



IMPACT OF THE INSTALLATION OF TWO WWTPS ON THE MICROBIOLOGICAL QUALITY OF SEAWATER IN THE ANZA-TAGHAZOUT COAST (NORTH OF AGADIR-MOROCCO)

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ABSTRACT

Background Marine environment plays a socio-economic role of great importance in Morocco. Its coasts which incorporate one of the richest biodiversity in the world suffered from large volumes of liquid waste of several industrial and domestic units. These effluents unfortunately are discharged into the marine environment. Agadir coastline located in the southwest of Morocco is among the famous economical ones of the African continent, but it is characterized by industrial pollution (Canning factories, By-product factories), old and New ports discharges, tourist factories (hotel discharges, bathing activities) and domestic wastewater. All of these factors have caused heavy microbiological pollution on these coasts (2500 m³ / day is a volume of untreated industrial and urban discharges from Anza city). To solve this problem, two wastewater treatment plants (WWTP): Anza and Aourir were installed in Agadir city. **Objectives** This study aimed to assess the impact of the installation of these two WWTP onto abiotic and fecal indicator bacterial (FIB) parameters in seawater and to estimate quality of the seawater bathing areas. **Methods** This study was conducted in the period from January 2017 to December 2018 in three studied beaches belonging to Anza-Taghazout coastline. Six abiotic parameters were measured in situ: Temperature, dissolved oxygen, electrical conductivity, salinity, Total Dissolved Solid and pH and two bacteriological analyzes: fecal coliforms (FC) and fecal streptococci (FS). **Results** Physico-chemical results show seasonal variations and bacteriological results in three beaches are within Moroccan Standards of bathing water quality and are all ranked B. Pearson correlation test carried out between the abiotic factors and the levels of FIB showed a significant positive correlation between FS and temperature and a significant negative correlation with TDS, while FC show a significant positive correlation with DO₂. **Conclusion** Seasonal variations of physico-chemical results are probably due to climate conditions, and classification of our studied beaches compared to old studies in the same ecosystem is probably related to the installation of the two WWTPs. The fecal contamination in these beaches has three origins: animal origin, mixed contamination origin predominantly human and mixed contamination origin predominantly animal.

Keywords: Abiotic parameters, Bathing water quality, Contamination origin, Correlation, Fecal coliform, Fecal streptococci.

1. INTRODUCTION

The marine environment plays an important role in the social and economic field of the Agadir region. At the harbor of Agadir, the coastal and artisanal fishing sector reached 30026 tons in 2017 and generated a turnover of 260212000 Dirhams [1]. For the tourism sector, tourist arrivals to this region are 1033358 tourists with 5451124 overnight stays [1]. The population of greater Agadir reached 638890 in 2017 [1]. All these anthropogenic activities (e.g. industrialization, harbors, tourism and recreational human activities) and some natural events (e.g. rainfalls) generate a strong microbiological pollution of the sea which receives treated and non-treated wastewater. This pollution is all the more dangerous since wastewater is loaded with many microorganisms, some of which are pathogenic for public health such as parasites (helminth eggs of *Ascaris*) [2], enteric viruses of Humans [3] and bacteria responsible for gastroenteritis, urinary tract infections and neonatal meningitis produced by *Escherichia coli* serotype O157: H7 [4,5].

In 1996, Agadir's coastline received a significant contribution of wastewater discharges from this city and its perimeter with a flow of 24 million m³ [6]. This flow continues to increase and the volume of urban wastewater discharges in Morocco will multiply to reach 900 million m³ in 2020 instead of 500 million m³ in 2000 [7]. Currently, 77% of the wastewater of greatest Agadir is collected and treated, but still remain important problems such as the collection and treatment of wastewater in the northern zone of Agadir, seat of the harbor, Anza urban and Anza Industrial zones [8]. To solve this problem, two wastewater treatment plants (WWTPs) have been recently installed: Anza WWTP and Aourir WWTP that have become operational since 2016.

This marine sector of Agadir has experienced numerous studies in the field of ecotoxicology [9,10,11], in chemical pollution such as trace metals and pesticides used in agriculture [12,13,14,15], in bacteriological pollution [16-6], and in sewage contamination [17,18]. On the bacteriological level, the studies undertaken before the installation of the two mentioned treatment stations have shown a strong bacterial load by total Mesophilic Aerobic Bacteria, Reducing Sulfite Bacteria and by the fecal bacteria such as total and fecal coliforms and fecal streptococci.

Since the study of fecal indicator bacteria (FIB), which cause serious health problems for swimmers [19], is highly recommended by the Moroccan standards for the quality of bathing water according to the standard Moroccan national

law for NM 03 7. 200, transposed from European Directive 76/160 / EEC and UNEP / Guidelines for the sanitary surveillance of marine bathing water, we have undertaken this study, which is the first one of its kind in the north marine sector of Agadir after the installation of the WWTPs mentioned above. It aims i) the monitoring of Fecal Indicator Bacteria (FIB) and abiotic parameters in three beaches, ii) the study of differences between seasons, years and between beaches by ANOVA One-way.

Our results will be compared with those of previous studies in order to assess the impact of the installation of the two WWTPs on the microbiological quality of marine waters in the Anza-Taghazout coastline where the main beaches of the north Agadir area are located.

2. MATERIALS AND METHODS

3.1 Study area

The study area covers 25 km north of Agadir. It includes three beaches on the Moroccan Atlantic coastline (Figure 1).
 - Anza beach (B₁) located at 10 km in the north of Agadir city (Latitude: 30.44948 N and Longitude: -9.661578W). The beach is approximately 0.8 km long and 0.03 km² of surface. It is a common recreational destination for an average of thousands of visitors per day during the bathing season (June-August). This beach is located near the Anza WWTP (0.9 Km) whose outfall is located approximately 2.5 km further out in the Atlantic Ocean. It is also characterized by artisanal fishing (fishing rod) and the collection of mussels.

- Aourir Beach (B₂) (Latitude: 30.502211 N and Longitude: -9.683547W) which receives the treated discharge of Aourir WWTP, is located near to the Aourir river estuary which constitute a source of pollution especially during rainfall periods. This beach is 0.6 km long and 0.04 km² of surface. It is characterized by surfing, swimming and people bring their pets like sheep (overgrazing) and dogs which pollute the beach by their waste. Also, during the early morning hours, birds gather near the shoreline and their feces may be a source of elevated fecal indicator levels in recreational water [20].

- Imourane Beach (B₃) (latitude: 30.507923 N and longitude: -9.687625 W) is characterized by the existence of a camping car for the rest of tourists, multiple hotel units and a landing point of small boats. It is also characterized by the existence of cane fishermen and people who collect mussels. It should be noted that beaches (B₂) and (B₃) have never been subjected to an environmental study.

