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Research Article

# Effect of Anthropogenic Activities on the Microbiological Quality of Lobia Creek in Southern Ijaw of Bayelsa State, Nigeria

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## Abstract

The negative impact of human activities on the aquatic environment and biodiversity within the maritime field poses a serious threat on public health. Lobia Creek in Bayelsa State constitutes the main source of rural water supply and is used for various human activities. This study was therefore aimed at determination of the impact of human activities on the microbiological properties of the Creek. Surface water samples were collected from five stations (control, drinking, abattoir, Jetty, and toilet) along the Creek in sterile sample bottles. The samples were transported in an ice packed cooler to the Microbiology laboratory of the Rivers State University for microbiological analyses using standard techniques. The isolated bacteria were also identified after gene amplification and sequencing. The data collected were statistically analyzed using Tukey Kramer statistical tool. Results of microbiological analysis showed that counts of total heterotrophic bacteria ranged from $0.36 \pm 0.04 \times 10^5$ to $1.44 \pm 28.28 \times 10^6$ CFU/ml, total fungi ranged from $0.7 \pm 0.01 \times 10^3$ to $2.1 \pm 0.42 \times 10^4$ CFU/ml, while total Coliform count ranged from $1.8 \pm 0.28 \times 10^4$ to $7.6 \pm 0.28 \times 10^4$ CFU/ml feacal Coliform count ranged from $0.7 \pm 0.21 \times 10^4$ to $3.8 \pm 0.57 \times 10^4$CFU/ml. Statistical analysis showed significant difference across the stations of the Creek indicating negative impact of human activities. The bacterial isolates molecularly identified included *Lysinibacillus macroides*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Escherichia coli* and *Shewanella algae*. The fungi identified were *Penicillium* sp, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Candida krusei*, *Rhizopus* spp. and *Mucor* spp. The presence of *E. coli* indicates faecal contamination of the creek water. The presence of *Shewanella algae* which causes ear, skin and soft tissue infections in the water samples is worrisome. The impact of human activities on Lobia creek therefore poses a serious threat on public health and the immediate environment especially as most of the bacteria and fungi isolated in this study are potential pathogens of microbial infection. The findings in this study should strengthen efforts to ensure strict compliance to policies, personal hygiene and proper waste management practices to avoid outbreak of water-borne disease.

**Keyword:** Lobia Creek; Microbiological Quality; *E. coli*; *Shewanella algae*; *Candida albicans*

## Introduction

Water is an indispensable and multipurpose natural resource and exists in the three states of matter; gaseous, liquid and solid phases [1]. Water is an abundant natural resource, crucial for the sustenance in all aspects of life and it is a valuable resource that needs to be well-cared-for [2]. The chemical composition of surface water is derived from atmospheric, soil, and rock source. The relative distribution of the chemical composition to surface waters from each of these sources is a function of the climate being modified increasingly by human activities. According to [3] clean, safe and adequate freshwater is vital for the survival of all living organisms and proper functioning of ecosystems, communities and economies. Declining water quality has become a global issue of concern as human populations grow, industrial and agricultural activities expand, and climate change threatens to cause major alterations to the hydrologic cycle [4].

About 75% of the earth's surface is covered with water but fresh water accounts for only less than 2.7% [2]. The increasing human pop- ulation, urbanisation, rapid industrialisation and expanding food production are all putting pressure on water resources, especially in developing countries as a source of provision for safe drinking and irrigation purpose. The challenge of water quality has become a global issue, in many developing countries. According to [5], in most developing countries including Nigeria, anthropogenic activi- ties of different kind such as bathing and washing, indiscriminate dumping and direct discharge of untreated waste including human feaces around the shoreline and into nearby rivers, streams and into creek, contaminate surface water directly and in turn contrib- ute to increase in microbial pollution. Other negative effects in- clude nutrient enrichment, deterioration of the water quality, and destruction of spawning grounds for aquatic and marine life [6].

