GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Assessment of Microbial Composition and Heavy Metals in Wetland Soils of the Niger Delta, Nigeria

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GSC Advanced Research and Reviews, 2025, 25(03), 252-259

Publication history: Received 28 October 2025; revised on 02 December 2025; accepted on 05 December 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.25.3.0364

Abstract

Wetland ecosystems rely heavily on soil microorganisms to drive nutrient cycling and regulate biogeochemical processes. Despite their ecological importance, the composition and functional networks of microbial communities in Niger Delta wetlands remain poorly characterized. In this study, microbial composition of wetland soils from Akwa Ibom, Bayelsa, and Rivers States were investigated using both culture-dependent and culture-independent approaches. Archaeal communities were represented predominantly by Euryarchaeota (39.41%), Thaumarchaeota (32.96%), Candidatus Thermoplasmatota (16.61%), Crenarchaeota (9.86%), and Candidatus Korarchaeota. Microbial population densities revealed total heterotrophic bacteria ranging between 1.7×10^{-6} and 2.3×10^{-8} cfu/g, phosphate-solubilizing bacteria between 1.2×10^{-5} and 1.4×10^{-7} cfu/g, and nitrogen-reducing bacteria between 3.0×10^{-4} and 1.6×10^{-7} cfu/g. Culture-based identification highlighted taxa such as *Bacillus cereus*, *Bacillus subtilis*, *Clostridium* sp., *Desulfovibrio* sp., *Micrococcus* sp., *Serratia* sp., *Pseudomonas* spp., *Staphylococcus* sp., *Pseudomonas putida*, *Nitrobacter* sp., *Chromatium* sp., *Nitrosomonas*, and *Azotobacter* sp. Molecular analysis further revealed nitrogen-fixing potential, with *Klebsiella pneumoniae subsp. pneumoniae HS11286*, *Enterobacter mori*, and *Roseomonas rhizosphaerae* amplifying the nifH gene. Heavy metal concentrations were quantified, with iron (349.64 ± 0.15–539.98 ± 0.90 mg/kg) dominating, followed by zinc (18.56 ± 0.13–56.41 ± 0.50 mg/kg), copper (4.19 ± 1.20–8.14 ± 0.70 mg/kg), chromium (2.42 ± 0.06–3.99 ± 0.13 mg/kg), and nickel (1.12 ± 0.04–2.11 ± 0.06 mg/kg), while cadmium and vanadium consistently remained below detection limits (<0.01 mg/kg). These findings provide the first comprehensive baseline of microbial and heavy metal characteristics of Niger Delta wetland soils, offering critical insights for sustainable management and agricultural productivity.

Keywords: Wetland; Heavy Metal; Archaea; *Nifh* Gene; Bacterial Densities.

1. Introduction

Wetlands are dynamic ecosystems that develop under conditions of periodic or continuous flooding, which lead to soil saturation and the establishment of anaerobic conditions that limit organic matter decomposition. The resulting accumulation of partially decomposed material contributes to soil formation in these habitats. Functionally, wetlands are recognized as critical ecological systems due to their central role in biodiversity support, greenhouse gas regulation, and climate stability (Sui et al., 2021). In many developing regions, particularly Nigeria and other African countries, wetlands are increasingly exploited for agriculture and irrigation (Mohammed et al., 2024), thereby expanding their socio-economic importance while simultaneously heightening their vulnerability to anthropogenic pressures.

Wetland soils are composed of diverse minerals, organic matter, and fragmented rocks that undergo environmental transformations and act as reservoirs for various contaminants. Among these, heavy metals are of particular concern because of their toxicity, environmental persistence, and potential to bioaccumulate through food chains (Akinwale et al., 2024). Anthropogenic activities such as industrial discharge, agricultural intensification, and urbanization have

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significantly elevated the risk of heavy metal pollution in wetlands (Ali et al., 2020). While trace elements like copper (Cu) and zinc (Zn) are essential for plant growth, excessive accumulation of toxic metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and nickel (Ni) poses serious ecological and health hazards (Alengebawy et al., 2021; Okon et al., 2017). In wetland environments, these metals are often immobilized in sediments through adsorption and precipitation; however, changes in environmental conditions may remobilize them into soils and water, leading to secondary contamination (Ali et al., 2020). Consequently, sediment heavy metal concentrations are widely used as indicators of wetland environmental quality (Adnan et al., 2022).

