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Journal of Biological Research and Biotechnology
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Molecular Characterization of Plastic Bio-Degrading Bacteria Isolated from River Ethiope

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Abstract

Plastic pollution is a global problem worsened by the widespread use and durability of plastics, affecting all levels of life. Many African nations, including Nigeria have initiated policies to reduce plastic pollution. This study investigates plastic biodegrading bacteria isolated from water and sediment samples collected from five locations (Umege, Urhouka, Ekrejeta, Oko-erire, Urhouvie) along river Ethiope in June 2023. Physicochemical analyses assessed parameters such as pH, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), nitrate, sulphate and selected heavy metals, additionally, DNA extracted from pure cultures of isolated bacteria were amplified with RAPD-PCR using primer OPA07 and selected molecularly identified isolates monitored for biodegradation of different plastic densities over a 35day period. Physicochemical results indicated water and sediment pH ranged from 5.3 to 5.9 and 5.8 to 6.4 respectively, with detectable levels of copper, iron and zinc while cadmium and lead were undetected in water samples. Random amplified polymorphic DNA-Polymerase chain reaction (RAPD-PCR) analysis revealed seven clades, of the thirty-one bacteria isolates, seven (22.58%) were putatively identified via 16S rRNA sequencing as *Enterobacter bugandensis*, *Lysinibacillus cresolivorans*, *Kosakonia pseudosacchari*, *Bacillus weidmannii*, *Lysinibacillus sphaericus*, *Bacillus tropicus* and *Lysinibacillus tabacifolli* demonstrated plastic degrading capabilities with *Bacillus tropicus* being the most effective. Further research could uncover innovative bioremediation strategies and identify additional plastic-degrading microorganisms from diverse genera or strains, aimed at combating and potentially eradicating plastic pollution globally.

Key Words: Plastic pollution; Biodegradation; Bacterial isolates; Random amplified polymorphic DNA (RAPD); 16S rRNA sequencing

Received: 2nd March 2026 / Accepted: 11th April 2026 / **Published online:**30th April 2026

© The Author(s), under exclusive license to Bio-Research-Journal-of-Biological-Research-and-Biotechnology (JBRB), ISSN: 2705-3822; **print-ISSN:** 1596-7409 (Online); *Open Access This article is available at <https://bio-research.com.ng/index.php/home>*, 2026.

DOI: <https://dx.doi.org/10.4314/br.v24i1.21>

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INTRODUCTION

Plastic pollution has intensified due to the excessive use of synthetic plastics and their remarkable durability, making it one of the most pressing global environmental threats (Kurniawan et al., 2023). In 2010, Africa struggled with waste mismanagement which led to the accumulation of 4.4 million metric tonnes of waste due to inadequate infrastructure across the continent (Nwafor & Walker, 2020). Traditional waste management methods such as landfills, incineration, and recycling have limitations, showing the need for better solutions (Blair & Mataraarachchi, 2021). While most West African countries have implemented policies to help reduce the use of lightweight plastic bags (Dumbili & Henderson, 2020), Nigeria is yet to fully adopt and implement a similar strategy despite having the largest population (195.9 million) and economy in Africa (Goodman et al., 2020). Microbial degradation of plastics, involves breaking down plastics through enzymatic activities by microorganisms and has emerged as a promising eco-friendly solution (Amobonye et al., 2021; Gricajeva et al., 2022). Several studies have shown that microbial metabolic capabilities hold the potential to biodegrade various synthetic plastics (Amobonye et al., 2021). Microorganisms display remarkable metabolic versatility and can effectively degrade various chemical compounds, including specific types of plastics (Qin et al., 2021). Despite this potential, further research is needed to understand the underexplored and precise biochemical mechanisms of plastic biodegradation (Wu et al., 2023). Research has demonstrated the capacity of pure microbial strains to degrade polymers, and understanding these metabolic pathways can enhance biodegradation efficiency (Priya et al., 2022). Microbial bioremediation thus holds substantial promise for addressing plastic pollution sustainably (Oliveira et al., 2020). Leveraging on the natural metabolic capabilities of microbes, effective waste management strategies can be developed to mitigate environmental impact, conserve resources, and protect ecosystems (Najar et al., 2024). The study was designed to isolate, identify and molecularly characterize microorganisms capable of biodegrading plastics. Isolated plastic bio-degraders may contribute to developing innovative approaches for reducing plastic waste, promoting recycling, and fostering more sustainable production and consumption patterns.

Methodology

Study site

The study was conducted in River Ethiope, located in Umuaja, Ukwuani Local Government Area, Delta State, Nigeria. Five sampling points were selected with coordinates: Umeghe: Point A (Latitude: 5.872777651°, Longitude: 6.176932819 °), Urhouka, Abraka: Pont B (Latitude: 5.868233 °, Longitude: 6.174341°), Ekrejeta, Abraka: Point C (Latitude: 5.865229°, Longitude: 6.169389°), Oko-Erere: Point D (Latitude: 5.861136°,

Longitude: 6.164174°), and Urhuovic, Abraka: Point E (Latitude: 5.850561°, Longitude: 6.157043°).

Sample collection

In June 2023, during the rainy season, water and sediment samples were collected from five points along the stretch of River Ethiope. Sediment samples were placed in new unused black sterile containers, tagged, and sealed, while water samples were collected into labeled brown sterile bottles. Both types of samples were transported to the laboratory on ice within 24 hours.

Physicochemical analysis

Physico-chemical analyses were conducted on both water and sediments samples from each sampling point. Horiba U50G Multi water sampler was used to measure electrical conductivity, pH, dissolved oxygen (DO) and total dissolved solids (TDS) in surface water. Standard methods (APHA-AWWA-WEF 2005) were employed for other assessments. Winkler's method was used to measure biological oxygen demand (BOD) and dissolved oxygen (DO) and HACH COD reactor (Model DRB200) was used in determining chemical oxygen demand (COD). Water chloride levels were measured by the Argentometric method 4500-C1-B. Nitrates (NO₃⁻), phosphates (PO₄⁻), and sulphates (SO₄⁻) levels were determined colorimetrically. Heavy metals concentrations in water and sediment samples were analyzed using an Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Analyst 200 model with an air-acetylene flame, using appropriate hollow cathode lamps at specific wavelengths for each metal.

