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Contribution to the Study of Bacterial Biodiversity in Atlantic and Mediterranean Coastal Waters in Morocco

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Abstract:- Located at the north-western tip of Africa, Morocco occupies a particular and unique geographical position as it is the only African country to have two Atlantic and Mediterranean facades participating in its richness in aquatic biodiversity. The importance of this wealth plays a significant role in economic and social development. This work proposes "a study of Bacterial Biodiversity in Atlantic and Mediterranean coastal waters in Morocco" by Physico-chemical, bacteriological methods aiming to analyze the current state of Bacterial Biodiversity in its waters. This study concerned seawater from 15 cities on the Moroccan coast at 18 sites spread over the Atlantic and Mediterranean coasts. The Physico-chemical results show fluctuations during the study period with a maximum peak during the summer season. The bacteriological results have shown the existence of 19 bacterial genus dominated by the vibrion and 86 species indicating a vast bacterial variety participating in the Moroccan marine biodiversity especially near the urban areas of large cities following the diversion excessive wastewater in their coasts without prior treatment and the convergence of the majority of big rivers towards these beaches.

Keywords:- *Seawater; Biodiversity; Physico-chemical; Bacteriology; Molecular.*

I. INTRODUCTION

Biodiversity is generally defined as a representation of species diversity within an ecosystem that also includes genetic diversity [1]. Climate-induced geographic changes are an essential factor in changing global biodiversity. Speed explains the spatial displacement of marine species [2], [3]. So far, 200,000 marine animal species have been described, ten times more than terrestrial species; about 20 000 species of marine plants (algae) and a much smaller number of microorganisms, namely viruses, fungi and bacteria have been identified. And regularly nowadays, new organisms are being discovered, despite all his efforts, 99% of Marine bacteria remain to be discovered, and the oceans become a vast world to explore [4]. The landscape of the planet's biodiversity has been Forged in the oceans, and only a few species have successfully passed through the marine universe and then diversified [5], [6].

Taking advantage of its privileged geographical position and like all other countries in the world, Morocco presents itself as an environmental heritage extremely rich in natural sources, it extends over a distance of about 3 500 km [7], from the city of Saïdia on the eastern Mediterranean to the southern Atlantic town of Lagouira (see figure 1) . It is an ecosystem of the great biological richness of fauna and flora that characterize its biodiversity [8], giving it a remarkable range of very varied bioclimates. The country to the north is under the influence of Mediterranean climate (512 km of coastline), to the west is under the oceanic influence (2,943 km of coastline), to the south and southeast is under the continental influence and in the centre and under the Saharan influence [9]. This diversity of terrain and climate corresponds to great bioecological diversity, as well as a wide range of natural environments: formations of forest, pre-Saharan and Saharan areas. The result is a wide diversity of species and genetics, which can be classified into three major types of ecosystems: terrestrial ecosystems, continental waters as well as Marin and coastal ecosystems. These ecosystems also contain a unique and specific microbial population for Morocco, as evidenced by recent discoveries of new bacteria for science [9].

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Fig. 1: Map of Morocco

II. OBJECTIVE

The objective of this work is to study the biodiversity of pathogenic and non-pathogenic microorganisms in Moroccan Atlantic and Mediterranean coastal waters by studying the physicochemical, microbiological and molecular parameters to analyze the biodiversity of our heritage marine microbial and the factors influencing its wealth.

III. MATERIALS AND METHODS

A. Samplings and Sampling sites

Sampling was carried out during the period 2021 and 2022 and beginning of 2023 the seaside season from June to December by taking in sterile barrels (5 litres) of seawater at 30 cm below the surface [10], [11]; The study sites were chosen based on the importance of the frequentation, the nature of the sites (relief, shape of the shore ...) and the particular risks of pollution which may exist (rejection of sewage, mouths of rivers, ports, etc.). 15 cities were the subject of our study, spread on the coasts of the Atlantic and the Mediterranean coasts of Morocco from north to south as shown by the geographical coordinates as shown in table 01 (**Nador, Al Houceima, Martil, Madiaq Tanger, Larache, Kenitra, Rabat, Mohamadia, Casablanca, El Jadida, El Oualidia, Safi, Agadir et Laayoune**). In total, we have chosen 18 sampling sites with 2 sites in the city of Laayoune and 3 sites at the level of the city of Casablanca due to the demographic, economic and the broad extent of their coastlines influence. 36 samples were collected comprising 18 samples for bacteriological and molecular analyzes and 18 for Physico-chemical analyzes. The samples were transported from the sites studied to the analytical laboratory in an insulated cooler between 2 and 5 ° C (ISO 8245 1999) [12] so that the Physico-chemical parameters and the initial germ content in the samples would not be changed or modified [13].

Table 1: Geographical coordinates of sampling sites.

