Characterization of bathing water on the Casablanca coast

[1]Hanane Idali, [1]Abdelilah Fahde, [2]Yassine Schahrakane, [3]Abderrazzak Rachidi

[1] Laboratory of Health and Environment, Faculty of Sciences Ain Chock, Hassan 2 University, Casablanca, Morocco; [2] Laboratory of Immunology and Biodiversity, Faculty of Sciences Ain Chock, Hassan 2 University, Casablanca, Morocco; [3] Laboratory of Chemistry and Bromatology, National Office of Food Sinity Security, Casablanca, Morocco. Corresponding author: H. Idali, idali.hanane27@gmail.com

Abstract. The coast of Casablanca is located on the Atlantic coast of Morocco, it has sectors of human activities and economic activities that seek the coast location, as a preferential or necessary site: seaports, refining and chemical industry, modern thermal power stations, sea fishing, marine aquaculture, seaside tourism and marinas. The pursuit of these activities and their development require the persistence of a certain coastal balance. The definition of the coast will be limited to the area of land-sea contact and interaction between marine and continental effects. This space represents a precious and limited resource because it corresponds to this narrow zone of contact between the continental domain and the underwater world. Any abusive occupation results in the final consumption of this resource, without the possibility of reproduction. On the other hand the rational and light exploitation allows this space to perpetuate itself without losing its quality. The objective of this work is to assess the state of pollution of the Casablanca coast through different studies (physico-chemical, microbiological and determination of heavy metals) on different matrix (algae, water and mussels). We assessed the pollution status of bathing water on the Casablanca coast on five sites (Paloma, Zenata, Hank, Sidi Abd Rahman and Dar Bouazza) through different physico-chemical studies (determination of nitrate, nitrite, orthophosphate, BOD_5, dissolved oxygen, temperature, pH), microbiological (looking for coliforms, streptococci and clostridium) and determination of heavy metals by the analysis of three metallic elements (lead, cadmium and zinc) at the level of two species, animal (*Mytilus edulis*) and plant (*Corallina mediterranea*). The comparative analysis of the average levels found for the different studies showed that the contamination did not reach worrying levels except for the microbiological analysis which showed the presence of contamination of faecal origin.
Key Words: sea water, Casablanca coast, pollution, physico-chemical parameters, microbiology, atomic absorption, heavy metals, *Mytilus edulis*, *Corallina mediterranea*.

Introduction. The Casablanca coastline, located on the Atlantic coast of Morocco, is part of the coastal Meseta stretching from Rabat to El Jadida (Egis BCEOM International et al 2011).

The coastal zone of Casablanca, several tens of kilometers long, includes the city of Casablanca. This coastal area is characterized by a morphology alternating sandy beaches, beaches with rocky plateaus, rocky areas. Some portions of these beaches are bordered by dunes a few meters high (Egis BCEOM International et al 2011).

Aquatic organisms depend for their growth and reproduction on the quality of the waters in which they live. In general, the quality of water is determined on the basis of quantitative and qualitative criteria such as, the presence in sufficient quantities of certain nutrients, the oxygen content, the pH, the temperature or even the presence of substances known for their toxicity (metals, pesticides, phenols, etc.) (S.E.E.E. 2007).

Therefore this study aims to analyze the various physico-chemical parameters (nitrate, nitrite, orthophosphate, biochemical oxygen demand (BOD_5), dissolved oxygen, temperature and pH), microbiological parameters (research and enumeration of coliforms, streptococci and clostridium), as well as the analysis of three heavy metals (lead, cadmium and zinc). This study was based on two species, animal (*Mytilus edulis*) and plant (*Corallina mediterranea*).

The objective of this work is to assess the state of pollution of the Casablanca coast through different studies on different matrix (algae, water and mussels). However, the final objective remains the fight against pollution and the preservation of public health in bathing areas.

Material and Method. The samples were collected along the Casablanca coast (Figure 1) in the zones of Dar Bouaza, Sidi Abd Rahman, Paloma, Hank and Zenata, in 2017, once per season.

Figure 1. Sampling areas on the Casablancan coast (Google August 20, 2019).

We sampled water for physico-chemical and microbiological analyses; then the species *M. edulis* and *C. mediterranea* were sampled for the determination of heavy metals. The choice of species was made according to accessibility as well as availability in the five zones, since sedentary species are considered as bio-indicators, this criterion was used to reflect the state of the environment. .

We took 1 g of flesh of *M. edulis* and 1 g of *C. mediterranea*, in a Teflon bottle, then we added a mixture of reagent of nitric acid/hydrogen peroxide (5:2), then we closed the bottles and put them in the mini microwave oven for mineralization for 45 min. The content was then collected in conical tubes and adjusted with ultra pure water up to 50 mL then stored in the freezer until analysis with Atomic Absorption Spectrometry (AAS).

For the physico-chemical analysis, the water samples were taken in 1L plastic sampling bottles, which were previously cleaned with a 1% HNO_3 solution, rinsed abundantly with distilled water and dried. When the samples were taken, they were rinsed with station water. Water temperature (°C), hydrogen potential (pH), electrical conductivity (EC) and dissolved oxygen (DO), were measured in situ using a multi parameter water quality sensors (Mp65 type). The other parameters: BOD_5 (dilution method), nitrates (NO3-) (sodium salicylate spectrometric method), nitrites ($NO2^-$) (sulfanilamide colorimetric method) and orthophosphate (PO_4^{3-}) (ammonium molybdate method) were analyzed in the laboratory following Oubraim (2002) within 24 hours of their transport in a cooler at 4°C.

