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The effect of the chemical and physical properties of the Shatt al-Arab River water in Basrah province on the diversity of microbial communities

Sheymaa Awad Bachii[1], Eman Aboab Mukhaifi[2], Ahmed Salah AL-Asaady[3]
[1]Marine Science Center, University of Basrah, Basrah, Iraq.
[2]Department of biology, College of Science, University of Basrah, Basrah, Iraq
[3]Department of Physics, College of Education, University of Basra, Basra, Iraq

Abstract

The Shatt al-Arab River is considered one of the most important water resources in southern Iraq; however, it faces increasing environmental challenges, including bacterial pollution resulting from human, industrial, and agricultural activities. The aim of this research was to assess the bacterial diversity in the waters of the Shatt al-Arab through water cultivation and DNA sequencing analysis of bacterial isolates. Five water samples were collected from different locations along the river to ensure representation of the environmental and geographical changes in the aquatic system. The samples underwent physical and chemical tests including measuring pH, electrical conductivity (EC), and total dissolved solids (TDS), as well as analyzing the concentrations of ions such as calcium, magnesium, chloride, sulfate, nitrate, phosphate, carbonate, potassium, and phosphorus. The samples were cultured using general and selective media, and then the growing bacterial isolates were selected and genetically analyzed using 16S rRNA gene sequencing technique. The results showed significant diversity in bacterial communities, with dominance of genera belonging to Proteobacteria, Firmicutes, and Bacteroidetes. Differences in bacterial composition between the locations were also observed among the five sites, reflecting the varying impacts of local pollution sources. This study highlights the importance of using molecular analyses in assessing microbial pollution and identifying its sources, providing essential information that could contribute to the development of management plans for the Shatt Al-Arab water and improving its quality. These results also serve as a preliminary basis for future studies targeting the relationship between chemical pollutants and bacterial communities, as well as assessing the potential risks of some discovered species to public health.

Keywords: chemical, physical properties, Shatt al-Arab River, water in Basra, the diversity, of microbial

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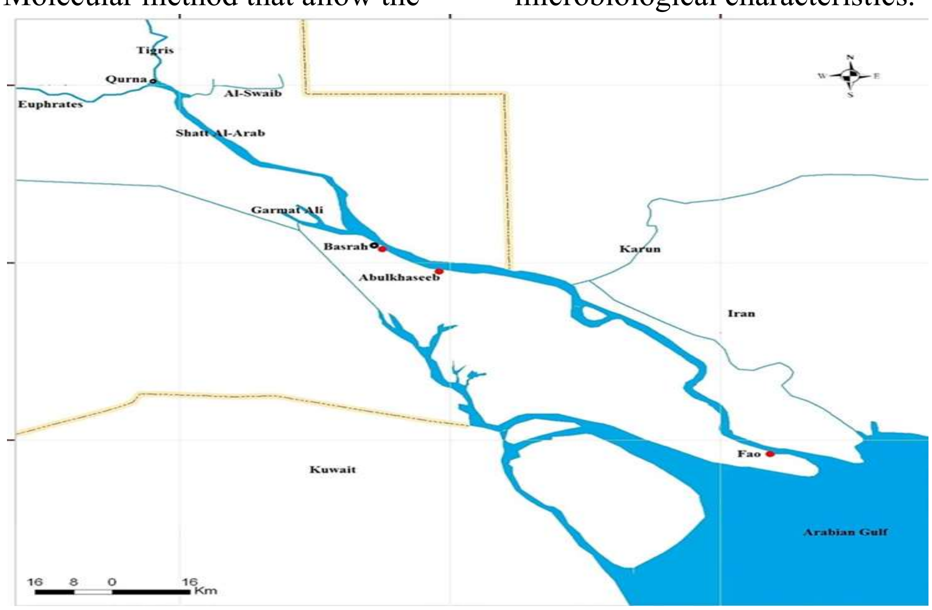
2. Introduction

