



Impact of Indiscriminate Waste Disposal into Otuoke River on the Abundance of Pathogenic Heterotrophic Bacteria in the River

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ABSTRACT

This study examines the impact of indiscriminate disposal of municipal waste on the abundance of pathogenic heterotrophic bacteria in the Otuoke River. Samples were collected from six locations. The number of colonies for each of the samples and the mean counts of the microbial colonies were taken for their colony-forming units (CFU). Different colonies based on their cultural characteristics on Nutrient Agar were sub-cultured. The Most Probable Number (MPN) method was used to quantitatively and qualitatively analyze the water samples. The data for this research were presented using descriptive statistics. The results show that Hospital road waterside has the highest number of bacterial colonies with a CFU/ml of 19.6×10^7 . While Abenedi Creek, has the lowest microbial concentrations with a CFU/ml of 9.2×10^7 . Although Abenedi creek is lower compared to other locations, but it still indicates a notable microbial presence. This study establishes a clear link between indiscriminate municipal waste disposal and bacterial contamination in the Otuoke River. Thus, emphasizing the need for effective waste management and public health strategies.

ARTICLE'S INFO

Article No.: 051725086

Type: Research

Full Text: [PDE](#), [PHP](#), [EPUB](#), [MP3](#)

DOI: [10.15580/gjemps.2025.1.051725086](https://doi.org/10.15580/gjemps.2025.1.051725086)

Accepted: 20/05/2025

Published: 16/06/2025

Keywords: Indiscriminate Disposal of Waste, Abundance of Pathogenic Bacteria, Otuoke River

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Article's QR code



INTRODUCTION

Municipal waste, often referred to as urban waste, encompasses a wide variety of materials discarded by households, businesses, and institutions. This waste includes organic matter, plastics, metals, glass, and other refuse that, if not properly managed, can lead to significant environmental pollution (Akhtar *et al.*, 2018). Improper disposal of municipal waste into water bodies poses a major ecological and public health threat, contributing to the contamination of rivers, lakes, and oceans (Khan *et al.*, 2020). A notable example is the indiscriminate disposal of waste into the Otuoke River, which has raised concerns regarding water quality and the proliferation of pathogenic heterotrophic bacteria. These microorganisms, which feed on organic substances, can cause illnesses in humans and animals (Liu *et al.*, 2019). Species such as *Escherichia coli*, *Salmonella*, and *Vibrio cholerae* are frequently detected in polluted waters and are indicators of fecal contamination and potential health risks (Ofori *et al.*, 2021). Their prevalence in river water can be influenced by factors like organic matter content, temperature, and nutrient levels (Zhang *et al.*, 2022). The Otuoke River, located in Ogbia Local Government Area, Bayelsa State, Nigeria, is a critical water source for domestic use, agriculture, and recreation. However, unregulated waste dumping has led to severe environmental degradation. Studies demonstrate that such practices introduce organic pollutants, heavy metals, and pathogenic microorganisms into the water, decreasing water quality and endangering both human health and aquatic ecosystems (Adewale *et al.*, 2021). Municipal waste originates from residential, commercial, and industrial sources, and its composition is highly diverse, including biodegradable materials like food waste, non-biodegradable plastics, metals, and hazardous substances (Ndubuisi *et al.*, 2017). When improperly disposed of into water bodies without adequate treatment, these wastes provide nutrients that support bacterial proliferation (Fang *et al.*, 2018). Bacterial contamination occurs via several pathways: organic waste supplies nutrients for bacteria (Osei *et al.*, 2020), fecal matter introduces enteric pathogens, and physical disturbance from waste dumping can resuspend sediments harboring dormant bacteria, further spreading contamination (Gao *et al.*, 2019). The presence of pathogenic heterotrophic bacteria in the Otuoke River has significant public health implications. Waterborne diseases such as cholera, dysentery, and typhoid fever are common in regions where water is contaminated with fecal matter (WHO, 2017). The local population, relying on the river for drinking, bathing, and washing, faces increased risk of infection. Additionally, irrigation of crops with contaminated river water can facilitate the spread of pathogens, further threatening food safety (Okafor *et al.*, 2020). Addressing waste disposal issues in developing countries like Nigeria is challenging due to inadequate infrastructure, low public awareness, and weak

regulatory enforcement. Implementing comprehensive waste management strategies—including improved collection systems, treatment facilities, and environmental education—is vital to reducing environmental contamination and protecting public health (Zhou *et al.*, 2023).

