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ANTIMICROBIAL PROPERTIES AND BACTERIAL CONTAMINATION IN WESTERN CASPIAN SEA COASTAL WATERS: A STUDY OF COLIFORM, E. COLI, AND ENTEROCOCCUS

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ABSTRACT

This study evaluates the antimicrobial properties and bacterial contamination of coastal waters in the western Caspian Sea, focusing on Coliform, Escherichia coli (E. coli), and Enterococcus bacteria. Water samples were collected from four coastal locations—Neftchala, Sumgayit, Bilgah, and Pirallahi—and analysed to determine bacterial concentrations against the minimum acceptable rates for safe water quality. The analysis showed variability in bacterial contamination levels across the sites, with Neftchala displaying undetectable levels of Coliform and E. coli. At the same time, Sumgayit, Bilgah, and Pirallahi exhibited varying concentrations, some exceeding the minimum acceptable rate thresholds. These findings provide insights into the potential antimicrobial properties of certain coastal waters and emphasise the need to investigate environmental and anthropogenic factors influencing microbial presence. Further research is necessary to explore the natural processes contributing to bacterial inhibition in these waters and to develop effective water management strategies for the Caspian Sea region.

1. Introduction

The Caspian Sea, the world's largest enclosed inland body of water, is a vital ecological and economic resource for the surrounding countries. Understanding the antimicrobial properties and bacterial contamination levels of its coastal waters is crucial for water quality management and public health. Bacteria such as Coliform, Escherichia coli (E. coli), and Enterococcus serve as critical indicators of microbial contamination in aquatic environments. Their presence often results from anthropogenic activities such as untreated sewage discharge, agricultural runoff, and industrial pollution, leading to significant ecological and health concerns [1,2]. Monitoring these bacteria in coastal waters provides insights into the environmental health and potential risks associated with waterborne pathogens [3,4].

Microbial contamination in coastal waters can be heavily influenced by local environmental conditions and human activities. For example, studies conducted on the southern Caspian Sea coast have documented varying levels of E. coli contamination in water samples from areas influenced by agricultural and urban runoff, highlighting the importance of location-specific monitoring [2]. In the western Caspian Sea, locations such as Neftchala, Sumgayit, Bilgah, and Pirallahi present a diverse range of contamination levels, which reflect different environmental and anthropogenic influences [4]. This variability underlines the need for continuous monitoring and assessment of bacterial contamination and its implications for public health and marine ecosystems [1,5]

Antimicrobial resistance (AMR) among waterborne pathogens is a growing concern, particularly with the increasing detection of resistant strains in marine environments. Enterococcus species, for instance, have shown resistance to multiple antibiotics, complicating infection control measures and necessitating the study of resistance patterns in coastal waters [1]. Research has demonstrated that waters with frequent human interaction, such as recreational beaches, often harbour resistant bacterial strains, indicating the potential role of such environments in the dissemination of AMR [6,7]. Studies on the molecular detection of antibiotic resistance and virulence gene determinants in Enterococcus species from coastal waters further emphasise the need to address AMR as a critical factor in water quality [4,8-10].

Recent investigations have explored the potential of natural and synthesised antimicrobial agents to reduce microbial contamination in marine settings. For example, deep-sea water has been shown to possess natural antimicrobial properties that can inhibit the growth of E. coli and Staphylococcus aureus, suggesting a promising alternative to chemical-based disinfection methods [6]. Similarly, the exploration of synthesised compounds, such as dipentylammonium (Z)-3-carboxylate complex, has revealed effective bactericidal properties against a range of pathogens, highlighting the potential of these agents for environmental and public health applications [3,7]. The application of such natural and synthetic agents offers a sustainable approach to water management, reducing the reliance on traditional chemical treatments and potentially mitigating the spread of resistant strains [3,8].