City	Geographic coordinates of sampling sites		
I- Nador	35°6’49.24’’N	2°42’58.47’’W	(beach Kariat Arkamane)
II- Al houceima	35°14’40.98’’N	3°55’33.00’’W	(beach Quemado)
III- Martil	35°37’22.15’’N	5°16’18.42’’W	(beach Martil)
IV- Madiaq	35°43’18,31’’N	5°20’12.10’’W	(beach Kabila)
V- Tanger	35°46’34.01’’N	5°47’19.86’’W	(beach Tanger)
VI- Larache	35°12’18.59’’N	6°09’01.64’’ W	(beach Peli grosa/ river Loukous)
VII- Kenitra	34°15’27.51’’N	6°40’41.45’’W	(beach Mehdia/ river Sebou)
VIII- Rabat	34°02’09.32’’N	6°50’11.12’’W	(beach Rabat/ river Abi Raqraq)
IX- Mohamadia	33°42’26.43’’N	7°23’21.22’’W	(beach Mohamadia/Port)
X- Casablanca	(1) 33° 38’ 06’’ N	7° 30’ 38’’ W	(large collector of beach Zenata)
	(2) 33°36’24.00’’N	7°38’5.16’’ W	(mosque Hassan II)
	(3) 33°35’20.82’’N	7°40’54.80’’W	(beach Ain Diab/ coffee shop Nzaha)
XI- El jadida	32°18’50.01’’N	9°15’02.08’’W	(beach El jadida)
XII- El oualidia	32°44’2.40’’N	9°02’31.20’’W	(lagoon El oualidia)
XIII- Safi	32°18’58.47’’N	9°15’03.15’’W	(beach Safi)
XIV- Agadir	30°25’11.66’’N	9°36’34.34’’W	(beach Agadir)
XV- Laayoune	(1) 28°03’00.05’’N	12°13’29.05’’W	(lagoon Naila)
	(2) 27°05’37.23’’N	13°25’18.75’’W	(beach Laayoune).

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IV. TECHNIQUES USED

A. Physico-chemical parameters :

The Physico-chemical analysis includes the measurement of Temperature, pH, Conductivity, Turbidity, Salinity, dissolved O2 that were analyzed in situ via Mettler Toledo, a type of calibrated multiparametric portable device [14]. Other parameters were analyzed in the laboratory such as the determination of nitrites by the Zambelli Method [15] and finally the measurement of the Biological Oxygen demand in 5 days (BOD5) according to the method of international standards ISO 5815-1 [16], [17].

B. Bacteriological tests:

The bacteriological analysis required the recovery of the germs by filtration of 100 ml of seawater on a porous membrane (0.45 µm), the standard method ISO 6579 has been applied: 1993 [10]. The latter is deposited in a Brain Heart Infusion (pre-enrichment) broth at different concentrations of NaCl (2.5 g / L, 5 g / L and 10 g / L) to increase the chances of growing marine bacteria... For the isolation of the strains, transplant on the solid medium BHI corresponding to the same concentration of NaCl (2,5 g / l; 5 g / l and 10 g / l) and finally identification of the bacteria cultivated by the colouring of GRAM control, test of oxidase, catalase and then the revelation of their biochemical characters through the API20 gallery [18].

C. Molecular Component :

Salmonella DNA is extracted by the rapid heat method (Boiling-prep) [19], 20 ml of seawater has been centrifuged at 10,000 rpm for 20 minutes, then the pellet is washed with a solution of distilled water, the pellet is recovered and resuspended in 200 .mu.l of molecular biological water, lysed by thermal action in a water bath at 100.degree. C. for 10 min and then centrifuged at 13 000 g (12,000 rpm) for 5 min, the supernatant is recovered and stored at -20 ° C until use [20]. For the extraction of DNA from the other cultivable strains, we used the technique of chloroform phenol Alcohol-Isoamylic PCA [21], verification of the quality of the DNA extracted by electrophoresis on agarose gel and quantification by spectrophotometric dosage at 260 nm. PCR amplification from 1 µl of genomic DNA was performed in the presence of the final concentration of Tampon Gotaq at 5x; 2.5 mM MgCl 2; 0.2 mM of each dNTP (Qiagen); 0.08 µM of ribosomal universal identification primers of m16 s bacteria (27f: 5'-AGAGTTTGATCCTGGCTCAG-3 'and 1492r: 5'-GGTTACCTTGTTACGACTT-3 'and 5U / µl of GoTaq DNA polymerase (Promega, France) in a final volume of 15 µl. The program used for the PCR is 35 cycles with a Hybridization T ° of 55 ° C: 95°C-2'/ (94°C, 40''/55°C, 40''/ 72°C-1') x 35 / 72°C-10' / 4°C-5'). The PCR product was monitored by electrophoresis on 1% agarose gel under a voltage of 120 volts for 32 minutes [22].

