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# ISOLATION AND EVALUATION OF NICKEL-TOLERANT BACTERIA FROM AQUATIC PLANT ROOTS FOR NICKEL BIOSORPTION IN LOKOJA, KOGI STATE

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**ABSTRACT**

Nickel contamination in aquatic ecosystems poses increasing ecological and public-health risks, particularly in rapidly urbanizing riverine environments. This study aimed to isolate and evaluate nickel-tolerant bacteria associated with the roots of aquatic plants for their potential application in nickel biosorption within Lokoja, Kogi State, Nigeria. Water and root samples of *Eichhornia crassipes*, *Nymphaea lotus*, and *Limnocharis flava* were collected from three pollution-prone riverbank locations. Standard microbiological procedures were used for bacterial enumeration, isolation, and phenotypic characterization, while nickel tolerance was assessed using $NiCl_2$-supplemented media. Biosorption assays were conducted using dried bacterial biomass across varying nickel concentrations (5–20 ppm) and pH values (3–8). Data were analyzed using one-way ANOVA at $p < 0.05$. Four isolates: *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Bacillus subtilis*, exhibited significant nickel tolerance (MIC > 3.0 mg/mL). *P. aeruginosa* demostrated the highest biosorption efficiency (84.63% at 20 ppm), with maximum removal for all isolates occurring at pH 7. Physicochemical analysis of water indicated moderate but increasing pollution downstream. These findings suggest that water plant root-associated bacteria, notably *Pseudomonas* spp. possess substantial potential for effective nickel bioremediation. It is therefore recommended that these isolates be further evaluated in pilot-scale bioremediation trials, particularly in resource-limited or nickel-impacted water systems.

**Keywords**: Aquatic plants; Bacterial isolates; Biosorption; Nickel contamination

## INTRODUCTION

Human activities continually expose both people and the environment to harmful pollutants. Industrial development over the past century has expanded at an extraordinary pace, increasing the pressure on the planet's natural resources and worsening global pollution concerns (Aruna Devy and Vasudevan, 2025). The environment is now contaminated by many pollutants, including inorganic ions, organic compounds, radioactive materials, gaseous pollutants, and nanoparticles (Walker *et al.*, 2012; Briffa *et al.*, 2020; Thangavelu *et al.*, 2022). Among these, heavy metals have drawn particular attention due to their toxicity, persistence, and tendency to accumulate in living organisms (Laoye *et al.*, 2025). Several heavy metals such as manganese, lead, chromium, nickel, cadmium, zinc, copper, aluminum, mercury, and iron are classified as priority environmental pollutants under the European Directive 2010/75/EU (Briffa *et al.*, 2020; Lau *et al*., 2021)

Nickel, one of the most widely used heavy metals, is frequently released into the environment through industrial activities (Genchi *et al*., 2020). Electroplating, alloy production, mining, metal refining, and smelting industries discharge significant quantities of nickel into surrounding water bodies and soils (Genchi *et al*., 2020; Oladimeji *et al*., 2024). Nickel is widely used across many sectors such as construction, electronics, transportation, and aeronautics, and as a result, large amounts eventually enter the ecosystem, contaminating water and threatening both human and ecological health (Genchi *et al*., 2020; Begum *et al*., 2022). In riverine communities where multiple human and industrial activities converge around major water bodies, the likelihood of nickel entering aquatic environments is even higher. Heavy metal contamination is a global concern, and nickel is particularly problematic due to its toxicity and non-degradable nature (Briffa *et al.*, 2020). Exposure to nickel concentrations above the World Health Organization drinking water limit of 0.5 mg/L has been associated with serious health risks (WHO, 2021). Nickel is classified as a human carcinogen and has been associated with embryotoxic and teratogenic effects (Léonard *et al.*, 1981; Saini *et al.*, 2013). At high levels, nickel exposure may lead to dermatitis, gastrointestinal distress, respiratory problems, nausea, vomiting, cyanosis, and general weakness (Genchi *et al*., 2020; Gates *et al*., 2023). These health challenges highlight the urgent need to address nickel contamination, especially in communities that rely directly on natural water sources for domestic use, agriculture, and fishing (Levická and Orliková, 2024). Traditional methods for removing heavy metals from water, such as ion exchange, membrane filtration, evaporative recovery, and chemical precipitation, are often expensive and may generate hazardous sludge requiring special disposal (Ayach et al., 2024). Because of these limitations, researchers are increasingly exploring eco-friendly alternatives. Biosorption has emerged as a promising technique for heavy metal removal due to its low cost, efficiency, and environmental compatibility (Yaashikaa *et al*., 2021). Microorganisms such as bacteria, algae, yeasts, and fungi possess structural and chemical components that enable them to bind and immobilize heavy metals. Their high surface area, rapid adsorption kinetics, and natural resilience in polluted environments make them attractive biosorbents for water treatment (Aslam *et al.*, 2025).

Aquatic plants have been found to play an important role in natural water purification systems by interacting closely with surrounding microorganisms. Their roots provide a unique habitat rich in diverse microorganisms, including bacteria that are naturally adapted to heavy-metal-stressed environments (Demarco *et al.*, 2023). Bacteria often possess specialized mechanisms that allow them to resist, transform, and sequester toxic metals such as nickel, enabling them to survive in contaminated environments (Mathivanan *et al.*, 2021). Harnessing these naturally evolved capabilities

provides a cost-effective and sustainable biological strategy for mitigating heavy metal pollution. In locations such as Lokoja, where rivers and floodplains support abundant aquatic vegetation, root-associated bacteria may serve as important natural agents for mitigating heavy metal contamination and restoring ecological balance. These plant–microbe interactions create biologically active zones capable of transforming, immobilizing, or reducing toxic metals in the surrounding water. Considering the increasing environmental concerns, the high cost and waste-generation problems associated with conventional remediation technologies, and the need for sustainable, nature-based solutions, investigating nickel-tolerant bacteria inhabiting aquatic plant root zones is both timely and highly relevant. Such microorganisms offer the potential to provide safe, inexpensive, and efficient strategies for lowering nickel concentrations in polluted water bodies. Their application in Lokoja, and in similar environmentally stressed regions, could contribute significantly to long-term water quality improvement and ecosystem recovery. Therefore, the aim of this study was carried out to isolate and evaluate nickel-tolerant bacteria from the roots of aquatic plants for their potential to biosorb nickel in aquatic systems in Lokoja, Kogi State.