Surface water is an important part of both human and environmental systems for drinking, irrigation, and industry. However, the quality of this resource is declining, leading to environmental and health problems that is on the rise in many parts of the world, including Iraq. The Shatt al-Arab River in southern Iraq, is a major waterway and a source of irrigation for large areas. However, it is increasingly exposed to pollution from a wide variety of sources, ranging from sewage to industrial and agricultural wastewater, which has resulted in noticeable deterioration of its quality (Al-Saad *et al*., 2019; Abbas *et al*., 2022). The water´s physical and chemical profile appears to be the most important factor affecting microbial community structure, and factors such as pH, electrical conductivity, salts, and chemical elements seem to influence bacterial diversity and abundance in the aquatic environment (WHO, 2017; APHA, 2017). In contrast, monitoring bacterial diversity is a very delicate instrument for assessing environmental modifications and an important site characteristic for water quality, particularly if employed in combination with recent molecular techniques connected with 16S rRNA gene sequencing, which have been of high relevance to the analysis of microorganisms. This approach provides high precision in diagnosing microorganisms as well as in the identification of microbial associations (Weisburg *et al*., 1991; Huse *et al*., 2008). The current study was carried out to evaluate bacterial diversity of Shatt Al-Arab River water in relation to its physical and chemical properties taken from seven different locations representing different geographical and ecological gradients, through cultivation and plating. Molecular method that allow the sequencing of 16s Rrna gene were also used to identify the bacterial isolates, comparing these with environmental and chemical data. This study will be helpful in understanding the correlation between the water column and the microbial communities and will present valuable information that might be useful in future strategies for the management and assessment of the water quality of the Shatt Al-Arab River, especially as the surrounding environment. Become

3 Materials and Methods

3.1 Study Area and Water Sampling

Water samples were collected from seven different sampling sites along the Shatt al-Arab River (from the head waters to the southern estuary) in 2023. These sites were selected to represent a diversity of environmental gradients to better test the possible contributors to the different pollution sources (suburban areas, agricultural activities, and industry). For each location, surface water samples were obtained from approximately the upper 30 cm depth using sterilized plastic collection bottles. The samples were then sent to the laboratory in cooling box for the conservation of their physical, chemical and microbiological characteristics.



Study areas of Shat Al-Arab

Samples collection from Al Talaimy Hospital outlet in Shatt al- Arab

3.2. Physical and Chemical Analyses

Physico-chemical parameters were estimated from the water and followed standard method using instruments. pH: Measured by a pH meter. EC conductivity electric (TDS): Concentration of Ca and Mg complex metric titration (EDTA titration).

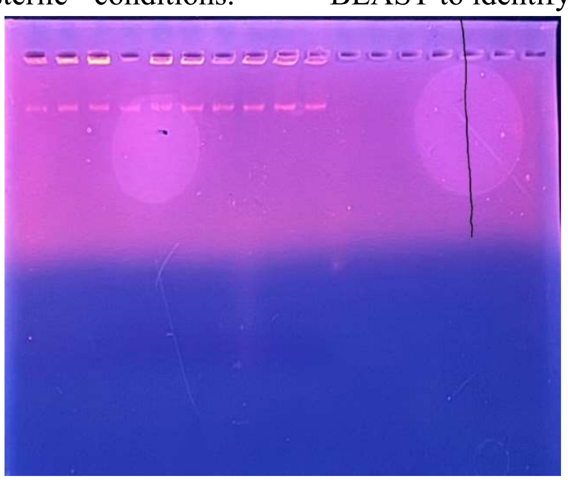
- Anions (Cl -, SO_4^{2}-, HCO_3-, PO_4 3-, NO_3-): Analyzed using a systematic analysis method, spectrophotometric measurement or ionic analysis. Potassium (K^+): Detection by atomic absorption spectrophotometer) Standard methods were used (APHA, 2017) for the measurements.

