MICROBIOLOGICAL ASSESSMENT OF SEAWATER QUALITY AT MISURATA BEACHES, LIBYA

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ABSTRACT

Bacterial contamination in marine waters is a significant environmental and public health concern, as it can have adverse effects on human health and marine ecosystems. This type of pollution often originates from sources such as untreated wastewater, leading to elevated levels of harmful bacteria in the water and increasing the risk of infections and disease transmission. This study aimed to assess the levels of bacterial pollution in the seawater of Misurata City's beaches, Libya, during 2021 by collecting samples from three coastal sites (Tuba, Jannat, and Yeader) during the months of April and June. The results revealed varying degrees of bacterial contamination at Jannat and Yeader, with Jannat showing the highest level of pollution with a statistically significant difference. Moreover, bacterial contamination was notably higher in June compared to April. Analysis showed that 56.11% of the samples were contaminated with Escherichia coli, 66.66% with Total Coliform bacteria, and 57.22% with Staphylococcus aureus. Additionally, members of the Enterobacteriaceae family were detected, including Klebsiella spp. at 18.3%, Pseudomonas spp. at 9.4%, while Proteus spp. and Salmonella spp. were the least prevalent at 5.5% each. No bacterial contamination was detected at the Tuba site, likely due to its distance from pollution sources. The study concluded that the discharge of untreated wastewater is a primary source of contamination and recommends effective wastewater treatment prior to discharge into the marine environment to mitigate health risks and environmental impacts.

KEY WORD: Seawater Pollution, Bacterial Contamination, Escherichia coli, Total Coliform, Staphylococcus aureus, Wastewater, Coastal Water Quality**.**

INTRODUCTION

Bacterial pollution in seawater is one of Coastal water quality is a key environmental and public health concern worldwide, particularly in developing countries where wastewater management is limited. Bacterial contamination in seawater is one of the serious environmental impacts that threaten human health and marine organisms, thereby reducing the benefits that coastal environments provide to society. Although surface waters polluted by sewage pose a threat to water and food security, as well as to human health and the ecosystem, pollution in the coastal environment is causing increasing concern. Assessing the sources and levels of pollution is a fundamental requirement for managing it safely and sustainably [1]. Marine environments are increasingly affected by human activities, such as the discharge of sewage into marine ecosystems. Fecal pollution that enters the sea through such activities poses risks of spreading microbial diseases and promoting the dissemination of antibiotic-resistant bacteria[2]. Wastewater is a complex mixture of domestic and industrial sewage and surface runoff, containing organic matter, nutrients, and a wide range of microorganisms, including pathogens [3]. water contaminated with fecal pollutants can pose a significant risk to human health, as a number of intestinal pathogens are often associated with feces in cases of direct contact with water[4]. Pathogenic bacteria may be transmitted to humans through contact with or ingestion of contaminated water [5].

According to the World Health Organization (WHO), waterborne diseases such as cholera, typhoid, and hepatitis are responsible for approximately 2 million deaths annually in rural areas of third-world countries, the majority of which occur among children under the age of five[6]. Accordingly, biological, physical, and chemical treatment to improve the sanitary quality of wastewater is critically important in these regions. While water has a natural ability to neutralize pollution, once contamination becomes excessive, water loses its capacity for self-purification[7].

The beaches of Misrata city were selected due to their vital role as an urban and industrial center along the Libyan coast, where there are potential sources of wastewater pollution that impact the marine environment and public health. The region also suffers from a notable lack of previous studies related to the assessment of bacterial contamination, particularly on Misrata’s beaches, highlighting the importance of this study. This research was distinguished by the use of selected bacterial indicators to analyze bacterial pollution through a seasonal assessment (April and June), providing up-to-date data that contribute to understanding and evaluating water quality and supporting effective environmental and public health decision-making. Therefore, this study aimed to assess bacterial pollution in seawater from Misurata beaches (Libya), using selected bacterial indicators to evaluate potential wastewater contamination.

Study Area:

Misurata is located in the northwest of Libya, approximately **208 km** east of the capital, Tripoli. It is bordered to the west by the city of Zliten, to the east by Sirte, to the south by Bani Walid, and to the north by the Mediterranean Sea. Misurata is the third-largest city in Libya and lies at a latitude of **32.22°N** and a longitude of **15.06°E**, as shown in Figure (1).

MATERIALS AND METHODS:

A field survey was conducted to monitor the outfalls through which wastewater is discharged into the sea in Misurata City, and their locations were determined using a GPS Etrex h device, manufactured by GARMIN (USA).

