Original Research Article

Evaluation of the physicochemical and bacteriological study of the three sites in AIN SEBAA-ZENATA region after the installation of the anti-pollution station of the east coast of Grand Casablanca

ABSTRACT

The Moroccan coastline has been subjected exceptionally to the growth of urban agglomerations as well as to the negative effect of various industrial activities, together with the contributions of watersheds, wadis, and streams that dump their sewage and the waste generated by cities and their periphery.

Aim: First of all, we carry out an evaluation of the Physicochemical, bacteriological state of the bathing water sample of the beaches of Ain Sebaâ-Zenata through the search of the bacteriological and chemical contaminants in three sites by reinforcing them to the national norms and standards which govern the quality of the bathing water, on the other hand, we compare these results with those found before the inauguration of the anti-pollution station East of Greater Casablanca.

Study design: Study of the seawater quality of three swimming beaches.

Place and Duration of Study: Seawater was collected on 15/04/2017 from 3 sites in the Ain Sebaa-Zenata(Douar Abdellah Belhaj Beach, El Nahla Beach, Zenata Beach) region of the city of Casablanca after the installation of the anti-pollution station.

Methodology: Our job articulates on two axes: On the one hand, the study and the analysis of the physicochemical, bacteriological quality of the seawater after the installation of EST Casablanca anti-pollution station at the level of three sites (Douar Abdellah Belhaj Beach, El Nahla Beach, Zenata Beach). On the other hand, the comparison of these results with others already carried out before the development of this station via the same characteristics: Physico-chemical, bacteriological.

Results: In the light of the acquired results, a correlation was pointed out between the Physicochemical and bacteriological examination in comparison with the standards in the three studied sites, which were previously recognized as heavily contaminated bathing areas before the installation of the station. It supports the conclusion that this anti-pollution system has a beneficial and advantageous effect and confirms its cleansing role.

Conclusion: the current physico-chemical and bacteriological results of seawater in the Ain Sebaaa - Zenata region after the installation of the anti-pollution station show a significant improvement in the quality of this bathing area as well as the beneficial effect of this plant, which performs a very considerable purification function.

Key words: Seawater, Anti-pollution station, Physico-chemical, bacteriological, molecular, Comparison.

1. INTRODUCTION

In Morocco, the marine environment plays a socio-economic role of great importance. The opening of its coast on two facades (Atlantic and Mediterranean) stretching about 3500 km of coastline [1]. On the Mediterranean Sea, from Cap Spartel to Saïdia, the coast stretches for 500 km and just under 3,000 km on the Atlantic, from Cap Spartel to Lagouira offers a strategic position [2] Indeed, the Moroccan coasts introduce the advantage of being among the richest in the world with a broad diversity of ecosystems. [3]Thus, a good part of this area constitutes a privileged place for the installation of many agglomerations and industrial units.

As a result, approximately 60% of the population and more than 70% of industries are concentrated in the coastal urban centres. Thus, these ribs are exposed to chronic contamination, due to direct discharges, mainly of domestic and industrial origin that is discharged without any prior treatment. The Casablanca region is located on the Atlantic coast, in the central west of Morocco and covers an area of 1,140.54 km², which offers it a strategic position. In recent years, it has experienced critical fishing and seaside activities, which has led to a high level of microbial pollution in seawater [1].

To reduce marine pollution due to industrial and urban discharges in the Casablanca region, which is a highly industrialized and urbanized city where most of the country's socio-economic activities are concentrated, namely industry, fishing, navigation ... Lydec has inaugurated an anti-pollution station (SAP-EST) in Grand Casablanca, putting into service a system of domestic and industrial wastewater treatment [4].

2. MATERIAL AND METHODS

2.1 Choice of sampling sites

The selection of sampling sites was based on the geographical coordinates of the previous study [5]and their representativeness of the environment concerning the sources of pollution. We have chosen: Site 1: Beach of Douar Abdellah Belhaj in front of the Sherifian society. (33° 7’ 11’’ N - 7° 32’ 57’’ W) ; Site 2 : Beach of EL NAHLA (33° 37’ 23’’ N - 7° 31’ 55’’ W) ; Site 3 : Beach of ZENATA I (33° 38’ 6’’ N - 7° 30’ 38’’ W).

