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Bacteriological Assessment of Fecal Contamination in the Sediments of the Gulf of Annaba (Southern Mediterranean): A Preliminary Investigation

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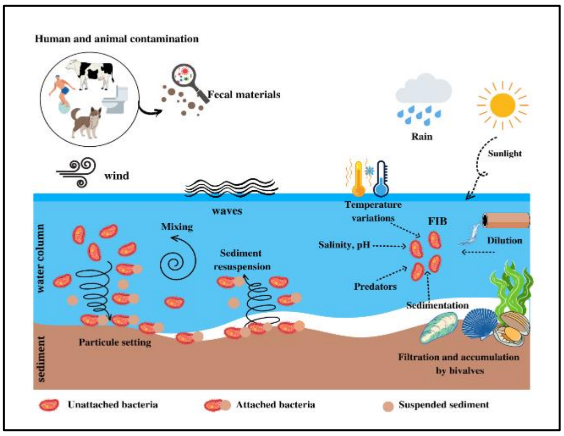
GRAPHICAL ABSTRACT



ABSTRACT

This study investigated the bacteriological and physicochemical quality of seawater and sediment samples collected from four sampling sites in the Gulf of Annaba (Northeastern Algeria) over a one-year period. Culture-based techniques were used to quantify and assess Fecal Indicator Bacteria (FIB) and potentially pathogenic bacteria. Additionally, various physicochemical parameters including temperature, pH, salinity, dissolved oxygen, and suspended solids were measured. The results revealed seasonal variations in the physicochemical variables, reflecting the influence of environmental conditions in the research area. The highest concentrations of FIB were observed in samples obtained from Sidi Salem and Rezgui Rachid, indicating a possible association with sewage contamination. Furthermore, the sediments collected from all sites exhibited higher levels of FIB and potentially pathogenic bacteria compared to the seawater samples, particularly during the summer and fall seasons.

1. INTRODUCTION

Coastal ecosystems are continuously subjected to significant anthropogenic impacts, including bacterial contamination from urban, agricultural, and industrial activities (Basili et al., 2021). This form of pollution can harbor harmful pathogenic bacteria, which can detrimentally impact human health and aquatic ecosystems, leading to severe environmental, economic, and health consequences, notably a deterioration in recreational water quality (Curran et al., 2022).

In polluted coastal waters, a wide range of bacterial species, including Fecal Indicator Bacteria (FIB), can be found alongside the most dangerous pathogens (Zhang et al., 2019). FIB, which are highly prevalent in human feces, tend to occur in higher

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concentrations in wastewater compared to many pathogens, making them reliable indicators for identifying sewage inputs (O'Mullan et al., 2019; González-Fernández et al., 2021).

Once introduced into coastal environments, FIB have a remarkable ability to persist, thereby increasing the potential for human disease transmission. Moreover, under specific hydrological conditions, these bacteria can migrate to nearby areas such as bathing and shellfish zones (Luna et al., 2016), thus, exposing humans to heightened susceptibility of contracting various illnesses, including skin diseases, gastrointestinal infections, acute respiratory infections, and allergies (Karbasdehi et al., 2017; Valério et al., 2022).

Since its implementation in 1993, the bathing water quality requirements derived from the Official Journal of the Algerian Republic (OJAR, 1993) has played a crucial role in evaluating the quality of coastal bathing waters by assessing the levels of FIB such as *Escherichia coli* (*E. coli*) and fecal streptococci (FS). These bacterial standards are established based on extensive research that estimates the risk of enteric bacteria-related diseases among bathers when exposed to different concentrations of these indicators in water (Aragonés et al., 2016).

Regrettably, the primary shortcoming of the previous standards lies in their restricted scope, as they solely focus on assessing the quality of coastal waters while neglecting the crucial role of sediments. Research studies conducted by Zimmer-Faust et al. (2017), Fang et al. (2018), and Chávez-Díaz et al. (2020) have consistently demonstrated the critical role of sediments in harboring bacteria and serving as a potential source of contamination.

Sediments play a crucial role in the fate of FIB due to several factors, including the abundant availability of organic carbon and nutrients, minimal temperature variations, reduced exposure to sunlight, and protection against predators (Fang et al., 2018). These favorable conditions in sediments contribute to the survival and potential proliferation of FIB (Chávez-Díaz et al., 2020). However, the presence of these bacteria can have significant implications for both human health and the overall quality of coastal ecosystems, particularly when sediments are disturbed and re-suspended. This re-suspension can arise from human activities, such as recreational water activities, as well as natural phenomena like tides and heavy rainfall, leading to high levels of contamination (Luna et al., 2012).

The Gulf of Annaba is a region of particular interest for environmental research due to its exposure to various natural and anthropogenic pressures (Amri et al., 2017). Previous monitoring surveys in this area have primarily focused on assessing the quality of seawater and marine organisms, such as bivalves (Hidouci et al., 2014; Kadri et al., 2015; Kadri et al., 2017; Boufafa et al., 2021), while largely overlooking the crucial role of sediments as a potential reservoir of fecal bacteria in coastal ecosystems. Consequently, our research aims to bridge this gap by undertaking the first comprehensive investigation to (1) evaluate the abundance and distribution of FIB and pathogenic bacteria in the sediments and (2) assess the impact of environmental variables in the seawater on the abundance of FIB in the Gulf of Annaba.

