



An Integrated Water Quality Assessment of the Rosetta Branch, River Nile

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ABSTRACT

The River Nile, Egypt's main source of drinking water, is increasingly impacted by untreated industrial effluents, agricultural runoff, and domestic wastewater discharge. This study evaluated the water quality along a 120 km stretch of the Rosetta branch using 75 samples collected from the branch and five major drains in the area. Comprehensive physicochemical and bacteriological analyses were conducted in December 2023. The findings revealed a clear deterioration in water quality attributed to drain discharges, reflected in elevated levels of ammonia, total dissolved solids, electrical conductivity, biological oxygen demand, total alkalinity, and turbidity, and a marked decline in dissolved oxygen. High counts of total and fecal coliforms, along with fecal streptococci exceeding the permissible limits, confirmed agricultural runoff and sewage effluents as the dominant pollution sources. The calculated Water Quality Index demonstrated a gradual decline in water quality from upstream to downstream, with the Sabal drain representing the most critical pollution hotspot. Correlation matrix analysis revealed several significant positive and negative relationships between physicochemical and bacteriological parameters. Among the 278 bacterial isolates identified, 59.35% originated from the Rosetta branch and 40.65% from the drain outlets. Newly isolated *Escherichia coli* strains were confirmed through 16S rDNA sequencing, and their nucleotide data were submitted to the NCBI GenBank (USA). The bioinformatics results supported the analytical outcomes, confirming the strong link between chemical indicators and microbial contamination. This study emphasizes the urgent need for effective wastewater treatment and continuous water quality monitoring to protect public health and preserve the ecological integrity of the Nile River.

1. Introduction

The Nile River represents Egypt's main freshwater supply and flows for nearly 950 km from downstream of the High Aswan Dam toward northern Egypt. Before reaching the Mediterranean Sea, the river bifurcates at the Delta Barrage into the Rosetta and Damietta branches, giving rise to the Nile Delta system that supports extensive agricultural and urban activities (**El-Rawy *et al.*, 2019; Woodward *et al.*, 2022; Omran and Azzam, 2025**).

Among these distributaries, the Rosetta branch represents a critical freshwater stream that stretches northward for about 239 km along the western boundary of the Delta, with an average width of 180 m and a depth ranging from 2 to 4m (**Nada *et al.*, 2021**). It terminates at the Edfina Barrage before reaching the Mediterranean Sea (**Azzam, 2015; Kristanti *et al.*, 2022**). Owing to its hydrological position, the Rosetta branch plays a vital role in agricultural irrigation, domestic water supply, industrial activities, fisheries, and recreational uses, rendering it indispensable to both the local and national economy (**Omran *et al.*, 2024; Salem and Zidan, 2025**).

Despite its strategic significance, the Rosetta branch faces significant environmental challenges owing to ongoing anthropogenic pressures (**Al-Afify *et al.*, 2022**). It receives over three million cubic meters of untreated or partially treated domestic and industrial effluents daily, along with substantial volumes of agricultural drainage (**Khalil *et al.*, 2023**). Major drains, including El-Rahawy, Sabal, El-Tahreer, Zaweit El-Bahr, and Tala, discharge into the branch, conveying agricultural residues and nutrients as well as untreated domestic wastewater from approximately fifty-five towns and villages along its course (**Azzam *et al.*, 2025**). These discharges significantly contribute to organic and microbial contamination, compromising water safety and increasing public health risks (**Elbahnasawy *et al.*, 2021; Kristanti *et al.*, 2022**).

In parallel, the Rosetta branch faces industrial pollution stressors, particularly along its eastern bank, where effluents from El-Maliya, Mobidat, and the Salt and Soda companies are discharged directly into the water (**El-DougDoug *et al.*, 2020; Olatunji *et al.*, 2024**). These industrial outfalls introduce chemical residues, heavy metals, and other potentially toxic substances that aggravate water quality deterioration (**Zhang *et al.*, 2023**). The combined impact of agricultural, domestic, and industrial effluents creates a complex pollution load, manifested in eutrophication, changes in

physicochemical properties, elevated microbial levels, and ecosystem imbalance (**Azzam *et al.*, 2024**).

The Rosetta branch constitutes a critical component of Egypt's freshwater network, underpinning agricultural productivity and regional socioeconomic activities. In this context, the present study assessed the water quality of the lake by jointly examining its physicochemical attributes and bacteriological indicators. Multivariate correlation analysis was applied to unravel the relationships among key contaminants and distinguish the dominant pollution drivers. Beyond conventional monitoring approaches, this study incorporated molecular validation of bacterial isolates using 16S rDNA sequencing in combination with Water Quality Index (WQI) modeling, allowing a comprehensive evaluation of spatial pollution patterns. By integrating drain-specific microbial signatures with WQI outcomes, this study identified major contamination hotspots and established an updated reference framework reflecting post-2020 conditions, with direct relevance for sustainable water resource management in the Nile Delta.

2. Material and Methods

2.1. Study Area and Sampling Procedure

The investigated reach covered nearly 120 km of the Rosetta branch of the River Nile, extending from the vicinity upstream of the El-Rahawy drain to areas downstream of the Tala drain (**Figure 1**). Fifteen locations were investigated, including five drain discharge points (El-Rahawy, Sabal, El-Tahreer, Zawiet El-Bahr, and Tala) and ten sites distributed along the Rosetta branch, positioned upstream and downstream of each outlet (**Table 1**). Sites located upstream of the discharge points were considered baseline references for evaluating the deterioration of downstream water quality (**Omran and Azzam, 2025**). Sampling was conducted in December 2023, in accordance with the Standard Methods for the Examination of Water and Wastewater (**APHA, 2017**). In total, seventy-five subsurface water samples were collected at approximately 50 cm depth using sealed polyethylene containers, preserved under cooled conditions, and transported immediately to the laboratory to ensure the integrity of physicochemical and bacteriological analyses.