In addition to contaminant dynamics, wetlands harbour rich microbial communities that regulate nutrient cycling and soil fertility. A single gram of soil may contain up to 10 billion microorganisms, including bacteria, archaea, fungi, protists, and viruses (Rosselló-Mora et al., 2001). Despite their ecological importance, less than one percent of these microorganisms have been isolated and characterized using culture-based techniques, leaving much of their diversity underexplored (Bodor et al., 2020). Advances in molecular biology and high-throughput sequencing (HTS) have revolutionized the study of microbial communities, enabling the characterization of phylogenetic and functional diversity through marker genes such as 16S rRNA, 18S rRNA, and ITS regions (Yang et al., 2016).

Microorganisms mediate key biogeochemical processes in wetland soils, including carbon and nitrogen mineralization, phosphorus and sulfur cycling, organic matter degradation, and nitrogen fixation (Meena et al., 2022). These processes directly influence soil fertility, plant growth, and ecosystem stability (Stefanowicz et al., 2021). For example, biological nitrogen fixation, primarily mediated by diazotrophic bacteria and archaea, transforms atmospheric nitrogen (N_2) into ammonia, thereby making it available to plants and other organisms (James, 2017). The enzyme complex nitrogenase, encoded by highly conserved nifH, nifD, and nifK genes, is central to this process and has been extensively used as a molecular marker for identifying and tracking nitrogen-fixing microorganisms across ecosystems (Fuhrman & Davis, 1997). Given the dual significance of wetlands as reservoirs for pollutants and hotspots of microbial diversity, evaluating the heavy metal and microbiological properties of wetland soils is essential for understanding their ecological status, resilience, and management needs. This study investigates these properties in selected South-South states of Nigeria, a region where agricultural intensification and oil exploration exert considerable pressure on wetland ecosystems.

2. Materials and method

2.1. Study Area

The study was conducted in wetlands of Akwa Ibom, Bayelsa, and Rivers States, Niger Delta, Nigeria, located at 3°2′N 36°25′71″, 3°2′N 20°19′14″, and 3°2′N 28°16′55″, respectively. The terrain is flat to gently sloping (0–3°) with uniform parent materials, and vegetation comprises grasses, legumes, ferns, raffia palms, and cultivated farmlands.

2.2. Sampling and Soil Collection

Soils (0–15 cm depth) were collected with sterile augers into labelled polyethylene bags and containers, transported on ice to the University of Uyo laboratories (August 2021). Microbiological assays were performed within 24 h, while samples for physicochemical analysis were air-dried and sieved (2 mm).

2.3. Microbiological Analysis

2.3.1. Dilution and Enumeration

Nine composite samples [L1–L3 (Akwa Ibom), BY1–BY3 (Bayelsa), R1–R3 (Rivers)] were serially diluted (Collins & Lynes, 1976). Heterotrophic bacteria were enumerated on nutrient agar using pour and spread plate methods; phosphate-solubilizers on Pikovskaya's medium; and free-living nitrogen fixers on nitrogen-free medium (Dahal, 2016). Incubation was at 28 °C for 24–48 h (heterotrophs, phosphate-solubilizers) or 5–7 d (nitrogen fixers). Colony counts were expressed as CFU g^{-1} soil.

2.3.2. Characterization of Isolates

Representative colonies were purified by repeated streaking and maintained on nutrient agar slants at 4 °C. Identification followed standard morphological and biochemical procedures (Cowan & Steel, 2003; Holt et al., 1994), including Gram staining, motility, catalase, urease, coagulase, citrate, H_2S production, sugar fermentation, and MR-VP tests.

2.3.3. DNA Extraction and PCR Amplification

Genomic DNA was extracted from pure cultures using the Quick gDNA™ Miniprep Kit (Zymo Research) and stored at −20 °C. The 16S rRNA V1–V3 regions were amplified with primers 27F/518R (Weisburg et al., 1991; Muyzer et al., 1993) in 30 µL reactions containing Taq polymerase, dNTPs, $MgCl_2$, and primers. PCR conditions were: 95 °C for 4 min; 30 cycles of 95 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. Amplicons were sequenced (Beckman Coulter, Inc.) and aligned with Clustal Omega (McWilliam et al., 2013). Phylogenetic affiliations were determined using BLAST (Altschul et al., 1997).

The nifH gene was amplified with PolF/PolR, IGK3/DVV, and Ueda19F/Ueda407R. Reactions (30 µL) contained Taq polymerase, $MgCl_2$, dNTPs, and primers. Touchdown PCR was applied for PolF/PolR (63–55 °C), while IGK3/DVV and Ueda19F/Ueda407R used annealing temperatures of 59 °C and 52 °C, respectively. Expected amplicon sizes were ~360 bp (PolF/PolR) and ~400 bp (IGK3/DVV, Ueda19F/Ueda407R). Sequences were aligned using MUSCLE (Edgar, 2004a,b).