2. METHODOLOGY

2.1 Description of the sampling zone

The Gulf of Annaba is one of the most valued regions in Algeria due to its strategic geographic position and socioeconomic importance. Situated in the extreme northeast of the country, it stretches approximately 40 km between Cap de Garde in the west (7°16'E - 36°68'N) and Cap Rosa in the east (8°15'E - 36°38'N) (Figure 1). This coastal area is permanently threatened by various anthropogenic activities, including the discharge of agricultural and industrial effluents, untreated wastewater, and fishing practices, etc. Additionally, it receives a substantial inflow of freshwater from Oued Bedjima and, notably, Oued Seybouse (Figure 1), which is the second longest river in Algeria with a catchment area of about 6,470 km^2 (Amri et al., 2017).

For this study, four sampling sites (Figure 1) were selected according to their proximity to the different sources of contamination in these areas, as well as their accessibility and importance in tourism. The characteristics of the selected sites are shown in Table 1.

2.2 Sampling strategy

Seawater and sediment samples were collected monthly early in the morning at each site over a one-year survey period from January to December 2018. To minimize bacterial exposure to solar irradiation, seawater samples were obtained from a depth of approximately 30-50 cm using sterilized glass bottles. Subsequently, sediment samples weighing approximately 50 g were collected by scraping the top few centimeters of the surface (2-5 cm) and carefully placed in sterile bags. All samples were immediately stored in a cool box maintained at a low temperature (4°C to 6°C) and transported to the laboratory for analysis within 2-3 h.

Directly after sampling, seawater environmental variables including temperature (T), pH salinity (Sal), and dissolved oxygen (DO) were measured *in situ* at each site using a multiparameter probe (Multi 340i/SET-82362, WTW®, Germany). Suspended solids (SS) were determined in the laboratory by the differential weighing method (Aminot and Kérouel, 2004).

Figure 1. Map showing the location of the Gulf of Annaba and sampling sites. The small map shows the overall location of Annaba with respect to Algeria and the Mediterranean Sea. The large map shows the exact locations of the four sampling sites (S1=Cap de Garde; S2=Rezgui Rachid; S3=Sidi Salem; S4=Lahnaya).

Table 1. Characteristics of the four sampling sites in the Gulf of Annaba

Name and coordinates of sampling site	Location in the study area and characteristics of the sampling sites
S1: Cap de Garde (36°96′N, 7°79′E)	Located in the western part of the study area and characterized by the presence of bathers in summer
S2: Rezgui Rachid (36°91′N, 7°76′E)	Located in the center of Annaba City and receives urban waste
S3: Sidi Salem (36°86′N, 7°76′E)	Located in the east of Annaba City, close to Wadi Seybouse and Bedjima and receives a mixture of industrial, urban agricultural and wastes
S4: Lahnaya (36°93′N, 8°20′E)	Located at 45 km from the city of Annaba and relatively unaffected by urban interference

2.3 Bacteriological analysis

2.3.1 Fecal indicator bacteria enumeration

A volume of 100 mL of seawater was directly analyzed without any pre-treatment. In the case of sediment samples, a detachment step was conducted prior to bacterial enumeration. Samples of approximately 10 g were homogenized and diluted in 90 mL of peptone water supplemented with 1 mL of Tween 80 ® (Biopack). The mixture was continuously stirred for 10 to 15 min to ensure thorough dispersion of the bacteria from the sediment particles.

For all samples, the density of FIB, including total coliform (TC), *E. coli*, and fecal streptococci were enumerated by a multiple tube dilution method using the three tube Most Probable Number (MPN) method (standard V 08-020 (1994)/ISO 7251 and 08-021 V (1993)/ISO 7402). The results were statistically expressed as MPN of bacteria per 100 mL or g according to the Mac Grady's tables.

2.3.2 Isolation and identification of potentially pathogenic bacteria

The isolation and identification of potentially pathogenic bacteria were carried out following the methods described by Rodier et al. (2009). Selected isolates were presumptively identified based on their morphological and cultural characteristics. Subsequently, they were further identified up to the species level using the Analytical Profile Index API 20E, API 20NE, and API staph systems, according to the manufacturer's recommendations. Additionally, enzymatic tests including oxidase, catalase, and staphylocoagulase tests were performed to confirm their characteristics and ascertain their enzymatic profiles. These enzymatic tests were conducted in accordance with standard protocols and guidelines.

The relative bacterial abundance is calculated by dividing the number of each bacterium by the total number of bacteria for each site and for each compartment. The results are expressed as a percentage.

2.4 Statistical analysis

Statistical analyses were carried out on the physicochemical and bacteriological variables using R software. Correlation analyses for seawater samples were estimated to analyze the intensity of the relationships between the data sets using the Spearman correlation coefficient. Finally, principal component analysis (PCA) was used as a descriptive method to characterize the pattern of the four sampling sites in the Gulf of Annaba.

3. RESULTS

3.1 Physicochemical variables

Five physicochemical variables were measured in all seawater samples during the sampling period. As expected, seawater temperature at the four study sites was strongly influenced by air temperature, reaching its highest value (27.7°C) in summer at S2 and its lowest value (11°C) in winter at S4 (Figure 2). Salinity showed a similar seasonal pattern to the temperature, where the maximum value was recorded at S4 (41 g/L) in summer (Figure 2). According to the Spearman correlation coefficient, these two variables were strongly correlated with each other ($r=0.76$, $p<0.0001$) (Table 2). In contrast, DO levels exhibited an inverse relationship with water temperature, with the highest values observed in winter and the lowest values during summer and fall (Figure 2). The highest correlation in this study was revealed between DO and temperature ($r=-0.85$, $p<0.0001$) (Tables 2).