Bacterial culturing and biochemical tests

Collected samples (water/sediment) were serially diluted to 10⁻⁶ and plated (0.1ml from the 10⁻³ dilution) onto nutrient agar in petri dishes. The plates were cultured at 37°C for 24 hours, thereafter; observed colonies sub-cultured until pure cultures were obtained. Morphological and biochemical characteristics including gram staining, oxidase, and catalase tests were conducted and pure cultures obtained were stored on agar slants at 4°C until use.

DNA extraction

DNA was extracted by boiling method; a loop full of pure isolates was resuspended in 300ul of sterile water, vortexed and boiled for 20 minutes. The mixture was then centrifuged at 13,000rpm for 10 minutes and supernatant was transferred into a fresh tube. The purity and DNA concentration was evaluated using a UV5 Nano Mettler Toledo nanodrop. The extracted DNA was stored at -20°C until use.

Random amplified polymorphic DNA (RAPD) analysis

RAPD-PCR amplification was performed with the following parameters: initial denaturation at 94°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension step at 72°C for 10 minutes using OPA 7 primer and 5X Solis BioDyne

PCR master mix (12.5mM MgCl₂) according to manufacturer's protocol. The amplicons were resolved on 1.5% agarose gel, viewed under UV light, and the image was digitally acquired.

Molecular identification of isolates

Polymerase chain reaction was conducted using extracted DNA from selected isolates, universal 16S primer pair and 5X Solis BioDyne PCR master mix (7.5mM MgCl₂) according to manufacturer's protocol with the following parameters: initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 40 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The amplicons were resolved on 1.5% agarose gel, viewed under UV light, and the digital image was acquired. The purified amplicons were sent for Sanger sequencing at Inqaba Biotech West Africa.

Evolutionary analysis

The evolutionary history was determined using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993). The bootstrap consensus tree was created from 100 replicates (Felsenstein, 1985) which represent the evolutionary relationship with branches that reproduced in less than 50% bootstrap replicates being collapsed. The percentage of the bootstrap next to the branches indicates the replicates in which the associated taxa clustered together (Felsenstein, 1985).

Plastic degradation test

The plastic degradation test on selected isolates was carried out according to Kästner et al. (1994) with modifications. Plastic samples of three different densities (Low, Medium and High) were obtained from commercially sourced plastic materials. Each plastic type was cut into uniform squares of approximately 1 x 1 cm using sterile scissors. The pieces were washed with distilled water, rinsed with 70% ethanol to remove surface contaminants and air-dried in a laminar flow hood. For each assay, 1g of sterile high, medium or low-density plastics was transferred into 100mL of sterile minimal salt medium (MSM), where the plastics served as the sole carbon source. The medium was

inoculated with 10mL of a 0.5 Mc Farlands standard of pure cultures. The liquid cultures were incubated at room temperature (24-28°C) with gentle shaking and optical density of 600nm was measured and recorded at seven-day intervals for thirty-five days using a UV-visible spectrophotometer to monitor microbial growth.

Results

Physicochemical analysis

The physicochemical analyses result for water and sediment samples are outlined in Tables 1 and 2, respectively.

Microbial and biochemical

A total of 31 pure bacterial isolates were obtained. Morphological analysis revealed that 16 of the isolates were short rods, 4 were fat rods, 1 long rod, 1 thin rod, 2 rods in sickle shape, 5 cocci, 1 rod in array, and 1 rod with slender spores. The margin of 19.44% of the isolates was filamentous or wavy, while 80.56% had even margins. 11.11% of the isolates displayed a raised appearance, with the majority (88.89%) being flat, 25% of the isolates were translucent, while 75% were opaque. Small and medium sized colonies were observed with varying colors: 30.56% white, 11.11% yellow, 11.11% milky, 44.44% cream, and 2.78% green. 91.67% exhibited a buttery texture, while 8.33% had slimy or sticky texture. Among the 31 isolates, only 1 (3.23%) was catalase negative, while 29 (93.55%) were oxidase negative and 26 (83.87%) were gram-positive.

Random Amplified Polymorphic DNA (RAPD)

The PCR amplification of bacterial isolate genomic DNA using RAPD primer resulted in a total of 66 alleles (Figure 2) across the isolates. Random amplified Polymorphic DNA (RAPD) amplification with OPA 7 primer displayed bands between 400bp to 1350bp on 1.5% agarose gel for all isolates. The phylogenetic tree (Figure 3) revealed the evolutionary relationship between the isolates, suggestive of common ancestry between them.

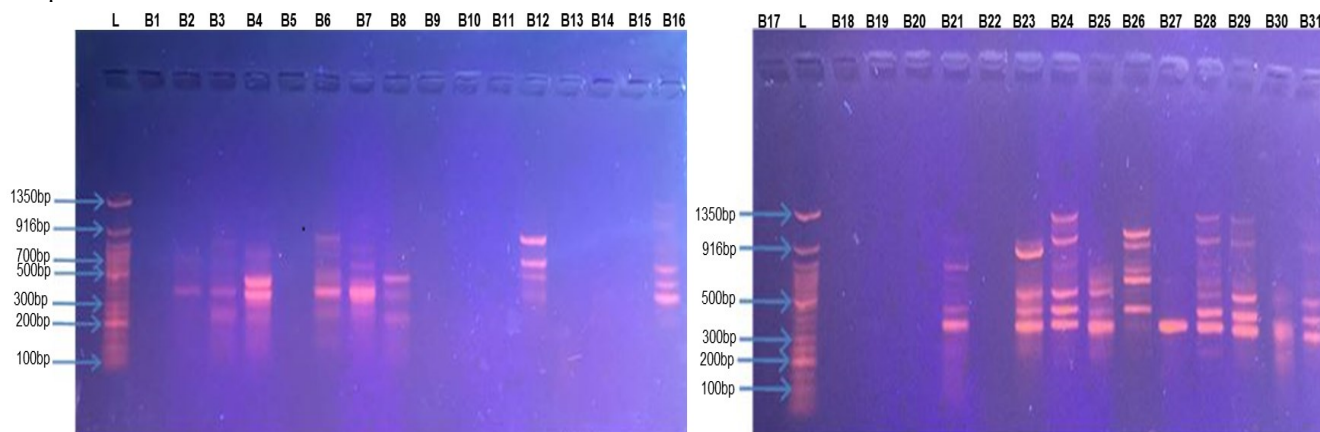


Figure 2: Agarose Gel showing RAPD results for isolates B1-B31 (L- Ladder (100bp))

