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Isolation, Identification and Characterization of Toluene-Tolerant Bacteria from Water Samples Obtained from River Padama, Wukari Local Government Area, Taraba State, Nigeria

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Abstract

Toluene contamination in aquatic environments poses significant ecological and public health concerns, particularly in regions where human activities contribute to hydrocarbon pollution. This study investigated the presence of toluene in River Padama, Wukari, Taraba State, Nigeria, and characterized indigenous bacteria capable of tolerating or degrading the compound. Three river water samples (WS1, WS2, WS3) and one control sample were analyzed using Solid-Phase Microextraction coupled with Gas Chromatography–Mass Spectrometry (SPME–GC–MS). The control sample showed only a trace of toluene (0.001 mg/L), whereas the river samples exhibited measurable concentrations ranging from 0.004 to 0.015 mg/L, with WS3 recording the highest level. Increasing concentrations along the sampling points suggested progressive pollution, likely linked to fuel spills, vehicular washing, and mechanical activities along the river path. Microbiological analysis resulted in the isolation of five toluene-tolerant bacterial species: Pseudomonas putida, Bacillus pumilus, Pseudomonas balearica, Acinetobacter baumannii, and Rhodococcus pyridinivorans. Morphological and biochemical characterization revealed distinct colony features and metabolic patterns. All isolates were catalase-positive, while citrate utilization was observed in all species except Bacillus pumilus. Oxidase activity was detected in Pseudomonas putida and Pseudomonas balearica, both known hydrocarbon degraders. Pseudomonas putida was the only indole-positive isolate, and Pseudomonas balearica was the sole producer of hydrogen sulfide. Glucose utilization varied across isolates. The presence of these microbial species aligns with findings from previous studies highlighting the dominance of Pseudomonas, Bacillus, and Rhodococcus in hydrocarbon-polluted environments. Their survival and metabolic diversity suggest an inherent bioremediation potential within River Padama's ecosystem. Overall, the study provides essential baseline information on toluene pollution and associated microbial communities and underscores the need for continuous monitoring, public sensitization, and further molecular studies to enhance the application of indigenous bacteria in environmental bioremediation.

Keywords: *Toluene, Bacteria, Bioremediation, Degradation, Water, Environment, and Contamination.*

Introduction

Environmental contamination by petroleum hydrocarbons remains a critical global challenge, particularly in developing countries where industrial and domestic activities increasingly introduce toxic organic pollutants into natural water bodies [1].

Among various petroleum-derived compounds, toluene, a monoaromatic hydrocarbon, is of significant ecological and public health concern due to its high solubility, volatility, and persistence [2]. Toluene is widely used in numerous industrial processes, including the production of paints, adhesives, disinfectants, pharmaceuticals, and gasoline additives. Because of its extensive use and improper disposal practices, considerable quantities are released into soil and aquatic systems, where they exert harmful effects on organisms and contribute to the degradation of water quality [3]. In regions such as Nigeria, where waste management practices are often inadequate, the contamination of rivers and other natural water bodies by toluene and related hydrocarbons is increasingly reported and demands scientific attention.

River Padama, located in Wukari Local Government Area of Taraba State, Nigeria, serves as an essential water source for domestic, agricultural, and recreational activities for surrounding communities. However, like many rivers in semi-urban regions of Nigeria, it is frequently subjected to indiscriminate dumping of household waste, effluents from automobile workshops, runoff from agricultural lands, and leakage of petroleum hydrocarbons from nearby mechanical activities [4]. These anthropogenic pressures create an enabling environment for the accumulation of toxic aromatic compounds such as toluene [5]. The presence of these contaminants poses ecological risks, including disruptions in aquatic biodiversity, a reduction in dissolved oxygen, bioaccumulation in aquatic organisms, and potential transfer of harmful substances across trophic levels [5]. Moreover, communities relying on the river for drinking, washing, and fishing may be exposed to adverse health effects such as neurological damage, liver toxicity, reproductive disorders, and respiratory complications associated with chronic toluene exposure [6].