Regarding the pH, the measurements remained relatively alkaline and consistent across the four seasons. The highest value (8.8) of this variable was recorded at S4 in spring (Figure 2). Maximum levels of suspended solids (0.4 mg/L) were reached twice, in the winter and the fall, at S2 and S3, respectively (Figure 2).

3.2 Bacteriological analysis of fecal indicator bacteria

The levels of TC, *E. coli*, and FS in both seawater and sediment samples at each sampling site and season are reported in Figure 3.

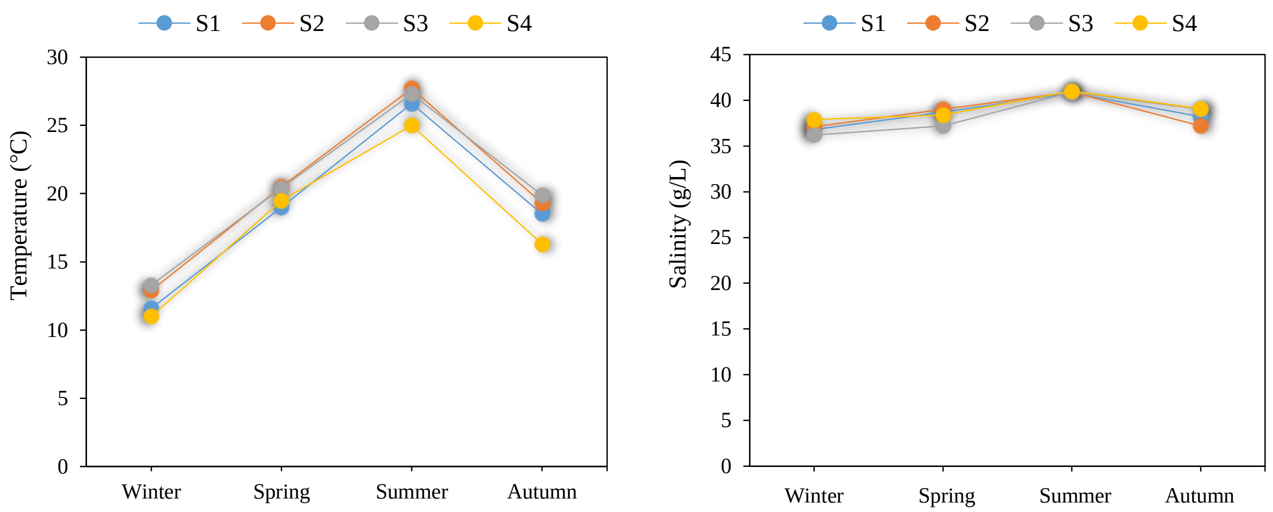

Figure 2. Results of the physicochemical analysis of seawater samples at the four sampling sites

