



Environmental and Microbial Impacts of Wastewater Discharges on the Nile Delta, Egypt

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ABSTRACT

This study presents a comprehensive spatiotemporal assessment of the Rosetta branch and its intersecting agricultural and municipal drainage outfalls using an integrated framework of physicochemical, nutrient, microbiological, and molecular indicators. This study provides one of the first integrated physicochemical, microbial, and bioinformatic assessments of drainage-driven risks in the Rosetta branch. Physicochemical analysis revealed that water temperature (27–33°C) was driven by climatic seasonality and was significantly positively correlated with pH ($r=0.69$, $P<0.05$). Although pH remained within regulatory thresholds, electrical conductivity and total dissolved solids were markedly elevated at drainage discharge points, signifying localized salinization risks for downstream irrigation. Furthermore, critical hypoxia was observed at most outfalls, where the dissolved oxygen levels fell below life-sustaining limits, reflecting high organic loading. Ammonia concentrations consistently exceeded the permissible limits and correlated strongly with biochemical oxygen demand and nitrate levels, confirming substantial organic pollution. Microbiological monitoring identified drainage outlets as contamination hotspots, characterized by elevated standard plate counts and significant spatial variability in total coliforms, fecal coliforms, and fecal streptococci. Water Quality Index classified these outlets as poor quality, with only marginal downstream recovery attributed to dilution and natural attenuation. Pathogen profiling via culture-based methods and 16S rRNA gene sequencing identified the predominance of *Escherichia coli* in the River Nile, whereas *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Salmonella* spp. were prevalent in the drainage effluents. Our results demonstrate that untreated drainage discharges are a major driver of environmental deterioration along the Rosetta branch, emphasizing the necessity of coordinated wastewater management strategies to safeguard the ecological integrity of the Nile Delta and associated public health.

INTRODUCTION

The Nile River is the primary freshwater source for Egypt, supporting agriculture, industry, and domestic needs across the densely populated Nile Delta (Azzam *et al.*, 2024). The Rosetta branch, one of the river's major distributaries, plays a pivotal role in regional water supply and irrigation networks (Mohamed *et al.*, 2026). However, rapid

urbanization, intensive agricultural practices, and insufficient wastewater treatment have increasingly exposed the river to diverse pollutants (**El-DougDoug *et al.*, 2020**). Agricultural runoff, municipal effluents, and industrial discharges introduce significant quantities of organic matter, nutrients, and microorganisms, raising concerns over water quality, ecosystem health, and public safety (**Elbahnasawy *et al.*, 2021**). Deciphering the interplay among these inputs within fluvial systems provides a scientific basis for sustainable water management decisions.

Variations in water quality across large river systems are shaped by a complex interplay of environmental conditions and human interventions, as physicochemical parameters govern the contaminant chemistry and microbial metabolism (**Omran *et al.*, 2024**; **Mwaheb *et al.*, 2026**). Elevated EC and TDS can compromise water usability, particularly for irrigation, while temperature and pH affect oxygen solubility and microbial proliferation (**El-Zeiny *et al.*, 2018**). Nutrients like ammonia and nitrate further exacerbate organic enrichment, often leading to hypoxic conditions that threaten aquatic life (**Azzam *et al.*, 2025**; **Dey *et al.*, 2026**). Monitoring these parameters provides essential insights into the extent and sources of pollution in surface water environments.

Microbial contamination is a critical indicator of water quality, reflecting inputs from sewage, livestock, and industrial sources (**Holcomb & Stewart, 2020**). Total coliforms, fecal coliforms, and fecal streptococci serve as reliable proxies for fecal pollution, whereas pathogenic bacteria such as *E. coli*, *P. aeruginosa*, *E. faecalis*, and *Salmonella* spp. pose direct risks to human and ecological health (**Omran & Azzam, 2025**). Traditional culture-based methods provide baseline microbial assessments, but molecular approaches, including 16S rRNA gene sequencing, offer higher specificity and sensitivity, allowing detection of genetically diverse strains and facilitating rapid source tracking of pathogens (**Crone *et al.*, 2020**).

Integrated water quality assessments combining physicochemical, nutrient, and microbiological indicators are increasingly recognized as essential for capturing the complex interactions governing riverine ecosystems (**Dessie *et al.*, 2024**). Water Quality Index (WQI) frameworks enable the classification of pollution hotspots and evaluation of cumulative impacts downstream of effluent discharges (**Abdou *et al.*, 2025**). Such holistic approaches inform risk assessments, identify priority areas for intervention, and support evidence-based strategies for water resource management.

Despite the recognized pressures on the Nile Delta, comprehensive studies integrating spatiotemporal physicochemical and microbial monitoring with molecular pathogen identification are limited. This study addresses this gap by evaluating the Rosetta branch and its intersecting agricultural and municipal drains. By linking physicochemical disturbances with microbial risk profiles and assessing pathogen diversity using molecular methods, this study provides a robust basis for guiding targeted wastewater management and protecting the ecological and public health integrity of the Nile Delta. Accordingly, this study aims to (i) assess the spatiotemporal variations in the

physicochemical and nutrient characteristics of the Rosetta branch in relation to agricultural and municipal drainage discharges; (ii) identify major microbial and pathogenic indicators using culture-based methods and 16S rRNA gene sequencing coupled with bioinformatic analysis; and (iii) evaluate the associated environmental and public health risks using an integrated multi-indicator framework.

MATERIALS AND METHODS

1. Study Area and Sampling Procedure

The study area encompassed an approximately 120-km stretch of the Rosetta branch of the River Nile, spanning from the upstream vicinity of the El-Rahawy drain to the downstream sections beyond the Tala drain (Fig. 1). Fifteen sampling stations were selected, comprising five major drainage outlets (El-Rahawy, Sabal, El-Tahreer, Zawiet El-Bahr, and Tala) and ten river sites located both upstream and downstream of each discharge point along the Rosetta branch. Upstream stations served as reference points for assessing downstream water quality degradation (Omran & Azzam, 2025). Field sampling was conducted during winter 2023, following the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Ninety-six subsurface water samples were collected at a depth of approximately 50cm using sterile polyethylene bottles, stored under cooled conditions, and promptly transferred to the laboratory to preserve the reliability of physicochemical and bacteriological analyses.

