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Bacteriological Examination of Ajumoda Stream and Reservoir in Iseyin Local Government of Oyo State, Nigeria

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Abstract: *The bacteriological quality of Ajumoda stream and reservoir in Iseyin Local Government, Oyo State, Nigeria was carried out to ascertain the quality of the water. Water samples were collected randomly from three different points of the stream for four weeks. Water samples were collected once in a week in the morning between 8:00am-10:00am for 4weeks. The bacteriological assessment was examined using the basic microbiological techniques. The total heterotrophic bacteria count, total coliform count and faecal coliform count were used to determine the bacterial contamination. Biochemical tests and gram reaction were used to identify the bacterial isolates. The mean total heterotrophic count, total coliform count and faecal coliform count across stations were* $66x10^4$ *(point A),* $225x10^6$ *(point B),* $34x10^2$ *(point C),* $48x10^4$ *(point A),* $138x10^6$ *(point B),* $26x10^2$ *(point C), and* $69x10^4$ *(point A),* $254x10^6$ *(point B),* $6x10^2$ *(point C) respectively. The bacteria isolated and characterized included Escherichia coli, Aeromonas hydrophilia, Bacillus megaterium and Flavobacterium aquatile. The coliform counts from all the studied points were higher and above WHO/EPA maximum limits for drinking water. The bacteriological analysis of the water indicated the presence of faecal contamination which suggests that the water is not fit for drinking without appropriate management.*

Keywords: *Bacteriological Analysis, THBC, TCC, FCC, Aeromonas hydrophillia, Flavobacterium aquatile*

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I. Introduction

Water constitute one of the most essential element supporting the life of innumerable animals, plants and human beings. The total quantity of fresh water on earth could satisfy all needs of the human population if it were evenly distributed and accessible. Now, water quality has become a global problem due to gradual deterioration of water quality [1], and as a result of increase in human population and urbanization [2]. Safety and quality of drinking water are always an important public health concern [3]. The raw water quality can be affected by human or animal activity either within that body of water or within its water shed. According to UNICEF report [4], about 800 million people in Asia and Africa are living without access to safe drinking water. Consequently, this has caused many people to suffer from various diseases [5]. However, inadequate quantity, poor quality of drinking water and poor sanitation are the main reasons in incidence and prevalence of diseases in the world [6].

Contamination of water has been frequently found associated with transmission of diseases causing bacteria, vibrio, salmonella, bacteria and parasitic dysentery and acute infection diarrhea caused by *E. coli* [7]. Polluted water is also an important vehicle for the spread of diseases such as Cholera, dysentery, typhoid fever, hepatitis which are some of the common water borne diseases that spread through contaminated water. Bacteriological assessment of water is a method of analyzing water to estimate the numbers of bacteria present and if needed to find out what sort of bacteria they are. It represents one aspect of water quality. A microbiological analytical procedures that uses samples of water to determines the concentration of bacteria, on which possible to inferences are drawn about the suitability of the water used for the examination of microbial load. This process is used routinely to confirm that water is safe for human consumption [8].

Comprehensive evaluations of microbial quality of water require survey of all the pathogens that have potential for human infections [9]. The essential parameters to be examined for drinking water quality according to World Health Organization includes faecal and total coliform count, *E. coli* examination, chlorine residual, turbidity, pH, dissolved oxygen content and temperature [10]. These guidelines are essential determinants to reduce or eliminate the risk of water pollution. While the drinking water resources, contaminated with agricultural, industrial and sewage waste are dangerous and less usable for human consumption and for agricultural purposes. Hence, it is essential to check the quality of water from various sources in view of this present study is design to examine the bacteria content of Ajumoda river in iseyin local government area to ascertain the level of contamination, hence the risk associated with their consumption. Therefore, this research

work was carried out to examine the bacteriological assessment of Ajumoda stream and reservoir in Iseyin, Oyo State.

II. Materials and Methods

Description of Sampling Site

The study area was situated in Ajumoda Yaara Koso area in Iseyin Local Government of Oyo State Nigeria. Its Geographical coordinates are 7°58°N of the equator and 3°36°E of the Greenwich meridian [11]. The sampling stations selected were three based on the activities occurring in the water body.

Point A: This station is located in the concrete reservoir, not treated, there were shaded trees on it, fetching by people for drinking and other domestic activities because it is less polluted.

Point B: This station is a stream, located outside the reservoir receiving domestic waste, washing with detergents and other soaps, runoff from nearby houses. The area was surrounded by carpet grass (*Axonopus fissifolius*)

Point C: This station is located in the reservoir, shaded with trees, always treated with chlorine and distributed in water pipe for people of Iseyin and its environ.

Sample Collection

Water samples were collected once in a week in the morning between 8:00 am-10:00 am for four (4) weeks. The samples were collected from 3 different points of the river. Point A (protected surface water), Point B (received storm water runoff) and Point C (partially treated water).