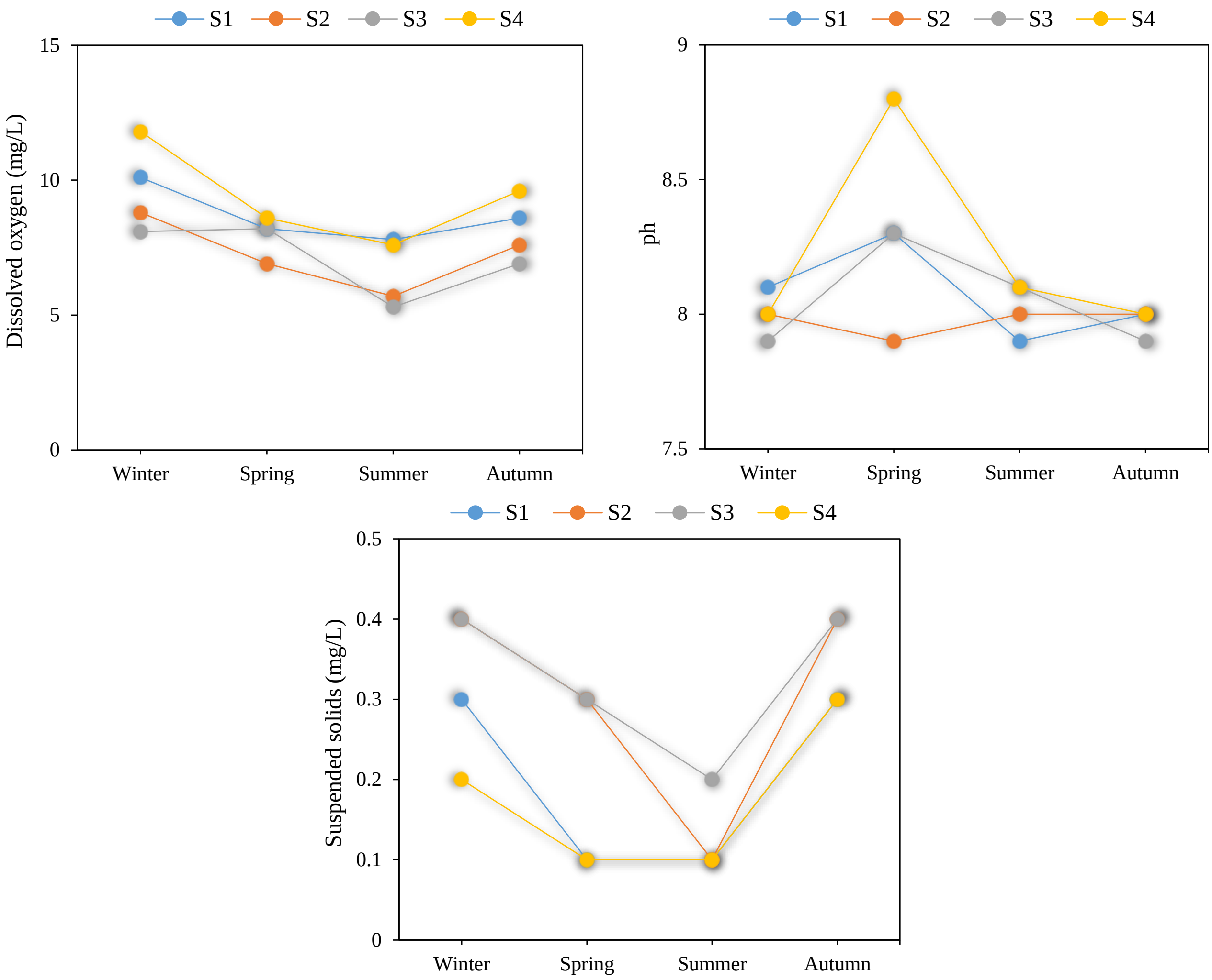

Figure 2. Results of the physicochemical analysis of seawater samples at the four sampling sites (cont.)

Total coliforms were consistently detected throughout the research period. The abundance of these bacteria was above the regulatory limits established by the Algerian legislation for recreational water (500 MPN/100 mL of water) (OJAR, 1993) at all sampling sites and seasons, with the exception of S4, where the lowest levels were observed in spring (340 TC/100 mL) and autumn (380 TC/100 mL) (Figure 3).

E. coli concentrations ranged from 23.3 *E. coli*/100 mL at S4 in spring to 853.3 *E. coli*/100 g at S3 in autumn (Figure 3). FS were consistently present in all seawater samples collected throughout the study year (Figure 3), mainly due to their high resistance to severe environmental stress. The highest amount of these bacteria (1500 FS/100 mL) was detected in seawater from S3 (Figure 3).

Unlike water, FIB were found in all sediment samples, and were obviously and comparatively higher than in seawater samples (Figure 3). Levels of 1.8×10^5 TC/100 g and 1.4×10^5 TC/100 g were recorded at S3 and S2 in spring and summer, respectively. The sediments in all the sites, were heavily contaminated by *E. coli*; values of 1.3×10^5 *E. coli*/100 g and 1.1×10^5 *E. coli*/100 g were obtained in winter at S3 and summer at S2, respectively (Figure 3). The lowest concentration of *E. coli* (2.1×10^4 *E. coli*/100g) was detected at S4 in winter. Regarding FS, the highest level was recorded in summer at S2 (1.5×10^5 FS/100 g) (Figure 3).

3.3 Identification of potentially pathogenic bacteria

During the study period, a total of 164 bacteria were isolated and identified. Investigation of the occurrence and abundance of potentially pathogenic bacteria showed higher sediment (56.76% of isolates) than seawater (40.23% of isolates) contamination in the Gulf of Annaba.