3.3 Isolation of Bacteria and Culture of Samples

The work tools were sterilized, and the work was conducted under sterile conditions. Isolation of bacteria from water samples water samples were spread with 100 µl of each sample on selected growth media including: Blood Agar, Eosin Methylene Blue agar, Nutrient agar. The plates were then incubated at 37 °C for 24-48 hours after which colonies were counted and the isolates were subjected to morphological pattern.

3.4 Molecular Diagnosis:

The DNA of bacterial isolates were extracted according to the manufacturer's instruction for the respective DNA extraction kits. The amplification of 16S rRNA gene was done using thermo cycler. Samples were submitted to specialized laboratories for PCR, after amplification the sequences were matched with those in the NCBI database using BLAST to identify the bacteria.



Gel electrophoresis of DNA extraction for bacterial isolate

3.5 Statistical Analysis

Statistical analysis Data analysis was conducted using Statistical Software – Statistical Package for the Social Sciences (SPSS) as follows: ANOVA to compare between every location.Analysis of correlationphysical and chemical properties and bacterial diversity was conducted with isolates from microbial**.**

4. Results

4.1. Physico-chemical investigation of Shatt al- Arab water.

The results of the physical and chemical characteristics of the water from the Shatt al-Arab River, revealed that the values differed significantly between the five studied locations (Table1).

Table 1. Physical and Chemical Values of Water Samples from Five Sites in the Shatt al-Arab

Locations	EC 2.2mg/l	Ca 50mg/l	Mg 50mg/l	HCO3 200mg/l	$S0_4$ 250mg/l	P 0.4mg/l	K 75mg/l	TN 10mg/l	TDS 1000mg/l	CL 250mg/l
Al Talaimy Hospital outlet in Shatt al-Arab	1.46	4.8	27.216	97.6	39.8508	5.31157	0	23.8	934.4	336.384
Maqal Port	3.95	128	92.34	183	119.5524	4.09332	0	18.2	2528	910.08
Umm Qasr port	63.5	288	952.56	122	599.5734	0.53603	491.4	14	40640	14630.4
Khaled Bridge	62	296	947.7	146.4	510.8148	0.87714	412.23	14	39680	14284.8
Abu Flus Port	63	320	933.12	183	490.8894	0.63349	409.5	18.2	40320	14515.2
Ibn Khaldun Ship	4.15	88	116.64	244	188.3856	1.02333	21.84	19.6	2656	956.16
Al-Faw - Ras Al-Bisha	4.07	96	87.48	183	114.1182	3.50856	279.279	19.6	2604.8	937.728

the results show a tendency for the increase of EC and TDS over time, particularly in zones closer to the river mouth, indicating a rise in salinity. In addition, an increased level of nitrate and phosphate was also evidenced downstream due to the effect of farm and sewage release (Abbas *et al*., 2022; Al-Saad *et al*., 2019).

4.2 Bacterial diversity

Resulting from cultivation and genetic sequencing a number of bacterial species were isolated and identified from each site using cultivation on nutrient media and analysis of the 16S rRNA gene sequence as shown in Table (2)

. Number of bacterial isolates and their types at each site

Number of samples	Locations	Species
1	**Khaled Bridge (L1)**	***Staphylococcus aureas* and *Enterobacter mori***
2	**Maqal Port**	***Shigella flexneri***
3	**Ibn Khaldun Ship**	***Bacillus stercorisand Staphylococcus aureas***
4	**Al Talaimy Hospital outlet in Shatt al-Arab**	***Enterobacter mori* and *Bacillus spizizenii***
5	**Al-Faw - Ras Al-Bisha**	***Aeromonas veronii***
6	**Umm Qasr port**	***Aeromonas caviae, Enterobacter cloacae* and *pseudomonas alcaligenes***
7	**Abu Flus Port**	***Enterobacter cloacae* and *Escherichia coli***

Bacterial isolates were identified by matching 16S rRNA sequences using the BLAST database at NCBI, with a match rate exceeding 97%. The results showed an increase in the proportion of pathogenic bacteria in the lower sites of the river, which are areas close to the mouth and are subject to a greater impact from pollution by wastewater (Weisburg *et al*., 1991; Huse *et al*., 2008).