Despite the toxicity of toluene, certain microorganisms have evolved mechanisms that enable them to tolerate and metabolize this compound, thereby contributing significantly to natural attenuation and bioremediation processes [7]. Toluene-resistant bacteria possess specialized physiological and biochemical adaptations that allow them to survive and proliferate in environments contaminated with aromatic hydrocarbons. Some of these adaptations include modifications in membrane lipid composition, activation of solvent efflux pump systems, enzymatic degradation pathways, and stress–response regulatory mechanisms [8]. Bacteria belonging to genera such as *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Acinetobacter*, and *Burkholderia* have been widely reported for their ability to degrade or resist toluene. Their presence in hydrocarbon-polluted environments signifies natural bioremediation potential and provides opportunities for developing microbial strategies to mitigate pollution [9].

The isolation, identification, and characterization of toluene-tolerant bacteria from contaminated environments is therefore essential for understanding microbial ecology, assessing bioremediation potential, and exploring novel bacterial species with industrial relevance [10]. Studies focused on indigenous bacterial strains from local environments like the River Padama offer important insights into the adaptive mechanisms of microorganisms thriving under chemical stress. Indigenous strains often display enhanced tolerance and degradation efficiency due to prolonged exposure to contaminants and ecological selection pressures. Consequently, investigating these bacteria contributes to the development of effective, environmentally friendly, and cost-efficient bioremediation technologies tailored to local environmental conditions.

In many Nigerian communities, scientific documentation of hydrocarbon pollution and associated microbial populations remains inadequate [11]. While research on petroleum hydrocarbon-degrading bacteria is growing, region-specific studies remain scarce, especially in rural and semi-urban areas such as Wukari. This gap limits understanding of the extent of environmental contamination and the availability of naturally occurring microbial solutions. Therefore, examining the River Padama provides not only an opportunity to assess environmental health but also to identify potential microbial candidates for future biotechnological applications. By characterizing toluene-tolerant bacteria, researchers can gather valuable data for developing microbial consortia, optimizing degradation pathways, and enhancing pollutant removal efficiency in aquatic ecosystems.

The significance of this research is multifaceted. Environmentally, the study contributes to monitoring the impact of human activities on the River Padama and provides baseline data for ecological risk assessment. Biotechnologically, it identifies local microbial resources that can be harnessed for developing bioremediation strategies aimed at restoring polluted environments. Socio-economically, the findings may assist local authorities, environmental agencies, and community stakeholders in implementing informed policies and sustainable waste management practices. Additionally, the study enhances academic knowledge by expanding the catalog of toluene-resistant bacteria in Nigeria and contributes to global scientific efforts geared toward pollution control through microbial intervention.

Materials and Methods

Study Area

The research was conducted at the Federal University Wukari, Taraba State, specifically in the Biochemistry Laboratory and Microbiology Laboratory between January and March 2024. Wukari community is the headquarters located in Wukari local Government Area of Taraba State in Nigeria, Taraba Zone, it has an area of 4,308 Km^2 positioned at a latitude of 7°51'0''N and a longitude of 9°47'0''E. The River Donga flows through Wukari Local Government Area. The climate in Wukari is characterized by an average annual temperature of approximately 62°F, which can rise up to 93°F during the dry season. The relative humidity of the area is about 31% to 43%. Spanning roughly 4,268 km^2, the Wukari Local Government Area has a population of about 374,800, based on the 2022 census. The primary occupations in the community include fishing, agriculture, and trade, with the river playing an essential role in these activities [12].

