Investigation of Indicators of Bacterial Pollution Using Environmental Genomics and Water Quality Index for the Purpose of Living Organisms in the Tigris River South of Mosul City

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Abstract: This study was conducted to investigate the presence of Total Plate Count (TPC) and Fecal Coliform (FC), and to isolate and identify some types of bacteria present in the Tigris River water, south of Mosul. The study area extended from Hammam Al-Alil to Al-Qayyarah (60 km). The presence of these bacteria is evidence of organic pollution in the river. The water quality index was used to assess the living organisms in the river, taking into account the physical and chemical factors specific to this model, represented by river temperatures, pH, and dissolved oxygen measurements. Traditional tests were also conducted to assess coliform bacteria by calculating the number of fecal coliform bacteria, which includes three stages: Presumptive Test, Confirmed Test, and Completed Test. The results of the physical and chemical tests for the living organisms in the river showed that the water temperature ranged between 10-24.5 °C and the concentrations of the indicator values. The acidity ranged between 6.4-7.9 and the dissolved oxygen results indicated a range of (6.4-10.3) mg/L. The range of phosphate and nitrate concentrations was between (0.01-0.14) and (0.001-0.99) mg/L, respectively. The results were within the Iraqi standard determinants and international specifications for surface water. The Tigris River water, south of Mosul, ranged between (moderate - good). The results of the bacteriological study also showed that the total number of bacteria ranged between (6-700) cells/100 ml, and colon bacteria ranged between 0-180 cells/100 ml, in addition to the isolation and identification of each of the bacteria (Acinetobacter radioresistens, Bacillus firmus, Pseudomonas aeruginosa

1 INTRODUCTION

Wisdom says that water is life. For years, water has been described as the womb that embraces life, as it constitutes a large percentage of the composition of living organisms. Although it is essential for life, when it is polluted, it can threaten it and destroy various organisms within it [1]. Water is a primary means of transmitting pathogens resulting from the discharge of human, animal, industrial and agricultural waste into it. Among the pathogens are typhoid, dysentery, polio, cholera, parasitic worms and others [2]. Reports from the World Health Organization indicate that 80% of diseases that affect humanity are caused by water pollution with pathogenic microorganisms, a problem that many countries in the world, including developed countries, still suffer from [3]. It indicates that there is a steady increase in the rate of infections with Rotavirus sp, which causes gastroenteritis, and infections with Salmonella bacteria, which causes typhoid, and Campylobacter bacteria [4]. Microbiological analysis of water is a safety valve for many epidemics and diseases that may result from contamination with human or animal waste. Bacteriological analysis of water is often relied upon to search for indicators of contamination, especially coliform bacteria, which are part of the intestinal bacteria family and live in various natural environments, in addition to living in the intestines of humans and animals. They can be found in waste, nutrient-rich water, soil, and rotting plant parts. Therefore, they may not be a sufficient indicator of water pollution. However, detecting Escherichia coli bacteria in potable water poses a significant risk, as it is one of the most important indicators of fecal pollution, and its presence in water confirms the recent contamination [5]. Clostridium perfringens bacteria is also one of the indicators of fecal pollution, the possession of this type of bacteria spores leads to its resistance to different

environmental conditions,. Fecal streptococcus is an important indicator of fecal contamination [6]. The aforementioned types of bacteria are indicators of the potential presence of pathogenic bacteria, due to the presence of these bacteria in the human intestine. Furthermore, these bacteria remain in water for a longer period and are present in greater numbers than pathogenic bacteria. They are also easier to isolate and purify [7].

2 MATERIALS AND METHODS

2.1 Water Sample Collection

The study was conducted on the Tigris River from its source in Mosul to the Qayyarah district, southeast of Mosul, within the administrative borders of Nineveh. The study spanned a total length of 66 km. Four main sites were identified along the river: Hammam al-Alil district, al-Safina village, al-Hood al-Fawqani village, and al-Qayyarah district, as shown in Figure 1 and Table 1. Samples were collected, one sample per site per month, for a period of twelve months, from February 2024 to January 2025. Clean polyethylene containers were used, and the containers were washed with the sample water in the field and laboratory.

2.2 Physical and Chemical Tests of Water Samples

Five factors were used to calculate the water quality index for aquaculture purposes: pH, dissolved oxygen, phosphate, nitrate, and temperature. Measurements were made according to standard and international methods [8]. Methods for measuring physical and chemical tests were followed in [9].

2.3 Bacteriological Tests of Water Samples

Water samples were collected in pre-sterilized glass bottles using an autoclave at a pressure of 1.5 pounds and a temperature of (121)°C for 15 minutes [10].

2.4 Culture Media

Prepared according to supplier's instructions (LABM) and sterilized in an autoclave at 121°C and 15 psi for 15 minutes. The media included Nutrient Agar ,MacConkey Broth, Peptone Water, and Eosin Methylene Blue Agar.