## MATERIALS AND METHODS

### Study Area

The study was carried out in Lokoja, Kogi State, Nigeria, a critical, rapidly expanding riverine city strategically located at the confluence of the River Niger and the River Benue, roughly centered at 7°48′N,6°44′E (Figure 1). The region features a tropical wet and dry climate, characterized by high annual temperatures (25–35 °C) and significant seasonal rainfall (up to 1,500 mm/year), which controls the water volume and pollutant dynamics of the two major rivers. The Lokoja Local Government Area had a population of approximately 195,261 in the 2006 census, with recent metropolitan area estimates rising sharply to around 931,000 in 2025 (Lokoja, Nigeria Metro Area Population, 2025). The population is ethnically diverse, with primary occupations focused on fishing, agriculture, trade, and civil service. The river system is intensively utilized for transportation, irrigation, and household water use. Sampling focused on three highly active riverbank locations: Ganaja, Kpata Market, and Nataco, chosen specifically for their continuous exposure to high levels of anthropogenic pollution, including domestic waste, agricultural runoff, and small-scale industrial discharges.

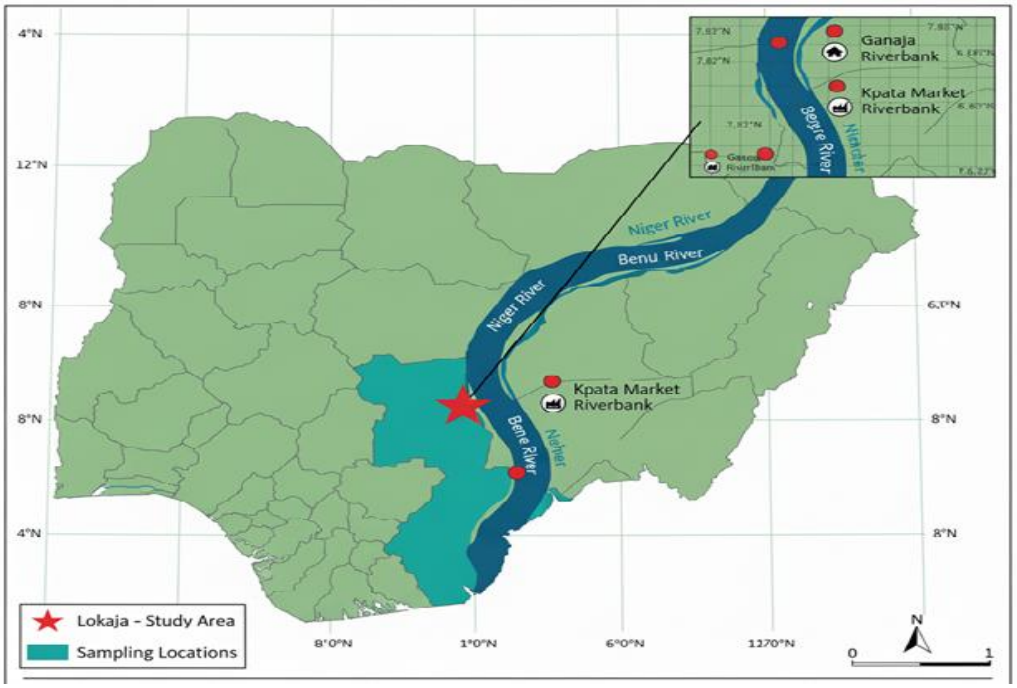


Figure 1: Map of Nigeria Showing Kogi State and Study Area Locations in Lokoja

### Collection of Water Samples

Water samples were collected concurrently with the plant samples to establish the physicochemical profile of the aquatic environment. Sterilized 1-Liter plastic bottles were utilized for sample collection. Following the guidelines established for water collection (APHA, 2017), water was carefully collected at a depth of approximately 15–20 cm below the surface at each of the three sampling locations (Ganaja, Kpata Market, and Nataco). The bottles were filled completely, minimizing headspace, and immediately sealed. The collected samples were then transported promptly on ice to the laboratory to minimize alteration of their chemical properties prior to physicochemical analysis.

### Collection and Processing of Aquatic Plant Samples

Fresh roots of the three target aquatic plant species, *Eichhornia crassipes*, *Nymphaea lotus*, and *Limnocharis flava*, were collected from the three designated sampling locations: Ganaja, Kpata Market, and Nataco. Intact plants were handpicked from the riverbanks to ensure the root systems remained as undisturbed as possible. Following removal from the water, the roots were immediately placed into sterile polyethylene bags to prevent external contamination. The entire collection process adhered to standard aseptic collection procedures outlined by the American Public Health Association (APHA, 2017). All collected samples were promptly transported on ice to the laboratory and processed within 2 hours of collection to maintain the viability of the bacterial populations. Upon

arrival at the laboratory, the collected plant roots were first processed to remove loosely adhering particles. The roots were washed gently with sterile distilled water to eliminate excess soil and debris without disturbing the firmly attached microbial community. Subsequently, approximately 10 g of the cleaned roots were excised aseptically and cut into small fragments. These root fragments were then introduced into 90 mL of sterile physiological saline (0.85% NaCl) and thoroughly homogenized using a sterile blender or mortar and pestle to release the associated bacterial populations (Madigan *et al.,* 2018). This resulting homogenate represented the initial $10^{-1}$ dilution of the root sample and was immediately subjected to serial dilution before being used for subsequent bacterial enumeration and isolation procedures.