For microbiological analyzes: 20 glass vials with a volume of 500 mL were sterilized by autoclaving for at least 15 minutes at 121ºC. In order to highlight any pollution of the faecal type, various tests were carried out, namely: the enumeration and the search for bacteria of faecal origin such as coliforms, faecal streptococci and *Clostridium* sulphite-reducers. The identification of bacteria was carried out using culture media, the composition of which makes it possible to demonstrate a physiological (fermentation) or enzymatic (research for the presence of urease) activity.

The methods used for the various bacteria count tests followed Rodier et al (2009), Tordjman-Valency (2016) and Hassi (2014): the methods of enumeration in solid medium (method by incorporation or by spreading); method by filtration; and most probable number (MPN) method.

Results

Determination of heavy metals. The results were structured in the form of graphs which show the average contents obtained in lead, cadmium and zinc in the two selected species (*M. edulis* and *C. mediterranea*) during the year 2017. The average contents were expressed in the form of concentrations in milligrams of pollutants per kilograms of the matrix studied.

Figure 2 shows that in case of *M. edulis*, during 2017, the lead contents reached a value of around 0.008 mg kg^{-1} in Paloma, while the lowest content, around 0.00 mg kg^{-1} obtained in Zenata. The cadmium contents recorded showed the presence of this metal in some zones with a maximum content at the Hank and Dar Bouazza of 0.903 mg.kg^{-1} and 0.835 mg kg^{-1} respectively and minimum at the level of all the rest of the zones of 0.014 mg kg^{-1}. The zinc contents recorded reached a high content of the order of 1.0978 mg kg^{-1}. This value was obtained in Paloma, while the lowest content, of the order of 0.1158 mg kg^{-1} obtained in the Hank area.

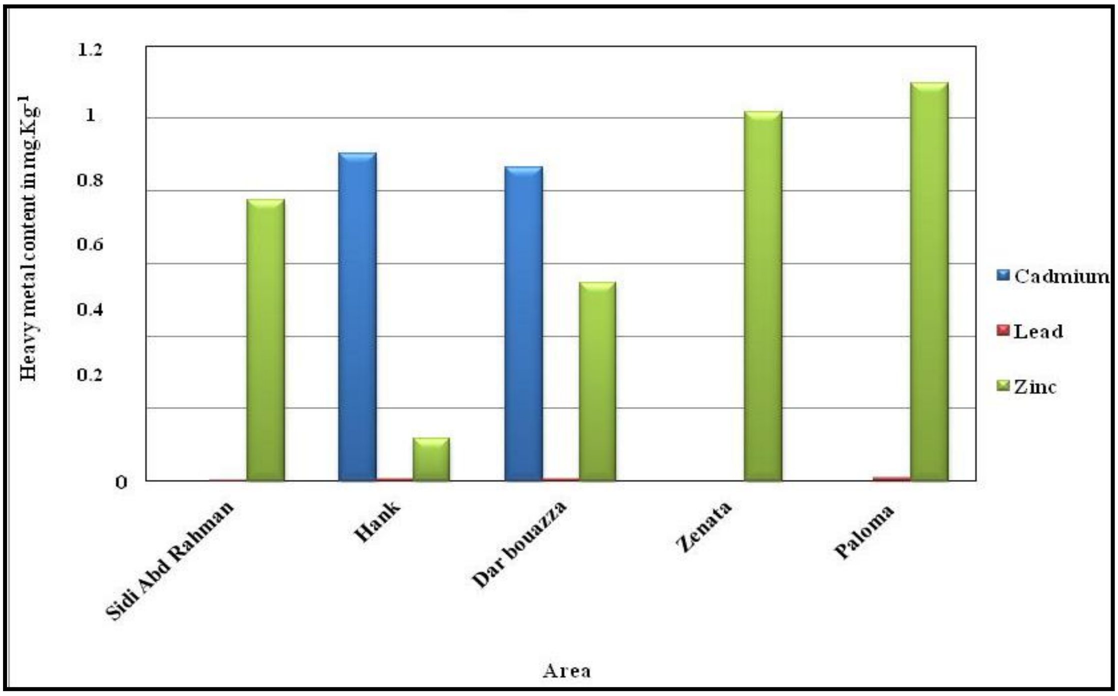

Figure 2. Heavy metal content in *Mytilus edulis* in 2017 in the five selected areas.

Regarding the species *C. mediterranea*, lead concentrations are almost absent for all stations except for Hank station which shows a concentration of 0.0313 mg kg^{-1}. The maximum concentration of cadmium is noted in the Paloma and Hank stations with the same content 0.9913 mg kg^{-1}. Overall, the accumulation of zinc in *C. mediterranea* from the stations studied does not show any significant differences depending on the sites with the exception of the Zenata station, which have concentrations significantly different from the others and which reach 1.733 mg kg^{-1}. Figure 3 shows that the most concentrated metal in *C. mediterranea* in 2017 at all stations is zinc with a maximum at the Zenata station.