2.3.4. 16S Metagenomic Analyses

Community DNA Extraction

Genomic DNA was extracted from 0.25 g of each soil sample using the ZymoBIOMICS™ DNA extraction protocol (Zymo Research). Briefly, samples were homogenized in ZR BashingBead™ Lysis Tubes with 750 µL of lysis buffer and subjected to bead-beating, followed by centrifugation at 10,000 × g for 1 min. Supernatants were passed through Zymo-Spin™ IV filters, and DNA was subsequently bound to Zymo-Spin™ IIC columns with soil DNA binding buffer. Sequential washes were performed with DNA pre-wash and DNA wash buffers, and elution was carried out with 100 µL of elution buffer. The eluates were further purified through Zymo-Spin™ IV-HRC filters to remove PCR inhibitors, yielding high-quality genomic DNA extracts (Poly et al., 2001).

PCR Amplification and Sequencing

Extracted DNA was amplified using a universal primer set (341F/785R) targeting the V3–V4 region of the 16S rRNA gene in a 96-well thermal cycler (2E™ UK). PCR products were gel-purified, end-repaired, and ligated with Illumina-specific adapters (NEBNext Ultra II DNA Library Prep Kit). Following quantification, samples were indexed with NEBNext Multiplex Oligos (Dual Index Primers Set 1) and purified with AMPure XP beads. Libraries were sequenced on an Illumina MiSeq platform (MiSeq v3 kit, 600 cycles), generating ~20 Mb per sample (2 × 300 bp paired-end reads). Bioinformatics analyses, including taxonomic assignment, were conducted using BLAST v2.24 (NCBI) and CLC Genomics Workbench v7.5.1 (Poly et al., 2001).

2.4. Heavy Metal Analysis

Soil samples were oven-dried at 80 °C for 48 h, gently disaggregated, and sieved to <63 µm. For digestion, 2.0 g of soil was treated with aqua regia (0.3 mL HNO_3 [65%] and 6.0 mL HCl [37%]). The mixture was heated to near dryness, cooled, and subsequently diluted with 20 mL of 5 M HNO_3. After overnight stabilization, samples were filtered, transferred to 100 mL volumetric flasks, and made up to volume with 0.5 M HNO_3 (Abiaobo et al., 2017; Binning & Baird, 2001). Concentrations of Fe, Zn, Ni, Cu, Pb, Cd, Cr, and V were quantified using a flame atomic absorption spectrophotometer (Optima 3000, Perkin Elmer). Detection limits were 0.02 mg/kg for Cr, 0.05 mg/kg for Pb, 0.1 mg/kg for Fe, 0.01 mg/kg for Ni, 0.002 mg/kg for V, 0.01 mg/kg for Zn, and 0.01 mg/kg for Cu, with recovery rates of 95–100%.

2.5. Statistical Analysis

All data were analyzed using SPSS v20.0 (IBM Corp., USA). One-way ANOVA and Pearson correlation tests were performed on log-transformed datasets where appropriate. Results are expressed as mean ± standard deviation, and statistical significance was considered at $p < 0.05$.

3. Results and Discussion

3.1. Microbial Diversity of Wetland Soils in Akwa Ibom, Bayelsa, and Rivers States

Wetland ecosystems of the Niger Delta harbour diverse microbial assemblages that play essential roles in soil fertility, nutrient transformations, and global biogeochemical cycles. The present study combined culture-based and metagenomic approaches to investigate bacterial and archaeal communities across wetlands in Akwa Ibom, Bayelsa, and Rivers States.

3.2. Bacterial Abundance and Community Structure

Culturable bacterial counts varied significantly among the sites, with total heterotrophic bacteria ranging from 1.7×10^6 to 2.3×10^8 cfu/g, phosphate-solubilizing bacteria from 1.2×10^5 to 1.4×10^7 cfu/g, and nitrogen-fixing bacteria between 3.0×10^4 and 1.6×10^7 cfu/g. Heterotrophic bacteria were consistently the most abundant group, reflecting their broad metabolic capacity to utilize diverse organic substrates. Similar trends have been reported in soils and sediment where heterotrophs dominate microbial populations due to their ecological versatility (Umana *et al*., 2017 a, b; Udosen and Umana, 2018).

