The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

IDENTIFICATION AND ESTIMATION OF PATHOGENIC BACTERIAL CONTAMINATION INDICATORS IN KHABUR RIVER, WITHIN ZAKHO DISTRICT, KURDISTAN REGION OF IRAQ

IBRAHEM AHMED HUSSEIN *

Department of Environmental Science, Faculty of Science, University of Zakho, Kurdistan Region-Iraq. Email: ibrahim.hussein@uoz.edu.krd

MUSTAFA ISMAIL UMER

Department of Soil and Water, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq. Email: mustafa.umer@uod.

Abstract

The Khabur River is the main source of water for the city of Zakho. So, the study's goal was to assess its environmental status in order to maximize its usage. In the current study, six sites were chosen to evaluate the microbial pollution indicators, such as Heterotrophic Plate Count (HPC), Total Coliform (TC), Faecal Coliform (FC), and *E. coli*. Pathogenic bacteria were also isolated directly from the river. The recorded values were ranged as follows: HPC (7.3×102 -780×102 cfu/ml), TC (170-3200 cell/100ml), FC (79-1840 cell/100ml), and *E. coli* (12-540 cell/100ml). The highest values were recorded in the warm seasons compared to the cold ones, due to the appropriate temperature for bacteria and the frequent use of water by people. It was found that the river is exposed to continuous pollution by pathogenic bacteria as a result of sewage water that is directly discharged into the river and soil erosion on the banks of the river. 129 isolates of bacteria were identified and belonged to 33 species. The Kirby-Bauer disk diffusion procedure was done to examine the susceptibility of isolated bacteria to 21 different antibiotics. The Multiple Antibiotic Resistance (MAR) Index was calculated for the isolated bacteria, and the highest values were found for the bacteria *Streptococcus agalactiae, Salmonella typhi, Proteus penneri, Pseudomonas aeruginosa* and *Aeromonas* spp. Also the efficacy of the antibiotics used was determined. Amikacin, ciprofloxacin, Imipenem, Levofloxacin, and Gentamycin were recorded the highest value compared to the rest antibiotics, and their efficacy was 98.41, 97.27, 93.84, 91.66 and 90.9, respectively.

Keywords: Khabur, Zakho, Pathogenic Bacteria, HPC, Coliforms, MAR Index.

INTRODUCTION

Most people who live in developing countries still get their water and throw away their trash in surface waters. A lot of people in this area get their drinking water from open or polluted sources, which can lead to outbreaks of diseases that are spread through water (Shafi *et al.*, 2013, Adesakin *et al.*, 2020). The effective management of resources, particularly water, is a substantial challenge in the pursuit of urban sustainability. Nonetheless, a significant portion of the global population continues to face challenges in obtaining clean water. Water is an essential resource that plays a fundamental role in all aspects of human life, including but not limited to drinking, food preparation, recreational activities, and the maintenance of hygiene. Water can also act as a disease vector. Waterborne infections rank among the major preventable causes of mortality, especially for children under five, with an estimated 1.6 million fatalities reported to the World Health Organization (WHO, 2021).

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

The Khabur River plays a critical role in supporting agricultural activities, providing freshwater resources, and fostering diverse ecosystems. The Khabur River encounters different challenges that affect its sustainability and the safety of the communities dependent on it, one of the primary hurdles faced by Iraq is the issue of water shortage, which is mostly caused by the impact of climate change. Additionally, the flow rate of the Khabur River, originating from Turkey, has been significantly reduced. This is of great concern as almost half of Iraq's surface water resources are derived from upstream rivers in neighboring countries such as Turkey and Iran, passing via the Kurdistan Region (Al-Qassas, 1999) Heavy metals, pesticides, and fertilizers have contaminated the Khabur River as a result of industrial, agricultural, and recreational activity combined with inadequate sewage treatment facilities (Al-Muqdadi *et al.*, 2016, Gulistan *et al.*, 2023). All living things depend on water, and the sustainability of ecosystems and human health depend on the quality of this resource. One of the main objectives of environmental and public health authorities is guaranteeing the safety of water for use in recreational and culinary purposes. Scientists and environmentalists use a variety of indicators to evaluate the quality of water (José *et al.*, 2021). The term "Biological Indicators" refers to the comprehensive range of biotic and abiotic reactions that are linked to changes occurring within a particular environment. They are frequently used to identify and distinguish between positive and negative effects in natural environments. These indicators can be utilized to determine ecological modifications caused by pollution incidents, which might potentially influence the existing biodiversity (Trishala *et al.*, 2016). Bacterial indicators are assemblages or species of bacteria that indicate contact with pollutants when their abundance in an ecosystem exceeds specific numerical thresholds. Bacteria are definitely useful for gauging pollution levels in a wide range of ecosystems. The HPC is a standardized method used to quantify the population of aerobic or facultative anaerobic heterotrophic microorganisms, in water sources (Mmuoegbulam *et al.*, 2019).

In the field of drinking water microbiology, the HPC approach has a long history. HPC was employed as an indirect measure of water safety as well as a gauge for how well water treatment plants were operating. Due to the introduction of fecal indicators, such as coliforms and enterococci, the use of HPC as a safety indicator decreased in the 20th century (Diduch *et al.*, 2016). Coliform bacteria are essential for evaluating the quality of water. The presence of these organisms plays as a proactive mechanism, alerting authorities to the possibility of pollution and enabling them to implement remedial actions in order to safeguard human health and ecological systems. Although coliforms do not possess inherent toxic properties, their existence can serve as an indicator of the potential presence of more hazardous infections (Savichtcheva & Okabe, 2006). Total coliform bacteria play a crucial role in assessing water quality due to several reasons such as their abundance in nature, indication of fecal pollution, response to environmental changes. Coliforms and E coli have many applications of assessment of drinking water safety, recreational water quality and environmental impact assessment (Niyoyitungiye *et al.*, 2020). Microbiological indicators for fecal pollution detection reveal the presence of pathogenic microorganisms. The use of fecal contamination indicators has emerged as a practical alternative to the challenges associated with identifying and measuring the waterborne pathogens responsible for waterborne diseases (Bireshwar, 2022).The identification of

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

Escherichia coli in river water serves as a robust indicator of the presence of fecal contamination, hence posing a potential hazard to public health. This is due to the fact that fecal matter can harbor a diverse array of pathogens, including bacteria, viruses, and protozoans. As a result, E. coli is now a common bioindicator used to evaluate the quality of water (Rochelle-Newall *et al.*, 2015). Antibiotic contamination from human activity has made aquatic environments, particularly rivers, as a reservoir for the spread of antibiotic-resistant microorganisms. The health concerns connected with waterborne bacteria in humans and animals are increased when they are exposed to antibiotic-resistant bacteria (Salikan *et al.*, 2020). So, the main objectives of this study were to isolate waterborne pathogenic bacteria, their contamination indicators, and to evaluate their resistance to antibiotics.