**Enumeration and Identification of Bacterial Colonies in Samples**

The total bacterial count associated with the aquatic plant roots was determined by preparing root homogenates in physiological saline and subjecting them to serial dilutions ranging from $10^{-1}$ to $10^{-6}$.An aliquot of each dilution was then inoculated onto sterile Nutrient Agar (Oxoid) using the spread-plate technique. The inoculated plates were subsequently incubated at 30 °C for a period of 24–48 hours to allow for optimal bacterial growth. Following incubation, the resulting colonies were counted, and the total viable bacteria were calculated and expressed as Colony-Forming Units per gram (CFU/g) of fresh root tissue (Cappuccino and Sherman, 2014). Representative colonies differing in morphology (shape, texture, margin, pigmentation, and elevation) were selected. Each isolate was purified by repeated streaking on fresh agar plates until a uniform colony type was obtained (Cappuccino and Sherman, 2014). Pure cultures were stored on nutrient agar slants at 4 °C. Each bacterial isolate was examined for colony characteristics, Gram reaction, and a panel of biochemical tests following standard microbiology procedures (Cappuccino and Sherman, 2014). Probable identities were assigned using the Bergey's Manual of Systematic and Determinative Bacteriology (Holt *et al.*, 1994).

**Physicochemical Analysis of Water Samples**

The physicochemical properties of the water samples were analyzed according to the standard procedures outlined by the American Public Health Association (APHA, 2017). Some parameters were measured directly at the sampling locations. Temperature was recorded in situ using a calibrated mercury-in-glass thermometer (Brannan® Laboratory Thermometer, UK). The pH of the samples was measured immediately using a portable digital pH meter (Hanna Instruments HI98107, USA), while Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were determined using a combined conductivity/TDS meter (Jenway 4510 Conductivity/TDS Meter, UK). Other parameters were analyzed in the laboratory. Dissolved Oxygen (DO) was determined using the Winkler titrimetric method after chemical fixation of the samples at the field site. Biochemical Oxygen Demand ($BOD_5$) was measured by incubating the water samples in a temperature-controlled laboratory incubator (Gallenkamp BOD Incubator, Model Sanyo MIR-153, UK/Japan) at 20°C for five days. Chemical Oxygen Demand (COD) was analyzed using the dichromate reflux method with a digital digestion block (HACH DRB200, USA). Nutrients such as Nitrate ($NO_3^-$) and Phosphate ($PO_4^{3-}$) were quantified using a UV–visible spectrophotometer (Spectrumlab 752S UV–Vis Spectrophotometer, China). The concentration of Nickel (Ni) was measured using flame Atomic Absorption Spectrophotometry (AAS) (Buck Scientific model 210VGP AAS, USA).

**Screening for Nickel-Tolerant Bacteria**

The bacterial isolates obtained from the roots of aquatic plants were screened to determine their tolerance to nickel ions ($Ni^{2+}$) using a modified procedure based on Malik (2004) and Nies (2016). Nutrient Agar (NA) was prepared, sterilized, and cooled to approximately 45 °C, after which Nickel Chloride ($NiCl_2$) was added to obtain final nickel concentrations of 0.5, 1.0, 2.0, and 3.0 mg/mL. The media were mixed thoroughly to ensure uniform distribution of the nickel and poured into sterile Petri dishes. Each purified bacterial isolate was streaked onto the plates containing the different nickel concentrations and incubated at 30°C for 24–48 hours. Following incubation, bacterial growth was visually assessed and graded as +++ (heavy growth), ++ (moderate growth), + (light growth), or – (no growth). Isolates that produced visible growth at the highest concentration (3.0 mg/mL) were further evaluated to determine their Minimum Inhibitory Concentration (MIC). Any isolate capable of growing at concentrations above 3.0 mg/mL was classified as nickel-resistant. From this screening, four isolates with the codes: EC-2, EC-3, EC-5, and NL-3, demonstrated the highest nickel tolerance (>3.0 mg/mL) and were selected for subsequent quantitative biosorption experiments.

**Biosorption Assay**

The biosorption assay was carried out using a modified procedure as described by Oyewole *et al.* (2019), with additional adjustments suitable for bacterial biomass preparation. The biosorption procedures began by cultivating each nickel-tolerant bacterial isolate in Nutrient Broth and incubating the cultures at 30°C for 24 hours. After incubation, the cells were harvested by centrifugation at 5000 rpm for 10 minutes, and the resulting biomass was washed twice with sterile distilled water to eliminate residual medium components. The washed biomass was then dried at 60°C to a constant weight to obtain the final biosorbent material. To evaluate the effect of initial nickel concentration on biosorption capacity, exactly 0.1 g of the dried biomass was introduced into 100 mL of aqueous nickel solutions containing initial $Ni^{2+}$ concentrations of 5, 10, 15, and 20 ppm. Each mixture was then agitated on a mechanical shaker at 150 rpm for 24 hours at room temperature to ensure optimal contact between the metal ions and the biosorbent surface. After shaking, the biomass was separated by filtration, and the remaining nickel concentration in the filtrate ($C_t$) was determined using Atomic Absorption Spectrophotometry (AAS). The biosorption efficiency (percentage removal) was calculated using the equation:

$$\% \text{ Removal} = \frac{Co - Ct}{Co} \times 100\%$$

Where: $C_o$ represents the initial nickel concentration and $C_t$ is the final concentration after biosorption.