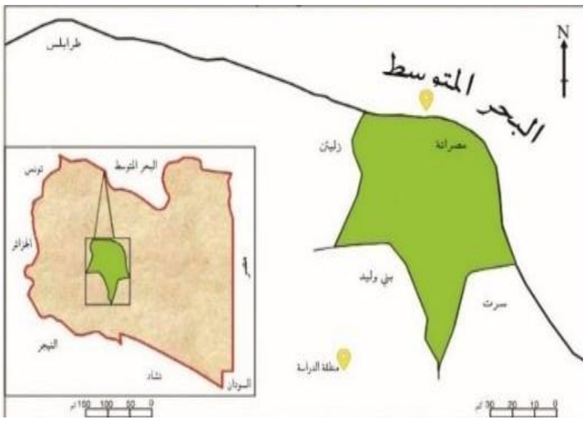

Figure 1: Location of Misurata City.

There are three wastewater outfalls in the city, located approximately 4500 meters away from the city center.

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Received: 20 August 2025 Accepted: 25 September 2025 Published: 15 October 2025

Figure (2) shows an aerial image of the study locations in Misurata City.

Wastewater Outfalls: The city has three wastewater outfalls to the sea:

1-Jannat Outfall: Located at latitude (32.410452N) and longitude (15.083116E).

2-Yeader Outfall: Located at latitude (32.402528N) and longitude (15.127919E).

3-Tuba Outfall: Located at latitude (N32.411283) and longitude (15.046390E).

Figure 2: Designated Locations for Water Discharge Outlets to the Sea in Misrata City.

Sample Collection:

In this study, a total of 180 seawater samples were collected from three locations along the beaches of Misrata city in two phases: the first phase in April, and the second phase in June, with 30 samples collected in each month from each of the three locations, as shown in Table (2). The samples were distributed as follows: 10 samples from the North of the outlet, 10 samples from the East of the outlet, and 10 samples from the West of the outlet. The distance between each sample and the next was approximately 25 meters, as illustrated in Figure (3).

Figure 3: Sample Collection Locations from Seawater in the Study Area

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The water samples were collected at 10:00 AM, and the seawater sampling was conducted as follows:

1-Samples were taken using deep pipettes from a depth of 30 cm below the water surface.

2-The samples were placed in sterile, tightly sealed plastic bottles with a capacity of 100 ml.

3-Approximately 10 ml of space was left empty in each bottle to allow for proper mixing, homogenization, and shaking of the sample before testing.

4-Each plastic bottle was labeled with all required information, including: sample number, day, date, and time of collection.

5-The samples were stored in an ice-filled cooler and transported to the laboratory for microbiological analysis.

Culture Media Used:

SS Agar, Blood Agar, MacConkey Agar, Baird Parker Agar, and Tryptone Bile Glucuronic Agar. The MPN method was also used for detecting the coliform group.

Diagnostic testes:

Gram Stain, API 20 E, Indole Test, Oxidase Test.

Statistical Analysis:

The laboratory results obtained were statistically analyzed using One-Way Analysis of Variance (One-Way ANOVA). In addition, Two-Way Analysis of Variance (Two-Way ANOVA) was used to determine whether there were statistically significant differences in the seawater sample results at a P-value < 0.05. Additionally, a One-Sample Test was used to compare the study results with standards set by the World Health Organization (WHO), the European Union (EU), and the United States Environmental Protection Agency (USEPA). The mentioned statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software.

RESULTS AND DISCUSSION:

In this study, several types of bacteria were detected as indicators of seawater contamination with wastewater. These included total coliform bacteria (Total Coliform), Escherichia coli (E. coli), as well as Gram-positive bacteria, such as Staphylococcus aureus. Additionally, some enteric bacteria (Enterobacteriaceae) were identified, which included a group of Gram-negative bacteria, namely Pseudomonas spp., Klebsiella spp., Proteus spp., and Salmonella spp., to assess the extent of bacterial contamination in the seawater at the beaches of Misrata city, Libya.

Comparison of E. coli, Staphylococcus aureus, and Total Coliform Bacteria Levels between April and June in the Jannat Area:

The comparison was conducted using One-Way Analysis of Variance (One-Way ANOVA) to study the relationship between bacterial levels during the months of April and June in the Jannat area, as shown in Table (1).

Table 1: Comparison of Bacterial Levels between April and June in the Jannat Area.

Bacteria	Month	Number	Mean	Std. Deviation	P-value
***Total Coliform* MPN**	June	30	826.97	466.8	0.867
	April	30	806.8	461.84	
***Staph. aureus* CFU/ml**	June	30	193.0	191.18	0.018
	April	30	100.66	80.9825	
***E. coli* CFU/ml**	June	30	113.233	440.24	0.088
	April	30	63.40	70.759	

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The study results, as shown in Table (1), revealed that the p-values for *E. coli* and *Total Coliform* were greater than 0.05, indicating no statistically significant differences between April and June in the Jannat area.
However, for *Staphylococcus aureus*, the p-value was less than 0.05, indicating a statistically significant difference between the two months. June was found to be more contaminated, with a mean count of 193 CFU/ml and a standard deviation of ±191.18 in the Jannat area.