MATERIALS AND METHODS

Study Area

Khabur is a river that rises in Turkey and flows through Iraq to join the Tigris at the tripoint of Turkey, Iraq and Syria. The Zakho communities along the Khabur River's path depend significantly on the river for their socioeconomic survival. The river basin is well-known for producing wheat, barley, and it is an essential source of water for irrigation and drinking (Al-Muqdadi et al., 2016). Moreover, the river has been subject to pollution from agricultural runoff, untreated sewage, and industrial effluents, resulting in water quality degradation. Such pollution can have detrimental impacts on the river's ecosystems, affecting biodiversity and the health of aquatic species (Gulistan *et al.*, 2023). To study the bacterial population of Khabur River in Zakho city, six locations were picked; first one (Tawki) was located more than 10 km before the river entering the city (37.126904″N, 42.766273″E) which account as monitoring point, It is encircled by agriculture areas on either bank of the river. The second (Pira Dalal) (37.136246″N, 42.696153″E), third (Pira Shebanike) (37.145065 ″N, 42.680746″E) and fourth (Xasta Shebanike) (37.145156″N, 42.680981″E) locations are existing within the city, where civil sewage is thrown directly into the river. Far from the city center (about 5 km), the fifth station (Cham korek) (37.158812″N, 42.619264″E) were chosen, which contains a garbage dump. Finally to assess the river's capacity for self-purification in response to microbial pollutants generated by urban activities within the city, The Sixth location (Bacheka) (37.141944″N, 42.535671″E) was chosen on the Iraqi-Turkish border (about 15 km) from the city center, it surrounded by agricultural lands on both sides of the river.

ISSN 1533-9211

DOI: 10.5281/zenodo.10394577

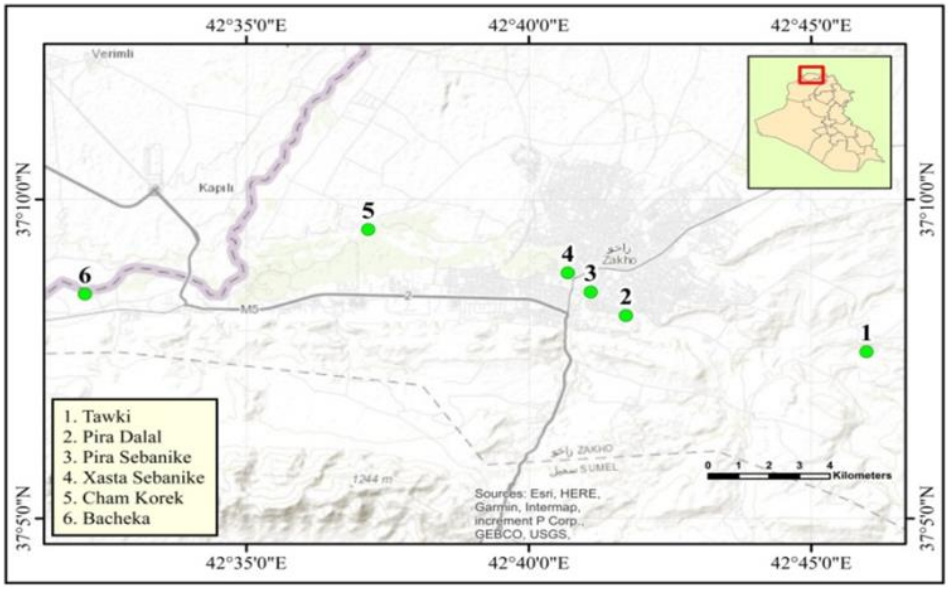

Figure 1: Studied Area

Bacterial Population Analysis

Heterotrophic plate count (HPC)

According to APHA, 2017, the enumeration of Heterotrophic Bacteria was conducted using the Serial Dilution and pour plate count; the samples were diluted serially up to 10^{-5} by mixing 1 ml of the water sample with 9 ml of sterile physiological saline solution 0.85%. Subsequently, 1 ml from each dilution was aseptically transferred onto double separate sterile Petri dishes. Then molten nutrient agar (45°C) was added to each Petri dish. The dishes were incubated at 37 C for 24-48 hrs. After incubation period the developed colony enumerated and results reported as colony forming unit cfu/ml.

$$\text{Colony forming unit cfu/ ml} = \frac{\boldsymbol{No.of\ colony\ \times dilution\ factor}}{\boldsymbol{volum\ of\ inculated\ sampl}} \quad (1)$$

Total coliform (TC)

Coliforms are Gram-negative, facultative anaerobic, non-spore-forming rods that capable to ferment lactose to acid and gas at 35-37 °C within 24- 48 hrs. For estimation of total coliform in water sample, multiple fermentation tube technique also called most probable number test, used to estimate the coliform density in a given sample. The method divided into three sequencing steps: Presumptive test (used lauryl tryptose broth), The procedure consist of 15 tubes (contain Durham tube to indicate gas production), the tubes divided into three groups, every group composed of 5 tubes, first and second groups contains single strength of lauryl tryptose broth, the third group contains double strength of the previous media, the three groups inoculated with 0.1, 1.0 and 10 ml of sample respectively. Second step called confirmed test (used brilliant green lactose broth) in this step, all positive tube (acid+gas) re-inoculated into

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

brilliant broth and incubated in the same manner, after incubation the positive tubes inoculated in the tubes of Endo agar as completed test, number of positive tubes used as code to estimate the most probable number of total coliform from special table of MPN. The total coliform reported in cell/100 ml (APHA, 2017).

Faecal coliform (FC)

For fecal coliform, EC Medium with MUG Broth was utilized to detect the presence of coliform bacteria in the water. It was a selective liquid media designed to detect coliform bacteria, especially E. coli. It contains Bile salts to inhibit both gram-positive and spore-forming bacteria while enhancing E. coli only. Then, Coliforms detected by the growth at 37°C, while Escherichia coli are noted by the growth at 44.5°C as recommended by (APHA, 2017). From all positive presumptive tests, tubes of EC medium inoculated and incubated at 44.5°C for 24 hrs. After incubation the number of positive tubes used to estimate the faecal coliform by using statistical tables, and reported as cell/100 ml.

Escherichia coli

EC medium with MUG is a selective enrichment media of *E coli* 0157, which is a strain of E. coli well known as *E. coli* O157:H7, and causes a severe intestinal infection in humans as recommended by APHA, 2017; the all positive tubes were selected to streak on Eosin Methylene Blue Agar, and incubated at 37°C for 24 hrs. E. coli's presence was detected through the observation of metallic green bacterial colonies appearing on the EMB media's surface (Sipriyadi *et al*., 2021).

Isolation and Identification of pathogenic bacteria

Two strategies used to isolate and identify pathogenic bacteria from water; first one is done by used selective media like MacConkey agar, to isolate gram negative bacteria and blood agar for isolation of gram positive bacteria in addition to gram negative bacteria and nutrient agar which is basic media. The second strategy is used specific selective and enrichment media, for staphylococcus species, Mannitol salt agar plates were streaked by 1 ml of the samples, for *Salmonella* spp. and *Shigella* spp., 1 ml of each sample was introduced into buffered peptone water (enrichment media), after incubation for 24 hrs at 37 °C, a loopful of inoculum from the previous media was streaked on Xylose Lysine Deoxycholate (XLD) Agar (Frederick et *al*., 2011).

For isolation of vibrio, 1 ml of each sample was added to alkaline peptone water (enrichment media) and incubated at 37 C for 24 hrs., after incubation a loopful of inoculum from the previous media was streaked on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar (Angela *et al*., 2011). For streptococcus spp the Bile Esculin agar used as selective media. Gram stein applied to differentiate between gram positive and gram negative bacteria as well as to detect the overall shape, size of bacterial cells and their arrangement. The morphology of developed colonies on different agar was taken into consideration like shape, size, pigmentation, and diffusion on agar. Many biochemical tests were applied for identification of bacteria species like, catalase, oxidase, IMViC tests (I for Indole, M for Methyl Red, V for Voges-Proskauer,

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

and C for Citrate), urease, Triple Sugar Iron test, motility.

