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A Physicochemical, Bacteriological and Molecular Evaluation State of the Coast of Ain Sebaa-Zenata in Morocco

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Abstract: ***In Morocco, the Atlantic littoral focuses on the majority of economic activities which creates an important phenomenon of coastal development. It records an average density of 162 inhabitants / km2 between Kenitra and the big city of Casablanca [1]. The coast of Casablanca is a national wealth and remains the Moroccan industrial region by excellence thanks to its port, the main economic core of the kingdom. However, there are [at this coastal strip] several sanitary sewers from urban agglameration and industrial units of Ain Sebaa-Zenata, which pose a threat to the health of bathers [2]. The spatio-temporal study we have conducted, through bacteriological contaminants and chemicals research, is a contribution to the evaluation of the physico-chemical, bacteriological and molecular state of the bathing beach water of Ain Sebaa-Zenata. The study, by conventional techniques has been focused on three sites I, II and III while the molecular analysis by PCR (Polymersae Chain Reaction) was performed only on two locations II and III during sampling frame. The results obtained by the conventional techniques have shown the existence of physico-chemical and biological indicators which are oriented to bacterial contamination by germs in the conventional sites I, II and III while the molecular study has been able to detect the presence of pathogenic bacteria such as Salmonella Typhimurium in the II and the III site, which were put under the microscope. These parameters were compared to the national and international standards governing the quality of bathing water. According to these standards, it was found out that the physico-chemical, bacteriological and molecular state of the water, from the three sites, which was studied is not compliant with the international bathing standards.***

Keywords: Littoral, AIN SEBAA - ZENATA, Physicochemical, Bacteriological

1. Introduction

The region of the big city of Casablanca has known since the 20th century a very important economic development, coupled with high population growth and rapid urbanization. This development is accompanied by various environmental problems [1]. In particular, the release of untreated domestic and industrial waste into the ocean which has several negative consequences on the environment and human health. Noting that, 57.8% of wastewater is dumped in the Atlantic coast and Mediterranean (-8% of liquid waste is treated) and 70% of the Moroccan coastal cities do not have their sewerage systems connected to wastewater treatment plants; some are ineffective and the rejects are being made exclusively in the sea and wadis without treatment (only 26 wastewater treatment plants are functional in 235 centers with a sewerage system) [1].

Marine pollution poses a threat to human health, animals and marine life (nearly 270 species are threatened and many resources are either on the verge of extinction, has completely disappeared), therefore the alarm bell is set ringing, for pollution has become a topical issue and it affects virtually every ecosystem, including the marine environment and the anarchic proliferation of algae, the growth of some bacterial species contaminate both water and edible species [3].

Pollutants are classified into 3 types:

-Urban domestic wastewater

Consolidating domestic wastewater that is being released by commercial and public facilities. The water is rich in chemical and biological agents.

-Industrial Wastewater

Industrial wastewater: combining physical agents representing inert, inactive and suspended materials. More organic chemicals that are directly related to food industry.

-Agricultural Wastewater

Combining fertilizers, animal wastes and pesticides that contaminate ground and surface water bodies [4].

Note that the bathing water is a highly regulated area and the national implementation of European directives (NDE) has led to a very high protection for bathers. In this context, knowledge of chemical threats has received satisfactory results so as to prevent the danger and the evaluation of the physico-chemical state of seawater to detect industrial pollution threats incurred As microbiological hazards which are based on enumeration of faecal coliforms (Escherichia coli) and enterococci (faecal streptococci) in accordance with the national standards (NM03.7.200) transposed the European directives (76/160 / EEC) and the WHO guidelines / UNEP. On the practical microbiological risks are difficult

to assess by conventional techniques which have cultural problems and the long period devoted to the identification cultivable germs (on average 48 hours) [2]- [5].

In this study we chose the area of AIN SEBAA / ZENATA because it is an area of population and industrial agglomeration responsible for directly discharging wastewater on the sea without any treatment.