Fig. 1. Localisation géographique des sites du prélèvement (Google Heart, visited on 21/11/2019).

2.2 Samples

The samples were taken on 15/04/2017. We place ourselves, equipped with a barrel of 5 litres sterile, in the wave breaking zone against the current, then we immerse in the water at 20 cm. The surface [[6], [7]is filled in three-quarters and closed immediately. The samples were sent from the sampling sites in a cooler (4 to 5 ° C) to the laboratory [8].

2.3 Analysis techniques

2.3.1 Physico-chemical parameters

For each sample, on the site, we measured water temperature, electrical conductivity, pH, turbidity, water salinity, and dissolved oxygen using a Mettler Toledo multiparametric measuring instrument. Other analyzes such as BOD5 (Biological Oxygen Demand in 5 days) were performed in the laboratory as described in the international standards ISO 5815-1 [9]and the determination of nitrite by the method of Zambelli [10].

2.3.2 Bacteriological parameters

The search and enumeration of bacteria of faecal origin (total coliforms on TTC Tergitol medium, faecal coliforms on TTC Tergitol medium and faecal streptococci medium on Slanetz and Bartley medium) and the detection of pathogenic bacteria (spores of Clostridium Sulfito-reducers on SPS medium and Salmonella on SS and Hektoen medium) were carried out by the membrane filtration technique (0.45 µm) using the standard method ISO 6579: 1993 [11], [12].

2.3.3 Molecular Parameters

DNA extraction is carried out by the rapid method of Boiling preparation [13]with small volumes, the molecular confirmation of Salmonella is carried out by the gene amplification technique (PCR)nestled in a 1Kb DNA fragment of the chromosome-specific InvA invasion gene (Salmonella Typhimurium No. M90846.1) using the F-5'accacgctttttttttcgtctttcgtctg3' and anti-sense R-5gagactacgcaaga3 According to a program that contained 35 cycles and a hybridization of 62oC: (95ºC - 5' / (94ºC, 45''/ 62ºC, 30''/ 72ºC,1') x35 / 72ºC - 10' / 4ºC - 5') [14].

3. RESULTS AND DISCUSSION

3.1 Physico-chemical results

According to the physicochemical study of the three sites: we were able to deduce that the parameters are very close to the recommended standards [15], specific parameters gave acceptable results in relation with the guide values, such as the pH (figure 3) which varies between 7.5 and 8.3 and according to Šolić and Krstulović - 1992, these values are influenced by the carbon dioxide content of the atmosphere (measured as CO2 partial pressure (pCO2)) which affects the content of the ocean surface, and in return affects the pH of seawater [16]. The temperature (Figure 2) has known values of 18.2 and 19.3 ° C which remains around the optimum value; moreover, the temperature measurement makes it possible to correct other parameters of water analysis whose values are related to the temperature (conductivity and salinity in particular). Studies show that anthropogenic CO2 increasing leads to ocean and atmospheric warming and ocean acidification (Caldeira 2005), Caldeira K (2005) and Hans-O (2014) proposed a model suggesting that the seawater temperature continues to rise from 2.0 to 4.5 ° C [17], [18] and that by the end of the century, the pH decreases by 0.4 unit [19]. While we observed an increase in the levels of each turbidity (Figure 5) (22 to 32, standard: 0.5 to 10 UNT) and conductivity (Figure 4) (minimum: 56 ms/cm, maximum: 60.2 ms/cm), which indicates significant mineralization of seawater. Yet, the conductivity gives an appreciation of the ion content of seawater and therefore controls its electrical capacity [20], however, this increase in turbidity coincided with the completion of the rainy season, this increase has been

