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BACTERIOLOGICAL AND PHYSICOCHEMICAL EVALUATION OF RIVER ELA, EDO STATE NIGERIA: WATER QUALITY AND PERCEIVED COMMUNITY HEALTH CONCERNS

OLATUNJI, E.O[1] AND ANANI, O.A.*[2]

[1]Department of Biological Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria

[2]Laboratory of Ecotoxicology and Forensic Biology, Department of Biological Science, Faculty of Science, Edo University, Iyamho, Edo State, Nigeria.

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Email: cybert2004@gmail.com

ABSTRACT

This study was conducted with the aim of assessing the bacteriological and physicochemical properties of River Ela. The bacteriological analyses were done using standard microbiological methods. The physicochemical parameters were analyzed using standard procedures. The results of this study revealed that the pH was acidic (5.51-5.84). All physicochemical parameters investigated were far above the WHO (World Health Organization) standard thresholds exempting sulphate and nitrate. The results of the Total Heterotrophic Bacteria Count was 3.7 x 10^5 cfu/ml for the upstream, 11.2 x 10^5 cfu/ml for the midstream and 6.1 x 10^5 cfu/ml for the downstream. The results of the Total Coliform Count was 2.3 x 10^3 cfu/ml for the upstream, 4.3 x 10^5 cfu/ml and 2.8 x 10^3 cfu/ml for the downstream. These counts obtained, exceeded the WHO safe limits. The bacterial isolated were *Salmonella* sp. (13.33%), *Citrobacter* sp. (6.67%), *Staphylococcus aureus* (13.33%), *Escherichia coli* (6.67%), *Pseudomonas* sp. (20.00%), *Bacillus* sp. (20.00%) and *Klebsiella* sp. (20.00%) for the first batch. While for the second batch isolates were *Salmonella* sp. (11.11%), *Pseudomonas* sp. (16.67%), *Bacillus* sp. (16.67%), *Citrobacter* sp. (5.56%), *Klebsiella* sp. (16.67%), *Staphylococcus aureus* (16.67%) and *Escherichia coli* (16.67%). The results of the antibiotics resistance testing carried out, revealed that *Bacillus* and *Salmonella* species were resistance to Chloramphenicol while *Staphylococcus aureus* was resistant to Streptomycin and *Pseudomonas* sp. was resistant to Sparfloxacin. Findings from this study revealed that River Ela was highly contaminated physically, chemically and bacteriologically. It is therefore advisable to boil the water before consumption.

Keywords: Sparfloxacin, Water, Health Risk, Bacteria, Era Esan, Ela River, Thresholds.

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INTRODUCTION

The environment of freshwater have been confirmed to be altered in varied forms; via some amplified processes such as subversive impurity leakages, bio-turbation, surface water and sediment flood and humans activities (Anani and Olomukoro, 2018).Humans use river water for different purposes; for drinking, irrigation, recreational opportunities, and habitat for economically important fisheries (Leroy et al., 2002). Basically, the intrinsic nature of river has been perceived to be sole receptor of any source generated wastes. The role of a river is not mainly to carry industrial wastes but its ability to self-purify. However, this is usually incredibly exploited! Diverse areas of the world have stated health issues related with the prolong use of contaminated river water, which range from diarrhea dysentery, premature birth abortion, viral hepatitis and gastric and duodenal ulcers amongst others. Majority of the people that live in riverine areas depend on water from the river for drinking and domestic purposes (Shuaib, 2004). Wu et al., (1999) reported that about 700 million people which are partly the population in China use water contaminated with different levels of animal and human excreta with total coliform bacteria beyond maximum permissible range by 28% in urban areas and 86% in rural areas.

There has been significant damages of rivers with pollutants, rendering the water unsuitable for useful purposes. More than 12% of urban dwellers in Africa depend on contaminated river waters for their household needs (Ologbosere et al., 2016). Shaltout and Khalil (2005) reported that more than 70% of some African citizens (Sudanese) get their source of water from surface waters, which are usually polluted by industrial and agricultural chemicals. More than 40% of Nigerians rely on polluted wells or surface waters for their household uses (Ologbosere et al., 2016). The regular use of heavily contaminated water for a long duration usually results in health issues which does not conform to the millennium development goals of World Bank and perceived to be a non-sustainable means of livelihood.