Comparison of E.coli, Staphylococcus aureus, and Total Coliform Levels Between April and June in the Yeader Area:

The comparison was conducted using One-Way Analysis of Variance (One-Way ANOVA) to study the relationship between bacterial levels during the months of April and June in the Yeader area, as shown in Table (2).

Table (2) Comparison of Bacterial Levels Between April and June in the Yeader Area

Bacteria	Month	No.	Mean	Std. Deviation	P-value
***Total Coliform* MPN**	June	30	738.92	519.413	< 0.05
	April	30	10.54	11.856	
***Staph. aureus* CFU/ml**	June	30	74.66	96.444	0.015
	April	30	28.33	31.95	
***E. coli* CFU/ml**	June	30	47.36	60.94	< 0.05
	April	30	0.8	1.03	

The results of the study, as shown in Table (2), indicated that the p-value was less than 0.05, which suggests that there were statistically significant differences between April and June in the levels of *E. coli*, *Total Coliforms*, and *Staphylococcus aureus* in the Yeader area. June was found to be more contaminated with bacteria.

Comparison of the levels of E. coli, Staphylococcus aureus, and Total Coliform between the study months and locations.

The comparison was conducted using Two-Way Analysis of Variance (Two-Way ANOVA) to study the relationship between bacterial levels according to the months of April and June and the study sites—Jannat, Al-Touba, and Yeader—as shown in Table (3).

Table (3) Comparison of the levels of *E. coli*, *Staph. aureus*, and Total Coliform between the months and study sites

Parameter	F-value	Mean Square	Sum of Squares (Type I SS)	P-value
Bacteria	52.53	24.82	49.64	< 0.05
sites	98.87	46.71	46.71	< 0.05
Months	41.48	19.60	19.60	< 0.05

Based on the comparison of bacterial levels between the sampling sites, the study results in Table (3) showed a P-value of less than 0.05. This indicates significant differences among the three sites. The Jannat site was the most contaminated, with a mean bacterial count of 350.67This may be due to the proximity of the area to direct sources of untreated or partially treated wastewater, in addition to high population density and urban

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development, which increase the amount of pollutants discharged into the sea. Moreover, the lack of water currents or weak water movement in the area may lead to the accumulation of pollutants and hinder effective water renewal. Added to this is the lack of environmental infrastructure, such as treatment plants or organized sewage systems, which exacerbates the problem of bacterial contamination in seawater.

A comparison was also conducted between April and June to study seasonal changes in bacterial colony counts. Table (3) shows that the P-value was less than 0.05, indicating significant differences between the two months.

The month of June had a higher bacterial prevalence, with a mean bacterial count of 221.57. This may be due to the fact that the high temperatures in June provide a more suitable environment for the growth and reproduction of bacteria, especially in coastal waters.

Increased human and tourist activities during the summer season, such as swimming and camping on beaches, also contribute to introducing additional pollutants into the marine environment. Additionally, the increased consumption of water and sewage during the summer leads to a higher amount of untreated or partially treated wastewater flowing into the sea, while the reduced capacity of the water to self-renew under these hot environmental conditions exacerbates the accumulation of pollutants, thereby increasing the severity of bacterial contamination in seawater.

A comparison was also made between the types of bacteria to determine which was more prevalent. According to Table (3), the P-value was less than 0.05, which shows significant differences among the bacterial types.

Total Coliform was the most widespread, with a mean bacterial count of 397.20 MPN/ml The high presence of coliform bacteria may be due to contamination of the water by sewage or animal waste.

The results of this study revealed the presence of total coliform bacteria (Total Coliform) in seawater, which in this study's findings regarding the presence of bacteria (Total Coliform) in seawater are consistent with other studies conducted in different countries, despite variations in contamination levels

The results were in agreement with Khairy et al. [8], but did not align with the study by Alibi et al. [9]

Comparison of Enterobacteriaceae Levels between Study Months and Sites:

The comparison was conducted using a one-way analysis of variance (ANOVA) test to study the relationship between the occurrences of bacteria from the family *Enterobacteriaceae* according to the study months and sites, as shown in Table (4).

Table (4) Comparison of *Enterobacteriaceae* Levels between Study Months and Sites

Parameter	Sites	Occurrences	Mean	Std. Deviation	P-value
Area	Yeader	34	4.25	3.53	**0.878**
	Jannat	36	4.50	2.82	
Month	June	45	5.62	3.7	**0.108**
	April	25	3.12	1.8	

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The results from Table (4) revealed that the P-value was greater than 0.05. This indicates no significant differences in Enterobacteriaceae levels across the study months and sites. This stability may be due to the presence of continuous or constant pollution sources, indicating that the environmental factors affecting the proliferation of Enterobacteriaceae did not change significantly during the study period, which reflects a relative stability in the aquatic environment for this bacterial group. "The findings of this study regarding the presence of bacteria in seawater are consistent with those of other studies conducted in different countries, despite variations in contamination levels, and are in agreement with Alaa El-Din [10].