**Effect of pH on Biosorption**

The pH optimization experiment was conducted to identify the ideal pH conditions for maximizing nickel removal efficiency by the bacterial biomass, following the approach reported by Pandey *et al.* (2007) with slight modifications. For this assay, 100 mL nickel solutions were prepared at a fixed concentration of 20 ppm, representing the highest concentration used in the earlier biosorption trials. The pH of each solution was then systematically adjusted across a range of pH 3 to 8 (i.e., 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0) using 1 M HCl to decrease the pH and 1 M NaOH to increase it. Precise

pH adjustment was critical because pH strongly influences the surface charge and metal-binding characteristics of the bacterial biosorbent as well as the chemical speciation of $Ni^{2+}$ ions in solution. Once each solution reached a stable pH value, the biosorption experiment was performed as previously described: 0.1 g of dried bacterial biomass was added to each pH-adjusted 100 mL sample, followed by agitation at 150 rpm for 24 hours at room temperature to ensure sufficient contact and equilibrium. After shaking, the mixtures were filtered, the remaining nickel concentration (Ct) in the filtrate was measured using Atomic Absorption Spectrophotometry (AAS), and the percentage removal was calculated.

**Data Analysis**

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation. One-way ANOVA was used to assess differences among treatments, followed by Tukey's HSD post-hoc test to determine significant pairwise differences at $p < 0.05$.

**RESULTS AND DISCUSSION**

The physicochemical assessment of water samples from the three riverbank locations revealed measurable variations across parameters (Table 1). The spatial variations observed in the physicochemical parameters across the three riverbank locations in Lokoja demonstrate the influence of anthropogenic activity intensity and hydrological interactions on river water quality. Temperature showed a progressive rise from Ganaja (28.6 °C) to Nataco (30.1 °C), remaining within WHO limits. Similar temperature ranges have been reported in other Nigerian rivers with moderate thermal loading (Ogungbile *et al.,* 2023; Adejuwon and Akinola, 2025). The increasing pattern here agrees with the report of Edegbene *et al*. (2025), who attributed downstream warming to reduced shading, shallower depths, and effluent discharges. On the other hand, the relatively higher temperature at Nataco sampling location may reflect increased commercial and industrial activities around the motor park region, which has historically been associated with thermal pollution in other studies (Raji *et al.,* 2015). The pH gradient, from slightly acidic conditions at Ganaja (6.42) to near-neutral values at Nataco (7.15), suggests differential buffering capacities along the river course. This observation is consistent with patterns reported for the Benue and Niger River systems, where pH tends to increase in areas receiving urban runoff and low-acid industrial effluents (Uchendu and Edogbo, 2025). The values remain within acceptable limits and also align with the pH neutrality trend noted in Bonny/New Calabar Estuary (Onojake *et al.,* 2017). In contrast, slightly more alkaline conditions documented in the Esinmirin River (Adejuwon and Akinola, 2025) indicate spatial hydrochemical variability across ecological zones. The acidic pH at Ganaja may reflect organic inputs, decaying vegetation, or acidifying compounds, as similarly reported by Ubuoh *et al*. (2023). Electrical conductivity (EC) and total dissolved solids (TDS) increased significantly downstream, with Nataco area presenting the highest values (215.7 µS/cm; 146.4 mg/L). This pattern is in concordance with previous observations that densely populated or commercial zones tend to exhibit higher ionic loads due to increased wastewater seepage (Edeki *et al.,* 2023; Uzamere *et al.,* 2023). Although the values here remain below WHO/FEPA thresholds, the progressive rise suggests growing mineralization, similar to the conductivity intensification reported in industrial-adjacent rivers in Kano State (Uchendu and Edogbo, 2025). Tula *et al.* (2022) also reported that electrical conductivity generally reflects the geochemical signature of a catchment, implying that variations here may result from changes in geology coupled with anthropogenic contributions. Dissolved oxygen (DO) declined steadily from Ganaja (6.82 mg/L) to Nataco (4.76 mg/L). This inverse relationship with BOD strongly suggests increasing organic pollution downstream. Comparable DO depletion patterns have been documented in several Nigerian rivers subjected to domestic and market effluents, including the Nworie River (Udechukwu *et al.,* 2025) and the interconnected coastal lagoons studied by Jolaosho *et al.* (2025). The low DO at Nataco falls below WHO minimum guidelines, indicating stressed ecological conditions. Similar declines in oxygenation were reported by Maduka and Ephraim-Emmanuel (2019) in oil-bearing communities of the Niger Delta, where organic-rich discharges suppressed aerobic capacity. Looking at the pattern,the comparatively high DO at Ganaja suggests limited pollution loading and greater water aeration. The BOD and COD values followed expected trends for rivers receiving progressive anthropogenic discharges, with the highest values at Nataco region (BOD: 6.34 mg/L; COD: 23.9 mg/L). While COD values remained within acceptable limits, BOD slightly exceeded the guideline threshold, highlighting increasing biodegradable organic content. This pattern agrees with the findings of Uzamere *et al.* (2023), who documented elevated BOD in segments of the New Calabar River proximal to effluent points. Edegbene *et al.* (2025) similarly observed higher BOD in urban-impacted versus rural river segments, attributing it to municipal waste inputs. However, unlike the extremely high organic loads reported for some Niger Delta water bodies (Onojake *et al.,* 2017; Ubuoh *et al.,* 2023), the Lokoja values here remain moderately elevated, suggesting early-stage pollution rather than severe degradation. Nitrate and phosphate concentrations exhibited slight but significant increases from Ganaja to Nataco. Although well within WHO limits, their spatial patterns are consistent with nutrient enrichment linked to agricultural runoff and domestic wastewater (Tula *et al*., 2022; Jolaosho *et al.*, 2025). Particularly, the elevated phosphate at Nataco may be associated with detergents, food-processing activities, and surface washing around the busy transport hub. Similar nutrient-loading dynamics have been described in the Esinmirin River (Adejuwon and Akinola, 2025) and the River Benue (Edegbene *et al*., 2025). Nickel levels (0.021–0.046 mg/L) were below WHO limits but showed a notable downstream rise, reflecting potential trace-metal inputs. This pattern parallels observations from Kano industrial rivers (Uchendu and Edogbo, 2025), where vehicular activity, metal corrosion, and effluent discharges contributed to elevated Ni levels. Although concentrations in this study are lower than those reported for the Niger Delta estuarine waters (Onojake *et al.,* 2017), the increasing gradient suggests a growing metal load that warrants continuous monitoring.