Study Area

The study was carried out in Otuoke, Ogbia Local Government Area, Bayelsa State, located in the Niger Delta region of Nigeria, the area is characterized by a rich biodiversity of plant species, some of which are traditionally consumed as food. Otuoke is located at coordinates of 4°45' and 6°18'E (Ogunlesi *et al.*, 2012). Otuoke's vegetation is that of a lowland rainforest. The temperature in this region is around 30-32°C during the day and drops slightly to 24-26°C at night. The community typically experiences significant rainfall from April to October each year. The area is characterized by lush, dense forests, with a wide variety of tree species, including mahogany, iroko, obeche, and numerous other hardwoods. The Otuoke River is a tributary of the Orasi River. It extends from Mbiama to Ogbia town and linked up to Nembe and Brass. The river also links with the Ekoli River through Owubio creek and links with Kolo creek at Elebele and Otuogidi. The river's major distributary is Akoloman creek. With the establishment of the Federal University in the Community, the population of the university has sky rocket, this leads to increased indiscriminate waste generation in Otuoke Community. The people in the community are mostly farmers and fishermen. Some are into trading, while others are working as civil servants in the state and federal parastatals.

Sample Collection

Samples were collected from hospital road waterside, highness's road waterside, beside Engoye Hotel, Onuakpo, inside Ologakpo Creek and close to Abenidi (Control) Creek. They were collected directly at the points where municipal wastes entered the river. These sites helped measure the immediate impact of waste disposal on bacterial populations. The samples were collected from various points downstream of the disposal sites to assess how far the impact of waste disposal spread along the river.

Media and Reagent Preparations

The preparation of microbiological media involved the following procedures: Nutrient Agar (NA): 28 grams of the medium was dissolved in 1000 milliliters of distilled water, heated until fully dissolved, then sterilized by autoclaving at 121°C for 15 minutes. After cooling to approximately 45°C, it was poured into sterile Petri dishes and allowed to solidify before use (Atlas, 2010). Salmonella Shigella Agar (SSA): 63 grams was added to

1000 milliliters of distilled water, mixed thoroughly, heated with frequent agitation, and boiled for one minute. The medium was then aseptically poured into sterile Petri dishes and allowed to solidify (Barrow & Feltham, 2003). Mannitol Salt Agar (MSA): 111.02 grams was suspended in 1000 milliliters of distilled water in a conical flask, heated until completely dissolved, and sterilized by autoclaving at 121°C under 15 psi for 15 minutes. After cooling to about 50°C, it was poured into Petri dishes to solidify (Holt *et al.*, 19994). Eosin Methylene Blue (EMB) Agar: 36 grams was suspended in 1000 milliliters of distilled water, mixed until uniform, heated to boiling to fully dissolve, and sterilized via autoclaving at 121°C under 15 psi for 15 minutes. It was then cooled to 45–50°C, agitated to oxidize the methylene blue and suspend precipitates, then poured into sterile Petri dishes. The plates were allowed to solidify and reach room temperature before inoculation (Cappuccino & Sherman, 2014; Dworkin & Falkow, 2020). Simmons Citrate Agar (Slant): 7 milliliters of the medium was dispensed into 12-milliliter test tubes, sterilized by autoclaving at 121°C under 15 psi for 15 minutes, then cooled in a slanted position to form agar slants (Dworkin & Falkow, 2020). Tryptone Broth: 15 grams was suspended in 1000 milliliters of distilled water. Then, 7 milliliters of this suspension was transferred into 12-milliliter test tubes and autoclaved at 121°C under 15 psi for 15 minutes (Cheesbrough, 2006). MR/VP Broth: 7 milliliters was dispensed into 15-milliliter test tubes and sterilized at 121°C under 15 psi for 15 minutes (Cheesbrough, 2006). MacConkey Broth: 10 milliliters were distributed into sets of five tubes containing inverted Durham tubes, then sterilized at 121°C under 15 psi for 15 minutes (Cheesbrough, 2006). These steps ensure proper sterilization and preparation for microbiological analyses.

The Qualitative and Quantitative Analysis

The Most Probable Number (MPN) method was used to quantitatively and qualitatively analyze the water samples. MPN is commonly used in estimating microbial populations in water, soil, and agricultural products (WHO, 2017).