Countries throughout the world are concerned with the effects of unclean drinking water because water borne diseases are a major cause of morbidity and mortality [7]. Clean drinking water is important for overall health and plays a substantial role in infant and child health and survival. The World Health Organization [7] estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters and seafood. Persons with compromised immune systems, such as those with AIDS, are especially vulnerable to water- borne infections, including those infections that are self-limiting and typically not threatening to healthy individuals [8]. Throughout the less developed part of the world, the proportion of households that use unclean drinking water source has declined, but it is extremely unlikely that all households will have a clean drinking water source in the foreseeable future [9].

In Nigeria, a vast majority of people living along the course of water bodies still source and drink from rivers, streams and other water bodies without any form of treatment irrespective of the state of these water bodies [1]. These natural waters con- tain a myriad of microbial species, many of which have not been cultured, much less identified. The number of organisms present varies considerably between different water types, and it is gener- ally accepted that sewage polluted surface waters contain greater number of bacteria than unpolluted waters [7]. Polluted surface waters can contain a large variety of pathogenic microorganisms including viruses, bacteria and protozoa. These pathogens, often of feacal source, might be from point sources such as municipal wastewater treatment plants [10] and drainage from areas where livestock are handled [11] or from non-point sources such as domestic and wild animal defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff [12]. Feacal contamination of water is globally recognized as one of the leading causes of waterborne diseases. The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development.

The river water quality is influenced by a range of factors such as weather, runoff, and waste discharge which result in changes in water quality parameters [13]. This can be observed in the variation of the impact that human activities can have on receiving waters. Water is used for domestic, industrial and agricultural purposes by humans. The sources of water supply in nature include ground and surface water lakes, ponds, rivers, streams, springs and rain [14]. Accesses to safe drinking water become imperative; hence, water needs to be conserved and valued. Water quality assessment is mostly used to measure the magnitude of water resources pollution [15]. Water quality assessment is the complete process of evaluation of the physical, chemical and biological nature of water based on human effects and intended uses. Globally, numerous deaths have been reported due to water resources not meeting the health criteria in terms of their constituents' concentration [16]. Improved water resource management ensures that water resources have less risk of contamination and the water is suitable for both human lives and the environment as large Variation in water quality is caused by natural and anthropogenic activities [17]. The spatial extent of pollution is critical as the mixing of pollutants occurs over a given distance [18]. It is vital to have accurate measurements for variability between sites and collection sessions in water quality of a river or any other watercourse. The risk associated with pollution depends on both the extent of the temporal and spatial variation of the pollutant [19].

Despite that it is an essential commodity; access to safe drinking water in many parts of the world has been threatened basically due to the contamination of water by human activities.

## Aim of the Study

This study was aimed at evaluating the effect of human activities on the microbiological quality of the surface water of Lobia Creek in Bayelsa State, Nigeria which is used for various domestic and industrial purposes.

## Materials and Methods

### Description of the study area

This research was carried out on surface water samples collected from Lobia creek in Southern Ijaw region of Bayelsa State located in the Niger Delta [20]. The Lobia creek is about 85km long with several communities located along its banks. The communities engage in similar economic activities and so they generate similar waste and adopted the same method of disposal. The sampling stations were along the Lobia Creek in area where Lobia communities are located with a coordinate of 4° 30 '00'N and 5° 50 '00'E. The Map of the study area is shown in figure 1 below.