**Table 1: Physicochemical Properties of Water Samples from Three Locations in Lokoja, Nigeria**

| Parameter | Ganaja Riverbank | Kpata Market Riverbank | Nataco Riverbank | WHO/FEPA Standard |
|---|---|---|---|---|
| Temperature (°C) | $28.6 \pm 0.40^{a}$ | $29.3 \pm 0.51^{ab}$ | $30.1 \pm 0.61^{b}$ | ≤ 35 |
| Ph | $6.42 \pm 0.12^{a}$ | $6.88 \pm 0.15^{b}$ | $7.15 \pm 0.18^{c}$ | 6.5–8.5 |
| Electrical Conductivity (EC) (µS/cm) | $142.5 \pm 6.80^{a}$ | $178.3 \pm 9.40^{b}$ | $215.7 \pm 10.6^{c}$ | ≤ 1000 |
| Total Dissolved Solids (TDS) (mg/L) | $96.2 \pm 5.10^{a}$ | $118.7 \pm 6.31^{b}$ | $146.4 \pm 7.01^{c}$ | ≤ 500 |
| Dissolved Oxygen (DO) (mg/L) | $6.82 \pm 0.27^{a}$ | $5.94 \pm 0.21^{b}$ | $4.76 \pm 0.18^{c}$ | ≥ 5 |
| Biochemical Oxygen Demand ($BOD_5$) (mg/L) | $3.12 \pm 0.14^{a}$ | $4.86 \pm 0.22^{b}$ | $6.34 \pm 0.27^{c}$ | ≤ 6 |
| Chemical Oxygen Demand (COD) (mg/L) | $12.5 \pm 1.10^{a}$ | $18.2 \pm 1.60^{b}$ | $23.9 \pm 2.10^{c}$ | ≤ 30 |
| Nitrate ($NO_3^-$) (mg/L) | $2.14 \pm 0.18^{a}$ | $3.27 \pm 0.21^{b}$ | $4.02 \pm 0.26^{c}$ | ≤ 50 |
| Phosphate ($PO_4^{3-}$) (mg/L) | $0.42 \pm 0.04^{a}$ | $0.68 \pm 0.07^{b}$ | $0.93 \pm 0.08^{c}$ | ≤ 5 |
| Nickel (Ni) (mg/L) | $0.021 \pm 0.003^{a}$ | $0.034 \pm 0.004^{b}$ | $0.046 \pm 0.005^{c}$ | ≤ 0.07 |

*(Values are Mean ± SD; different superscripts within the same row indicate significant differences at $p < 0.05$.)*

The total bacterial counts obtained from the roots of the three aquatic plants showed variation across the sampling sites (Table 2). For *Eichhornia crassipes*, the highest count was recorded at the Kpata Market riverbank ($5.20 \times 10^6$ CFU/g), followed by Ganaja ($4.81 \times 10^6$ CFU/g) and Nataco ($4.51 \times 10^6$ CFU/g). *Nymphaea lotus* similarly showed its highest count at Kpata Market ($3.93 \times 10^6$ CFU/g), with lower values at Ganaja ($3.62 \times 10^6$ CFU/g) and Nataco ($3.41 \times 10^6$ CFU/g). For *Limnocharis flava*, bacterial counts were also highest at Kpata Market ($3.11 \times 10^6$ CFU/g), compared to Ganaja ($2.81 \times 10^6$ CFU/g) and Nataco ($2.60 \times 10^6$ CFU/g). Significant differences ($p < 0.05$) were observed within each plant species across the locations. The differences in total bacterial counts recorded across the three riverbank locations suggest that local environmental conditions significantly shape the microbial populations associated with aquatic plant roots**.** In this study, *Eichhornia crassipes* consistently supported the highest bacterial loads at all sites, followed by *Nymphaea lotus* and *Limnocharis flava*. This general pattern agrees with earlier findings by Tanaka *et al*. (2011) and Crump and Koch (2008)**,** who emphasized that variations in nutrient input, hydrology, and site disturbance significantly influence the abundance and composition of root-associated bacteria. The relatively higher bacterial counts observed at Kpata Market appear to be driven by the heavy nutrient enrichment, frequent human activity, and organic waste entering the river at that point. This explanation is supported by Martínez-Martínez *et al*. (2023)**,** who noted that *Typha* roots exposed to human disturbance harbored richer and denser microbial communities. Similarly, Ali *et al.* (2025) found that *E. crassipes* grown in nutrient-rich waters hosted more diverse bacterial assemblages, which is consistent with the present observations. On the other hand, the relatively lower counts at Nataco may reflect reduced organic matter or better water quality. This pattern is in line the findings of Ahmed Abdullahi *et al*. (2024)**,** who reported that cleaner or less nutrient-loaded aquatic environments support fewer root-associated bacteria. Thus, the spatial trends in the present study are in strong agreement with earlier reports that nutrient availability is a major driver of microbial density. Furthermore, the differences observed among the plant species also agrees with earlier. *E. crassipes* possesses a large, fibrous, and complex root system, which provides extensive surface area for microbial attachment. This structural advantage likely explains why it consistently recorded the highest bacterial loads. Similar relationships between root morphology and microbial colonization were described by Rahmi *et al*. (2024) and Sofo *et al*. (2020)**,** whose studies showed that plants with more elaborate root architectures tend to harbor larger microbial communities. The present results therefore align well with these earlier findings.