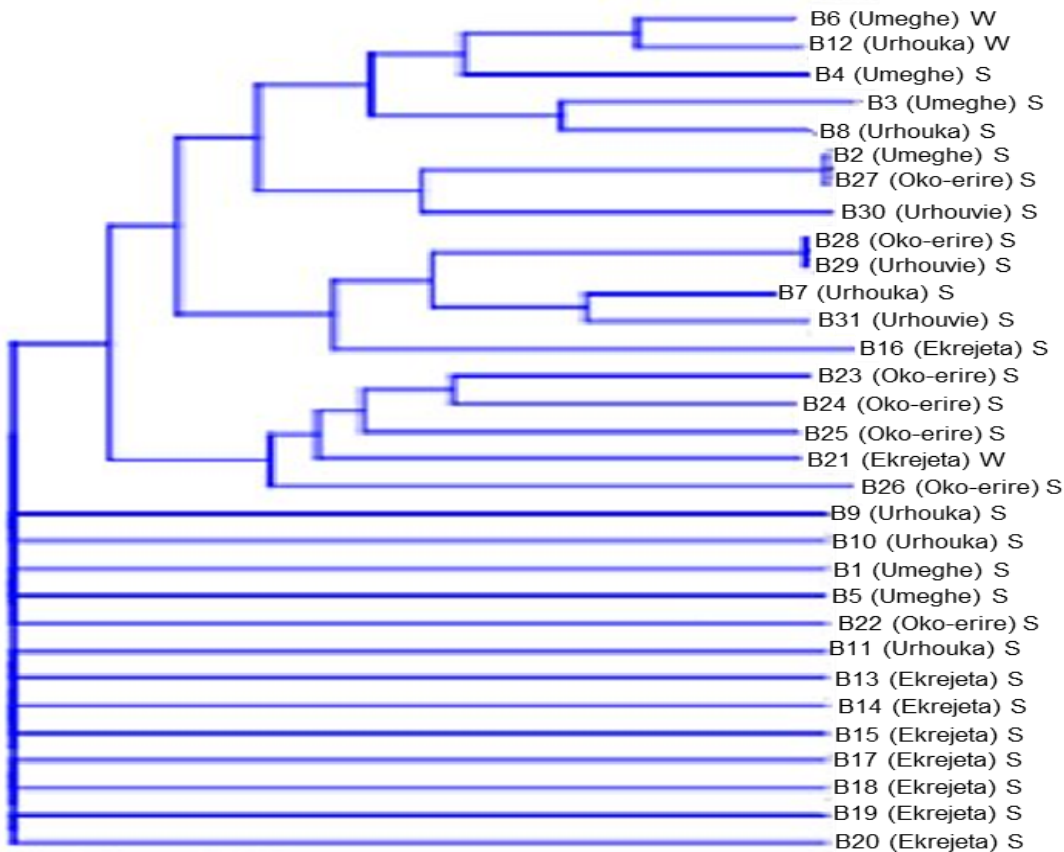


Figure 3: Phylogenetic analysis of RAPD-PCR OPA 7 amplicons of bacteria isolates (S-sediment; W-water)

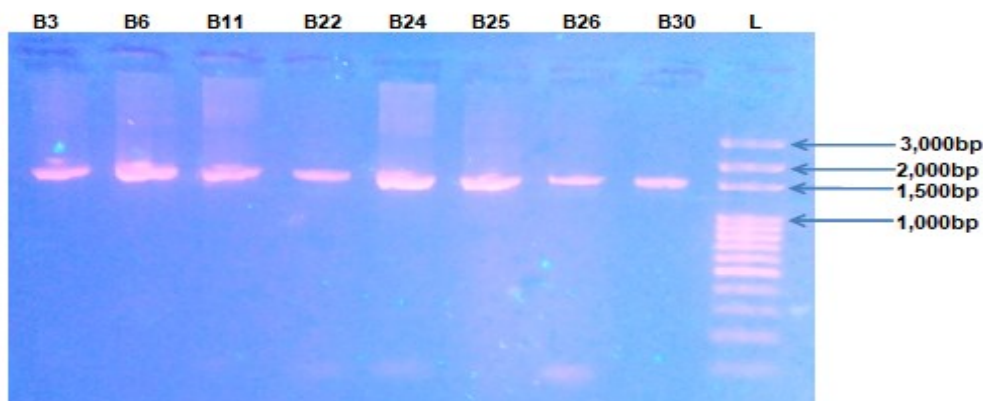


Figure 4: PCR Amplicons of 16S rRNA of selected isolates (L- Ladder (100bp))

Polymerase chain reaction (PCR) and sequencing

The observed amplicons of 16S RNA gene in the eight isolates displayed bands on 1.5% agarose gel, matching the expected band size of 1500bp confirming the presence of bacterial DNA. The clear and uniform intensity in the bands, indicating good DNA quality and PCR efficiency. The 16S rRNA sequences obtained in this study ranged between 677 and 1165 base pairs (bp) in length, which was adequate for comparison with data reported at NCBI database for unidirectional sequencing. Typically, the full length of the 16S rDNA gene is up to 1500 bp (Clarridge, 2004). Unfortunately, sequences for B26 were too short for use in conclusive identification.