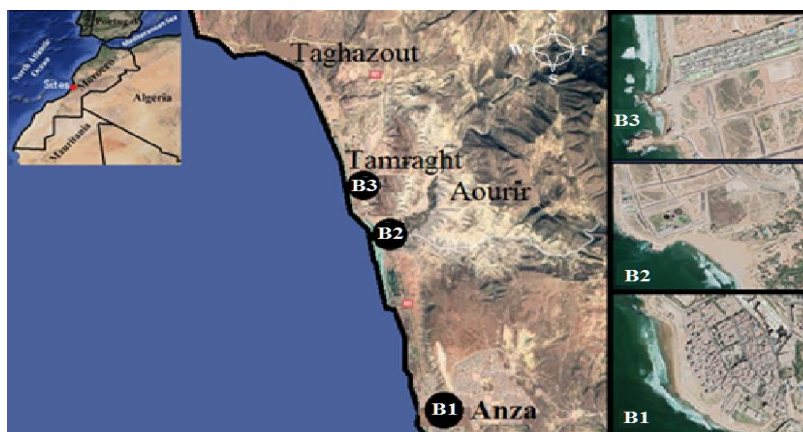


Figure 1: Geographic location of the study area Anza beach (B₁), Aourir beach (B₂) and Imourane beach (B₃).

3.2 Sampling procedure

Homogenous samples of 1L of seawater were taken manually in sterile glass bottles from each site with frequency of nine samples for each season during two cycles 2017 and 2018. Samples were stored in an icebox at a temperature of 4°C in obscurity to avoid any disinfection effects of sunlight and changes to microbial presence. All samples were transported to the laboratory for analysis within 24h after sampling [21].

3.3 Abiotic parameters

Temperature (T), pH, electrical conductivity (EC), salinity (Sa), Total Dissolved Solids (TDS) and dissolved oxygen (DO₂) were measured in situ using a portable multi-parameter Orion Star™ A329 Thermo SCIENTIFIC®.

3.4 Fecal indicator bacteria (FIB) analysis

FIB colonies counts were obtained using the membrane filtration method (ISO, 2000). Seawater samples were passed through a sterile membrane filter of cellulose nitrate (0.45 μm, 47 mm, diameter, Millipore, Sartorius®, Germany), and placed on different selective culture media. Lactose TTC Agar with Tergitol 7® for FC bacteria and Bile Esculin Azide Agar for FS bacteria. Selective culture media for FC were incubated at 44°C for 24h and at 37°C for 48h for FS. Colonies with a

golden sheen were taken to be FC whilst dark colonies were considered as FS. Each of the two FIB is expressed in terms of a count of colony forming units per 100 mL (CFU/100 mL).

3.5 Classification of bathing water quality

The quality of the bathing water of the supervised beaches concerns the research of microbiological parameters, fecal coliforms and fecal streptococci in accordance with the Moroccan national standard (NM 03.7.200), transposed from the European Directive (76/160/EEC) and the (WHO/UNEP,1996) Guidelines for Sanitary Monitoring of Marine Bathing Waters. The Moroccan standard classifies bathing water into four categories: A, B, C and D (Tables 1 and 2).

Table 1. Standard values for bathing water classification established in Moroccan National Standard (NM 03.7.200).

Microbiological parameters	Guide value (CFU/ 100mL)	Mandatory values (CFU/ 100mL)
Fecal coliforms (FC)	100	2000
Fecal streptococci (FS)	100	400

Table 2 : Moroccan classification of bathing water.

Good quality (class A)	Medium quality (class B)	Poor quality (class C)	Bad quality (class D)
80% of FC \leq 100 UFC/100 mL 95% of FC \leq 2000 UFC/100 mL 90% of FS \leq 100 UFC/ 100 mL	95% of FC \leq 2000 UFC/100 mL And the conditions relating to the guide numbers not being wholly or partly verified	5% -33% of FC > 2000 UFC/100 mL	More than 33% of FC > 2000 UFC/100 mL
Conform to the Moroccan standards		No conform to the Moroccan standards	

3.6 Fecal contamination origin

Fecal contamination flora was quantified using the ratio (R) of Borrego and Romero, (1982) which related concentration of fecal coliform with concentration of fecal streptococci in the following relationship: $R=FC/FS$. The ratio (R) may be interpreted according to five intervals of origin contamination: Animal origin if $R < 0.7$; humane origin if $R > 4$; mixed origin with animal predominance if $0.7 \leq R \leq 1$; uncertain origin if $1 \leq R \leq 2$ and mixed origin human predominance if $2 \leq R \leq 4$ [22].

3.7 Statistical analysis

The means and standard deviations were used to present the seasonal variations of the different studied parameters. Values of 0 CFU/100 mL in bacteriological studies were substituted by 1 CFU/100 mL according to directive 2006/7/EC (EEC, 2006). All raw bacteriological data was log transformed to remove large data variations and to minimize the difference between them. One-way ANOVA and Tukey HSD post-hoc test was used to test a difference among two years, difference between seasons and difference between beaches. The relationship between fecal bacteria and other abiotic factors was analyzed using Pearson correlation test. The statistics were performed with StatSoft, Inc. (2014). STATISTICA (data analysis software system), version 12 (www.statsoft.com).

3. RESULTS

3.1 Abiotic factors

Seasonal variations of abiotic factors at the three studied beaches and ANOVA One-way results are shown successively in Figure 2, Tables 3 and 4. The maximum temperature was observed in the spring (25.88 ± 0.71 °C) while the minimum value was detected in winter (15.13 ± 0.84 °C). The one-way ANOVA analysis showed statistically highly significant differences ($p < 0.001$) between seasons for the three ranges subject of this study. The difference of pH registered between seasons were significantly higher ($p < 0.01$) for all studied beaches except for Imourane beach (B_3) in 2018 which was not significant ($p > 0.05$). The maximum value of pH (8.38 ± 0.09) was recorded in summer season in beach (B_1), whereas the lowest value (7.73 ± 0.49) was recorded in winter in the same beach. Salinity was in euhaline salinity range ($30 < \text{salinity} < 40$ g/L) and showed a significantly higher difference between seasons ($p < 0.01$) in all studied beaches except Imourane beach (B_3) ($p > 0.05$). Salinity was higher in autumn season (37.33 ± 0.27 g/L) comparatively to the lowest value (32.51 ± 1.73 g/L) observed in winter. Annual average of salinity increase between 2017 and 2018 from 35.54 ± 2.03 g/L to 36.78 ± 0.61 g/L. Seasonal variation of EC in the studied beaches presents significantly very higher differences ($p < 0.001$) between seasons. EC presents the highest value (57.42 ± 0.70 mS/cm) in autumn season compared to low value (47.01 ± 0.36 g/L) registered in winter season. Study of EC rate between 2017 and 2018 show an increase from 53.72 ± 3.41 in 2017 to 56.46 ± 0.69 mS/cm in 2018. TDS concentration during the two monitoring cycles ranged from minimum value (26.90 ± 0.04 mg/L) registered in summer to maximum value (28.46 ± 0.61 mg/L) observed in autumn. This seasonal variation showed a significantly high difference ($p < 0.01$) in the studied beaches except Anza beach (B_1) ($p > 0.05$). With regard to the dissolved O_2 concentrations, a minimum value (7.01 ± 0.49 mg/L) was recorded in the three studied beaches in autumn, while the winter season showed a maximum value (9.62 ± 0.94 mg/L). DO_2 concentration was significantly very high ($p < 0.001$) in 2018 for both studied beaches. The study of the difference between 2017 and 2018 shows that there is a very significant increase ($p < 0.001$) for salinity, EC and TDS, while the pH