The objective of this work is to study the impact of pollution from discharges of domestic wastewater and their negative consequences on the marine environment by evaluating ,at first, the physico-chemical and bacteriological status bathing water of some ribs in the region of Ain Sebaa and Zenata I, known as marine pollution zone and, secondly, the use of molecular techniques including PCR (Polymerase Chain Reaction), to accompany the microbiological study and verify the absence or presence of hardly cultivable bacterial pathogen.

2. Material and Methods

2.1 Location Sites

The three study stations are located at the beach of Ain Sebaâ / Zenata I, are distributed from south to north from Sherifian Company of Fertilizers and Chemicals (SCE). Geographical points are given by a Garmin eTrex type GPS.

Table 1: Geographic coordinates of the three study sites.

Sites	Geographic coordinates of the selected stations
Site I	33° 7’ 11’’ N 7° 32’ 57’’ W
Site II	33° 37’ 23’’ N 7° 32’ 55’’ W
Site III	33° 37’ 6’’ N 7° 30’ 38’’ W

Figure 1: Location of study sites

2.2 Sample

With the sterile balloon placed in the wave breaking zone against the current, the balloon is immersed in water at 20 cm from the surface. It is filled in three quarters and closed immediately.

2.3 Chronology of samples

To make a comparison of the bacteriological quality and chemical water depending on seasons and daily activities. All samples from the various sites have been realized over two months at different times of the day.

2.4 Techniques used

2.4.1 The physico-chemical parameters

Temperature, pH, conductivity, turbidity, salinity, dissolved O2 were analyzed using the Mettler Toledo type of portable device other parameters were analyzed in the laboratory such as the determination of nitrite and measurement of BOD5.

2.4.2 Bacteriological tests

Based on the filtration method of 100 ml of seawater on membrane (diameter: 0.45 µm). Then the count of the colonies after culture in Tergitol Triphenyl-Tetrazolium-chloride (TTC) medium cast in Petri dishes and incubation for 24 hours at 37 ° C for Total Coliforms and 24 hours at 44 ° C for Fecal Coliforms. The Fecal Streptococci after filtration on a membrane, the filter is deposited on SLANETZ medium and BARTLEY. Incubation is at 37 ° C for 24 hours. In contrast, Salmonella, Choleric Vibrion and Clostridium Sulfite Reducer, which are pathogenic germs with serious health effects, will undergo a qualitative examination after enrichment on media such as Buffered Peptone Water (EPT) for Salmonella a two-way crop, that of Bouillon de Selenite and incubation for 24 hours at 37 ° C. in Salmonella-Shigelle medium and also by the Bouillon Rappaport route and culture for 24 hours at 37 ° C. on Hektoen medium. Choleric Vibrion was investigated by the enrichment method with Alkaline Peptone Water (E.P.A) and cultured for 24 hours at 37 ° C in selective Thiosulfate Citrate Bile Sucrose (TCBS) medium. Clostridium sulphito-reductant is investigated by the heat resistance technique and cultured for 24 hours at 37 ° C. in Sulfate Polymixen Sulfadiazine SPS medium. [7]-[15].

2.4.3 Molecular study

The DNA is extracted by the rapid heat method (Boiling-prep), adapted to small volumes: centrifuge 20 ml of seawater at 10,000 rpm for 10 min and then wash the pellet with a solution of distilled water (ED), recovery of the pellet which is suspended in 200 µl of molecular biological water, lysed by thermal action in a water bath at 100 ° C for 10 min, followed by centrifugation at 13,000 g (12,000 rpm). min) for 5 min, the supernatant is recovered which is stored at -20 ° C until use.

Reaction Mixture

PCR used to amplify a 1Kb of DNA fragment of the gene of invasion common to all Salmonella invA chromosome (No. M90846.1 Salmonella Typhimurium) specific to Salmonella using primers: F- 5'accacgctctttcgtctgg direction 3 'and antisense 5'gaactgactacgtagacgctc R 3'. 4 U DNA, 100 .uM each primer, 5 U of Taq polymerase, 10% 10X buffer; 25 mM of MgCl2;. 5 mM dNTP and complete by molecular biology water [2]. To validate the results in parallel using a molecular weight marker (100 bp DNA ladder << Invitrogen >>, Cat No. 10488-058). DNA extract of Salmonella Typhimurium penta-resistant types (ACTeStSul) is used as a positive control, a DNA extract of Escherichia coli V517 (E. coli), a DNA extract of Staphylococcus Aureus and sterile H2O solution is used as a negative control.