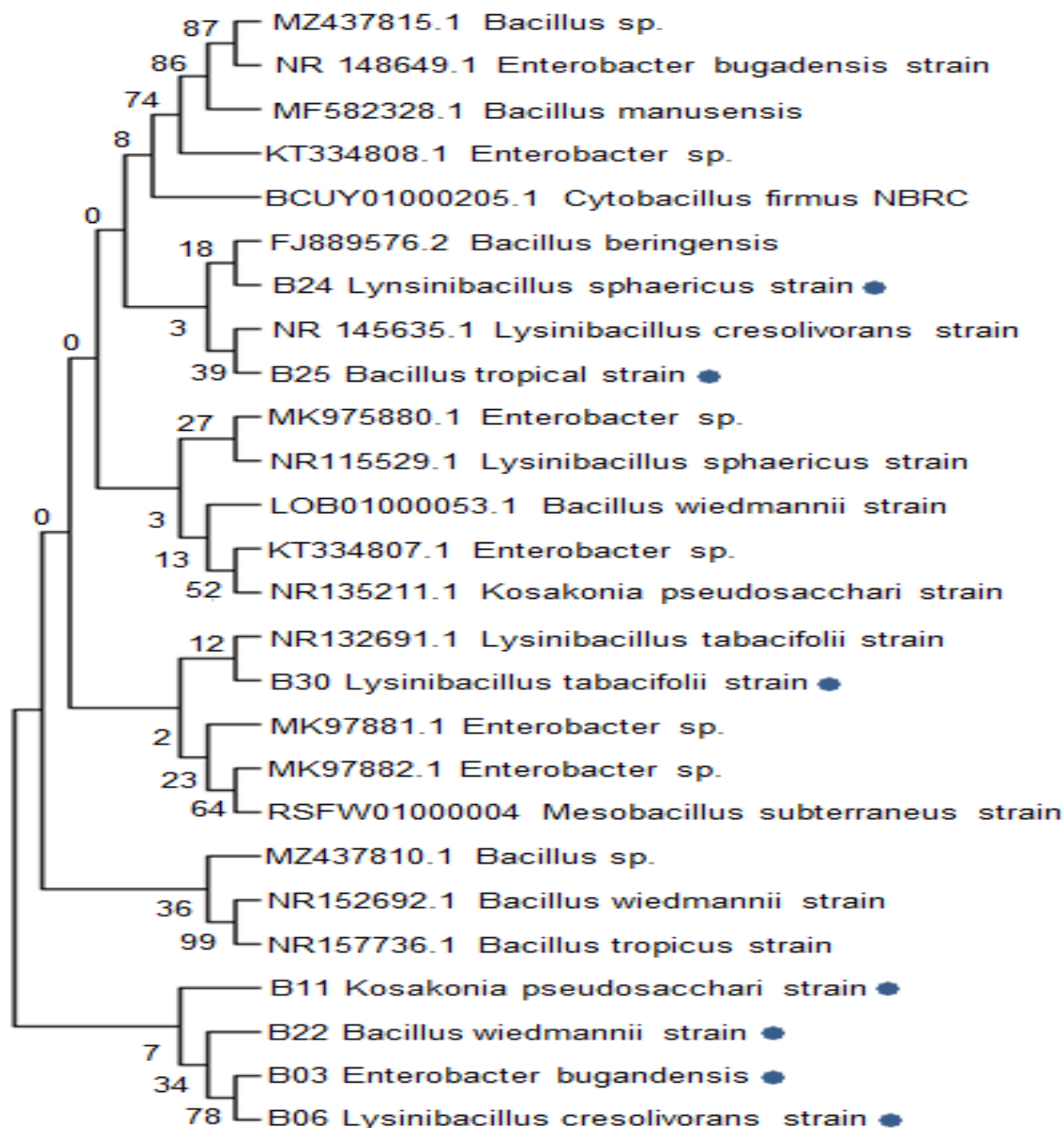
Evolutionary analysis

The phylogenetic tree (Figure 5) shows the evolutionary relationship between the selected isolates and isolates identified as already known plastic bio degraders obtained from other studies.

Plastic degradation

Optical density measurements at 600nm revealed the process by which biodegradation occurred in the low, medium and high density plastics. The different growth phases which are the lag, log, stationary and decline phases were expressed differently in the isolates (Figure 6) and

this reflects the differences in the enzymatic activity of the isolates and their adaptation.



● Selected Isolates from this study

Figure 5: Molecular phylogenetic Analysis of the 16S rRNA subunit of Selected isolates in this study and known plastic degraders in literature.