Presumptive Test

Ten milliliters of the medium was dispensed into sets of 5 tubes with inverted Durham Tubes, and the medium was sterilized at 15 psi pressure (121°C) for 15 minutes (American Public Health Association, 2017). After sterilization and cooling to 45–40°C, the inoculum was added according to its required amount: for the first set, 10ml of the inoculum was added to the first tube and serially diluted to the subsequent tubes. For the second set, 1ml was added, and for the last set, 0.1ml was added. The tubes were incubated for 24–48 hours at 37°C for general bacteria and at 44°C specifically for faecal coliforms and *E. coli* (Barrow & Feltham, 2003).

Confirmatory Test

Eosin Methylene Blue (EMB) Agar was prepared and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was poured into sterile Petri dishes and allowed to solidify. This was followed by inoculation of all positive tubes using a wire-loop. A loopful of each culture was streaked onto the plates using the streak plate technique and incubated at 37°C for 24–48 hours (APHA, 2017).

Completed Test

Using Nutrient Agar Slants and Gram Staining: A loopful of culture from the positive tubes was inoculated onto Nutrient Agar Slants and incubated for 24–48 hours at 35°C. Gram staining was performed for identification of Gram-negative, non-spore-forming rods: smears of the isolates were prepared on clean glass slides, heat fixed, stained with crystal violet, washed with distilled water, flooded with Gram's iodine solution, decolorized with 95% alcohol, counter-stained with safranin, air-dried, and observed under oil immersion (Barrow & Feltham, 2003).

Biochemical Characterization and Gram Reaction

Methyl Red (MR) Test: Using organisms from 18–24 hours old pure cultures, each bacterial isolate was inoculated into prepared Methyl Red broth. The tubes were incubated at 37°C for 24 hours. After incubation, 2–3 drops of methyl red indicator were added to the tubes, and immediate observation was made for red coloration indicative of mixed acid fermentation (Dworkin & Falkow, 2020).

Voges-Proskauer (VP) Test: Using organisms from 18–24 hours old pure cultures, each bacterial isolate was inoculated into prepared Voges-Proskauer broth. The tubes were incubated at 37°C for 24 hours. Following incubation, 1ml each of Barritt's reagents A and B were added to the tubes, gently shaken, and allowed to stand for 15–30 minutes to detect acetoin production (Cheesbrough, 2006).

Indole Test: Isolates aged 18–24 hours were added to Tryptophan broth in test tubes and incubated for 24 hours. After incubation, Kovac's indole reagent was added to each tube, and results were observed after 10–15 minutes for the production of indole (APHA, 2017).

Citrate Test: Test organisms were inoculated into prepared Simmons Citrate Agar slants using an inoculating needle and incubated for 24 hours at 37°C. The slants were observed for the presence or absence of blue coloration, indicating citrate utilization (Cheesbrough, 2006).

Oxidase Test: Using the paper method, one or more colonies from pure cultures were rubbed onto filter paper. Kovac's oxidase reagent was added to each and

observed for the presence or absence of blue coloration within 10 seconds, indicating the presence of cytochrome c oxidase (Cheesbrough, 2006).

Catalase Test: A small amount of 18-24 hours old isolate was transferred onto a clean glass slide, and hydrogen peroxide (3%) was added. The production of bubbles within 5-10 seconds indicated a positive catalase test (Dworkin & Falkow, 2020).

Coagulase Test: Using the slide method, drops of normal saline were placed on clean glass slides corresponding to the number of isolates. A sterile inoculating loop was used to emulsify one or more colonies of pure isolates in the saline, and plasma was added. Clumping or agglutination of the mixture within 10-15 seconds indicated a positive coagulase test (Cheesbrough, 2006).

Gram's Reaction: Thin smears of isolates from 18-24 hours old pure cultures were heat-fixed on clean glass slides, stained with crystal violet, flooded with Gram's iodine, decolorized with 95% ethanol, counter-stained with safranin, air-dried, and observed under a microscope using oil immersion for Gram reaction (Cheesbrough, 2006; Dworkin & Falkow, 2020).

Data Presentation and Analysis Method

The data for this research was presented using descriptive. Descriptive statistics was utilized to summarize and describe the data through the use of tables. This approach allows for a clear and concise presentation of the microbial contamination levels and bacterial isolates found across various sampling locations in Otuoke, Bayelsa State.

