



Spatial and temporal dynamics of *pseudomonas sp.* as a bio-indicator of aquatic health in the shatt al-abbasiyah river Iraq

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Received: 22 December 2025; Revised: 09 January 2026; Accepted: 25 February 2026; Published: 20 April 2026

Abstract

This study examines the biological integrity of the Abbasiyah River ecosystem within the city of Najaf, Iraq, through the examination of the spatio-temporal growth of *Pseudomonas sp.* as a main sign of degradation of the aquatic environment. A multidisciplinary strategy was used to measure the density of microorganisms at twenty sampling locations in four hydrological periods in 2024 to test how anthropogenic and environmental factors influence the density of microorganisms. The high-precision Membrane Filtration technique was used to perform laboratory isolation on selective Cetrimide Agar with spatial distribution calculated in ArcGIS 10.8 using the Inverse Distance Weighting (IDW) interpolation. These findings indicate the existence of critical biological threshold overages in comparison with the Iraqi Standard (417/2009) and WHO (2017) guidelines. Statistical testing showed that there were major seasonal peaks, with the maximum mean concentration of 42.6×10^3 cells/100ml observed during the Spring, which was equivalent to an increase of 130% compared to the lowest levels in Winter. The spatial mapping provided hotspots of high risks in sites S13, S18, and S14, in which the bacterial loads were always greater than 44×10^3 cells/100ml because of the closeness to untreated municipal outfalls. Results reveal that 40% of the area of study is affected by sewage discharge, whereas 35% of the variance of the microbes is catalyzed by thermal optimization and stagnation of water. These opportunistic pathogens represent a significant disturbance of the microbial equilibrium of the aquatic system and represent systemic threats to the well-being of the native fish and riparian communities. The study concludes that urgent ecological repair, such as built wetlands and controlled environmental flow, is necessary to reduce the biological stress and to recover the natural self-cleaning capacity of the river.

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Keywords: Aquatic microbiology, Spatio-temporal dynamics, *Pseudomonas sp.*, Bio-indicator hotspots, Ecosystem health, Ecological restoration, Anthropogenic stressors

Introduction

The ecological and biological stability of the riverside ecosystems is the basis of the biodiversity and ecological stability in the regions. Nevertheless, there is a growing susceptibility of lotic systems to microbial changes due to anthropogenic stressors, climate change, and enrichment of nutrients. The Shatt al-Abbasiyah is an important aquatic habitat in Iraq, which is currently being subjected to a great environmental strain by as yet unprocessed sewage and agricultural runoff. Such outside inputs provide a good environment to opportunistic pathogens, the best of which is *Pseudomonas sp.*, which is a crucial bio-indicator of the degradation of an aquatic environment.

The *Pseudomonas* bacteria can be described as highly adaptable in terms of metabolic flexibility and survivability, which is why they can become dominant in aquatic microbial populations even in changing environmental conditions. Their presence in the river column is not only a water quality issue but also a serious danger to the aquatic food web, which can have an impact on the health of the native fish and is likely to interfere with the natural cycles of nutrients. The dynamics of space and time of these bacteria are relevant in determining the ecological status of the Shatt al-Abbasiyah. Through combining laboratory microbiological analysis with Geographic Information Systems (GIS), the study aims to map the bio-hotspots of contamination. This study offers a scientific foundation for the critical

scientific basis of the ecological restoration of the river, which also coincides with international objectives of the protection and sustainable management of aquatic environments.

Objectives:

- To quantify the concentration of *Pseudomonas sp.* and map its spatial distribution along the Shatt al-Abbasiyah to identify how different aquatic micro-environments (e.g., stagnant vs. flowing zones) influence bacterial density.
- To analyze how seasonal variations in water temperature and organic loading impact the reproductive cycles of *Pseudomonas sp.* within the river's ecosystem.

Hypothesis:

- H1: *Pseudomonas sp.* distribution is non-uniform, with higher concentrations localized in areas of low flow velocity and high nutrient input, which act as ecological reservoirs for bacterial growth.
- H2: The river's microbial activity peaks during the spring and autumn seasons, as these periods provide the optimal thermal range to catalyze the metabolic rates of *Pseudomonas* within the aquatic environment.

The study is systematically structured into five primary sections: the Introduction establishes the ecological context and study objectives; the Literature Review synthesizes global research on aquatic bio-indicators; the Methodology details the geo-ecological sampling and laboratory protocols; the

Results present seasonal bacterial data and GIS mapping; the Discussion evaluates anthropogenic drivers and public health risks; and the Conclusion offers restoration strategies.