The PCR product was purified using ExoSAP-IT (Exonuclease I and Shrimp Phosphatase Alkaline in Buffer). The sequencing reaction was carried out with the MicroSEQkit® 500 16S rDNA according to the principle of incorporation of a fluorophore-labelled dideoxynucleotide. The sequences were then obtained using an ABI Prism Model 377 Automated Sequencer; after 5% polyacrylamide gel electrophoresis, the data collection was carried out using software (Sequence analysis), then corrected on the Finch TV and then analyzed in the BLAST GenBank nucleotide library by alignment on their closest related sequences [23].

V. RESULTS

A. Results of physic-chemical parameters:

In terms of the Physico-chemical results for all the sites studied, we found that the temperature values meet the standards (<25) with a fluctuating profile and a higher rate (25.7 ° C) it was recorded in summer in the lagoon Naila de Laayoune while the lowest value was observed during the winter on the Tangier beach (16.8 ° C) (Table 2). Measurements of the pH of seawater show values ranging from 8.1 in winter at site 2 in Casablanca City and 8.7 in summer at Quemado Beach in Al Houceima City (Table 2). This result fluctuates around the optimal value of the seawater pH (8.3), indicating the buffer effect of the sea. The salinity and the electrical conductivity of the seawater of the studied coasts show a significant temporal variation, the highest was detected in the summer of 2018 of the order of 36 mg /l for the salinity in the Casablanca site 1, and 58.2 ms/cm for the conductivity in the 2 site of the Laayoune beach. In return, the following minimum values were recorded in February 2017 (salinity = 34.2 g / l in El Oualidia Lagoon, conductivity = 50 ms/cm in Mehdia / wad Sebou Beach of Kenitra City) (see Table 2). It indicates a permanent increase in the conductivity, showing strong mineralization of the water of the beaches studied.

During the study period, the recorded turbidity rate is high and shows temporal fluctuations in all sites studied, with a maximum of 37.2 NTU in 2017 in Casablanca Site 1 and a minimum of from 11.4UTN in summer 2018 to Al Houceima, and it has also been noted that all the Mediterranean sites have meagre Turbidity rates compared to those of the Atlantic. It should be noted a sharp increase in the value of nitrites that exceeds 0.2 mg / l, especially in summer in the Atlantiques sites of major cities such as Casablanca (site 1: 0.28 mg / l) and some sites on the Mediterranean coast such as Martil beach (0.24 mg / l) indicating the presence of significant pollution, but sites 1 and 2 in Laayoune city reveal minimum values of the order of 0.008 mg / l and 0.007 mg / l in summer then 0.004 mg / l and 0.005 mg / l in winter; on the other hand, in all the sites, Nitrite levels are declining considerably in winter. The dissolved O2 experienced an increase in winter with a maximum value of 47% raised at site 1 in Laayoune city, but in summer almost all values decreased to a minimum value of 26% in the study site of the city of Safi. The BOD5 reports very high rates whether it is in summer or winter for all the sites with a dominance of the maximal values observed in summer in Safi of 1002 mg of O2 / l, however, in Laayoune city, it was raised in winter a minimum rate of 2.5 mg O2 / l.

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Table 2: Evolution of physico-chemical parameters in the sites studied.

Site		Temperature °C		pH		Conductivity ms/cm		Turbidity UTN		Salinity mg/l		Dissolved O_2 %		Nitrites mg/l		BOD5 mg d'O_2/l	
		Eté	Hiver	Eté	Hiver	Eté	Hiver	Eté	Hiver	Eté	Hiver	Eté	Hiver	Eté	Hiver	Eté	Hiver
I		23,5	17,6	8,5	8,3	54,5	52,5	13,3	15,4	34,4	32,5	31,5	33	0,23	0,17	101	72
II		22,5	17,7	8,7	8,3	55,8	53	11,4	15,5	33,2	341,5	35	38	0,22	0,18	123	88
III		24,5	17,4	8,6	8,5	53,2	51,2	12,5	13,5	35	33	38	40,1	0,24	0,20	108	91
IV		25,2	17,2	8,52	8,2	56,8	52	14	16,5	33	32	34,2	37,2	0,18	0,14	121	90
V		22,2	16,8	8,6	8,3	55,7	54	15,1	17,2	34	31	32	35	0,12	0,08	87	62
VI		24	18	8,7	8,2	52,7	49,5	23	33	32,1	31,5	43,3	44,5	0,18	0,17	64,5	61,2
VII		23,2	17,5	8,5	8,1	54,5	50	22	29	34,4	33,2	39,2	40,3	0,17	0,15	66,5	63,5
VIII		23,5	17,5	8,75	8,52	55	50,8	23,5	27	33,5	30,2	40,2	41	0,21	0,19	228	187
IX		24,5	17,9	8,69	8,33	54	53,9	24,5	36	33,2	32,6	41,5	43,4	0,20	0,22	292	114
X	1	20,9	17,7	8,3	8,3	58	56	29,4	37,2	36	35	30	45,5	0,28	0,25	60	56
	2	21,9	17,3	8,45	8,15	56	54	28,7	38,9	33,5	32	39	43,8	0,19	0,17	82	78
	3	20,5	17,5	8,3	8,4	53,5	49	31	36	32	30,5	37	42,8	0,17	0,15	67	62
XI		23	17,5	8,23	8,26	54,5	53	25,6	32,7	32,7	341,3	33,4	35	0,14	0,13	450	381
XII		24	17	8,28	8,29	58	56	23	26,5	36,1	34,2	38	40	0,33	0,30	485	373
XIII		25	18	8,38	8,45	55,9	52,3	32,3	34,3	33,2	31,3	26	36,2	0,29	0,27	1002	987
XIV		23	19	8,56	8,2	56	51	29,9	36	32	30,3	37	40	0,19	0,17	299	280
XV	1	24,5	22,5	8,31	8,34	57,1	54,5	21	29	34	31	38	47	0,006	0,004	3,2	2,8
	2	25,7	22	8,23	8,25	58,2	56,3	24,3	28,1	35	33	36	45	0,008	0,005	3,1	2,5