Identification by VITEC 2 compact

To confirm the identified bacteria by previous tests, the VITEC 2 compact from BIOMERIEUX was used, VITEK is an automated microbiology system utilized in clinical and environmental laboratories for microbial identification (ID) and antimicrobial susceptibility testing (AST). Developed by bioMérieux, the VITEK system significantly enhances the accuracy, speed, and efficiency of microbial detection and analysis. Individual bacterial colonies grown on nutrient agar were re-suspended in 2.5 mL of sterile 0.45% saline solution in order to bring the bacterial suspension to a turbidity level equivalent to the (0.5-0.63 McFarland). After that the bacterial suspension was inoculation into the ID cassette for identification by VITEK 2 (Lucky *et al*., 2021).

Antibiotic Susceptibility

The Kirby-Bauer disk diffusion procedure and Mueller-Hinton agar was used to examine the sensitivity test of isolated bacteria, totally 21 commercial available antibiotics used in this research, for each bacterial species, certain antibiotics were chosen based on previous research, The diameter of the inhibition zone was correlated with a standardized interpretation chart, provided by the Clinical and Laboratory Standards Institute (CLSI, 2021), the interaction divided into Resistant (R), Intermediate (I) and Sensitive (S).

Multiple Antibiotic Resistance (MAR) Index

The MAR Index is a quantitative measure used to assess the level of antibiotic resistance in bacterial populations from clinical and environmental samples. This index has extensive application in microbiology to evaluate the resistance profiles of bacteria from non-clinical sources, such as water, soil, and wildlife. Calculation of the MAR Index involves determining the resistance of a bacterial isolate to a panel of antibiotics and then using this information to calculate a numerical index. The formula for calculating the MAR Index is:

$$\text{MAR Index} = (\sum_{n=1}^{n}(A/B)/C \qquad (2)$$

Where: A= total number of antibiotics to which the isolate is resistant

B= total number of tested antibiotics,

C= total number of species isolates

In this equation, A greater MAR Index signifies an elevated degree of multi-antibiotic resistance, implying that the bacterial specimen has faced and endured exposure to multiple antibiotics within its surroundings. The MAR index serves as a method for assessing health risks, where a value ≤0.2 signifies a low level of antibiotic usage, whereas a value exceeding 0.20 indicates a high level of antibiotic utilization (Salikan *et al*., 2020, Laith, and Najiah, 2013).

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

Antibiotic Efficacy

Antimicrobial efficacy refers to the ability of antimicrobial agents, such as antibiotics, antiseptics, disinfectants, or other substances, to effectively inhibit the growth or kill microorganisms, including bacteria, viruses, fungi, and protozoa. This effectiveness is typically measured by the agent's ability to prevent the proliferation of harmful microorganisms and reduce the risk of infections or diseases (Hassan *et al*., 2017)

Antibiotic efficacy calculated using the following formula: $AE = \left(\frac{S}{T}\right) \times 100$ (3)

Where, S is number of bacterial species that are sensitive to a certain antibiotic.

T is the total numbers of bacterial species were tested against the same antibiotic.

Statistical Analysis

SPSS Software was used to analyses the microbial indicator data; one-way analysis of variance (ANOVA) was applied to find out any statistically significant among bacterial indicators values in the study sites. Additionally, Pearson correlation analysis was conducted to figure out the relationship between previous indicators.

RESULTS AND DISCUSSION

Heterotrophic Plate Count (HPC)

The obtained values of HPC were ranged from (7.3-780) $\times 10^2$ cell/ml in station 1 and 3 respectively. As show in the figure 2, the annual average of HPC was higher in the stations inside the city (3,4,5) more than the stations outside the city which reflects the impact of pollution sources within the city on the condition of the river, and the highest annual average in station 3 (146.97×10^2 cell/ml) is due to its proximity to the sewer pipes that discharge urban waste containing bacteria directly into the river. According to pollution degree, the stations was ordered as follow S3>S5>S4>S6>S2>S1 as shown in figure (2). The study Adeba *et al*., 2019 on Wabe River in Ethiopia was record HPC less than the present study and it was ranged from (4.5-79) $\times 10^2$ cfu/ml. The figure (3) shows seasonal fluctuation in HPC, Autumn season record elevated of HPC, this is happened as a combination of elevated the river flow (increase river bank erosion) and comfortable temperature for bacteria metabolism and reproduction. The lowest value was recorded in summer, due to decrease the water temperature. The statistical analysis in table (1) showed that there was no significant difference between the study stations due to the presence of various sources of bacteria in the study stations, such as agricultural areas outside the city, municipal waste, and tourist uses within the city. The Pearson correlation coefficient also showed a significant difference between HPC and total coliform bacteria, as both come from surrounding sources. It is clear from Table (2) that there is a weak relationship between the HPC and both FC and *Escherichia coli* suggesting that HPCs are not a reliable indicator of fecal contamination, but sudden changes in HPC levels can signal changes in water quality (Anna *et al.,* 2020).

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

Table 1: Statistical analysis of impact of location on bacterial indicator

Parameters	Stations	Mean	Standard deviation	+ SE	Sig. (p)
HPC cfu/100ml $\times 10^2$	1	68.04[a]	96.64	27.89	NS 0.392
	2	84.78[a]	126.73	36.58	
	3	203.89[a]	293.52	84.73	
	4	99.96[a]	147.88	42.68	
	5	117.66[a]	138.81	40.07	
	6	89.28[a]	88.99	25.68	
TC cell/100mL	1	484.16[c]	381.15	110.03	<0.001
	2	469.16[c]	237.38	68.52	
	3	1490[a]	465.57	134.40	
	4	985.83[b]	642.26	185.40	
	5	879.16[b]	475.21	137.18	
	6	444.66[c]	88.64	25.58	
FC cell/100ml	1	256.58[b]	128.35	37.05	<0.001
	2	302.5[b]	200.82	57.97	
	3	1180[a]	567.54	163.83	
	4	610.83[b]	551.84	159.30	
	5	579.16[b]	498.36	143.86	
	6	266.16[b]	89.28	25.77	
E. coli cell/100ml	1	63.33[c]	47.51	13.71	<0.001
	2	92.66[bc]	84.83	24.48	
	3	231.16[a]	150.36	43.40	
	4	111.83[bc]	70.95	20.48	
	5	158.58[ab]	135.21	39.03	
	6	77.33[bc]	57.32	16.54	