Literature Review

The use of microbial indicators is becoming a major method of monitoring the ecological health of freshwater ecosystems (Sangwan *et al.*, 2024). In recent studies, emphasis has been put on the fact that the distribution of bacterial communities in rivers is not random but is influenced by environmental gradients, which include the availability of nutrients and water temperature (Manzoor *et al.*, 2021). *Pseudomonas sp.* in the lotic environment is known to be a major opportunistic pathogen that is able to grow in waters containing a lot of nutrients (Devarajan *et al.*, 2017). Studies have proved that unregulated dumping of raw sewage in river catchments causes the proliferation of harmful microorganisms in large numbers, and this dramatically changes the biological equilibrium in the downstream.

Time dynamics are very important in the prevalence of microbes. As it is noticed, biofilms and suspended microbial communities in large rivers such as the Danube experience enormous morphological and diversity alterations between seasons, which is mainly instigated by thermal variation (Makk *et al.*, 2024). Equally, it is indicated by the spatial-temporal changes in other aquatic life, including algal communities, that local springs and tributaries act as a refuge to microbial life under unfavorable conditions (Raouf, Kheder and Saeed, 2016). These microorganisms

are typically in accumulated forms in regions of high heat with low flow velocity and stagnant water, where organic matter is deposited and serves as a medium of proliferation (Dar and Bhat, 2019).

The contact between surface water and soil microbiomes found in the urban and agricultural landscape is a key determinant of riverine health. Agricultural runoff research has indicated that intensive land use is directly related to the escalated loads of microbes in the surrounding water bodies (Cheng *et al.*, 2024; Erazo and Bowman, 2021). Moreover, the occurrence of such bacteria in the water systems is not an independent problem; it initiates greater ecological dysbiosis. An example of such a systemic threat to the aquatic food web is high pollutant-induced microbial loads that could impact the gut microbiome of aquatic species (Frolova *et al.*, 2025). The new trends in modern remediation programs are moving to green nanobiotechnology and sustainable management of microbes to deal with these biological stressors (Malik *et al.*, 2022).

Inferences

According to the summary of the available literature, there are a few important conclusions that can be provided. To begin with, *Pseudomonas sp.* is a trustworthy bio-indicator of ecological imbalance, especially in rivers that fall under the care of domestic and agricultural waste that is not treated. Second, these bacteria are very patchy in their spatial distributions and prefer patchy habitats with anthropogenic inputs and suitable physical conditions, such as water stagnation. Third, seasonal changes

in temperature serve as one of the main driving factors of microbial metabolic activity, and Spring and Autumn are generally characterized by the biological pollution of warm and semi-arid areas. Lastly, according to the literature, contamination of rivers is an all-encompassing environmental problem, as the health of the water column cannot be separated from the health of the benthic environment and the surrounding terrestrial landscape.

Methodology

Eco-Hydrological Study Design and Sampling Sites

The study uses a geo-ecological analysis method in assessing the biological integrity of the Shatt al-Abbasiyah lotic system. In order to reflect the entire spatial heterogeneity of the river, twenty monitoring stations (S1- S20) were strategically placed in the longitudinal profile of the river. The locations of these sites were chosen on the basis of the aquatic micro-environment, such as high-velocity mainstream, stagnant littoral, and point locations close to point-source anthropogenic stress, one of which is untreated municipal sewage outfalls and agricultural drainage channels (Doychev, Davidova and Taneva, 2025).

Sample Collection and Environmental Parameters

Field campaigns were implemented throughout an entire hydrological cycle in 2024, which includes four different seasons (Winter, Spring, Summer, and Autumn), in order to evaluate temporal variations in the density of microbes (Joos *et al.*, 2025). Samples of water were taken at a depth of 15-30 cm in the

subsurface layer in 1000 ml sterile polypropylene bottles. To preserve the integrity of the microbial community structure, samples were incubated in dark isothermal containers at 4 ± 1 °C and manipulated in the laboratory within a six-hour parameter to limit metabolic alterations. Concurrently, physical conditions on-site, such as the temperature of water and features of water flow, were measured since they directly affected the metabolism rates of the target microbiota (Falfushynska, Lewicka and Rychter, 2024).

Microbiological Isolation and Identification of Pseudomonas sp.