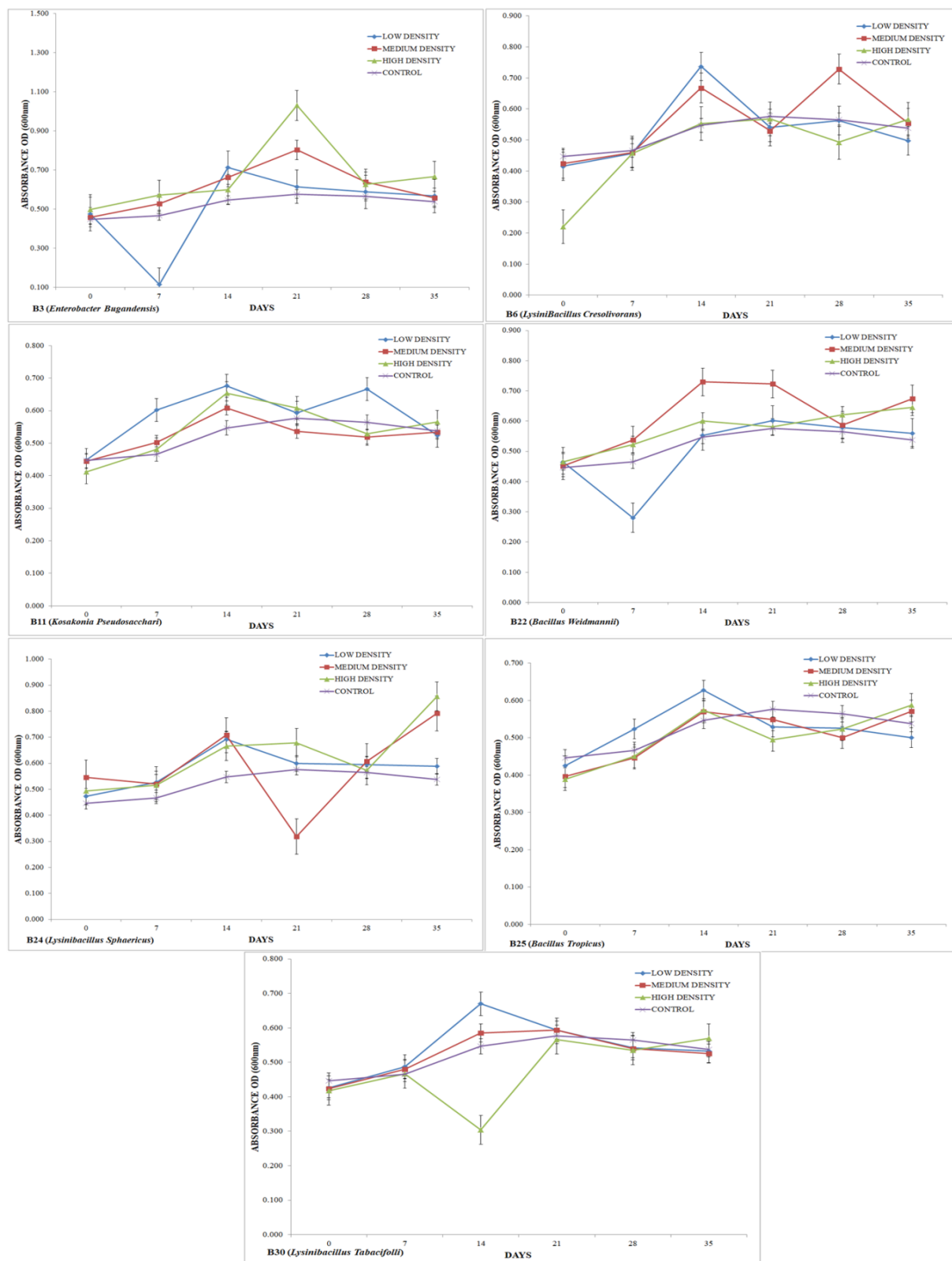


Figure 6: Bacteria growth phases of isolates used for plastic degradation

Plastic weight

On day 35, the weights of plastic densities used in the degradation test were recorded. Four out of seven isolates reduced the weight of low and medium-density plastics, with B11 and B22 demonstrating significant reduction in

low-density plastic weight, B24 and B25 for medium-density plastics and B25 for high-density plastics. Additionally, it is worthy to note that B25 reduced weights

in two out of three plastic densities, indicating their potential as plastic degraders.

Table 3: Difference in plastic weight between day 0 and day 35 of the degradation test

SAMPLE ID	LOW DENSITY	MEDIUM DENSITY	HIGH DENSITY
B3	0.0018	0.0012	0.0022
B6	0.0104	0.0000	0.0035
B11	-0.0016	0.0393	0.0063
B22	-0.0690	0.0738	0.0081
B24	0.0015	-0.0810	0.0053
B25	0.0017	-0.0188	-0.0134
B30	0.0032	0.1321	0.1732
Control	0.0002	0.0002	0.0046

Discussion

The physicochemical analysis conducted on both soil and water samples revealed a low pH at all five sampling points, ranging from 5.8 in Ekrejeta to 6.4 in Umege for sediment samples and 5.3 in Urhouka to 5.9 in Oko-erire for water samples. These readings indicate that both the water and sediment are slightly acidic, falling below the could recommended standard range set by the WHO (World Health Organization, 2022). The acidity be attributed to several factors, including industrial discharge and anthropogenic activities, which may render the water and sediment harmful to aquatic life, humans, and the environment. According to Sunday et al. (2014), the pH levels of most natural water sources typically fall within the range of 6.5 to 8.5, deviating from the neutral point of 7.0. Such variations may result from fluctuations in the CO₂/bicarbonate equilibrium, which can greatly affect the aquatic ecosystems. Total dissolved solids varied from 22 (Umege) to 36 (Oko-erire). Total suspended solids were lowest at Umege, Ekrejeta, and Oko-erire (0.10) and highest at Urhouka and Urhuovie (0.20). Total solids ranged from 22.1 (Umege) to 36.1 (Oko-erire). The low levels of total dissolved solids (TDS), total suspended solids (TSS), and total solids (TS) across all sampling points indicate good water quality suitable for harboring/sustaining aquatic life and potential domestic use. According to Chokor (2021), variations in TSS, TDS, and TS values might arise from slight changes in temperatures, pH, and other environmental factors, leading to the precipitation of particles out of solution or the dissolution of suspended solutes. Low Nitrate levels in both samples ranging from 0.09 (Umege) to 0.15 (Oko-erire) in water samples and 0.17 (Urhuovie) and 0.13 (Umege) in sediment samples, rules out cyanosis should the water be consumed by humans. The low levels of sulfate in sediments may account for it not being detected in water samples which support the conclusion that the water quality is good, although sulfate levels typically do

not affect adults, but children can be sensitive to elevated sulfate levels. According to the WHO's guidelines from 2021, individuals unaccustomed to drinking water with high sulfate levels may experience diarrhea.

Notably high Biological Oxygen Demand (BOD) values observed at all sampling points, indicative of significant degree of organic pollution poses substantial threat to aquatic life and signifies poor water quality, likewise elevated Chemical Oxygen Demand (COD) values at all sampling points, suggests pollution at a moderate level. According to the WHO, the standard for COD of good quality water should be less than 10mg/L. Higher COD levels may indicate a greater presence of microorganisms contributing to organic matter decomposition. Dissolved oxygen (DO), used as an indicator of surface water health, was within acceptable range across all sampling points, signifying adequate oxygen levels, and good water quality that support aquatic life and align with WHO recommendation (WHO, 2021).