Further research on antimicrobial agents derived from marine environments, including deep-sea water, has demonstrated their ability to inhibit a broad spectrum of bacteria, providing a potential solution for microbial control in coastal waters [4-6]. Additionally, understanding the mechanisms underlying the natural antimicrobial properties of seawater can inform the development of new biotechnological applications for environmental safety [7].

This study aims to assess the bacterial contamination and potential antimicrobial properties of coastal waters in the western Caspian Sea, focusing on Coliform, E. coli, and Enterococcus bacteria. By comparing bacterial concentrations across different locations and assessing their alignment with the minimum acceptable rates for water quality, this research provides a comprehensive understanding of microbial dynamics in the region. The findings will contribute to developing more effective water management strategies and exploring the natural antimicrobial processes that may help maintain water quality in the Caspian Sea [4,6,7]

2. Methodology:

This study was conducted to evaluate the antimicrobial properties and bacterial contamination levels of coastal waters in the western Caspian Sea, explicitly focusing on the presence of Coliform, Escherichia coli (E. coli), and Enterococcus bacteria. Water samples were collected from four coastal locations—Neftchala, Sumgayit, Bilgah, and Pirallahi—representing different environmental conditions and levels of anthropogenic influence.

2.1 Sample Collection:

Water samples were collected from the four locations during a period of stable weather conditions to avoid variations due to rainfall or storm events. At each site, samples were taken from a depth of approximately 1 meter to ensure consistency. Sterilised glass bottles were used for sample collection, and each bottle was labelled with the location, date, and time of collection. The samples were immediately stored in an icebox to maintain a low temperature and were transported to the laboratory for analysis within one week. This timeframe ensured that microbial integrity was preserved while allowing for logistical considerations. Figure 1 illustrates the sites from which water samples were collected.

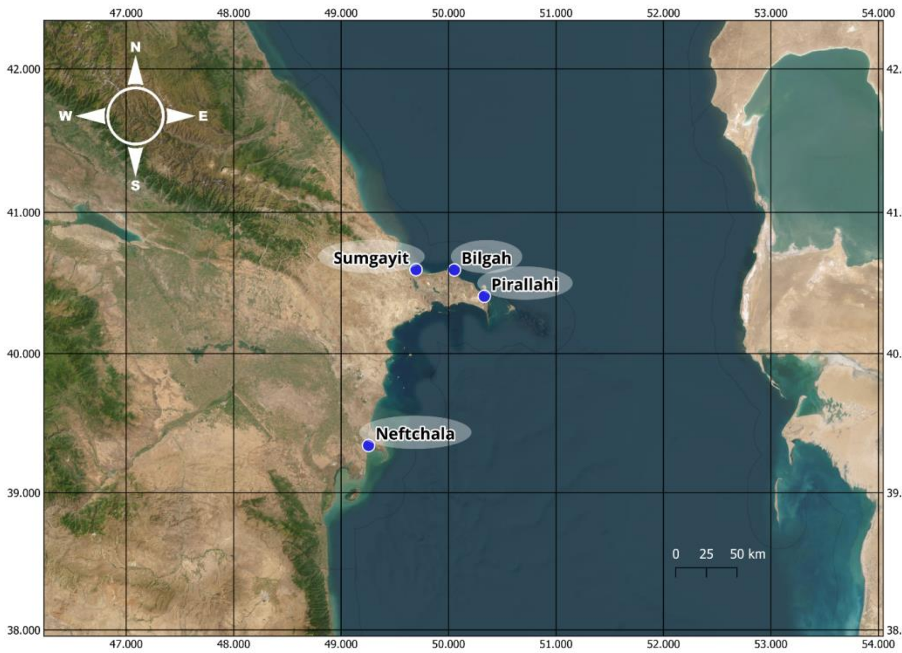

Fig 1. *Locations of conducted experiments on the map*

2.2 Bacterial Analysis:

The concentrations of Coliform, E. coli, and Enterococcus bacteria in the water samples were determined using a Biotrak-4250 analyser conducted under the supervision of the Ministry of

Ecology and Natural Resources of the Republic of Azerbaijan. The analysis was carried out at a temperature of 37°C for 24 hours under fully sterile conditions in a controlled environment. For each analysis, 1 ml of the faecal water sample was mixed with 9 ml of nutrient media, specifically BiMedia 155A, BiMedia 160C, and BiMedia 330A, to provide the optimal conditions for bacterial growth and detection. BiMedia 155A was used for Coliform, BiMedia 160C for E. Coli and BiMedia 330A for Enterococcus.