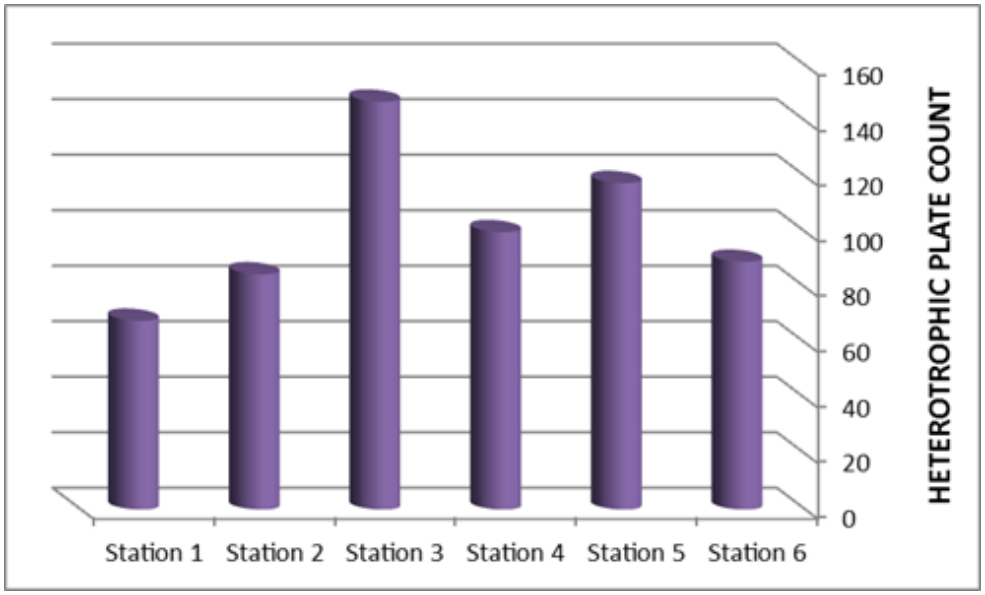

Figure 2: Annual HPC in the studied station

ISSN 1533-9211

DOI: 10.5281/zenodo.10394577

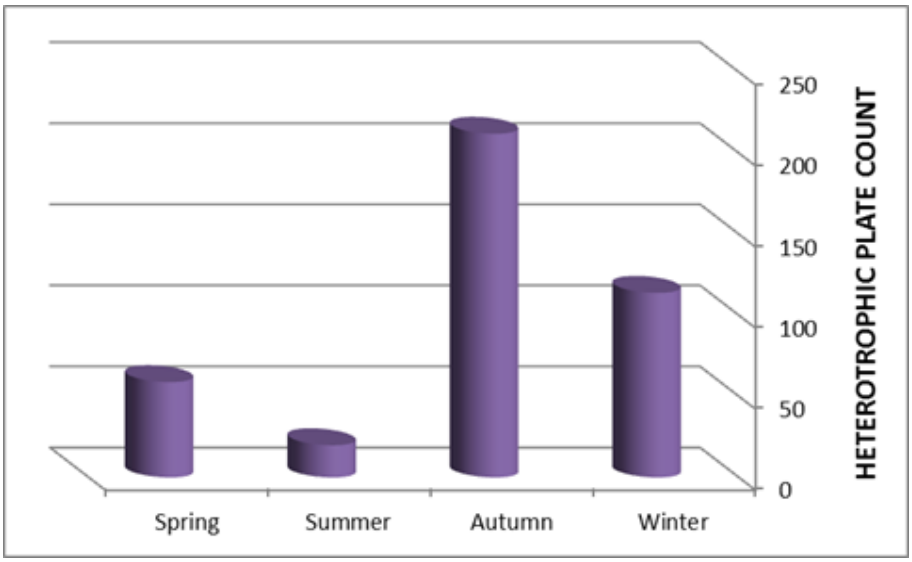

Figure 3: Seasonal HPC in the studied station

Total Coliform (TC)

The results of total coliform bacteria were positive in all study samples. The recorded values ranged from 170 cells/100 ml in station 1 in June and 3200 cells/100 ml in station 3 during September. In the study of Hawraa and Mrooj, 2018 on Hilla River they were recorded values of TC more than the present study, it was ranged between (3.02-7.74) $\times 10^3$ cfu/100ml. In the same manner, Figure (4) shows that the stations inside the city were recorded annual rates higher than the stations outside the city. This is due to the diversity of sources that discharge coliform bacteria into the city, including urban waste, sewage discharge and storm water. The highest rate was recorded at station 3 due to discharge domestic waste directly into the river. This was confirmed by statistical analysis, which found a significant difference between the study sites. This is corresponding to study of Wisam and Wasan, 2018 which revealed that the Basrah Center station had the highest percentage of TC compared to other stations. The order of the stations according to the degree of pollution was: S3 > S4>S5 > S1 > S2 > S6. The study also found that total coliform bacteria levels were highest during the spring and autumn seasons as observed in figure (5). This is likely due to the fact that the water level is higher during these seasons, which can lead to the erosion of riverbanks and the introduction of bacteria into the water, the suitable temperature in these seasons also affects the increase of bacterial reproduction, similar results was recorded by Salman *et al.*, 2013 Al- Hilla River, Iraq. While In Winter the low temperatures reduce bacterial activity and reproduction. In summer, the decrease in the number of bacteria is because of the high temperature. The Pearson correlation coefficient in Table (2) showed a significant relationship between HPC and TC at the $P \leq 0.05$ level. This proves that both groups may come from environmental sources in addition to human activities.