3. Results and Discussion

3.1 Results of physicochemical analysis

Table 2: Evolution of physical and chemical parameters in the three study sites

	04/03/2013	25/03/2013	15/04/2013	29/04/13
Water temperature (C °)				
Site I	17,7	19,2	22,3	23
Site II	17,2	19,5	22,5	21,2
Site III	17,8	19,7	22,7	22,9
pH				
Site I	7,02	6,72	6,92	7,4
Site II	8,03	6,75	7,17	8,3
Site III	8,22	6,79	7,01	8,52
Conductivity (mS / cm)				
Site I	64,5	64,4	63	64
Site II	63,9	63,7	62,5	67
Site III	64,5	64	64,2	66,2
Turbidity (NTU)				
Site I	32,3	31,2	22	8,2
Site II	32	31,9	21,7	9,3
Site III	43,2	32	18,2	16,5
Salinity (mg / l)				
Site I	43,3	41,5	42,3	44
Site II	42,9	40,2	43	43,5
Site III	42,8	43,2	42,6	45
Dissolved oxygen (%)				
Site I	34,2	32,5	43,2	40,2
Site II	20,5	25,2	32,2	43,5
Site III	29,5	28,7	35,1	44,4
Nitrite NO2 (mg / l)				
Site I	0,14	0,17	0,016	0,18
Site II	0,04	0,22	0,20	0,22
Site III	0,24	0,10	0,022	0,26
BOD5, mg (O2 / l)				
Site I	4,2	4,5	6,8	7,2
Site II	62	76	83	91
Site III	69	85	105	122

The pH and temperature meet the standards, then we observe a permanent increase in conductivity, indicating significant mineralization of water, BOD5 indicates a significant bacterial load, increased nitrite and lower values of dissolved oxygen showing poor oxygenation [1].

3.2 Results of bacteriological

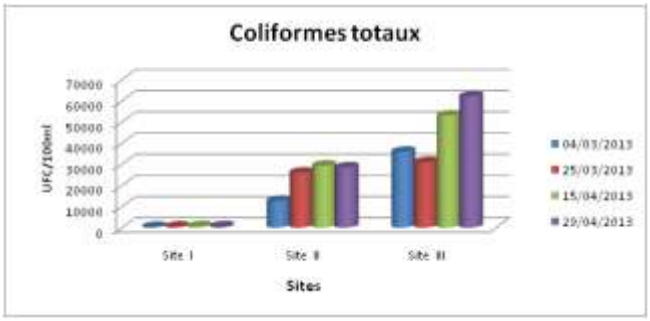

Figure 2: Spatiotemporal evolution of total coliforms in the 3 sites.

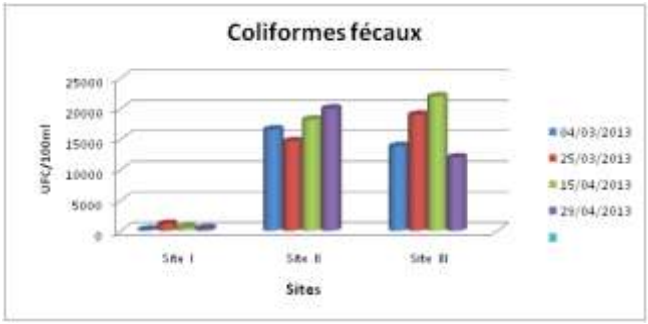

Figure 3: Spatiotemporal evolution of faecal coliforms in 3 sites.

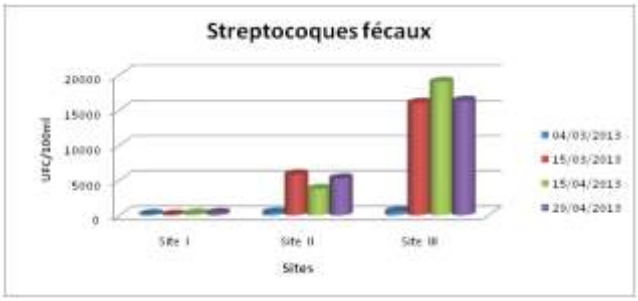

Figure 4: Spatiotemporal evolution of faecal streptococci in 3 sites.