Table 1: Water sampling sites from the Tigris River within Nineveh Governorate.

Location	Location coordinates
43°16'06.5"E 36°10'38.7"N	**1**\ Hammam Al-Alil
43°21'01.2"E 35°58'48.1"N	**2**\ Al-Safina
43°18'46.1"E 35°51'27.4"N	**3**\ Al-Hood
43°17'12.7"E 35°46'31.1"N	**4**\ Al-Qayyarah

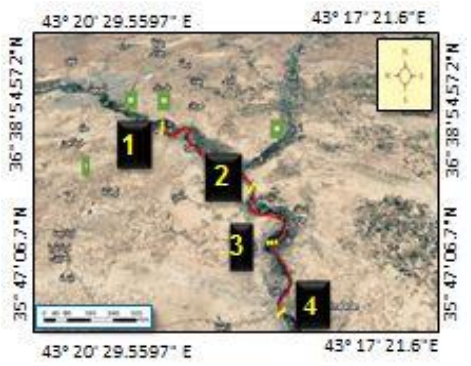

Figure 1: Main studied sites on the Tigris River south of Mosul (Google Earth).

2.5 Solutions, Reagents, and Dyes

They were prepared according to the instructions [9]. Such as normal saline, Kovac's reagent.

2.6 Detection of Cacteria in Water Samples

A series of tests was performed to detect the presence of bacteria. The following tests were performed.

2.6.1 Total Plate Count (TPC)

A dilution was made for each sample (up to 1-10 dilutions, 2-10 dilutions). 1 ml of the sample was taken and placed in a sterile Petri dish. The previously sterilized, cooled Nutrient Ager medium was poured over the sample. The plates were placed upside down in an incubator at 37°C for 24-48 hours. The number was then calculated using the following equation: TPC cell.ml-1 = Reciprocal of dilution × number of colonies growing on the medium

2.6.2 Counting the Number of Faecal Coliform Bacteria

The number of fecal coliform bacteria was calculated using the Multiple Tube Method and the Most Probable Number (MPN) mentioned by the American Association for Public Health, which includes three stages [10]**:**

2.6.2.1 Presumptive Test

Nine test tubes containing MacConkey Broth medium were inoculated with three concentrations (10, 1, and 0.1) ml of the sample, respectively. They were then incubated in a water bath at 44°C. After incubation, the tubes showed a gas accumulation and a yellow color, indicating the presence of fecal coliforms in the water sample. The results were recorded, and using the MPN table, the most probable number of coliforms present per 100 ml of the sample was determined [10].

2.6.2.2 Confirmed Test

The test was performed by taking a full loop from the positive tubes for the presumptive test and inoculating them onto previously prepared and sterilized Eosin Methylen Blue agar (EMB) using the schematic method. These tubes were then incubated upside down at 37°C for 24-48 hours to determine if the bacteria that formed the gas were negative. Whether the bacteria are Gram-positive (i.e., within the coliform group) or Gram-positive; this medium is capable of inhibiting the growth of Gram-positive bacteria, while the appearance of small, round colonies with dark centers surrounded by a dark green metallic sheen is evidence that the bacteria belong to the coliform group.

2.6.2.3 Completed Test

In this test, test tubes containing previously prepared and sterilized peptone water medium were inoculated by taking a full loop from the positive tubes in the hypothetical test. The tubes were incubated at 44°C for 24-48 hours. Drops of Kovacs reagent were then added. A red ring was observed on the surface of the medium, indicating the growth of E. coli and a number of different species and strains of Enterobacteriaceae. Referring to the MPN table, we can arrive at the results to obtain the most probable number of E. coli bacteria per 100 ml of the water sample.

2.7 Isolation and Identification

2.7.1 Isolation

Bacterial species were isolated from water samples using a full loop and cultured on Nutrient agar and MacConkey agar.

2.7.2 Cultural Identification

The morphological characteristics of the isolated colonies were studied after purification on selective culture media. These included the shape, texture, size, color, and borders of the isolated colonies [12].

2.7.3 Confirmation of Identification Using Molecular Methods

Polymerase Chain Reaction (PCR) was used to confirm the identification of bacterial isolates by following the following steps:

2.7.4 DNA Extraction

The Genomic DNA mini kit from Geneaid was used to extract DNA from Gram-positive and Gram-negative bacteria. The initial migration was followed by DNA detection, followed by Polymerase Chain Reaction (PCR), which included Primer sequence Table 2.

Table 2: Primer sequence.

Source	Result	Sequence Primer	Gene
NCBI	1300 pb	F GACCTCGTTTAGTTCACAGA	16Sr RNA
		R CACACGCTGACGCTGACCA	

Primers were prepared according to the manufacturer's instructions.