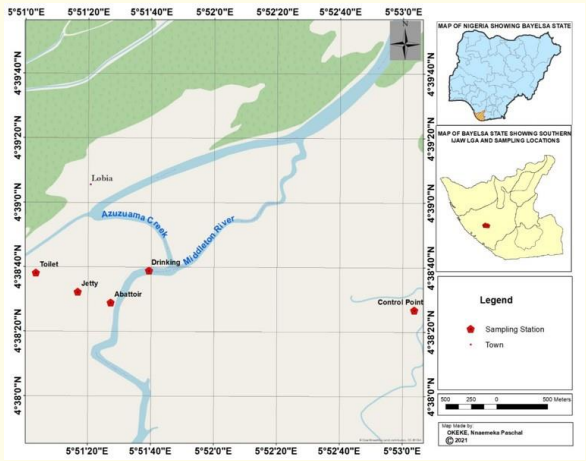


**Figure 1:** Map of the study area showing the sampling stations.

### Sample collection

Creek water samples for microbiological analysis were collected from five different stations (toilet, Jetty, abattoir, drinking, and control which were also designated as Stations 1, 2, 3, 4, and 5 respectively) along the creek at monthly intervals for a period of six months from August 2020 to January 2021. A total of 150 creek water samples were collected during the study. Sample bottles were sterilized at 150°C for 2 hours, wrapped in aluminium foil prior to the collection of water samples. Being that accessibility to the stations were very unique, samples were collected by special means. Samples were collected from station 1 (Toilet station) using sterile bottle with a rope from the hole in the middle of the toilet, at the station 2 (Jetty station) samples were collected from concrete step down the jetty, station 3 (Abattoir) samples were collected using a sterile bottle with rope while at stations 4 and 5 (Drinking and Control) samples were collected after paddling a canoe to these stations.

### Microbiological analyses of water samples

### Serial dilution

One millilitre each of the water samples were separately added to 9 ml of normal saline (diluents). After thorough shaking, further 10-fold (v/v) serial dilutions were made by transferring 1 ml of the diluted water sample to freshly prepared normal saline diluents to a range of $10^{-3}$ dilutions [21].

### Enumeration and isolation of total heterotrophic bacteria (THB)

Total Heterotrophic Bacteria was enumerated as described by [21]. Bacterial Colonies that appeared on the nutrient agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from $10^{-3}$ dilutions were counted and the means were calculated and expressed as colony forming unit per millilitre using the formula below:

$$\text{CFU/ml} = \frac{\text{number of colonies}}{\text{volume plated}(0.1)} \times \text{Dilution} \quad [1].$$

While discrete colonies that developed on the nutrient agar plates were sub-cultured on freshly prepared nutrient agar plate in order to isolate pure cultures.

### Total coliform counts (TCC)

Total Coliform Counts were enumerated as described by [21]. Bacterial Colonies that appeared on the MacConkey agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from $10^{-2}$ dilutions and incubated at 37ºC for 24 hours were counted and the mean expressed as CFU/ml [22].

### Feacal coliform counts

Feacal Coliform Counts was enumerated as described by Prescott *et al.* [21]. Bacterial Colonies that appeared on the Eosin

Methylene Blue (EMB) agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from $10^{-2}$ dilutions and incubated 45.5ºC for 24 hours were counted and the mean expressed as CFU/ml [22].

### Total fungal counts

This was determined using Sabouraud Dextrose Agar (SDA) amended with Tetracycline to suppress bacterial growth [23]. The spread plate technique as described by Prescott *et al.* [21] was adopted. An aliquot zero point one (0.1 ml) milliliter from $10^{-2}$ dilution of the serially diluted samples were inoculated onto predried SDA agar plates in duplicates. The inocula were then spread evenly on the surface of the media using a flamed bent spreader. The plates were then incubated at room temperature (25°C) for 5 days after which the colonies that developed were counted and the mean of total Fungal counts were recorded accordingly.

### Purification and maintenance of Isolates

After incubation, pure isolates were obtained by picking (with sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar in plates and incubated at room temperature for 24 hours until pure distinct colonies developed.

### Identification of bacterial isolates

### Biochemical characterization

Pure bacterial isolates were identified by the method described by Collins *et al.* [24] and Cheesebrough [25]. Pure bacterial iso- lates were subjected to Biochemical tests which include oxidase test, Catalase test, Indole test, methyl red test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate test, Sugars fermentation test and Triple sugar iron agar test. Bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology [26].