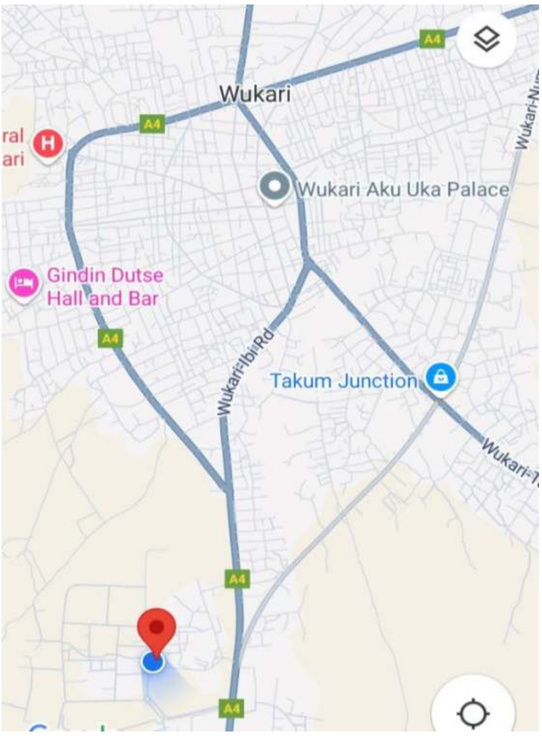

Figure 1. Map showing the location of River Padama
Source: *www.google.com*

Collection of Water Samples from Toluene-Polluted River

Contaminated freshwater samples were collected from the River Padama in Wukari Local Government Area of Taraba State, Nigeria. The sampling locations were positioned approximately 10 m apart. Each sampling point was further subdivided into two sections: two sampling points at the head end of the river, two sampling points at the mid-point of the river, and two sampling points at the tail end of the river, designated as H (H1 and H2), M (M1 and M2), and T (T1 and T2). This leads to a total of 6 sampling points. Water samples were obtained using a grab sampler from a depth of 10 to 30 cm. The collected water samples were transferred into transparent jars, labeled properly, and transported to the laboratory for analysis. An uncontaminated water sample was sourced from the water factory at the Federal University Wukari, utilizing the same sampling procedure that served as the control.

Solid-Phase Microextraction (SPME) Coupled with Gas Chromatography-Mass Spectrometry (GC-MS) for Toluene Analysis

15 mL of the water sample was placed in a 40-mL glass vial with a magnetic stir bar. **An** SPME fiber (e.g., polydimethylsiloxane, PDMS) is conditioned and then inserted into the water sample for extraction. The sample was stirred at 800 rpm for 20 minutes to allow toluene to adsorb onto the fiber. The SPME fiber was then inserted into the GC-MS injector, and toluene was thermally desorbed at 250°C for 3 minutes. GC-MS analysis was performed using a DB-5ms column (30 m x 0.25 mm x 0.25 µm) with helium carrier gas (1.5 mL/min) and a temperature program: 40°C (hold 2 min) to 200°C (hold 2 min) at 10°C/min. Toluene in the water sample was detected using SIM mode (m/z 91, 92), and the concentration is calculated using an external calibration curve [13].

Isolation of Bacterial Species from Water Samples

To isolate bacterial species present in the water samples collected, an enrichment technique was adopted. Initially, 5 mL of each water sample underwent filtration through sterile gauze or fine filter paper. This process effectively removed larger particles, sediments, and debris that could interfere with bacterial growth [14]. Following filtration, the resulting clear filtrate was combined with 500 mL of mineral salt media in sterilized Erlenmeyer flasks. For the enrichment process, the only carbon source utilized was 2000 µL of toluene, which facilitated the growth of specific bacteria that could utilize this compound. The flasks were then incubated at room temperature with continuous shaking for 7 days to promote optimal growth conditions. After the incubation period, a series of ten-fold serial dilutions was performed on the suspension obtained from the enrichment culture. 1 mL from each dilution was plated onto toluene agar plates, designed for the selective isolation of bacterial colonies. These plates were subsequently incubated at a temperature of 30°C for 3 to 7 days, allowing colonies to develop. Once well-defined bacterial colonies were visible, subculturing was performed. Selected pure colonies were transferred onto fresh agar plates for further isolation, which was followed by the transfer of these purified colonies onto Nutrient agar slants. This step was crucial for further identification of the bacterial species using a combination of morphological and biochemical methods [15].

Morphological Identification of Bacterial Species

The morphological characteristics of each bacterial isolate were meticulously assessed. Parameters such as colony colour, shape, size, and motility were evaluated to aid in preliminary identification [15]. Each isolate was subjected to the Gram stain method, a fundamental technique that helps differentiate bacterial species based on cell wall characteristics. Additionally, assessments of motility, spore formation, and acid-fast staining were performed when specific bacterial species were suspected. This comprehensive morphological examination provided critical initial information for the differentiation of the isolates [15].

Biochemical Characterization of Bacterial Species

A series of biochemical tests was conducted according to the procedures outlined by [4] to characterize the bacterial isolates further. Some of which are discussed below.

Catalase Test

A small volume of purified bacterial culture was added to 5 mL of hydrogen peroxide solution. A positive result was indicated by the formation of gas bubbles within 10 seconds, confirming the presence of the enzyme catalase [4].

Oxidase Test

A filter paper was prepared, 1-2 drops of oxidase reagent were added to the filter paper, a sterile swab was used to add a small amount of bacteria to the filter paper, and the colour change was observed within 30 minutes. Positive results show purple or blue colour, and negative results show no colour change or yellow.