attributed to the flow of rivers and muddy runoff that discharged their contents into the shoreline during the study period, and the installation in this region of large sewage collectors in Casablanca city injecting water from these coasts with faecal waste and nutrients, it should be noted that very turbid water prevents the penetration of light and reduces the effect of microbial destruction by UV light from the sun [21], which explains the presence of bacteria and their resistance [22]as well as their high presence during the winter. Concerning salinity (Figure 6), values of 40 mg /l were reported at site 2, 41 mg / l in site 1 and 42 mg/l in site 3; many authors talk about climate change that has a direct impact on the whole land including the oceans, among the filed scenarios is that are expected is that the salinity changes due to the global warming phenomenon, the reduction of salinity or its increase depends on the latitude of the region [23]. This explains the variations in salinity found in the sites studied. BOD5 (Figure 8), which represents a moderately high bacterial load raging between 3 mg of O2 /l at site 1 and 60 mg of O2 //1 at site 2, this test is, currently, called the closed bottle; however, there is much scientific research on BOD5 in freshwater and wastewater [24], unlike seawater, where there has been little information of BOD5, which most of the dissolved organic matter is resistant to microbial oxidation [25]. There was also a slight increase of nitrites (Figure 9) of the order of 0.088 mg /l, however BRANDHORST (1959) stated that the high concentrations of nitrite in water are a result of the lack of nitrifying bacteria [26], confirming the presence of bacteria in all the seawater sites studied with a higher rate in the site 3 indicating to a higher bacterial load. We also recorded a decrease in dissolved oxygen values (Figure 7) up to 61% in site 2 and 72% in site 1 and despite this decrease, it remains significant and showing poor oxygenation in both sites, on the other hand, proper relative oxygenation has been reported as high as 92% for site 3. The consumption of dissolved O2 in seawater provides valuable information concerning the biological consumption of living organisms [27]; which converges towards the possibility of the presence of microorganisms in the seawater of these sites. Therefore, and according to the current Physico-chemical results of the sites studied after the installation of the EST anti-pollution station of Casablanca, there is a marked improvement of all the values measured under the same spatiotemporal conditions compared to the results found in a subsequent study we conducted prior to the installation of this sewage treatment plant project [5]. This proves its effectiveness in restoring the parameters of the quality control of seawater for swimming.