Morphological, cultural, and biochemical profiling identified 13 bacterial taxa, including *Bacillus cereus, B. subtilis*, *Clostridium* sp., *Desulfovibrio* sp., *Micrococcus* sp., *Serratia* sp., *Pseudomonas* sp., *Staphylococcus* sp., *P. putida*, *Nitrobacter* sp., *Chromatium* sp., *Nitrosomonas*, and *Azotobacter* sp. Sequencing of selected isolates further identified *Bacillus marasmi, Klebsiella pneumoniae subsp. pneumoniae HS11286, Enterobacter mori*, and *Roseomonas rhizosphaerae*, all carrying the nifH gene, a conserved marker for nitrogen fixation. This highlights the functional potential of wetland soils to sustain nitrogen inputs, though possession of nifH does not always translate to active nitrogenase expression (Udosen et al., 2018; Braun et al., 1999).

The coexistence of autotrophic groups (Nitrosomonas, Nitrobacter, Chromatium) with heterotrophic and facultative bacteria suggests strong interactions between carbon, nitrogen, and sulfur cycles. Variations in phosphate-solubilizing and nitrogen-fixing bacterial counts between sites likely reflect local differences in soil nutrients and organic matter inputs, consistent with the principle that functional microbial diversity shifts with community structure (Tribedi & Sil, 2013).

3.3. Archaeal Composition and Functional Roles

Metagenomic analysis revealed five archaeal phyla across the wetlands: Euryarchaeota, Thaumarchaeota, Candidatus Thermoplasmatota, Crenarchaeota, and Candidatus Korarchaeota. Archaea accounted for 1.12% of total microbial reads (1631 sequences). Among them, Euryarchaeota (39.41%) was the dominant lineage, with methanogenic taxa underpinning methane emissions and organic matter turnover in wetlands. This aligns with global reports of Euryarchaeota prevalence in aquatic sediments (Lang et al., 2018). Thaumarchaeota (32.96%) was the second most abundant phylum, particularly enriched in Bayelsa soils (61.30%). Known for ammonia oxidation, their dominance under low ammonium conditions (<15 μM) suggests that nitrogen availability strongly shaped their distribution (Pratscher et al., 2011; Kim et al., 2021).

Crenarchaeota (9.86%) exhibited strong site variation, with lowest abundance in Bayelsa soils (3.39%). This may be linked to soil pH, as prior studies showed that acidic soils (pH 4.5–6.0) suppress certain Crenarchaeota groups (Lehtovirta et al., 2009). Candidatus Thermoplasmatota contributed 16.61%, representing metabolically versatile taxa associated with organic matter degradation. Interestingly, Candidatus Korarchaeota was detected exclusively in Rivers State wetlands (1.16%), expanding the known ecological distribution of this rare archaeal lineage beyond geothermal systems (Reigstad et al., 2009).

Together, bacterial and archaeal assemblages reveal the Niger Delta wetlands as reservoirs of functional microbial diversity. Heterotrophic bacteria dominate decomposition and nutrient recycling, while diazotrophic groups contribute to nitrogen enrichment. Simultaneously, archaeal methanogens (Euryarchaeota) drive methane emissions, and ammonia-oxidizing archaea (Thaumarchaeota) regulate nitrogen turnover. Site-specific patterns in microbial abundance—shaped by soil pH, nutrient availability, and hydrological inputs—underscore the sensitivity of wetland microbial ecology to local environmental gradients.

3.4. Heavy Metal Concentration of Wetland Soils

The concentrations of selected heavy metals in wetland soils across Akwa Ibom, Bayelsa, and Rivers States are summarized in Table 4.2. The observed ranges were as follows: iron (Fe), 349.64 ± 0.15 to 539.98 ± 0.90 mg/kg; zinc (Zn), 18.56 ± 0.13 to 56.41 ± 0.50 mg/kg; copper (Cu), 4.19 ± 1.20 to 8.14 ± 0.70 mg/kg; chromium (Cr), 2.42 ± 0.06 to 3.99 ± 0.13 mg/kg; nickel (Ni), 1.12 ± 0.04 to 2.11 ± 0.06 mg/kg; while cadmium (Cd) and vanadium (V) were consistently below detection limits (<0.01 mg/kg). The general order of abundance was: Fe > Zn > Cu > Cr > Ni > Cd ≈ V. Wetlands are widely recognized as sinks for heavy metals, largely due to sedimentation, adsorption onto organic matter, and ligand exchange processes (Olatunde et al., 2022). However, the mobility and bioavailability of these metals are strongly influenced by soil physicochemical parameters, particularly pH and redox status (Okon et al., 2017). Thus, wetland contamination not only threatens ecosystem integrity but also raises concerns for food security and water resource safety in adjoining communities.