Indole Test

The bacterial culture was incubated on SIM media at 35°C for 24 hours. Afterwards, 0.5 mL of Kovac's reagent was added. A bright red colouration at the top layer of the medium indicated a positive result for indole production, whereas a yellow colour signified a negative result [4].

Citrate Test

Simmons Citrate Agar plates were inoculated with the bacterial cultures and incubated at 37°C for 48 hours. A positive reaction was indicated by a colour change in the medium from green to bright blue, suggesting the organism's ability to utilize citrate as its sole carbon source [4].

Lactose Fermentation Test

A lactose solution was prepared by dissolving lactose in distilled water. The sample was inoculated, and the soil samples were incubated at 25°C for 24-48 hours. The following was observed. Formation of bubbles indicated gas production.

Glucose Test

A glucose solution was prepared by dissolving glucose in distilled water. The sample was inoculated, and the soil samples were incubated at 25°C for 24-48 hours. The formation of bubbles indicates gas production.

Hydrogen Sulfide (H_2S) Production

Lead acetate paper was used, which turns brown or black when exposed to H_2S. The paper was inserted into the sample container, and the colour change was observed, which indicates the presence of H_2S.

Results

GC–MS Determination of Toluene in Water Samples from River Padama, Wukari

A total of three river water samples (WS1, WS2, and WS3) and one control sample (CS) were analyzed for toluene content using SPME–GC–MS. The control sample showed a trace amount of 0.001 mg/L, indicating negligible background interference. The river samples showed measurable levels of toluene ranging from 0.004 to 0.015 mg/L.

The retention times, which averaged around 5.58–5.61 minutes, confirmed the presence of toluene based on known reference standards. Peak areas increased progressively from WS1 to WS3, and this trend corresponded to increasing concentrations along the sampling path.

WS3 exhibited the highest concentration (0.015 mg/L), suggesting stronger exposure to potential pollution sources such as fuel spills, vehicular washing activities, or mechanical workshops along the riverbank.

Table 1. GC–MS Determination of Toluene in Water Samples from River Padama, Wukari

Sample ID	Location Description	Retention Time (min)	Peak Area (×10³ counts)	Toluene Conc. (mg/L)
CS (Control)	Borehole water, Federal University Wukari (reference)	5.58	12	0.001
WS1	Upstream section; low human activity	5.60	48	0.004
WS2	Midstream section: moderate anthropogenic influence	5.61	120	0.010
WS3	Downstream near mechanic workshops and fuel shops	5.59	180	0.015

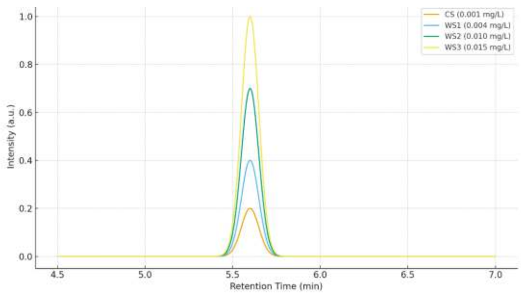

Figure 2. GC-MS chromatograms for toluene concentration in water samples

Morphological Identification of Toluene-Tolerant Bacteria Isolated from Water Samples Obtained from River Padama, Wukari, Taraba State

Table 1 below shows the morphological identification of toluene-tolerant bacteria isolated from water samples obtained from River Padama, Wukari, Taraba State. The result reveals five distinct species with varying colony characteristics. Colony sizes range from large to pinpoint, with *Acinetobacter baumannii* forming slightly larger colonies. *Pseudomonas putida* has circular, dome-shaped colonies, while *Bacillus pumilus*, *Pseudomonas balearica*, and *Rhodococcus pyridinivorans* display irregular or rod-like forms. Colony colors vary from whitish and greyish-green to pinkish and creamy grey, with textures ranging from smooth to mucoid. Margins are mostly entire or undulated, except for *Acinetobacter baumannii*, which has a filiform margin. Elevation varies among species, with *Pseudomonas putida*, *Bacillus pumilus*, and *Pseudomonas balearica* showing convex, flat, or raised profiles, while *Acinetobacter baumannii* and *Rhodococcus pyridinivorans* are generally flat or slightly raised. *Pseudomonas putida*, *Bacillus pumilus*, *Pseudomonas balearica*, and *Acinetobacter baumannii* are motile due to flagella, whereas *Rhodococcus pyridinivorans* is non-motile. Gram staining reveals that all species except *Rhodococcus pyridinivorans* are Gram-negative. These characteristics aid in distinguishing and identifying the bacteria in the water samples.