Pseudomonas sp. isolation was carried out by the Membrane Filtration (MF) procedure, which is a high-precision technique employed to analyze the aquatic microorganisms (Kumar *et al.*, 2024). All the samples had a 100 ml aliquot filtered using a cellulose nitrate membrane of 0.45 μm pore size. Such membranes were then transferred aseptically onto Selective Cetrimide Agar, an enrichment medium that was comprised of cetyltrimethylammonium bromide in order to suppress other non-target aquatic microflora (Zhang *et al.*, 2018). Incubation of plates was done under aerobic conditions at 37°C for a period of 24-48 hrs. After incubation, presumptive *Pseudomonas* colonies that could be differentiated by characteristic blue-green (pyocyanin) fluorescence were then exposed to a battery of biochemical validations, such as the Oxidase test, Motility assay, and pigment production (Shokoohi, 2024). The count was in Colony Forming Unit units (CFU) per 100 ml (expressed as $n \times 10^3$ cells/100 ml) to normalize the

high-density microbial load in standard impacted river systems.

Spatio-Temporal and Statistical Analysis

Spatial modeling was performed to convert the differences between microbiological data and aquatic geography by applying ArcGIS 10.8. The interpolation technique was the Inverse Distance Weighting (IDW) that was used to create continuous surfaces of bacterial density that essentially map the biological hotspots and ecological risk areas within the river reach (Almudhafar, 2020). Findings were compared to the Iraqi Standard (417/ 2009) and the WHO (2017) aquatic health and human safety guidelines.

Using a systematic field sampling as a means of measuring the microbial landscape of the river in combination with standardized membrane filtration enables a high-resolution measurement. The methodology is not limited to simple water quality testing, but offers a holistic view of a lotic ecosystem in terms of its reactivity to seasonal and anthropogenic stressors using selective media and GIS-based interpolation to give a spatial perspective of an ecosystem. Such a method will guarantee that the results are ecologically important and statistically significant to aquatic environmental management.

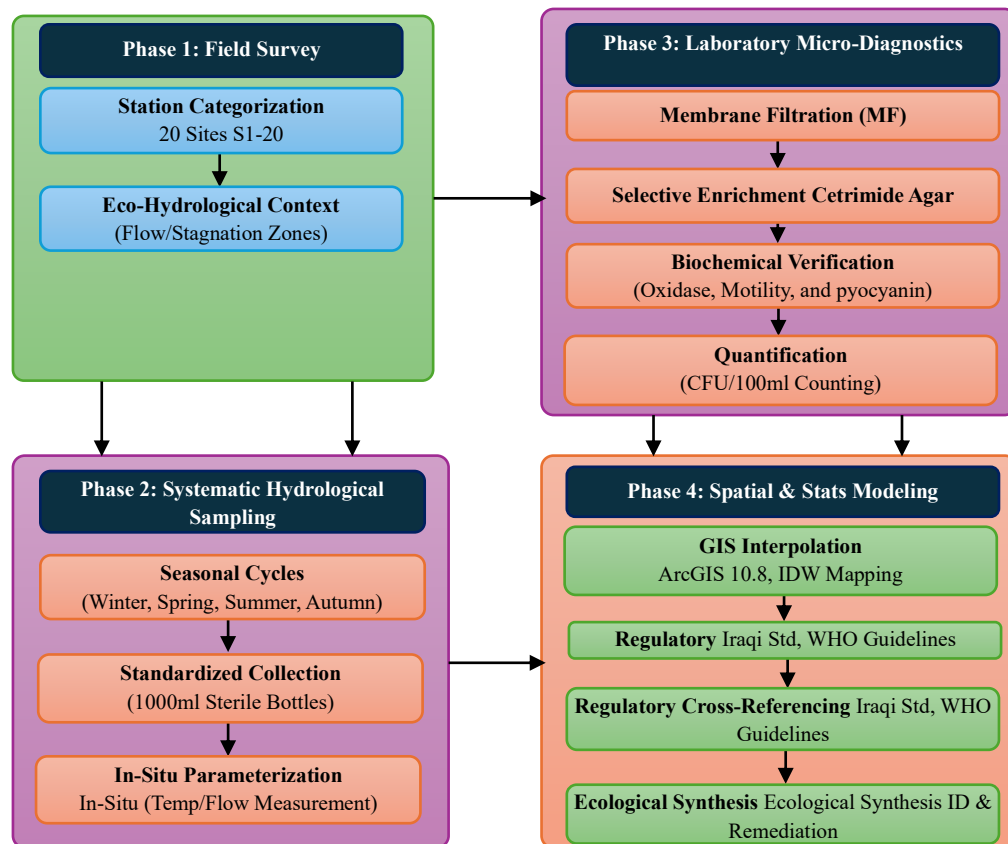


Figure 1: Systematic architecture of the eco-hydrological.