**Table 2: Total Bacterial Counts (CFU/G Root) Of Aquatic Plant Roots from Three Locations in Lokoja, Nigeria**

| Aquatic Plant | Ganaja Riverbank ($\times 10^6$) | Kpata Market Riverbank ($\times 10^6$) | Nataco Riverbank ($\times 10^6$) |
|---|---|---|---|
| Eichhornia crassipes | $4.81 \pm 0.30^{a}$ | $5.20 \pm 0.41^{b}$ | $4.51 \pm 0.22^{a}$ |
| Nymphaea lotus | $3.62 \pm 0.25^{a}$ | $3.93 \pm 0.33^{b}$ | $3.41 \pm 0.24^{a}$ |
| Limnocharis flava | $2.81 \pm 0.21^{a}$ | $3.11 \pm 0.15^{b}$ | $2.60 \pm 0.23^{a}$ |

*(Values are Mean ± SD; different superscripts within the same row indicate significant differences at $p < 0.05$.)*

The recovered bacterial isolates were subsequently subjected to phenotypic characterization, which revealed distinct colony morphologies, Gram reactions, and biochemical profiles (Tables 3a and 3b). Based on these characteristics, the isolates were identified as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Micrococcus luteus*, *Micrococcus varians*, and other *Bacillus* species, each distributed among the roots of the different aquatic plants. The frequent occurrence of Bacillus, Pseudomonas, and Micrococcus in both aquatic and terrestrial plant roots agrees with the findings of Tanaka *et al.* (2017) and Makino *et al.* (2022), who isolated similar species from aquatic plants and emphasized their strong ability to colonize roots. The recovery of *B. subtilis*, *B. cereus*, and *B. megaterium* further supports the observations of Meneguzzi *et al.* (2024)**,** who described these species as important plant-associated bacteria with growth-promoting traits. The occurrence of *Pseudomonas fluorescens***,** *P. putida*, and P. aeruginosa is also consistent with reports by Vílchez *et al*. (2021) and Carroll *et al*. (2020)**,** who showed that *Pseudomonas* species attach rapidly to root surfaces and form stable rhizosphere populations. Their widespread recovery here suggests that they are well adapted to the aquatic root environment, especially in nutrient-rich or disturbed sites. The detection of *Micrococcus luteus* and *Micrococcus varians* agrees with Abedinzadeh *et al.* (2019)**,** who found Micrococcus species in maize roots and suggested that they thrive in environments enriched with plant root exudates. Their presence in this study may be attributed to niche specialization or competitive interactions within the root microhabitats, similar to the complex microbial dynamics described by Kim *et al*. (2025**)**. When comparing the three

plant species, the pattern of microbial diversity partly aligns with the results of Raharja *et al.* (2025)**,** who reported that plant species and growth stages influence microbial assemblages. However, the present findings differ slightly because our study compared locations rather than developmental stages, which may explain the variation. The predominance of *Bacillus* and *Pseudomonas* across samples supports their well-known ecological roles. Studies by Xi *et al*. (2022) and Zaki *et al.* (2025) reported that these bacteria contribute to mineral weathering, nutrient cycling, and stress alleviation in plant roots. Equally, Rana *et al.* (2023) demonstrated similar functions in endophytic bacteria from groundnut roots. The functional consistency across studies suggests that the isolates in the present work may also play important roles in enhancing plant growth and contributing to natural water purification processes.

**Table 3a: Phenotypic Characteristics of Bacterial Isolates Obtained from Aquatic Plant Roots in Lokoja**

| Isolate | Colony Morphology | Gram | Catalase | Oxidase | Citrate | Motility | Indole |
|---|---|---|---|---|---|---|---|
| EC-1 | Cream, circular, entire, raised, smooth | + | + | – | + | + | – |
| EC-2 | Yellowish, irregular, undulate, flat, dry | + | + | – | + | – | – |
| EC-3 | White, circular, entire, convex, mucoid | – | + | + | – | + | – |
| EC-4 | Pale cream, filamentous, lobate, flat, rough | + | + | – | + | + | – |
| EC-5 | Off-white, circular, entire, convex, smooth | – | + | + | – | + | – |
| EC-6 | Yellow, circular, entire, raised, moist | + | + | – | + | – | – |
| NL-1 | Creamy, entire, convex, smooth | – | + | + | – | + | – |
| NL-2 | White, irregular, lobate, flat, dry | + | + | – | + | – | – |
| NL-3 | Cream, circular, entire, convex, slimy | – | + | + | – | + | – |
| NL-4 | Yellow, circular, entire, raised, smooth | + | + | – | + | – | – |
| LF-1 | Cream, circular, entire, convex, shiny | + | + | – | + | + | – |
| LF-2 | White, flat, filamentous, rough | + | + | – | + | + | – |

**Key:** EC: *Eichhornia crassipes;* NL: *Nymphaea lotus;* LF: *Limnocharis flava* + = Positive reaction; – = Negative reaction; Alk/Alk = Alkaline slant / Alkaline butt (no sugar fermentation); $H_2S$ = Hydrogen sulfide production

**Table 3b: Phenotypic Characteristics of Bacterial Isolates Obtained from Aquatic Plant Roots in Lokoja**

| Isolate | Urease | TSI | MR | VP | Gelatin | Starch | Nitrate | Glucose | Lactose | $H_2S$ | Probable Identity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EC-1 | – | Alk/Alk | – | – | + | + | – | – | – | – | *Bacillus subtilis* |
| EC-2 | – | Alk/Alk | – | – | + | – | – | – | – | – | *Bacillus cereus* |
| EC-3 | – | Alk/Alk | – | – | – | – | + | – | – | – | *Pseudomonas putida* |
| EC-4 | + | Alk/Alk | – | – | + | + | – | – | – | – | *Bacillus megaterium* |
| EC-5 | – | Alk/Alk | – | – | – | – | + | – | – | – | *Pseudomonas aeruginosa* |
| EC-6 | – | Alk/Alk | – | – | – | – | – | – | – | – | *Micrococcus luteus* |
| NL-1 | – | Alk/Alk | – | – | – | – | + | – | – | – | *Pseudomonas fluorescens* |
| NL-2 | – | Alk/Alk | – | – | + | + | – | – | – | – | *Bacillus* sp. |
| NL-3 | – | Alk/Alk | – | – | – | – | + | – | – | – | *Pseudomonas aeruginosa* |
| NL-4 | – | Alk/Alk | – | – | – | – | – | – | – | – | *Micrococcus varians* |
| LF-1 | – | Alk/Alk | – | – | + | + | – | – | – | – | *Bacillus subtilis* |
| LF-2 | + | Alk/Alk | – | – | + | + | – | – | – | – | *Bacillus megaterium* |