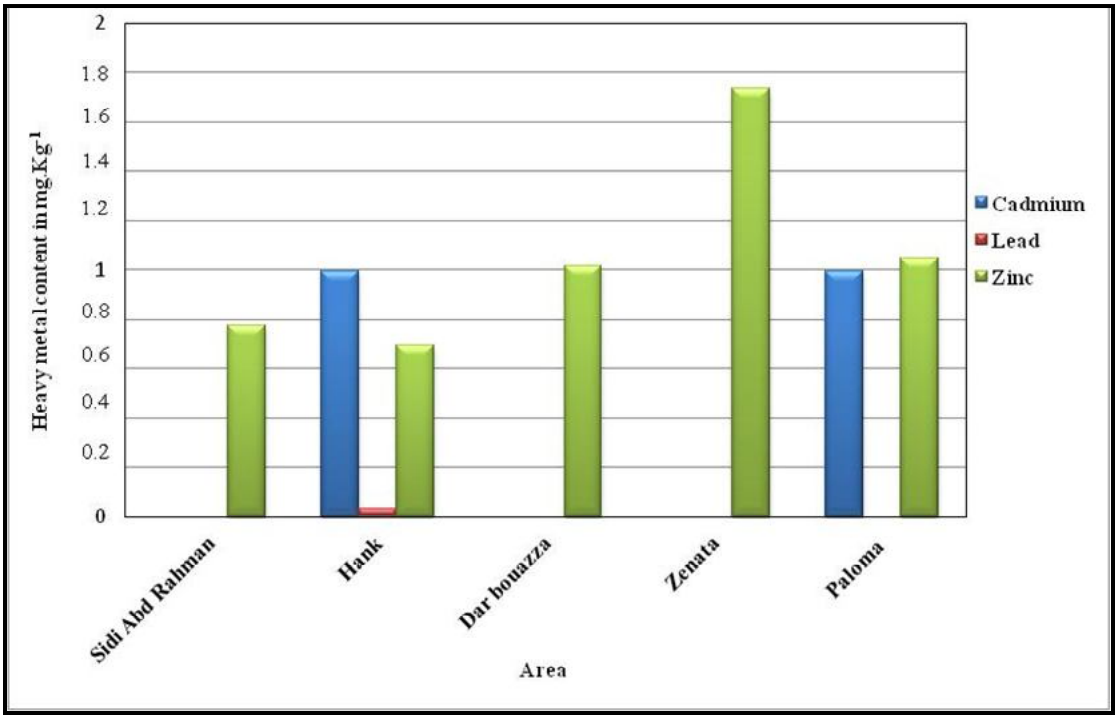

Figure 3. Heavy metal content in *Corallina mediterranea* in 2017 in the five selected areas.

Physico-chemical study. It concerned the determination of the various parameters in water, in situ (temperature, DO, EC and pH) and in the laboratory (nitrates, nitrites, BOD_5 and ortho-phosphates). Water sampling was carried out seasonally in the five zones.

Figure 4 shows that the temperature values of the water in the five zones analyzed during the study period vary between minimum values of 6°C recorded for the sample of the Dar Bouazza zone for the winter season, and maximum values of 24°C recorded for the Paloma zone sample for the Summer season.

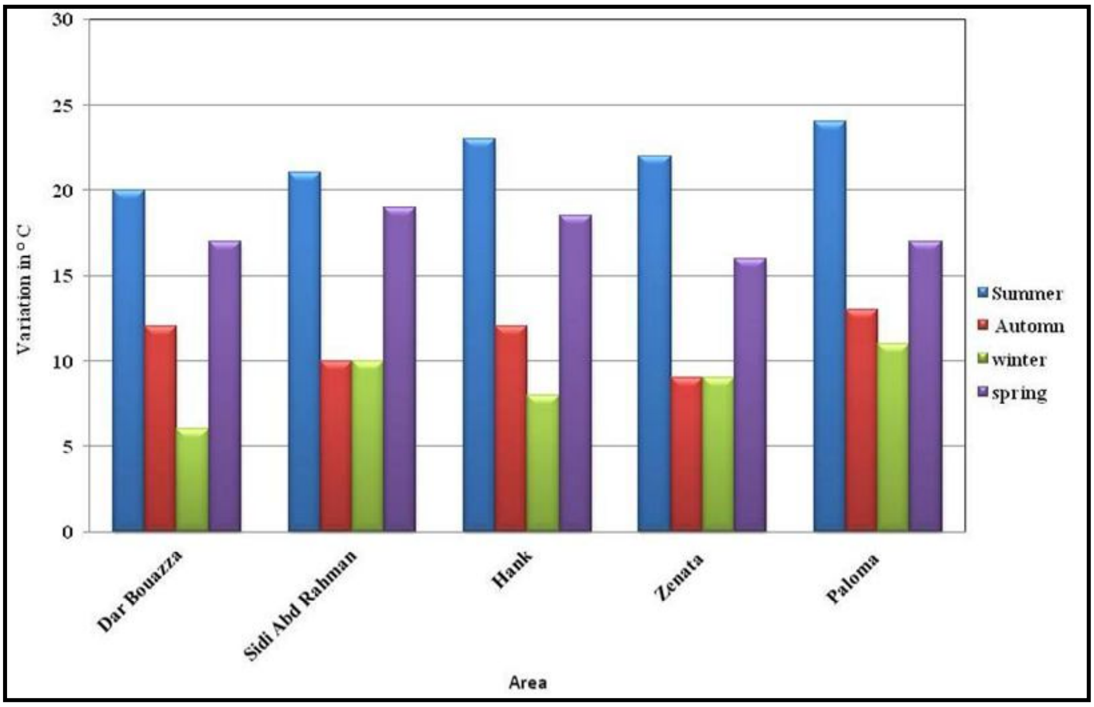

Figure 4. Temperature variation during the seasons in 2017.

WHO has set a guide value for temperature, which is 25°C (Moussa Moumouni Djermakoye 2005). In fact, the measured temperature values which vary from 6 to 24°C comply with the standards.

Figure 5 shows that the pH recorded during our study period varies between 5.69 and 8.65. The standards fix the pH between 6 and 9 (Bélaid 1993); in comparison with our results all the stations respect the standards and present an alkalinity for the majority of the areas except a slight acidification at the Dar Bouazza station in winter.