A PCR machine was used to amplify the 16S rRNA gene using specific forward and reverse primers, and the DNA concentration was adjusted for all study samples. DNA was then extracted from the agarose gel, and the resulting PCR bands were purified and sent for nucleotide sequencing using a Geneaid analysis kit. The nucleotides of the amplified fragments were then identified using DNA sequencing technology to identify and identify the species of the organism based on matching the gene sequences obtained with the gene sequences recorded for species and strains at the National Center for Biotechnology

Information (NCBI). The results were analyzed using the BLAST program.

3 RESULTS AND DISCUSSION

The results of the physical and chemical factors used to calculate water quality for aquatic habitats (CCME WQI) as shown in Table 3 and Figure 2 showed that temperatures ranged between 10-24.5 °C. High temperatures encourage the growth of microorganisms such as fungi, bacteria, and viruses, which can cause diseases for consumers, especially diarrhea in children and viral hepatitis in the hot months of the year [12]. The pH values ranged between in Table 3 (6.4-7.9).

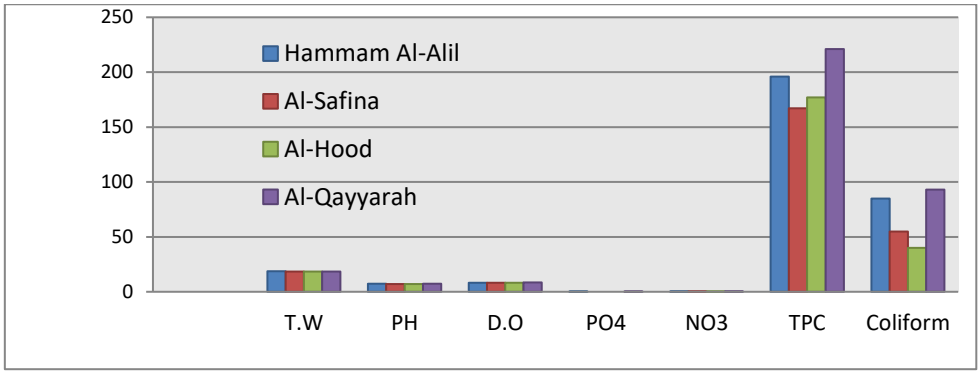

Figure 2: Physical and chemical factors guide water quality not for aquatic purposes.

Table 3: Averages, minimum and maximum concentrations of the physical and chemical factors under study.

Locations factors		Hammam Al-Alil	Al-Safina	Al-Hood	Al-Qayyarah
T. Water	Mean	18.6	18.3	18.4	18.5
	max	24.0	24.0	24.5	24.5
	min	10.0	10.1	10.4	10.4
PH	Mean	7.3	7.1	7.0	7.2
	max	7.9	7.7	7.6	7.8
	min	6.7	6.5	6.4	6.6
D.O mg/L	Mean	8.1	8.2	8.3	8.5
	max	10.2	10.2	10.3	10.2
	min	6.4	6.6	6.7	6.5
PO_4-3, mg/L	Mean	0.1	0.0	0.0	0.1
	max	0.14	0.12	0.11	0.13
	min	0.03	0.01	0.01	0.02
NO_3^-, mg/L	Mean	0.420	0.30	0.266	0.37
	max	0.990	0.69	0.650	0.88
	min	0.030	0.00	0.001	0.01
TPC ml/ 100	Mean	196	167	177	221
	max	650	580	600	700
	min	16	13	6	14
Coliform Bacteria ml/100	Mean	85	55	40	93
	max	165	130	90	180
	min	4	2	0	5

The results indicated that the river water tends towards alkalinity, as well as the lack of fluctuations in the pH values of the river water, which is attributed to the ability of Iraqi water and soil to neutralize the acidity (ANC) due to the high concentration of alkalinity in the water, as well as the high carbonate compounds CO3 in the bottom sediments [13], [14]. (Dissolved Oxygen (DO) and the results of Table 3 indicated that its value ranged between 6.4-8.5 mg/L, which indicates that the raw water is well-ventilated and free from causes of taste and odor resulting from anaerobic degradations [9], [15]. The results Table 3 of nitrate concentrations, which ranged between 0.001-0.990 mg/L, showed a variation in the extent of the decrease. The reason for the decrease in the nitrate value may be attributed to its consumption by floating algae and algae present in the area [16]. Phosphorus is one of the important nutrients for cell components, as it is an essential element for the growth, maintenance and repair of body tissues. It is necessary, along with calcium and magnesium, for healthy growth and bone formation in infants and children [17]. Its concentrations ranged between In our study, between 0.01-0.14, nitrates and phosphates were among the Iraqi standard determinants and international water specifications, as shown in Table 4, as the quality of the Tigris River water south of Mosul ranged between moderate - good and protected waters, rarely threatened, weak and deviating from the required level when compared with the rest of the rivers, especially in the southern part of Iraq, as it suffers from weakness.