Anthropogenic threats to water bodies were often connected with human health, especially disease causing organisms and oxygen-demanding wastes (Savita, 2016). Rajaram and Ashutosh (2008) stated that industrial wastes were one of the main causes of irreversible degradation going on in surface water system. Organic pollution caused by oxygen demanding wastes is common amongst surface water (Yingrong et al., 2017). The natural process of biological disintegration and chemical oxidation that takes place within water courses utilizes dissolved oxygen. Decomposition of materials is a usual process in all aquatic ecosystems and is a role of decomposers such as aerobic bacteria and fungi (Filkersilasie, 2011).

Nonetheless, serious consequences to aquatic biota may result if the common natural mechanisms that clean or self-purify the water are overloaded by large entry of pollutants. Severe oxygen depletion can give rise to the loss of many desirable aquatic biota and also create a stinking anaerobic system.

The increasing problem of river pollution has made it necessary to monitor the quality of water Enerijiofi et al., (2003) as well as its health status. Regions with dense human populations are the verge of risk! Consequent of this, the main objective of this study is to evaluate the bacteriological, physicochemical properties and perceived health concerns of River Ela in Edo State, Nigeria.

Material and Methods

Study Area

The study area is River Ela with sampling geographical coordinates of latitude 6° 30' 0N and longitude 6° 22' 0E (Figure 1), flows into Ewatto River. River Ela is one of the major sources of economic hub of the community because of the ecosystem services it renders.

Sample Collection

Water samples were gotten from River Ela at three different points at about 9-10 am in a sterile bottle (2.5 L) with the bottle cap sterilized using ethanol and cotton wool and properly corked. The bottle was not filled to the top to allow air bubbles escape. The first point was station A which is the upstream, the second point is station B which is the midstream and the third point is station C which is the downstream. The water samples were gotten from these three stations on two consecutive occasions and taken to the laboratory for physicochemical and bacteriological analyses.

Determination of the physicochemical parameters

After sample collection, the physicochemical analyses were conducted on the water sample. These physicochemical analysis included; pH, electric conductivity, chloride, nitrate, sodium, potassium, total dissolved solid, biochemical oxygen demand, sulphate, dissolved oxygen, chemical oxygen demand, calcium, magnesium, phosphate and turbidity were determined using standard methods adopted from Enerijiofi et al., (2018).

Microbial Analysis

Isolation of Total Heterotrophic Bacteria and Coliform Count

A fivefold serial dilution was done using the water sample. 9 ml of distilled water was measured into five Mccartney bottles and 1ml of the water sample was homogenized in the first bottle labeled 10^{-1} the sample was transferred into the second bottle labeled 10^{-2} this method was repeated till the 10^{-5}. Pour plate were done using the two different media for the total heterotrophic bacteria and Coliform counts and the 10^{5} dilution used (Cheesbrough, 2016). It was incubated at 37 °C for 24 hours. The stock was also plated and incubated at the same temperature for 24 hours. Results were taken after 24 hours and the distinct

colonies isolated and stored at 4 °C for further uses (Avishai and Davidson, 2014).

Morphological Characterization of Bacteria

The bacterial isolate were characterized morphologically based on their shape, color (cream, white), texture (dry, moist, mucoid), size, elevation (raised, flat, convex), margin (entire, lobate, undulate) and opacity (transparent, opaque, translucent). Gram Staining was carried out (Sandle, 2004).

Biochemical Tests

This included; Catalase, Indole, Coagulase, Motility, Citrate utilization, Spore staining, Methyl red and Voges-Proskeur tests and Sugar fermentation tests (Clarke and Cowan, 1952)

Identification of Isolate

Characterization and identification of Isolates were carried out using Bergey's manual of Determinative Bacteriology as reference (Cheesbrough, 2006).

Antibiotic Susceptibility Test method
Agar well diffusion method

Muller Hinton agar medium was prepared according to the manufacturer's instruction; the dissolved medium was autoclaved at 121 °C for 15 minutes. The autoclaved medium was mixed well, allowed to cool and poured into a petri dish. The petri dishes containing Muller Hinton medium was coated with the bacterial strain. Wells were bored using a sterile borer and the antibiotics put in different concentrations. The plates were incubated at 37 °C for 24 hours. The antibacterial activity was determined by measuring the inhibition zone which was formed around the well (Cheeseborough et al., 2006).