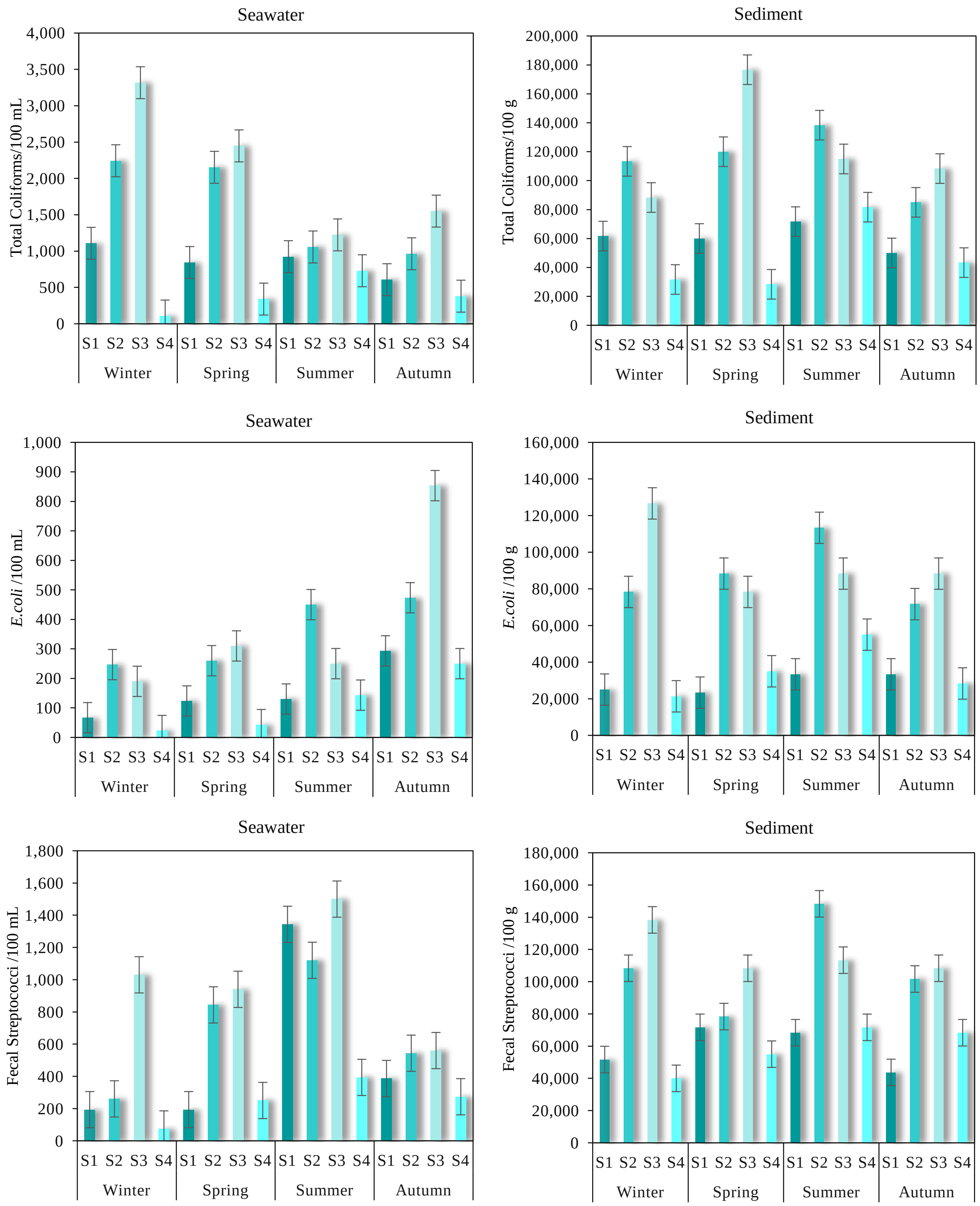

Figure 3. Spatial and temporal variations of FIB in seawater and sediments

Several potentially pathogenic bacteria, mostly associated with fecal and sewage contamination, were found in seawater and sediment samples. *E.coli* (36.6%) showed the maximum occurrence in all sampling sites and environmental samples, followed by *Aeromonas hydrophila* (5.49%), *Enterobacter cloacae* (5.48%), *Burkholderia cepacia* (4.27%), *Klebsiella pneumoniae*, *Vibrio parahaemolyticus* (3.66%), *Pseudomonas aeruginosa*, and *Staphylococcus sciuri*

(3.59%). The relative abundance of all species identified in this research is shown in Figure 4.

Figure 5 illustrates the seasonal distribution of potentially pathogenic bacteria in the Gulf of Annaba, indicating that the diversity of these species exhibited an increase during the warm season (summer and fall) in comparison to the cold season (winter and spring) in both compartments (seawater and sediment).

