Microbiological survey of the bathing water of moroccan beaches from Mehdia to Skhirat

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Abstract—The Moroccan coastline occupies a privileged place at the level of the entire coastline of the African continent. The quality of bathing water is a criterion increasingly demanded by the general public for the choice of its holiday resorts. Main objective of this present study is to find out the status of to find status of bathing water through physicochemical and microbiological examination.

Sampling was done from 8 beaches of Kenitra Mehdia, Nations, Rabat-Sale, Harhoura, Temara, Golden Sand, Val D'or and the beach of Skhirate Amphitrite. Bacteriological evaluations were done & presence of feacal Coliforms and/or Streptococci was considered as indicative of faecal pollution. Enumeration of faecal Coliforms and faecal Streptococci was done by filter membrane method on nutrient media Tergitol7 Agar, Litskey, Slanetz & Bartley. In addition to microbiological sampling of water, temperature and pH of the water were measured "in situ". Data related to the tide (high or low) and populations were collected.

Regarding bacterial load of beaches in present study it was found that at Mehdia beach, Nations beach, Rabat-Sale beach, Temara beach, Harhoura beach, Sable D'or beach and at Skhirat beach the contamination standard is exceeded in 30% of samples for CF and 20% for SF, 10% for CF and 0% for SF, 100% of the samples for CF and 70% for SF, 50% for CF and in none (0%) for SF, 20% for CF and 10% for SF, 30% for CF and 10% for SF and 40% for CF and 10% for SF respectively. But at Val D'or beach in none of sample exceed the guide value (VG) for faecal Coliform and fecal Streptococci. So the beaches of the Nations, Harhoura and Val D'or are classified A. The beaches of Mehdia, Temara, Golden Sand and Skhirat with medium quality waters are classified B. Only the beach of Rabat-Sale was found polluted and classified in category C.

Keywords: Coastline Pollution, Microbiology, Coliforms, Streptococci, Morocco.

I. INTRODUCTION

The Moroccan coastline is obviously a heritage of great importance for the country. It represents a Mediterranean facade stretching about 460 km from Tangier to Saidia and an Atlantic facade of 2500 km from Tangier to Lagouira.

It is a strategic area with a wide variety of natural and biological resources, including beaches, dunes, lagoons and wetlands. It is under increasing demographic pressure from urban agglomerations and the influence of various industrial, port and tourist activities.

Morocco has a strong tendency to urbanization estimated at 4.4% per year, which is accompanied by a phenomenon of coastalisation of the population. The expanding coastal agglomerations provide the bulk of urban growth with more than 60% of the total urban population of the country.[1]

The coastline is also the location of most industrial units; more than 80% of industries are located near coastal areas. The industries are highly concentrated along the Kenitra-Safi axis and especially in the Casablanca agglomeration. This axis concentrates nearly 62% of industrial units and nearly 70% of industrial employment.[1]

In addition, through the marine facilities located along the coast, transiting 98% of trade with the outside of this fact Moroccan water are experiencing intense shipping. Thus, hundreds of boats run daily along the Moroccan coast, including tankers and tankers that pose a permanent threat to marine pollution. Similarly, the important fisheries resources consist of more than 7137 animal species providing a fishing potential estimated at 1000 000 annual tons.[2]

In recent years, tourism policy has made seaside a priority option and this by the development of certain areas such as Tangier and Agadir which concentrate 70% of the hotel capacity approved. The surge in domestic demand on the seaside has also led to the proliferation of second home projects and the occupation of the public domain, which has led to the emergence of a real lack of health infrastructure and consequently a negative impact on several beaches.

Moreover, the quality of the beaches has become over the years a criterion increasingly used by the general public to choose its holiday beaches. This led the authorities to ensure the protection of beaches against pollution by wastewater and to establish a classification of beaches according to their quality.

The Main objective of this current research is to find out status of bathing water through physicochemical and microbiological examination.

II. METHODOLOGY

This descriptive study was conducted at beaches & of Biology and Health, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.

Sample of water for this study was taken from 8 beaches viz. Kenitra Mehdia, Nations, Sale, Harhoura, Temara, Sable D'or, Val D'or and Skhirat Amphitrite Beach.

Study method: It includes followings:-

Waters sampling: At each stage of the microbiological sampling, there is a risk of distorting the entire procedure, which is why sampling requires special care to avoid any contamination. Thus sterility and adequate temperature are of paramount importance in carrying out microbiological research. For optimal sampling, according to Moroccan standard NM03.07.006[1] followings were kept in mind. Then sampling was proceeded as follows.

- Sampling points are based on the extent of the beach in areas with the highest density of bathers;

- Sampling at the level of the upper layer of the body of water at a depth of 30 cm;

- Sampling time is fixed between 12h and 13h;

- Storage conditions of the samples designated for bacteriological analysis include storage in an insulated box at a temperature between 0 and 4 °C.

In addition to the water sampling, the following parameters were measured "in situ":-

- Temperature of the air using a mercury thermometer

- Water temperature using a pH meter probe

- The pH of the water using portable pH meter Hanna type Instrument

- The tide (high or low)

- Estimate of the population.