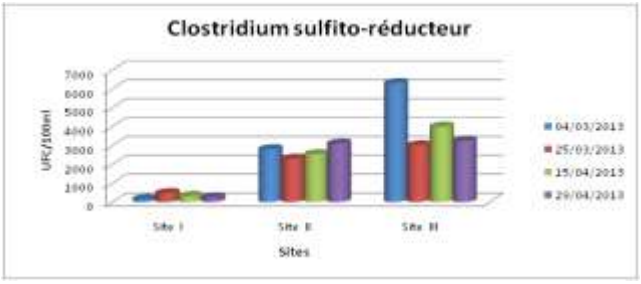

Figure 5: Spatiotemporal evolution of sulphite-reducing clostridia in 3 websites.

Bacteriological results show high levels of total coliforms, faecal, fecal streptococci and the presence of sulfite-reducing Clostridium spores with a very high rate especially in the site II and III which indicates an old fecal contamination [1].However conventional research by culture on usual media pathogenic bacteria such as Salmonella and Vibrio cholera patients in the study areas, are negative, which leaves us in doubt of the effectiveness of these techniques and to push our research by using other much more sensitive means of microorganism detection perspective namely PCR.

3.3 Results of molecular analysis

Thus shown on this gel photo, we see the presence of a band of expected size of 275 bp [6] at the level II and III sites, against the site I which has not been studied molecularly for two reasons: the first one, it has a low bacterial contamination from the classical bacteriological study, and for the second one we get there are specific primers for Salmonella.

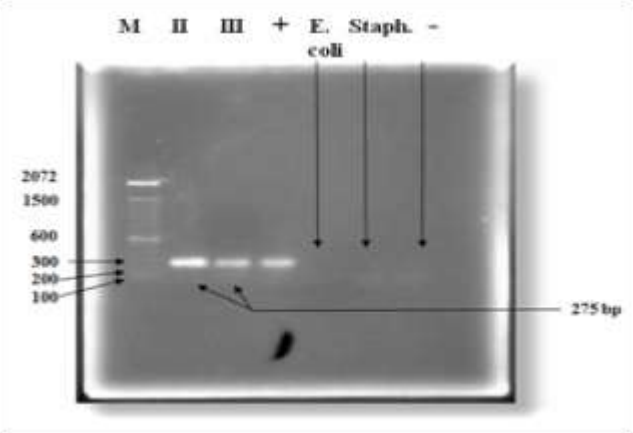

Figure 6: The electrophoretic profile of the bands obtained by PCR.

4. Conclusion

The area of Ain Sebaa-Zenata presents a demographic and industrial agglomeration responsible for discharges of waste water into the sea without any treatment. However, this seaside resort has numerous swimming beaches frequented by a significant number of holidaymakers throughout the year [9]. For this reason the evaluation of the physico-chemical state, bacteriological and molecular seawater beaches Ain Sebaa-Zenata was the subject of our study.

According to the national standards (NM03.7.200) and international (European Directive. 76/160 / EEC and Directives of the WHO / UNEP) [6], each setting realized, it appears that physico chemical and bacteriological state mainly in the area of our study do not always meet the standards. However, it is important to note that if less than 20 samples taken throughout the season on a point and a single crossing of the essential number in E. coli or fecal coliforms, enough for classification of the beach in category C c ' is to say non-compliant and that the water is polluted momentarily which can affect all coastal activities [6], generating a great danger to swimmers who remain insensible to the non-compliance of water in these beaches that have no ban on swimming panel. . After all, these results are correlated with all annual reports from 2002 to 2015 which were made by the Ministry of State in charge of Water and Environment in Morocco.

Note also that the search for pathogenic bacteria such as Salmonella by PCR was promising for the identification of these organisms that are difficult to cultivate using conventional techniques; this will help to better judge the quality of bathing areas knowing more than sixty percent (60%) of toxic infections worldwide are caused by Salmonella [10].

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