The Biotrak-4250 analyser was used to detect and quantify bacterial concentrations automatically. This equipment enables precise analysis of bacterial growth in the nutrient media, providing accurate results for Coliform, E. coli, and Enterococcus levels in the samples. After 24 hours of incubation at 37°C, the bacterial concentrations were recorded. Results were expressed in bacterial units per litre (unit/l) for each type of bacteria, based on the growth observed in the BiMedia-specific nutrient conditions.

This automated analysis method allows for a standardised and compassionate approach to monitoring bacterial contamination levels in coastal waters, ensuring that the measurements are reliable and reproducible across different samples and locations [4].

2.3 Determination of Minimum Acceptable Rate:

To assess whether the bacterial contamination levels were within safe limits, the concentrations of Coliform, E. coli, and Enterococcus were compared against the Minimum Acceptable Rates for water quality. These Minimum Acceptable Rates values serve as benchmarks for determining whether the observed bacterial levels pose a potential health risk. The values used in this study 2000 n/L for Coliform, 1000 n/L for E. coli, and 1000 n/L for Enterococcus—are based on the standards reported [4], which are specifically determined for the studied region and water type in the Caspian Sea context (see Table 1).

Additionally, the results were cross-referenced with the **WHO guidelines for safe recreational water environments**, which provide criteria for bacterial concentrations in recreational waters (see Table 2). For other uses, such as drinking water, the WHO sets much stricter standards (see Table 3). This dual reference allows for a more comprehensive understanding of how the contamination levels compare both regionally and internationally. [10-12]

***Table 2. Minimum Acceptable Rates based on** (Guliyeva et al., 2024)*

Bacteria Type	Minimum Acceptable Rates **Values**
Coliform	2000 n/L
E. coli	1000 n/L
Enterococci	1000 n/L

Table 3. WHO Guidelines for Recreational Water Quality

Bacteria Type	Excellent Quality	Good Quality	Poor Quality
Coliform	Not specified	Not specified	Not specified
E. coli	≤ 250 CFU/100 mL	251–500 CFU/100 mL	501–1,000 CFU/100 mL
Enterococci	≤ 40 CFU/100 mL	41–200 CFU/100 mL	201–500 CFU/100 mL

Table 4. WHO Guidelines for Drinking Water Quality

Bacteria Type	Acceptable Limit
Coliform	0 CFU/100 mL
E. coli	0 CFU/100 mL
Enterococci	0 CFU/100 mL

2.4 Statistical Analysis:

The bacterial counts obtained from the different locations were statistically analysed to determine significant differences in contamination levels across the study sites. Due to the limited sample size and the non-normal distribution of the data, the **Kruskal-Wallis test**, a non-parametric alternative to one-way ANOVA, was used to test for differences in bacterial concentrations (Coliform, E. coli, and Enterococcus) between the four locations—Bilgah, Pirallahi, Sumgayit, and Neftchala. The Kruskal-Wallis test ranks the data and compares the median ranks across groups. A p-value of less than 0.05 was considered statistically significant, indicating differences in bacterial contamination levels among the sites. If substantial differences were detected, further pairwise comparisons were conducted using post-hoc tests to identify specific groups that differed from each other.