Statistical Analysis

The study employed descriptive statistics which deals with presentation of numeric fact of data in either table or a graph form with the methodology of analyzing the data using Chi's square.

Results

The results of the physicochemical characteristics of Ela River

Table 1 revealed the physicochemical parameters from River Ela for the Upstream, Midstream and Downstream. The minimum and maximum values obtained were; pH (5.51-5.84), Electrical conductivity (75-156 µS/cm), Chloride (9.75-20.28 mg/l), Total Suspended Solid (0.07-0.14 mg/l), Total Dissolved Solid (38.25-79.56 mg/l), Turbidity (0.39-0.81 mg/l), Chemical Oxygen Demand (15.75-72-63.96 mg/l), Dissolved Oxygen (2.70-5.62 mg/l), Biological Oxygen Demand (0.08-2.30 mg/l), Sulphate (3.83-7.96 mg/l), Nitrate (2.25-4.68 mg/l), Phosphate (0.75-1.56 mg/l), Calcium (3.38-7.02 mg/l), Magnesium (0.83-1.72 mg/l), Sodium (9.00-18.72 mg/l) and Potassium (6.68-9.37 mg/l).

The results of the Bacteriological characteristics of Ela River

Table 2 showed the total heterotrophic bacteria count for the Upstream, Midstream and Downstream with the downstream value (11.2 x 10^5 cfu/ml) greater than the midstream (6.1 x 10^5

cfu/ml) and upstream (3.7 x 10^5 cfu/ml). There was no significant difference in Bacteria and Coliform Count from water samples from River Ela ($P > 0.05$).

Table 3 showed the distribution of isolates in the first and second batches of sample collected. *Pseudomonas*, *Bacillus* and *Klebsiella* species were conspicuously present in the three sampled stations in the two batches of samples collected. Also, *Staphylococcus aureus* and *Escherichia coli* were present in all the stations. Also, *Citrobacter* sp. was present in water samples in both batches at the downstream.

The Percentage occurrence of the isolates in the first and second batches from river Ela were calculated (Figures 2 and 3). For the first batch isolate *Pseudomonas*, *Bacillus* and *Klebsiella* species had the highest percentage frequency of occurrence of 20.00% while *Escherichia coli* and *Citrobacter* sp. had the least of 5.56%. For the second batch isolate *Pseudomonas* sp. *Bacillus* sp. *Klebsiella* sp., *Staphylococcus aureus* and *Escherichia coli* had the highest percentage of 16.67% while *Citrobacter* sp. has the least percentage of 5.56%.

For the first batch isolate *Pseudomonas*, *Bacillus* and *Klebsiella* species had the highest percentage of 20.00%. For the second batch isolate *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Staphylococcus aureus* and *Escherichia coli* had the highest percentage of 16.67%. *Salmonella* sp. been susceptible to gentamycin and ciprofloxacin but resistant to chloramphenicol, *Pseudomonas* sp. susceptible to pefloxacin but resistant to sparfloxacin, *Bacillus* sp. susceptible to augmentin, tarivid, Streptomycin, ciprofloxacin but resistant to chloramphenicol, *Citrobacter* sp. susceptible to pefloxacin, tarivid, streptomycin, amoxicillin but susceptible to augmentin, tarivid, septrin, ciprofloxacin, *Staphylococcus aureus* susceptible to augmentin, gentamycin, pefloxacin but resistant to streptomycin, *Escherichia coli* susceptible to streptomycin, chloramphenicol, ciprofloxacin but resistant to augmentin and amoxicillin.

Table 4 showed the antibiogram pattern of the isolate with *Salmonella* sp. been susceptible to gentamycin and ciprofloxacin but resistant to chloramphenicol, *Pseudomonas* sp. susceptible to pefloxacin but resistant to sparfloxacin, *Bacillus* sp. susceptible to augmentin, tarivid, Streptomycin, ciprofloxacin but resistant to chloramphenicol, *Citrobacter* sp. susceptible to pefloxacin, tarivid, streptomycin, amoxicillin but susceptible to augmentin, tarivid, septrin, ciprofloxacin, *Staphylococcus aureus* susceptible to augmentin, gentamycin, pefloxacin but resistant to streptomycin, *Escherichia coli* susceptible to streptomycin, chloramphenicol, ciprofloxacin but resistant to augmentin and amoxicillin.