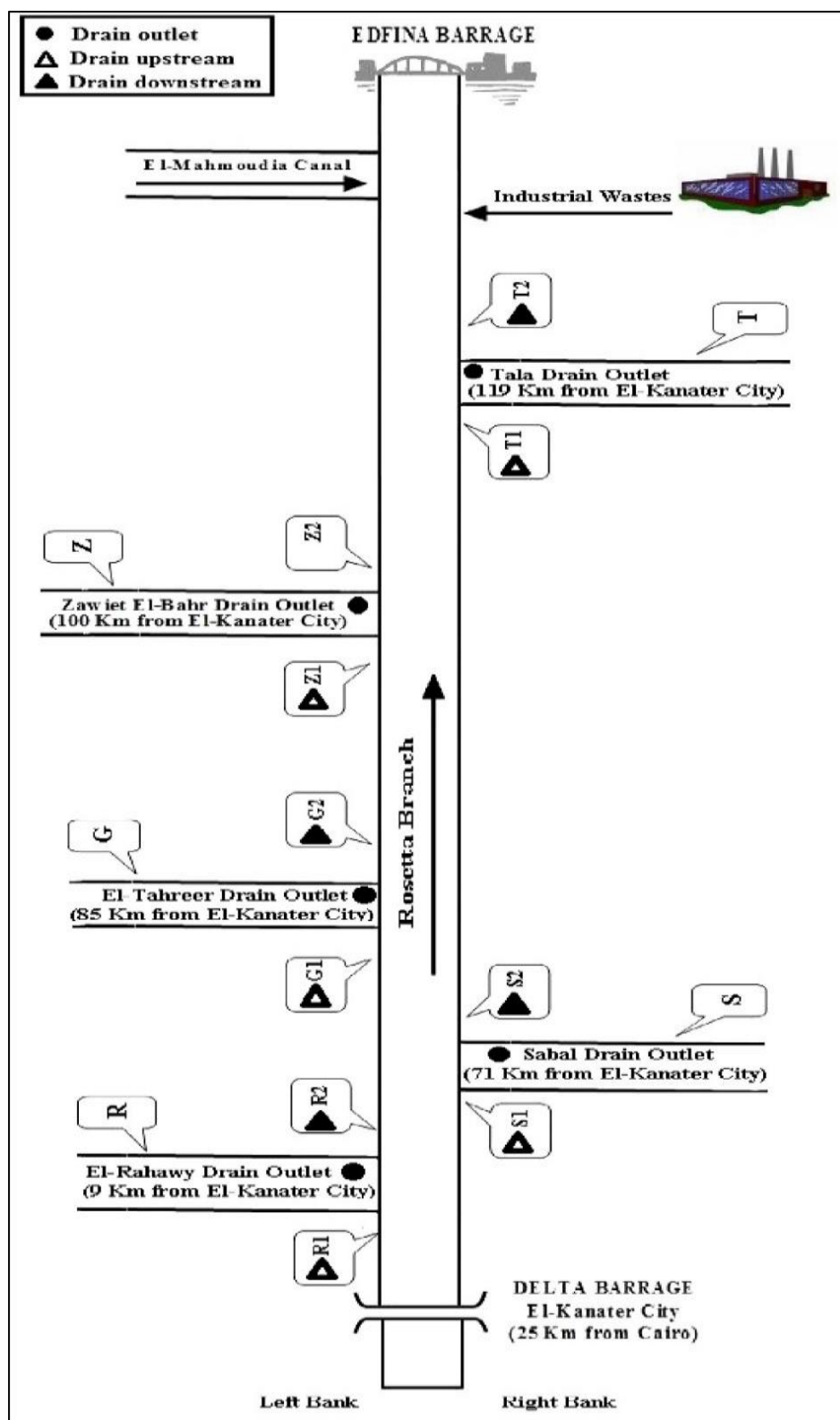


Figure 1. Schematic diagram showing the sampling sites (Drains & Rosetta branch).

Table 1. Sampling sites and coordinates along the Rosetta branch and drains outlets.

Site ID	Sampling location description	Latitude (N)	Longitude (E)
R1	Upstream section of Rosetta branch prior to El-Rahawy drain outlet	30.20840	31.03395
R	Discharge point of El-Rahawy drain at the left bank of Rosetta branch	30.20728	31.03297
R2	Downstream reach of the Rosetta branch after El-Rahawy drain outlet	30.20706	31.03010
S1	Upstream Rosetta branch before Sabal drain inflow	30.53264	30.84811
S	Outlet of Sabal drain at the right bank of Rosetta branch	30.53707	30.85197
S2	Downstream Rosetta branch following Sabal drain discharge	30.54140	30.85035
G1	Upstream Rosetta branch prior to El-Tahreer drain inflow	30.60653	30.79775
G	El-Tahreer drain discharge point at left bank	30.60685	30.79692
G2	Downstream Rosetta branch after El-Tahreer drain junction	30.60747	30.79652
Z1	Upstream of Rosetta branch before Zawiet El-Bahr drain outlet	30.71419	30.76534
Z	Outlet of Zawiet El-Bahr drain at the left bank of the Rosetta branch	30.71460	30.75528
Z2	Downstream Rosetta branch after Zawiet El-Bahr drain discharge	30.71924	30.76098
T1	Upstream section of the Rosetta branch prior to Tala drain inflow	30.81616	30.81044
T	Tala drain outlet located at the right bank of the Rosetta branch	30.81715	30.81327
T2	Downstream reach of Rosetta branch following Tala drain discharge	30.81954	30.81170

2.2. Physicochemical Assessment

Physicochemical characteristics of collected samples were determined according to internationally recognized analytical protocols (APHA, 2017). In situ measurements of temperature, pH, electrical conductivity, dissolved oxygen, and total dissolved solids were obtained using a portable multiparameter probe (Hydralab Surveyor) and subsequently confirmed in the laboratory. Turbidity levels were measured using a HACH turbidimeter (model 16800). Ammonia (NH_3) was quantified using an ammonia-selective electrode (ORION 95–12) coupled to a benchtop ion analyzer (ORION 940), while biochemical oxygen demand was evaluated using a fast respiratory BOD system (ORION 890). Inorganic anions, including chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}), were determined using ion chromatography (Dionex DX-500). Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) alkalinities were analyzed using ion chromatography (Metrohm). Concentrations of major cations (Ca^{2+} , Mg^{2+} , Na^+ , and K^+) and trace elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, V, and Zn) were measured by inductively coupled plasma–optical emission spectrometry (ICP–OES) equipped with an ultrasonic nebulizer (PerkinElmer Optima 3000).