***Key:*** *EC: Eichhornia crassipes; NL: Nymphaea lotus; LF: Limnocharis flava + = Positive reaction; – = Negative reaction; Alk/Alk = Alkaline slant / Alkaline butt (no sugar fermentation); $H_2S$ = Hydrogen sulfide production*

Screening for nickel tolerance identified four bacterial isolates exhibiting minimum inhibitory concentrations greater than 3.0 mg/mL (Table 4). These high-resistance isolates included EC-2, EC-3, and EC-5 from *Eichhornia crassipes*, and NL-3 from *Nymphaea lotus*. Based on phenotypic identification, the resistant isolates were characterized as

*Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Bacillus subtilis*. This finding is in agreement with earlier reports showing that *Pseudomonas*, *Enterobacter*, and *Bacillus* are commonly associated with nickel-rich environments. According to the report of Costa *et al.* (2019), serpentine soils naturally enriched with Ni tend to select for Ni-tolerant *Pseudomonas* and *Bacillus*. Similarly, Elgamal *et al.* (2018) documented *P. aeruginosa* strains capable of thriving in heavily polluted Egyptian soils with high nickel burdens. The consistency between our results and these previous findings suggests that these genera possess conserved physiological mechanisms for nickel resistance, including metal efflux systems, oxidative-stress mitigation, and plasmid-encoded resistance determinants. According to the report of Elgamal *et al.* (2018), plasmid-mediated resistance may also explain the exceptionally high tolerance observed in our *P. aeruginosa* isolate.

**Table 4: Nickel-Tolerance Bacterial Isolates (MIC > 3.0 Mg/Ml) Selected for Biosorption Studies**

| Isolate Code | Source Plant | MIC (mg/mL $NiCl_2$) | Probable Identity |
|---|---|---|---|
| EC-2 | *Eichhornia crassipes* | >3.0 | *Pseudomonas putida* |
| EC-3 | *Eichhornia crassipes* | >3.0 | *Pseudomonas aeruginosa* |
| EC-5 | *Eichhornia crassipes* | >3.0 | *Enterobacter cloacae* |
| NL-3 | *Nymphaea lotus* | >3.0 | *Bacillus subtilis* |

Biosorption assessment across increasing initial nickel concentrations showed progressive increases in removal efficiency for all isolates (Table 5). At 5 ppm, efficiencies ranged from 41.22 ± 1.03% for *Bacillus subtilis* to 52.14 ± 1.25% for *Pseudomonas aeruginosa*. At 10 ppm and 15 ppm, *P. aeruginosa* maintained the highest biosorption values (68.92 ± 1.18% and 78.51 ± 1.26%, respectively), while *B. subtilis* consistently recorded the lowest. At 20 ppm, *P. aeruginosa* again showed the highest efficiency (84.63 ± 1.42%), followed by *P. putida* (79.88 ± 1.21%), *E. cloacae* (72.55 ± 1.37%), and *B. subtilis* (69.80 ± 1.15%). Significant differences ($p < 0.05$) were observed among isolates at each concentration. The biosorption patterns observed in this study support the established role of *Pseudomonas* species as efficient metal biosorbents. *P. aeruginosa* displayed the highest removal capacity across treatments, reaching 84.63% removal at 20 ppm. This result is in agreement with the findings of Ansari and Malik (2007) and Alboghobeish *et al.* (2014), both of whom reported the efficient ability of *Pseudomonas* to adsorb divalent metal ions compared to many other bacteria. Similarly, Pagnucco *et al.* (2023) reported that *Pseudomonas* strains isolated from urban waters exhibited some of the strongest Ni biosorption efficiencies. The high performance of our isolates may be linked to their origin from aquatic plant roots, which are niches known to promote bacteria with abundant cell-surface functional groups, dense exopolysaccharide layers, and strong metal-binding ligands. *Bacillus subtilis* exhibited the lowest biosorption efficiency among the isolates. This observation partially agrees with the variable responses reported for *Bacillus* in previous studies. While Moeini *et al.* (2024) described strong biosorption potential in some *Bacillus* strains, Costa *et al*. (2019) emphasized wide physiological variability within the genus. The reduced performance of *B. subtilis* in our study may stem from its cell-wall features, such as lower densities of negatively charged functional groups or less extensive extracellular polymeric substances compared to Gram-negative bacteria like *Pseudomonas* and *Enterobacter*. Such structural features likely contribute to its weaker affinity for $Ni^{2+}$. Increasing nickel concentration resulted in increased removal efficiency, which is in agreement with the reports of Pagnucco *et al.* (2023) and Alboghobeish *et al*. (2014), who observed similar trends until sorption sites approached saturation. The absence of saturation within the tested range suggests that the isolates possess a high density of active binding sites, further supporting their suitability for bioremediation applications.