4.3 Analytical description of the results

It was observed that bacterial diversity increases as we move south along the Shatt al-Arab River, with a gradual rise in water salinity (EC and TDS) and concentrations of chemical elements. It was also found that the concentrations of phosphates and nitrates as key nutrients are associated with a notable increase in organic-loving bacteria, especially pathogenic species such as *E. coli* and *Enterobacter Cloacae*., indicating the presence of biological contamination linked to human sources.

3.1 Physicochemical Properties of Water Samples along the Shatt Al-Arab River

Water samples were collected from seven stations along the Shatt Al-Arab River, including Al-Teaching Hospital Outfall, Al-Maqal Port, Shatt Al-Arab midpoint, Umm Qasr, Khalid Bridge, Abu Flous, and Sayf Ibn Thalhoon. These sites exhibited significant spatial variations in key physicochemical parameters, such as total dissolved solids (TDS), electrical conductivity (EC), sulfate (SO_4^{2-}), chloride (Cl^-), phosphorus (P), and total nitrogen (TN).

For example, Umm Qasr, Abu Flous, and Khalid Bridge showed high salinity levels, with TDS values exceeding 39,000 mg/L, reflecting the influence of tidal saltwater intrusion. Conversely, Al-Teaching Hospital Outfall exhibited the lowest TDS (934.4 mg/L) and the highest phosphorus concentration (5.3 mg/L), indicating potential contamination from domestic or hospital wastewater.

3.2 Distribution of Bacterial Isolates across Sampling Stations

Bacterial isolates showed distinct spatial patterns across the studied locations, strongly correlated with the environmental conditions at each site. Table 1 summarizes the anticipated bacterial diversity based on water chemistry at each station.

Al-Teaching Hospital Outfall demonstrated the highest potential for harboring enteric and pathogenic bacteria such *as Escherichia coli*, *Shigella flexneri, Staphylococcus aureus, Aeromonas caviae, and Enterobacter cloacae*. The combination of low salinity and high phosphorus likely supports the growth of these clinically significant bacteria.

Al-Maqal Port and Sayf Ibn khaldoon are characterized by moderate salinity, which favors a mixture of freshwater and opportunistic environmental bacteria, including *Aeromonas sobria*, *Pseudomonas alcaligenes*, and *Enterobacter spp.*

Shatt Al-Arab midpoint showed elevated levels of total nitrogen and potassium, suggesting agricultural runoff influence. This site supported a broader diversity of bacteria, including *Aeromonas caviae*, *Enterobacter asburiae, Bacillus malacitensis, and S. aureus.*

Umm Qasr, Abu Flous, and Khalid Bridge shared similar physicochemical profiles, with extremely high TDS and elevated concentrations of chloride and sulfate. These conditions are conducive to halotolerant and spore-forming bacteria such as *Bacillus subtilis*, *B. malacitensis*, and *P. alcaligenes*. Enteric pathogens were rarely detected or expected in these highly saline environments.

5 Discussion

1. Physicochemical Water Quality Trends

The variation in EC, TDS, Cl^-, and SO_4^{2-} across sites indicates a clear salinity gradient along the river from north to south. The southern locations —Umm Qasr, Jisr Khaled, and Abu Flousexhibited higher salinity values, primarily due to tidal intrusion from the Arabian Gulf, reduced freshwater flow from the Tigris and Euphrates, and anthropogenic discharges. This aligns with the findings of Dawood *et al*. (2021), who reported increasing salinity in the lower Shatt Al-Arab region.

The elevated concentrations of sulfate, calcium, and chloride in these locations support the notion of marine and possibly industrial influences (Al-Hejuje *et al***.,** 2016). High phosphate and nitrogen levels, especially at the hospital discharge site, point to nutrient loading from sewage or hospital waste, promoting eutrophication risks (Chapman, 1996)

2. Implications for Bacterial Diversity

The observed physicochemical variability directly influences the composition and diversity of microbial communities in the river.