2.3. Microbiological Assessment

Water samples were analyzed within six hours of collection according to **the Standard Methods for the Examination of Water and Wastewater (APHA, 2017)**. Standard plate counts (SPC) were obtained at 22 °C and 37 °C using the pour plate technique (Method No. 9215 B). Total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) were quantified using the membrane filtration method with a stainless-steel, autoclavable manifold linked to an oil-free Millipore vacuum/pressure pump. Filtration was performed on sterile gridded Sartorius membranes (47 mm diameter, 0.45 µm pore size). Cultures were grown on selective media M-Endo agar LES, M-Fc agar, and M-Enterococcus agar following Methods 9222 B, 9222 D, and 9230 C, respectively, using dehydrated media (Difco, USA). Bacterial densities were reported as colony-forming units per 100 mL (CFU/100 mL) and calculated using standard formula:

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

2.4. Selection, Isolation, and Identification of Aquatic Bacterial Isolates

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*

The presence of *E.coli* was assessed following Standard Method 9213 D using m-TEC agar as a selective medium. Inoculated plates were incubated at 44.5 ± 0.2 °C for 24 h, after which colonies exhibiting yellow to yellow-brown coloration were considered presumptive *E.coli*. Further confirmation was achieved by subculturing the selected colonies onto Eosin Methylene Blue (EMB) agar, where characteristic pink colonies with a distinctive metallic sheen were observed.

2.4.2. *Pseudomonas aeruginosa*

Isolation of *P.aeruginosa* was carried out using Standard Method 9213 E on M-PA-C agar medium. Plates were incubated at 41.5 ± 0.5 °C for 72 h. Colonies measuring approximately 0.8–2.2 mm in diameter, displaying a flat morphology with pale peripheral margins and dark brown to greenish-black centers, were selected as presumptive *P.aeruginosa*. Confirmation was performed by streaking onto cetrimide agar, a selective medium that suppresses competing microflora and enhances the production of characteristic fluorescein and pyocyanin pigments.

2.4.3. *Staphylococcus aureus*

Isolation of *S.aureus* followed Standard Method 9213 B using Baird–Parker agar. After incubation at 35 °C for 48 h, colonies exhibiting a shiny black appearance surrounded by a narrow clear halo were selected. Confirmatory identification was conducted by subculturing onto mannitol salt agar, where the development of golden-yellow colonies indicated mannitol fermentation typical of *S. aureus*.

2.4.4. *Enterococcus faecalis*

Presumptive *E.faecalis* isolates were recovered using M-Enterococcus agar according to Standard Method 9230 C. Colonies exhibiting pink to red coloration were selected and purified for further identification.

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

Biochemical characterization of *Enterobacteriaceae* and other non-fastidious Gram-negative rods was performed using API 20E system (bioMérieux, France). The system comprises 20 microtubes containing dehydrated biochemical substrates, which were inoculated with standardized bacterial suspensions to rehydrate the media. Inoculated strips were incubated at 37 °C for 18–24 h. Metabolic reactions were interpreted based on color changes, either occurring directly or following the addition of specific reagents. Final identification was achieved by comparing the obtained biochemical profiles with the manufacturer's reference database, following the established interpretation guidelines (Juang and Morgan, 2001).

2.6. Water Quality Index (WQI)

The WQI is a numerical indicator 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, PO_4^{3-} , NO_3^- , 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.

2.7. Molecular Verification of Bacterial Identity by 16S rDNA Analysis

Three purified *E.coli* 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. Genomic DNA was extracted using DNA genomic kit (TKR101, Chromous DpvL. Ltd., Germany), and DNA integrity was verified using electrophoresis on a 0.8% (w/v) agarose gel. Amplification of 16S rDNA gene was performed using a thermal cycler (ABI 2720) in a total reaction volume of 100µL containing dNTPs (2.5mM), 10× PCR buffer, Taq DNA polymerase (3U), template DNA (10ng), and forward (5'-GGGGGATCAACGGACCTGT-3') and reverse (5'-TCCTTAGAGACCCACGGC-3') primers specific for G- bacteria (**Azzam *et al.*, 2023**). The PCR conditions were as follows: an initial denaturation step at 94°C/5min, followed by 35 amplification 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. Sequencing of amplified DNA was conducted using the BigDye Terminator Cycle Sequencing Kit (v3.1, Applied Biosystems) according to the manufacturer's instructions and analyzed using an Applied Biosystems genetic analyzer. All obtained 16S rDNA sequences were deposited in the NCBI/EMBL/DDBJ database and compared with reference records by BLAST alignment step to determine sequence similarity and to assign accession numbers. Phylogenetic relationships between the isolates identified in this study and related strains were inferred by constructing a phylogenetic tree using the ClustalW algorithm implemented in MEGA software (v. 11.0) (**Kumar *et al.*, 2016**).

2.8. 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 and molecular analysis, the following software tools were employed:

- Basic local alignment search tool (BLASTN and BLASTP).

- Clustalw program (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).

3. 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 2–8**.

Table 2. Physicochemical parameters (mean values) for samples obtained from drains outlet.

Parameters	Unit	R ^a	S	G	Z	T	LAW 48/1982 ^b
Temp.	°C	30	29	30	29	31	5 degrees above normal
pH	Unit	8.12	7.25	8.09	8.30	8.48	7-8.5
EC	mmohscm ⁻¹	1.008	1.676	1.016	1.027	1.057	-
TDS	mgL ⁻¹	701	910	710	830	953	not exceed than 500
Tur.	NTU	9.01	16.2	15	8.53	23.41	-
NH ₃	mgL ⁻¹	1.9	13	1.7	1.05	10	not exceed than 0.5
DO	mgL ⁻¹	0.54	0.91	0.81	0.95	0.86	not less than 5
BOD	mgL ⁻¹	110	36	9	102	40	not exceed than 10
COD	mgL ⁻¹	236	89	79	240	92	-
Cl ⁻	mgL ⁻¹	163.1	232	183	93.5	154	-
NO ₂ ⁻	mgL ⁻¹	1.21	0.51	1.00	0.56	4.5	-
NO ₃ ⁻	mgL ⁻¹	24.4	51.00	27.4	10.01	10.00	not exceed than 45
PO ₄ ³⁻	mgL ⁻¹	3.71	<0.2	<0.2	<0.2	<0.2	not exceed than 1
SO ₄ ²⁻	mgL ⁻¹	115	210	100.9	120.1	191	not exceed than 200
CO ₃ ²⁻	mgL ⁻¹	0	0	0	0	0	-
HCO ₃ ⁻	mgL ⁻¹	425	292	370	404	589	-
Total alka.	mgL ⁻¹	425	292	370	404	589	50-200
Ca ²⁺	mgL ⁻¹	66.37	84.12	57.14	71.6	87.16	-
K ⁺	mgL ⁻¹	12	13	12	18	23	-
Mg ⁺²	mgL ⁻¹	21.78	32.99	30.18	30.22	42.58	-
Na ⁺	mgL ⁻¹	119	185	110	110	190	-

^a Sampling sites R, S, G, Z, and T are depicted in Figure 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.