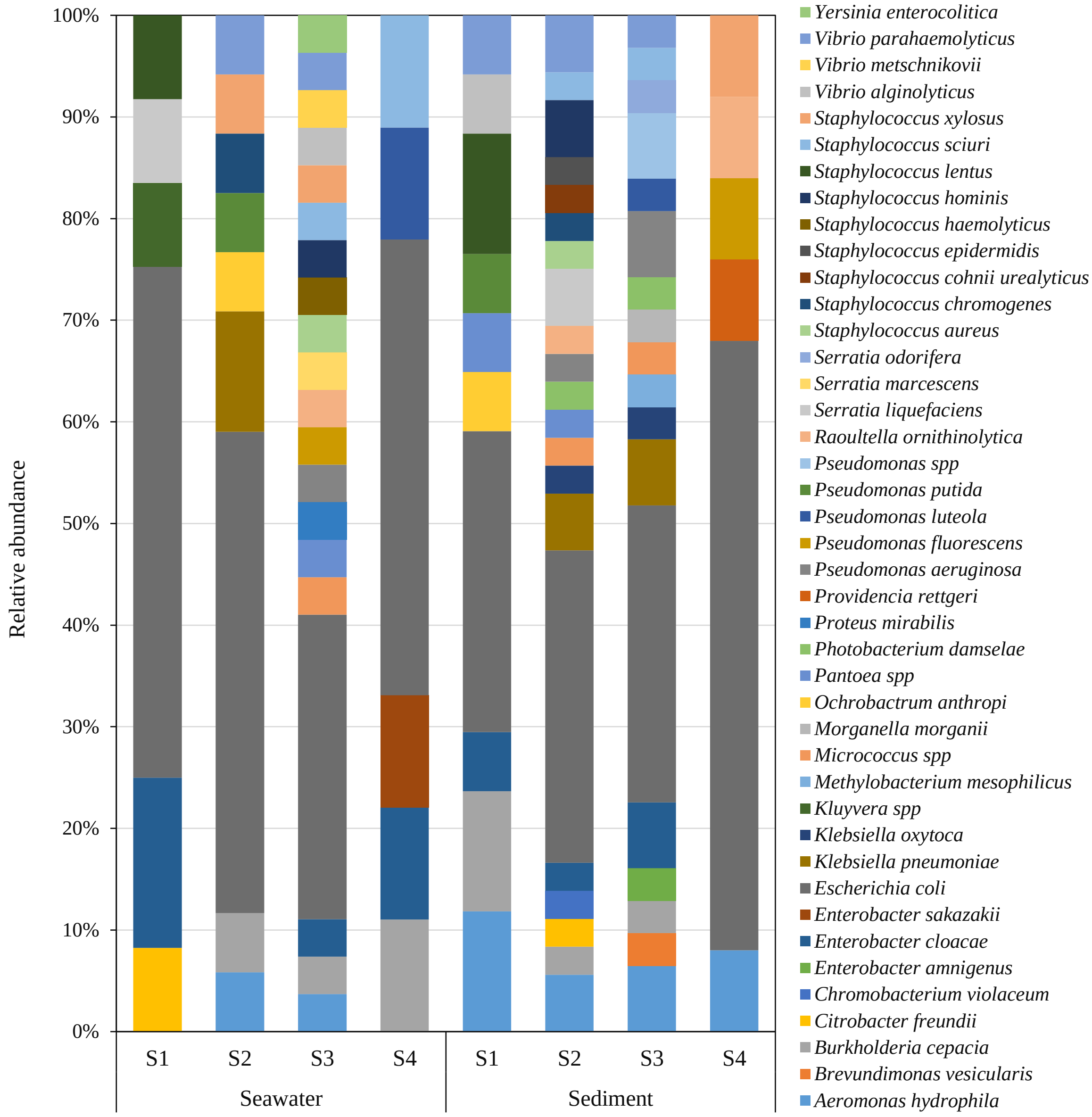

Figure 4. Relative abundance of potential pathogenic bacteria in seawater and sediments samples

3.4 Influence of physicochemical variables on FIB in the Gulf of Annaba

Spearman correlation analysis revealed that the levels of FIB were significantly related to physicochemical variables: seawater temperature, salinity, dissolved oxygen, and suspended solids (Table 2). No significant correlations were found between FIB and pH in all the sampling sites.

Dissolved oxygen, suspended solids, and seawater temperature were found to show the strongest correlations with the levels of FIB (Table 2). According to the correlation analysis, the highest and most significant correlations were found between FS and DO (r=-0.79, $p<0.0001$), as well as temperature (r=0.75, $p<0.0001$). TC showed a positive correlation with suspended solids (SS) (r=0.51, $p<0.05$), whereas *E. coli* exhibited a negative correlation with dissolved oxygen (r=-0.57, $p<0.05$) (Table 2).

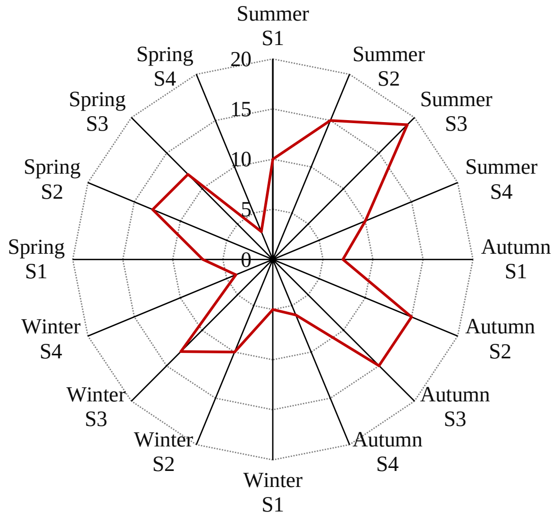

Figure 5. Seasonal distribution of potential pathogenic bacteria in seawater and sediments samples

3.5 Principal component analysis (PCA)

PCA analysis of seawater variables produced two main axes accounting for 74% of the total information in the data set (Figure 6).

The projection into the first axis accounting for 56.3% of the variance opposed the variables seawater temperature (r=-0.53), salinity (r=0.47), and FS (r=-0.41) to the variable DO (r=0. 46) (Figure 6). This suggests that the differences in these variables are most likely due to seasonal changes. Instead, PC2 accounted for 31% of the variation and exhibited negative associations with TC (r=-0.51), SS (r=-0.48), *E. coli* (r=-0.43), and positive correlation with pH (r=0.41) (Figure 6).

Table 2. Spearman's correlation matrix of the seawater quality

	T	Sal	pH	DO	SS	TC	EC
Sal	0.76 ***						
pH	0.00	-0.02					
DO	-0.85***	-0.59*	0.24				
SS	-0.52*	-0.66**	-0.47	0.18			
TC	0.14	-0.34	-0.29	-0.38	0.51*		
EC	0.41*	0.15	-0.34	-0.57*	0.45	0.44	
FS	0.75***	0.38	-0.39	-0.79***	-0.06	0.53*	0.55*