Microbiological analysis methods: For the prospecting of the biological quality of bathing waters, it is necessary to carry out bacteriological analyzes to search for microorganisms which are indicators of faecal pollution, especially faecal Coliforms and faecal Streptococci.

Coliforms: The term "coliform" refers to the different species belonging to the gram negative Enterobacteriaceae family, aerobic or optionally anaerobic, non-sporogonal and whose Main characteristic is the fermentation of lactose with gas production.[3]

Fecal Coliforms (CF): According to the World Health Organization,[4] the faecal Coliforms are Gram-, aerobic and facultative anaerobic, rod-shaped, non-sporulating bacteria that ferment lactose by producing gas, within 24 hours, at the same time they growth at 44°C on the medium Tergitol 7 Agar. Faecal Coliforms have a highly significant positive correlation with faecal contamination caused by humans or warm-blooded animals, and are therefore a good indicator of the health quality of coastal waters.[5] This is based on the fact that *Echerichia coli* can't survive for a long time in the environment. Its survival time depends on several physicochemical factors, temperature is the essential factor that affects its concentration. Other work[6] show that the density of faecal Coliforms and directly proportional to the biological oxygen demand (BOD_5). So their presence in the water always indicates a recent faecal contamination.

Fecal Streptococci (SF): Faecal Streptococci are slightly oval, Gram-positive, spherical cocci in the form of pairs or short chains during their growth on Slanetz and Bartely medium containing 2,3,5 triphenyl tetrazolium chloride (TTC). The group of fecal Streptococci normally comes from human intestines or warm-blooded animals and indicates faecal pollution when they are detected in seawater. Their mortality rate depends on salinity, temperature and solar radiation etc.

Enumeration of Fecal Coliforms: Enumeration of Fecal Coliforms done by Filter membrane method.

Filter membrane method: The membrane filter method is the method used for the enumeration of fecal Coliforms and fecal Streptococci. This method is suitable for the enumeration of faecal Coliforms and faecal Streptococci in coastal bathing waters of temperate seas. It has been established for sanitary surveillance of beaches.[7-10]

Principle: The method consists in filtering a volume of seawater sample taken under sterile conditions, according to the quantity of Coliforms estimated in the water sample. The filtration membrane should have a pore diameter of 0.45 μm optimal for the total retention of bacteria. The membrane is placed on the surface of the culture medium poured into Petri dishes and cultured. After incubation the cultures are examined for bacterial colonies with specific characteristics and count to deduce the bacterial concentration per 100 ml of water.
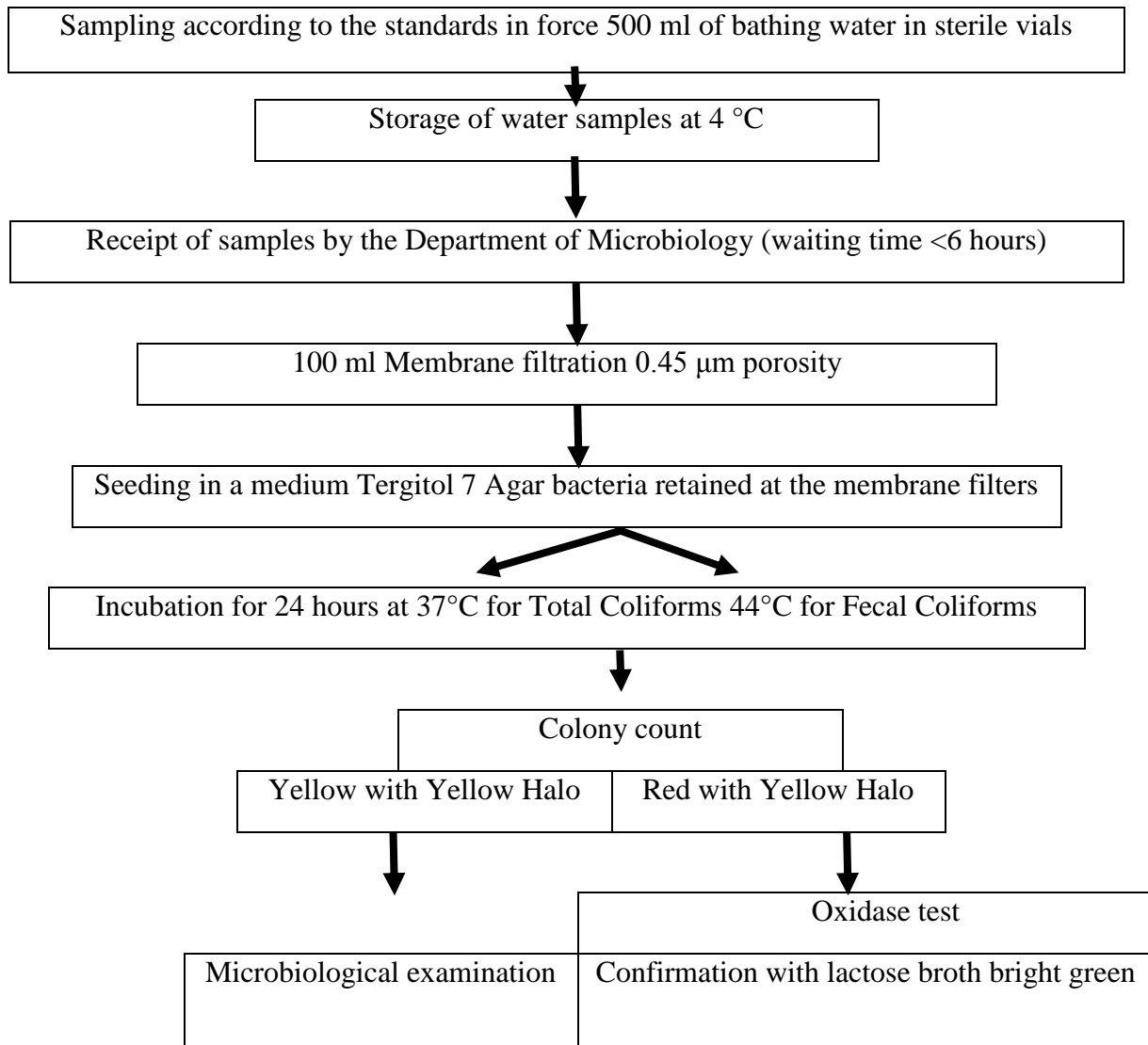
Interpretation: Sodium heptadecyl sulfate (Tergitol 7) inhibits unwanted secondary flora. The degradation of lactose to acid is revealed by a yellow turn of the pH indicator, bromothymol blue,