3.1. Physicochemical characteristics of water samples

3.1.1. 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 2 and 3**, water temperature ranged from 29–31°C at the drain outlets and 28–31°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 (**Figure 2**) showed a strong positive association between temperature and pH ($r = 0.71$, $p < 0.05$), 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)**.

Table 3. Physicochemical parameters (mean values) for samples obtained from Rosetta branch.

Parameters	Unit	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^b
Temp.	°C	30	28	30	29	28	30	31	29	28	31	5 degrees above normal
pH	Unit	7.66	7.93	8.01	7.34	7.55	7.31	8.12	7.91	7.76	8.01	7-8.5
EC	mmohscm ⁻¹	0.431	0.961	0.509	1.041	0.419	0.941	0.830	1.012	0.490	0.820	- ^c
TDS	mgL ⁻¹	276	503	366	850	221	550	419	750	267	771	not exceed than 500
Tur.	NTU	4.86	6.13	7.22	11.7	3.2	12	5.9	5.61	10.98	15.23	-
NH ₃	mgL ⁻¹	<0.01	0.74	1.03	10	0.16	1.1	0.38	1.01	0.815	13	not exceed than 0.5
DO	mgL ⁻¹	10.3	2.63	10.92	3.80	8.35	1.56	6.10	3.86	6.13	1.49	not less than 5
BOD	mgL ⁻¹	9	84	7	29	3	8	4	69	10	35	not exceed than 6
COD	mgL ⁻¹	28	109	25	75	28	62	27	107	30	86	-
Cl ⁻	mgL ⁻¹	30.7	145.8	74	178	31.6	149	57.3	77.2	63	110	-
NO ₂ ⁻	mgL ⁻¹	0.22	0.92	<0.2	<0.2	0.39	0.76	0.18	0.40	0.21	3.91	-
NO ₃ ⁻	mgL ⁻¹	3.2	18.2	<0.2	<0.2	3.10	10.83	3.41	8.13	0.801	6.00	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	27.9	109	65.3	112.5	30.1	64	51.39	103.5	37.5	142	not exceed than 200
CO ₃ ²⁻	mgL ⁻¹	0	0	0	0	0	0	0	0	0	0	-
HCO ₃ ⁻	mgL ⁻¹	200	320	145	226.1	192	216	231	336	163	407	-
Total alka.	mgL ⁻¹	200	320	145	226.1	192	216	231	336	163	407	20-150
Ca ²⁺	mgL ⁻¹	31.67	48.11	37.01	62.57	33.52	55	47.1	54.3	40.99	90.45	-
K ⁺	mgL ⁻¹	5	9	9	11	5	9	10	15	10	21	-
Mg ⁺²	mgL ⁻¹	11.04	19.98	19.24	28.71	12.25	26.11	21.98	27.9	12.64	35.10	-
Na ⁺	mgL ⁻¹	35	101	46	112	33	104	62	83	55	128	-

^a Sampling sites R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2 are depicted in Figure 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.

3.1.2. Values of pH

pH, which reflects hydrogen ion activity, regulates key biological and chemical processes in aquatic environments. As presented in **Tables 2 and 3**, all water samples exhibited pH values

within permissible limits, consistently above 6.5 typical of natural waters containing carbonates and bicarbonates (**Azzam, 2015**). Elevated pH levels likely result from photosynthetic activity, where CO₂ uptake by aquatic plants reduces carbonic acid concentration, thereby increasing pH (**Soliman *et al.*, 2023**).

3.1.3. 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 1.008 to 1.676 mmohscm⁻¹, with the highest values recorded at the Sabal, Tala, and Zawiet El-Bahr drains, while El-Tahreer and El-Rahawy outfalls exhibited comparatively lower readings (**Table 2**). 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 and Westcott, 1985**). Along the Rosetta branch, EC varied between 0.419 and 1.041 mmohscm⁻¹, with higher values consistently observed downstream of the drain discharge points (**Table 3**). Heatmap correlation matrix revealed a negative correlation with DO ($r = -0.39$, $p < 0.05$) and strong positive correlations with several other water quality parameters (**Figure 2**), consistent with previous studies (**Omran and Azzam, 2025**).

3.1.4. 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. **Table 3** presents the TDS levels in water samples from the Rosetta branch, which mostly remained within the acceptable limits. In contrast, TDS values at the drain outfalls exceeded acceptable thresholds, ranging from 701 to 953 mgL⁻¹ (**Table 2**). 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 and Westcott, 1985**). Consistent with these observations, TDS exhibited a strong positive correlation with both electrical conductivity and turbidity ($r = +0.99$, $p < 0.05$) in agreement with the findings of **Azzam and Faiesal (2019)**.

3.1.5. Turbidity

Turbidity reflects the concentration of fine suspended particles in water, primarily arising from colloidal materials such as clay, silt, organic detritus, plankton, and other microscopic organisms, along with suspended inorganic matter. It serves as an indicator of pollution intensity in surface waters (**Azzam and Ibrahim, 2021**). As shown in **Tables 2 and 3**, turbidity values ranged from 8.53 to 23.41 NTU in the drain outlets and from 3.2 to 15.23 NTU along the Rosetta branch. The observed downstream increase in turbidity along the branch likely results from the outlets of drains effluents. Turbidity exhibited positive correlations with most measured parameters (**Figure 2**), while showing negative correlations with DO ($r = -0.31$, $p < 0.05$) and pH ($r = -0.17$, $p < 0.05$).

3.1.6. Ammonia (NH₃)

NH₃ is a naturally occurring nitrogenous compound that originates mainly from the decomposition of organic matter, domestic sewage, and agricultural runoff (**Azzam, 2015**). In the present study, NH₃ concentrations showed pronounced spatial variability frequently exceeded the permissible limit of 0.5 mg L⁻¹ set by Law No. 48/1982. Concentrations ranged between 1.05 to 13 mg L⁻¹ at the drain outfalls (**Table 2**) and from <0.01 to 13 mg L⁻¹ along the Rosetta branch (**Table 3**), reflecting substantial organic pollution inputs. Ammonia toxicity is strongly influenced by pH, which ranged from 7.25 to 8.48 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 NH₃ and NO₃⁻, BOD, and bacteriological indicators ($r = +0.87$, $p < 0.05$), 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**).