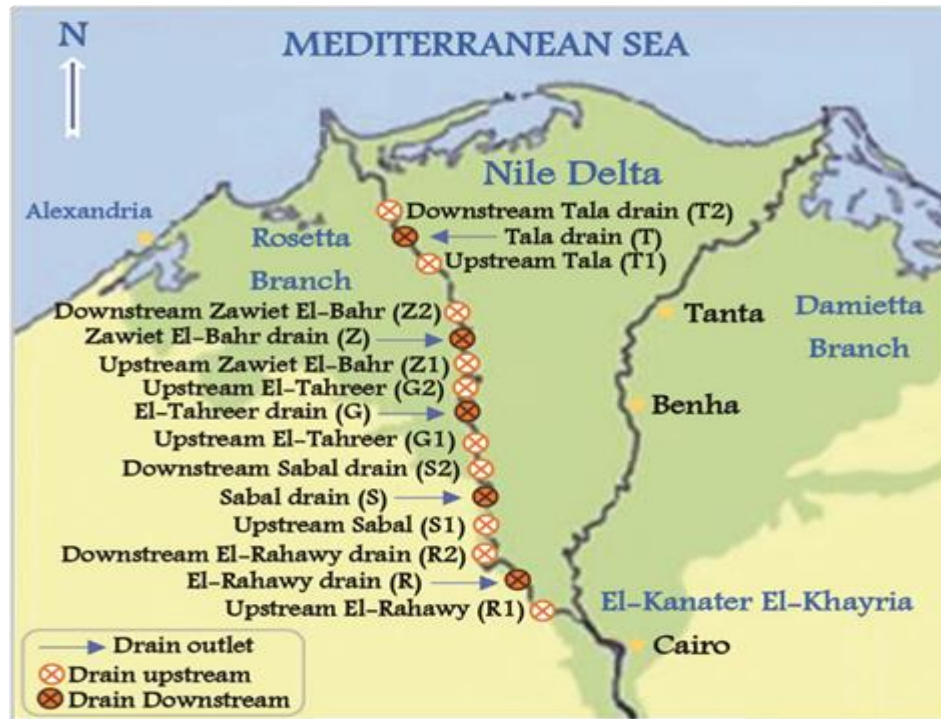


Fig. 1. Map of sampling sites (Drains and Rosetta branch).

2. Water Samples Assessment

2.1. Physicochemical Assessment

Water samples were subjected to a comprehensive physicochemical evaluation using internationally accepted standard procedures (APHA, 2017). Core water quality variables, including temperature, pH, electrical conductivity, dissolved oxygen, and total dissolved solids, were measured directly in the field using a portable multiparameter instrument (Hydralab Surveyor), followed by laboratory verification to ensure the consistency of the data. Turbidity was quantified using a HACH turbidimeter (Model 16800). Nitrogenous pollution in the form of ammonia was assessed electrochemically using a selective ammonia electrode (ORION 95–12) interfaced with an ion analyzer (ORION 940), whereas the organic load was evaluated through biochemical oxygen demand analysis employing a rapid respiratory BOD system (ORION 890). Dissolved inorganic anions, namely chloride, nitrite, nitrate, phosphate, and sulfate, were determined using ion chromatography (Dionex DX-500). Alkalinity components (carbonates and bicarbonates) were analyzed using a Metrohm ion chromatographic system. The concentrations of major dissolved cations and trace-level metals were quantified by inductively coupled plasma–optical emission spectrometry (ICP–OES; PerkinElmer Optima 3000) fitted with an ultrasonic nebulizer.

2.2. Bacteriological Assessment

Microbiological analyses were initiated within six hours of sample collection, in accordance with internationally standardized procedures (APHA, 2017). Heterotrophic standard bacterial counts were assessed using standard plate count assays incubated at 22°C and 37°C using a pour plate approach. Indicator bacteria, including total coliforms, fecal coliforms, and fecal streptococci, were enumerated by membrane filtration using a sterilizable stainless-steel manifold connected to an oil-free vacuum system. Sample aliquots were filtered through sterile gridded cellulose nitrate membranes (47mm diameter, 0.45µm pore size; Sartorius, Germany). Selective cultivation was performed on appropriate differential media for each bacterial group, namely M-Endo LES agar, M-FC agar, and M-Enterococcus agar, following established standard methodologies. Microbial counts were expressed as colony-forming units per 100mL (CFU100mL⁻¹) using the conventional enumeration procedure by the following equal:

$$\text{CFU/100mL} = \text{CFU} \times 100/\text{mL of filtered sample} \dots \dots \dots \text{Eq. (1)}$$

2.3. Water Quality Index (WQI)

WQI is a numerical factor expressed on a 100-point scale that was originally proposed by the National Sanitation Foundation (USA) to summarize water quality conditions. In the present study, WQI values were generated using a computerized model that integrated key physicochemical and microbiological variables, including DO, FC, pH, BOD, temperature, PO₄³⁻, NO₃⁻, and turbidity. By condensing complex datasets into

a single value, the WQI facilitates a straightforward interpretation of water quality status, which is categorized into five classes, ranging from very poor to excellent quality.

2.4. Selection, Isolation, and Identification of Bacterial Species

Aquatic bacterial groups of public health relevance were prioritized for isolation and identification due to their potential risks to human health, following the recommended procedures outlined by APHA (2017).

2.4.1. *Escherichia coli*

E. coli was detected using a selective culture approach based on standardized microbiological procedures. Water samples were cultured on m-TEC medium (Method NO. 9213D) and incubated at an elevated temperature ($44.5 \pm 0.2^\circ\text{C}$) for 24h to favor the growth of thermotolerant *E. coli*. Colonies displaying yellow to yellow-brown pigmentation were considered as presumptive isolates. Verification was subsequently performed by transferring representative colonies to Eosin Methylene Blue agar, where the development of pink colonies with a characteristic metallic sheen verified the identity of *E. coli*.

2.4.2. *Pseudomonas aeruginosa*

P. aeruginosa was isolated using a selective culturing strategy designed to favor its growth over that of competing microorganisms. Water samples were inoculated onto M-PA-C medium (Method NO. 9213E) and incubated at $41.5 \pm 0.5^\circ\text{C}$ for 72h. Colonies with flat morphology, pale edges, and dark brown to greenish-black centers (0.8–2.2 mm in diameter) were considered as presumptive isolates. Verification was subsequently performed by subculturing onto cetrimide agar, where the distinctive production of fluorescein and pyocyanin pigments confirmed the identification of *P. aeruginosa*.

2.4.3. *Staphylococcus aureus*

S. aureus was selectively isolated from water samples using a culture-based approach. Samples were plated on Baird–Parker agar (Method NO. 9213B) and incubated at 35°C for 48 hours. Colonies displaying a glossy black surface with a narrow clear zone were considered presumptive isolates. To confirm identification, representative colonies were transferred onto mannitol salt agar, where the appearance of golden-yellow colonies demonstrated mannitol fermentation characteristic of *S. aureus*.

2.4.4. *Enterococcus faecalis*

Presumptive *E. faecalis* isolates were obtained through selective culturing on m-Enterococcus agar (Method NO. 9230C). Colonies showing pink to red pigmentation were selected and purified for subsequent confirmatory analyses.

2.5. Identification by Analytical Profile Index (API) 20 Strips

Biochemical profiling of Enterobacteriaceae and other non-fastidious gram-negative rods was performed using the API 20E system (bioMérieux, France). Standardized bacterial suspensions were used to rehydrate the dehydrated substrates in microtubes, which were then incubated at 37°C for 18–24 hours. Metabolic activity was evaluated based on the observed color changes, either directly or after applying specific

reagents. Identification was finalized by matching the resulting biochemical patterns against a reference database according to the manufacturer's guidelines (**Juang & Morgan, 2001**).