ISSN 1533-9211

DOI: 10.5281/zenodo.10394577

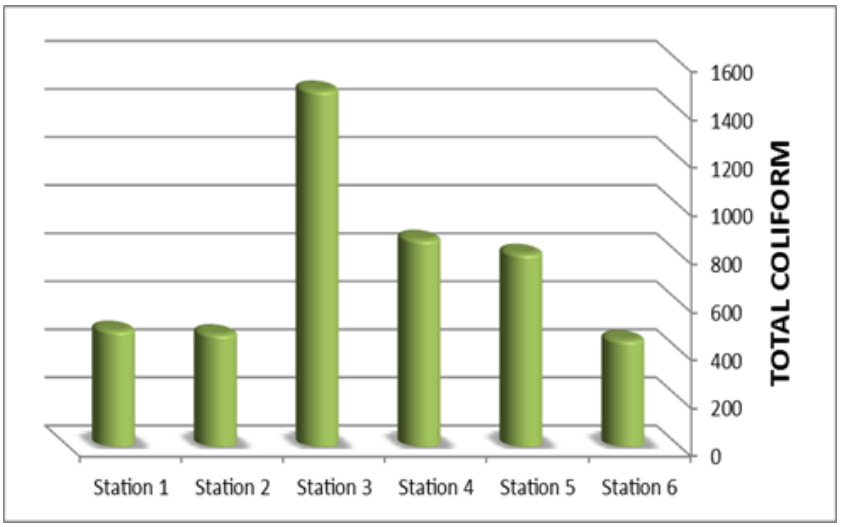

Figure 4: Annual TC in the studied station

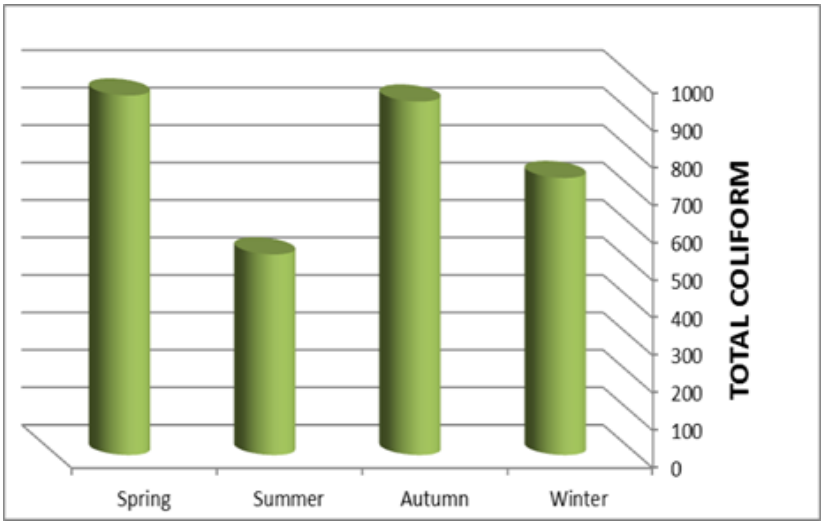

Figure 5: Seasonal TC in the studied station

Faecal Coliform

The recorded values of fecal coliform bacteria in the study sites ranged from the highest value recorded in the third site (1840 cell/ 100 ml) in October to the lowest value recorded in the first station (79 cell/ 100 ml) in June. The results of this study in figure (6) showed that fecal coliform bacteria levels were highest at the third site, which is located near municipal pollution sources, that add huge amounts of wastewater from nearby homes and businesses, which carry huge numbers of pathogenic bacteria into the river,. This was confirmed by the statistical analysis, which showed significant differences between the study stations. The stations are ranked from most polluted to least polluted as follows: S3>S4>S5>S2>S6>S1. The study also found that FC levels were higher in the summer and spring months than in the winter and fall

ISSN 1533-9211

DOI: 10.5281/zenodo.10394577

months as show in Figure (7), this is corresponding to study of Salman *et al*., 2013 Al- Hilla River, Iraq. This is likely due to the appropriate temperature for bacterial reproduction and the rise in water levels, which increase human activities on the river. The decrease in temperature in winter, which affects the metabolic activity of bacteria, as well as the lack of contact between the local population and the river, led to the recording of low rates of FC during the season, and summer recorded a low rate of fecal bacteria due to the decline in water levels to critical levels that reduced people's contact with the river. The Pearson correlation coefficient in table (2) showed a significant relationship between TC and FC at a significant level ≤ 0.01, because fecal bacteria are a subset of the TC group. Finally, and this is concern, due to the potential co-occurrence of pathogens (Crofts *et al*., 2017).

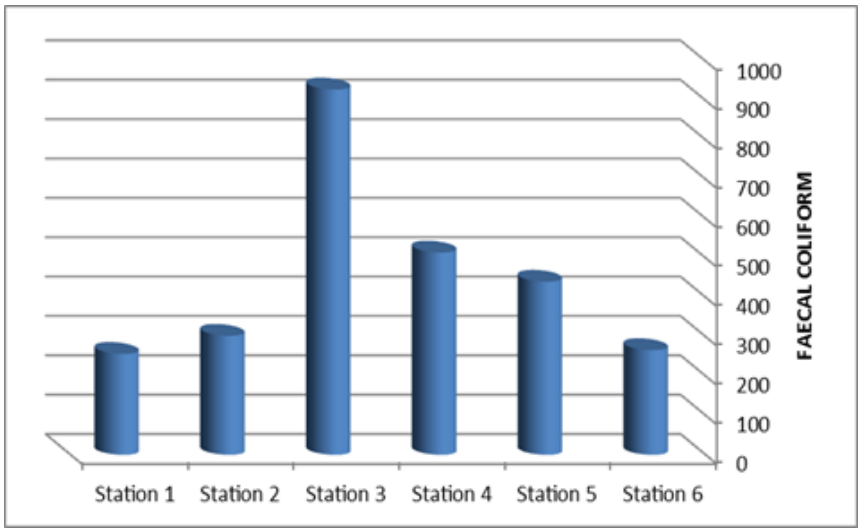

Figure 6: Annual FC in the studied station

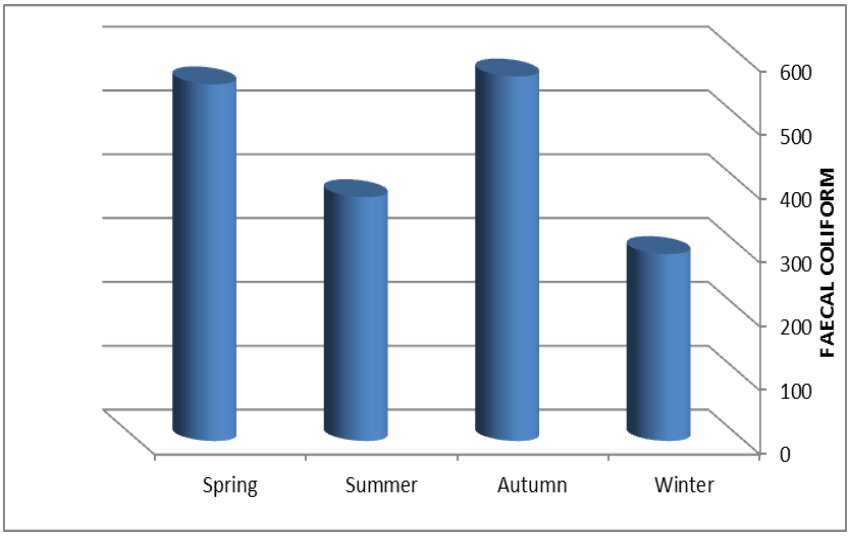

Figure 7: Seasonal FC in the studied station

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

Escherichia coli

Positive results of *E. coli* bacteria were detected in all samples, indicating continuous fecal contamination of the Khabur River in Zakho city. Consequently, the direct use of river water for drinking without proper treatment poses a significant risk. This is because *E. coli* bacteria are always accompanied by the presence of pathogenic bacteria (Rochelle-Newall *et al.*, 2015). The highest value of *E. coli* (540 cell/100ml) was recorded in the station 3 in August, potentially caused by its position at the Zakho's center. The lowest *E. coli* value (12 cell/100ml) was recorded in the station 6 in April, possibly due to dilution resulting from increased water levels in the river. Stations three and five recorded higher annual rates compared to other stations as observed in figure (8), which may be attributed to the proximity of station three to sewage pipes and the presence of a waste dump near station five. The order of stations based on the pollution level was as follows: (S3 > S5 > S4 > S2 > S6 > S1). The figure (9) shows that *E. coli* bacteria levels were highest during the warmer months of spring, summer, and fall. This is likely because warmer temperatures and increased water usage for domestic and recreational purposes create more favorable conditions for the bacteria to thrive., similar findings was observed in the study of Hussein *et al*, 2019 on Al-Diwaniyah River, Iraq. While the counts significantly decreased during the cold winter season, which affects the survival of bacteria in the river. Moreover, the reduced use of water during the winter and, consequently, the decreased influx of bacteria into the river confirm that using the Khabur River for drinking requires treatment during the warmer months more than in the winter season. Pearson correlation in table (2) revealed a significant relationship between *E. coli* and both Total Coliform (TC) and Fecal Coliform (FC) at significance levels of 0.05 and 0.01, respectively, affirming that *E. coli* is an essential component of FC.

The WHO, 2006 states these categories:

- Absence of E coli per/100 ml of water is to be safe to drink.
- A count ranging from 1 to 10 MPN/100 ml is assessed as low risk
- Ranging from 11–100 MPN/100 ml were categorized as low risk.
- Enumeration more than 100 MPN/100 ml is assessed hazardous.

In the present study: 48.62% (n=35) of samples was categorized as low risk and 51.38% (n=37) of samples were assessed as hazardous.