2.5 Assessment of Antimicrobial Properties:

The study assessed the potential antimicrobial properties of the coastal waters by analysing the bacterial contamination levels at each location—Bilgah, Pirallahi, Sumgayit, and Neftchala. The assessment was based on the comparison of bacterial counts (Coliform, E. coli, and Enterococcus) across these locations to identify any site-specific variations that may indicate natural antimicrobial effects. Lower bacterial counts in specific locations could suggest the presence of natural conditions or environmental factors that inhibit microbial growth. The findings were interpreted in the context of the Minimum Acceptable Rates from (Guliyeva et al., 2024) and cross-referenced with **WHO guidelines for recreational water quality** to provide a comprehensive understanding of the water quality and potential antimicrobial properties in the western Caspian Sea region. [10-12]

3. Results and Discussion

To evaluate the microbial safety and potential antimicrobial properties of coastal waters in the western Caspian Sea, this study analysed bacterial contamination levels across four different locations: Bilgah, Pirallahi, Sumgayit, and Neftchala. The presence of Coliform, E. coli, and Enterococcus bacteria was quantified and assessed relative to the region-specific Minimum Acceptable Rates provided by (Guliyeva et al., 2024) and the internationally recognised **WHO guidelines** for recreational water quality.

3.1 Bacterial Contamination Levels Across Locations:

The bacterial analysis across the four locations—Bilgah, Pirallahi, Sumgayit, and Neftchala—revealed varying levels of Coliform, E. coli, and Enterococcus bacteria. The concentrations of these bacteria were measured at specific time points to evaluate their presence in the water samples relative to the Minimum Acceptable Rates and WHO guidelines. As shown in Table 4, Bilgah exhibited Coliform levels of 58,000 unit/l, which is below the Minimum Acceptable Rate of 2000 unit/l, but E. coli levels were 55,000 unit/l, far exceeding the acceptable limit of 1000 unit/l. Pirallahi showed even higher bacterial concentrations, with Coliform levels reaching 114,000 unit/l and E. coli levels at 110,000 unit/l. Sumgayit also showed notable bacterial presence, with Coliform levels at 52,000 unit/l and E. coli at 48,000 unit/l. In contrast, Neftchala recorded bacterial levels below the detection limits for both Coliform and Enterococcus and 14,700 unit/l for E. coli, indicating a relatively better water quality that might naturally inhibit microbial growth.

Table 5. *Bacterial Contamination Levels at Different Time Points Across Locations*

Location	Bacteria Type	Environment (BiMedia Type)	Concentration (unit/l)	Minimum Acceptable Rate (unit/l)
Bilgah	Coliform	BiMedia 155A	58,000	2000
Bilgah	E. coli	BiMedia 160C	55,000	1000
Bilgah	Enterococcus	BiMedia 330A	0	1000
Pirallahi	Coliform	BiMedia 155A	114,000	2000
Pirallahi	E. coli	BiMedia 160C	110,000	1000
Pirallahi	Enterococcus	BiMedia 330A	0	1000
Sumgayit	Coliform	BiMedia 155A	52,000	2000
Sumgayit	E. coli	BiMedia 160C	48,000	1000
Sumgayit	Enterococcus	BiMedia 330A	0	1000
Neftchala	Coliform	BiMedia 155A	0	2000
Neftchala	E. coli	BiMedia 160C	14,700	1000
Neftchala	Enterococcus	BiMedia 330A	0	1000

3.2 Comparison with Minimum Acceptable Rates and WHO Guidelines:

The findings across the locations were compared to the Minimum Acceptable Rates, which were 2000 unit/l for Coliform, 1000 unit/l for E. coli, and 1000 unit/l for Enterococcus. Except for Neftchala, all other locations exceeded these thresholds, especially for E. coli and Coliform, indicating poor water quality that could pose health risks if used for recreational activities.

Additionally, these results were compared with the WHO guidelines for recreational water quality, which specify that for E. coli, concentrations should be ≤ 250 CFU/100 mL for excellent quality and up to 1,000 CFU/100 mL for poor quality. For Enterococci, ≤ 40 CFU/100 mL is considered exceptional, and up to 500 CFU/100 mL is poor quality. The contamination levels observed in Bilgah, Pirallahi, and Sumgayit far exceed these WHO limits, underscoring the need for immediate interventions and continuous monitoring to prevent potential health risks.