3.1.7. Dissolved Oxygen (DO)

The presence of sufficient dissolved oxygen supports aerobic microbial activity and organic matter breakdown, making it a fundamental indicator of water quality. However, low DO levels encourage the dominance of anaerobic microorganisms, leading to a decline in water quality and the generation of foul-smelling gases such as H₂S, NH₃, and CH₄ (**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 0.54 to 0.95 mgL⁻¹ (**Table 2**). Such depletion

indicates elevated loads of organic matter and nutrients (Soliman *et al.*, 2022). In contrast, DO levels in the Rosetta branch fluctuated between 1.56 and 10.92 mgL⁻¹ (Table 3), 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).

3.1.8. Biological 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 2 and 3), ranging from 9–110 mg L⁻¹ in drains and 3–84 mg L⁻¹ in the branch, frequently exceeding the permissible limits. This suggests substantial organic loading from domestic sewage, industrial discharge, and urban runoff (Nada *et al.*, 2021; Azzam *et al.*, 2022; Lin *et al.*, 2024). BOD levels were strongly correlated with bacterial indicators and negatively correlated with temperature ($r = -0.45$, $p < 0.05$), pH, and dissolved oxygen (DO) ($r = -0.80$, $p < 0.05$), reflecting oxygen depletion driven by microbial activity (Omran *et al.*, 2024).

3.1.9. Major Anions

As shown in Tables 2 and 3, chloride and sulfate concentrations at most sampling sites were within normal ranges. In the drain outfalls, Cl⁻ concentrations ranged from 93.5 to 232 mgL⁻¹ and SO₄²⁻ from 115 to 191 mgL⁻¹, while in the Rosetta branch, Cl⁻ varied between 31.6 and 178 mgL⁻¹ and SO₄²⁻ between 27.9 and 112.5 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). PO₄³⁻ concentrations were generally below 0.2 mgL⁻¹, except at El-Rahawy outlet, where a markedly higher value of 3.71 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 292 to 589 mgL⁻¹ at the drain outfalls and from 145 to 407 mgL⁻¹ in the Rosetta branch. Bicarbonate showed a weak positive

correlation with total coliforms ($r = +0.18$, $p < 0.05$). Total alkalinity values exceeded the permissible limits (200 mg L^{-1} for drains and 150 mgL^{-1} for the Rosetta branch) at all sites except Sabal upstream (145 mgL^{-1}), reflecting the clear influence of drain discharges on Nile water quality.

3.1.10. Major Cations

The measured concentrations of Ca^{2+} , K^+ , Mg^{2+} , 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 2 and 3**).

3.1.11. Trace Metals

Freshwater systems naturally contain trace metals at low levels, mainly from geological weathering, with additional contributions from sewage, industrial effluents and atmospheric fallout (**Soliman *et al.*, 2023**). The measured concentrations of the ten metals in the Rosetta branch and associated drain outfalls, shown in **Tables 4 and 5**, did not exceed the thresholds set by Egyptian Law 48/1982. This pattern suggests that domestic sewage and agricultural runoff are the dominant sources of pollution (**Azzam *et al.*, 2023**).

Table 4. Mean trace metal contents (mg L^{-1}) in water samples from drains outlet.

Parameters	R ^a	S	G	Z	T	LAW 48/1982 ^c
Al ^b	0.1095	0.0548	0.0978	0.0516	0.202	- ^d
Ba	0.0364	0.0516	0.0735	0.0921	0.083	-
Cr	<0.002	<0.002	<0.002	0.0551	<0.002	< 0.01
Cu	0.087	0.011	0.048	0.795	0.039	< 1.0
Fe	0.1229	0.0888	0.0163	0.1048	0.145	< 1.0
Mn	0.097	0.129	0.109	0.0924	0.187	< 1.5
Ni	<0.004	<0.004	<0.007	0.0268	<0.004	-
Se	<0.007	<0.007	<0.006	>0.006	<0.007	-
V	<0.005	0.0213	<0.005	0.0343	<0.001	-
Zn	<0.005	<0.005	<0.005	0.827	0.034	< 1.0

^a Sampling sites R, S, G, Z, and T are depicted in Figure 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 5. Mean trace metal contents (mg L⁻¹) in water samples from the Rosetta branch.

Parameters	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^b
Al	0.0022	0.0997	0.0103	0.0502	0.0147	0.0616	0.0081	0.0231	<0.007	0.107	-
Ba	0.0118	0.0303	0.0175	0.0498	0.003	0.0145	<0.007	<0.007	0.004	0.05	-
Cr	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.0293	0.039	<0.002	<0.002	<0.01
Cu	0.031	0.051	<0.002	<0.006	0.027	0.035	0.041	0.612	0.007	0.016	< 1.0
Fe	0.0061	0.086	<0.006	0.0509	<0.007	0.0091	<0.007	0.0193	<0.001	0.11	<1.0
Mn	0.007	0.091	<0.001	0.073	0.007	0.0813	0.0211	0.0543	<0.004	0.098	< 1.5
Ni	<0.004	<0.004	<0.004	<0.004	<0.007	<0.004	<0.004	<0.004	<0.004	<0.004	-
Se	<0.007	<0.007	<0.007	<0.007	<0.006	>0.006	>0.001	<0.001	<0.007	<0.007	-
V	<0.005	<0.005	<0.005	0.0029	<0.005	>0.005	>0.005	<0.007	<0.001	<0.001	-
Zn	<0.005	<0.005	<0.002	<0.005	<0.005	>0.005	>0.002	<0.006	<0.005	<0.005	< 1.0

^a Sampling sites R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2 are depicted in Figure 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.

3.2. 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 6 and 7**.

3.2.1. Standard Plate Counts (SPC)

SPC levels at 22°C and 37°C were generally high across the samples, reaching their highest concentrations at the El-Rahawy drain outlet. At the drain outfalls, SPC values ranged from 20×10^3 to 210×10^4 CFU mL⁻¹ at 22°C and from 7.0×10^3 to 68×10^4 CFU mL⁻¹ at 37°C. Along the Rosetta branch, SPC values fluctuated between 1.0×10^3 and 91×10^4 CFU mL⁻¹ at 22°C, and from 3.0×10^2 to 15×10^4 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**).