### Molecular identification

### DNA extraction (Boiling method)

Five milliliters (5.0 ml) of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000 rpm for 3 minutes. The cells were re-suspended in 500 µl of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 mins at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20°C for other downstream reactions [22].

### DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with two microlitre (2 µl) of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestral; the upper pedestral was brought down to contact the extracted DNA on the lower pedestral. The DNA concentration was measured by clicking on the "measure button and concentration of the extracted genomic DNA was displayed on a computer screen [27].

### 16S rRNA amplification

The 16s rRNA genes of the isolates were amplified using the universal primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix were made up of 12.5 µL of Taq 2X Master Mix from New England Biolabs (M0270); 1 µL each of 10 µM 16SrRNA gene forward primer (16SF GTGCCAGCAGCCGCGCTAA) and reverse primer (16SR: AGACCCGGGAACGTATTCAC); 3 µL of DNA template and then made up with 7.5 µL Nuclease free water.

### Cycling conditions for 16SrRNA gene

Initial denaturation at 94°C for 5 mins, followed by 36 cycles of denaturation at 94°C for 30sec, annealing at 56°C for 30 secs and elongation at 72°C for 45sec. Followed by a final elongation step at 72°C for 7 minutes. The product was resolved on a 1% agarose gel at 120V for 20 minutes and visualized on a blue light transilluminator [28].

### Sequencing

The PCR products were purified using absolute ethanol and then sequences on ABI 3500 Genetic Analyser (Thermofisher, USA) and all analysis were performed using Bio-Edit sequence editor and organisms were identified by aligning isolate sequences with those on NCBI via BLAST tool.

### Gel electrophoresis

Plasmids were separated by electrophoresis in 1% agarose (Sigma Aldrich, USA) at a voltage of 4.5 V/cm; buffer used was TAE

(Tris-AcetateEDTA) for 3 hours. Following electrophoresis, the gels were stained for 15 minute with ethidium bromide solution (1.0 µg/ml EtBr in 0.5 x TrisAcetate-EDTA (TAE)), and then observed under UV light. The image was registered and analyzed using Quantity One software, version 4.1 [27].

### Statistical analyses

Statistical analyses on data obtained were carried out using one way ANOVA and all pairs Tukey-kram.

## Results

The results of microbial counts: Total heterotrophic bacteria and fungi, Total coliform and Feacal coliform counts obtained from the five (5) sampled stations within Lobia Creek are presented in figures 2, 3, 4 and 5 respectively.

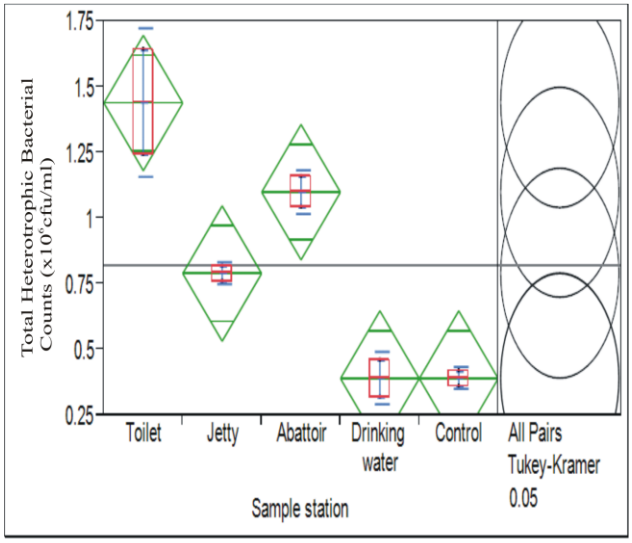


**Figure 2:** One-way Analysis of total heterotrophic bacteria of sample stations.

Figure 2 revealed that Total heterotrophic bacterial count from Creek water samples ranged from 0.36 ± 4.24 x $10^6$ CFU/ml to 1.44 ± 2.28 x 10 CFU/ml with station 1 (Toilet) having the highest bacterial load 1.44 ± 2.28 x $10^6$ CFU/ml followed by station 3 (Abattoir) 1.10 ± 0.49 x $10^6$ CFU/ml > station 2 (Jetty) 0.79 ± 0.24 x $10^5$ CFU/ml > station 4 (Drinking water) 0.39 ± 0.9 x $10^5$ CFU/ml > and least bacterial load 0.36 ± 4.24 x $10^5$ CFU/ml was recorded in station 5 (Control).