3.3 Statistical Analysis of Bacterial Contamination Levels:

The **Kruskal-Wallis H test** was used to evaluate differences in bacterial contamination levels (Coliform, E. coli, and Enterococcus) across the four locations: Bilgah, Pirallahi, Sumgayit, and Neftchala. This non-parametric test, appropriate for non-normally distributed data, assesses whether the median ranks of multiple groups differ significantly. The test statistic HIs calculated using:

$$H = \frac{12}{N(N+1)} \sum \left(\frac{{R_i}^2}{n_i} \right) - 3(N+1) \qquad (1)$$

where NIs the total number of observations, $R_i$is the sum of ranks for the i -th group and n_i is the number of observations in that group.

The test showed significant differences in **Coliform** and **E. coli** levels among the locations ($p < 0.05$), with post-hoc tests indicating higher contamination at Bilgah, Pirallahi, and Sumgayit compared to Neftchala. No significant differences were found for **Enterococcus** ($p > 0.05$), reflecting consistently low levels across all sites. These findings suggest localised factors driving bacterial contamination, requiring targeted interventions.

3.4 Implications for Water Quality and Management:

The results highlight significant microbial contamination in several coastal areas, with Bilgah, Pirallahi, and Sumgayit showing levels far beyond both regional and international safety guidelines. This suggests potential sources of pollution, such as untreated sewage discharge, agricultural runoff, or other anthropogenic influences that must be addressed to ensure safer water for recreational and public use. In contrast, Neftchala's relatively lower contamination levels indicate a more favourable water quality profile, which could be further investigated to understand the factors contributing to these conditions.

3.5 Recommendations and Future Research:

The findings call for targeted water quality management strategies to mitigate contamination sources and improve the overall safety of coastal waters in the Caspian Sea. Future research should involve more frequent and comprehensive sampling across different seasons and environmental conditions, coupled with chemical analyses to identify specific pollutants or natural antimicrobial compounds influencing bacterial growth.

4. Conclusion

This study provides a comprehensive assessment of bacterial contamination levels across four coastal locations in the western Caspian Sea—Bilgah, Pirallahi, Sumgayit, and Neftchala—by quantifying the presence of Coliform, E. coli, and Enterococcus bacteria and comparing them against established minimum acceptable rates and WHO guidelines for recreational water quality. The findings reveal significant microbial contamination in Bilgah, Pirallahi, and Sumgayit, where bacterial levels, particularly for Coliform and E. coli, substantially exceed both regional and international safety thresholds. These results suggest that these areas are heavily impacted by anthropogenic pollution, such as untreated sewage discharge, agricultural runoff, and possibly industrial activities, necessitating immediate intervention and targeted water management strategies to mitigate potential health risks.

In contrast, the comparatively lower bacterial counts observed in Neftchala indicate more favourable water quality conditions, which may be attributed to natural factors or reduced human impact. This highlights the potential for certain coastal areas to exhibit natural antimicrobial properties that warrant further investigation. The use of both regional and international guidelines provided a robust framework for evaluating contamination levels, emphasising the need for harmonised standards in assessing water quality for public health safety.

The study underscores the urgent need for enhanced monitoring and regulatory measures in the Caspian Sea region. Implementing regular, high-frequency water quality monitoring, coupled with advanced microbial source tracking techniques, could provide deeper insights into pollution dynamics and inform more effective mitigation strategies. Additionally, future research should focus on understanding the ecological and public health implications of such contamination, exploring the presence of antimicrobial resistance in marine pathogens, and investigating natural antimicrobial compounds that could offer sustainable solutions for water quality management.

Overall, this study contributes to the growing body of knowledge on coastal water quality in the Caspian Sea and provides critical insights for policymakers, researchers, and environmental managers. By addressing both immediate contamination issues and exploring sustainable long-term solutions, the region can move towards safer, cleaner, and healthier marine environments.

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