Sampling Strategy

Sterile bottles were used to collect water samples for bacteriological analysis. Sample containers were tightened carefully to ensure homogenized samples for laboratory analysis. The samples were placed immediately in a cold ice chest at temperature of 4^oC to prevent possible alteration of parameters and also ensure that microorganisms remain viable though dormant. Samples were transported immediately to laboratory for analysis to prevent death of the microbes.

Preparation of Agar

Nutrient agar, MacConkey agar and Eosin Methylene Blue (EMB) were prepared according to manufacturers specifications which stated as follows: About 28g of nutrient agar powder, 50g of MacConkey agar powder and 36g of Eosin Methylene Blue were weighed and dissolved separately in one litre or 1000ml of distilled water and sterilized by autoclaving at 121^oC for 15 minutes respectively. After inoculation with a specific time, it was poured into a plate where the samples were introduced for culturing and isolation of microorganisms.

Inoculation on *Media in the Plate*

The pour plate method was used. Ten–fold serial dilution of the water samples was prepared aseptically in physiological saline of 10^{-1} to 10^{-6} and 0.1ml of the dilution was placed on the nutrient agar plates. All incubations were conducted at 37°C for 24hours.

Total Heterotrophic Bacteria Count

After the inoculation of micro-organisms, plate containing 30-300 colonies were selected and counted. The number of colony forming unit per ml (cfu/ml) was calculated by multiplying the number of colonies by the dilution factor. Also sub-culturing were carried out so as to obtain a pure culture and was viewed under the microscope for identification. Bacteria isolates were also characterized on the basis of their colonial structure, morphological characteristics and gram staining reaction using the procedure of [12].

Determination of Total and Faecal Coliform

The membrane filtration method was used to determine the total and faecal coliform count according to International Organization for Standard. Serial dilutions of 10^{-1} to 10^{-6} were prepared. MacConkey agar was used for total coliform while Eosin Methylene Blue (EMB) was used for faecal coliform count. The membrane was removed from a sterile package and was placed into the funnel assemblage. The pouring lip of the sample container was flamed and the sample was poured into the funnel. The vacuum was turn on so as to allow the sample to draw completely through the filter. The forceps was flamed and was used to remove the membrane from the funnel. The membrane filter was placed into the prepared petri-dishes and incubated at 35^oC for Total coliforms and 44^oC for Faecal coliforms for 18-24 hours. After incubation the numbers of colonies were counted [13,14].

III. Results

Table 1: Mean Total Heterotrophic (cfu/ml) of the water Samples Collected from Ajumoda Stream and Reservoir.

Sampling period	Point A	Point B	Point C	WHO Standard	EPA Standard
Week 1	80×10^4	230×10^6	35×10^2	1.0×10^2	1.0×10^2
Week 2	50×10^4	150×10^6	31×10^2	1.0×10^2	1.0×10^2
Week 3	70×10^4	270×10^6	37×10^2	1.0×10^2	1.0×10^2
Week 4	65×10^4	250×10^6	33×10^2	1.0×10^2	1.0×10^2
Mean	66×10^4	225×10^6	34×10^2	1.0×10^2	1.0×10^2

Keys:
Point A- Protected source water
Point B- Storm water runoff
Point C- Partially treated drinking water
WHO – World Health Organization, EPA – Environmental Protection Agency.

Table 2: Mean Total Coliform Counts (cfu/ml) of the water Samples Collected from Ajumoda Stream and Reservoir

Sampling Periods	Point A	Point B	Point C	WHO Standard	EPA Standard
Week 1	40×10^4	90×10^6	32×10^2	zero/100ml	zero/100ml
Week 2	50×10^4	150×10^6	30×10^2	zero/100ml	zero/100ml
Week 3	45×10^4	190×10^6	20×10^2	zero/100ml	zero/100ml
Week 4	56×10^4	120×10^6	22×10^2	zero/100ml	zero/100ml
Mean	48×10^4	138×10^6	26×10^2	zero/100ml	zero/100ml

Table 3: Mean Faecal Coliform (cfu/ml) of the water Samples Collected from Ajumoda Stream and Reservoir

Sampling period	Point A	Point B	Point C	WHO Standard	EPA Standard
Week 1	80×10^4	270×10^6	10×10^2	zero/100ml	zero/100ml
Week 2	50×10^4	280×10^6	15×10^2	zero/100ml	zero/100ml
Week 3	70×10^4	200×10^6	NIL	zero/100ml	zero/100ml
Week 4	75×10^4	265×10^6	NIL	zero/100ml	zero/100ml
Mean	69×10^4	254×10^6	6.0×10^2	zero/100ml	zero/100ml