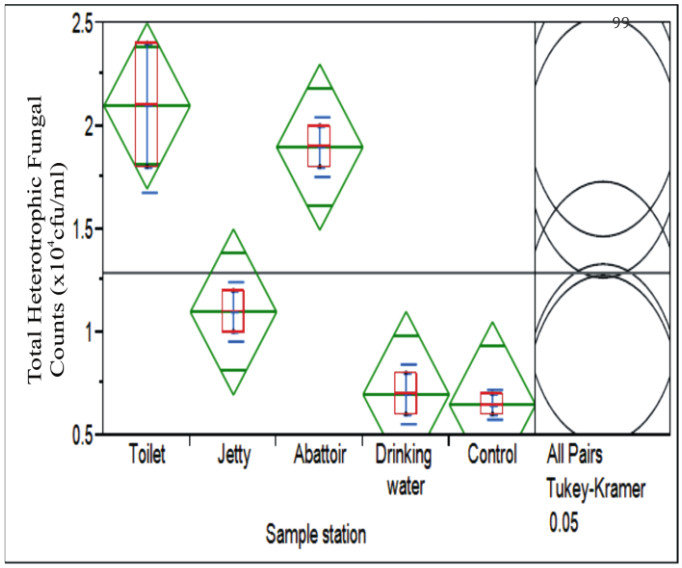


**Figure 3:** One-way Analysis of total fungi of sample stations.

Figure 3 revealed that Total fungal counts ranged as follows 0. 7 ± 0.01 x $10^3$ CFU/ml (Control station), < 0.7 ± 0.04 x $10^3$ CFU/ml (Drinking Station), < 1.1 ± 0.14 x $10^4$ CFU/ml (Jetty) < 1.9 ± 0.14 x $10^4$ CFU//ml (Abattoir), < 2.1 ± 0.42 x 10 CFU//ml (Toilet).

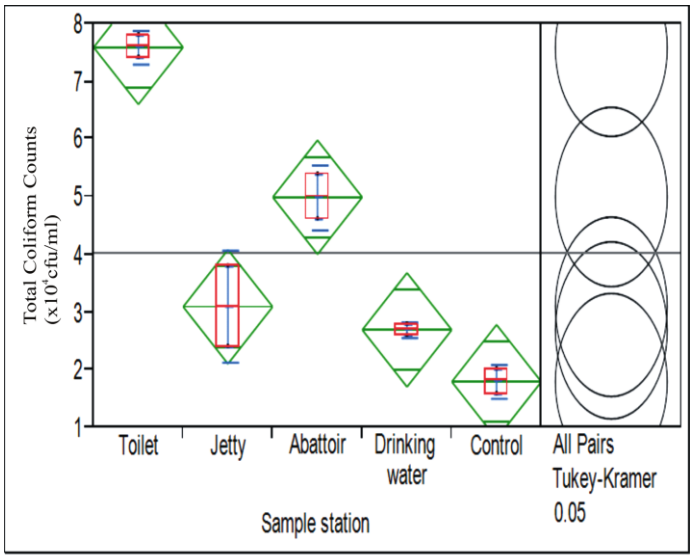


**Figure 4:** One-way analysis of total coliform of sample stations.

Figure 4 showed that Total coliform counts ranged from 1.8 ± 0.28 x $10^4$ CFU/ml to 7.6 ± 0.28 x $10^4$ CFU/ml with station 1 (Toilet)