Discussions

Quantification of the physicochemical characteristics of Ela River

The physiochemical properties carried out on the water sample from river Ela, in Ewatto, Edo State, Nigeria revealed that the pH range (5.51-5.84) was below WHO permissible limits of 6.5-8.5 (W.H.O 2003 and 2006). This indicates that the river is therefore considered to be slightly acidic.

This high level of acidity could be because of the acidic metabolite e.g. acetic and lactic acid present in it. The electrical conductivity was highest at the downstream because of the presence of inorganic dissolved solids like chloride and nitrate which agrees with the report of Alexandr et al., (2014). The chlorine level in water indicates pollution due to organic waste of animals. The chlorine content in the water is within the WHO limits. The Total Suspended Solid (TDS), Total Dissolved Solid values for the three stations were within WHO permissible limits. Turbidity was highest at the midstream as a result of surface run offs which agrees with the report of Okorafor et al., (2012). The high level of turbidity at the midstream is because of the different activities carried out at that point such as bathing, washing and swimming in the river which made the level of contamination high. The Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) had the highest values at the downstream as a result of organic and inorganic pollutants present in the river. The COD value at the downstream exceeded the acceptable concentration for unpolluted surface water quality which falls within 20 mg/l (Olatunji et al., 2011). The BOD_5 had a high value downstream because of the disposal of domestic waste in the river. The sulphate in water is significant to consider it's suitability to the public both for domestic and industrial use. It occurs naturally in water due to leaching from common minerals and domestic sewage increases (John, 2016). The sulphate values decreased significantly from the downstream to the upstream which were lower than the acceptable limit which is in accordance with report of Olutiola et al., (2000). The presence of magnesium and calcium in the river is due to geological formation of the water sample which could result in hardness of the river water. Hardness of water prevents the water from forming lather when used to wash clothes and can also increase the boiling point. Calcium and Magnesium showed values lower than the accepted limit.

The Findings from this study revealed that there was no significant difference between the physicochemical parameters in the water samples obtained from River Ela ($P > 0.05$). These findings are in conformity with what was obtained by Olatunji et al., (2011) and Omonigho, (2018).

Quantification of the Bacteriological characteristics of Ela River

In this study, the total heterotrophic bacteria count revealed that the midstream had the highest heterotrophic bacterial and coliform counts. It indicated a high level of human actions like contamination through surface run offs during rains; indiscriminate urine and faces disposal, washing of bikes, bathing and washing of clothes were carried out at this point. The bacterial load in the river was higher than the WHO (2003) standard limits. *Salmonella* sp., *Bacillus* sp., *Klebisella* sp., *Escherichia coli*, *Citrobacter* sp., *Bacillus* sp., *Pseudomonas* sp. and *Staphylococccus aureus* isolates were characterized from river Ela. There was dominance of gram negative bacterial isolate over the gram positive in agreement with Olatunji et al., (2011) and Enerijiofi et al., (2018).

The distribution of the isolates in this study showed that the downstream played host

to all bacteria isolates in both water sampled showing that downstream was polluted. This was also as stipulated by Wu et al., (1999). In both batches, *Pseudomonas*, *Bacillus* and *Klebsiella* species had the highest frequency of occurrence with 20.00% and 16.67% in batches 1 and 2 respectively which is in conformation with the findings of Okorafor et al., (2012). Some of the bacterial isolate found in river Ela were similar to those by Enerijiofi et al., (2018) and Omonigho et al., (2018).

The antibiogram of the isolate revealed varied pattern of susceptibility and resistance as regard to the antibiotic used. The findings of this study showed that augmentin antibiotic may have a strong potential in reducing the impacts of strains of microorganisms compared to other antibiotic used in this study. Moreover, gentamicin and amoxicillin were able to curtail the microbial isolates, this could be because the above listed antibiotics are not common across the counter, expensive and mostly not sold except with prescription from a physician.