2.6. Molecular Identification and Phylogenetic Analysis of *P. aeruginosa* Isolates

Three purified *P. aeruginosa* isolates with distinct morphological characteristics were selected for molecular identification. The isolates were cultivated in TSB supplemented with 2% NaCl (Merck, UK) then incubated at 37°C for 24h. DNA was extracted using TKR101 Kit, Chromous DpvL. Ltd., Germany, and purity of DNA was quantitatively determined using electrophoresis on a 1% (w/v) agarose gel. 16S rDNA gene was amplified by a thermal cycler (ABI 2720) in a total volume of 100µL containing 2.5mM dNTPs, 10× buffer, Taq DNA polymerase (3U), 10ng of template, and forward (5'-CAGACTGCGATCCGGACTAC-3') and reverse (5'-TGGTAGTCCACGCCGTAAAC-3') primers for G-bacteria (**Azzam *et al.*, 2025**). The denaturation step was adjusted at 94°C/5min, followed by 35 cycles of denaturation at 94°C/30s, annealing at 55°C/30s, and extension at 72°C/2min, with a final elongation step at 72°C/5min, and reaction products were sequenced following standard protocols by Applied Biosystems Ver. 3.1. Resulting sequences were deposited in public nucleotide databases and compared with reference entries using BLAST to confirm species identity. Phylogenetic relationships were inferred by aligning sequences with related strains and constructing a tree using the ClustalW algorithm in MEGA software (v.11.0) (**Kumar *et al.*, 2016**).

2.7. Statistical and Computational Data Analysis

Each experiment was conducted in triplicate, and results are reported as mean values. Statistical computations were performed using the SPSS statistical package (version 23). Data interpretation involving multiple variables was performed through correlation coefficient matrixes to assess the relationships between physicochemical and bacteriological parameters. Pearson correlation analysis was applied following confirmation of data normality, with correlations deemed statistically significant at $P < 0.05$. This approach facilitated the identification of key interactions among water quality indicators that influence overall water quality. For bioinformatics data analysis, the following advanced software and programming language were applied:

- Basic alignment approach (BLASTN).
- Clustalw software (version 1.74).
- DNAMAN software (Lynnon Biosoft, Quebec, Canada, v10.0.0)
- Molecular Evolutionary Genetic Analysis (MEGA X) software (US, v11.0).
- CLC Genomics Workbench (Qiagen, Netherlands, v24.0.2).

RESULTS AND DISCUSSION

A comprehensive overview of physicochemical and bacteriological data from the drain outlets and Rosetta branch sites during winter 2023 is shown in Tables (1–7).

1. Physico-Chemical Assessment of Water Samples

1.1. Temperature

Temperature plays a crucial role in regulating chemical reaction rates, metabolic processes, and interactions between pollutants, parasites, and other pathogens in aquatic systems. As presented in Tables (1, 2), water temperature ranged from 28–33°C at the drain outlets and 27–32°C along the Rosetta branch. These variations were primarily influenced by climatic conditions, sampling time, and sunlight duration, indicating that temperature was largely governed by ambient air rather than thermal effluents. The correlation matrix (Fig. 2) showed a strong positive association between temperature and pH ($r=0.69$, $P<0.05$), likely due to the hydrolysis of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions at elevated temperatures, generating hydroxyl (OH^-) ions that increase pH. A comparable trend was reported by **Azzam *et al.* (2022)** and **Mohamed *et al.* (2026)**.

1.2. pH

pH, which reflects hydrogen ion activity, regulates key biological and chemical processes in aquatic environments. As presented in Tables (1, 2), all water samples exhibited pH values within permissible limits, consistently above 6.5 typical of natural waters containing carbonates and bicarbonates (**Azzam, 2015; Mohamed *et al.*, 2026**). Elevated pH levels likely result from photosynthetic activity, where CO_2 uptake by aquatic plants reduces carbonic acid concentration, thereby increasing pH (**Soliman *et al.*, 2023**).

Table 1. Physicochemical parameters (mean values) for samples obtained from drains outlet.

Parameter	Unit	R ^a	S	G	Z	T	LAW 48/1982 ^b
Temp.	°C	28	28	29	30	33	5 degrees above normal
pH	Unit	7.2	7.65	8.45	8.1	7.75	7-8.5
EC	mmohscm ⁻¹	1.369	1.382	0.889	0.911	1.808	-
TDS	mgL ⁻¹	950	873	568	411	1120	not exceed than 500
Tur.	NTU	11.32	18.06	12.01	10.32	10.36	-
NH ₃	mgL ⁻¹	2.1	11	1.4	0.69	15	not exceed than 0.5
DO	mgL ⁻¹	0.32	1.55	1.40	0.96	0.91	not less than 5
BOD	mgL ⁻¹	119	30	11	87	13	not exceed than 10
COD	mgL ⁻¹	236	89	79	40	92	-
Cl ⁻	mgL ⁻¹	215.1	146.9	116	86	179.6	-
NO ₂ ⁻	mgL ⁻¹	1.3	0.72	0.78	0.43	0.63	-
NO ₃ ⁻	mgL ⁻¹	30.5	45.60	12.5	7.1	10.5	not exceed than 45
PO ₄ ³⁻	mgL ⁻¹	0.19	<0.2	<0.2	<0.2	<0.2	not exceed than 1
SO ₄ ²⁻	mgL ⁻¹	127	151	80.4	89.2	109	not exceed than 200

CO ₃ ²⁻	mgL ⁻¹	0	0	0	0	0	-
HCO ₃ ⁻	mgL ⁻¹	236	249	219	238	315	-
Total alka.	mgL ⁻¹	236	249	219	238	315	200
Ca ²⁺	mgL ⁻¹	69.71	90.2	52.69	46.5	91.41	-
K ⁺	mgL ⁻¹	18	18	10	10	16	-
Mg ⁺²	mgL ⁻¹	26.04	32.01	17.73	17.33	32.6	-
Na ⁺	mgL ⁻¹	170	167	100	91	182	-

^a Sampling sites R, S, G, Z, and T are depicted in Fig. (1) and detailed in Table (1); ^b LAW 48/1982 is the Egyptian legislation designed to protect the River Nile and other waterways from pollution. ^c -, no standard guideline exists.

1.3. Electric Conductivity (EC)

EC indicates the capacity of water to carry an electric current and primarily depends on the concentration of dissolved ions, especially mineral salts. In the current study, EC measured at the drain outlets ranged from 0.889 to 1.808 mmohscm⁻¹, with the highest values recorded at the Sabal, Tala, and El-Rahawy drains, while El-Tahreer and Zawiet El-Bahr outfalls exhibited comparatively lower readings (Table 1). These elevated EC levels indicate substantial inputs from land runoff and industrial effluents, potentially creating salinity-related hazards for irrigation when drainage water is reused improperly (acceptable EC < 700 mmohscm⁻¹; Ayres & Westcott, 1985). Along the Rosetta branch, EC varied between 0.405 and 0.925 mmohscm⁻¹, with higher values consistently observed downstream of the drain discharge points (Table 2). Heatmap correlation matrix revealed a negative correlation with DO ($r=-0.44$, $P<0.05$) and strong positive correlations with several other water quality parameters (Fig. 2), consistent with previous studies (Omran & Azzam, 2025; Mohamed *et al.*, 2026).

Table 2. Physicochemical parameters (mean values) for samples obtained from Rosetta branch.