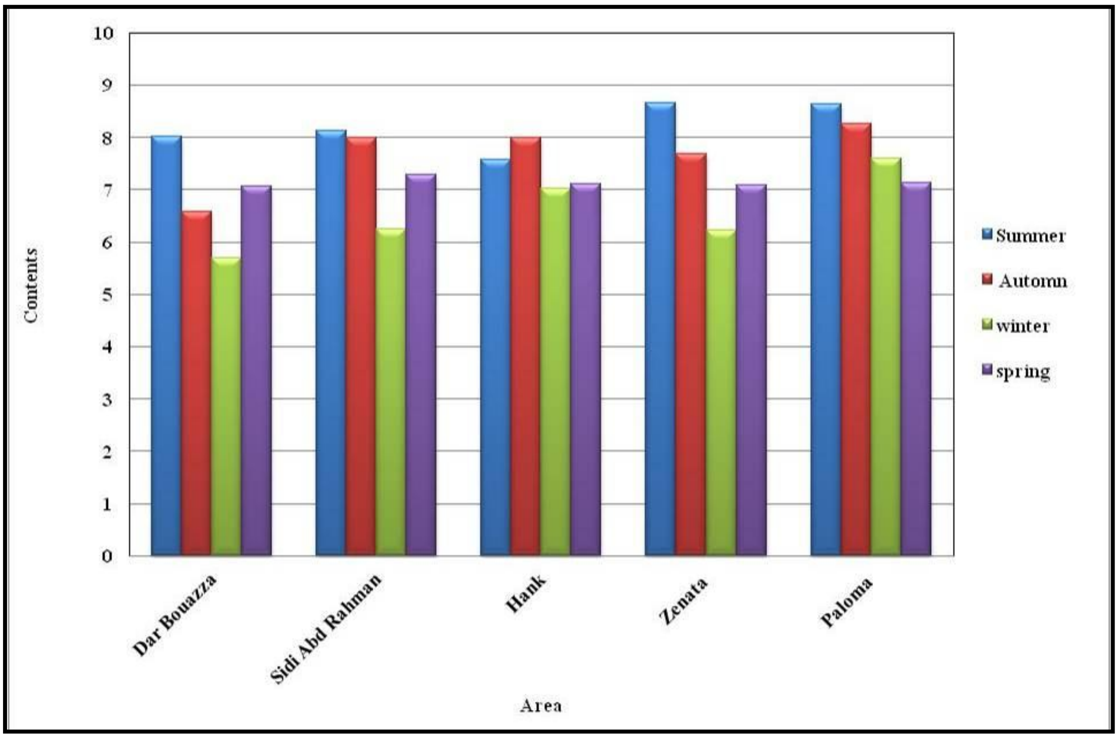

Figure 5. pH determination in 2017.

Figure 6 shows that our seawater is saturated with DO, the values in general are greater than 8.8 mg L^{-1}, the low values are around 7.65 mg L^{-1} at the Zenata station in spring.

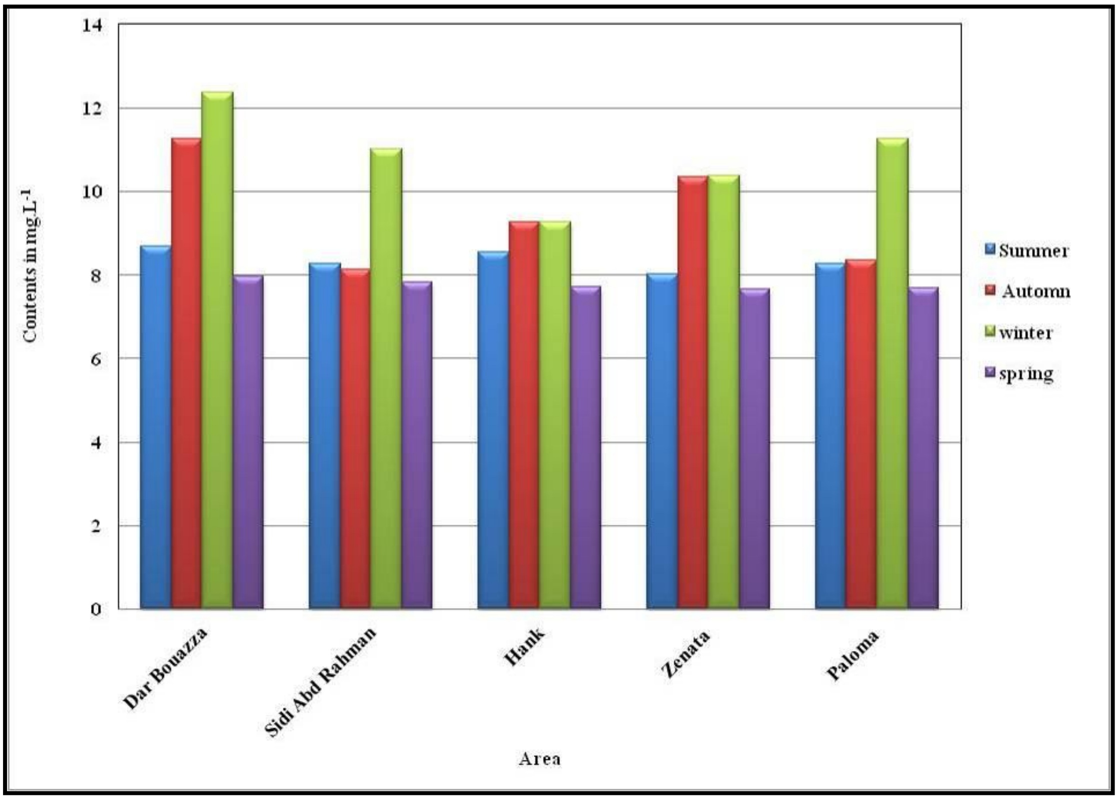

Figure 6. Determination of dissolved oxygen in 2017.

Figure 7 shows that the BOD_5 values vary from 0 to 30 depending on the zones and the seasons. Compared with the standards, the areas studied are mostly of poor to very poor quality. The decrease or increase in BOD_5 is due to the temperature variation which affects all biological processes, to the presence of microorganisms, to the poverty or the richness of the environment in nutritive salts (nitrogen and phosphate derivatives) and the presence of inhibitory substances which have a toxic effect on the activity of microorganisms (Moussa Moumouni Djermakoye 2005).