B. Bacteriological results:

The bacteriological results of the sites studied show a certain diversity of bacteria in the different sampling areas dominated by enterobacteria species. In winter, all the studied sites have a large and diverse bacteriological load, including pathogenic and non-pathogenic germs, notably in large cities such as Casablanca (see Table 3). It may be due to the increase of the demographic and industrial agglomeration, not to mention the various wastewaters in the sea waters of these cities without prior treatment. While the sites of the Mediterranean littoral and even in winter, have a low bacterial load compared to Atlantic coastal sites with the presence of some pathogens germs. In the summer, however, there was a decrease in this bacterial load at all the studied sites with shallow values inflated at the sites in Laayoune city littoral. Other strains could not be identified by the API20 gallery, which led us to make the molecular tool.

Table 3: The Bacteria identified by the API20 gallery in the studied sites.

Species / Site	E. coli	Klebsiella pneumonaee	Aeromonas hydrophila	Klebsiela oxytoca	Proteus sp.	Morganella morganii	Staphylococcus haemolyticus	Staphylococcus hominis,	Enterobacter cancerogenus	Proteus Mirabilis	Bacillus subtilis	Enterobacter sp	Acinetobacter baumannii	Shigella sp.	Serratia	Staphylococcus aureus
I	+++	+++	++	++	++	++	+++	+	++	++	+	++	+	++	+	++
II	++	-	++	+	++	++	++	+	+	+++	+	++	++	-	+	++
III	++	++	++	++	++	-	+	++	+++	++	++	+++	++	++	++	++
IV	+	--	+	++	+	++	++	++	++	+++	+	+	-	-	+	+
V	++	++	+++	+++	+	+++	++	+	+	++	++	+++	++	+++	-	+++
VI	+++	++	+++	++	++	++	++	+	+++	++	-	++	++	++	++	++
VII	+++	+++	++	++	+	+	+++	++	+	+++	+++	++	++	-	+	+
VIII	+++	++	+++	+++	++	+++	++	+	+++	+	++	+++	+	++	+++	+++
IX	+++	+++	+++	+++	++	+++	++	+++	++	+++	++	++	+++	+++	+++	++
X	+++	++	++	++	++	++	+++	++	+++	++	+++	+++	-	++	-	++
XI	++	++	+++	++	+++	++	+	+++	+++	+++	++	++	++	++	++	++
XII	+++	++	+	++	-	+	++	+++	++	+++	+++	+++	++	+++	+	+
XIII	+++	+++	++	+++	+++	++	+++	+	+++	+++	+++	+++	+++	+++	++	++
XIV	+++	++	++	++	+	++	++	+	++	+++	++	++	+	++	+	+
XV	++	+	+	-	-	++	+	-	-	+	+	++	-	-	+	-

+++ : Very abundant.
++ : Less abundant.
+ : Not very abundant

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C. Molecular results:

The PCR concerned all the isolated strains, identified or not by the API20 gallery.