Table 4: Result of Biochemical Characteristics of the bacteria

Sample	Gram Stat.	Rxn	Cell morph	Cat	Oxi	Casein Hyd	Galatin Hyd	Methyl Red	Voges proskae	Nitrate Rdn	Growth 60°	Growth 30°	Coa	Urease	Growth At PH 3.9	Growth At PH 9.2	Growth NaCl	Identity
PB	-	R	+	-	-	-	+	+	-	-	-	+	-	-	-	-	*E. coli*
PB	-	R	+	-	+	+	+	+	-	-	-	+	-	-	-	-	*A. hydrophilia*
PC	-	R	+	-	+	+	+	+	-	-	-	+	-	-	-	-	*A. hydrophilia*
PC	-	R	+	-	-	-	+	+	-	-	-	+	-	-	-	-	*E. coli*
PA	+	R	+	+	+	+	-	-	+	-	-	-	-	-	-	+	*B. megaterium*
PC	-	R	+	-	+	+	-	-	-	-	+	-	-	-	+	-	*F. aquatile*
PA	-	R	+	-	+	+	+	+	-	-	-	+	-	-	-	-	*A. hydrophilia*

Key:
- Negative, +Positive, R- Rod in shape, PA- Point A, PB -Point B, PC- Point C

Table 5: Result of Sugar Fermentation Reaction of the Isolates

Sample statn	Citrate Utilization	Moti lity	Indole test	Glu cose	Fruc tose	Mal tose	Lac tose	Suc rose	Galac tose	Xy lose	Dul citol	Man itol	Identity
PB	-	+	+	+	+	+	+	d	(+)	d	D	+	*Escherichia coli*
PB	-	+	+	+	+	+	(+)	d	d	+	-	+	*Aeromonas hydrophilia*
PC	-	+	+	+	+	+	(+)	d	d	+	-	+	*Aeromonas hydrophilia*
PC	-	+	+	+	+	+	+	d	(+)	d	D	+	*Escherichia coli*
PA	+	+	-	+G	+	+	+	+	(+)	+	+	+	*Bacillus megaterium*
PC	+	-	-	+	+	+	-	+	-	-	-	+	*Flavobacterium aquatile*
PA	-	+	+	+	+	+	(+)	d	d	+	-	+	*Aeromonas hydrophilia*

Key:
- Negative, + Positive, (+) Weekly positive, +G positive with gas, d Delayed positive

IV. Results

The result showed the total heterotrophic bacteria counts recorded in Ajumoda stream and reservoir with the mean heterotrophic count as $66x10^4$ (point A), $225x10^6$ (point B), and $34x10^2$ (point C). In week 1 point A has the highest cfu/ml ($80X10^4$) while the least was shown in week two ($50x10^4$). Point B has the highest cfu/ml in week three ($270x10^6$), followed by week four ($250x10^6$), week one ($230x10^6$) and the least was shown in week two ($150x10^6$) while the average mean was $225x10^6$. Point C has mean coliform count of $34x10^2$ while the highest coliform count in week three ($37x10^2$) with the least ($31x10^2$) in week two respectively (Table 4.1).

From table 2, it was revealed that the total coliform counts recorded in Ajumoda stream and reservoir with the mean total coliform count as $48x10^4$ for point A, $138x10^6$ for point B, and $26x10^2$ for point C. In week 4 point A has the highest cfu/ml ($56x10^4$) while the least was revealed in week one ($40x10^4$). Point B has the highest cfu/ml in week three ($190x10^6$), while the least value occurred in week one ($90x10^6$). Point C hast the highest total coliform count which was recorded in week one ($32x10^2$) while the least value appeared in week three ($20x10^2$) respectively.

It was also revealed that the Faecal coliform counts recorded in Ajumoda stream and reservoir had the mean Faecal coliform count as $69x10^4$ for point A, $254x10^6$ for point B, and $6.0x10^2$ for point C. In week1 point A had the highest Faecal coliform counts ($80x10^4$) while the least shown was in week two with value of ($50x10^4$). Point B reveal the highest cfu/ml in week two ($280x10^6$), while the least value occured in week three ($200x10^6$). Point C had the highest Faecal coliform count recorded in week two ($15x10^2$) while no Faecal coliform counts was recorded in week three and four respectively (Table 3).

Table 4 above shows the results of biochemical characteristics of the bacteria isolates. All isolates are rod-like in shape. There are two isolates from point B. Both reacted positively to catalase, methyl red, voges proskae, coagulase and pH at 9.2 while they reacted negatively to other test parameters, except for the second isolate that reacted positively to casein hydrolysis and galatin hydrolysis. There are three isolates from point C in which the first two isolates reacted the same way as in point B but for the third isolate, it reacted positively to catalase, casein hydrolysis, galatin hydrolysis, growth at 30^oC and pH at 3.9 while it reacted negatively to other tests. There are two isolates from point A. the first one reacted positively to gram reaction, catalase, oxidase, casein hydrolysis, galatin hydrolysis, nitrate reduction, growth at pH 9.2 and growth on Nacl while it reacted negatively to other test parameters. The second isolate reacted positively to catalase, casein hydrolysis, galatin hydrolysis, methyl red, voges proskae, coagulase and pH at 9.2 while it reacted negatively to other tests.