and T show a significantly very high decrease ($p < 0.001$). No significant difference was observed in DO_2 concentrations ($p > 0.05$) between 2017 and 2018 (Table 5). With regard to the study of difference between three studied beaches (B_1); (B_2) and (B_3), no significant differences ($p > 0.05$) were remarked in pH, T, EC, TDS, salinity and DO_2 in 2017. In 2018, no significant differences were observed in T, salinity and DO_2 , while, significant differences were noted for TDS, pH and EC ($p < 0.05$) (Table 6).

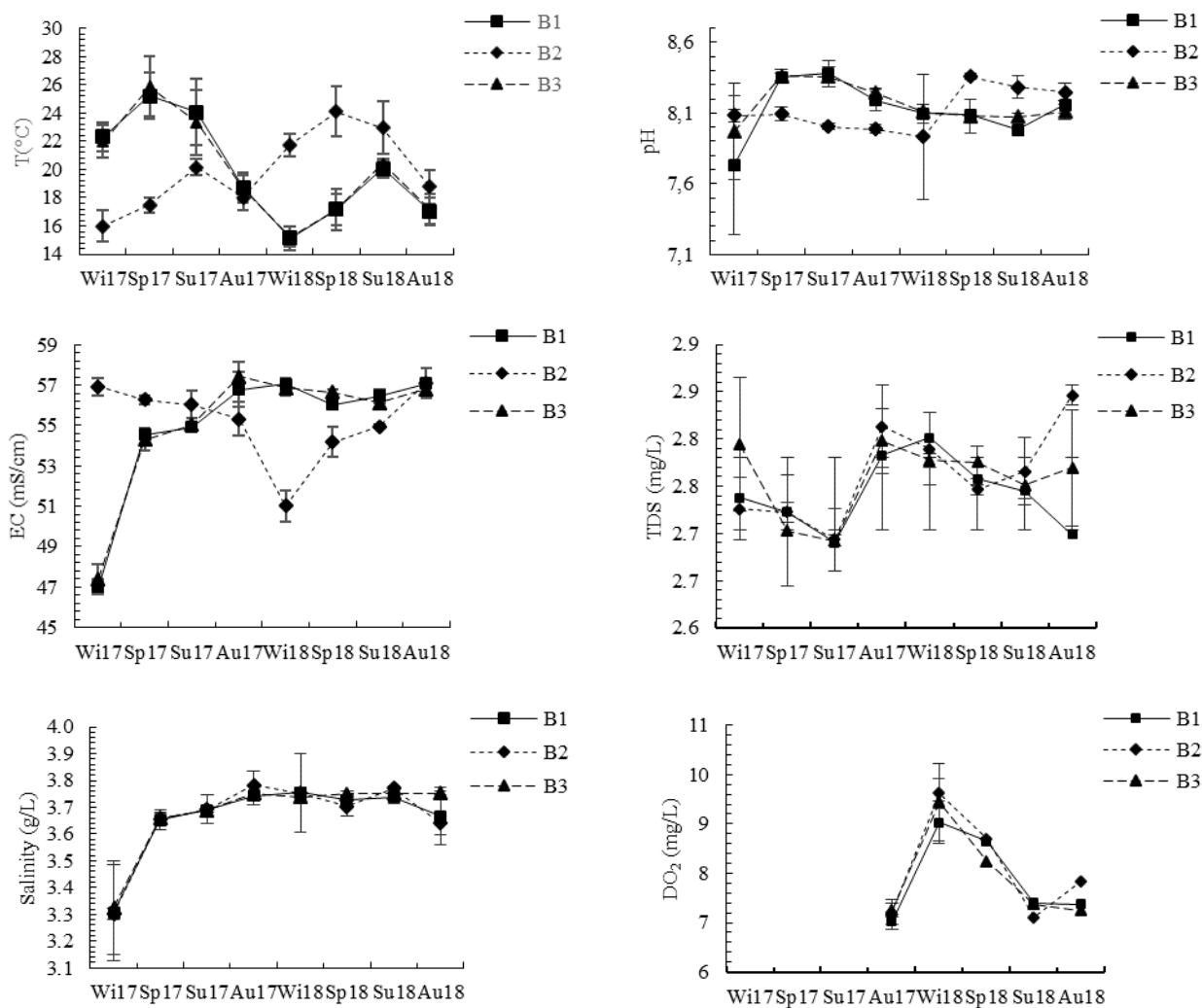


Figure 2: Seasonal variation of temperature (T), pH, electrical conductivity (EC), Total Dissolved Solids (TDS), salinity and dissolved oxygen (DO_2). Values are expressed as the Mean \pm SD.

Table 3: Results of one-way ANOVA comparing the effects of seasons on the variations of abiotic factors.

Year	Year 2017					Year 2018				
	Variables	Beaches	F	P	Sig	Beaches	F	P	Sig	
pH		B_1	09.81	0.0001	**	B_1	12.43	1.00E-05	**	
		B_2	06.62	0.0013	**	B_2	19.26	0	**	
		B_3	04.80	0.0071	**	B_3	00.43	0.7305	NS	
T		B_1	21.21	0	**	B_1	29.52	0	**	
		B_2	17.43	0	**	B_2	32.50	0	**	
		B_3	15.15	0	**	B_3	30.76	0	**	
EC		B_1	365.40	0	**	B_1	07.74	5.00E-04	**	
		B_2	339.60	0	**	B_2	23.68	0	**	
		B_3	90.73	0	**	B_3	07.18	0.0008	**	
TDS		B_1	03.77	0.201	NS	B_1	10.60	0	**	
		B_2	06.61	0.0013	**	B_2	04.49	0.0097	**	
		B_3	21.85	0	**	B_3	10.98	4.00E-05	**	
Sal		B_1	25.054	0	**	B_1	05.70	0.003	**	
		B_2	32.458	0	**	B_2	06.12	0.0021	**	
		B_3	34.396	0	**	B_3	01.36	0.2711	NS	
DO_2		B_1	--	--	--	B_1	12.89	1.00E-05	**	
		B_2	--	--	--	B_2	19.38	0	**	
		B_3	--	--	--	B_3	12.04	2.00E-05	**	

P: p-value, **F:** Factorial, **Sig:** Significance, (**NS:** Not Significant) : $p > 0.05$, (*: Significant Differences) : $p \leq 0.05$, (**: Significantly Higher Differences): $p \leq 0.005$ or $p \leq 0.001$.