3.2.2. Total Coliforms (TC)

TC densities exhibited considerable spatial variation, ranging from 117×10^2 - 133×10^3 CFU mL⁻¹ at the drain outfalls and from 100 - 48×10^3 CFU mL⁻¹ along the Rosetta branch. The highest TC counts were recorded at the Sabal drain outlet and its downstream site. Except for El-Tahreer and Zawiet El-Bahr drains, all other monitored drains and more than 73% of the Rosetta branch

sampling sites were within the limits set by the **US EPA (2012)**, which recommends a maximum TC concentration of 5000 CFU mL⁻¹. However, when compared to the more stringent limits proposed by **Niyoyitungiye *et al.*, (2020)** 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.

Table 6. Bacteriological indicators (mean values) for samples obtained from drain outlets.

Parameters	R ^a	S	G	Z	T	LAW 48/1982 ^c
SPC at 22°C (CFU mL ⁻¹) ^b	210 x 10 ⁴	178 x 10 ⁴	62 x 10 ³	20 x 10 ³	30 x 10 ⁴	- ^d
SPC at 37°C (CFU mL ⁻¹)	68 x 10 ⁴	29 x 10 ⁴	11 x 10 ³	7 x 10 ³	6 x 10 ⁴	-
TC (CFU100mL ⁻¹)	90 x 10 ³	133 x 10 ³	20 x 10 ²	17 x 10 ²	11 x 10 ³	-
FC (CFU100mL ⁻¹)	14 x 10 ³	45 x 10 ³	7 x 10 ²	5 x 10 ²	4 x 10 ³	-
FS (CFU100mL ⁻¹)	11 x 10 ³	36 x 10 ³	5 x 10 ²	2 x 10 ²	7 x 10 ³	-

^a Sampling location, designated as R, S, G, Z, T, are depicted in Figure 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 7. Bacteriological indicators (mean values) for samples obtained from the Rosetta branch.

Parameters	R1 ^a	R2	S1	S2	G1	G2	Z1	Z2	T1	T2	LAW 48/1982 ^c
SPC at 22°C (CFU mL ⁻¹) ^b	42 x 10 ²	70 x 10 ⁴	1000	91 x 10 ⁴	23 x 10 ²	21 x 10 ³	13 x 10 ²	45 x 10 ²	1000	25 x 10 ³	- ^d
SPC at 37°C (CFU mL ⁻¹)	10 x 10 ²	11 x 10 ⁴	5 x 10 ²	15 x 10 ⁴	9 x 10 ²	7 x 10 ³	4 x 10 ²	11 x 10 ²	300	10 x 10 ³	-
TC (CFU100mL ⁻¹)	440	19 x 10 ³	800	48 x 10 ³	477	10 x 10 ²	250	800	100	12 x 10 ²	-
FC (CFU100mL ⁻¹)	51	4 x 10 ³	200	9 x 10 ³	56	200	30	100	20	4 x 10 ²	-
FS (CFU100mL ⁻¹)	13	9 x 10 ²	100	21 x 10 ²	49	70	14	32	8	110	-

^a Sampling location, designated as R1, R2, S1, S2, G1, G2, Z1, Z2, T1, and T2, are depicted in Figure 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.

3.2.3. Fecal Coliforms (FC)

FC counts at the drain outfalls exhibited marked variation, with values ranging from a maximum of 45 x 10³ CFU mL⁻¹ at Sabal drain to a minimum of 500 CFU mL⁻¹ at Zawiet El-Bahr drain. In the Rosetta branch, FC concentrations varied between 20 and 9 × 10³ CFU mL⁻¹. Notably, El-Tahreer drain was the only site that met the international standards set by **US EPA (2012)**, which recommend a maximum FC limit of 2000 CFU mL⁻¹. Guideline compliance was observed at around 80% 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.

3.2.4. Fecal Streptococci (FS)

FS counts varied considerably, ranging from a maximum of 36×10^3 CFU/mL⁻¹ at Sabal drain to a minimum of 200 CFU/mL⁻¹ at Zawiet El-Bahr drain. In contrast, FS levels in the Rosetta branch fluctuated between 8 and 21×10^2 CFU/mL⁻¹. In general, sites in both the drains and the Rosetta branch that recorded FS counts exceeding 1000 CFU/mL⁻¹ were found to be within the international standard limits recommended by US EPA (2012) except for El-Rahawy and Sabal drains and its downstream. The upward trend in TC, FC, and FS counts downstream appears linked to drain effluents, consistent with earlier reports (Azzam *et al.*, 2017; Omran and 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 (Azzam and Faiesal, 2019; El-DougDoug *et al.*, 2020; Elbahnasawy *et al.*, 2021; Omran *et al.*, 2024). 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).

3.3. Statistical Analysis

Pearson's correlation analysis indicated a strong positive relationship among the bacteriological parameters, including SPC, TC, FC, and FS, with $r = +0.95$ ($p < 0.05$), as illustrated in Figure 2. Comparable patterns of interdependence among microbial indicators have been documented in earlier studies on the Nile River and its drainage systems (Azzam, 2015; Soliman *et al.*, 2022). Notably, significant positive correlations ($r > 0.77$, $p < 0.05$) were also observed between these bacteriological parameters and NH₃, BOD, and nitrate, whereas moderate positive associations ($r > 0.65$) were detected with EC, TDS, turbidity, and major cations. In contrast, bacteriological indicators were inversely related to DO ($r = -0.82$, $p < 0.05$), suggesting that oxygen depletion is linked to elevated microbial contamination in the studied water system (Omran *et al.*, 2024).