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

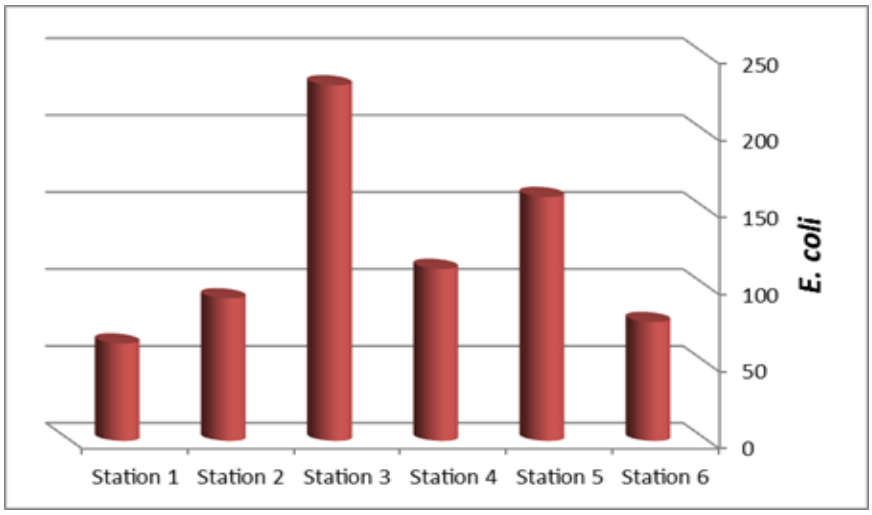

Figure 8: Annual *E. coil* in the studied station

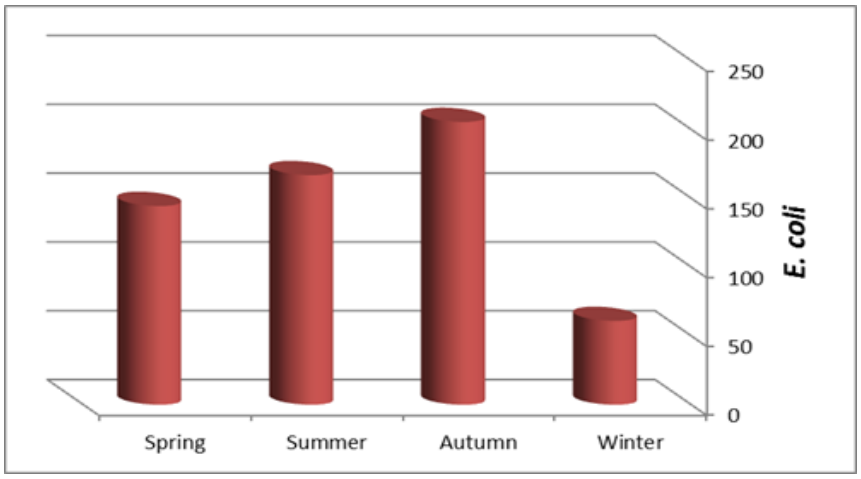

Figure 9: Seasonal *E. coli* in the studied station

Table 2: Pearson Correlation among microbial indicators

	HPC.	TC.	FC.	*E coli*
HPC.		0.284*	0.176	0.114
TC.			0.833**	0.272*
FC.				0.374**
** Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.				

Isolation and Identification

By using basic, enrichment, and selective media, 129 bacterial isolates were obtained from the six study sites. Among these, 18.6% (n=24) of the isolates were Gram-positive, represented by four genera. Gram-negative bacteria made up the majority of isolates, with 81.4% (n=105) isolates belonging to 13 different genera, listed in Table (3). The isolated bacteria also can be

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

classified into two groups based on where there source: Autochthonous and Allochthonous. Autochthonous bacteria are defined as bacterial communities that are indigenous and intrinsic to the ecological system of a river. In contrast, allochthonous bacteria are introduced into the river ecology from external sources, originating from terrestrial or human-made environments, and subsequently transmitted into the river ecosystem. In the current study 41.86% (n=54) of isolates were autochthonous bacteria belonging to six species mentioned in table 3. In contrast, 58.14% (n=75) of isolates were allochthonous bacteria belonging to 11 species mentioned in the previous table.

Table 3: Isolated bacteria, Auto= Autochthonus, Allo= Allochthonus, S=Station

	BACTRIA	**Auto**	**Allo**	**S 1**	**S 2**	**S 3**	**S 4**	**S 5**	**S 6**	**TOTAL**
1	*Aeromonas sobria*	+		1		4	3		1	9
2	*Aeromonas veronii*	+		1	1	2	2	1	2	9
3	*Aeromonas hydrophila/puncata caviae*	+			2	1	2	2	1	8
4	*Aeromonas hydrophila*	+		2					1	3
5	*Proteus mirabilis*		+	2	1	2	1	3	1	10
6	*Proteus vulgaris*		+			1		1		2
7	*Proteus peneri*		+				1			1
8	*Citrobacter freundii*		+	1	2	2		2	1	8
9	*Serratia marcescens*		+				2	1		3
10	*Serratia liquefaciens group*		+				1			1
11	*Alcaligenes faecalis ssp faecalis*		+				1			1
12	*Raoultella planticola*		+			1		1		2
13	*Klebsiella pneumoniae*		+	2	2	4	5	2	1	16
14	*Klebsiella oxytoca*		+				1	1		2
15	*Pseudomonas aeruginosa*		+	1	1	2	3	1	2	10
16	*Pseudomonas putida*		+		1			1		2
17	*Escherichia coli*		+	2	1	2	3	2	2	12
18	*Salmonella typhi*		+			1		1		2
19	*Salmonella paratyphi*		+			1				1
20	*Shigella boydii*		+		1					1
21	*Pantoea spp.*		+					1		1
22	*Comamonas testosteroni*	+					1			1
23	*Enterococcus faecalis*	+					1	1		2
24	*Enterococcus gallinarum*	+				1				1
25	*Kocuria kristinae*	+			1		1			2
26	*Streptococcus pneumoniae*	+						1	1	2
27	*Streptococcus pseudoporcinus*	+			1		1			2
28	*Streptococcus agalactiae*	+						1		1
29	*Staphylococcus aureus*	+			1	2	3	1	1	8
30	*staphylococcus vitulinus*	+						1		1
31	*staphylococcus lentus*	+		1				1		2
32	*staphylococcus simulans*	+			1				1	2
33	*staphylococcus sciuri*	+							1	1
Isolation for each station				13	16	26	32	26	16	**129**

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

MAR (Multiple Antibiotic Resistance) Index

The MAR Index is a quantitative measure ranging from 0 to 1, where higher values signify a higher level of antibiotic resistance (Krumperman, 1983). After calculating the MAR index of the 129 bacterium isolates, it was discovered that 66.66 % (n=86) of isolates had a MAR index value more than 0.2. That is meaning there is a high probability of contamination in areas where antibiotics are commonly used (Rotchell and Paul, 2016). In the case of study the sources of antibiotic in river may be from wastewater discharge and agricultural runoff (Berendonk *et al.*, 2015). It appear form table (4) That *Streptococcus agalactiae, Salmonella typhi, Proteus penneri, and Pseudomonas aeruginosa* had the highest MAR index values, measuring 0.63, 0.62, 0.58, and 0.5, respectively. All of these bacteria are recognized as pathogenic.The study also found that all isolates of *Aeromonas* spp had high MAR rates (ranging between 0.44-0.58). This is a particularly concerning finding because *Aeromonas* spp is a common bacterium that is found in water and is also common waterborne bacteria. In the study of El-Hossary *et al*, 2023, they found that the (MAR) index of Aeromonas spp. Isolated from fish in Egypt was ranged between 0.142–0.642 with 64.2% of the isolates having MAR values equal to 0.642. The study of Salikan, *et al*, 2020 discovered a correlation between the amount of antibiotic use in the surrounding areas and the MAR index of bacteria from the rivers.