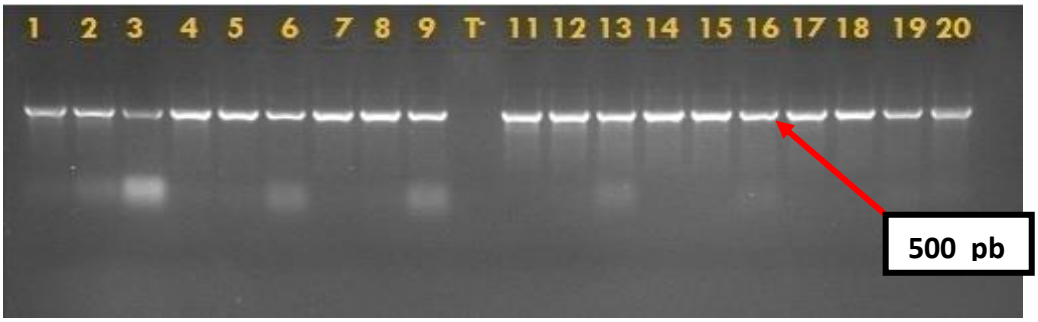

Fig. 2: PCR results presented on a 1% agarose gel.

Figure 2 shows an example of the results of the PCR performed. The PCR fragments were sequenced. A total of 86 sequences were obtained and filed in GenBank under the access numbers listed in Table 4.

Table 4: Alignment of sequences and their homologies

N° Echan.	Strain	GenBank no.	16S rDNA identification (closest neighbor)	Sequence similarity (%)
1	A1.2	KU195332.1	Escherichia coli	99 %
2	Ha15	MG238582.1	Klebsiella pneumoniae	99 %
3	201709CJKYOP-60	MH093817.1	Vibrio sp.	99 %
4	MF457871.1 Klebsiella sp. strain 211B	U85838	Klebsiella sp.	97 %
5	298.2.1	KX454095.1	Bacillus subtilis	100 %
6	0013	KP236158.1	Shewanella algue	100 %
7	DK1	MK053775.1	Paenibacillus sp.	99 %
8	C40 16S	MF521962.1	Lysinibacillus fusiformis	99 %
9	XH219	KY623288.1	Staphylococcus hominis	100 %
10	11	NR_152638.1	Paenibacillus aquistagni	99 %
11	X6-1 16S	MK053775.1 KT152825.1	Paenibacillus sp	95 %
12	YL15 16S	KP859510.1	Shewanella sp.	99 %
13	1CCIP2	KM025387.1	Staphylococcus aureus	99 %
14	201709CJKYOP-59	MH093816.1	Vibrio sp.	99 %
15	LC2016-1	MF589233.1	Shewanella sp.	99 %
16	0102 16S	KP236237.1	Shewanella indica	100 %
17	HS5-MRL 16S	KX128922.1	Lysinibacillus fusiformis	96 %
18	16S SDT1S3	JQ045822.1	Serratia sp.	94 %
19	QY170324	MK156693.1	Vibrio alginolyticus	99 %
20	CSMCRI 1072	JQ665335.1	Vibrio parahaemolyticus	98 %
21	SPJ9 16S	MG575436.1	Vibrio parahaemolyticus	98 %
22	SD-6 16S	KF994954.1 KT759091.1 KT759091.1	Aeromonas hydrophila	99 %
23	SGS 16S	MK121204.1 MK121204.1	Shewanella sp.	100 %
24	S110 16S	KR902615.1	Bacillus thuringiensis	99 %
25	CHS 3 16S	KR148956.1	Shigella sp.	100 %
26	H1	AM235884.1	Staphylococcus sp.	100 %
27	FRM72 16S	KX233853.1	Enterobacter hormaechei	99 %
28	YB-3	MF661927.1	Bacillus sp.	99 %
29	GPUS12 16S	MF398407.1	Acinetobacter baumannii	99 %
30	Oa17A 16S	MG461561.1	Paenibacillus alvei	97 %

