± 0.22	4.99±0.59
p-value	0.115	0.003	0.075	0.018	0.458	0.481
t-value	-2.007	-6.472	2.397	3.895	0.821	0.776
Remarks	NS	S	NS	S	NS	NS

***this was done in replicate**

Table 4: Comparison of periodic variations of physicochemical parameters in July

	pH	Temperature (^0C)	Salinity (%)	Total Dissolved Solid (mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.76 ± 0.10	29.50 ± 0.20	7.44 ± 0.47	9.12 ± 0.53	4.18 ± 0.27	5.97 ± 0.61
Evening	6.91 ± 0.05	30.13 ± 0.55	6.31 ± 0.29	10.03 ± 0.48	4.69 ± 0.48	5.75 ± 1.13
p-value	0.077	0.135	0.023	0.090	0.179	0.355
t-value	-2.365	-1.872	3.584	-2.222	-1.629	-1.046
Remarks	NS	NS	S	NS	NS	NS

***this was done in replicate**

Table 5: Seasonal comparison of heavy metals among the seasons

	Cadmium (Cd) mg/l	Mercury (Hg)	Lead (Pb) mg/l	Arsenic (As) mg/l	Chromium (Cr) mg/l	Nickel (Ni) mg/l
November (A)	-0.058 ± 0.384	0.373 ± 0.025	0.097 ± 0.009	-0.168 ± 0.122	-0.030 ± 0.003	-0.216 ± 0.026
April (B)	-0.072 ± 0.008	0.272 ± 0.028	0.087 ± 0.011	-0.205 ± 0.414	-0.026 ± 0.006	-0.249 ± 0.044
July (C)	-0.066 ± 0.006	0.257 ± 0.039	0.077 ± 0.012	-0.203 ± 0.044	-0.025 ± 0.005	-0.241 ± 0.030
p-value	0.764	0.008	0.146	0.821	0.460	0.508
F-value	0.281	12.235	2.702	0.204	0.887	0.760
Remarks	NS	S	NS	NS	NS	NS
Post hoc (Tukey) A and B		0.017 (S)				
A and C		0.009 (S)				
B and C		0.837 (NS)				

***this was done in replicate**

Table 6: Periodic comparisons of heterotrophic plate count (HPC) (CFU/ml)

	November (10^{5})	April (10^{5})	July (10^{6})
Morning	5.08 ± 0.34	5.44 ± 0.31	6.14 ± 0.33 (10^{6})
Evening	5.49 ± 0.53	5.66 ± 0.33	6.39 ± 0.23 (10^{5})

***this was done in replicate**

Table 7: Periodic variations of total coliform count (TCC) and total *E. coli* in the morning hours

Days	November		April		July	
	TCC MPN/100 ml	**TEC MPN/100 ml**	**TCC MPN/100 ml**	**TEC MPN/100 ml**	**TCC MPN/100 ml**	**TEC MPN/100 ml**
1	11	3 (27.3)	21	3 (14.3)	21	3 (14.3)
2	14	3 (21.4)	11	3 (27.3)	20	3 (15.0)
3	21	3 (14.3)	14	4 (28.6)	14	4 (28.6)
4	11	4 (36.4)	11	3 (27.3)	39	3 (8.0)
5	15	3 (20.0)	20	7 (35.0)	150	3 (2.0)

*** this was done in replicate**

Table 8: Periodic variations of total coliform count (TCC) and total *E. coli* in the evening hours

Days	November		April		July	
	TCC MPN/100 ml	**TEC MPN/100 ml**	**TCC MPN/100 ml**	**TEC MPN/100 ml**	**TCC MPN/100 ml**	**TEC MPN/100 ml**
1	14	3 (21.4)	11	3 (27.3)	20	7 (35.0)
2	11	3 (27.3)	11	3 (27.3)	11	3 (27.3)
3	7	3 (42.9)	20	7 (35.0)	15	3 (20.0)
4	11	3 (27.3)	9	4 (44.4)	9	4 (44.4)
5	9	4 (44.4)	7	3 (42.9)	14	3 (21.4)

***this was done in replicate**

Table 9: 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	10µg	≤ 12 mm	Polymyxins