RESULTS

Table 1: Colony forming unit/ml

Sample	No. Of Colonies	CFU/ML
Hospital Road Waterside	196	19.6×10^7 cfu/ml
Highness Road Waterside	168	16.8×10^7 cfu/ml
Beside Engoye hotel waterside	184	18.4×10^7 cfu/ml
Onuakpo	157	15.7×10^7 cfu/ml
Inside Ologakpo Creek	164	16.4×10^7 cfu/ml
Close to Abenidi (Control) Creek	92	9.2×10^7 cfu/ml

Table 1 presents the number of colony-forming units (CFU) per milliliter (ml) of samples collected from various locations. Hospital road waterside has the highest number of colonies with 196 colonies, translating to a CFU/ml value of 19.6×10^7 . This indicates a very high concentration of viable microorganisms in the sample from Hospital road. Highness road waterside sample has 168 colonies, resulting in a CFU/ml value of 16.8×10^7 . While slightly lower than Hospital road, this also indicates a high microbial concentration. With 184 colonies and a CFU/ml of 18.4×10^7 . Beside Engoye hotel shows a high level of microbial presence, close to the values observed at Hospital road. Onuakpo sample has 157 colonies,

corresponding to a CFU/ml of 15.7×10^7 . This is the lowest among the locations with over 150 colonies, yet still indicates a significant microbial presence. Inside Ologakpo Creek sample here has 164 colonies, translating to 16.4×10^7 CFU/ml, indicating a high concentration of microorganisms, similar to Highness's road waterside. Close to Abenidi (Control) Creek samples has the lowest number of colonies, with 92, resulting in a CFU/ml of 9.2×10^7 . Although lower compared to other locations, it still indicates a notable microbial presence.

Table 2: Bacteria isolated and their occurrence

Sample	Isolates
Hospital road waterside	Escherichia coli Psuedomonas aeruginosa Staphylococcus aureus Klebsiella Spp.
Highness house waterside	Hydrogen sulfide producing Salmonella Spp. Escherichia coli Psuedomonas aeruginosa Staphylococcus aureus
Beside Engoye hotel waterside	Escherichia coli Psuedomonas aeruginosa Staphylococcus aureus Klebsiella Spp.
Ōnuakpo waterside	Escherichia coli Psuedomonas aeruginosa
Inside Ologakpo Creek	Escherichia coli Psuedomonas aeruginosa Staphylococcus aureus Klebsiella
Close to Abenidi (Control) Creek	Escherichia coli Psuedomonas aeruginosa Klebsiella Spp.

Table 2 outlines the types of bacteria isolated from various locations. *Pseudomonas aeruginosa* is found in all locations, indicating a common presence and potential for various infections. *Staphylococcus aureus* is Present in four of the six locations, indicating a significant occurrence. *Klebsiella Spp* is found in four of the six locations. Hydrogen sulfide producing *Salmonella Spp* is

only found at Highness road waterside, indicating specific contamination in this location. The data indicates a widespread presence of pathogenic bacteria across the sampled locations, with *E. coli* and *Pseudomonas aeruginosa* being the most common. This suggests significant health risks related to these areas, particularly from fecal contamination and potential infections

Table 3: MPN Analysis Result.

Sample	Combination of positives			MPN Count	95% Confidence	
	10ml	1ml	0.1ml		Lower	Upper
Hospital Road Waterside	5	4	4	350	160	820
Back of Highness's Waterside	4	4	2	47	15	120
Ngoye Road Waterside	4	3	1	33	15	77
Onuakpo Waterside	3	2	2	20	6.8	40
Inside Ologakpo Creek	4	4	2	47	15	120
Close to Abenidi (Control) Creek	1	2	0	6	6	18

The results from the MPN (Most Probable Number) analysis provide valuable insights into the microbial contamination levels at various sampling locations. The MPN analysis reveals a count of 350 with a 95% confidence interval ranging from 160 to 820. This

indicates a very high level of microbial contamination at this location. The wide confidence interval suggests variability in the microbial count, implying that the actual concentration could change significantly.



Figure 1: Mixed and pure cultures on special media.

Figure 1 shows the mixed culture of bacteria from Otuoke River Samples grown on Nutrient Agar demonstrating the diverse microbial community present in the river.

Table 4: Biochemical characterization of the isolates

ORGANISM	Salmonella Spp.	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Klebsiella pneumoniae
TEST					
Catalase	+	+	+	+	+
Oxidase	-	-	+	-	-
Coagulase	-	-	-	+	-
Methyl Red	+	+	-	+	-
Voges-Proskauer	-	-	-	+	+
Citrate	-	-	+	+	+
Indole	-	+	-	-	-
Grams Reaction	-	-	-	+	-

Table 4 provides a biochemical characterization of five bacterial isolates: *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Each organism is tested for various biochemical reactions. The results are given as positive (+) or negative (-) for each test.