Table 1: Physicochemical results of the water samples

	Units	Station A (Upstream)	Station B (Midstream)	Station C (Downstream)	WHO (2003 and 2006) (Limit)
pH	-	5.84	5.55	5.51	6.5-8.5
Electrical Conductivity	µS/cm	75	132	156	500
Chloride	mg/l	9.75	17.16	20.28	500
Total Suspended Solid	mg/l	0.07	0.12	0.14	1000
Total Dissolved Solid	mg/l	38.25	67.32	79.56	500
Turbidity	NTU	0.39	0.81	0.69	5
Chemical oxygen demand	mg/l	15.75	27.72	63.96	NI
Dissolved oxygen	mg/l	2.70	4.22	5.62	14
Biochemical oxygen demand	mg/l	0.16	0.08	2.30	NI
Sulphate	mg/l	3.83	6.73	7.96	150
Nitrate	mg/l	2.25	3.96	4.68	50
Phosphate	mg/l	0.75	1.32	1.56	200
Calcium	mg/l	3.38	5.94	7.02	50
Magnesium	mg/l	0.83	1.45	1.72	30
Sodium	mg/l	9.00	15.84	18.72	NI
Potassium	mg/l	6.68	9.37	7.96	NI

LEGEND: NI (Not indicated), Station A (Upstream), Station B (Midstream) and Station C (Downstream).

Table 2: Total Heterotrophic Bacteria Count and Total Coliform Count

	Sample ID	THBC ($x10^5$cfu/ml)	TCC ($x10^5$cfu/ml)
Station A	Upstream	3.7	2.3
Station B	Midstream	11.2	4.3
Station C	Downstream	6.1	2.8

World Health Organization Standard for THBC is 100cfu/ml while for TCC is 10cfu/ml in drinking water. Statistics showed bacteria have no significant effect on river Ela in THBC and TCC.

Table 3: Distribution of the isolates in first and second batches

		Salmonella sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Citrobacter* sp.	*Klebsiella* sp.	*Staphylococcus aureus*	*Escherichia coli*
				First Batch				
Station A	Upstream	-	+	+	-	+	-	-
Station B	Midstream	+	+	+	-	+	+	-
Station C	Downstream	+	+	+	+	+	+	+
				Second Batch				
Station A	Upstream	-	+	+	-	+	+	+
Station B	Midstream	+	+	+	-	+	+	+
Station C	Downstream	+	+	+	+	+	+	+

(+) means - isolate present in the sample and (-) means -Isolates absent in the sample

Table 4: Antibiogram pattern of the isolates measure in (mm)

	Augmentin	Gentamycin	pefloxacin	Tarivid	Streptomycin	Septrin	Chloramphenicol	Sparfloxacin	Ciprofloxacin	Amoxacillin
Salmonella sp	I	S	I	I	I	I	R	I	S	I
Pseudomonas sp	I	I	S	I	I	I	I	R	I	I
Bacillus sp	S	I	I	S	S	I	R	I	S	I
Citrobacter sp	I	I	S	S	S	I	I	I	I	S
Klebsiella sp	S	I	I	S	I	S	I	I	S	I
Staphylococcus aureus	S	S	S	I	R	I	I	I	I	I
Escherichia coli	S	I	I	I	S	I	S	I	S	R

LEGEND: Augmentin (AU), Gentamycin (CN), Pefloxacin (PEF), Tarivid (OFX), Streptomycin, Septrin (SCT), Chloramphenicol (CH), Sparfloxacin (CPX). Less than 14 (R – Resistant), Between 14 and 17 (I – Intermediate), Greater than 17 (S – Susceptible).