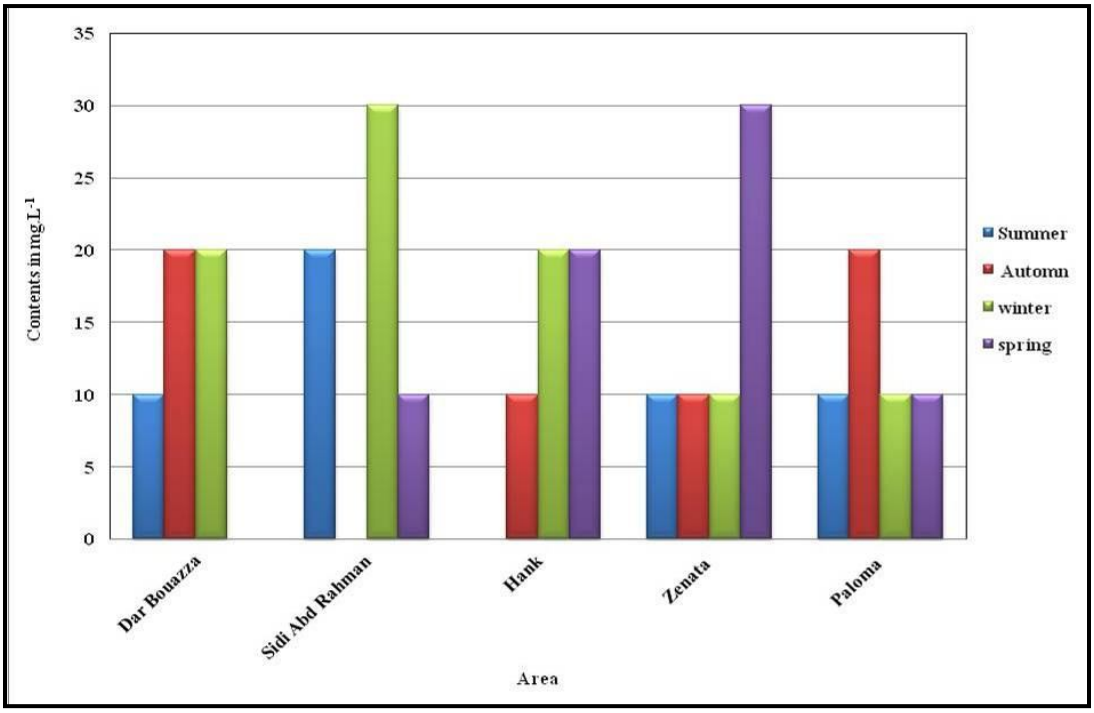

Figure 7. BOD_5 assay in 2017.

Figure 8 shows that the contents of orthophosphates vary from 0 to 0.005 mg L^{-1}, with the absence of this element during the spring season for all stations. The presence of phosphates is influenced by discharges from collective sanitation and treatment plants, sometimes also marked by the presence of nitrates and micro-organisms (Direction départementale des territoires et de mer de Vendée 2011).

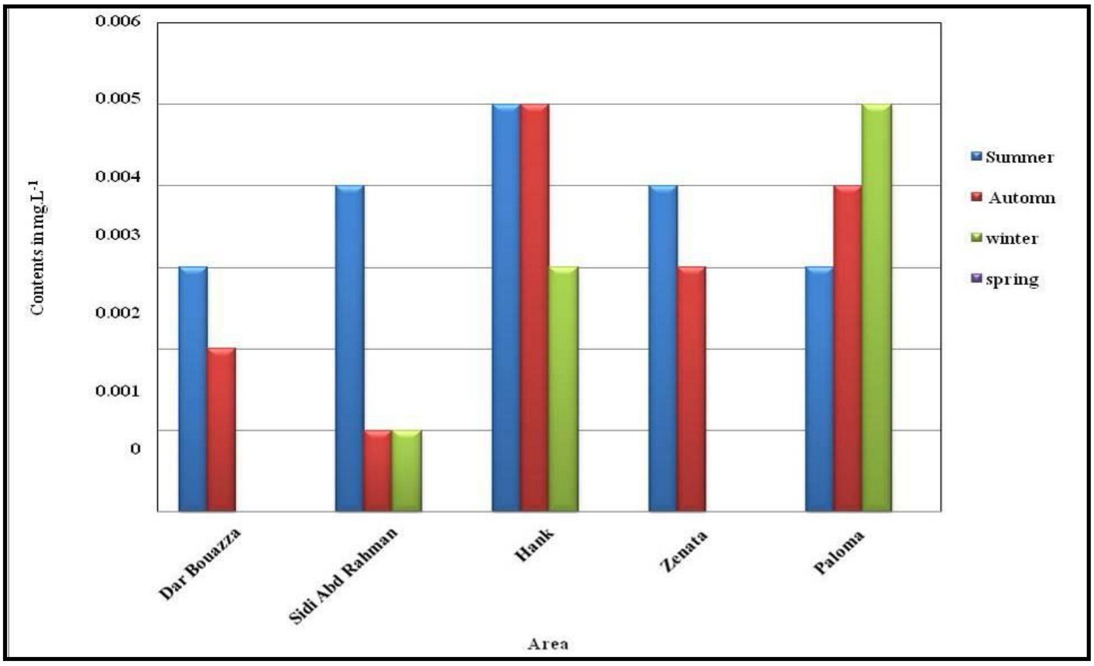

Figure 8. Dosage of orthophosphates in 2017.

Figure 9 shows that the electrical conductivity values range from 10.06 to 50.26 µS cm^{-1}. According to the relationship between conductivity and mineralization (Moussa Moumouni Djermakoye 2005) which tells us about the degree of mineralization of water where each ion acts by its concentration and its specific conductivity, we see that the conductivity of our water is between 0 and 100 µS cm^{-1}, which means very low mineralization and therefore a dilution of the ions in the water.