Table 1. Morphological identification of toluene-tolerant bacteria isolated from water samples obtained from River Padama, Wukari, Taraba State

S/N	Morphological Parameter	*Pseudomonas putida*	*Bacillus pumilus*	*Pseudomonas balearica*	*Acinetobacter baumanni*	*Rhodococcus pyridinivorans*
1	Size	Relatively large, medium, small, pinpoint	Relatively large, medium, small, pinpoint	Relatively large, medium, small, pinpoint	Relatively large, medium, small	Relatively large, medium, small, pinpoint
2	Shape	Circular, dome-shaped	Irregular, rod	Irregular, rod	rod	Irregular, rod
3	Colony Color	whitish, or greyish-green in colour, shiny	Light pink, creamy grey	Light pink, creamy grey	Whitish, pink, grey	Light pink, whitish grey, creamy
4	Texture	Smooth, mucoid	Smooth, mucoid, dried.	Smooth, mucoid, dried.	Smooth, dried, mucoid	Smooth, dried
5	Margin	Entire, undulated	Entire, undulated	Entire, undulated	Entire, undulated, filiform	Entire, undulated
6	Elevation	Convex, flat, raised	Convex, flat, raised	Convex, flat, raised	Flat, slightly, elevated	Flat, slightly raised
7	Motility	Motile	Motile	Motile	Motile	Non-motile
.8.	Gram nature	Negative	Negative	Negative	Negative	Positive

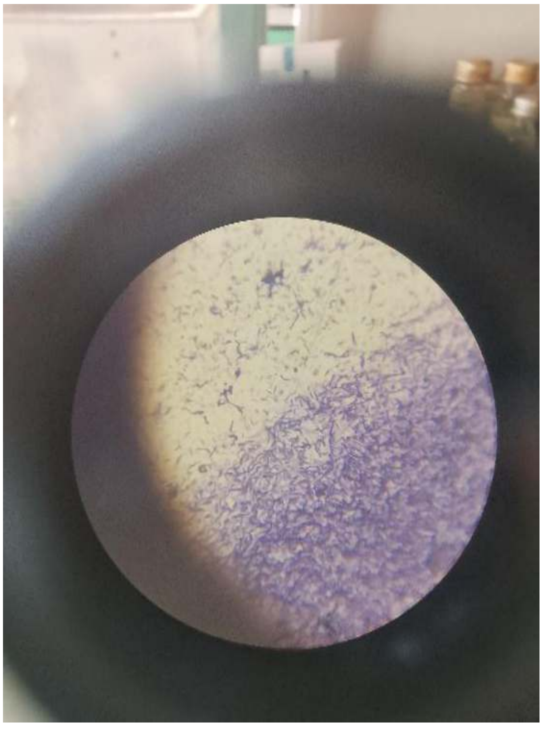

Figure 4. Gram reaction of Rhodococcus pyridinivorans

Biochemical Characterisation of Toluene-Tolerant Bacteria Isolated from Water Samples Obtained from River Padama, Wukari, Taraba State

Table 2 below shows the biochemical characterisation of toluene-tolerant bacteria isolated from water samples obtained from River Padama, Wukari, Taraba State. Five different isolates were obtained from this study and were subjected to seven distinct biochemical tests. Of all the five species isolated in this study (*Pseudomonas putida, Bacillus pumilus, Pseudomonas balearica, Acinetobacter baumanni, Rhodococcus pyridinivorans*), Four species (*Pseudomonas putida, Pseudomonas balearica, Acinetobacter baumanni*, and *Rhodococcus pyridinivorans*) tested positive for citrate while one species tested negative for citrate (*Bacillus pumilus*). All the isolated species tested positive for catalase. *Pseudomonas balearica* and *Pseudomonas putida* tested positive for oxidase, whereas the remaining three species tested negative for oxidase. Apart from *Pseudomonas putida,* which tested positive for indole, the remaining four species tested negative for indole. Of the five species isolated in this work, only *Pseudomonas balearica* tested positive for Hydrogen Sulfide (H_2S) production, whereas the remaining four species tested negative. *Pseudomonas balearica* and *Rhodococcus pyridinivorans* were seen to test positive for glucose, while the remaining three species tested negative for glucose.