biphenyl chloride 2,3,5 tetrazolium (TTC) is reduced very rapidly by almost all Coliforms except *Escherichia coli* and *Enterobacter aerogens* which give a red coloring.

Yellow colonies with yellow halo *Escherichia coli*

Red with possibly yellow halo Coliforms without *E. coli*

Plate 1: Protocol for the detection and enumeration of fecal Coliforms.

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┌─────────────────────────────────────────────────────────────────┐
│ Sampling according to the standards in force 500 ml of bathing   │
│ water in sterile vials                                           │
└─────────────────────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────┐
│ Storage of water samples at 4 °C                 │
└─────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────────────────────┐
│ Receipt of samples by the Department of Microbiology             │
│ (waiting time <6 hours)                                          │
└─────────────────────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────┐
│ 100 ml Membrane filtration 0.45 μm porosity      │
└─────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────────────────────┐
│ Seeding in a medium Tergitol 7 Agar bacteria retained at the     │
│ membrane filters                                                 │
└─────────────────────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────────────────────┐
│ Incubation for 24 hours at 37°C for Total Coliforms 44°C for     │
│ Fecal Coliforms                                                  │
└─────────────────────────────────────────────────────────────────┘
                              │
┌─────────────────────────────────────────────────┐
│                  Colony count                    │
├────────────────────────┬─────────────────────────┤
│ Yellow with Yellow Halo│ Red with Yellow Halo     │
└────────────────────────┴─────────────────────────┘
         │                           │
                              ┌─────────────────────────────────────┐
                              │ Oxidase test                         │
┌────────────────────────┬─────────────────────────────────────────┐
│ Microbiological        │ Confirmation with lactose broth bright   │
│ examination            │ green                                    │
└────────────────────────┴─────────────────────────────────────────┘
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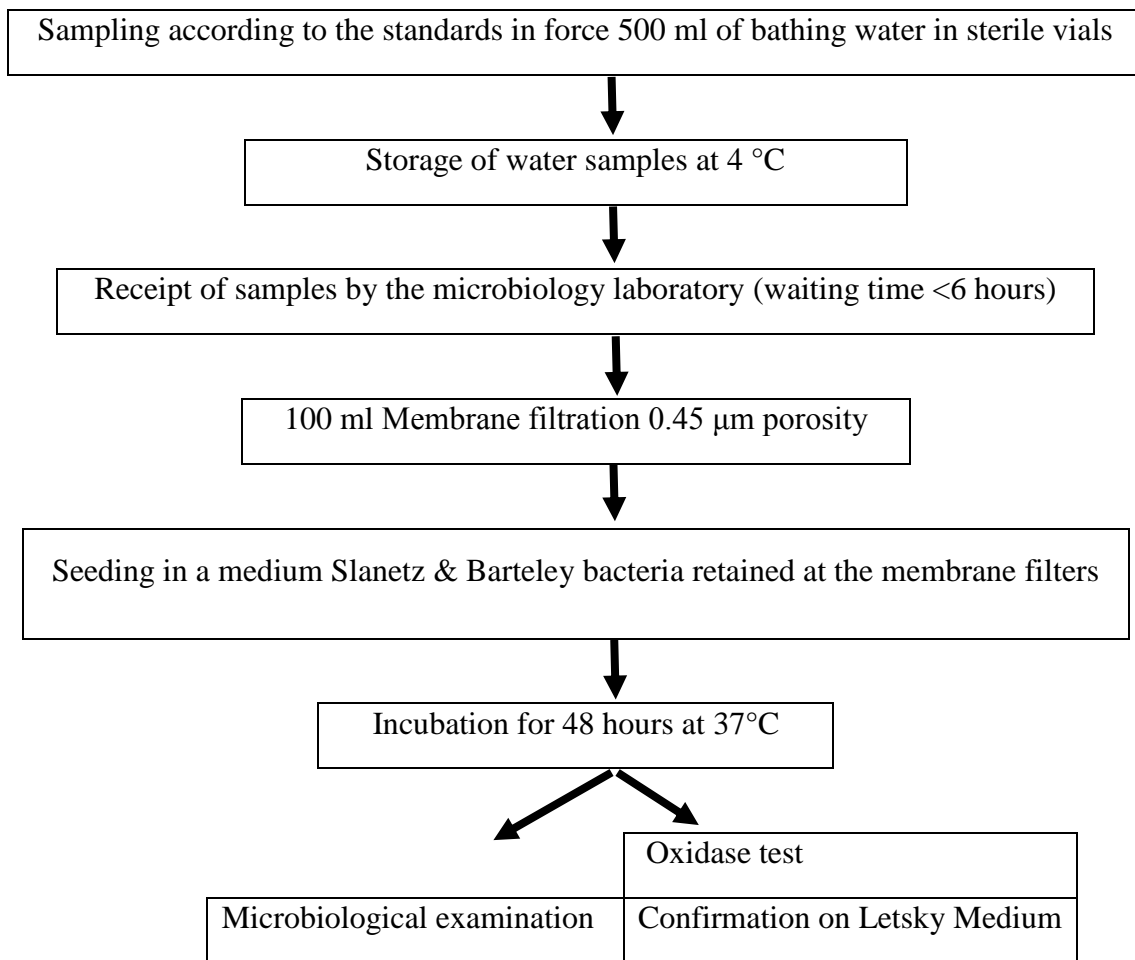
Confirmatory test: Faecal coliform colonies presumed on Tergitol 7 Agar medium are confirmed on bright green bilious lactose medium at 37 ± 1 ° C for 24-48h.