Table 4: Results of Post-hoc test Tukey HSD (Mean±SE) comparing the effect of seasons on abiotic and bacteriological parameters

Variables		pH	T	EC	TDS	Sal	DO ₂	FS	FC	
2017	B ₁	Win	07.72±0.09 ^a	22.3±0.61 ^a	47.0±0.22 ^a	27.4±0.19 ^a	32.5±0.39 ^a	-	17.00±105.27 ^a	18.00±221.52 ^a
		Spr	08.35±0.09 ^b	25.2±0.61 ^b	54.5±0.22 ^b	27.2±0.19 ^{a,b}	36.0±0.39 ^b	-	267.83±105.27 ^a	1020.83±221.52 ^a
		Sum	08.37±0.09 ^b	24.1±0.61 ^{a,b}	54.9±0.22 ^b	26.9±0.19 ^{a,b}	36.0±0.39 ^b	-	338.61±85.95 ^a	104.22±180.87 ^{a,b}
		Aut	08.19±0.09 ^b	18.7±0.61 ^c	56.8±0.22 ^c	27.8±0.19 ^b	36.93±0.39 ^b	7.01±0.23 ^a	213.27±85.95 ^a	769.00±180.87 ^b
	B ₂	Win	07.97±0.07 ^a	18.7±0.71 ^a	47.4±0.23 ^a	26.9±0.2 ^a	32.51±0.37 ^a	-	35.17±23.40 ^a	14.50±8.58 ^a
		Spr	08.24±0.07 ^b	21.9±0.71 ^b	54.3±0.23 ^b	27.2±0.2 ^a	36.08±0.37 ^b	-	45.83±23.40 ^a	30.78±8.58 ^a
		Sum	08.36±0.07 ^b	23.3±0.71 ^{b,c}	55.0±0.23 ^b	27.3±0.2 ^a	36.42±0.37 ^b	-	62.72±19.11 ^a	34.11±7.01 ^a
		Aut	08.36±0.07 ^b	25.8±0.71 ^c	57.4±0.23 ^c	28.1±0.2 ^b	37.33±0.37 ^b	7.21±0.23 ^a	79.50±19.11 ^a	43.00±7.01 ^a
	B ₃	Win	07.93±0.08 ^a	18.7±0.59 ^a	51.0±0.26 ^a	27.0±0.11 ^a	32.75±0.32 ^a	-	16.00±81.38 ^a	34.17±39.66 ^a
		Spr	8.25±0.08 ^{a,b}	21.7±0.59 ^b	54.2±0.26 ^b	26.9±0.11 ^a	36.08±0.32 ^b	-	21.67±81.38 ^a	78.00±39.66 ^a
		Sum	08.28±0.08 ^b	22.9±0.59 ^{b,c}	54.9±0.26 ^b	28.0±0.11 ^b	36.41±0.32 ^b	-	410.06±66.45 ^a	123.67±32.38 ^a
		Aut	08.36±0.08 ^b	24.1±0.59 ^c	57.1±0.26 ^c	27.8±0.11 ^b	37.01±0.32 ^b	7.25±0.23 ^a	11.33±66.45 ^b	35.89±32.38 ^a
2018	B ₁	Win	08.08±0.01 ^a	16.0±0.31 ^a	56.9±0.23 ^a	28.1±0.12 ^a	37.04±0.16 ^a	9.03±0.23 ^a	20.00±23.32 ^a	80.00±59.69 ^a
		Spr	08.09±0.01 ^a	17.5±0.31 ^b	56.28±0.23 ^{a,b}	27.6±0.12 ^{a,b}	36.76±0.16 ^{a,b}	8.64±0.23 ^a	193.78±23.32 ^{a,b}	286.44±59.69 ^{a,b}
		Sum	08.01±0.01 ^b	20.1±0.31 ^b	56.0±0.23 ^b	27.5±0.12 ^{b,c}	36.89±0.16 ^b	7.40±0.23 ^b	244.67±23.32 ^{b,c}	115.00±59.69 ^{a,b}
		Aut	07.99±0.01 ^b	18.0±0.31 ^c	55.3±0.23 ^b	27.0±0.12 ^c	36.16±0.16 ^b	7.38±0.23 ^b	108.50±23.32 ^c	34.00±59.69 ^b
	B ₂	Win	08.09±0.01 ^a	15.1±0.35 ^a	57.0±0.10 ^a	27.9±0.10 ^a	37.03±0.23 ^a	9.62±0.24 ^a	61.11±17.97 ^a	69.33±9.97 ^a
		Spr	08.09±0.01 ^b	17.2±0.35 ^b	56.0±0.10 ^b	27.5±0.10 ^{a,b}	36.55±0.23 ^{a,b}	8.70±0.24 ^{a,b}	53.17±17.97 ^a	13.89±9.97 ^a
		Sum	07.98±0.01 ^b	20.0±0.35 ^b	56.5±0.10 ^c	27.7±0.10 ^b	37.21±0.23 ^b	7.11±0.24 ^{b,c}	172.00±17.97 ^a	46.11±9.97 ^{a,b}
		Aut	08.16±0.01 ^c	17.1±0.35 ^c	57.1±0.10 ^c	27.9±0.10 ^b	35.90±0.23 ^b	7.84±0.24 ^c	10.72±17.97 ^b	19.22±9.97 ^b
	B ₃	Win	08.11±0.03 ^a	15.2±0.38 ^a	56.8±0.12 ^a	27.7±0.12 ^a	36.87±0.06 ^a	9.44±0.29 ^a	163.83 ± 98.39 ^a	26.00±19.46 ^a
		Spr	08.08±0.03 ^a	17.2±0.38 ^b	56.6±0.12 ^b	27.5±0.12 ^a	37.00±0.06 ^a	8.25±0.29 ^a	496.06±98.39 ^{a,b}	66.00±19.46 ^{a,b}
		Sum	08.08±0.03 ^a	20.4± 0.38 ^b	56.1±0.12 ^b	27.7±0.12 ^a	37.03±0.06 ^a	7.37±0.29 ^a	293.33±98.39 ^{a,b}	80.44±19.46 ^{a,b}
		Aut	08.11±0.03 ^a	17.2± 0.38 ^c	56.8±0.12 ^b	28.6±0.12 ^b	37.01±0.06 ^a	7.24±0.29 ^b	40.17±98.39 ^b	128.22±19.46 ^b

Values having the same letter in the same column are not significantly different.