Table 4: MAR index of isolated bacteria

Bacteria	No. of Isolates	MAR Index	Bacteria	No. of Isolates	MAR Index
Aeromonas sobria	9	0.5	*Escherichia coli*	12	0.17
Aeromonas veronii	9	0.58	*Salmonella typhi*	2	0.62
Aeromonas hydrophila /puncata/caviae	8	0.51	*Salmonella paratyphi*	1	0.41
Aeromonas hydrophila	3	0.44	*Shigella boydii*	1	0.37
Proteus mirabilis	10	0.24	*Enterococcus faecalis*	2	0.25
Proteus vulgaris	2	0.12	*Enterococcus gallinarum*	1	0.3
Proteus peneri	1	0.58	*Pantoea spp.*	1	0.08
Citrobacter freundii	8	0.41	*Alcaligenes faecalis ssp faecalis*	1	0.35
Serratia marcescens	3	0.33	*Streptococcus pneumoniae*	2	0
Serratia liquefaciens group	1	0.08	*Streptococcus pseudoporcinus*	2	0.33
Kocuria kristinae	2	0.39	*Streptococcus agalactiae*	1	0.63
Raoultella planticola	2	0.16	*Staphylococcus aureus*	8	0.33
Comamonas testosteroni	1	0.16	*staphylococcus vitulinus*	1	0.16
Klebsiella pneumoniae	16	0.35	*staphylococcus lentus*	2	0.04
Klebsiella oxytoca	2	0.33	*staphylococcus simulans*	2	0.41
Pseudomonas aeruginosa	10	0.5	*staphylococcus sciuri*	1	0
Pseudomonas putida	2	0.16			

Antibiotics Efficacy

Rivers play an essential role in ecosystems by acting as channels for the movement of many different materials, such as bacteria and antibiotics. The release of medicines and antibiotic residues into rivers via wastewater discharge, agricultural runoff, or other forms of pollution fosters the growth of antibiotic resistance. Bacterial populations residing in such habitats may

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

encounter sub-lethal levels of antibiotics, so creating a selective force that promotes the survival and spread of antibiotic-resistant strains (Pruden *et al.*, 2013). The efficacy of used antibiotics in this study showed that the Amikacin, ciprofloxacin, Imipenem, Levofloxacin and gentamycin has high effectiveness among used antibiotics in this study, and their efficacy were 98.41%, 97.27%, 93.84%, 91.66% and 90.9% respectively. The efficacy of other antibiotics showed in table (5).

Table 5: Antibiotic Efficacy, T is the total No.of bacterial species were tested against a certain antibiotic. S is No. of bacterial species that are sensitive to the same antibiotic

	ANTIBIOTICS	T	S	EFFICACY
1	Amikacin (AK-10)	126	124	98.41
2	Ciprofloxacin (CIP-10)	110	107	97.27
3	Imipenem (IPM-10)	65	61	93.84
4	Levofloxacin (LEV-5)	24	22	91.66
5	Gentamicin (CN-10)	121	110	90.9
6	Nitrofurantoin (F-100)	25	22	88
7	Tobramycin (TOB-10)	59	51	86.44
8	Ceftazidime (CAZ -30)	101	87	86.13
9	Cefepime (FEP -10)	47	38	80.85
10	Meropenem (MEM-10)	75	56	74.66
11	Trimethoprim (SXT-25)	91	66	72.52
12	Piperacillin (PRL-100)	50	34	68
13	Cefixime (CFM-5)	115	69	60
14	Cefotaxime (CTX-10)	66	39	59.09
15	Azithromycin (AZM-15)	39	21	53.84
16	Clindamycin (DA-10)	53	22	41.5
17	Vancomycin (VA-30)	56	22	39.28
18	Ampicillin (AM-25)	128	34	26.56
19	Erythromycin (E-10)	82	19	23.17
20	Penicillin (P-10)	74	15	20.27
21	Oxacillin (OX-10)	65	13	20

CONCLUSIONS

1. Khabur River experiences many fluctuations during the year due to climate change, the temporal and spatial distribution of rainfall, and the reduction in the amount of flowing water due to dams built in neighboring countries.
2. The Khabur River faces continuous pollution by pathogenic bacteria throughout the year, so causing a potential health hazard to individuals if the water is utilized without appropriate treatment.
3. The presence of pathogenic bacteria in the river is attributed to multiple sources, primarily due to human activities. To lower their risks, serious steps must be taken like treating wastewater before it is released and avoiding overusing of antibiotics in clinic and in agriculture.

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

4. Bacterial indicators of pollution exhibit elevated values during warm seasons in comparison to cold ones. This indicates that the river needs further treatment during the warmer months before using it for various purposes.
5. The proliferation of fecal pollution bacteria during warm seasons can be attributed to the favorable temperature conditions that promote bacterial growth and development.
6. Some isolated bacteria have acquired antibiotic resistance due to pollution and the misuse of antibiotics in clinics and in agriculture, which are carried away into the river and provide an opportunity for bacteria to acquire resistance
7. The efficacy of the antibiotics used against the isolated bacteria varied, some of which, such as Amikacin, ciprofloxacin, Imipenem, Levofloxacin and gentamycin, have high efficacy among the antibiotics used in this study.

References

1) Adeba T, Fedesakebede T, Malaiyarasa P (2019) Assessments Of The Total Heterotrophic Bacterial Population Density From Wabe River, South-Central Ethiopia. IJIPSR 7:1-13. https://doi.org/10.21276/IJIPSR.2019.07.01.765

2) Adesakin T, Oyewale A, Bayero U, et al. (2020) Assessment of bacteriological quality and physico- chemical parameters of domestic water sources in Samaru community, Zaria, Northwest Nigeria. *Heliyon* 6: 1-13. https://doi.org/10.1016/j.heliyon.2020.e04773

3) Al-Muqdadi, S, Omer, M, Abo R, et al. (2016) Dispute over Water Resource Management—Iraq and Turkey. Journal of Environmental Protection 7: 1096-1103. https://doi.org/10.4236/jep.2016.78098

4) Al-Qassas, M (1999) Draught: Deterioration of land in arid areas. J. Alim Al-Ma'arifa 242: 67–74. (In Arabic) https://www.neelwafurat.com/itempage.aspx?id=lbb385237-382388&search=books

5) American Public Health Association APHA (2017) Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 23rd Eds. Editors: E.W. Rice, R.B. Baird, A.D. Eaton. Washington DC. USA. https://www.amazon.in/Standard-Methods-Examination-Water%20-Wastewater/dp/087553287X

6) Angela D , Valentina T, Lucia N, et al. (2011) Comparison between thiosulphate-citrate-bile salt sucrose (TCBS) agar and CHROM agar Vibrio for isolating Vibrio parahaemolyticus. Food Control 22: 124-127. https://doi.org/10.1016/j.foodcont.2010.06.013