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31	DGT5 16S	KX768307.1	Bacillus sp	98 %
32	Drngp 16S	EU855791.1	Isolat de Lysinibacillus	99 %
33	BAB-5843 16S	KX350217.1	Lysinibacillus sp.	94 %
34	B 16S	MH844557.1	Enterobacter cancerogenus	97 %
35	USC-26001	MK156693.1	Vibrio alginolyticus	96 %
36	CSMCRI 1072	JQ665335.1	Vibrio parahaemolyticus	99 %
37	16S WEED7	EF584106.1	Bactérie Vibrionaceae	99 %
38	CO4 16S	MG099654.1	Vibrio sp.	99 %
39	SD-6 16S	KF994954.1	Aeromonas hydrophila	99 %
40	DS2015	KR866070.1 KR866070.1	Morganella morganii	99 %
41	JLT199 16S	KX989247.1	Vibrio sp.	98 %
42	OFM3	MH542324.1	Enterobacter cloacae	100 %
43	P * 29 16S	EU586317.1 EU586317.1	Paenibacillus sp.	89 %
44	OLM52 16	MH542268.1	Enterobacter cloacae subsp	99 %
45	LTB3 16S	KC210850.1	Shewanella algue	100 %
46	16S 16S4	MH361599.1	Shewanella chilikensis	96 %
47	211B	MF457871.1	Klebsiella sp.	97 %
48	201709CJKYOP-32	MH093789.1	Vibrio sp.	99 %
49	EC1704-1	MG602205.1	Escherichia coli	99 %
50	CLC-F19	MH518201.1	Staphylococcus sp.	94 %
51	BR-SR 16S	MH137743.1	Shewanella algue	96 %
52	AYQ-1 16S	MH934954.1 MH934954.1 MH934954.1	Proteus mirabilis	97 %
53	FDAARGOS_575	CP033732.1 CP033732.1 CP033732.1	Staphylococcus hominis	99 %
54	201709CJKYOP-48	MH093805.1 MH093805.1 CP033732.1 CP033732.1	Vibrio sp	99 %
55	SWFU2928	JN935016.1	Bacillus amyloliquefaciens subsp.	99 %
56	H14	MH750681.1	Proteus sp.	97 %
57	HV6-S 16S	MK000713.1	Staphylococcus aureus,	99 %
58	AMI-3 16S	MK184207.1	Paenibacillus macerans,	90 %
59	16S SWTPB26	MG892813.1	Lysinibacillus macroides	98 %
60	DGT5	KX768307.1	Bacillus sp.	98 %
61	B8 16S	KY038189.1	Staphylococcus lugdunensis	99 %
62	JLT1942	KX989275.1	Vibrio sp.	93 %
63	OLM44 16S	MH542264.1	Staphylococcus haemolyticus	98 %
64	AP73 16S	MG564754.1	Vibrio parahaemolyticus,	99 %
65	IRB2AS6w	KU892723.1	Algues Shewanella	96 %
66	H78 16S	MF372630.1	Staphylococcus hominis,	97 %
67	NR7 16S	EU784844.1	Staphylococcus sp	95 %
68	384	JQ012981.1	Photobacterium sp	96 %
69	AN38	JQ409380.1	Vibrio nereis	98 %
70	NCTC8267	LR134153.1	Salmonella enterica subsp	94 %
71	F44 16S	MH559818.1	Klebsiella pneumoniae	98 %
72	HPC149 16S	HM551121.2	Enterobacter sp	96 %

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73	JLT1926	KX989269.1	Vibrio sp.	98 %
74	FCC058	KF360268.1	Cronobacter sakazakii	98 %
75	PRK1	KY050735.1	Bacillus sp	88 %
76	SGJ326	KF360275.1	Cronobacter sakazakii	84 %
77	11 ARN	NR_152638.1	Paenibacillus aquistagni	96 %
78	RM4 16S	FJ855135.1	Acinetobacter baumannii	95 %
79	thr-KHG 16S,	KX276172.1	Bacillus aryabhattai,	88 %
80	OLM44 16S	MH542264.1	Staphylococcus haemolyticus	94 %
81	NCTC6086	LR134187.1	Salmonella enterica	96 %
82	BGWA1 16S	MH793469.1	Enterobacter hormaechei	95 %
83	16S OG32	FR839342.1	Enterobacter sp	98 %
84	OS12 16S	JQ905072.1	Bacillus circulans	82 %
85	RCB810	KT261022.1	Escherichia coli	97 %
86	ZLynn1000-46	KY316464.1	Bacillus sp	99 %

After sequence alignment, we noticed that the bacterial strains that were identified by the API20 gallery are the same as those found by the analysis of their sequences. Thanks to this technique, we could also identify the bacteria that were not revealed by the classical gallery. In total, we identified 86 bacterial strains housed in 19 genera (see Table 4) and dominated mainly by the genus *Vibrion (sp, alginolyticus, parahaemolyticus, nerei)* which represents 17.43% of the identified bacteria with sequence similarity that varies from 96 to 100% followed by *Staphylococcus (sp, aureus, haemolyticus, hominins, lugdunensis)* and *Bacillus (sp, circulans, aryabhatta, amyloliquefaciens subsp, subtilis)* which occur with a percentage of 12.78% of the identified strains. And a sequence similarity that varies successively between 94 to 100%, 82 to 99%.

The genus *Shewanella (sp., Algae, chilikensis, indica)* occurs with a sequence correlation between 96 to 100% and 9.29% of the isolated bacteria. *Paenibacillus (sp, aquistagni, alvei, macerans)* exposed sequence similarity from 88 to 99% and then 6.97% of identified strains. *Enterobacter (Hormaechei, Cloacae subs, Cloacae, Carcinogenus)* were identified with 5.81% of the detected bacterial flora with a similar sequence of 95-100%.