Parameter	Unit	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^b
Temp.	°C	28	27	29	28	30	29	32	30	32	32	5 degrees above normal
pH	Unit	7.89	7.31	7.90	8.01	7.81	7.32	7.60	7.73	8.02	8.11	7-8.5
EC	mmohscm ⁻¹	0.46	0.751	0.405	0.735	0.547	0.609	0.556	0.701	0.501	0.925	- ^c
TDS	mgL ⁻¹	251	492	272	601	350	390	356	398	246	683	not exceed than 500
Tur.	NTU	6.45	10.33	9.05	12.02	3.27	11.03	7.51	9.06	8.12	9.01	-
NH ₃	mgL ⁻¹	0.03	0.61	0.96	9	0.49	0.71	0.40	0.52	0.96	11	not exceed than 0.5
DO	mgL ⁻¹	9.11	1.82	6.01	4.01	5.31	2.95	5.83	3.11	5.02	4.07	not less than 5
BOD	mgL ⁻¹	10	43	4	10	7	3	9	14	8	11	not exceed than 6
COD	mgL ⁻¹	21	60	28	31	26	31	14	29	11	33	-
Cl ⁻	mgL ⁻¹	31.8	101.7	28.1	150.2	44.7	59	48.5	61	84.2	141.3	-
NO ₂ ⁻	mgL ⁻¹	0.2	0.86	0.2	0.53	0.25	0.52	0.27	0.39	0.15	0.48	-
NO ₃ ⁻	mgL ⁻¹	0.4	19.1	0.6	0.49	4.6	6.7	4.4	6.3	0.95	7.9	not exceed than 45
PO ₄ ³⁻	mgL ⁻¹	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	-
SO ₄ ²⁻	mgL ⁻¹	32.9	78	34	126	41.9	63.2	42.9	74.1	28.3	101	not exceed

												than 200
CO ₃ ²⁻	mgL ⁻¹	0	0	0	0	0	0	0	0	0	0	-
HCO ₃ ⁻	mgL ⁻¹	185	202	170	201	208	210	214	227	198	274	-
Total alka.	mgL ⁻¹	185	202	170	201	208	210	214	227	198	274	150
Ca ²⁺	mgL ⁻¹	37.21	50.11	43.17	75.1	42.18	44.91	42.7	43.9	45.7	95.67	-
K ⁺	mgL ⁻¹	6	11	9	13	7	8.5	7	10	9	15	-
Mg ²⁺	mgL ⁻¹	10.42	16.23	11.53	24.98	12.05	15.26	13.12	15.81	11.86	21.08	-
Na ⁺	mgL ⁻¹	40	110	44	155	51	63	48	77	40	151	-

^a Sampling sites R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2 are depicted in Fig. 1 and detailed in Table 1. ^b LAW 48/1982 is the Egyptian legislation designed to protect the River Nile and other waterways from pollution. ^c -, no standard guideline exists.

1.4. Total Dissolved Solids (TDS)

TDS comprise both organic and inorganic constituents, many of which are undesirable due to their potential to impart color, taste, and odor to water, exert osmotic stress on aquatic organisms, or introduce carcinogenic compounds particularly halogenated species. TDS values at the drain outfalls exceeded acceptable thresholds, ranging from 873 to 1120 mgL⁻¹, except for El-Tahreer and Zawiet El-Bahr drain outfalls (Table 1). While, Table (2) presents the TDS levels in water samples from the Rosetta branch, which mostly remained within the acceptable limits, except for Sabal and Tala downstream sites. Such elevated levels suggest potential irrigation challenges associated with unauthorized drainage reuse, as TDS concentrations should remain below 450 mgL⁻¹ to avoid salinity hazards (Ayres & Westcott, 1985). Consistent with these observations, TDS exhibited a strong positive correlation with both electrical conductivity and turbidity ($r = +0.97$, $P < 0.05$) in agreement with the findings of Azzam and Faiesal (2019).

1.5. Turbidity

Turbidity indicates the concentration of fine particles suspended in water, including colloidal substances such as clay, silt, organic debris, plankton, and other microscopic organisms, as well as inorganic matter. It is commonly used to measure pollution in surface water (Azzam & Ibrahim, 2021). In this study, the turbidity levels at the drain outlets ranged between 10.32 and 18.06 NTU, whereas along the Rosetta branch, they varied from 3.27 to 12.02 NTU (Tables 1 and 2). The gradual increase in turbidity downstream is likely associated with input from drain effluents. Moreover, turbidity showed positive correlations with most of the monitored parameters, whereas it was negatively correlated with dissolved oxygen ($r = -0.35$, $P < 0.05$) and pH ($r = -0.21$, $P < 0.05$) (Fig. 2).

1.6. Ammonia (NH₃)

In the present study, NH₃ concentrations showed pronounced spatial variability frequently exceeded the permissible limit of 0.5 mg L⁻¹ set by Law 48/1982. Concentrations ranged between 0.69 to 15 mg L⁻¹ at the drain outfalls (Table 1) and from 0.52 to 11 mg L⁻¹ along the Rosetta branch (Table 2), reflecting substantial organic pollution inputs. Ammonia toxicity is strongly influenced by pH, which ranged from 7.20 to 8.45 during the study period. Corresponding NH₃ levels exceeded USEPA (1999) guideline values for most sites, except for upstream locations near El-Rahawy, El-

Tahreer, and Zawiet El-Bahr. Pearson correlation analysis revealed strong positive relationships between NH_3 and NO_3^- , BOD, and bacteriological indicators ($r = +0.91$, $P < 0.05$), along with a negative correlation with DO (Fig. 2), highlighting the combined influence of sewage discharge and agricultural drainage on ammonia enrichment at the studied sites (Soliman *et al.*, 2022).

1.7. Dissolved Oxygen (DO)

Adequate levels of DO promote the activity of aerobic microbes and the decomposition of organic matter, making DO a key indicator of water quality. In contrast, reduced DO concentrations favor anaerobic microorganisms, which can deteriorate water quality and produce odorous gases, such as H_2S , NH_3 , and CH_4 (Elbahnasawy *et al.*, 2021). DO concentrations varied across the sampling sites according to pollution intensity and site characteristics. Nearly all samples collected from the drain outfalls fell below the permissible limit of 5 mgL^{-1} specified by Law 48/1982, ranging from 0.32 to 1.55 mgL^{-1} (Table 1). Such depletion indicates elevated loads of organic matter and nutrients (Soliman *et al.*, 2022). In contrast, DO levels in the Rosetta branch fluctuated between 1.82 and 4.01 mgL^{-1} , falling below the permissible limit of 5 mgL^{-1} , except for upstream locations near El-Rahawy, Sabal, Zawiet El-Bahr, and Tala (Table 2), reflecting spatial variations in water quality. DO concentrations are strongly influenced by temperature and salinity declining with higher salinity and rising as temperature decreases. The observed depletion in DO likely results from microbial decomposition of organic-rich effluents discharged directly from the drains (Lin *et al.*, 2024).

1.8. Biochemical Oxygen Demand (BOD)

BOD, which represents the oxygen consumed by aerobic microorganisms during organic matter decomposition, serves as a key indicator of organic water pollution (Azzam *et al.*, 2024). Elevated values were observed at most drain outfalls and along impacted Rosetta branch sites (Tables 1, 2), ranging from 11 – 119 mg L^{-1} in drains and 7 – 43 mg L^{-1} in the branch, frequently exceeding the permissible limits except for upstream locations near Sabal and El-Tahreer. This suggests substantial organic loading from domestic sewage, industrial discharge, and urban runoff (El-Meihy, 2018; El Sayed *et al.*, 2020; Azzam *et al.*, 2022). BOD levels were strongly correlated with bacterial indicators and negatively correlated with temperature ($r = -0.48$, $P < 0.05$), pH, and dissolved oxygen (DO) ($r = -0.77$, $P < 0.05$), reflecting oxygen depletion driven by microbial activity (Omran *et al.*, 2024).