Interpretation:

Cloudy medium and presence of gas under the bell Fermentation of lactose Coliforms.

Enumeration of Fecal Streptococci: For Enterococci on membrane filters, selective Agar is used according to Slanetz and Bartley. This medium contains an abundant amount of nutrients to ensure good growth. On the other hand, all secondary flora is inhibited by azide.

Plate 2: Protocol for detection and enumeration of Fecal Streptococci.

Sampling according to the standards in force 500 ml of bathing water in sterile vials

↓

Storage of water samples at 4 °C

↓

Receipt of samples by the microbiology laboratory (waiting time <6 hours)

↓

100 ml Membrane filtration 0.45 μm porosity

↓

Seeding in a medium Slanetz & Barteley bacteria retained at the membrane filters

↓

Incubation for 48 hours at 37°C

↓   ↓

| Microbiological examination | Oxidase test |
| | Confirmation on Letsky Medium |

Interpretation: Enterococci colonies reduce TTC to red-colored formazan, which allows early identification of yellow Escherichia coli colonies. Pink to brown colonies with a diameter of 0.5 to 2 mm are usually fecal Streptococci.

Confirmatory test: Fecal Streptococci colonies presumed on Slanetz & Bartley medium are confirmed on Litskey medium at 37 ± 1 ° C for 24-48h. Tubes with a disorder are considered positive and taken into account for the enumeration of fecal Streptococci.

Method of classification of bathing waters: For the classification of bathing waters four categories are distinguished namely: **A**, **B**, **C** and **D** on the basis of the overrun of the Moroccan standards[1] for monitoring the quality of bathing water (Table 1) and the international standards and guidelines for the sanitary monitoring of bathing waters.[11-12]

Table 1
Guide values and imperative values set by the Moroccan standard NM 03.7.200[1]

Parameters	Guide Value VG UFC/100mL	Imperative Value VI UFC/100mL
Fecal Coliforms	100	2000
Fecal Streptococci	100	400

* Category A: Good quality waters for swimming.

- At least 80% of Escherichia coli or faecal coliform results are less than or equal to the guideline value of 100 bacteria / 100mL;

- At least 95% of the results in E. coli or faecal Coliforms are less than or equal to the mandatory value of 2000 bacteria / 100 mL;

- At least 90% of fecal Streptococci results are less than or equal to the guideline value of 100 bacteria / 100 mL.

* **Category B**: Medium quality waters for swimming.

- The water quality is medium when the imperative number set by the directive for E. coli and faecal Coliforms of 2000 CFU / 100mL is respected in less than 95% of the samples.

* **Category C**: Waters temporarily polluted.

- Water monitoring points for which the frequency of exceeding the mandatory number for E. coli or faecal Coliforms is between 5% and 33.3% is considered to be temporarily polluted. This pollution can be the subject of immediate or medium-term measures to permanently improve the quality of water.