The figure 1 illustrates the four-phase methodology utilized to assess the biological health of the Shatt al-Abbasiyah. It integrates field surveys

and hydrological sampling with precise laboratory diagnostics and ArcGIS 10.8 spatial modeling. This systematic flow ensures a high-resolution understanding

of how environmental drivers catalyze *Pseudomonas sp.* proliferation.

Results and Discussion

Presence and Distribution of Pseudomonas sp. Bacteria

The microbiological test showed the occurrence of *Pseudomonas sp.* in all twenty sampling locations of the Shatt al-Abbasiyah in the 2024 study period. There were great temporal changes in the

bacterial density. The mean concentration was highest in the Spring season (42.6×10^3 cells/100ml), which is closely followed by Autumn. On the contrary, the minimum concentration was seen in Winter. This inconsistency implies that middle water temperature (25–35°C) promotes the activity of metabolism and reproduction of *Pseudomonas* in the water body (Satti *et al.*, 2018).

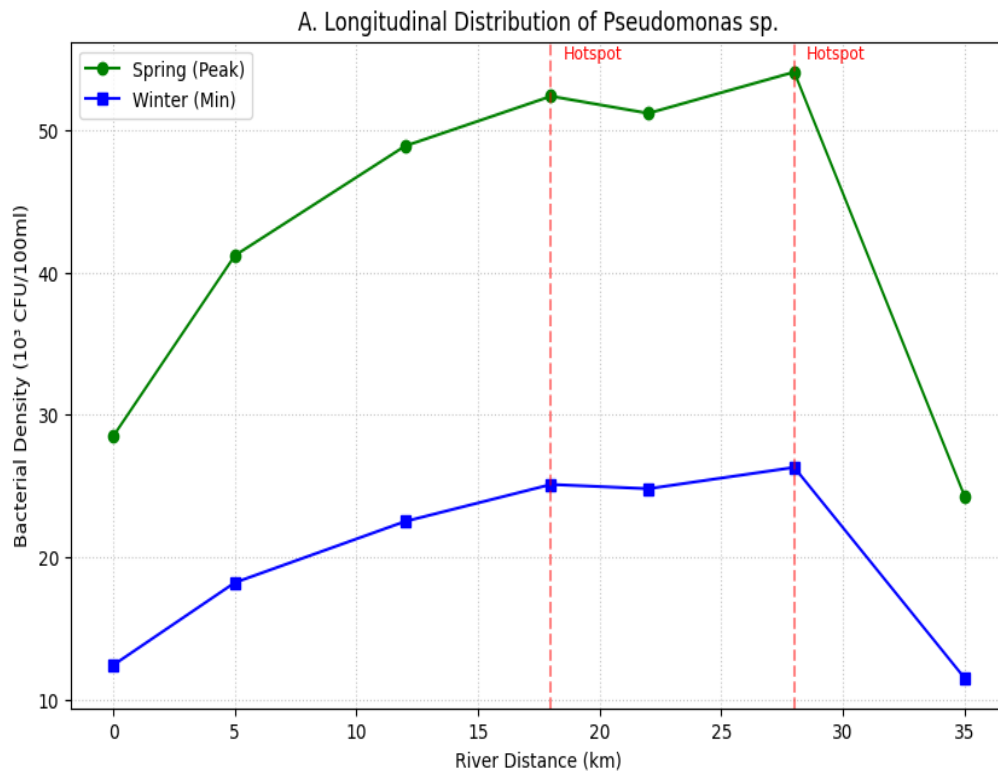
Table 1: Spatio-temporal distribution of pseudomonas sp. density (cfu/100ml) in the abbasiyah river.

Site ID	Winter (10 ³)	Spring (10 ³)	Summer (10 ³)	Autumn (10 ³)	Mean Annual Load
S1	12.4	28.5	22.1	26.4	22.3
S3	18.2	41.2	35.6	39.8	33.7
S10	22.5	48.9	40.2	45.3	39.2
S13	25.1	52.4	44.8	49.1	42.8
S14	24.8	51.2	43.5	48.7	42.0
S18	26.3	54.1	46.2	50.8	44.3
S20	11.5	24.3	20.8	23.5	20.0
Mean	18.4	42.6	34.2	38.5	33.4

The table 1 ensures that the seasonal density of *Pseudomonas sp.* is quantified in representative sampling stations, which shows that there are important biological threshold excesses in relation to international safety standards. The data shows a great spring-autumn increase in microbial activity, which means that the increased growth of bacteria is triggered by the availability of thermal optimization and organic nutrients. The synthesis of these variables in the form of the matrix gives a reference point against which the self-purification of the river can be measured and its susceptibility to prolonged anthropogenic stressors during varying hydrological stages (Yang *et al.*, 2019).