Table 5 above shows the results of sugar fermentation reaction of the bacteria isolated from the water samples collected from three different sampling points of Ajumoda stream and reservoir. The isolates includes, *Escherichia coli, Aeromonas hydrophilia, Bacillus megaterium* and *Flavobacterium aquatile.* The two isolates from point B are *Escherichia coli* and *Aeromonas hydrophilia. Escherichia coli, Aeromonas hydrophilia* and *Flavobacterium aquatile* are isolated from point C while *Bacillus megaterium* and *Aeromonas hydrophilia* are isolated from point A. *E. coli* reacted negatively to citrate utilization, delayed positive to sucrose and xylose, weekly positive to galactose while it reacted positively to other tests. *A. hydrophilia* reacted negatively to citrate utilization and ducitol, weekly positive to lactose, delayed positive to sucrose and galactose while it reacted positively to other tests. *B. megaterium* reacted negatively to indole test, positive with gas to glucose, weekly positive to galactose and reacted positively to other test parameters. *F. aquatile* reacted posively to citrate utilization, glucose, fructose, maltose, sucrose and mannitol but reacted negatively to other tests.

V. Discussion

The mean total heterotrophic counts from the three sampling points of Ajumoda stream and reservoir and the result were higher than the values reported by [15] in Ogoniland. The values across stations also exceeded the limits of WHO standard for heterotrophic bacteria count in potable water where THC should not be more than $1.0x10^2$ cfu/ml as in conformity with the reprt of [16]. The highest values of THC recorded in point B in week 3 indicated heavy anthropogenic activities compared to other stations, since the primary source of these bacteria in water are animal and human activities. THC were higher during week3 in all sampling points, which may be due to the heavy rainfall pattern around middle of october. Similar observation was made by [15, 17] who also reported heavy bioloads during rainy season in their study areas. The result in this study agree with the report of [18] that reported high bacteria count from Ebutte river, even though the THC reported by the author were lower compared to the present study. The higher value reported in the present study is lower compared to the report of [19] who reported high level of total heterotrophic bacteria count on Tigris river with the value that range from (468-9100 Cfu/ml).

None of the sampling points of the water sources complied with World Health Organization standard for coliform in water. The total coliform of all samples were extremely higher than the WHO standard for coliform bacteria in water which is zero (0) total coliform per 100ml of water [14]. Similar observation of higher values exceeding WHO limits was reported by [19] in Tigris River. However, the lowest total coliform count

(cfu/ml) were reported in point C across weeks of examination, this may be due to addition of chlorine for water treatment, this result agrees with the reports of [20, 21] who conducted similar studies in Gudu stream, Abuja and Ebutte River, Edo State, Nigeria.

The alarming high number of faecal coliforms obtained from the water samples (point A and point B) indicates high level of faecal contamination of the water which potentially poses a high health risk for the inhabitant of the community. This agrees with [22] that stated that water used for washing and bathing should definitely have FCC (cfu/ml) and that high coliform counts are an indication of high faecal contamination. The lowest FCC reported in Point C in week 1and 2 and 0cfu/100ml during third and fourth week indicated less pollution of the reservoir. This disagree with the report of [12] on Uke River who reported high Faecal coliform count above WHO standard level. Reduction in the FC at point C during week 3 may be due to restriction of human activities into the reservoir. The isolates reaction to biochemical tests indicated presence of *Escherichia coli, Aeromonas hydrophilia which are gram-negative bacteria* that implies the occurrence of faecal contamination in sampled water. *A. hydrophilia* has been known to be pathogenic in huma and fishes. Higher percentage occurrence of E-coli was also reoorted by [15, 19] and was related to cause gastrointestinal diseases in human. The *Bacillus megaterium* observed in this study have been associated with no pathogenic in human and *Flavobacterium aquatile* has devastating impact on human health such as systemic infection in man and mortality in fishes, both are present in points A and C, their presence indicated contamination of water body [23].

VI. Conclusion

The study revealed that Ajumoda reservoir and stream had high coliform counts and that the high faecal coliform counts showed that the water is more contaminated with human excrement and detergent as a result of cloth washing and particles. Monitoring of the reservoir is imperative if the water is to be used for drinking purposes. However, control of human activities to prevent faeces and refuse from entering water body as the key to avoid bacterial contamination of the water. In a nutshell, it is evident that water borne diseases are due to improper disposal of refuse and contamination of water by sewage and surface runoff.

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