* p≤0.05; ** p≤0.01; *** p≤ 0.001

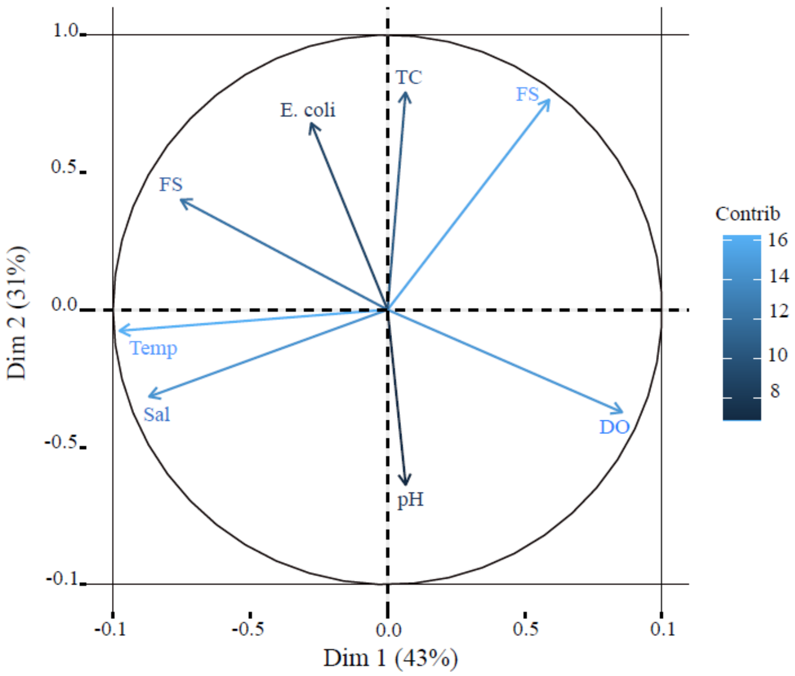

Figure 6. Principal component analysis performed on data from seawater samples. Correlation of environmental variables with the first axis of the standard PCA. Temp: seawater temperature, Sal: salinity, DO: dissolved oxygen, SS: Suspended solids. *E. coli*: *Escherichia coli*, TC: total coliforms and FS: fecal streptococci.

4. DISCUSSION

4.1 Physicochemical variables

The measurements of physicochemical variables of seawater samples showed that temperature, salinity, pH, DO, and SS present seasonal fluctuations in all sites at the Gulf of Annaba. Peak values for seawater temperature coincided with the summer-fall period. As previously observed, variations of this abiotic variable are broadly related to the local climatic conditions, particularly to ambient temperature (Gutiérrez-Cacciabue et al., 2014).

Salinity, similar to water temperature, exhibited its highest values during the warm season, as shown in Figure 2. This pattern is likely attributed to the combined effects of elevated temperatures, which promote significant evaporation, and reduced precipitation, resulting in decreased freshwater inflow (Mihanović et al., 2021). The inflow of freshwater into the sea through the Oueds plays an important role in seawater dilution and, consequently, the reduction in salinity, particularly during winter. Notably, our findings are consistent with the observations reported by Lamine et al. (2019) in Morocco.

The pH levels in the Gulf were predominantly alkaline, fluctuating between 7.3 and 8.9 (Figure 2). It is worth noting that, according to Chapman (1996), pH values within the range of 6 to 9 are generally considered safe for aquatic life and fisheries.

The annual cycle of dissolved oxygen in the Gulf revealed a pronounced oxygenation of the Gulf water during the cold season, primarily due to the decrease in water temperature and the occurrence of high wind speeds. These factors contribute to continuous mixing of the water mass, resulting in an enrichment of dissolved oxygen during the winter season (Hébert and Légaré, 2000). Conversely, the low levels of this variable observed during the dry season indicate elevated bacterial growth and oxygen consumption, alongside a reduction in the self-purification capacity of the seawater (Rodier et al., 2009).

Regarding suspended solids, their concentrations showed higher values during winter in comparison to summer. These variations appear to be related with climatic conditions, particularly the increased precipitation during winter. The abundance of rainfall leads to soil leaching and substantial allochthonous inputs, contributing to elevated suspended solids in the Gulf.

4.2 Bacteriological analysis

4.2.2 Seawater

The spatial-temporal variations of FIB revealed a distinct seasonal pattern across the four study sites. Significantly higher levels of these bacteria were observed during the summer and fall seasons (Figure 3) probably due to the influence of high temperatures, which are recognized as one of the key environmental factors promoting the persistence of FIB. Barreras et al. (2019) suggest that elevated temperatures can prolong the duration of FIB presence in the water, a hypothesis supported by our study. Notably, our findings demonstrated significant positive correlations between FIB and temperature, as confirmed by Spearman's correlation and multivariate analysis ($p \leq 0.05$) (Table 2). These results are in line with the studies conducted by Lamine et al. (2019) and Chávez-Díaz et al. (2020).

Moreover, in areas designated for swimming, where the average depth of the seawater column does not exceed 2 meters, sediments are constantly in motion due to factors such as recreational activities of bathers and coastal currents. This dynamic environment facilitates the migration of a portion of FIB present in the sediments into the water column, leading to elevated levels of contamination (Garrido-Pérez et al., 2008).

As expected, the central zone sites of the Gulf (S2 and S3) exhibited the highest concentrations of fecal bacteria (Figure 1). These sites are characterized by high population density, animal husbandry, and extensive anthropogenic activities, including industries, recreational practices, coastal tourism, and the presence of fishermen and swimmers, among others (Kadri et al., 2017; Boufafa et al., 2021). Importantly, it should be noted that, according to the Algerian Bathing Water executive decrees (OJAR, 1993), the seawater at these two sites is considered to have an unacceptable sanitary quality. In contrast, the strong hydrodynamic conditions, coupled with limited sewage discharge at S1 and S4, have contributed to relatively lower levels of fecal contamination compared to the other two sites (Boufafa et al., 2021).