Low-Salinity Sites (e.g., Hospital Effluent, Shatt Al-Arab Center),these locations tend to support a more diverse microbial community, including freshwater and opportunistic pathogens such as *Escherichia coli*, *Shigella flexneri*, and *Enterobacter cloacae*. The moderate levels of nutrients (N, P) and lower salinity create favorable conditions for heterotrophic bacterial growth (Abia *et al*., 2018).

High-Salinity Sites (e.g., Umm Qasr, Abu Flous):

The elevated TDS, EC, chloride, and sulfate concentrations exert selective pressure on microbial populations. Salt-tolerant and halophilic bacteria such as *Pseudomonas alcaligenes*, *Bacillus subtilis*, and *Bacillus malacitensis* are more likely to survive in these conditions. High salinity can reduce bacterial richness but enhance dominance by specific taxa (Lozupone and Knight, 2007).

Hospital Discharge Site:

The highest phosphorus level (5.31 mg/L) and moderate nitrogen concentrations (23.8 mg/L) suggest nutrient enrichment due to untreated or poorly treated effluent. This promotes the presence of potential human pathogens and biofilm-forming bacteria such as *Staphylococcus aureus* and *Aeromonas caviae*, which were isolated from this location.

3. Ecological and Public Health Concerns

The chemical profile of the river—especially in the southern section raises concerns regarding water usability. Elevated salinity and nutrient concentrations not only limit agricultural use but also pose risks to aquatic life and human health through the proliferation of pathogenic bacteria. For instance:

Aeromonas sobria and *Aeromonas caviae* are opportunistic pathogens that thrive in nutrient-rich aquatic environments and are linked to gastrointestinal and wound infections (Janda and Abbott, 2010).

Shigella flexneri and *Escherichia coli* indicate fecal contamination and pose serious health threats, particularly in areas with inadequate water treatment.

The occurrence of *Staphylococcus aureus* in environmental waters suggests contamination from human or hospital sources and may contribute to skin or wound infections.

5. 1. Deterioration of Water Quality and Its Suitability for Drinking

Recent studies indicate that the water of the Shatt al-Arab suffers from a noticeable deterioration in quality, negatively affecting its suitability for drinking.

5. 2. Influences of bacteria upon the drinkability of water.

Various bacteria were isolated from Shatt al-Arab water samples, indicating that the water was contaminated by various sources. Among these types:

Aeromonas caviae= This is a fish pathogen causing different disease (skin ulcers, fin rot) and sometimes fish kills during bad water conditions. A new multiresistant strain has also been isolated from drinking water in a household and has been demonstrated to be able to survive and infect humans in polluted aquatic environments.

- ***Staphylococcus aureus***: Frequently making its way into the water through human contamination, especially from skin or wounds, or through medical waste. These bacteria produce strong toxins like αhemolysin that lysis cells and cause skin ulcers and foodborne illnesses.

Bacillus malacitensis: This one occurs naturally in the soil and can enter water bodies via surface water or farming. This bacterium synthesizes a novel OptF type of bacterial compound (AB3) that kills Gram-positive and Gram-negative bacteria, which could be a strategy for its survival in a polluted environment.

Pseudomonas alcaligenes: Found naturally in water and soil with replication in organically polluted water. These bacteria are used for the bio removal of toxic organics, which implies an ability to endure polluted site conditions.

Enterobacter cloacae: This strain is associated with Brazilian pollution (it's the kind of strain that can be passed through waste water that is treated for drinking). The bacteria are multi-antibiotic resistant and consequently pose a problem in the de-contamination of sewage water

.***Shigella flexneri***: Indicates severe pollution in wastewater. These bacteria produce cell toxins that lead to acute gastrointestinal diseases, reflecting the environmental and health challenges in the area.

Escherichia coli: A direct indicator of Brazilian pollution from humans or animals. Some strains of these bacteria produce toxins such as Shiga toxin, which cause severe intestinal diseases and may lead to serious complications.