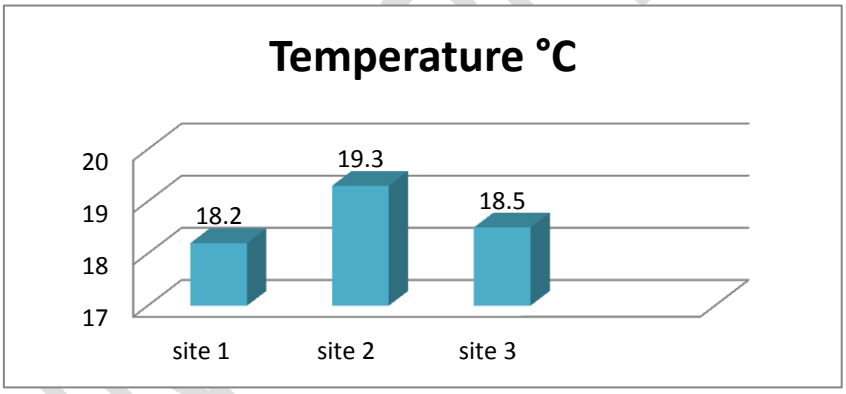

Fig. 2. Graphical representation of seawater temperature at Site 1, 2, 3

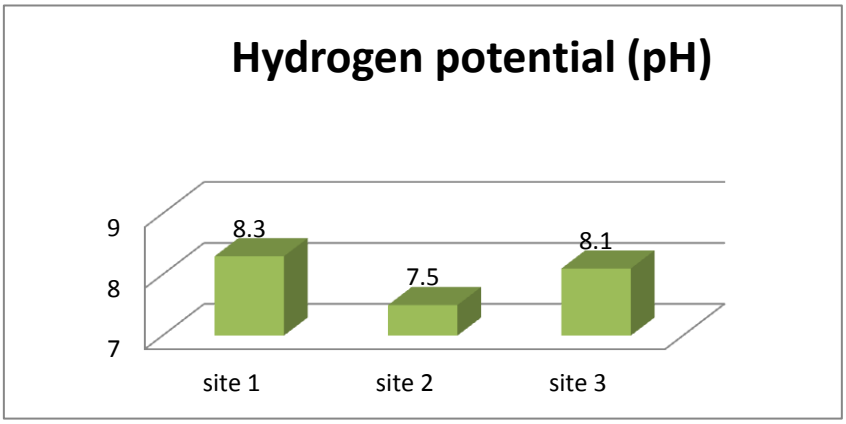

Fig. 3. Graphical representation of the pH of seawater at Site 1, 2, 3

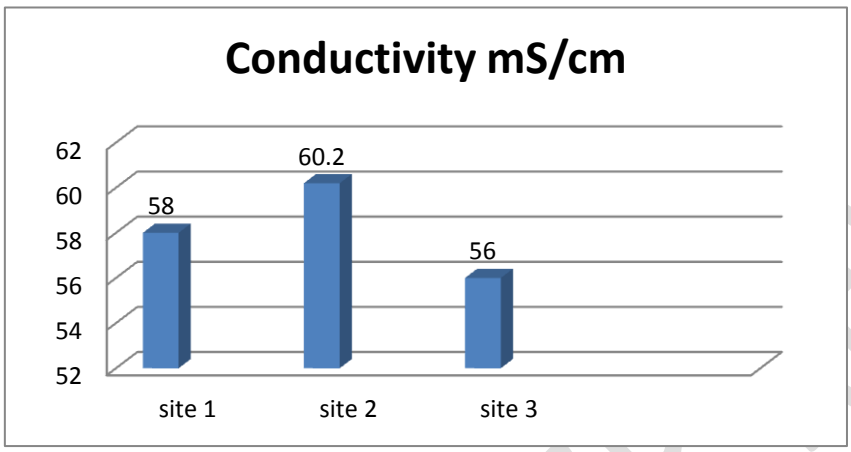

Fig. 4. Graphical representation of seawater conductivity at site 1, 2, 3