4.2.3 Sediments

FIB densities were shown to be heavily concentrated in sediments compared to seawater samples (Figure 3), confirming their prolonged survival and persistence in this compartment. Our results corroborate previous studies conducted at different marine beaches worldwide, which have consistently reported significantly higher average concentrations of FIB in sediments compared to water samples (Crabill et al., 1999; Davies and Bavor, 2000; Karbasdehi et al., 2017). This difference in concentration could be explained by significant dilution of FIB within the water column due to mixing processes, resulting in lower concentrations. Additionally, adsorption and sedimentation processes play a crucial role in removing bacteria from suspension and facilitating their accumulation in bottom sediments (Rozen and Belkin, 2001; Mote et al., 2012).

Our results also revealed a correlation between high bacterial loads during the warm season and elevated FIB levels in sediments. This suggests that the variations in bacterial concentrations were most likely related to the temperature of the sediments, particularly the warming of the surface layer, which creates favorable conditions for bacterial growth. These findings are consistent with the studies conducted by Whitman et al. (2014) and Abreu et al. (2016). According to Zhang et al. (2015), higher temperatures during the summer can stimulate increased concentrations of fecal indicators. This poses a potential concern for bathers, especially children, who tend to have more frequent and active interactions with sediment, making them more susceptible to fecal bacteria (Abreu et al., 2016).

In contrast, the important concentrations of FIB during winter would be probably due to the high levels of organic matter resulting from untreated wastewater drained by heavy rainfall. Our findings support earlier research that highlights the role of organic matter in the persistence and long-term survival of these indicators in sediments (Malham et al., 2014; Perkins et al., 2014; Chávez-Díaz et al., 2020). A study conducted by Craig et al. (2004) demonstrated higher survival levels of *E. coli* in sediments compared to water, and that the presence of substantial organic matter in sediments further enhanced the survival of this bacteria.

The association of bacteria with sediments has been demonstrated to circumvent the negative effects of environmental stresses, such as protection from UV light, salinity, and seasonal variations, resulting in higher survival of indicator bacteria (Zimmer-Faust et al., 2017). Bacteria attach themselves to clays, such as montmorillonite, and utilize the abundant organic and mineral elements present in sediments as a source of nutrients (Malham et al., 2014). This attachment to sediments protect bacteria from predators and bacteriophage attacks. Consequently, sediments can be considered as important accumulation zones for FIB, and in extreme cases, they can even serve as naturalized habitats (O'Mullan et al., 2019).

4.3 Pathogenic bacteria

Pathogenic bacteria were abundant in seawater and sediment samples collected from the Gulf of Annaba, which is comparable to previously reported contaminated coastal areas (Karbasdehi et al., 2017; Chávez-Díaz et al., 2020). Similar to FIB, these species were higher in sediments than in seawater samples, which validates their significant role in assessing pathogen's abundance in these coastal environments.

As can be seen in Figure 4, biochemical tests revealed the presence of different potentially pathogenic species. While many of these species belong to the family *Enterobacteriaceae*, commonly found in the gut microbiota of humans and animals, some are environmental bacteria that are typically isolated from clinical settings. Several studies have reported the association of these species with human outbreaks, including meningitis, urinary and pulmonary tract infections, nosocomial infections, and gastroenteritis (Wang et al., 2020; Hespanha et al., 2021; Soumastre et al., 2022). According to Mohammed et al. (2012), in the lack of optimal indicators of non-fecal health hazards, certain bacteria such as Pseudomonas, may be beneficial in assessing the health conditions of the coastal environments.

Following the *Enterobacteriaceae* family, *Staphylococcus* spp., predominated in seawater and sediment samples, especially in heavily contaminated sites (S2 and S3). These bacteria are considered opportunistic pathogens, and certain strains have the potential to cause illness (including skin infections), even in healthy individuals (Whitman et al., 2014). Interestingly, *Staphylococcus* spp. exhibited higher abundance in the sediment than in the surrounding water during the summer, which is consistent with findings from other studies (Esiobu et al., 2004; Shah et al., 2011).

The results of our study also revealed that *Vibrio* spp. showed the highest occurrence during the summer, which is in agreement with several studies carried out on sediment and seawater samples (Abia et al., 2016; Baron et al., 2017; Rincé et al., 2018; Debnath et al., 2019). According to Arab et al. (2021), the significant increase in water temperature and salinity during the summer season creates favorable conditions for the proliferation of *Vibrio* spp., which can cause several infectious diseases and pose a significant threat to the health of bathers.

5. CONCLUSION

The findings of the current study demonstrated the significant role played by sediment as a reservoir of FIB and potentially pathogenic bacteria in the Gulf of Annaba. It has been confirmed that the bacteriological quality of both seawater and sediment samples in the study area was strongly influenced by a range of anthropogenic activities, environmental factors, and seasonal variations. In particular, it was observed that during the hot season, the concentration of FIB often exceeded the maximum permissible limits recommended by Algerian regulations for recreational water. Notably, the site of Sidi Salem was found to be the most contaminated, followed by Rezgui Rachid, due to the presence of multiple untreated wastewater outfalls and domestic animals in the vicinity. Consequently, regular and stringent monitoring of water quality in the Gulf is crucial to mitigate the risks to public health and the environment.

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