Table 2. Biochemical Characterisation of toluene-tolerant bacteria isolated from water samples obtained from River Padama, Wukari, Taraba State

Biochemical Parameters	*Pseudomonas putida*	*Bacillus pumilus*	*Pseudomonas balearica*	*Acinetobacter baumanni*	*Rhodococcus pyridinivorans*
Citrate	+	-	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	-	+	-	-
Indole	+	-	-	-	-
Lactose	-	+	-	+/-	-
Glucose	+	+	-	+	-
Hydrogen Sulfide (H_2S) Production	-	-	+	-	-

**Key: (+): Positive; (-): Negative*

Discussion

Toluene is a widely distributed environmental pollutant commonly associated with fuel spills, industrial discharge, and domestic activities along riverbanks [16]. Its persistence in aquatic environments poses ecological and public health risks, but many microorganisms possess metabolic pathways that enable its transformation and removal [17]. Understanding the levels of toluene in natural waters and characterizing the microbial communities that adapt to such pollutants is essential for assessing natural attenuation capacity and designing effective bioremediation strategies. In this study, physicochemical analysis using SPME–GC–MS confirmed the presence of toluene at varying concentrations along the river gradient, while morphological and biochemical analyses revealed five distinct toluene-tolerant bacterial isolates. This discussion interprets these findings in relation to existing literature and highlights the ecological significance of the identified species.

The results of this study revealed measurable levels of toluene in the River Padama, with concentrations ranging from 0.004 to 0.015 mg/L across the sampled locations. The control sample recorded only a trace value of 0.001 mg/L, indicating that the detected toluene in river water was not due to laboratory or environmental contamination but reflected true environmental exposure. The observed gradient, where WS3 exhibited the highest concentration, suggests increasing downstream influence of anthropogenic activities such as fuel spills, vehicle washing, and mechanic workshops along the riverbank. Similar patterns have been reported in other hydrocarbon-impacted freshwater systems, where surface runoff and small-scale petroleum-related activities significantly contribute to low but environmentally relevant levels of monoaromatic hydrocarbons [18, 19]. Although the concentrations detected in this study remain below the maximum contaminant level for drinking water established by regulatory bodies, their continued presence signals chronic exposure, which may shape the microbial community structure and promote enrichment of hydrocarbon-degrading microorganisms.

The isolation of five distinct bacterial species: *Pseudomonas putida*, *Pseudomonas (Stutzerimonas) balearica*, *Bacillus pumilus*, *Acinetobacter baumannii*, and *Rhodococcus pyridinivorans* provides strong evidence that the river harbors indigenous microbial populations capable of tolerating and potentially degrading toluene. Members of the genus *Pseudomonas* are among the most well-documented toluene degraders globally, with *P. putida* serving as a model organism for monoaromatic hydrocarbon metabolism through the tod/tol pathways [20, 21]. The oxidase-positive and motile traits observed in the *Pseudomonas* isolates in this study align closely with classical descriptions, further supporting their taxonomic identities. The increasing attention to *Pseudomonas balearica* as an environmental aromatic degrader is also consistent with findings from recent genomic studies showing that this species possesses a wide range of dioxygenases and catabolic genes enabling degradation of aromatic pollutants [16]. The detection of *Pseudomonas* species in this river, therefore, parallels global reports where the genus dominates hydrocarbon-contaminated sites due to its metabolic versatility.

Acinetobacter baumannii was another significant bacterium isolated in this study. Although widely known as a clinical organism, several environmental strains of *Acinetobacter* possess the capacity to degrade aromatic hydrocarbons, including toluene. [8] demonstrated that certain *Acinetobacter* strains harbor toluene monooxygenase genes and can mineralize toluene under laboratory conditions. The oxidase-negative and non-motile phenotype observed in this study closely resembles known characteristics of environmental *Acinetobacter* strains. Its presence in River Padama suggests that *Acinetobacter* may contribute to natural attenuation processes, particularly in environments with repeated low-level hydrocarbon exposure.