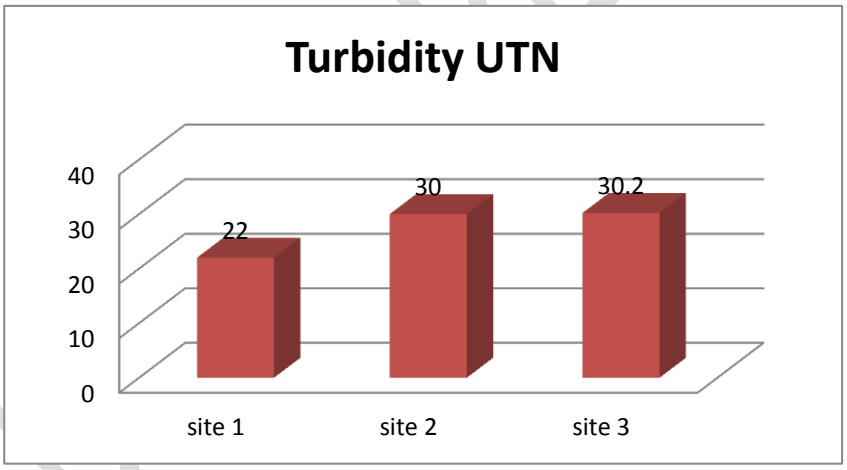

Fig. 5. Graphical representation of seawater turbidity at Site 1, 2, 3

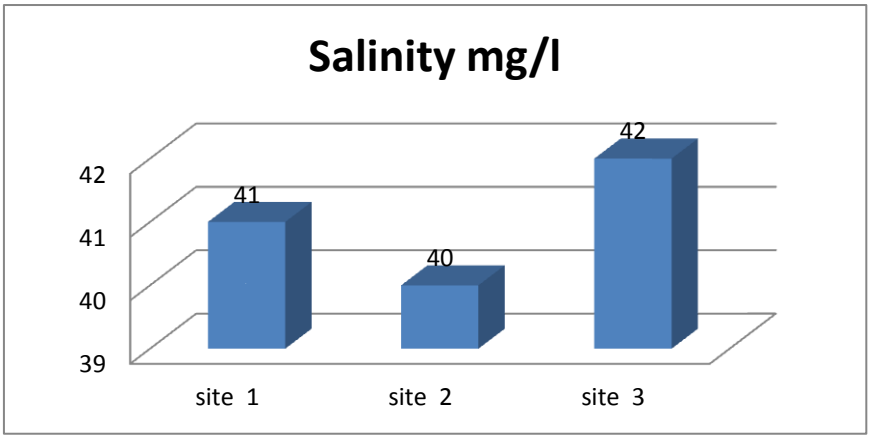

Fig. 6. Graphical representation of seawater salinity from Site 1, 2, 3

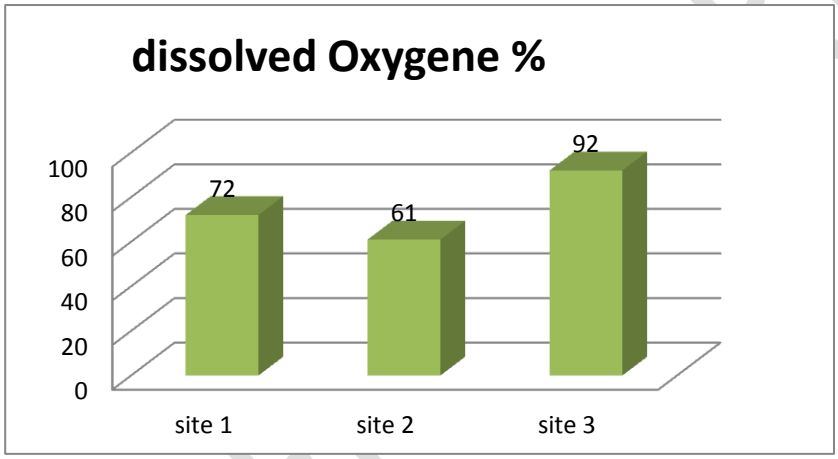

Fig. 7. Graphical representation of dissolved oxygen in seawater from Site 1, 2, 3