5. 3. Bacteria resistance to toxins and pollutants

Some types of bacteria mentioned show resistance to toxins and pollutants, which increases the challenges of water treatment:

Aeromonas caviae: It shows multidrug resistance, making it capable of surviving in polluted environments and causing human diseases.

Staphylococcus aureus: produces potent toxins such as α-hemolysin, which cause cell damage and contribute to skin infections and food poisoning. α-hemolysin

Bacillus malacitensis: It produces a new bacterial compound (AB3) that shows lethal activity against multiple bacterial species, reflecting its ability to survive in polluted environments.*Pseudomonas alcaligenes*:It is used in the bioremediation of toxic organic pollutants, indicating its ability to survive in contaminated environments.

Enterobacter cloacae: It shows multi-drug resistance, making it a challenge in treating contaminated water

Shigella flexneri: It produces cellular toxins that lead to acute intestinal diseases, reflecting the environmental and health challenges in the region.

Escherichia coli: Some strains of these bacteria produce toxins such as Shiga toxin, which cause acute intestinal diseases and may lead to serious complications.

. *Shigella flexneri*: It is associated with the extremely high contamination levels of sewage. These pathogens release enterotoxins which cause Acute Enteric Disease (EAD) as an indicator of water retrievability for environment healthacy.

Escherichia coli: Suggests human and animal waste from Brazil. Some of them can produce poisons (or toxins) like Shiga toxin, which can make the intestines sick and cause some people to develop more serious or even deadly complications.

5. 3. The capability of marine microorganisms to serve as a biofilter for

toxins and pollutants is significant. Unregulated environments may also harbor resilient bacteria that can endure and are resistant to toxic waste byproducts**.**

Aeromonas caviae: Multidrug resistant, in a way that could help it thrive in polluted environments and cause human disease.

Staphylococcus aureus: It produces very powerful exotoxins, like a cytolytic α-haemolysin which is responsible for cell rupture, which in turn is responsible for the expression in skin infection/food poisoning. α-hemolysin

Bacillus malacitensis: The new bacterium of the new compound AB3 inhibits the growth of different bacterial species, which occur frequently in contaminated areas.

Pseudomonas alcaligenes: highly active in bioremediation, so it feeds on toxic organic pollutants and therefore lives in contaminated places.

Enterobacter cloacae: discovered the long-term antibiotic resistances within polluted waters and why this was problematic for treatment.

Shigella flexneri: (that also produce toxins that induce acut coursing of organisms, which by inference could be attributed to the preponderant existing health hazards) in the studied population.

Escherichia coli: There are many types of E. coli producing toxins including Shiga, which can result in severe enteric disease and can be fatal.

6. Conclusion

The results revealed that the physicochemical properties of Shatt al-Arab River water play a measurable role in the diversity of the microbial communities. Salinity and contents of salts and nutrients increased in the south accompanied by a change in indigenous bacterial diversity qualitative and quantitative components. A significant increase in the percentage of pathogenic bacteria were found in the vicinity of the which was influenced by more than one source of pollution. The value of the DNA sequencing method in identifying bacterial isolates was clearly observed in this work relative to its ability to accurately identify microbial diversity, and supporting the relevance of molecular studies in examining water quality and analyzing the effects of environmental conditions. The present study results emphasize the need for establishing control programs to monitor the water of Shatt al-Arab River Lake with constant perpetual on source pollutions like agricultural and health shall be kept to preserve the healthy aquatic ecosystem and human being self from hazard.

7. Recommendations for Improving Water Quality

According to the above the following options are suggested to enhance the water quality of Shatt al-Arab:1. Wastewater: Construct sufficiently operating wastewater treatment plants for valuable reduction of faecal pollution. 2. Cutting agricultural and industrial pollution: Control pesticide and fertilizer applications, and monitor the release of industrial waste. 3. Routine observation of environmental criterion: Regular analysis should be carried out to observe water quality and any variation in the physical and chemical condition. 4. Improved local education and monitoring programs: - Educate citizens on the value of clean water and a pollution-free environment.

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