- It is important to note that if less than 20 samples are taken during the whole season on one point, a single exceeding of the imperative number in E. coli or faecal Coliforms, is enough to cause the classification of the beach in category C.

* **Category D**: Poor quality waters.

During this study, several bathing water samples were taken at the main 8 beaches of the study area. Survey includes bi-monthly site visits, climate surveys, and physicochemical measurements and microbiological analyzes. Sampling of water was taken bimonthly except in January, May & August. These samples were subjected to microbiological analyzes (faecal Coliforms and faecal Streptococci) as per above described methods.

III.   RESULTS

In this study it was found that air & water temperature and PH at various beaches were with almost same variation. Temperature ranges from 16^0C to 32^0C being higher from May to September in all 8 studied beaches. Ph ranges from 7 to 8.68. (Figure 1)

When month wise variation of distribution of foecal Coliforms and Streptococci was analyzed it was found with significant (p<0.001) variation for both type of bacterias. Likewise beach wise distrubution of foecal Coliforms and Streptococci was further evaluated and both were found significantly(p<0.001) vary with variation in beach. (Figure 2&3 and Tables 2)

The relatively high percentages of exceedance for faecal coliform guideline values were recorded at the beaches of Mehdia, Rabat-Sale, Temara, Sable d'Or and Skhirat with over 30% exceedance. The highest percentages of contamination not faecal Coliforms were detected in the Rabat-Sale beach (100%). The highest level for Streptococci is found in the Rabat-Sale beach (70%). (Figure 3&4 and Tables 3)