Table 5: Results of ANOVA One way and Tukey HSD tests comparing the effect of years on abiotic factors

Variables	T	pH	EC	TDS	Sal	DO ₂
2017	17.58±3.00 ^a	8.20±0.31 ^a	53.72±3.41 ^a	27.38±0.65 ^a	35.54±2.03 ^a	8.17±0.65 ^a
2018	22.31±2.00 ^b	8.07±0.07 ^b	56.46±0.69 ^b	27.70±0.49 ^b	36.78±0.61 ^b	10.64±1.13 ^a
P	0.00000	0.00006	0.00000	0.00007	0.00000	0.15633
F	184.54	16.760	67.231	16.357	37.339	2.0323
Sig	**	**	**	**	**	NS

P: p-value, F: Factorial, Sig: Significance, (NS: Not Significant) : p > 0.05, (*: Significant Differences) : p ≤ 0.05, (**: Significantly Higher Differences): p ≤ 0.005 or p ≤ 0.001. Values having the same letter in the same column are not significantly different.

Table 6: Results of ANOVA One way and Tukey HSD test comparing the effect of beaches on abiotic factors

Variables	Year 2017					Year 2018				
	Beaches	Mean±SD	F	P-value	Sig	Beaches	Mean±SD	F	P-value	Sig
pH	B ₁	8.16±0.38 ^a	0.44	0.64	NS	B ₁	8.04±0.06 ^a	4.37	0.014	*
	B ₂	8.23±0.25 ^a				B ₂	8.08±0.07 ^{a,b}			
	B ₃	8.20±0.29 ^a				B ₃	8.09±0.08 ^b			
T	B ₁	22.57±3.04 ^a	0.53	0.53	NS	B ₁	17.9±1.76 ^a	0.721	0.488	NS
	B ₂	22.47±3.33 ^a				B ₂	17.35±2.04 ^a			
	B ₃	21.89±2.63 ^a				B ₃	17.49±2.19 ^a			
EC	B ₁	53.31±3.84 ^a	0.82	0.43	NS	B ₁	56.14±0.89 ^a	6.804	0.0016	**
	B ₂	53.33±3.84 ^a				B ₂	56.65±0.52 ^b			
	B ₃	54.30±2.34 ^a				B ₃	56.61±0.45 ^b			
TDS	B ₁	27.33±0.66 ^a	0.19	0.82	NS	B ₁	27.50±0.52 ^a	5.034	0.008	**
	B ₂	27.38±0.73 ^a				B ₂	27.73±0.36 ^{a,b}			
	B ₃	27.42±0.56 ^a				B ₃	27.85±0.51 ^b			
Sal	B ₁	35.47±2.07 ^a	0.02	0.97	NS	B ₁	36.71±0.57 ^a	2.771	0.067	NS
	B ₂	35.58±2.14 ^a				B ₂	36.67±0.84 ^a			
	B ₃	35.56±1.92 ^a				B ₃	36.97±0.18 ^a			
DO ₂	B ₁	7.01±0.54 ^a	0.31	0.73	NS	B ₁	8.11±1.01 ^a	0.468	0.627	NS
	B ₂	7.21±0.76 ^a				B ₂	8.31±1.18 ^a			
	B ₃	7.24±0.68 ^a				B ₃	8.07±1.22 ^a			

P: p-value, F: Factorial, Sig: Significance, (NS: Not Significant) : p > 0.05, (*: Significant Differences) : p ≤ 0.05, (**: Significantly Higher Differences): p ≤ 0.005 or p ≤ 0.001. Values having the same letter in the same column are not significantly different.

3.2 Bacteriological results

During the two cycles of 2017 and 2018, 24 surveys were conducted in three studied beaches from January 2017 to December 2018. One-way ANOVA results and seasonal variations of FIB at three studied beaches are shown successively in table 7, table 8 and Figure 2. The concentrations of fecal coliforms in seawater samples show a variable trend during 2017-2018 survey, with values ranging from a maximum value of 1020.83 ± 795.83 UFC / 100mL registered in spring season of 2017 to a minimum value of 13.89 ± 4.77 UFC / 100mL detected in spring season of 2018. Table 7 shows that no significant difference was observed in FS concentrations for both (B₁) and (B₂) in 2017 (p > 0.05) while, remaining results of FS were significantly higher difference (p < 0.01). For the concentrations of fecal streptococci, it ranged from a

minimum value (10.72±4.25 UFC / 100mL) in autumn season 2018 to a maximum value (496.06 ± 464.19 UFC / 100mL) in spring season 2018 (Figure 2). With regard to study of difference of FC concentrations between seasons showed in Table 7, it should be noted that seasonal variations showed a significantly higher difference (p <0.01) in all studied beaches except for (B₂) and (B₃) beaches in 2017 which were not significant (p > 0.05).

Study of difference between three studied beaches concerning FC and FS concentrations are shown in Table 8. For FS concentrations, a significant difference between beaches is registered during 2017 cycle and a significantly higher difference (p <0.01) during 2018 cycle. Whilst, the FC concentrations showed a significantly higher difference (p <0.01) between all studied beaches during 2017 and 2018. For the latter group of bacteria, a significant difference was also observed between years (Table 9).

During the 2017 cycle, more than 95% of FC results are lower than mandatory values (2000 CFU / 100mL) for beaches (B₁) and (B₃), while more than 95% of FC results are below guideline values (100 CFU / 100mL) for beach B₂. For the FS results, all three beaches showed values that exceed the guideline values (100 CFU / 100mL) with 20% for beaches (B₂) and (B₃) and 50% for beach (B₁). So, all three beaches during 2017 cycle are ranked B (Table 10).

Concerning 2018 cycle, more than 95% of FC results are lower than mandatory values (2000 CFU / 100mL) for three studied beaches. For the FS results, all three beaches show values that exceed the guideline values (100 CFU / 100mL) and don't respect the 90% percentile. So, all the three beaches during 2018 cycle are ranked also B (Table 10).