Heavy metals such as iron (Fe), copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) were also analyzed in both water and sediment samples. Iron, copper, and zinc were found at high and moderate levels, while lead was not detected in water samples but was present at moderate levels in sediment samples. Cadmium was absent in both water and sediment samples. Zinc (Zn) was the most abundant heavy metal in both water and sediment samples, while cadmium (Cd) was the least abundant. The relative abundance of heavy metals followed this order: Zn > Fe > Cu > Pb = Cd, as both lead and cadmium were not detected in significant amounts. The presence of these heavy metals is a source of concern as they may be bio-accumulated in fish from this river; thus, a survey on edible fauna should be conducted to assess potential risks associated with heavy metal consumption. The acidity of both water and sediment can be influenced by heavy metals; although they do not directly impact the environment's acidity, they can lead to changes in pH levels under certain conditions (Zhang et al., 2018).

The phylogenetic tree (Figure 5) illustrates the evolutionary relationships between the isolated bacteria and known plastic-degrading strains, revealing similarities with strains from *Bacillus* and *Enterobacter* genera, both known for their plastic biodegradation capabilities. The clustering pattern shows the genetic similarity between our isolates and their taxonomic grouping within different genera. B24 (*Lysinibacillus sphaericus*), B25 (*Bacillus tropicus*), and B30 (*Lysinibacillus tabacifolii*) displayed close relationship to these known strains with high bootstrap values noted at specific nodes confirming the reliability of these groupings (Naveed et al., 2024). This suggests that the isolated bacteria species share similar plastic-degrading abilities with the known bio degraders and their potential capability in biodegrading plastics. This finding expands our knowledge of their ecological and

metabolic roles as well as their potential applications in environmental bioremediation (Lv et al., 2024). After establishing phylogenetic relationships among these isolates, the study assessed the plastic-degrading capabilities of selected bacteria isolates from river water and sediment samples based on previous research suggesting that rivers host organisms capable of breaking down plastics (Ali et al., 2021). The results of a plastic biodegradation study using high, medium and low-density plastics show that plastic polymers can serve as carbon sources in terms of reduced weight loss of plastics which demonstrates the relationship between optical density measurements and biodegradation. This observation aligns with the work of Damayanti et al. (2020), who similarly reported a positive correlation between microbial growth and polyethylene degradation. Comparable studies, such as Ojha et al. (2017) and Awasthi et al. (2022), have also shown that an increase in microbial biomass typically coincides with enhanced biodegradation, supporting the relationship observed between OD measurements and weight loss in this study. Isolate B25 (*Bacillus tropicus*) showed the highest plastic biodegrading ability although, B11 (*Kosakonia Pseudosacchari*), B22 (*Bacillus Wiedmannii*) and B24 (*Lysinibacillus sphaericus*) also exhibited high degradation of the plastic densities. The reduction in plastic weight over time, serves as an indicator of microbial action on these plastic materials. Microorganisms degrade polyethylene by targeting its carbon backbone using extracellular enzymes, resulting in weight reduction through biodegradation processes.

Similar genera have been implicated in polyethylene breakdown in several studies. *Bacillus spp.* has widely been reported as potent polyethylene degraders (Das & Kumar, 2015), while *Lysinibacillus* species have also shown LDPE-degrading activity comparable to what was recorded in this study (Kumar et al., 2021). The weight loss observed in the present study is consistent with the degradation trends reported by Navarro-Pérez et al. (2022), who noted that polyethylene-degrading bacteria often show density-dependent degradation efficiency due to differences in polymer branching and crystallinity. Furthermore, Zhang et al. (2022) reported that ultraviolet (UV) radiation alters the properties of polyethylene, making it more susceptible to bacterial attachment and degradation, hence these isolates would have thrived in their natural environment. Unequal cytoplasmic membrane permeability of bacteria, may have contributed to the observed decrease in the optical density of some of the isolates, which have to stabilize pH efficiently before they can metabolize properly prior to kick starting the degradation process, similar observation was reported by Damayanti et al. (2020). The ability of bacteria to degrade plastic can help with good management of plastic waste in the future. The results of a plastic biodegradation study using high, medium and low density plastics showed that plastic polymers can serve as carbon sources since they were being degraded (Damayanti et al., 2020). This highlights the relationship between microbial growth and biodegradation potentials in isolates. The plastic

degradation test gives good understanding of the growth dynamics of the isolates, their metabolic activity and their biodegradation capabilities on these plastic densities. The isolates exhibited different growth phases and degradation including the lag, log, stationary and decline phases, which are important for understanding the process of biodegradation and differ greatly among the isolates and the plastic densities (Navarro-Pérez et al., 2022). The lag phase (Day 0 to Day 7) which is the initiation period for biodegradation when the bacterial cells adapt to the medium and plastic substrates. During this time, metabolic enzymes necessary for degradation are synthesized but the degradation activity that occurs at this phase is limited. B3 (*Enterobacter bugandensis*), B24 (*Lysinibacillus sphaericus*) and B30 (*Lysinibacillus tabacifolii*) had a clear lag phase, as indicated by low optical density (OD) values until day 7. This low optical density values suggest slow acclimatization to the plastic environment. The control samples showed minimal OD changes confirming the absence of biological activity as no isolates are present in them. Studies by Awasthi et al. (2022) and Kumar et al. (2021) similarly describe prolonged lag phases when bacteria encounter polyethylene as the sole carbon source, due to the need to synthesize appropriate catabolic enzymes. The log phase (Day 7 to Day 14) showed an exponential growth phase which is usually characterized by a rapid increase in OD, indicating active bacterial growth and plastic degradation. B3 (*Enterobacter bugandensis*) and B24 (*Lysinibacillus sphaericus*) peaked sharply showing a sharp increase in OD, particularly on low- and medium-density plastic substrates, indicating enhanced metabolic activity and efficiency in the biodegradation process. Similarly, there is a sharp increase in the OD of B6 (*Lysinibacillus cresolivorans*) and B22 (*Bacillus wiedmannii*) on medium-density plastic substrate, demonstrating their capability to effectively degrade plastics with moderate density. Comparable exponential increases were reported in *Pseudomonas*, *Enterobacter*, and *Bacillus* species degrading LDPE (Ojha et al., 2017; Das & Kumar, 2015). The strong performance of B6 and B22 on MDPE parallels findings by Kumar et al. (2021) showing that *Lysinibacillus* spp. preferentially degrade medium-density polymers due to intermediate crystallinity. Between day 14 to Day 28, the phase where most isolates entered a stationary phase, where there is a balance between bacterial growth and substrate availability, usually indicated by stabilized OD values. The OD values of B25 (*Bacillus tropicus*) and B11 (*Kosakonia pseudosacchari*) plateaued across all plastic densities, indicating reduced availability of degradable material or nutrient limitations in the medium. Longer stationary phase was observed with medium-density plastics for isolates like B30 (*Lysinibacillus tabacifolii*), reflecting the complex structure of medium-density plastics, which slows degradation. This is consistent with the report of Navarro-Pérez et al. (2022), who observed plateauing biomass after 2–4 weeks of polyethylene degradation due to substrate limitation. The decline phase (Day 28 to Day 35), marked by a slight decrease in OD, characterised by reduced