In this study, cadmium and vanadium were found at negligible levels (<0.01 mg/kg), values far below the UNEP (2013) threshold of 0.35 mg/kg and the FAO/WHO (2011) guideline value of 0.8 mg/kg for agricultural soils. The absence of Cd likely reflects its minimal contribution from local parent materials or basement rock composition. This finding is consistent with Chibuike et al. (2019), who reported negligible Cd levels in wetland soils. Chromium concentrations (2.42–3.99 mg/kg) were highest in Rivers State and lowest in Akwa Ibom, yet all values remained well below the UNEP (2013) limit of 70 mg/kg. Similarly, nickel concentrations (1.12–2.11 mg/kg) were substantially lower than the FAO/WHO permissible limit of 35 mg/kg, suggesting limited risk from Ni contamination in the studied wetlands.

Zinc concentrations ranged between 18.56 and 56.41 mg/kg, also within safe thresholds (<300 mg/kg; MAFF, 1992). However, these values were considerably higher than those reported for wetlands at the Federal University of Agriculture, Abeokuta, Nigeria (1.64–2.37 mg/kg; Olatunde et al., 2022). Such differences likely reflect regional variations in land use, hydrology, and anthropogenic inputs. Copper concentrations (4.19–8.14 mg/kg) were below the UNEP threshold of 15 mg/kg, contrasting with the markedly higher levels (51.20 mg/kg) reported for Katanga wetland soils, Uganda (Nabulo et al., 2008). Geographic and climatic differences, as well as differential anthropogenic pressures, may explain this disparity.

Iron was by far the most abundant element (349.64–539.98 mg/kg), exceeding the UNEP range of 0.16–22.3 mg/kg for soils. Elevated Fe levels can be attributed to the natural abundance of iron in the Earth's crust, leaching from iron-rich parent rocks, and anthropogenic activities such as industrial effluents, waste disposal, and corrosion of iron-containing materials. Such enrichment is consistent with observations in other heavily industrialized or densely populated wetland systems. Correlation analysis revealed strong positive associations between chromium and iron ($r = 0.997$, $p < 0.05$), and between copper and iron ($r = 0.998$, $p < 0.05$). These strong relationships suggest that the metals may share common geogenic or anthropogenic sources, possibly linked to geological substrates or effluent discharges. The metal concentrations detected in these wetlands largely fall within international safety thresholds, with the exception of iron, which exhibited consistently elevated levels. This finding underscores the dual role of wetlands as both natural regulators and potential reservoirs of metal contaminants. Long-term monitoring is therefore essential to assess cumulative impacts on ecological health and potential risks to human populations dependent on wetland resources.

4. Conclusion

Wetland ecosystems of the Niger Delta are reservoirs of diverse microbial assemblages and variable heavy metal loads, both of which underpin their ecological functioning and environmental vulnerability. The bacterial community was dominated by metabolically versatile heterotrophs, alongside functionally significant diazotrophs, nitrifiers, and sulphur-metabolizing taxa. Archaeal lineages, particularly methanogenic Euryarchaeota and ammonia-oxidizing Thaumarchaeota, further highlight the critical roles of wetlands in greenhouse gas dynamics and nutrient cycling. These microbial groups not only sustain soil fertility but also mediate essential transformations that link carbon, nitrogen, and sulphur cycles. Site-specific differences in community structure reflected the influence of soil pH, nutrient status, and hydrological inputs, underscoring the sensitivity of microbial ecology to local environmental gradients. Heavy metal analyses revealed consistently low concentrations of cadmium, vanadium, chromium, nickel, zinc, and copper, all within international safety thresholds. However, elevated iron levels—substantially above UNEP standards—indicate enrichment from both geogenic and anthropogenic sources. Strong correlations between iron, copper, and chromium suggest shared origins, possibly linked to parent rock leaching and effluent discharges. While the wetlands currently act as sinks for most trace metals, the persistence of high iron concentrations raises concerns about long-term accumulation and ecological risks. These findings emphasize the dual ecological significance of Niger Delta wetlands: as dynamic microbial hotspots sustaining global biogeochemical processes, and as vulnerable systems prone to contaminant loading. Maintaining their integrity requires integrated management that balances resource use with ecosystem conservation. Long-term monitoring of microbial communities and trace metals is essential, not only for safeguarding wetland biodiversity and soil fertility but also for ensuring the sustainability of ecosystem services critical to surrounding communities.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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