Table 7: Results of one-way ANOVA comparing the effects of seasons on the variations of FS and FC bacteria

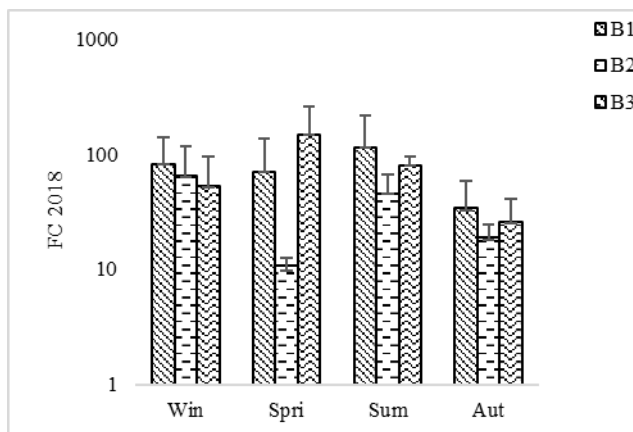
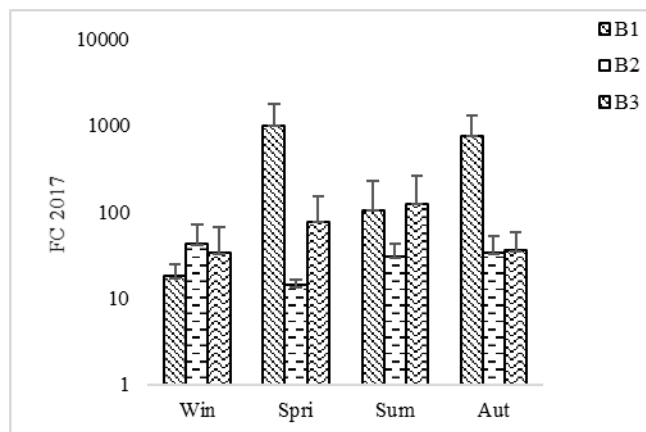
Years		Year 2017			Year 2018			
Variables	Beaches	F	P	Sig	Beaches	F	P	Sig
FS	B ₁	1.9442	0.1473	NS	B ₁	17.903	0	**
	B ₂	0.8289	0.4901	NS	B ₂	14.653	0	**
	B ₃	8.2238	0.0005	**	B ₃	3.92	0.0172	*
FC	B ₁	5.7229	0.0038	**	B ₁	3.4061	0.0293	*
	B ₂	1.9471	0.1468	NS	B ₂	6.634	0.0013	**
	B ₃	1.5709	0.2203	NS	B ₃	4.6998	0.0079	**

P: p-value, F: Factorial, **Sig**: Significance, (**NS**: Not Significant) : p > 0.05, (*: Significant Differences) : p ≤ 0.05, (**: Significantly Higher Differences): p ≤ 0.005 or p ≤ 0.001.

Table 8: Results of one-way ANOVA comparing the effects of beaches on the variations of FS and FC bacteria

Years		Year 2017					Year 2018				
Variables	Beaches	Mean±SD	F	P	Sig	Beaches	Mean±SD	F	P	Sig	
FS	B ₁	222.53±270.15 ^a	4.36	1.50E ⁻⁰²	*	B ₁	141.73±109.51 ^{a,b}	6.539	2.10E ⁻⁰³	**	
	B ₂	54.43±56.82 ^b				B ₂	74.25±79.45 ^a				
	B ₃	133.95±263.52 ^{a,b}				B ₃	248.34±330.07 ^b				
FC	B ₁	469.73±662.03 ^a	11.81	0	**	B ₁	128.86±196.68 ^a	5.15	7.00E ⁻⁰³	**	
	B ₂	30.96±22.04 ^b				B ₂	37.13±36.42 ^b				
	B ₃	70.30±99.97 ^b				B ₃	75.16±67.03 ^{a,b}				

P: p-value, F: Factorial, **Sig**: Significance, (**NS**: Not Significant) : p > 0.05, (*: Significant Differences) : p ≤ 0.05, (**: Significantly Higher Differences): p ≤ 0.005 or p ≤ 0.001. Values having the same letter in the same column are not significantly different.



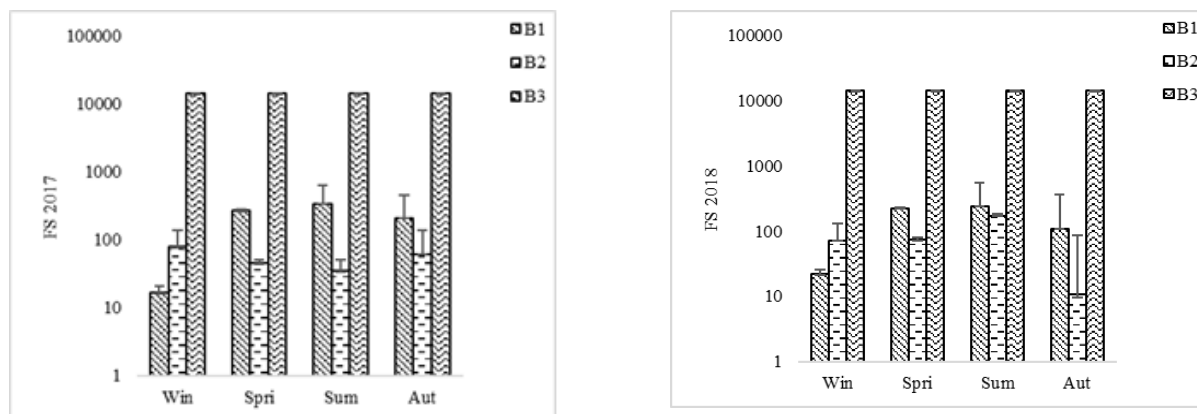


Figure 2: Seasonal variation of FC and FS concentrations in bathing water of the three beaches (The values presented are Mean ± SD (CFU/100mL).

Table 9: Results of one-way ANOVA comparing the effects of years on the variations of FS and FC bacteria

Variables	FS	FC
2017	136.92 ± 228.53 ^a	190.33 ± 431.23 ^a
2018	154.77 ± 216.35 ^a	80.38 ± 126.43 ^b
P	0.57474	0.01241
F	0.31588	6.3691
Sig	NS	*

P: p-value, F: Factorial, Sig: Significance, (NS: Not Significant) : p > 0.05, (*: Significant Differences) : p ≤ 0.05, (**: Significantly Higher Differences): p ≤ 0.005 or p ≤ 0.001. Values having the same letter in the same column are not significantly different.

Table 10: Classification of bathing water quality in the studied beaches

CYCLES	2017			2018			
	Beaches	B ₁	B ₂	B ₃	B ₁	B ₂	B ₃
Concentrations of FC per 100mL	CF80	50% ≤ 100*	100% < 100*	80% < 100*	66.66% < 100*	75% < 100*	91.66% < 100*
	CF95	100% < 2000**	100% < 2000**	100% < 2000**	100% < 2000**	100% < 2000**	100% < 2000**
Concentrations of FS per 100mL	SF90	50% ≤ 100*	80% < 100*	80% < 100*	41.66% < 100*	66.66% < 100*	50% < 100*

(*):Guide value and (**):Mandatory value.