bacterial growth due to substrate depletion, accumulation of toxic byproducts, or cell death, was observed in medium-density plastics cultures of isolates B30 (*Lysinibacillus tabacifolii*) and B22 (*Bacillus wiedmannii*) with a significant drop in OD values, which suggests that a large portion of the degradable plastic has been metabolized, or an onset of deliterious feedback mechanism(s). The peaked OD₆₀₀ values, such as for B3 (*Enterobacter bugandensis*) with low-density plastics (1.500 OD₆₀₀) and B6 (*Lysinibacillus cresolivorans*) with medium-density plastics (0.900 OD₆₀₀), highlight the optimal degradation period, which varies depending on plastic density and isolate efficiency. The reduction in OD for B30 and B22 reflects substrate exhaustion and possible accumulation of toxic intermediates, a pattern also reported by Skariyachan et al. (2017) during advanced stages of HDPE degradation. Isolates B3 (*Enterobacter bugandensis*) and B24 (*Lysinibacillus sphaericus*), efficiently degraded low-density plastics, as evidenced by clear OD peaks, B6 (*Lysinibacillus cresolivorans*) and B30 (*Lysinibacillus tabacifolii*) had higher OD values reflecting their ability to degrade medium-density plastics while the OD values of B25 (*Bacillus tropicus*) and B11 (*Kosakonia pseudosacchari*) were less pronounced, indicating the structural resistance of high-density plastics to microbial degradation. The degradation weight loss results (Table 3) further support the findings from the OD measurements. B3 (*Enterobacter bugandensis*) and B24 (*Lysinibacillus sphaericus*) the weight of low-density plastics, consistent with their high OD peaks. Similarly, B6 (*Lysinibacillus cresolivorans*) and B30 (*Lysinibacillus tabacifolii*) notably reduced the weight of medium-density plastics, aligning with their proliferation observed in the OD data. High-density plastics generally showed less weight loss across all isolates, confirming their resistance to microbial degradation.

Conclusion

The increase in plastic pollution poses severe threats to human health, aquatic ecosystem and the environment at large, emphasizing the urgent need for effective plastic-degrading organisms. Ongoing researches across various water bodies have identified numerous microorganisms with plastic-degrading capabilities, offering promising solutions to mitigate and potentially eliminate the problem of plastic pollution. In this study, isolates B3 (*Enterobacter Bugandensis*), B6 (*Lysinibacillus Cresolivorans*), and B30 (*Lysinibacillus tabacifolii*) exhibited high efficiency at degrading low and medium density plastics, this suggests that the combination of multiple organisms during biodegradation may help improve their degradability which would help with improved bioremediation strategies aimed at combating and potentially eradicating plastic pollution globally.

Conflict of interest

The authors have no competing interests.

Authors' contributions

AOA: Conceptualization, experiment design and manuscript editing. MVN and OAA: Experiment and manuscript writing. OOE and ATM: Experiment and data analysis, and all authors approved the final paper.

Acknowledgements

Authors thank 2021 Tertiary Education Trust Fund National Research Fund Intervention No TETF/DR&D-CE/NRF2021 to do this study. Also Dr. V. F. Doherty for Lab facility.

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Tables

Table 1: Physicochemical analysis results

Parameters	Umege	Abraka		Oko-Erire	Urhuovie	W.H.O Recommended Limit		
		Urhouka	Ekrejeta			Abraka	MIN	MAX
Water Samples	pH	5.7	5.3	5.6	5.9	5.7	6.5	8.5
	TDS	22	26	32	36	35	500	-
	TSS	0.1	0.2	0.1	0.1	0.2	30	
	TS	22.1	26.2	32.1	36.1	35.2		
	HARDNESS	ND	ND	ND	ND	ND	30	200
	NITRATE (NO ₃)	0.09	0.11	0.13	0.15	0.14	5	30
	SULPHATE (SO ₄)	ND	ND	ND	ND	ND	200	400
	BOD	13	14	12	11	10	-	-
	COD	27	30	25	24	22	-	-
	DO	6	7.2	6.1	5.6	6.21	-	-
	CADMIUM (Cd)	ND	ND	ND	ND	ND	-	0.01
	COPPER (Cu)	0.14 mg/l	0.174 mg/l	0.19 mg/l	0.22 mg/l	0.188 mg/l	0.005	1.5
	IRON (Fe)	0.421mg/l	0.522 mg/l	0.571 mg/l	0.65 mg/l	0.563 mg/l	0.1	1
	LEAD (Pb)	ND	ND	ND	ND	ND	NIL	NIL
	ZINC (Zn)	0.61 mg/l	0.732 mg/l	0.763 mg/l	0.81 mg/l	0.732 mg/l	5	15
Sediment Samples	pH	6.4	6	5.8	5.9	6.1		
	NITRATE (NO ₃)	0.13	0.15	0.16	0.14	0.17		
	SULPHATE (SO ₄)	5.1	4.3	3.9	4.7	5.3		
	CADMIUM (Cd)	ND	ND	ND	ND	ND		
	COPPER (Cu)	0.244mg/kg	0.072mg/kg	0.284mg/kg	0.121mg/kg	0.144mg/kg		
	IRON (Fe)	0.731mg/kg	0.316mg/kg	0.853mg/kg	0.362mg/kg	0.441mg/kg		
	LEAD (Pb)	0.001mg/kg	0.003mg/kg	0.002mg/kg	0.004mg/kg	0.003mg/kg		
ZINC (Zn)	0.965mg/kg	0.036mg/kg	1.364mg/kg	0.573mg/kg	0.641mg/kg			