1.9. Chemical Oxygen Demand (COD)

COD is widely applied as an important parameter for assessing organic contamination in aquatic systems (Lv *et al.*, 2024). Similar to the BOD assay, COD analysis provides valuable information on the potential ecological impacts of organic matter, including compounds that are resistant to biological degradation (Azzam *et al.*, 2022; Mohamed *et al.*, 2026). As shown in Tables (1, 2), COD concentrations at the drainage outlets varied considerably, ranging between 40 and 236 mgL^{-1} , whereas

samples collected along the Rosetta branch exhibited lower values, fluctuating from 11 to 60 mgL⁻¹. Importantly, COD levels in all examined surface and drainage water samples exceeded the permissible threshold of 10 mg L⁻¹ stipulated by Egyptian Law NO. 48/1982. The concurrently high BOD and COD concentrations recorded at the investigated sites along the Rosetta branch indicate substantial organic pollution, most likely associated with intensive anthropogenic pressures in the surrounding area.

1.10. Major Anions and Cations

As shown in Tables (1, 2), chloride and sulfate concentrations at most sampling sites were within normal ranges. In the drain outfalls, Cl⁻ concentrations ranged from 86 to 215.1 mgL⁻¹ and SO₄²⁻ from 80.4 to 151 mgL⁻¹, while in the Rosetta branch, Cl⁻ varied between 28.1 and 150.2 mgL⁻¹ and SO₄²⁻ between 28.3 and 126 mgL⁻¹. Elevated Cl⁻ levels at some sites suggest inputs of sewage and industrial effluents rich in chlorides, as Cl⁻ concentrations should not exceed 50 mgL⁻¹ (Azzam, 2010; Mohamed *et al.*, 2026). PO₄³⁻ concentrations were generally below 0.2 mgL⁻¹, except at El-Rahawy outlet, where value of 0.19 mgL⁻¹ was recorded. These findings remain within the permissible limits (1 mgL⁻¹). NO₃⁻ levels were within acceptable limits (≤ 45 mgL⁻¹) in all drains, except at the Sabal outlet, where higher values were attributed to the oxidation of NH₃⁺ to NO₃⁻ and NO₂⁻ through nitrification by aerobic bacteria. NO₃⁻ concentrations were within permissible limits at all sites along Rosetta branch. Carbonate ions were not detected in any of the collected samples, whereas bicarbonate (HCO₃⁻) concentrations ranged from 219 to 315 mgL⁻¹ at the drain outfalls and from 170 to 274 mgL⁻¹ in the Rosetta branch. Bicarbonate showed a weak positive correlation with total coliforms ($r = +0.15$, $P < 0.05$). Total alkalinity values exceeded the permissible limits (200 mg L⁻¹ for drains and 150 mgL⁻¹ for the Rosetta branch) at all sites, reflecting the clear influence of drain discharges on Nile water quality. On the other hand, the measured cations concentrations of Ca²⁺, K⁺, Mg²⁺, and Na⁺ in the water from both the drains and Rosetta branch complied with the established limits. Sodium was the predominant cation, with calcium, potassium, and magnesium present at lower levels (Tables 1 and 2).

1.11. Trace Metals

Freshwater contains low levels of trace metals from both natural and human sources (El-Meihy, 2018; Soliman *et al.*, 2023). Analysis of ten metals in the Rosetta branch and drain outlets showed concentrations below the Egyptian legal limits (Tables 3, 4). Domestic wastewater and agricultural runoff were identified as the main contributors to metal pollution, which is consistent with previous studies (El Sayed *et al.*, 2020; Azzam *et al.*, 2023).

Table 3. Mean trace metal contents (mg L⁻¹) in water samples from drains outlet.

Parameter	R ^a	S	G	Z	T	LAW 48/1982 ^c
Al ^b	0.0852	0.1150	0.0922	0.0371	0.0853	- ^d
Ba	0.071	0.068	0.057	0.008	0.066	-
Cr	0.0238	0.0301	0.0138	0.0368	0.0091	< 0.01
Cu	0.0226	<0.008	0.049	0.055	0.0093	< 1.0
Fe	0.0340	<0.006	<0.006	0.042	<0.006	< 1.0
Mn	0.0392	0.0221	0.0156	0.0331	0.0084	< 1.5
Ni	<0.004	<0.002	<0.002	<0.004	<0.004	-
Se	<0.007	<0.007	<0.007	<0.007	<0.007	-
V	0.0419	0.0293	0.0632	0.0249	0.0135	-
Zn	0.0501	0.005	0.0118	0.0637	0.0091	< 1.0

^a Sampling sites R, S, G, Z, and T are depicted in Fig. (1) and detailed in Table (1). ^b The metals analyzed include Al (aluminum), Ba (barium), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Ni (nickel), Se (selenium), Sn (tin), V (vanadium), and Zn (zinc). ^c LAW 48/1982 is the Egyptian legislation designed to protect the River Nile and other waterways from pollution. ^d -, no standard guideline exists.

Table 4. Mean trace metal contents (mg L⁻¹) in water samples from the Rosetta branch.

Parameter	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^b
Al	0.0337	0.069	0.0471	0.0951	0.0548	0.0613	0.0192	0.024	0.0154	0.0391	-
Ba	0.029	0.053	0.051	0.037	0.031	0.064	0.032	0.054	0.019	0.028	-
Cr	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.0293	0.039	<0.002	<0.002	<0.01
Cu	0.0153	0.0201	<0.008	<0.005	0.018	0.033	0.0311	0.043	<0.004	0.0017	< 1.0
Fe	0.016	0.031	<0.006	<0.006	<0.006	<0.006	0.018	0.036	<0.006	<0.006	<1.0
Mn	0.0178	0.0315	0.0074	0.0209	0.0085	0.0099	0.0129	0.0326	0.0022	0.0053	< 1.5
Ni	<0.004	<0.004	<0.002	<0.002	<0.002	<0.004	<0.004	<0.004	<0.004	<0.004	-
Se	<0.007	<0.007	<0.007	<0.007	<0.005	<0.007	<0.007	<0.007	<0.007	<0.007	-
V	0.0103	0.0355	0.0181	0.025	0.0124	0.0585	0.0142	0.0181	0.004	0.011	-
Zn	0.0271	0.0347	<0.005	<0.005	0.0041	0.0073	0.0233	0.0529	0.0027	0.0058	< 1.0

^a Sampling sites R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2 are depicted in Fig. (1) and detailed in Table (1). ^b The metals analyzed include Al (aluminum), Ba (barium), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Ni (nickel), Se (selenium), Sn (tin), V (vanadium), and Zn (zinc). ^c LAW 48/1982 is the Egyptian legislation designed to protect the River Nile and other waterways from pollution. ^d -, no standard guideline exists.

2. Bacteriological Profile of River and Drain Samples

Microbial composition significantly influences Rosetta branch quality degree. The results from the ten sampling sites along the branch and five major drains are presented in Tables (5, 6).

Table 5. Bacteriological indicators (mean values) for samples obtained from drain outlets.