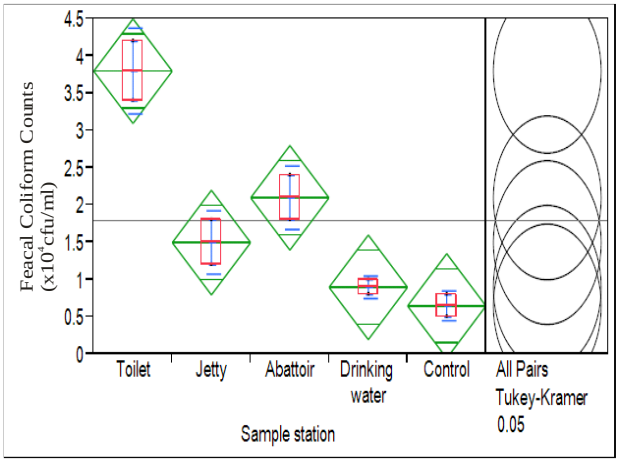


**Figure 5:** One-way analysis of feacal coliform of sample stations.

having the highest coliform load (7.6 ± 0.28 x $10^4$ CFU/ml) followed by station 3 (5.0 ± 0.57 x $10^4$ CFU/ml), station 2 (3.1 ± 0.99 x $10^4$ CFU/ml), Station 4 (2.7 ± 0.14 x $10^4$ CFU/ml) and least coliform counts were recorded in station 5 (1.8 ± 0.28 x $10^4$ CFU/ml).

Figure 5 revealed that feacal colifom counts ranged from 0.7 ± 0.21 x $10^3$ CFU/ml to 3.8 ± 0.57 x $10^4$ CFU/ml with station 1 (Toilet) having the highest feacal coliforms load (3.8 ± 0.57 x $10^4$ CFU/ml) followed by station 3 (2.1 ± 0.42 x $10^4$ CFU//ml), station 2 (1.5 ± 0.42 x $10^4$ CFU/ml), Station 4 (0.9 ± 0.14 x $10^3$ CFU/ml) and least coliform count was recorded in station 5 (0.7 ± 0.21 x $10^3$ CFU/ml).

The morphological and Biochemical characteristics of the bacterial isolates from the sampled stations of Lobia Creek revealed that the following bacterial isolates; *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Micrococcus* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia* sp., *Shigella* sp., *Shewanella* sp., *Staphylococcus* sp., and *Vibrio* sp., were isolated in this study. While *Penicillium* sp., *Aspergillus flavus., Candida albicans., Aspergillus flavus., Aspergillus niger., Rhizopus* sp., *Mucor* sp., *Candida krusei, Fusarium* sp., and *Saccharomyces* sp., were the fungal species isolated across the five sampled stations of the Lobia creek.

The results of the evolutionary relationship between the bacterial isolates and their gene bank relatives after the genomic identification using gene amplification and sequencing of the bacterial isolates is as shown in the phylogenetic tree in figure 6 below.