3.3 Fecal contamination origin

Results of fecal contamination origin of three studied beaches are shown in Table 11. Contamination of (B₂) and (B₃) during the two cycles 2017 and 2018 have an animal origin (R lower than 0.7). Whilst, (B₁) showed mixed contamination predominantly human during 2017 cycle (2 ≤ R ≤ 4) and showed mixed contamination predominantly animal during 2018 (0.7 ≤ R ≤ 1).

Table 11: Ratio (R) of fecal contamination origin in seawater

Beaches	B ₁	B ₂	B ₃
2017	2.11	0.57	0.52
2018	0.91	0.5	0.30

3.4 Correlations between fecal bacteria and abiotic factors

Correlations between abiotic factors (pH, T, EC, TDS, Sal and DO₂) and fecal bacteria of seawater (FC and FS) are mentioned in Table 12. pH, EC and Salinity were not significantly correlated with FS and FC concentrations for the three studied beaches. Whilst, FS concentrations have significant positive correlation with Temperature (r=0.3506; p=0.003) and significant negative correlation with TDS (r=-0.3023; p=0.010) in (B₁). In addition, FC concentrations have significant positive correlation with DO₂ (r=0.3012; p=0.010) in (B₂). It must be noted that all remaining correlations were not significant.

Table 12: Correlations between fecal bacteria and abiotic factors (Pearson correlation test)

Beaches	Variables	pH	T	EC	TDS	Sal	DO ₂
B ₁	FS	r=0.1161 p=0.331	r=0.3506 p=0.003	r=0.0683 p=0.569	r=-0.3023 p=0.010	r=0.1950 p=0.101	r=-0.1704 p=0.152
	FC	r=0.1422 p=0.233	r=0.1308 p=0.273	r=0.1193 p=0.318	r=0.0540 p=0.652	r=0.0926 p=0.439	r=-0.1121 p=0.348
B ₂	FS	r=-0.0666 p=0.579	r=-0.0089 p=0.941	r=0.0114 p=0.924	r=0.0070 p=0.954	r=0.0100 p=0.934	r=-0.1443 p=0.227
	FC	r=-0.0287 p=0.811	r=-0.1217 p=0.308	r=-0.0104 p=0.931	r=-0.0183 p=0.879	r=-0.0564 p=0.638	r=0.3012 p=0.010
B ₃	FS	r=0.1514 p=0.204	r=-0.0249 p=0.836	r=0.1403 p=0.240	r=0.0771 p=0.520	r=0.1966 p=0.098	r=-0.0867 p=0.469
	FC	r=0.0995 p=0.405	r=0.1786 p=0.133	r=0.0089 p=0.941	r=-0.1225 p=0.305	r=0.1327 p=0.267	r=0.0383 p=0.750

p: p-value ; r: correlation coefficient (Correlation is significant at the 0.05 level)

4. DISCUSSION

Many published works reported that abiotic factors have a great influence on various cells activities and undergoes the action of many others external factors. Variability of temperature in the three sites could be explained by direct influence of regional climat characterised by a hot season in both spring and summer and a cold season in winter. In addition, minimum value of pH registred in winter could be explained by a dilution of seawater due to the heavy rains recorded during the winter season. While increase of pH registred in the region coincides with periods of increasing of phytoplankton during summer season [23]. The variations of pH can also be caused by the pollution of seawater by industrial discharges existing near to those beaches and by runoff discharges [24]. The means annual values of pH ($pH_{2017} = 8.20$ and $pH_{2018} = 8.07$) are close to the seawater pH value 8.3 [25].

The results of salinity show a low values recorded during the winter season which may be explained by the dilution of seawater by rainwater. While the high salinity recorded in the summer season would be due to the evaporation of seawater under hot temperature recorded during this season. The annual average increase in salinity observed between 2017 and 2018 could be explained by the decrease in wastewater discharges rejected without treatment into marine waters [17] following the installation of the two WWTPs Anza and Aourir.

EC also show a significant variation between seasons with a decrease in winter and an increase in summer. The decrease of the EC during the first three months of 2017 could be explained by dilution of seawaters by the contribution of rainwater [26]. Whilst, the elevated rate of EC between 2017 and 2018 translates the wealth and richness of marine waters by ionized mineral salts and by dissolved salts in the water [27,28]. Also, Ben Charrada *et al.*, (1997) relate increase of both EC and salinity in summer to seawater evaporation [29].

Concerning TDS, its concentration and composition in seawater were determined by several factors such as atmospheric precipitation, geology of the drainage and evaporation-precipitation phenomenon [30]. Seasonal variations of TDS concentrations result also from industrial effluents, changes to the water balance by limiting inflow, increased water use, increased precipitation or by salt-water intrusion [31].

Furthermore, our results about DO_2 are in accordance with the studies of Hebert *et al.*, (2000), who suggest that cold water is more concentrated in oxygen than hot water [32] and in desaccord with the results got by Chaouay *et al.*, (2016) [26]. Also, the high level of dissolved oxygene could be due to movement and turbulence of water masses during elevating level of waves. The drop in dissolved oxygen could be explained by its consumption by aerobic bacteria that degrade the contaminants i.e fertilizers, particulate matter and industrial wastes, that comes from raw wastewater, purified wasetwater or the discharges from river [33].

In the present work, monitoring of bacteriological contamination in three sites shows a significant seasonal variation and all of the three beaches during the two cycles conform to the Moroccan standards of bathing waters. The high concentration of FIB bacteria found in our work can be explained by the effluents from sewage treatment plants, industrial and from municipal one [34]. Also, fecal pollution of seawater by FC and FS may originate from wildlife, farming activities, human activities and from watershed [35]. Our obtained results are in contradiction with the results of the work established by Mimouni *et al.*, (2002) found in Anza beach [16]. These authors found that concentration of FC is 8.10^4 CFU/100 mL and concentration of FS is 7.9×10^3 CFU/100 mL conversely to our mean annual results (from 80.38 ± 126.34 to 190.33 ± 431.23 CFU/100 mL for FC and from 136.92 ± 228.23 to 154.77 ± 216.35 CFU/100 mL for FS. The situation of Anza beach in Mimouni *et al.*, (2002) studies is due to wastewater outlets in this beach classified in the category D [16]. This finding was consistent with that of Chouay *et al.*, (2014) who have given the same category (category D) to Anza beach in their studies [6]. They found that concentrations of Total coliforms, Fecal coliforms and Sulfito-reducing bacteria in Anza beach varied from 1.6×10^3 to 4×10^5 CFU/100mL, from 3×10^2 to 2.5×10^4 CFU/100mL and from 4×10^4 to 6.5×10^4 CFU/100mL respectively and which are also very high comparing to ours. Thus, the improvement of bathing water quality in this sector from comparing our results to those of Mimouni and Chouay may be due to the positive effect of the installation of the two WWTPs of Anza and Aourir on the marine environment [16-6]. These finding results in our work are in accordance with those found by Amorim *et al.*, 2014 [36]. There sampling done in Castelo do Queijo beach in Portugal, prohibited the swimming during the 2007 bathing season, presents a reduction in fecal contamination and presents good water quality during the study period (*E. coli* present a value of 59 ± 58 CFU/100ml and Intestinal enterococci has a value of 6 ± 6 CFU/100mL) [36]. They explained this change to the remoteness of the studied site from pollution sources and higher forcing by the hydrodynamic variables of the ocean [36].