Parameter	R ^a	S	G	Z	T	LAW 48/1982 ^c
SPC at 22°C (CFU/mL) ^b	142 x 10 ⁴	210 x 10 ⁴	62 x 10 ³	34 x 10 ³	25 x 10 ⁴	- ^d
SPC at 37°C (CFU/mL)	53 x 10 ⁴	69 x 10 ⁴	23 x 10 ³	9 x 10 ³	9 x 10 ⁴	-
TC (CFU/100mL ⁻¹)	60 x 10 ³	115 x 10 ³	78 x 10 ²	21 x 10 ²	16 x 10 ³	-
FC (CFU/100mL ⁻¹)	17 x 10 ³	32 x 10 ³	4 x 10 ²	9 x 10 ²	3 x 10 ³	-
FS (CFU/100mL ⁻¹)	5 x 10 ³	9 x 10 ³	1 x 10 ²	4 x 10 ²	1 x 10 ³	-

^a Sampling location, designated as R, S, G, Z, T, are depicted in Fig. (1) and detailed in Table (1). ^b SPC refers to the standard plate count; TC denotes total coliform; FC represents fecal coliform; FS indicates fecal *streptococci*. ^c LAW 48/1982 pertains to the Egyptian legislation aimed at safeguarding the River Nile and other water bodies from pollution. ^d -, guideline not available.

Table 6. Bacteriological indicators (mean values) for samples obtained from the Rosetta branch.

Parameter	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^c
SPC at 22°C (CFU _{mL} ⁻¹) ^b	30 x 10 ²	72 x 10 ⁴	1400	93 x 10 ³	11 x 10 ²	28 x 10 ³	15 x 10 ²	49 x 10 ²	1000	33 x 10 ³	- ^d
SPC at 37°C (CFU _{mL} ⁻¹)	12 x 10 ²	8 x 10 ⁴	7 x 10 ²	13 x 10 ³	7 x 10 ²	5 x 10 ³	7 x 10 ²	12 x 10 ²	200	15 x 10 ³	-
TC (CFU100mL ⁻¹)	150	25 x 10 ³	500	21 x 10 ³	400	19 x 10 ²	350	600	94	7 x 10 ²	-
FC (CFU100mL ⁻¹)	21	900	200	3 x 10 ³	71	250	68	200	16	1 x 10 ²	-
FS (CFU100mL ⁻¹)	5	200	37	480	12	110	23	45	9	24	-

^a Sampling location, designated as R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2, are depicted in Fig. (1) and detailed in Table (1). ^b SPC refers to the standard plate count; TC denotes total coliform; FC represents fecal coliform; FS indicates fecal streptococci. ^c LAW 48/1982 pertains to the Egyptian legislation aimed at safeguarding the River Nile and other water bodies from pollution. ^d -, guideline not available.

2.1. Standard Plate Counts (SPC) Bacteria

SPC levels at 22°C and 37°C were generally high across the samples, reaching their highest concentrations at the Sabal drain outlet. At the drain outfalls, SPC values ranged from 34×10^3 to 210×10^4 CFU_{mL}⁻¹ at 22°C and from 9.0×10^3 to 69×10^4 CFU_{mL}⁻¹ at 37°C. Along the Rosetta branch, SPC values fluctuated between 10.0×10^2 and 72×10^4 CFU_{mL}⁻¹ at 22°C, and from 2.0×10^2 to 8×10^4 CFU_{mL}⁻¹ at 37°C. The elevated SPC values, particularly near drain outlets, indicate significant microbial contamination likely associated with sewage and organic waste discharges, reflecting deteriorated water quality conditions (Azzam *et al.*, 2017).

2.2. Total Coliforms

TC densities exhibited considerable spatial variation, ranging from 21×10^2 - 115×10^3 CFU_{mL}⁻¹ at the drain outfalls and from 94 - 25×10^3 CFU_{mL}⁻¹ along the Rosetta branch. The highest TC counts were recorded at the Sabal drain outlet and its downstream site. Except for Zawiet El-Bahr drains, all other monitored drains and more than 80% of the Rosetta branch sampling sites were within the limits set by the USEPA (2012), which recommends a maximum TC concentration of 5000 CFU mL⁻¹. However, when compared to the more stringent limits proposed by Mohamed *et al.* (2026), a maximum of 1000 CFU_{mL}⁻¹ for surface waters designated for drinking water supply some sites exhibited elevated TC concentrations, indicating potential microbiological contamination risks.

2.3. Fecal Coliforms

FC counts at the drain outfalls exhibited marked variation, with values ranging from a maximum of 32×10^3 CFU_{mL}⁻¹ at Sabal drain to a minimum of 400 CFU_{mL}⁻¹ at El-Tahreer drain. In the Rosetta branch, FC concentrations varied between 16 and 3×10^3 CFU_{mL}⁻¹. Notably, El-Tahreer and Zawiet El-Bahr drains were the only sites that met the international standards set by the USEPA (2012), which recommend a maximum FC limit of 2000 CFU_{mL}⁻¹. Guideline compliance was observed at around 70% of the sites

along the Rosetta branch. Nevertheless, when compared with the more stringent guideline of 200 CFU/mL⁻¹ for surface waters intended for drinking purposes (**Azzam, 2015**), the findings indicate that most samples were bacteriologically unsafe for human consumption.

2.4. Fecal Streptococci

FS counts varied considerably, ranging from a maximum of 9×10^3 CFU/mL⁻¹ at Sabal drain to a minimum of 100 CFU/mL⁻¹ at El-Tahreer drain. In contrast, FS levels in the Rosetta branch fluctuated between 5 and 480 CFU/mL⁻¹. In general, sites in both the drains and the Rosetta branch that recorded FS counts not exceeding 1000 CFU/mL⁻¹ were found to be within the international standard limits recommended by **USEPA (2012)** except for El-Rahawy and Sabal drains outlets. The upward trend in TC, FC, and FS counts downstream appears linked to drain effluents, consistent with earlier reports (**Azzam et al., 2017; El Sayed et al., 2020; Omran & Azzam, 2025**).

Water quality assessments revealed that all studied drains contributed to chemical and microbial pollution, with El-Rahawy, Sabal, and Tala showing the most pronounced contamination. The observed spatial variability likely stems from a combination of industrial, agricultural, and domestic inputs (**El-DougDoug et al., 2020; Elbahnasawy et al., 2021; Omran et al., 2024; Mohamed et al., 2026**). Elevated organic and bacterial loads at El-Rahawy and Sabal notably reduced the suitability of the Rosetta branch for potable use, as reflected in higher NH₃, TDS, EC, turbidity, BOD, and total alkalinity, and lower DO (**Azzam et al., 2014**). Pollution gradually decreases along the branch owing to self-purification and dilution (**Soliman et al., 2023**).

3. Water Quality Index (WQI)

WQI showed clear spatial variation among the upstream, outlet, and downstream sites of the examined drains (Fig. 2). Upstream locations exhibited WQI values ranging from 42.53 ± 1.50 to 66.74 ± 1.30 , corresponding to medium water quality. In contrast, outlet sites had significantly lower WQI values between 23.92 ± 0.20 and 31.25 ± 0.20 , classifying them as poor-quality zones. This sharp decline reflects the direct contribution of untreated domestic and agricultural effluents from drains into the Rosetta branch. Downstream sites displayed WQI values of 28.86 ± 1.99 to 41.84 ± 1.95 , indicating that water quality remained compromised despite partial dilution with branch water at these sites. The minimum WQI (23.92 ± 1.00) was recorded at the El-Rahawy drain outlet, highlighting it as a critical pollution source for the lake. The WQI pattern demonstrated a progressive deterioration of water quality downstream of major drain discharges, emphasizing the cumulative impact of anthropogenic inputs on the Rosetta branch ecosystem. These trends are consistent with prior observations of water quality degradation near agricultural and industrial discharges in the Nile Delta (**El-Zeiny et al., 2018; Omran & Azzam, 2025**). Moreover, WQI values showed a strong correlation with the measured physicochemical and microbiological parameters, with fecal coliforms

identified as the primary driver influencing the index (Azzam *et al.*, 2023; Mohamed *et al.*, 2026).