DISCUSSION OF FINDINGS

The data from the CFU/ml counts indicate that Hospital road waterside has the highest microbial contamination with a CFU/ml value of 19.6×10^7 , followed by beside Engoye hotel waterside (18.4×10^7 CFU/ml) and Highness road waterside (16.8×10^7 CFU/ml). Inside Ologakpo also shows significant contamination with a

CFU/ml value of 16.4×10^7 . Onuakpo has a lower contamination level at 15.7×10^7 CFU/ml, and Close to Abenidi has the lowest microbial load at 9.2×10^7 CFU/ml. The bacterial isolates and their degree of occurrence provide insights into the types of bacteria present in each location. Hospital road, beside Engoye hotel waterside, and Inside Ologakpo all have *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella spp.*

These bacteria indicate fecal contamination (*E. coli*), environmental and healthcare-associated contamination (*Pseudomonas aeruginosa*), and potential infections from *Staphylococcus aureus* and *Klebsiella spp.* (Ashbolt, 2004; Fouz, & Mounier, 2018). Back of Highness house waterside also has a similar profile but includes Hydrogen sulfide producing *Salmonella spp.*, indicating foodborne illness risks (Scallan *et al.*, 2011). Onuakpo has fewer bacterial isolates, showing only *E. coli* and *Pseudomonas aeruginosa*, while Close to Abenidi has *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella spp.*, indicating a slightly diverse but still significant contamination (Pang *et al.*, 2019). The MPN analysis provides a quantitative estimate of microbial concentration and supports the CFU/ml findings. Hospital road waterside has the highest MPN count of 350 indicating severe contamination (APHA, 2012). Back of Highness house waterside and Inside Ologakpo both have an MPN count of 47, suggesting moderate contamination levels. Beside Engoye hotel waterside shows a slightly lower MPN count of 33, while Onuakpo has an even lower count of 20, indicating less but notable contamination (Hachich *et al.*, 2012). Close to Abenidi has the lowest MPN count of 6, suggesting minimal contamination. These findings have significant implications for public health and sanitation practices in the study area. The high levels of microbial contamination, particularly at Hospital road waterside and beside Engoye hotel waterside, suggest a high risk of infectious diseases. The presence of pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella spp.* indicates the potential for outbreaks of gastrointestinal and respiratory infections, as well as healthcare-associated infections (Ashbolt, 2004; Tong *et al.*, 2015). The detection of hydrogen sulfide-producing *Salmonella spp.* at the Back of Highness house waterside further raises concerns about foodborne illnesses (Scallan *et al.*, 2011; WHO, 2017).

CONCLUSION

The microbial analysis across various locations reveals significant levels of contamination, with Hospital road waterside and beside Engoye hotel waterside showing the highest CFU/ml and MPN counts. These areas also have a diverse range of pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella spp.*, indicating severe health risks due to fecal and municipal waste

contamination. The relationship between the indiscriminate disposal of municipal waste into the Otuoke River and the abundance of heterotrophic bacteria is clearly established through this study. The waste disposal practices contribute significantly to the microbial load in the river, introducing pathogenic bacteria that pose severe health risks. The high counts of CFU/ml and MPN in heavily contaminated areas such as Hospital road waterside and beside Engoye hotel waterside are directly linked to the disposal of municipal waste into the river. The presence of fecal coliforms, including *Escherichia coli*, indicates that waste disposal leads to fecal contamination, further exacerbating the health risks to the local population. Effective waste management and stringent environmental regulations are imperative to reduce microbial contamination and protect public health in Otuoke community and its environs. Public health campaigns should be conducted to educate community inhabitants on the risks of microbial contamination of the river water due to indiscriminate waste disposal into the river. The importance of hygiene and regular environmental sanitation should be emphasized. Regulations regarding waste disposal, water quality, and environmental protection should be implemented and enforced.

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Cite this Article: Onu, B; Preye, TM (2025). Impact of Indiscriminate Waste Disposal into Otuoke River on The Abundance of Pathogenic Heterotrophic Bacteria in The River. *Greener Journal of Environmental Management and Public Safety*, 13(1): 28-35, <https://doi.org/10.15580/gjemps.2025.1.051725086>.