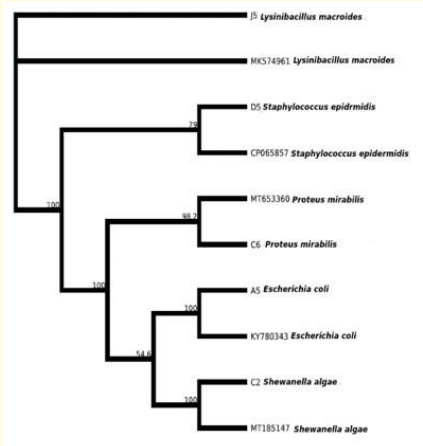


**Figure 6:** Phylogenetic tree showing the evolutionary distances between the bacterial Isolates.

## Discussion

The present study has revealed the microbiological properties including the molecular identification of some bacterial isolates of the surface water samples from Lobia creek. The microbial counts revealed that the mean total heterotrophic bacterial and fungal counts, total coliform counts and feacal coliform counts obtained from five (5) sampled stations were significantly high across the five stations. Station 1 recorded the highest microbial load followed by fish abattoir dump point while control point which is free of any noticeable anthropogenic activities recorded the lowest count. Mean separation using all pair Turkey Kramer showed that there were significant differences at ($P < 0.05$) between the control and all the other stations. This indicated that the various human activities in all the other stations had a significant effect on the microbial quality. Hence, the high microbial load recorded in this study could be attributed to the various human activities across the stations. The result is in correlation with the work reported by Mandri *et al.* [29] and Khan., *et al.* [30] who worked on surface water at different stations of various anthropogenic activities and obtained similar results. These could be attributed to certain physiochemical factors like temperature, pH, Dissolved oxygen, Biological oxygen demand etc which favours some organisms in a polluted water body [31].

According to the report of Obire *et al.* [1] low DO values from sampling zone and stations was as a result of the presence of or-

ganic matter and nutrient which also enhanced the proliferation and increased microbial growth that degrade organic matter. High bacterial counts from leachate and an adjacent stream due to high content of organic matter were also reported by Obire and Aguda [5]. The high microbial counts reported for the different stations in this study indicated the high level of pollution influenced by the various anthropogenic activities on the banks of Lobia creek.

Microorganisms of enteric origin are one of the most common pathogens encountered in the aquatic environments, including discharged municipal wastewater effluents as well as surface water. In this study, eleven bacterial isolates belonging to the genera and their percentage of occurrence in parenthesis are as follows; *Staphylococcus* sp (22%), *Enterobacter* sp (17%), *E. coli* (14%), *Vibrio* sp (6%)„ *Pseudomonas* sp (9%), *Klebsiella* (12%), *Shigella* sp (5%), *Serratia* sp (3%), *Proteus* sp. (8%), *Shewanella* sp (2%), *Micrococcus* sp (2%), and seven fungal isolates, namely, *Penicillium* sp (22%), *Candida* sp (8%), *Mucor* sp (16%), *Aspergillus* sp (12%), *Rhizopus* spp (2%), *Fusarium* sp. (10%), and *Saccharomyces* sp.(5%) were obtained from the samples analyzed over the sampling period. The presence coliforms in the water system are universally accepted to indicate contamination, and possible presence of other pathogenic organisms [32]. *E. coli* is a subgroup of feacal coliforms used as an indicator of feacal contamination, although vast majority of *E. coli* are completely harmless, some strains of the bacteria have acquired genetic capabilities which enable them to encode virulence factors [33]. Pathogenic *E. coli* strains cause diverse forms of bacterial induced illnesses with symptoms ranging from mild diarrhoea to severe complication and even death [34]. The presence of faecal coliform in the drinking water and control samples indicated that Lobia creek is highly polluted with faecal matter and potential pathogens which suggested that water is not potable and safe for domestic use.

## Conclusion and Recommendations

The levels of the microbiological parameters determined for the Lobia creek surface water has helped to ascertain the water quality and hence the potability. The dynamic nature of the creek water and human activities along the bank of the creek, irrespective of the stations considered accounts for the even distribution and fluctuations in levels of parameter examined. The results obtained in this study showed that human activities around and within Lobia creek have significant negative effect on microbiological characteristics of the creeks which makes it unfit for human consumption. The presence of coliforms especially faecal coliforms across all the five stations including the drinking and control points indicated high level of faecal contamination of the water body. The presence of *E. coli* indicates faecal contamination of the creek water. The presence of *Shewanella algae* which causes ear, skin and soft tissue infections in the water samples is worrisome. The impact of human activities on Lobia creek therefore poses a serious threat on public health and the immediate environment especially as most of the bacteria and fungi isolated in this study are potential pathogens of microbial infections. The Lobia creek water is therefore microbiologically unsafe for drinking as the inhabitants in communities along the banks of the creek consume the water without any form of treatment. The findings in this study should strengthen efforts to ensure strict compliance to policies, personal hygiene and proper waste management practices to avoid outbreak of water-borne disease. The Government should provide safe and secure source of water for drinking and other domestic purposes to avoid emergence of certain microbial infections.

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