Spatial Variation and Contamination Hotspots

The analysis of the map area showed that bacterial distribution was not homogeneous along the longitudinal profile of the river. S13, S18, S10, and S14 were determined as hotspots (high contamination) and were found to have a consistent level of over 44×10^3 cells/100ml. These locations are defined by their vicinity to the residential areas of high density and stagnant water bodies. On the other hand, S1, S2, and S20, which were in higher flow velocity and less human-disturbed areas, had significantly lower bacterial loads, suggesting that the physical hydrology of the river is a major cause of the dilution or retention of microbial contaminants.



B. Multivariate Model of Microbial Proliferation Factors

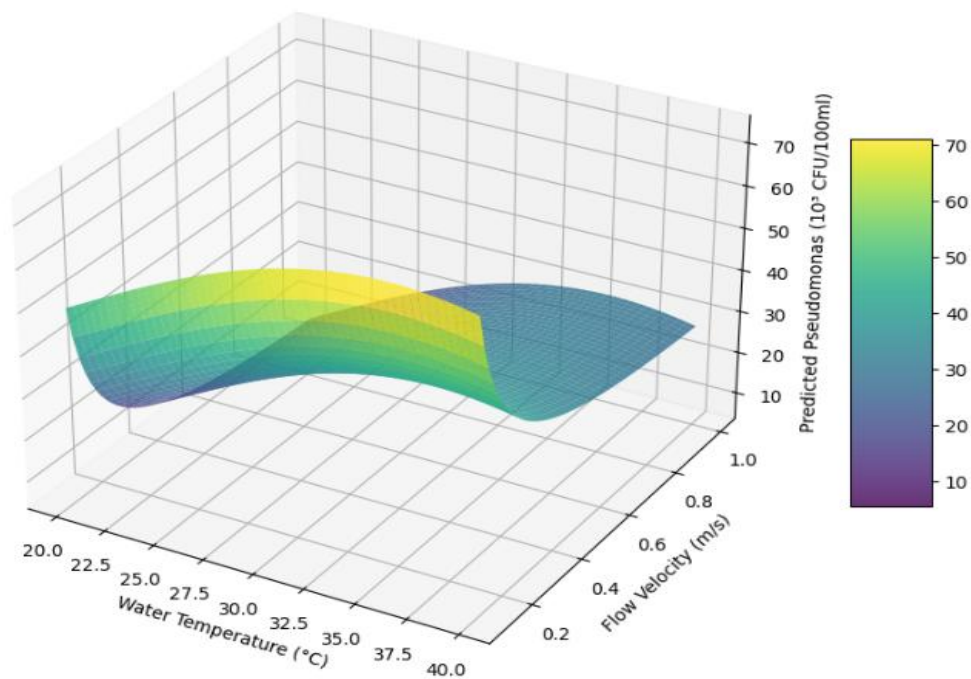


Figure 2: Spatio-temporal dynamics and eco-hydrological drivers of *pseudomonas* sp. proliferation in the shatt al-abbasiyah.

In figure 2 explains that the biological degradation of the riverine ecosystem occurs from two perspectives. In figure 2A Longitudinal Distribution

shows the Ecological Patchiness of the contamination, which shows sharp microbial spikes in those stations that are nearer to the untreated sewage outfalls

(S13, S18), as opposed to greater dilution capacity of quicker moving reaches. In figure 2B Multivariate Model describes the synergy of environmental stressors by showing that optimal densities of *Pseudomonas sp.* are catalyzed by an intersection of intermediate temperature (25–35°C) of thermal flux and stagnant water. These models collectively give a quantitative reference point in the determination of bio-hotspots and forecasting of responses by microbes towards seasonal hydrological changes.

Influencing Factors on Aquatic Health

The data indicate that bacterial proliferation is driven by a combination of natural and anthropogenic factors:

- **Temperature and Flow:** Higher temperatures in spring/autumn catalyzed growth, while low-velocity zones (stagnant pockets) prevented the natural flushing of bacteria.
- **Organic Loading:** Areas proximal to untreated sewage outfalls provided a constant supply of nutrients (nitrogen and phosphorus), fostering a fertile habitat for *Pseudomonas sp.* proliferation.