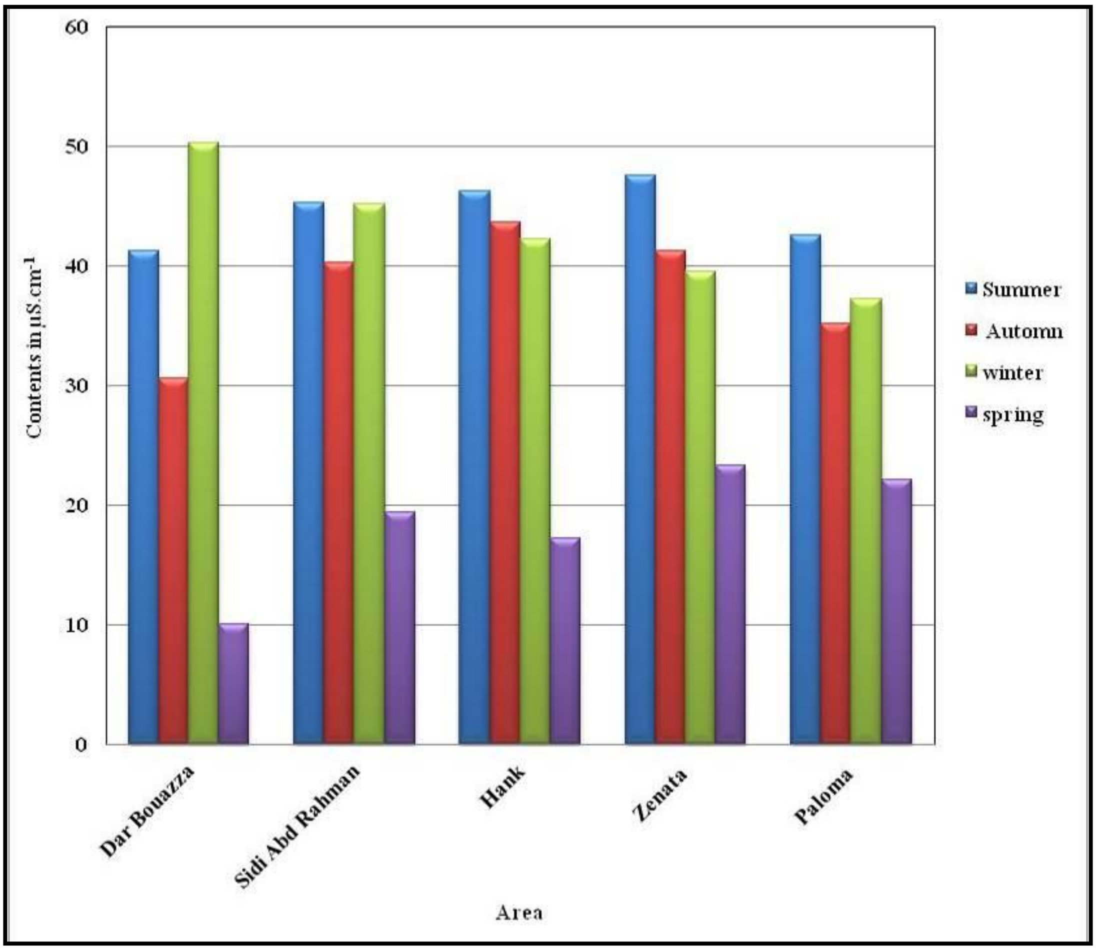

Figure 9. Conductivity assay in 2017.

The nitrate contents vary from 0.292 mg L^{-1} in spring to Sidi Abd Rahman to 0 mg L^{-1} in all the rest of the stations during the same season (Figure 10)

Nitrates and phosphates are essential nutrients for plant growth (phytoplankton and large algae) in coastal ocean waters. But, brought in excess, they risk bringing exaggerated and problematic growths which one qualifies as eutrophication provoking in particular sometimes localized deoxygenations and fish mortalities (Direction départementale des territoires et de mer de Vendée 2011), which is not the case in our results for these two elements.

Figure 11 shows that the nitrites values range from 0.006 to 0.187 mg L^{-1} at Dar Bouazza and Hank respectively. In general, the results exceed the standards, especially in summer and spring. Knowing that Zenata is considered to be a contaminated station throughout the year, a high concentration of nitrites indicates pollution of organic origin (Moussa Moumouni Djermakoye 2005).