Figure 1
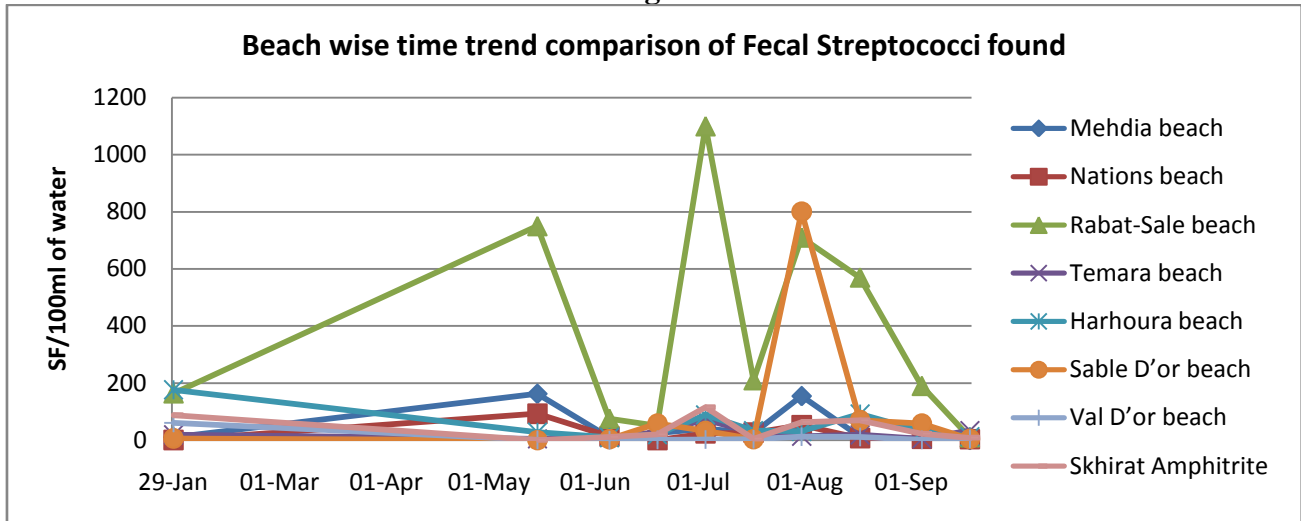**Comparison of air & water temperature and PH at various Beaches**
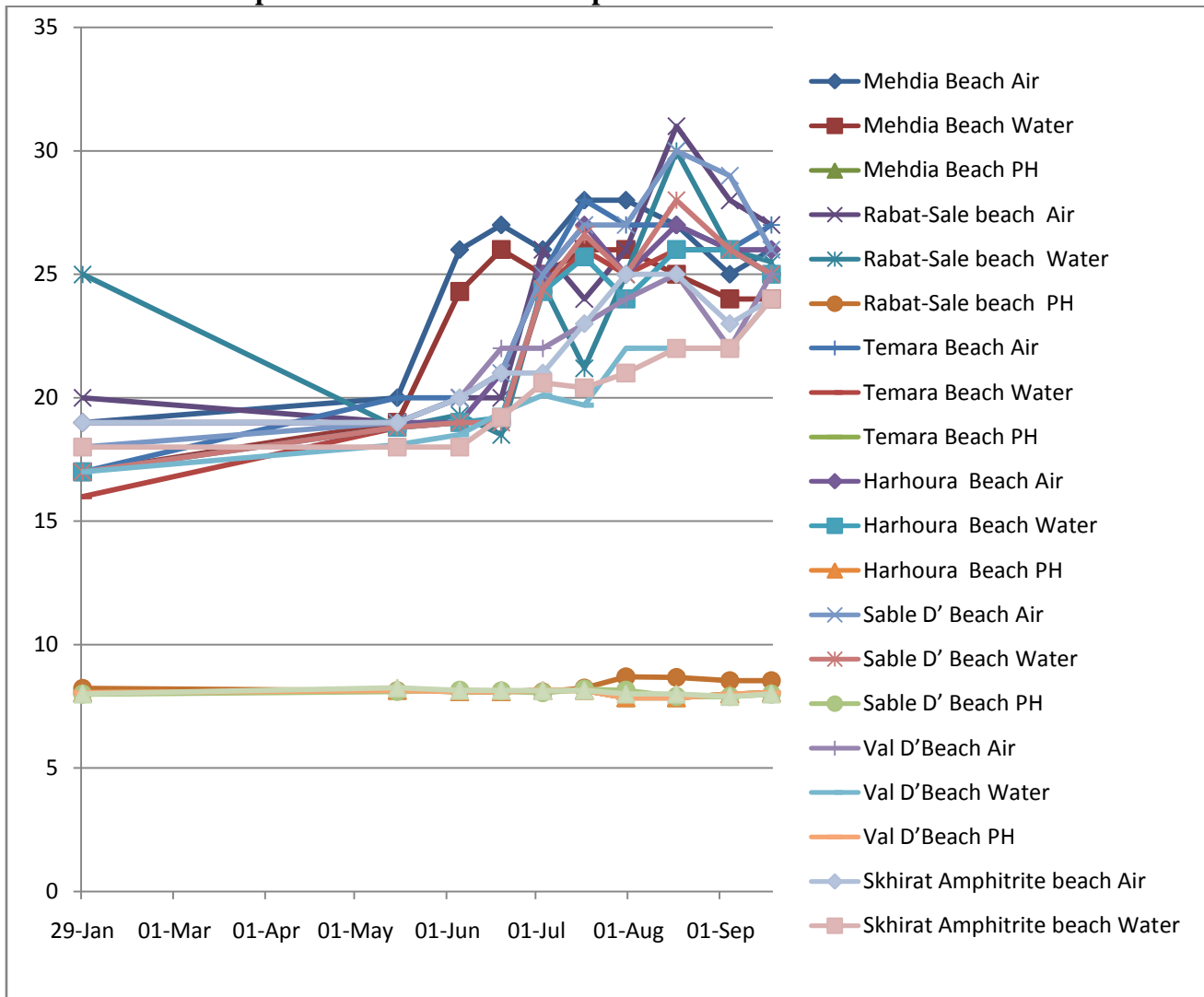
Figure 2
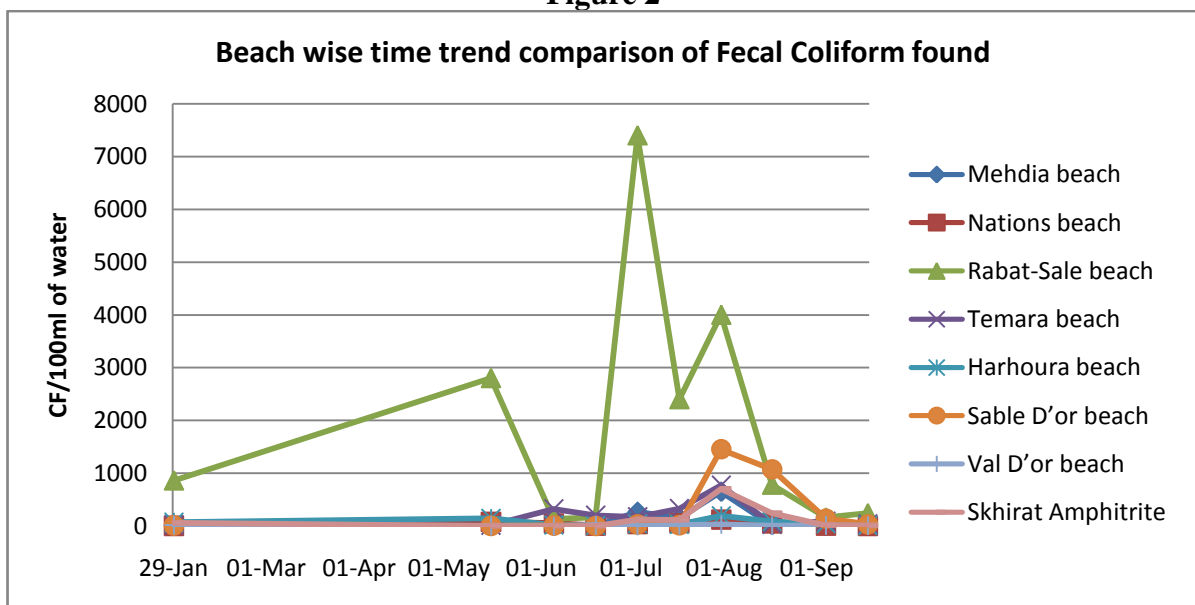
Figure 3