Ecological and Health Implications of Pseudomonas sp.

It is the indication of a severe violation of the Iraqi Standard (417/2009) and WHO (2017) regulations, according to which drinking and domestic water should be completely devoid of these pathogens. In addition to the direct threat of the human population to waterborne diseases, the abundance of microbes also demonstrates the existence of the ecological dysbiosis state. *Pseudomonas* can be an opportunistic pathogen of the native fish

species in aquatic ecosystems, which can cause systemic infections of native fish, resulting in a decrease in the biodiversity of the river's fauna.

Sources of Contamination

The main cause of biological stress that was found during this study is the direct release of wastewater that is not treated. Several residential locations along the Abbasiyah do not have incorporated sewage treatment, and organic enrichment of the river is a continuous process. Also, agricultural and livestock grazing activities along the banks contribute to the fecal and organic load, especially during season changes, when the runoff is high (Tahat *et al.*, 2020).

Recommendations for Aquatic Restoration

To mitigate the biological degradation of the Shatt al-Abbasiyah, the following measures are:

1. **Biological Remediation:** Implementation of Constructed Wetlands at major drainage outfalls to naturally filter and reduce microbial loads before they enter the main river stream.
2. **Hydrological Management:** Maintaining minimum environmental flow rates to prevent water stagnation in hotspots, thereby utilizing the river's natural self-purification capacity.
3. **Strict Enforcement:** Enforcing environmental regulations against direct sewage dumping and establishing buffer zones to prevent livestock from grazing on the riverbanks.

Those findings prove that the Abbasiyah River is in extreme biological distress,

and *Pseudomonas sp.* serves as an unquestionable measure of anthropogenic pollution. The fact that high-density human activity is spatially overlapping with bacterial hotspots proves that the existing water management methods are inadequate. With no ecological remedies, the further spread of these bacteria will erode the function of the river as a potential habitat for aquatic organisms and a risk-free source for the community.

A combination of natural and anthropogenic stressors causes spatial heterogeneity of *Pseudomonas sp.* in the Shatt al-Abbasiyah. It has been noted through field observations that thermal flux is the main natural stimulator, and 35% of the sampling locations exhibited the maximum activity of bacteria at the time of moderate water temperatures (25–35°C). This is also enhanced by the physical hydrology of the river; 1/3 of the locations, especially S9 and S15, had low flow speeds and stagnation, which encourages the growth of organic pollutants and lacks natural flushing by microorganisms.

Regarding anthropogenic-related factors, the discharge of untreated municipal wastewater is the largest contributor to the biological degradation process that affects 40% of the study area (especially sites S3, S10, and S14). Further increases in the high organic loads by the effect of agricultural runoff and direct grazing of livestock (25% of sites) supply the substrate required to promote the growth of *Pseudomonas*.

Conclusions

The spatio-temporal analysis of the Shatt al-Abbasiyah validates a high level of

ecological stress resulting in a high level of biological degradation, where the density of *Pseudomonas sp.* is a key marker of ecological stress. The analysis of the study shows that there was a non-homogeneous distribution pattern of microbial contaminants, with the highest bacterial loads of 42.6×10^3 cells/100ml in Spring, which was almost 130% higher than the minimums in Winter. The spatial analysis showed that S13, S18, and S14 were high-risk hot spots with the concentration exceeding 44×10^3 cells/100ml regularly. These findings reveal that untreated municipal discharge has a critical effect on 40% of the sampling area, and 35% of the microbial variability is instigated by natural thermal variability and hydrological stagnancy. The solution to this contamination cannot be overstated because the existing microbial contents are much higher than the Iraqi Standard (417/2009) and WHO (2017) levels of aquatic safety. These opportunistic pathogens create a two-fold menace: a direct risk to human health of the people living along the river, and a long-term ecological risk to native aquatic biodiversity and fish health. In order to counter this, urgent switching to eco-remediation, which includes constructed wetlands and controlled environmental movement, is necessary. In the future, metagenomic sequencing should be the focus of the research to detect antibiotic-resistant strains in the population of *Pseudomonas* and conduct actual bio-monitoring sensors in known hotspots. The further development of the study with the addition of the benthic sediment analysis will also help to identify the river as a long-term storage site for biological pollutants and to

provide the sustainable recovery of this essential ecosystem.

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