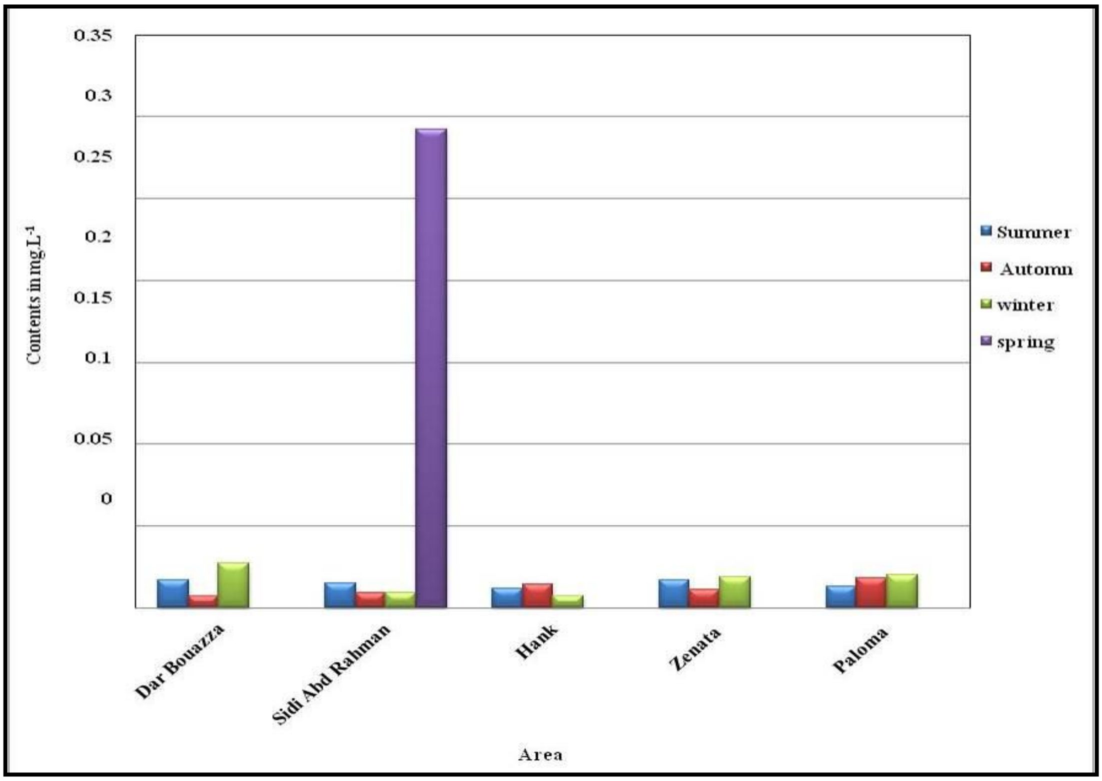

Figure 10. Determination of nitrates in 2017.

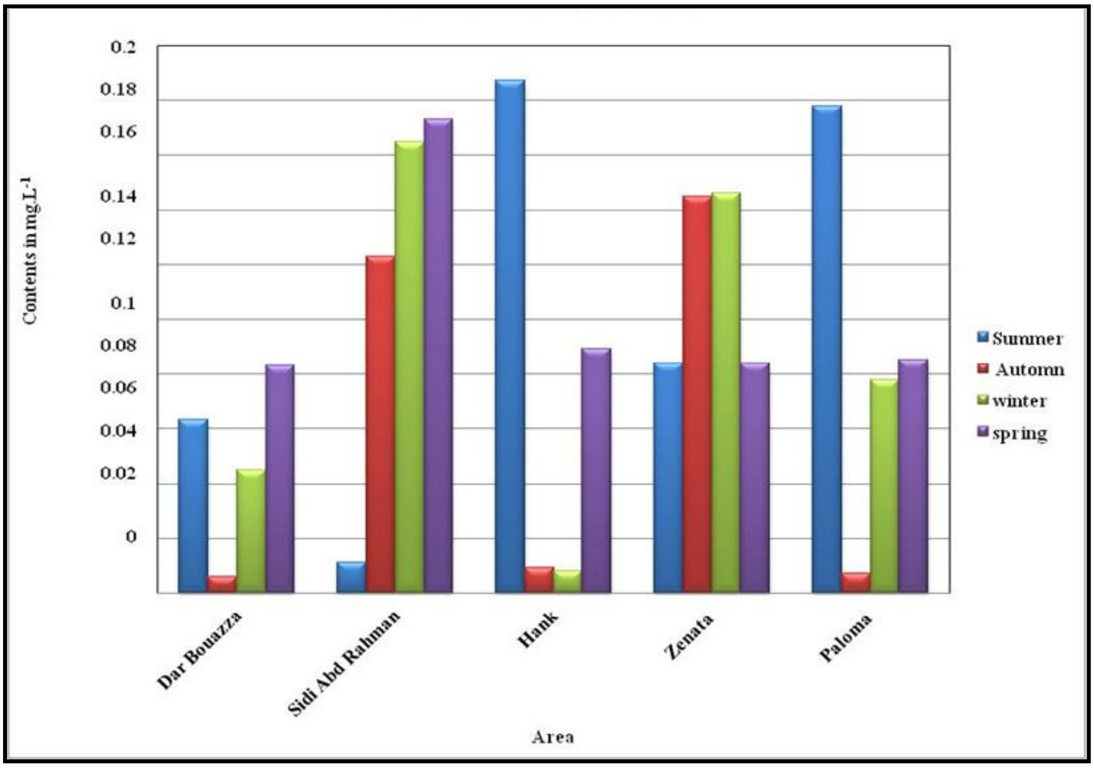

Figure 11. Determination of nitrites in 2017.

Microbiological study*.* The water samples were taken from the five areas seasonally to quantify the rate of microbial load, by researching and counting coliforms and fecal streptococci as well as the sulphite-reducing *Clostridium*.

For the results of total germs we can see that the highest values were recorded during the spring and this at the Zenata station. These rates reached the maximum value of 48 x 10^4 CFU mL^{-1}. This means organic contamination at this station. The lowest values were observed at the same station in autumn and at the Dar Bouazza station in summer at 0 CFU mL^{-1} (Figure 12). The total mesophilic load is not a parameter for assessing the bacteriological quality but can nevertheless tell us about the degree of potability of the waters and any food product (Tourab 2013).