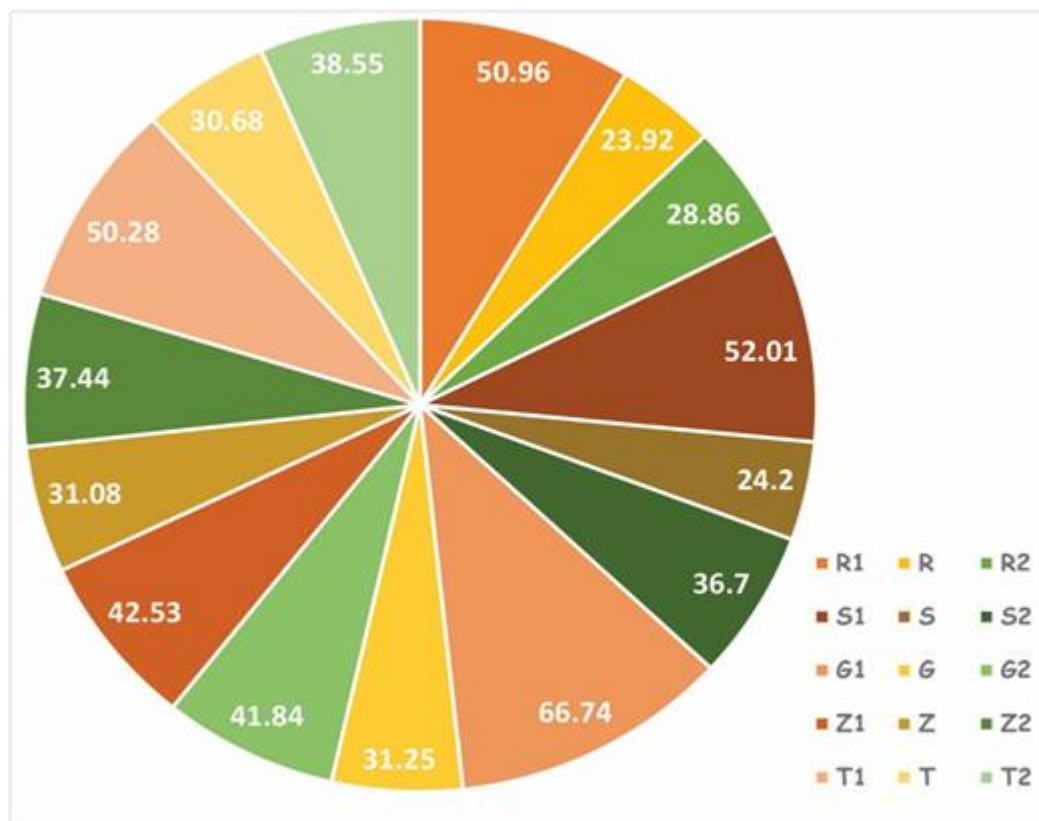


Fig. 2. Water Quality Index values (mean $\pm$ SD) for the studied water samples.

4. Bacterial Diversity and Identification in River Nile and Drainage Water Samples

A total of 310 bacterial strains were recovered from the analyzed water samples. Taxonomic assignment was achieved for 298 isolates (96.13%), whereas 12 isolates (3.87%) remained unclassified. The identified bacteria comprised six species belonging to four families. These families included Enterobacteriaceae, Pseudomonadaceae, Staphylococcaceae, and Enterococcaceae (Table 7). The majority of bacterial isolates were gram-negative (191 isolates, 64.09%), while gram-positive strains comprised 107 isolates (35.91%). In River Nile samples, *E.coli* was predominant (16.2%), with *P.aeruginosa* and *S.aureus* detected at 7.80 and 10.40%, respectively. The predominance of *E.coli* reflects recent fecal contamination, likely derived from domestic and agricultural runoff (Azzam *et al.*, 2014; Mohamed *et al.*, 2026).

Table 7. Distribution and relative abundance of bacterial taxa detected in the River Nile and drains outfalls.

Bacterial species	The River Nile		Drains	
	count	Abundance (%)	count	Abundance (%)
<i>E. coli</i>	48	16.2	21	7.0
<i>C. freundii</i>	0	0	5	1.63
<i>Salmonella</i> sp.	0	0	2	0.67
<i>P. vulgaris</i>	37	12.30	12	4.0
<i>P. aeruginosa</i>	22	7.80	44	14.60
<i>S. aureus</i>	30	10.40	17	5.50
<i>E. faecalis</i>	41	13.50	19	6.4
Total	178	60.2	120	39.8

The relatively high proportions of *P. vulgaris* and *E. faecalis* further indicate elevated organic pollution and the presence of enteric microorganisms associated with anthropogenic discharges. Conversely, drain outlets exhibited a distinct microbial composition, with *P. aeruginosa* (14.60%) and *E. coli* (7.0%) being the dominant species, followed by *E. faecalis* (6.40%). The abundance of *P. aeruginosa* and *E. coli* suggests strong influence from industrial and hospital effluents, as these species commonly thrive in nutrient-rich and polluted environments. *C. freundii* (1.63%) and *Salmonella* spp. (0.67%) were detected exclusively in drain outlets, indicating that these drains act as reservoirs for pathogenic bacteria. The occurrence of *Salmonella* was restricted to drainage points, indicating fecal contamination linked to anthropogenic or livestock sources, particularly poultry operations, while its absence along the Rosetta branch highlights spatial differences in pathogen distribution (Soliman *et al.*, 2022; Gwenzi *et al.*, 2023).

High densities of *P. aeruginosa* and *S. aureus* in water bodies pose public health risks, as exposure to contaminated water may enable pathogen transmission via mucosal contact or through skin lesions (Ezzat & Azzam, 2020; Mohamed *et al.*, 2026). The observed disparity in bacterial community composition between the River Nile and its drains underscores the role of drain effluents as major contributors to the microbiological degradation of the Nile system. The continuous discharge of untreated or partially treated wastewater likely explains these contamination patterns, emphasizing the urgent need for improved wastewater management and continuous microbial monitoring to protect water quality and public health.