ND-Not Detected

Table 2: Identification of isolated bacteria strains and other strains, their GenBank accession number and Sequence similarity

Sample id	Location	Source	Query length (bp)	Identified genus	Genbank description	Query cover (%)	E-value	Sequence similarity (%)	Accession no
B3	Umeghe	Sediment	1162	<i>Enterobacter Bugandensis</i>	<i>Enterobacter Bugandensis</i> 247BMC	13	3e-37	84.21	NR_148649.1
B6	Umeghe	Water	1154	<i>Lysinibacillus Cresolivorans</i>	<i>Lysinibacillus Cresolivorans</i> strain SC03	80	3e-22	87.50	NR_145635.1
B11	Urhouka	Water	925	<i>Kosakonia Pseudosacchari</i>	<i>Kosakonia Pseudosacchari</i> strain JM-387	31	2e-89	87.07	NR_135211.1
B22	Oko-erire	Sediment	1165	<i>Bacillus Weidmannii</i>	<i>Bacillus Weidmannii</i> strain FSL W8-0169	50	3e-99	78.02	NR_152692.1
B24	Oko-erire	Sediment	1124	<i>Lysinibacillus Sphaericus</i>	<i>Lysinibacillus Sphaericus</i> strain IAM 13420	25	4e-80	86.62	NR_115529.1
B25	Oko-erire	Sediment	1098	<i>Bacillus Tropicus</i>	<i>Bacillus Tropicus</i> strain MCCC 1A01406	69	0.0	86.75	NR_157736.1
B30	Urhovie	Sediment	677	<i>Lysinibacillus Tabacifolii</i>	<i>Lysinibacillus Tabacifolii</i> strain NBRC 103108	10	3e-06	81.69	NR_132691.1

Appendix

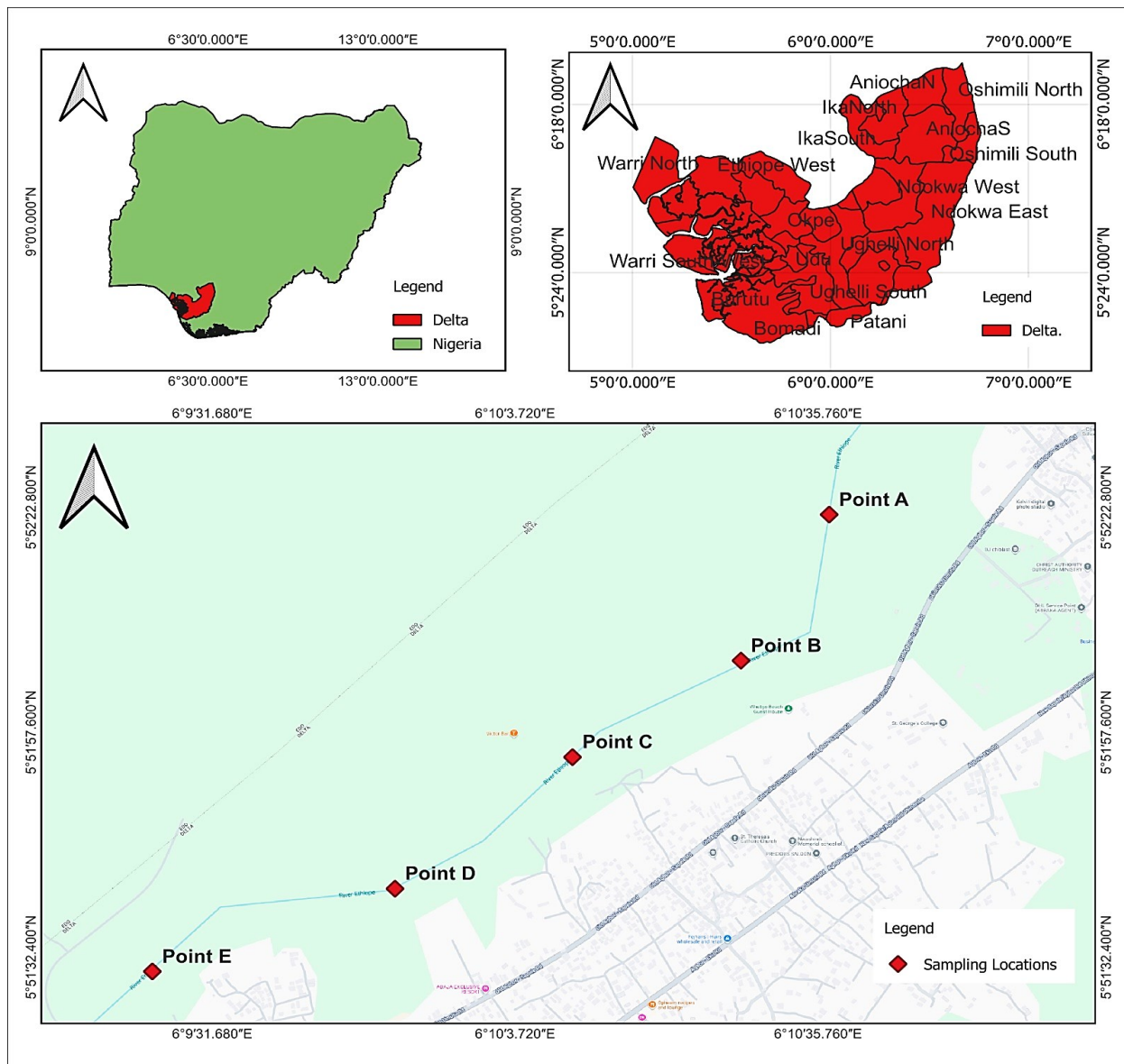


Figure 1: Sample collection sites