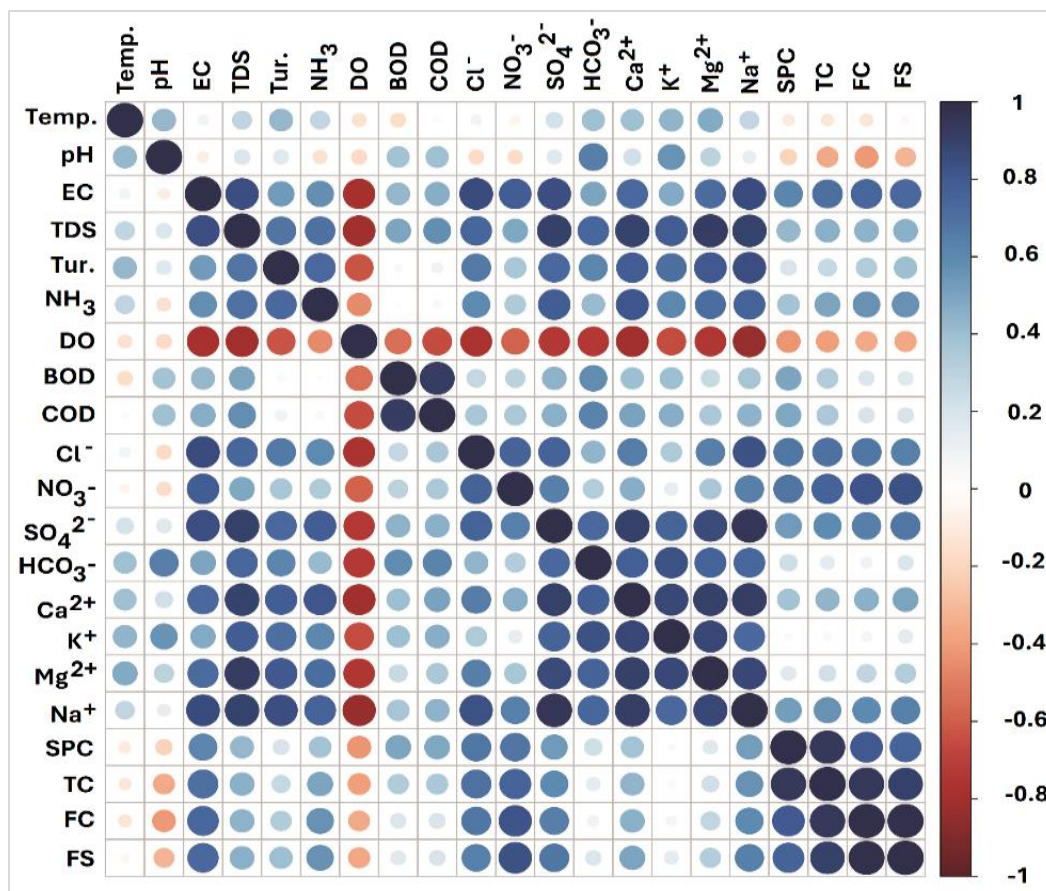


Figure 2. Heatmap of Pearson correlation matrix illustrating the interrelationships among the physicochemical and microbiological parameters assessed at the 15 sampling sites. Abbreviations: Temp., temperature; EC, electrical conductivity; TDS, total dissolved solids; Tur., turbidity; NH_3 , ammonia; DO, dissolved oxygen; BOD, biochemical oxygen demand; COD, chemical oxygen demand; Cl^- , chloride; NO_3^- , nitrate; SO_4^{2-} , sulfate; HCO_3^- , bicarbonate; Ca^{2+} , calcium; K, potassium; Mg^{2+} , magnesium; Na, sodium; SPC, standard plate count; TC, total coliform; FC, fecal coliform; FS, fecal streptococci.

3.3. Water Quality Index (WQI)

WQI showed clear spatial variation among the upstream, outlet, and downstream sites of the examined drains (**Figure 3**). Upstream locations exhibited WQI values ranging from 59.07 ± 1.12 to 62.29 ± 1.45 , corresponding to medium water quality. In contrast, outlet sites had significantly lower WQI values between 32.12 ± 0.98 and 40.28 ± 1.25 , 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 37.17 ± 1.35 to 46.76 ± 1.52 , indicating that water quality remained compromised despite partial dilution with

branch water at these sites. The minimum WQI (32.12 ± 0.98) was recorded at the Sabal 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 (Omran and 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).

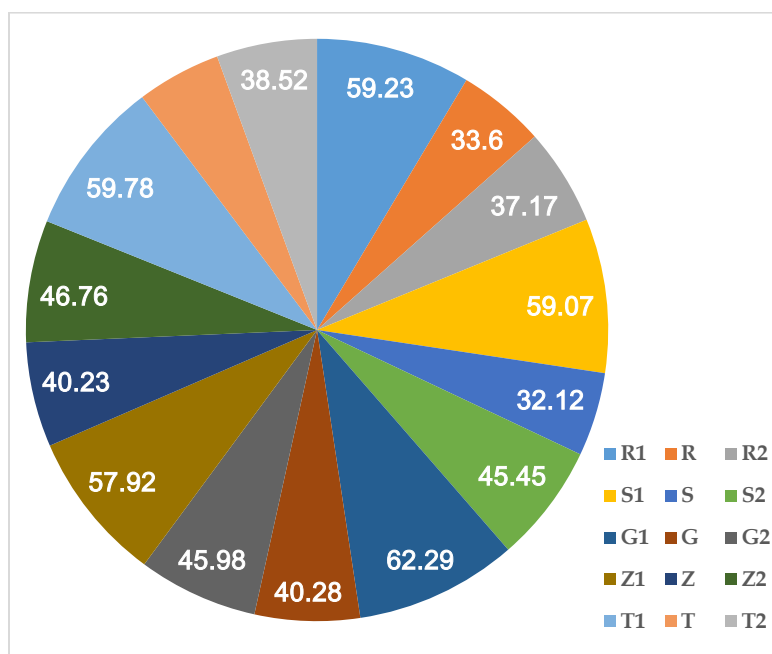


Figure 3. Water quality index values (mean $\pm$ SD) for the studied water samples.

3.4. Bacterial Diversity and Identification in River Nile and Drain Water Samples

In this study, 292 bacterial isolates were obtained from the water samples. Of these, 278 isolates (95.2%) were successfully assigned to known taxa, whereas 14 isolates (4.8%) could not be identified and were designated as unknown taxa. As detailed in **Table 8**, the confirmed isolates included six bacterial species distributed across four primary families: *Enterobacteriaceae*, *Pseudomonadaceae*, *Staphylococcaceae*, and *Enterococcaceae*. The majority of bacterial isolates were gram-negative (188 isolates, 67.6%), while gram-positive strains comprised 90 isolates (32.4%). In River Nile samples, *E.coli* was predominant (18.7%), with *P.vulgaris* and *E.faecalis*

detected at 14.74% and 12.23%, respectively. The predominance of *E.coli* reflects recent fecal contamination, likely derived from domestic and agricultural runoff (Azzam *et al.*, 2014). 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* (12.23%) and *S.aureus* (10.43%) being the dominant species, followed by *E.coli* (6.47%). The abundance of *P.aeruginosa* and *S.aureus* suggests strong influence from industrial and hospital effluents, as these species commonly thrive in nutrient-rich and polluted environments. *C.freundii* (2.52%) and *Salmonella spp.* (1.14%) 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).