3.1 Total Plate Count (TPC)

The total plate count included all types of aerobic, anaerobic, and facultative Gram-negative bacteria capable of fermenting lactose. Therefore, it is an important indicator of water safety and the extent of water contamination [18], [15]. The results in Table 3 showed that the total bacterial count ranged between 6-700 cells/100 ml. The reason for the variation in counts is due to the different factors affecting the presence of bacteria, the pH value, and the soil surrounding the river [19].

3.2 Fecal Coliform Bacteria

Intestinal bacteria are one of the most important causes of waterborne diseases. E. coli is the most prominent and widespread of these bacteria. They are naturally found in the intestines of humans and warm-blooded animals, and are therefore abundant in human and animal feces. This indicates water contamination if the number of coliform bacteria ranges, as shown. In Table 3 between 0-180 cells/100 ml, these pathogenic bacteria cause a group of infections inside and outside the intestine [20]. The Tigris River water is classified as category (A2) based on the classification provided by [21]. who classified surface water required for use into three categories, Table 5.

3.3 Molecular Diagnosis

The use of molecular diagnostic techniques is important and necessary due to their speed, particularly the use of Polymer Chain Reaction (PCR) technology. The study of the bacterial genome and comparison of its nitrogenous base sequences with those available in Gen Bank identified three bacterial species.

3.4 Cultural Diagnosis

Pseudomonas aeruginosa colonies on MacConkey agar were shown to be non-lactose fermenters. Bacillus bacteria were also isolated on nutrient agar and appeared white, chalky, dry, and rough to the touch.

Acinetobacter radioresistens was also isolated on nutrient agar and appeared as pink, lactose-fermenting colonies.

3.5 Molecular Diagnosis

This reaction was performed on purified genomic DNA samples extracted from the bacterial isolates using a primer specific for the 16srRNA gene.

Table 4: Iraqi and international standard specifications for water.

WHO 2006, 2003	Aquatic life	Physical and chemical characteristics
6.5-9.5	6.5-9	PH
–	15	Temp
50	13	mg/L NO_3
0.4	0.3	PO^{-4} mg/L
>5	5	DO mg/L

Table 5: Twort classification of surface water.

Maximum number of fecal colon cells allowed per 100 ml	Category
20	A1
2000	A2
20000	A3

3.6 Results of the Specialized PCR Reaction for Genomic DNA Samples

This reaction was performed on purified genomic DNA samples extracted from bacterial isolates. The strain was isolated using a primer specific for the 16s rRNA gene. After isolating the bacterial species from the samples under study and extracting their genomes [11], PCR was performed to confirm their molecular identification by detecting the 16s rRNA gene. The nitrogenous base sequences of each isolate were analyzed, and the results were compared at the National Center for Biotechnology Information (NBCI) using the Blast program. The results showed similarity between the three isolates, as shown in Table 6.

Table 6 shows the genera of isolated bacteria and the percentages of partial identification of the isolated bacterial species.

Table 6: Genera and identification percentages of isolated bacterial strains.

Reverse number	Molecular diagnosis and similarity ratio
MT367790.1	*Acinetobacter radioresiste* %100
MT457466.1	*Bacillus firmus* %100
ON032863.1	*Pseudomonas aeruginosa* %100

4 CONCLUSIONS

This study confirms the presence of moderate organic pollution in the Tigris River waters south of Mosul. The total bacterial count (TPC) ranged from 6–700 cells/100 ml, and fecal coliform counts (FC) ranged from 0–180 cells/100 ml. Species of health and environmental significance, such as Acinetobacter radioresistens, Bacillus firmus, and Pseudomonas aeruginosa, were isolated. Although the physical and chemical parameters (temperature, pH, dissolved oxygen, phosphate, and nitrate) were within the permissible limits according to Iraqi and international standards, the presence of bacteriological contaminants highlights the need for enhanced treatment procedures and continuous monitoring along the 60 km stretch from Hammam al-Alil to Qayyarah.

The relative distance from direct pollution sources and natural dilution processes have contributed to raising water quality levels to the "good" range in some locations. However, the continued discharge of untreated domestic and industrial waste threatens the sustainability of water resources and the health of local communities. Therefore, it is recommended to:

1) Install monitoring and biological filtration stations at critical points along the river.
2) Develop awareness and regulatory programs to reduce the discharge of sewage and industrial waste.
3) Conduct periodic studies that include analyzing additional environmental factors and monitoring long-term water quality developments.
4) These measures will ensure the preservation of the quality of the Tigris River and protect human health and aquatic ecosystems south of Mosul.

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