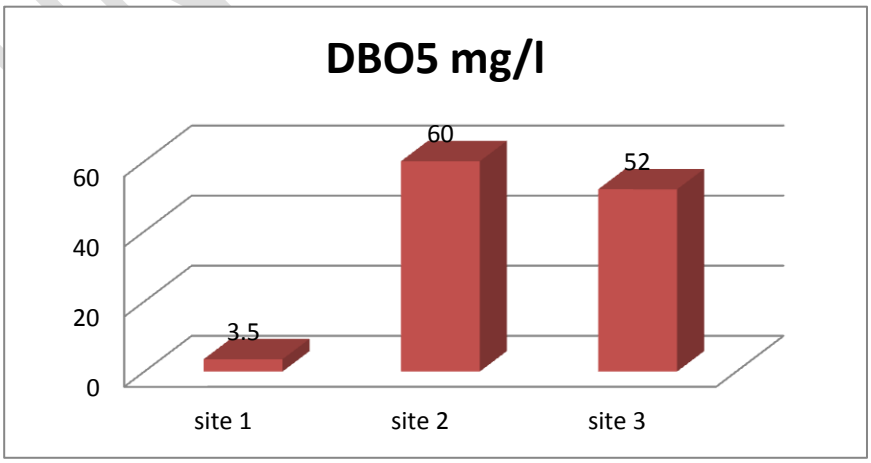

Fig. 8. Graphical representation of the BOD5 of seawater from Site 1, 2, 3

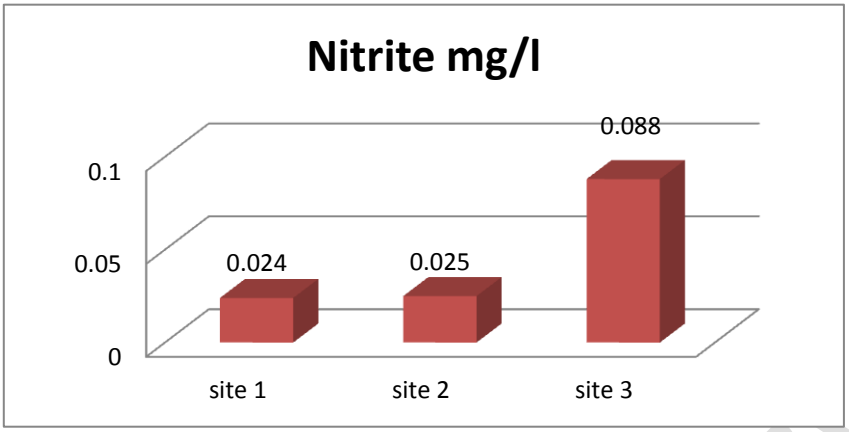

Fig. 9. Graphical representation of nitrites in seawater from Site 1, 2, 3

3.2 Bacteriological results

As for the bacteriological results at the three sites, the values obtained were consistent with the guideline values for faecal coliforms with 60 CFU / 100 ml in site 1, 23 CFU / 100 ml in site 2 and 45 CFU. / 100 ml in site 3 (Table 1), and for faecal streptococci whose count varied between 1 CFU /100 ml in the site1,2 and 8 CFU / 100 ml in site 3 (Table 1). That proves that all the sites studied are largely compliant with the national standard (NM 03.7.200), transposed from the European Directive (76/160 / EEC) and the WHO / UNEP Directives and applicable to the sanitary surveillance of marine bathing waters [28] which tolerates guideline values (GV) in the order of 100 CFU in 100 ml of filtered seawater for both faecal coliforms and fecal streptococci, conversely, imperative values (VI) per 100 ml of filtered seawater, they are of the order of 2000 CFU / 100 ml for faecal coliforms and 400 CFU / 100 ml for faecal streptococci (Table 2). In parallel with the enumeration of bacteria, which, allows the assessment of the quality of the bathing water of the monitored beaches. W have isolated other bacterial strains such as total coliforms and sulfite-reducing Clostridium to get an idea about the bacterial load and to date the biological pollution.

On the other hand, the isolation of Salmonella is a part of the protocol to verify the quality of bathing areas because the presence of only one of these bacteria condemns the closure of the concerned beach to avoid the risk of bathers contamination. We have noted in the study areas that total coliform enumeration ranged from 40 CFU / 100 ml in site 2, 73 CFU / 100 ml in site 3 and 140 CFU / 100 ml in site 1 with the absence of certain pathogenic germs such as Clostridium sulfite-reducer spores and Salmonella (Table 1). Furthermore, and based on these bacteriological results which are currently found, it is clear that they show a considerable decrease in all indicators of faecal contamination by the enumeration of both faecal coliforms and faecal streptococci that have become compliant with national standards. (NM 03.7.200) however, before the installation of the anti-pollution station in the EST of Casablanca, the bacteriological quality of this region was deteriorated as a result of untreated wastewater, which shows the beneficial effect of the anti-pollution station on the quality of seawater bathing areas of the region of Ain Sebaa - Zenata.

Table 1. Enumeration of bacteria isolated in site 1, 2 and 3

Microbiological Parameters	Site 1	Site 2	Site 3
fecal coliforms UFC/100ml	60	23	45
fecal streptococci UFC/100ml	1	1	8
total coliforms UFC/100ml	140	40	73
Clostridium sulfite-reducing spores UFC/100ml	0	0	0
Salmonella UFC/100ml	0	0	0

However, to better verify the bacteriological results, we propose to carry out a molecular detection and identification study through PCR (Polymerase Chain Reaction). This study focuses mainly on the search for germs that are difficult to cultivate, such as Salmonella, since standard bacteriological research tests have given negative results. In this perspective, many studies have proved the effectiveness of the method of molecular biology by PCR for the rapid and promising search for Salmonella[29], [30] as the classical identification techniques through the demonstration of deceptive biochemical traits. That can last three days to have a complete identification, yet, it is needed to look for another alternative.