7) Anjali M. and Sharma H (2022) Microbs for Humanity and its Applications. Chapter 7: E. coli as an Indicator of Organic Pollution in Water: A Review: Daya Puplshing House. Astral Internatinal Pvt. Ltd. New Delhi. India: 147-166. https://www.researchgate.net/publication/359578762

8) Anna R, Joanna B and Dorota K (2020) Heterotrophic Plate Count for Bottled Water Safety Management, Mdpi journal, Processes 739: 1-10. https://doi.org/10.3390/pr8060739

9) Berendonk T, Manaia C, Merlin C, et al. (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13: 310-317. https://doi.org/10.1038/nrmicro3439

10) Bireshwar B. (2022) Total Coliform and Fecal Coliform Bacterial Estimation Assessing Water Quality of Lake Saheb Bandh. International Journal of Aquatic Science. 13: 77-90. https://www.journal-aquaticscience.com/article_147370.html

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

11) Clinical and Laboratory Standards Institute (CLSI) (2021) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100, 31st ed. 41. https://clsi.org/media/z2uhcbmv/m100ed31_sample.pdf

12) Crofts T, Gasparrini, A & Dantas G. (2017) Next-generation approaches to understand and combat the antibiotic resistome. Nat Rev Microbiol 15: 422-434. https://doi.org/10.1038/nrmicro.2017.28

13) El-Hossary D, Mahdy A, Elariny E, et al. (2023) Antibiotic Resistance, Virulence Gene Detection, and Biofilm Formation in Aeromonas spp. Isolated from Fish and Humans in Egypt. Biology 421:1-14. https://doi.org/10.3390/biology12030421

14) Frederick A, Nurul H and Rusul G (2011). Comparison of Media for the Isolation of Salmonella (XLD and Rambach) and Listeria species (ALOA and Palcam) in Naturally Contaminated Duck Samples. Internet Journal of Food Safety 13: 20-25. https://www.researchgate.net/publication/281022324

15) Gulistan A, Mustafa I and Musher R (2023) A Study On The Contamination of Khabur River With Heavy Metals Due To Spatial And Seasonal Discharged Wastewater In The Iraqi Kurdistan Region. Science Journal of University of Zakho, 11: 298–305. https://doi.org/10.25271/sjuoz.2023.11.2.1060

16) Hassan R, Eldegla H, Elmorsy F, et al. (2017) Egyptian pediatric association Gazette clinical and microbiological characteristics of healthcare-associated infections in a tertiary care pediatric hospital. Egyptian Pediatric Association Gazette 65: 127–131. https://doi.org/10.1016/j.epag.2017.09.001

17) Hawraa M and Mrooj S (2018) Evaluating The Water of The Hilla River Through The Study of Physical and Chemical Properties and Microbial Content. JUBPAS 26: 18–31. https://www.journalofbabylon.com/index.php/JUBPAS/article/view/1369

18) Hussein K, Al-Bayati S and Al-Bakri S (2019) Assessing Water Quality for Al-Diwaniyah River, Iraq Using GIS Technique. Engineering and Technology Journal 37: 256-264. https://dx.doi.org/10.30684/etj.37.7A.6

19) José L, Elkyn R and Mario P (2021) A systematic review of microorganisms as indicators of recreational water quality in natural and drinking water systems. Journal of Water and Health. 19: 20–28. https://doi.org/10.2166/wh.2020.179

20) Krumperman P (1983) Multiple Antibiotic Resistance Indexing of Escherichia coli to Identify High-Risk Sources of Fecal Contamination of Foods. Applied and Enivironmental Microbiology 46: 165-170. https://doi.org/10.1128/aem.46.1.165-170.1983

21) Laith, A and Najiah M (2013) Aeromonas hydrophila: antimicrobial susceptibility and histopathology of isolates from diseased catfish, Clarias gariepinus (Burchell). J Aquac Res Development 5: 1-7. https://doi.org/10.4172/2155-9546.1000215

22) Lucky H, Enty T, Hans P, et al. (2021) Performance of TDR-300B and VITEKVR 2 for the identification of Pseudomonas aeruginosa in comparison with VITEKVR-MS. Journal of International Medical Research 49: 1–12. https://doi.org/10.1177/0300060521989893

23) Mmuoegbulam A, Unimke A, Andy I, et al. (2019) Effect of variations in heterotrophic plate count methods on bacterial estimation in some sachet water in Calabar Metropolis. World Journal of Advanced Research and Reviews 1: 18–27. https://doi.org/10.30574/wjarr.2019.1.2.0019

24) Niyoyitungiye L, Giri A and Ndayisenga M (2020) Assessment of Coliforms Bacteria Contamination in Lake Tanganyika as Bioindicators of Recreational and Drinking Water Quality. South Asian Journal of Research in Microbiology 6: 9-16. https://doi.org/10.9734/sajrm/2020/v6i330150

25) Pruden, A, Larsson D, Amézquita A, et al. (2013) Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment. Environ Health Perspect 12: 878-885. http://dx.doi.org/10.1289/ehp.1206446

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10394577

26) Rochelle-Newall E, Nguyen T, Le T, et al. (2015) A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. Front. Microbiol 6: 1-15. https://doi.org/10.3389/fmicb.2015.00308

27) Salikan N, Yern K and Zain, N (2020) Isolation of Antibiotic Resistant Bacteria from Rivers in Terengganu, Malaysia. International Journal of Life Sciences and Biotechnology 3: 241- 257. https://doi.org/10.38001/ijlsb.711948

28) Salman J, Al-Azawey A, Hassan F (2013) Study of Bacterial Indicators in Water and Sediments from Al-Hilla River, Iraq. Hydrol Current Res 13:1-5. https://dx.doi.org/10.4172/2157-7587.S13-001.

29) Savichtcheva O and Okabe S (2006) Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application. Water Research 40: 2463-2476. https://doi.org/10.1016/j.watres.2006.04.040

30) Shafi S, Kamili A, Shah M, et al. (2013) Coliform bacterial estimation: A tool for assessing water quality of Manasbal Lake of Kashmir, Himalaya. Afr. J. Microbiol Res. 7: 3996-4000. https://doi.org/10.5897/AJMR2013.5863

31) Shields R, Clancy C, Press E, et al. (2016) Aminoglycosides for treatment of bacteremia due to carbapenem-resistant Klebsiella pneumoniae. Antimicrob Agents Chemother 60:3187–3192. https://doi.org/10.1128/AAC.02638-15

32) Trishala K, Parmar D and Agrawal Y (2016) Bioindicators: the natural indicator of environmental pollution. Frontiers in Life Science 9: 110-118. https://doi.org/10.1080/21553769.2016.1162753

33) Wisam A and Wasan A. (2018) Coliform bacteria as a tool for assessing water quality of Shatt Al-Arab River in southern Iraq. International Journal of Technical & Scientific Research Engineering 1: 26-31. https://www.ijtsre.org/papers/2018/ev1c1/B.pdf

34) World Health Organization WHO (2021) Progress on household drinking water, sanitation and hygiene 2000-2020: five years into the SDGs: 1-164. https://www.who.int/publications/i/item/9789240030848

35) World Health Organization WHO (2006) Guidelines for Drinking-Water Quality, 3 Eds, vol.1. WHO. Geneva, Switzerland. https://www.unisdr.org/files/2034_VL206905.pdf