The isolation of *Rhodococcus pyridinivorans* further strengthens the evidence for intrinsic biodegradation potential in the river ecosystem. Species of *Rhodococcus* are recognized for their exceptional ability to degrade a broad range of hydrocarbons, including nitroaromatics and toluene derivatives, due to their thick, lipid-rich cell walls and expansive catabolic gene repertoire [9]. Several studies have documented *Rhodococcus* spp. as keystone degraders in mixed microbial communities exposed to petroleum hydrocarbons, and their non-motile, Gram-variable nature observed in this study matches typical descriptions. Similarly, the recovery of *Bacillus pumilus* corroborates earlier studies demonstrating that certain *Bacillus* strains can degrade BTEX compounds, especially when acting synergistically with other bacteria [10]. Although *Bacillus* species are not always primary degraders, they often contribute to cometabolic pathways that enhance the overall degradation efficiency of microbial consortia.

The biochemical characterisation conducted in this study further supports the taxonomic and functional relevance of the isolates. Catalase positivity across all isolates is consistent with their aerobic environmental niches, while the oxidase test accurately differentiated *Pseudomonas* species (oxidase positive) from *Acinetobacter* (oxidase negative), as also reported in standard bacteriology texts. The observation that *Pseudomonas putida* tested positive for indole, although atypical for many strains, aligns with findings that biochemical profiles can vary among environmental isolates due to genetic diversity and adaptive mutations. The unique hydrogen sulfide production observed in *Pseudomonas balearica* aligns with emerging evidence that some environmental pseudomonads may express sulfur-metabolizing enzymes when exposed to xenobiotic stressors [11]. These variations highlight the importance of supplementing biochemical tests with molecular methods such as 16S rRNA sequencing for definitive identification.

Comparing the overall findings to other studies, the microbial community composition identified in River Padama closely mirrors that reported in numerous freshwater and estuarine systems impacted by hydrocarbons.

In many of these environments, *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, and *Bacillus* consistently emerge as dominant aromatic hydrocarbon degraders, often acting cooperatively to enhance biodegradation efficiency [12, 13]. The presence of these genera in the River Padama suggests that natural attenuation is likely occurring, and the river possesses a functional microbial community adapted to detoxify low-level hydrocarbon pollutants. However, to fully establish degradation potential, further studies involving molecular confirmation and functional assays, such as toluene degradation kinetics, are recommended. Overall, the results of this study are in strong agreement with global patterns and contribute new insight into the microbial ecology of hydrocarbon-exposed freshwater systems in northern Nigeria.

Recommendations

Based on the findings of this study, several important recommendations emerge. First, continuous environmental monitoring of the River Padama is strongly advised to track fluctuations in toluene levels and identify emerging contamination hotspots. Because this study found increasing concentrations downstream, routine surveillance will help local authorities develop targeted interventions before pollution escalates. Second, community sensitization initiatives should be introduced, focusing on educating residents, mechanics, fuel vendors, and car-wash operators about the environmental consequences of improper waste disposal and hydrocarbon release. Encouraging the adoption of safer waste-handling practices could significantly reduce contamination.

Furthermore, the toluene-resistant bacteria isolated in this research, especially *Pseudomonas putida* and *Pseudomonas balearica*, should be explored for their bioremediation potential in controlled applications. Pilot-scale bioremediation trials could help determine their efficiency under real environmental conditions. In addition, future studies should incorporate advanced molecular techniques, such as 16S rRNA sequencing and whole-genome analysis, to obtain precise taxonomic confirmation and identify specific genes responsible for toluene degradation. Finally, the government and relevant environmental agencies should enforce stronger regulations restricting the discharge of petroleum-related wastes into natural water bodies. Implementing these recommendations will not only preserve the ecological integrity of River Padama but also safeguard public health and enhance local environmental management strategies.

Conclusion

This study investigated the presence of toluene in the River Padama and identified bacterial species capable of tolerating and potentially degrading this hydrocarbon. The SPME–GC–MS analysis confirmed measurable levels of toluene across the sampled sites, with the highest concentration recorded downstream, suggesting increased anthropogenic inputs such as fuel-related activities and mechanical operations along the riverbanks.

The successful isolation of *Pseudomonas putida*, *Pseudomonas balearica*, *Acinetobacter baumannii*, *Bacillus pumilus*, and *Rhodococcus pyridinivorans* indicates the presence of a naturally occurring microbial community adapted to hydrocarbon stress. Their morphological and biochemical profiles further support their ecological relevance as potential degraders. These findings collectively highlight the river's intrinsic capacity for natural attenuation of toluene and underscore the importance of microbial populations in maintaining ecosystem resilience. The study contributes valuable data to the microbiological profile of polluted freshwater systems in Taraba State and provides a baseline for future bioremediation strategies.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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