Table 8. Distribution and relative abundance of bacterial taxa detected in Rosetta and drainage samples.

Bacterial species	River Nile		Drains	
	Isolate count	Relative abundance (%)	Isolate count	Relative abundance (%)
<i>Escherichia coli</i>	52	18.7	18	6.47
<i>Citrobacter freundii</i>	0	0	7	2.52
<i>Salmonella sp.</i>	0	0	4	1.144
<i>Proteus vulgaris</i>	41	14.74	9	3.24
<i>Pseudomonas aeruginosa</i>	23	8.28	34	12.23
<i>Staphylococcus aureus</i>	15	5.4	29	10.43
<i>Enterococcus faecalis</i>	34	12.23	12	4.32

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 and Azzam, 2020). The observed disparity in bacterial community composition between 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.

3.5. Genotypic characterization of Selected Bacterial Isolates

Genotypic approaches are increasingly being adopted in aquatic microbiology to enhance the accuracy of bacterial identification and reduce ambiguities related to phenotypic diversity (Azzam *et al.*, 2014; Guo *et al.*, 2025). Accordingly, the selected *E.coli* isolates were molecularly characterized to confirm the culture-based identification results. Three representative isolates (RS01, MS02, and RS03) were selected based on their consistent biochemical profiles and frequent occurrence among the recovered isolates. Genomic DNA purity, assessed spectrophotometrically at A260/A280, yielded acceptable ratios of 1.75, 1.93, and 1.90 for RS01, MS02, and RS03. Amplification of the 16S rRNA gene produced clear PCR amplicons of approximately 1465–1467 bp, confirming successful and specific amplification of the target gene. Sequence analysis and multiple sequence alignment (MSA) confirmed that all three isolates belonged to *E.coli*. Sequence data from this study were deposited in NCBI under accession codes PP824990.1–PP824992.1, and subsequent phylogenetic analysis of 16S rRNA genes revealed clustering consistent with the genetic relatedness of the isolates to previously reported *E.coli* strains (Figure 4).

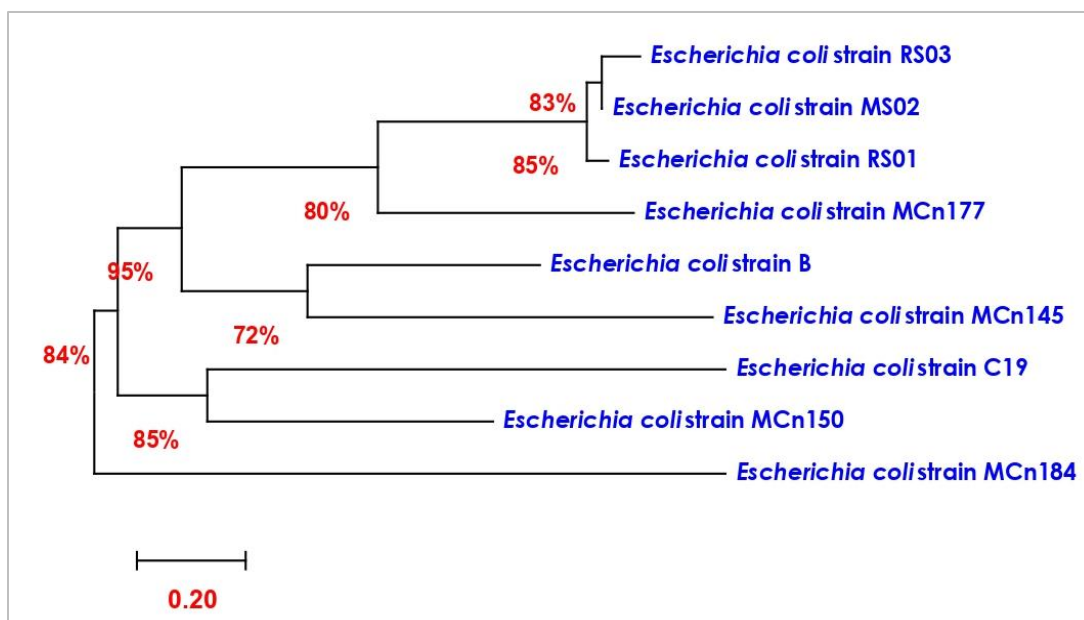


Figure 4. Phylogenetic clustering of *E.coli* isolates with closely related GenBank sequences using 16S rRNA gene data.

The observed sequence variation highlights the genetic heterogeneity of *E.coli* populations in aquatic environments and supports the usefulness of molecular tools for confirming bacterial

identity and exploring phylogenetic relationships. Molecular analysis serves as a confirmatory tool that complements conventional microbiological methods rather than replacing them (**Azzam *et al.*, 2023; Royer *et al.*, 2024; Xiao *et al.*, 2025**). The integration of culture-based identification with 16S rRNA gene sequencing enhances the confidence in bacterial detection and provides additional insights into genetic diversity, which is valuable for microbial source tracking and water quality assessment. Similar integrative approaches have been widely recommended for environmental water monitoring (**Elbahnasawy *et al.*, 2021; Gerace *et al.*, 2022; Oon *et al.*, 2023; Curry *et al.*, 2024; Azzam *et al.*, 2025; Cholet *et al.*, 2025**).

4. Conclusions

This study concluded that the water quality of the Rosetta branch of the River Nile is severely affected by untreated agricultural and domestic discharges from drains located along its course, as evidenced by elevated pollutant loads and widespread microbial contamination of the water. WQI results revealed a progressive deterioration in water quality from upstream to downstream sites, with the Sabal drain identified as the most critical pollution source, followed by El-Rahawy. Although limited self-purification capacity was observed, it was insufficient to counterbalance the continuous inflow of contaminants. The integration of bioinformatics tools in this study demonstrated their significant value for the early and accurate detection of microbial contamination, enabling precise identification and genomic characterization beyond the capabilities of conventional microbiological techniques. These findings highlight the urgent need to prioritize wastewater treatment at major drain outlets, particularly Sabal and El-Rahawy to prevent further degradation of the Rosetta branch. Integrating conventional monitoring with molecular approaches provides a robust and practical framework for the early detection of microbial risks and supports evidence-based decision-making for sustainable water resource management in Egypt. Enforcing water protection laws, alongside wastewater treatment and regular monitoring, is fundamental to sustaining the Nile River and minimizing risks to public health.

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