5. Genotypic Characterization of Selected Bacterial Isolates

Molecular, genotype-based identification techniques are extensively utilized in aquatic microbiology because of their enhanced precision and consistency relative to traditional phenotypic methods. These techniques effectively reduce the errors associated

with phenotypic diversity and taxonomic ambiguity (Azzam *et al.*, 2014; Mohamed *et al.*, 2026). Given the high incidence of microbial contamination detected in the analyzed water samples, *P. aeruginosa* isolates were selected for molecular confirmation of the detection strategy. Three predominant isolates (APS01, APS02, and APS03) were selected for 16S rDNA sequencing based on their consistent biochemical positivity, widespread prevalence, and confirmed DNA and PCR quality. Spectrophotometric assessment at A260/A280 indicated acceptable DNA purity, with ratios of 1.44, 1.75, and 1.92 for APS01, APS02, and APS03, respectively. Amplification products were resolved on 2% agarose gels, yielding distinct bands ranging from ~ 1374 to 1491 bp, thereby confirming successful and specific PCR amplification. Comparative nucleotide analysis was performed using MSA between the obtained sequences and the global reference strains. The resulting sequences were deposited in the NCBI GenBank database under the accession numbers PP967334.1, PP967337.1, and PP967338.1. All examined isolates were definitively identified as *P. aeruginosa* using 16S rDNA PCR analysis. This finding aligns with previous studies that demonstrated the efficacy of molecular tools in reducing the misidentification of *P. aeruginosa* in aquatic environments (Ezzat & Azzam, 2020). Conversely, culture-dependent methods may impede accurate source tracking and delay mitigation efforts (Zhang *et al.*, 2022). Molecular identification approaches offer superior sensitivity and specificity, often achieving accuracy rates of 95–100% (Crone *et al.*, 2020; Azzam *et al.*, 2023; Dey *et al.*, 2026). Phylogenetic reconstruction based on aligned sequences grouped the analyzed strains into eight distinct clades (Fig. 3). The three Egyptian isolates exhibited 88% similarity among themselves and ~ 97% similarity to several internationally reported *P. aeruginosa* strains retrieved from GenBank, including BQ10, MML1, CM101, NG15, MBC, and D2 strains.

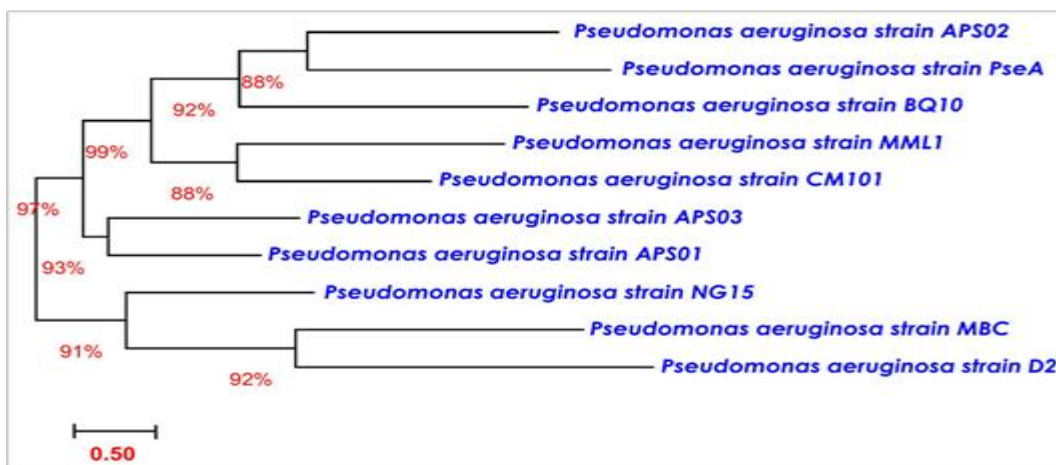


Fig. 3. Phylogenetic clustering of *P.aeruginosa* isolates with closely related GenBank sequences using 16S rRNA gene sequencing data.

Our findings demonstrated detectable genetic diversity among *P. aeruginosa* strains, as revealed by 16S rRNA gene sequencing. This diversity highlights the effectiveness of the bioinformatics workflow applied in this study, where the use of specific primers enabled the detection of a broad range of *P. aeruginosa* genotypes in aquatic systems, supporting the high specificity value (98%) obtained from the statistical evaluation. Therefore, employing multiple primers simultaneously offers greater analytical reliability than relying on individual primer sets alone (Silva *et al.*, 2025; Mohamed *et al.*, 2026). Although the concentration of initially extracted bacterial DNA plays a critical role in generating consistent and representative results, increasing the number of primers is a deliberate strategy to enhance DNA recovery and improve the yield of purified genetic material. This optimization increased the likelihood of capturing the genetic variability within the target population. Future studies are recommended to focus on expanding the molecular characterization of newly isolated aquatic bacteria, particularly from heavily contaminated drainage and surface waters. This approach may facilitate direct pathogen detection from environmental water samples, without the need for prior culture isolation, in agreement with previous investigations (Pang *et al.*, 2019; Doménech-Sánchez *et al.*, 2023; Azzam *et al.*, 2025; Mohamed *et al.*, 2026).

Sequence-based analysis of the 16S rRNA gene demonstrated clear genetic heterogeneity among the *P.aeruginosa* strains. The applied bioinformatics pipeline, together with the selected primer combinations, proved capable of capturing diverse strain profiles in aquatic matrices, which supports the high specificity level (99%) previously obtained through statistical assessments. Using more than one primer set concurrently improved detection robustness compared to single-primer assays (Ezzat & Azzam, 2020; Silva *et al.*, 2025). While the abundance of bacterial DNA remains a key determinant of analytical sensitivity, primer optimization in the present study was designed to enhance DNA yield and improve the concentration of the purified products. This methodological adjustment increased the likelihood of recovering representative genetic material from complex environmental sample. Future investigations should aim to broaden the molecular profiling of aquatic bacteria, with particular emphasis on drainage networks and river systems subjected to intense chemical and biological contamination. This approach may enable culture-independent molecular detection of waterborne pathogens, providing a more rapid and reliable alternative for environmental monitoring. Similar perspectives have been highlighted in recent molecular surveillance studies (Oon *et al.*, 2023; Curry *et al.*, 2024; Cholet *et al.*, 2025; Mohamed *et al.*, 2026).

6. Statistical and Computational Data Analysis

Pearson's correlation analysis indicated a strong positive relationship among the bacteriological parameters, including SPC, TC, FC, and FS, with $r = +0.98$ ($P < 0.05$), as illustrated in Fig. (4). Comparable patterns of interdependence among microbial indicators have been documented in earlier studies on the Nile River and its drainage

CONCLUSION

This study demonstrates that the Rosetta branch of the River Nile is heavily influenced by untreated agricultural and municipal drain discharges, which drive both physicochemical and microbiological degradation of water quality. Organic and nutrient

enrichment from these effluents contributes to elevated NH₃, BOD, TDS, and salinity levels, whereas DO depletion and increased turbidity further compromise water quality. Microbial assessments revealed high densities of coliforms, fecal indicators, and some pathogenic bacteria, with drains serving as hotspots for *P. aeruginosa*, *E. faecalis*, and *Salmonella* spp., highlighting substantial public health risks. Molecular analyses confirmed genetic variability among key bacterial strains and highlighted the superior reliability of multi-primer and bioinformatics approaches over conventional culture methods. WQI evaluations identified persistent poor-quality zones at drain outlets, with only partial downstream recovery, reflecting limited natural self-purification processes. Our findings highlight the critical role of drain effluents in shaping the water quality and microbial risk along Rosetta branch. Effective management strategies, including enhanced wastewater treatment, controlled drainage reuse, and routine microbial monitoring, are essential for mitigating pollution, protecting ecosystem health, and safeguarding public health. Expanding the molecular surveillance of aquatic bacterial communities offers a promising pathway for the rapid and accurate detection of waterborne pathogens in highly impacted freshwater systems.

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