Lysinibacillus (macroides, sp, fusiforms) is 4.64% of the total species detected and similar sequences that range from 94 to 99%. The genus *Klebsiella (pneumonia, sp)* is like *Lysinibacillus*, with 4.64% of the strains identified against the similarity of the sequences varies between 97 and 99%. *Escherichia (coli)* have percentage identification of 3.48% and similarity sequences ranging from 97% to 99%. With the same percentage of identification which is 2.32%, one has *Salmonella (enterica, enterica subsp), Acinetobacter (baumannii), Proteus (sp, mirabilis)* and *Cronobacter (sakazakii)* but they have similarities in sequence successively between 94 to 99%, 95 99%, 97% and 84%, 98%. Finally, we isolated *Serratia (sp), Shigella (sp), Morganella (morganii), Photobacterium (sp), Aeromonas (hydrophilic)* which have 1.16% of the identified bacteria and correlation sequences simultaneously of 94%, 100%, 99%, 96%, 99 %.

VI. DISCUSSION

During the study phase, the seawater temperature showed an almost similar annual variation in all the studied sites with a minimum recorded in winter and a maximum in summer. Consequently, water temperatures recorded at different sites generally indicate good water quality. The pH also shows an increase in summer and a decrease in winter. High pH values can be caused by spring plankton, flowering, which is responsible for the reduction of acidity due to an unbalanced pH with chlorophyll levels [24]. Another study indicates that low pH values (acidity) could be related to the rainfall period that causes a massive supply of continental waters loaded with suspended materials and CO2 [25] hence the increase in acidity in winter.

Salinity and conductivity also indicate an increase in summer and decrease in winter. The rise of the atmosphere temperature in summer generates the evaporation of the seawater, which leads to an increase in salinity [26]. In contrast, the rainwater carried by the wadis and released in the sea causes marine environment dilution, and consequently, the reduction of the salinity and the conductivity in winter.

The cycle of dissolved oxygen concentration is almost similar in all sites studied, with a decrease in summer and an increase in December (Autumn). Generally, dissolved oxygen is influenced by water temperature, tides, and water [27]. Winter seems to improve the oxygen content of seawater slightly because it limits bacterial growth and therefore minimizes oxygen consumption. According to Khattabi [28], the dissolved oxygen content is the result of a large number of biotic and abiotic factors, depends on the biological activity of the organism and the environment, that is to say, the balance between photosynthesis and environment, breathing, winds and temperature. Nitrite, a natural substance in the nitrogen cycle in aquatic environments, is generally occurred at low concentrations in marine environments. However, contamination of seawater by bacteria from industrial waste and poorly treated wastewater can result in denitrification by several facultative anaerobic bacterias that reduce nitrates to nitrites [29], [30]. This is in agreement with our results of nitrite measurements found in almost all the sites of our study, showing a significant increase in the value of nitrites exceeding 0.2 mg/l, indicating the presence of significant pollution at different levels for the two sites in

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Laayoune city whose values are significantly reduced, probably the consequence of the reduced urbanism, which is one of the main factors in the contamination of coastal waters [31]–[33].

Turbidity is associated with the occupancy of various organic particles, mainly: clay, colloids and plankton; it can be promoted by rainfall [34]. The measured results show a significant rise in all the studied sites where it reaches a maximum of 38.9 (UTN) in winter in site 2 of Casablanca city and with a minimum recorded in summer in Al Houceima city. BOD5 is an indicator of organic pollution; it exposes the level of biodegradability of the effluent [35]. There has been a significant increase in Safi city beach reaching a maximum of 1002 mg O2/l in summer but remains high compared to those found by Ekweozor. 2001 in Nigeria [36], Endamana et al. 2003 in Cameroon [37] and Raweh & al., 2011 [38]; and a minimum of 2.5 mg O2 / l in winter at the site of Laayoune city.

The results obtained from bacteriological analyses revealed the existence of 19 genera with predominantly *Vibrion*-like bacteria (Gram-negative bacilli, oxidase-positive) and 16.85% of all identified bacteria especially in winter on the sites of the Atlantic cities such as Larache, Kenitra, Rabat, Mohammedia, El Jadida, El Oualidia, Safi and Agadir. In summer, this dominance decreases remarkably in most of these cities; it was also noted that the detection of *vibrion* at the sites of the Mediterranean cities is almost nil. This result is probably related to the oligotrophic nature of the Mediterranean coastal zone and the effect of solar radiation and temperature in summer. Besides, it promotes and accentuates the phenomenon of self-purification of the oceans, thus negatively affecting the viability of the majority of bacteria in seawater [39]–[41]. Ramamurthy et al. (2014) [42] have shown that this family of bacteria can be transformed into a viable but uncultivable form under stress conditions, such as cold, oligotrophic environments, and/or at a high light intensity.

Secondly, after the *Vibrion*, our results unveiled the presence with equal (12,32%) the genus *Staphylococcus (species: Aureus, Hominis, Haemolyticus, sp)* and the *Bacillus (species: sp, subtilis, Circulans, Aryabhattai, Amyloliquefaciens sub sp).* However, *staphylococc*i are Gram-positive, oxidase-negative, catalase-positive cocci and are commonly associated with skin, mucous membranes, and the human body; certainly, 30% of the human population are healthy carriers [43]. *Staphylococcus Aureus* is the most pathogenic species, it causes many diseases of varying intensity, such as boils, abscesses, wound infections, there are in the environment of strains more dangerous strains by its resistance to antibiotics such as methicillin [44]–[48].