Comparison of the Numbers and Percentages of Bacteria from the Enterobacteriaceae family:

The comparison was performed using a One-Way Analysis of Variance (One-Way ANOVA) on the frequency of occurrence of bacteria belonging to the Enterobacteriaceae family, in order to determine whether there are statistically significant differences at a specified significance level (P-value) in the results obtained from the study samples (Table 5).

Table (5) Counts and percentages of bacteria belonging to the Enterobacteriaceae family

Type of Bacteria	Occurrences	Percentage (%)	Mean	Std. Deviation	P-value
Klebsiella spp	33	18.33	8.25	3.30	0.008
Pseudomonas spp	17	9.4	4.25	2.5	2.5
Proteus spp	10	5.5	2.5	2.5	0.57
Salmonella spp	10	5.5	2.5	1.0	1.0

The results of the study, as shown in table (5), indicate that the P-value was less than 0.05, which signifies a significant difference in the frequency of occurrence of bacteria belonging to the Enterobacteriaceae family. Among these, Klebsiella spp. was the most frequently detected, appearing 33 times (18.33%), followed by Pseudomonas spp., which appeared 17 times (9.4%). Both Proteus spp. and Salmonella spp. appeared equally, each with 10 occurrences, representing 5.5% of the total. This distribution reflects differences in the survival and growth abilities of these bacteria in the aquatic environment; Klebsiella spp. exhibits a high capacity to adapt to changing environmental conditions, whereas the presence of Pseudomonas spp., Proteus spp., and Salmonella spp. indicates their limited adaptability to marine environmental factors such as temperature and salinity. The results also indicate that continuous pollution sources play an important role in maintaining the presence of these bacteria, highlighting the need for ongoing environmental monitoring to assess potential health risks.

Comparison of Escherichia coli Results with International Standards:

The results obtained for *E. coli* counts (CFU/100 ml) were compared with the standard limits set by the World Health Organization (WHO), the European Union (EU), and the United States Environmental Protection Agency (USEPA) using a One-Sample Test.

The above table indicated statistically significant differences in *E. coli* bacteria, as the P-value was found to be less than 0.05. This means that the

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average E. coli counts at the Janat and Yeader sites exceeded the permissible limits according to the standards set by the World Health Organization[11]. The European Union[12]. and the United States Environmental Protection Agency[13].

Table (6) Comparison of the study results for E. coli with international standards

sites	S	B S V	Mean	MD	SD	P.Value
Jannat	WHO	>500	8831.66	8331.66	11296.0093	0.000
Jannat	EU	>500	8831.66	8331.66	11296.0093	0.000
Jannat	USEPA	235	8831.66	8596.66	11296.009	0.000
Yeader	WHO	>500	2408.33	1908.33	4876.032	0.004
Yeader	EU	>500	2408.33	1908.33	4876.032	0.004
Yeader	USEPA	235	2408.33	1173.33	4876.032	0.001

CONCUSION

The study concluded that bacterial pollution in seawater varies significantly across the three surveyed areas. Jannat Estuary recorded the highest levels of pollution due to the improper discharge of wastewater, in addition to the proximity of discharge pipes to the shoreline, which led to the spread of foul odors in those areas. In contrast, the Touba Estuary was the least polluted, with no bacteria detected during April and June, while the Yedder Estuary recorded moderate levels of pollution. The main cause of wastewater reaching the sea is attributed to the random connection between sewage networks and rainwater drainage systems, which poses a serious health risk to humans.
Exposure to such contaminated water may lead to diseases such as intestinal poisoning and skin infections. Accordingly, the study recommends urgent measures to improve wastewater treatment systems and ensure the protection of both the environment and public health.

RECOMENDATIONS

The study recommended strengthening environmental regulations and oversight concerning sewage outfalls to reduce pollution and protect public health. It also emphasized the importance of establishing integrated wastewater treatment plants equipped with specialized laboratories, and extending discharge pipes further offshore to minimize coastal pollution. The study highlighted the need to raise environmental awareness among citizens and to combat the random connection between sewage systems and rainwater drainage networks. It also called for avoiding swimming near polluted discharge areas, utilizing treated wastewater for agricultural irrigation and industrial purposes, and establishing national standards for the quality of marine water.
Finally, the study recommended conducting further research on bacterial contamination and concentrations of heavy metals in the seawater and marine fish in the Misrata region.

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