Also, many studies attribute decrease of bacteria charge in seawater to abiotic and biotic factors. For example, action of temperature and solar irradiation on seawater may decrease and eliminate significantly fecal bacteria such as Total coliforms, *Escherichia coli* and Enterococci [37]. Studies of Richard *et al.*, (2004) also suggest reduction of indicator bacteria e.i. *E. coli* in large freshwater bodies due to solar inactivation [38]. Curtis *et al.*, (1992) explained elimination of these bacteria to its ultraviolet fraction which cause membrane damage of bacterial cell by leaving them permeable to mineral salts and modify its osmotic pressure [39]. The other abiotic factor which is the origin of concentration decrease of fecal bacteria is salinity. High salinity may be a stress factor of fecal bacteria in seawater [40] and provoke a decrease of fecal coliforms rate [41].

Studies of relation between dissolved oxygen and bacterial load done by Hughes (2003) in Antarctic seawater, proved that high concentrations of dissolved oxygen could inactivate microbes by solar UV radiation [40]. When oxygen is present at ultra-violet found in solar radiation, photochemical damage to *E. coli* increased [42,43,44,45]. The combination of UV wavelength and oxygen allows the formation of highly reactive free radicals causing cellular damage [46]. In Addition, the reduction of survival of fecal coliform is influenced by basic pH values [47,48]. Climatological conditions may also change and influence load of fecal bacterian seawater. For example, results of Eregno *et al.*, (2016) study indicate existence of acceptable risk for the bacterial and parasitic pathogens one day after rainfall event [49]. It can be origin of contamination for swimmers in recreational beaches in urban areas which receiving heavy rainfall and sewage overflows. Concerning biotic factors, many bacterial antagonists such as protozoa are capable to consume bacteria of seawater and decrease its rate [50].

In the current study, many interpretations could probably be attributed to the human and animal origins of fecal contamination found in our three studied sites. Human origin in general may be due to touristic activities, bathing activities and rainfalls. On the other hand, the animal origin may be due to wild animals living along the maritime coasts and dogs of the tourists. It should be noted that our results are in agreement with those of Lamine *et al.*, 2019 on the Taghazout coasts and who found mixed contamination predominantly human [51]. They attributed this origin to several sources such as urban waste and runoff into the sea without treatment [51]. The concentrations of coliforms and fecal coliforms showed during bathing activities have an average of 10^5 CFU/100 ml and 6.10^3 CFU/100 ml respectively, emphasizing that the concentrations in young children are very high compared to young and older couples [52]. Recent studies have also indicated that recreational waters are also polluted with fecal pollution from bird droppings [53,54,55,56]. In addition, a comparison made by Martin and Gruber (2005) along the algae line between marine vegetation that contains dog excrement remains and marine vegetation without dog fecal waste showed high concentrations of enterococci (5×10^5 CFU/g) in the first experiment compared to the second (5×10^4 CFU/g) [57]. Similarly, Mary E. Wright *et al.*, 2009 found in a recreational beach located on the island of Virginia Key east of the coast of Miami, Florida, in the United States, concentrations of higher enterococci observed in dog feces (7.4×10^6 CFU/g) followed on average by birds excrements (3.3×10^5 CFU/g), while shrimp fecal mounds present lower levels of enterococci (2.0 CFU/g) [20].

Results of correlation test between abiotic factors and FIB showed a significant positive correlation between FS and T and a significant negative correlation with TDS. FC show a significant positive correlation with DO_2 . Recent study monitored by Lamine *et al.*, (2019) showed a significant positive correlation between FS and all the environmental parameters T ($r=0.4198$; $p=0.001$), pH ($r=0.4936$; $p=0.000$), TDS ($r=0.4956$; $p=0.000$), Sal ($r=0.4712$; $p=0.000$), EC ($r=0.4683$; $p=0.000$), DO_2 ($r=0.5025$; $p=0.000$) and rainfall ($r=0.4621$; $p=0.000$), but these abiotic factors were not significantly correlated with FC except with a rainfall which has a significant positive correlation ($r=0.2859$; $p=0.022$) [51]. In addition, study of bacteriological and Physical-chemical variables in the Bay of Gulluk (Aegean Sea) during the period from 2012 to 2013 doesn't show any significant correlation between fecal bacteria density and salinity, although they found that both FC and FS bacteries were positively correlated with temperature [58]. They related this pollution situation to recreational, domestic, and industrial activities [58]. Similar studies conducted in the Bay of İzmir (Turkey) in the eastern Aegean Sea by Kacar and Omuzbukan (2017) found a negative correlation between both FC and FS levels and abiotic factors. They determined that higher indicator bacteria ratios were observed during periods of intense rainfall and low temperature [59]. Janelidze *et al.*, (2011) don't found any correlations between fecal pollution indicators and physical-chemical parameters (salinity, temperature, pH and DO_2) in Georgian coast of the Black Sea during the period of 2006 to 2008 [60]. But a correlation has been found between an increasing anthropogenic impact on the Black Sea and increased fecal contamination of seawater with seasonal variations [60]. Another monitoring report in Bushehr (Persian Gulf, Iranian) coastal waters published by Karbasdehi *et al.*, (2017), found that total coliform and FC concentrations showed an inverse correlation with salinity, but they argue that density of indicator bacteria increases with decrease in salinity [61].

5. CONCLUSION

In conclusion, physical-chemical and bacteriological results of the present study highlighted the different variability between seasons and between sites concerning each measured parameter. This variability specially of bacterian load can be related to biotic and abiotic factors existing in this aquatic area. In addition, these results, which classify all of the three beaches conform to the Moroccan standards of bathing waters, showed the amelioration of the bathing water quality specially in Anza beach. This beach, as many other areas within Moroccan coasts, was negatively influenced many years ago by the discharges of industrial and domestic wastewater without any treatment. But actually, this beach is classified B due to the installation of two new wastewater treatment plants (WWTPs) Anza and Aourir. Hence, implement of these WWTPs improve the significance of the wastewater treatment, provide a good results for bathing water quality, swimming safety for the public and demonstrates to bathing water quality managers that similar action in other polluted coasts may be a good solution to ameliorate and restore the health state of the beach.

6. REFERENCES

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