The genus *Bacillus* (Gram-positive Bacillus, negative oxidase and catalase-positive) consists of several phylogenetically diverse species; they are ubiquitous in the terrestrial and marine environment [49]. As a result, there is a variety that lives on the continent and can reach the marine environment as a result of numerous environmental transfer factors. Another variety of exclusively marine origin that has spread in coastal seawater because of seabed deposits. It has been shown that the only difference between these two species is the fact that the in vitro propagation of marine Bacillus necessarily requires the presence of seawater [50].

The bacteria of the genus *Shewanella* are mobile bacilli, oxidase and catalase-positive, Gram-negative and non-fermentative; present worldwide, mainly in marine and other underwater environments [51]; these germs have also been reported in soil, fish, meat, poultry and dairy products [52]. Most human *Shewanella* infections have been reported in warmer regions, particularly in Southeast Asia, Southern Africa, Southern Europe and the Caribbean [52]–[54]. The genus *Paenibacillus* comprises more than 89 species of facultative anaerobes forming endospores, and are heterotrophic bacilli, flagellates, negative oxidase, positive catalase and low Gram-positive, which were previously included in the genus *Bacillus* and reclassified as a separate gender [55]. These bacteria have been detected, particularly, in water, soil, in some insects and as well as in clinical specimens [56].

The accelerated spread in the aquatic environment of antibiotic-resistant *Enterobacteriaceae* through wastewater has conceived a threat to public health [57, p. 1], [58]. Recently, CPK (Creatinine-Phospho-kinase) -producing bacteria have been reported in wastewater, urban lakes, rivers and streams, highlighting their adaptability to different aquatic environments which further increases epidemics [59], [60]. The genus *Lysinibacillus* are gram-positive bacilli, oxidase and catalase-negative, aerobic, sporulated with flagella. Most of its strains have been isolated from soil, others from organic solvents, fermented foods and surface waters [61]. The genus *Klebsiella*, *Escherichia*, *Acinetobacter*, *Proteus*, *Cronobacter*, *Serratia*, *Shigella*, *Morganella* and *Salmonella* belong to the *Enterobacteriaceae* family which are Gram-negative bacilli, Oxidase negative, catalase-positive (except *Shigella*: catalase-negative), Non-demanding (relaxed culture except for *Salmonella*), immobile or mobile, ferments glucose (with or without gas production), optional aero-anaerobes. They can affect the gastrointestinal tract by causing severe foodborne illness, especially when shellfish farms are present in polluted marine areas, which usually indicates the degradation of seawater quality by wastewater [62]. Furthermore, it should be noted that during a subsequent study conducted in 2014 on the three sites in the AIN SEBAA-ZENATA beach in Casablanca, we found that the isolation of Salmonella was critical by conventional techniques and in traditional culture environments, hence our use of molecular biology by PCR (Polymerase Chain Reaction) which allowed us to highlight the Salmonella genus [20].

Photobacterium species are Gram-negative, positive-oxidase, catalase-positive Cocco-bacilli that are distributed in the marine environment and derive their nomenclature from their ability to emit visible light [63], [64]. *Aeromonas hydrophila* is a Gram-negative, mobile, facultative anaerobic, oxidase and catalase-positive Bacillus; it causes serious infectious diseases such as gastroenteritis and wound infections to humans, it is also identified in many animals and one in a large number of aquatic species such as fish [65].

VII. CONCLUSION

During our study, we were able to identify 86 species (Gram-positive and negative bacilli, Gram-positive cocci) thanks to the API20 gallery and through the molecular tool. It was probably the first study to identify bacterial biodiversity in the majority of the Atlantic and Mediterranean cities in Morocco. Bacteria are of marine and/or terrestrial origin with a high bacterial load during the winter season, unlike in summer, where there is a decrease in the bacterial arsenal, probably due to the phenomenon of self-purification of the oceans and sun rays. It should also be noted that the use of PCR (Polymerase Chain Reaction) targeting the 16s RNA gene sequence typical to all bacteria is a promising technique for the identification of non-cultivable and/or non-identifiable strains of bacteria by classical technique. We have also pointed out that these species are housed in 19 genera dominated by Vibrion which can tolerate culture environments with 3% NaCl, hence the success of its isolation in the broth and agar of Brain Heart Infusion (BHI) with 2.5 g / l NaCl.

We have also found that the marine ecosystems are more or less affected by direct or indirect human activities related to economic development and population growth, further increasing biodiversity including pathogens and multi-resistant germs, which involve implementing emergency measures and management policies to safeguard our natural heritage.

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