Table 2. Moroccan standards (NM 03.7.200) for the bacteriological quality of marine bathing waters.

Microbiological Parameters	Guide values (VG) / 100 ml	Mandatory values (VI) / 100 ml
Fecal coliforms	100	2000
Fecal Streptococci	100	400

3. 3 Molecular Results

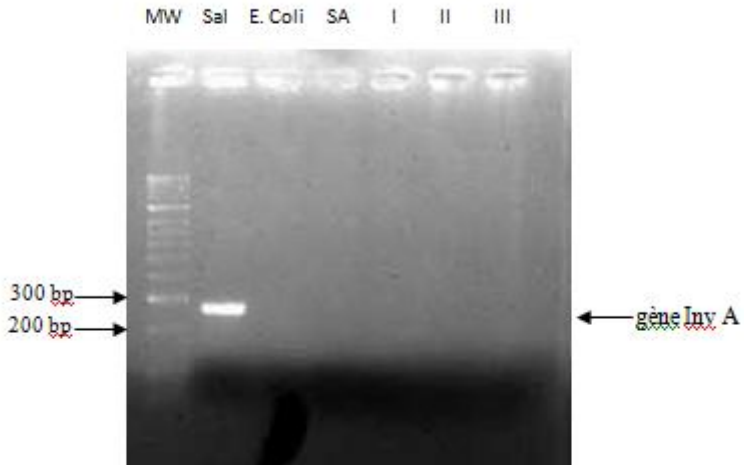

Fig. 10. Agarose gel electrophoresis 1.5% of the invA gene (275 bp), MW: Size marker (100 bp Leader), I: Site 1, II: site 2, III: site 3, E.Coli: Escherichia Strain Coli, Sal: Salmonella strain Typhimurium DT 104, SA: Staphylococcus strain Aureus.

The results of the PCR were presented on the agarose gel. The 275bp band provided for the Salmonella-positive control DNA is well-present, and there is no band for the DNA of the two negative controls (E. coli, Staph) which suggests that the PCR was conducted in right conditions, without contamination. As a result, the reliability of the results is entirely guaranteed. Besides, numerous studies have proved the efficacy of the search speed of pathogenic germs by molecular techniques such as PCR instead of using conventional techniques [31], [32] less promising and longer for identification. Some germs are difficult to cultivate.

Regarding the three sites studied, we find that there is no Inva gene band on the electrophoresis gel, which means the absence of Salmonella Typhimurium germ searched by PCR. In sites 1, 2 and 3 it was previously declared positive by the same technique in site 2 and 3 [5] which further confirms the beneficial effect of environmental protection after the inauguration of this anti-pollution station in the EST area of Grand Casablanca.

4. CONCLUSION

Since the commissioning of the eastern coast of East Casablanca (SAP-Est) anti-pollution system in 2015, wastewater from the entire area stretching from the port of Casablanca to the Mohammedia City, has been pre-treated before being discharged into the sea; this study is the first of its kind to include results after the inauguration of this station and at the same time relying on other subsequent and alarming results that previously demonstrated the seriousness of bacterial pollution as well as the degradation of the Physico-chemical quality which beaches were suffering from prior to this treatment project. The study, in one hand, allowed us to monitor the physicochemical and bacteriological quality of the seawaters in this region after the installation of this station, and on the other hand to verify the proper functioning of the wastewater treatment plant by comparing the results currently found during this study with those already achieved before the establishment of the station. In general, it has been found in our study that the seawaters of Zenata region comply with Moroccan standards. Concerning the physicochemical quality, we observed a decrease in the levels of each of the turbidity, conductivity, BOD5 and nitrites compared to the values obtained before the installation of SAP. At the level of the bacteriological study of the three sites, we were able to deduce that the values obtained for faecal coliforms and faecal streptococci following the guide values, which mean the absence of faecal contamination, we also noted the absence of pathogenic germs such as Salmonella and Clostridium- Sulfite-reducers.

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