Table 10: Antibiotic resistance of *E. coli*

Antibiotics	Andoni River (139)
Meropenem	0 (0 %)
Imipenem	1 (0.72 %)
Tigecycline	0 (0 %)
Cefotaxime	20 (14.39 %)
Gentamicin	4 (2.88 %)
Cefoxitin	14 (10.07 %)
Amoxicillin/ Clavulanic acid	21 (15.11 %)
Amikacin	3 (2.16 %)
Ciprofloxacin	9 (6.47 %)
Colistin	0 (0 %)

Table 11: 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, AMC	1	9.09
CTX, FOX, AMC	3	27.27
CTX, AMC, AK, CIP	3	27.27
CTX, CN, FOX, AMC	2	18.18
CTX, CN, FOX, AMC, AK, CIP	1	9.09
IPEM, CTX, CN, FOX, AMC, AK, CIP	1	9.09

Table 12: Antibiotic resistance index of *E. coli* isolated from Andoni River

MAR Index	Resistant Isolates (n=139)	% MAR Index
0.0	102	73.38
0.1	28	20.14
0.2	3	2.16
0.3	2	1.43
0.4	2	1.43
0.5	0	0
0.6	1	0.72
0.7	1	0.72

DISCUSSION

Temperature was the only physicochemical parameter that did not meet the recommended standard values. The temperature was greatest in the evenings and in April (Tables 2&3). This result may have been influenced by the intensity of sunlight in Nigeria during this season, as these results are not in conformity with the report by other researchers in Nigeria. In 2013, findings by Dimowo showed that dissolved oxygen (DO) and pH were above the maximum permissible limit for drinking water, however, his findings showed that temperature was within the permissible limit (Dimowo, 2013). Also in 2020, another research carried out reported that DO values were not within the recommended standard values, although, the researchers reported that temperature values were within the standard limit (Dauda and Olaofe, 2020).

The amounts of cadmium, arsenic, chromium, and nickel were found to be within the suggested levels in the current investigation, but those of mercury and lead were found to be above those limits (Table 4&5). Mercury concentrations were higher in April than in other months, while lead concentrations were higher in November. The results may have been affected by less rain in November and April. Although some reports conform to the results from this present work, other findings did not (Wangboje and Ikhuabe, 2015; Abui *et al.*, 2017; Ollor *et al.*, 2018; Aleru *et al.*, 2019a; Aleru *et al.*, 2019b; Aleru and Wachukwu, 2020; Edori and Iyama, 2020).

The highest count of heterotrophic bacteria observed was in the evening of July. Coliforms and *E. coli* were found in high numbers in both periods and seasons (Tables 6,7&8). This is an indication of faecal pollution, which makes the River unsuitable for drinking, domestic and agricultural uses. The results may have been affected by building toilets on the water body, bathing in the water body and other activities carried out there. The residents of Andoni should be encouraged to boil their water before use. Additionally, the building of toilets on water bodies should be discouraged. The practice of adequate hygiene is highly recommended. Apart from this study, other studies carried out in Nigeria have also reported the presence of pathogenic bacteria in water bodies (Raji and Oyeniyi, 2017; Ollor *et al*., 2018; Aleru *et al*., 2019a; Aleru *et al*., 2019b; Aleru and Wachukwu, 2020).

Additionally, according to this study, out of the 10 antibiotics used, resistance among Amoxicillin/ Clavulanic acid, Cefotaxime, and Cefoxitin was most frequent than the other antibiotics (Tables 9,10&11). Similar results have been reported in previous studies (Ollor *et al*., 2018; Aleru *et al*., 2019a; Aleru *et al*., 2019b). In this study, 139 isolates of *E. coli* were subjected to antibiotic profiling. Out of the 139 *E. coli* isolates, 102, 28, and 3 showed multiple antibiotic resistance indexes of 0.0, 0.1, and 0.2, respectively (Table 12).

CONCLUSION

Findings from this present study indicate that the Andoni River is highly polluted, especially with pathogenic bacteria. The results of the antibiotic profiling also show that the River is polluted with chemical pollutants, which may cause mutation in the bacteria. To solve these problems, residents of this area need to be educated on the health impacts of building toilets on water bodies. There is also a need to encourage the residents that water from the River should be treated before domestic use.

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