**Table 5: Biosorption Efficiency (%) of Nickel-Resistant Bacterial Isolates at Different Initial Nickel Concentrations**

| Isolate Code | 5 ppm (%) | 10 ppm (%) | 15 ppm (%) | 20 ppm (%) |
|---|---|---|---|---|
| Pseudomonas putida | $48.22 \pm 1.12^{a}$ | $62.35 \pm 1.45^{a}$ | $71.44 \pm 1.37^{b}$ | $79.88 \pm 1.21^{a}$ |
| Pseudomonas aeruginosa | $52.14 \pm 1.25^{a}$ | $68.92 \pm 1.18^{b}$ | $78.51 \pm 1.26^{a}$ | $84.63 \pm 1.42^{a}$ |
| Enterobacter cloacae | $44.83 \pm 1.10^{b}$ | $57.64 \pm 1.32^{c}$ | $66.90 \pm 1.41^{c}$ | $72.55 \pm 1.37^{b}$ |
| Bacillus subtilis | $41.22 \pm 1.03^{b}$ | $53.12 \pm 1.27^{c}$ | $61.30 \pm 1.22^{d}$ | $69.80 \pm 1.15^{b}$ |

*Different superscript letters within a column indicate significant differences ($p < 0.05$)*

Nickel biosorption varied considerably across pH levels for all selected isolates, showing a consistent rise in efficiency from acidic to neutral conditions (Table 6). At pH 3, removal was lowest, ranging from 17.33 ± 1.04% in *Bacillus subtilis* to 25.63 ± 1.18% in *Pseudomonas aeruginosa*. Biosorption increased at pH 5, where *P. putida* reached 48.30 ± 1.15%, *P. aeruginosa* 53.91 ± 1.34%, *Enterobacter cloacae* 44.33 ± 1.19%, and *B. subtilis* 40.92 ± 1.13%. Peak efficiencies were recorded at pH 7 for all isolates, with *P. aeruginosa* achieving the highest value at 80.42 ± 1.41%, followed by *P. putida* (74.55 ± 1.32%), *E. cloacae* (66.71 ± 1.30%), and *B. subtilis* (60.38 ± 1.27%). A decline occurred at pH 8, indicating reduced biosorption beyond the neutral range. Significant differences ($p < 0.05$) were observed across pH levels within each isolate, with the greatest contrast between acidic (pH 3–4) and neutral (pH 6–7) conditions. The pH-dependent pattern observed, reduced uptake in acidic media, maximum removal around neutrality, and diminished efficiency under alkaline conditions, agrees closely with trends reported in earlier studies. According to Díaz *et al*. (2022), nickel uptake by *Serratia marcescens* peaked between pH 6 and 7, a range comparable to the optimum identified in the present work. In the same vein, Moeini *et al*. (2024) identified similar pH optima for Ni biosorption in *Bacillus* species, emphasizing that neutral pH enhances the availability of negatively charged cell-surface sites required for metal binding. The reduced removal at acidic pH can be explained by proton competition for these negatively charged binding sites on the bacterial cell surface, a mechanism also reported by Díaz *et al*. (2022) when describing decreased Ni uptake in *S. marcescens* under low pH conditions. Conversely, the decline at alkaline pH is consistent with the explanation provided by Moeini *et al.* (2024), who noted that nickel hydroxide precipitation at high pH can reduce the amount of soluble $Ni^{2+}$

available for biosorption and create the appearance of diminished removal efficiency. The pH responses of the isolates follow the same basic chemical processes that control how nickel attaches to bacterial cell surfaces, a pattern also reported in studies of nickel–microbe interactions by Vahedi *et al*. (2017), where nickel exposure influenced bacterial surface behavior and biofilm activity.

**Table 6: Effect of Ph On Nickel Biosorption (%) By Selected Nickel-Tolerant Bacterial Isolates**

| Isolate Code | pH 3 | pH 4 | pH 5 | pH 6 | pH 7 | pH 8 |
|---|---|---|---|---|---|---|
| *P putida* | 22.41 ± 1.12$^{d}$ | 34.52 ± 1.21$^{c}$ | 48.30 ± 1.15$^{b}$ | 62.11 ± 1.28$^{a}$ | 74.55 ± 1.32$^{a}$ | 69.12 ± 1.20$^{b}$ |
| *P. aeruginosa* | 25.63 ± 1.18$^{d}$ | 38.14 ± 1.26$^{c}$ | 53.91 ± 1.34$^{b}$ | 68.25 ± 1.22$^{a}$ | 80.42 ± 1.41$^{a}$ | 73.88 ± 1.29$^{b}$ |
| *E. cloacae* | 19.42 ± 1.05$^{d}$ | 30.88 ± 1.17$^{c}$ | 44.33 ± 1.19$^{b}$ | 56.95 ± 1.25$^{a}$ | 66.71 ± 1.30$^{a}$ | 61.22 ± 1.18$^{b}$ |
| *B. subtilis* | 17.33 ± 1.04$^{d}$ | 28.14 ± 1.10$^{c}$ | 40.92 ± 1.13$^{b}$ | 52.66 ± 1.20$^{a}$ | 60.38 ± 1.27$^{a}$ | 55.41 ± 1.14$^{b}$ |

**Different superscript letters within each row indicate significant differences ($p < 0.05$)*

## CONCLUSION

This study showed that aquatic plant roots in Lokoja support diverse bacterial communities, some of which are highly tolerant to nickel and capable of removing it from contaminated water. Water quality results indicated increasing pollution pressure from Ganaja to Nataco, reflected in rising conductivity, nutrients, and declining dissolved oxygen. These changing environmental conditions also influenced bacterial abundance, with the highest microbial loads found at the more disturbed Kpata Market site. Several isolates belonging to *Pseudomonas*, *Enterobacter*, and *Bacillus* demonstrated strong nickel tolerance, and four of them showed high biosorption ability. *Pseudomonas aeruginosa* performed best across concentration and pH tests, while *Bacillus subtilis* showed the lowest removal efficiency. Biosorption consistently increased from acidic to neutral pH, confirming that cell-surface charge plays an important role in metal binding. These findings suggest that root-associated bacteria from common aquatic plants can serve as effective biological tools for nickel removal in freshwater systems. Their high tolerance and strong biosorption capacity show promise for developing low-cost, nature-based bioremediation strategies suitable for communities along the Niger and Benue rivers. The remarkable performance of *Pseudomonas* species highlights their potential as candidate strains for biofilters and constructed wetlands. Investigations into microbial consortia, biofilm-mediated removal, and the use of immobilized cells could further enhance nickel remediation efficiency. Continuous monitoring of trace metals in Lokoja watersheds is also recommended to guide environmental management and protect public health.

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