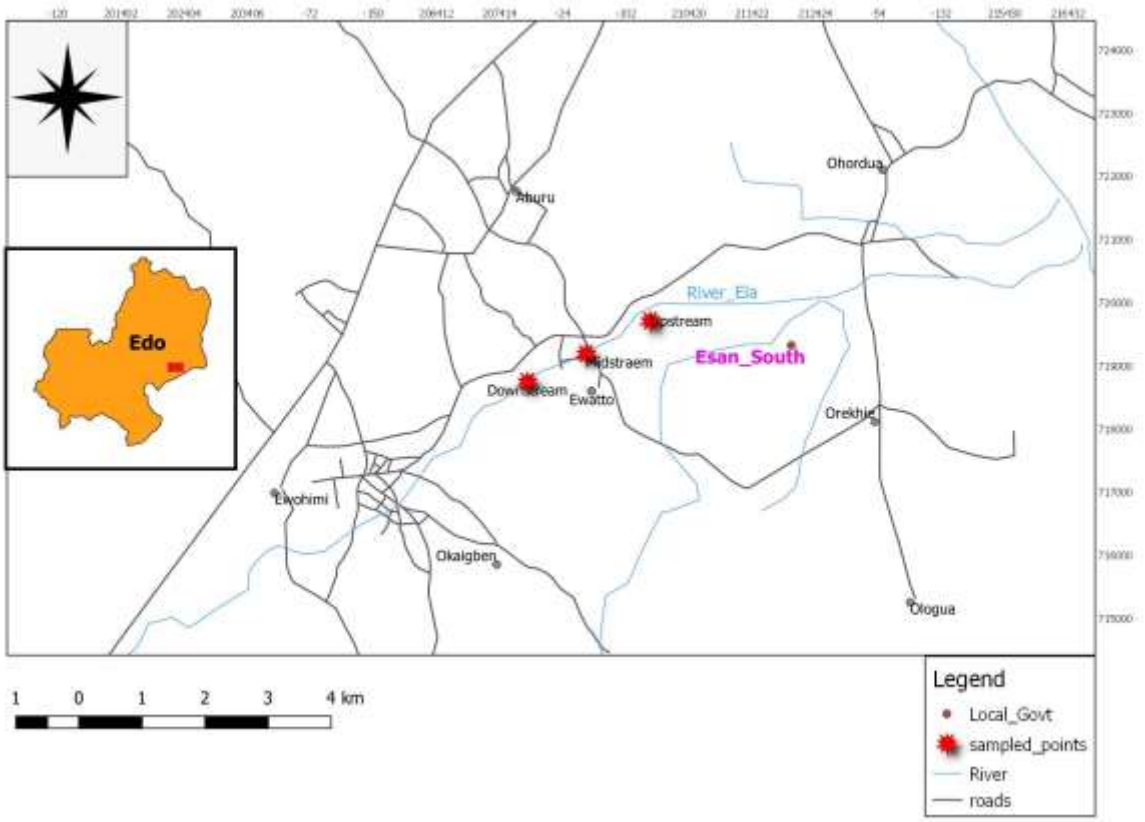

Figure 1: Map of Edo state showing sampling points

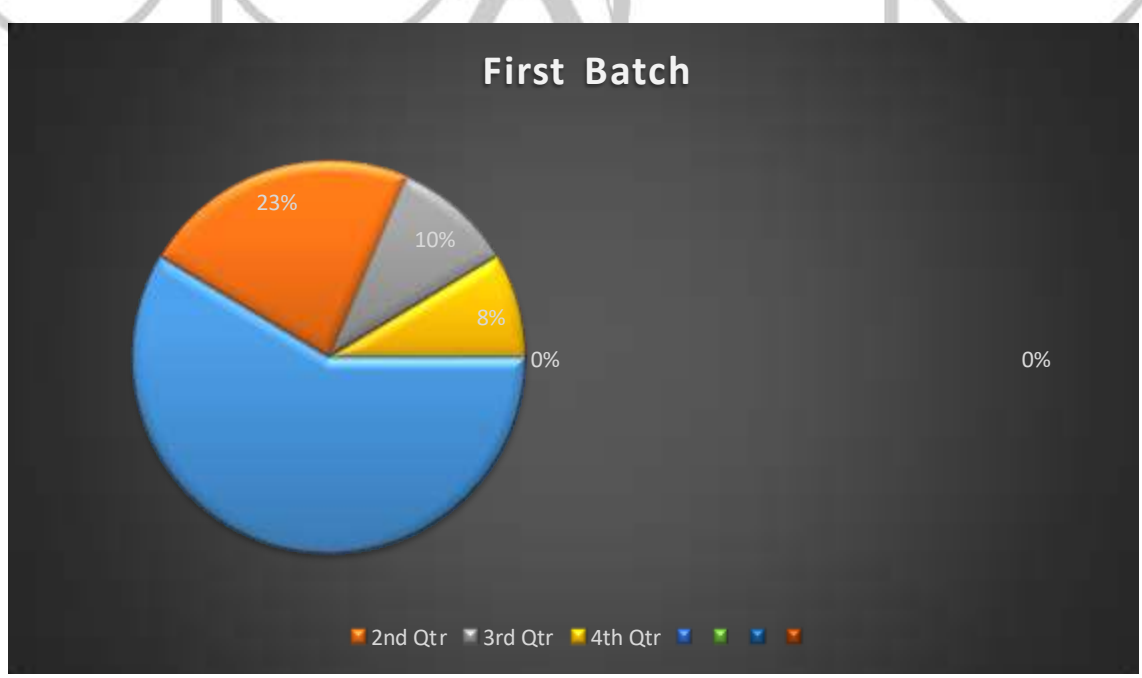

Figure 2: Percentage occurrence of Bacteria isolate in the First Batch

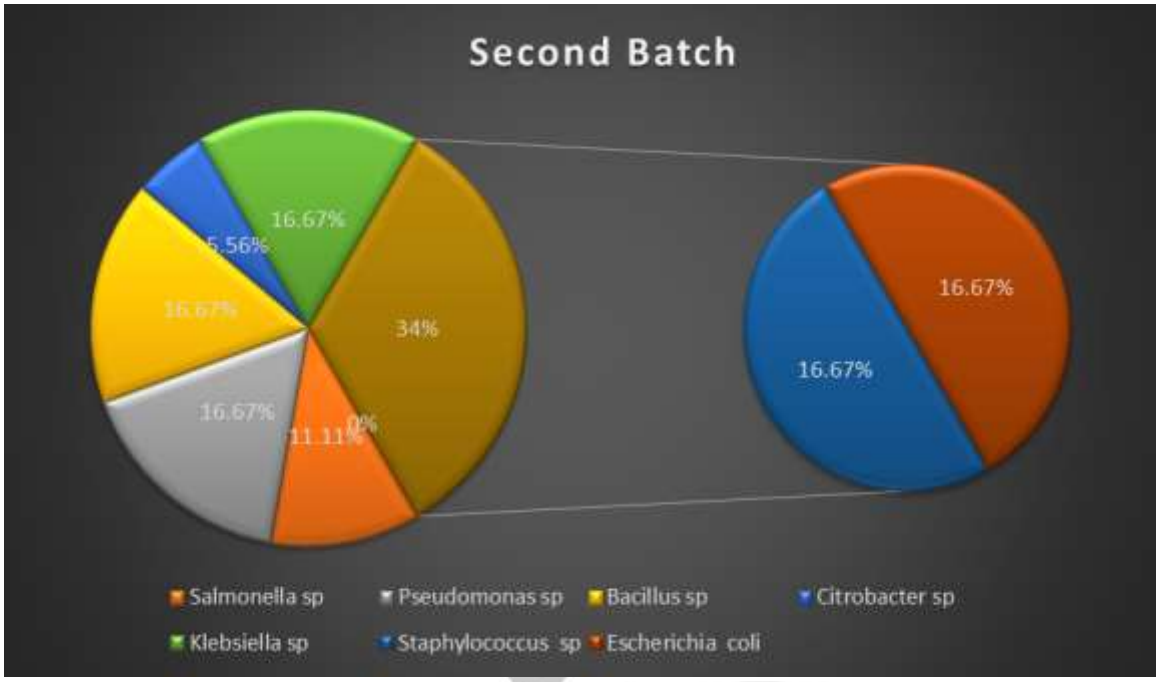

Figure 3: Percentage Occurrence of isolate in the second batch

Conclusion

It can be concluded that the bacteriological, physicochemical properties had significant impact on River Ela. It was obvious that the quality of water in River Ela had been compromised. This is because the water did not meet most of the standard limits of WHO. The Total Heterotrophic Bacteria Count and Coliform Counts exceeded the WHO standard limits for safe drinking water. It is recommended that the water be boiled before use to forestall waterborne diseases.

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