When association between type of tide with foecal Coliforms and Streptococci was analyzed it was found with significant (p<0.001) variation for both type of bacterias as per type of tide. Likewise when association between size of population with foecal Coliforms and Streptococci was analyzed it was found significant (p<0.001) for both type of bacterias. (Tables 2)

Table 2
Comparison of Foecal Coli form and Streptococci as per Tide & Population at various Beaches

		15-May	5-Jun	19-Jun	3-Jul	17-Jul	17-Aug	31-Jul	4-Sep	18-Sep	29-Jan
Mehdia Beach	Tide	Low	Low	High	High	Low	Rising	Low	Low	High	High
	Population	500	4200	5100	7000	7300	8000	8000	5500	3000	30
	CF/100mL	9	0	2	266	150	645	14	0	0	15
	SF/100mL	162	10	25	40	25	154	12	4	0	10
Nations beach	Tide	Low	Low	High	High	Low	Rising	Low	Low	High	High
	Population	14	34	70	900	1400	3000	3000	20	10	2
	CF/100mL	77	50	6	36	54	120	47	7	1	4
	SF/100mL	92	13	0	23	26	52	7	4	3	0
Rabat-Sale beach	Tide	Low	Low	High	High	Low	Rising	Low	Low	High	High
	Population	36	73	200	400	1700	2000	3000	300	50	9
	CF/100mL	2800	130	160	7400	2400	4000	780	160	240	860
	SF/100mL	750	75	50	1100	210	710	570	190	10	164
Temara Beach	Tide	Low	High	High	Low	Rising	Low	Low	High	Rising	High
	Population	16	700	800	1400	2500	2600	1200	17	6	0
	CF/100mL	12	320	200	170	329	770	30	90	40	18
	SF/100mL	4	9	28	71	25	13	18	4	33	18
Harhoura beach	Tide	Low	High	High	Low	Rising	Low	Low	High	Rising	High
	Population	15	300	300	700	800	850	1100	350	40	7
	CF/100mL	141	15	4	40	30	189	80	41	7	67
	SF/100mL	27	9	19	88	33	30	91	30	12	176
Sable D' beach	Tide	Low	High	High	Low	Rising	Low	Low	High	Rising	High
	Population	0	30	460	1450	1000	2100	1800	800	800	10
	CF/100mL	4	9	1	30	15	1450	1070	140	31	8
	SF/100mL	0	3	58	32	3	800	70	58	4	6
Val D' beach	Tide	Low	High	High	Low	Rising	Low	Low	High	Rising	High
	Population	7	60	350	600	600	750	1000	250	100	4
	CF/100mL	1	0	14	0	16	28	4	9	5	15
	SF/100mL	1	0	1	2	3	12	11	1	4	60
Skhirat Amphitrite beach	Tide	Low	High	High	Low	Rising	Low	Low	High	Rising	High
	Population	36	90	300	500	800	2000	2500	34	3	0
	CF/100mL	0	0	2	120	114	700	240	13	9	58
	SF/100mL	0	11	20	115	5	63	69	22	8	87

Regarding bacterial load of beaches in present study it was found that at Mehdia beach, the contamination standard is exceeded in 30% of samples for CF and 20% for SF, at Nations beach, the contamination is exceeded in 10% for CF and 0% for SF, at the Rabat-Sale beach, the contamination standard is exceeded in 100% of the samples for CF and 70% for SF, at Temara beach, the contamination threshold is exceeded in 50% for CF and in none (0%) for SF, at Harhoura beach, the contamination threshold is exceeded in 20% for CF and 10% for SF, at Sable D'or beach, contamination exceeds 30% for CF and 10% for SF and at Skhirat beach area the contamination of samples is exceeded in 40% for CF and 10% for SF. But at Val D'or beach no exceedance of the guide value (VG) for faecal coliform (0%) and fecal Streptococci (0%). So Val D'or beach, Nations beach and Harhoura beach is in category 'A'. And Sable D'or beach, Mehdia beach, Skhirat and Temara beach are in category 'B' whereas Rabat-Sale beach is in category 'C'. (Figure 4)

Figure 4
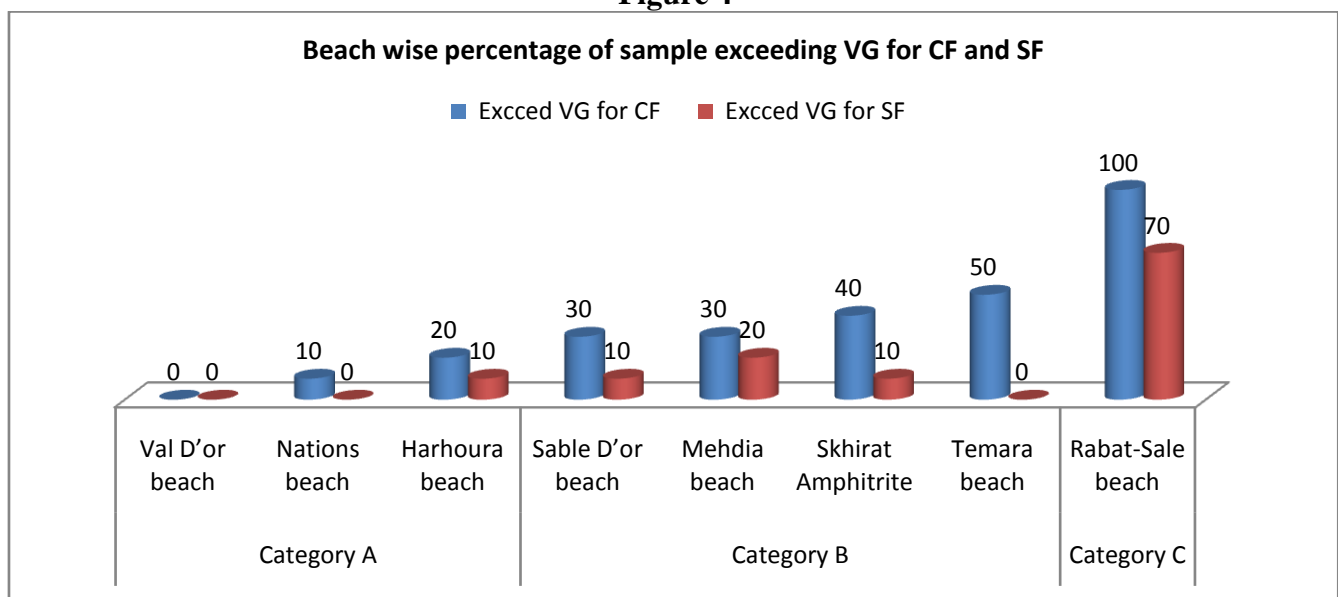
IV. DISCUSSION

In this study it was found that air & water temperature and PH at various beaches were with almost same variation. So atmospheric variation was not found in various breaches in Moracco. But month wise and breach wise variation of distribution of foecal Coliforms and Streptococci was found with significant ($p<0.001$) variation for both type of bacterias. Highest populated beach was Rabat-Sale followed by Temra, Skhirat, Mehdia, Sable D' Harhoura, Nations and Val D'. None of the sample of Val D' found with bacteria.

The beaches of the Nations, Val d'Or and Harhoura are of good quality for swimming due to the absence of wastewater discharges and urban activities and also the low pressure of summer visitors.

The waters sampled at the beaches of Temara, Sable D'or and Skhirat are of average quality for bathing because of a more pronounced contamination by the indicator germs of fecal contamination. This pollution and degradation of the environment are mainly related to the activities of summer visitors during the summer. The temperature and pH of water are favorable for the survival of bacteria.

Mehdia beach has an average quality for swimming. It is a special ecosystem due to the presence of the estuary of Sebou River which drains wastewaters from three major cities Fez, Meknes and Kenitra

estimated at more than 200 000 m^3 / day. In fact, the pollution generated is very impressive because of the agricultural, port and industrial activities of this river: sugar factories, paper mills, oil mills, tanneries, cement factories, textiles, refineries etc.

The temporal monitoring of the level of contamination of the waters of Rabat-Salé beach has led to the conclusion that this beach is not bathing. This observation could be interpreted by the diversity of the sources of pollution, the variations of the flow rate and finally the mortality of the bacteria. In fact, the sewage as soon as they arrive in the rivers is diluted by the rains. Similarly, domestic pollution is dampened by industrial and agricultural waste rich in heavy metals and pesticides that prevent the proliferation of microorganisms due to bacterial inhibition by toxicity and eutrophication.

The study area is subject to considerable urban pressure and is therefore influenced by the various industrial, port and tourist activities. It receives inputs from the watersheds of Sebou, Bou Regreg and Ikem rivers and the continental alluvium they carry as well as the wastewater outfalls of coastal agglomerations.

CJ Parmar etall[13] also found bacterial contamination of water of their study area i.e. 86 coastal water samples in southern California. Likewise SC Adberg etall[14] and Marieke Adank atall[15] also found foecal contamination in their study areas.

V.   CONCLUSION

It was concluded from study that at the beaches of Mehdia, Nations, Rabat-Sale, Temara, Harhoura, Sable D' and Skhirat, the contamination standard is exceeded in 30% of samples for CF and 20% for SF, 10% for CF and 0% for SF, 100% of the samples for CF and 70% for SF, 50% for CF and in none (0%) for SF, 20% for CF and 10% for SF, 30% for CF and 10% for SF and 40% for CF and 10% for SF respectively. But at Val D'or beach in none of sample exceed the guide value (VG) for faecal Coliform and fecal Streptococci. So the beaches of the Nations, Harhoura and Val D'or are classified A. The beaches of Mehdia, Temara, Golden Sand and Skhirat with medium quality waters are classified B. Only the beach of Rabat-Sale was found polluted and classified in category C. Bacterial concentration was found to be associated with the population.

CONFLICT OF INTEREST

None declared till now.

ACKNOWLEDGMENTS

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