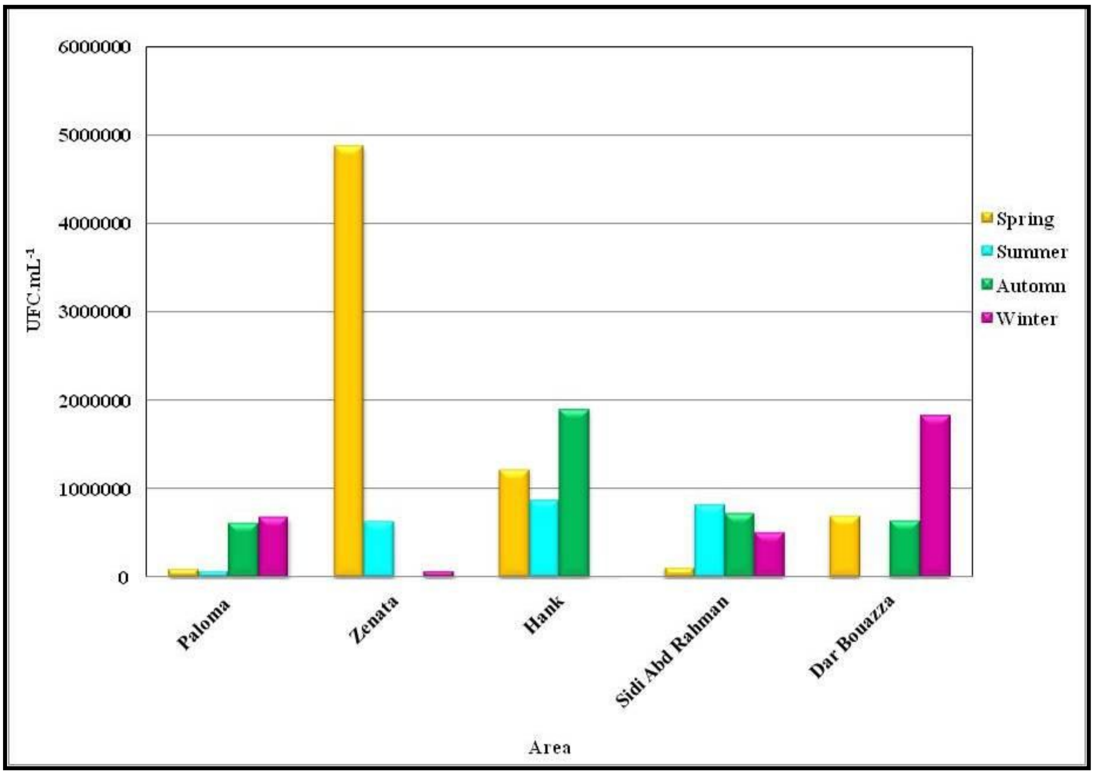

Figure 12. Total germ rate in 2017.

The total coliform results show us high values during the summer season with a maximum value of 25.10^4 CFU.mL^{-1} at the Sidi Abd Rahman station, while all the zones register an absence of coliforms during the winter with 0 CFU mL^{-1} (Figure 13).

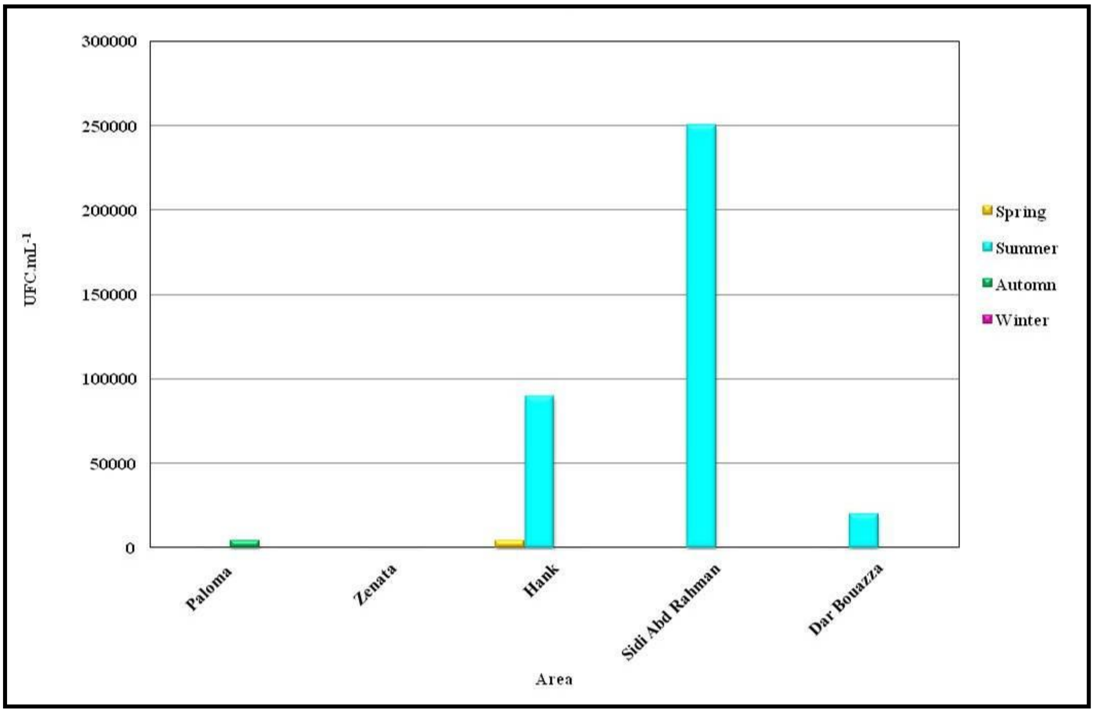

Figure 13. Fecal coliform rates in 2017.

Zenata station records the lowest rates of these germs during all seasons. The summer season shows the highest rates of proliferation of microorganisms in the majority of stations.

In comparison with the standards (Benderbouz & Felahi 2018) for the required quality of bathing water of 100 CFU.100mL^{-1}, the results obtained are for the majority of areas below the guide values and the limited values with the exception of the summer.

The increase in germination in Summer is probably due to the increased discharge of wastewater which can be the result of the high consumption of water and the activities of the inhabitants during this holiday season.

The presence of total coliforms in seawater is an indication of contamination by wastewater and a decrease in wind speed which induces poor self-purification of seawater (Benderbouz & Felahi 2018).

The decrease in the number of fecal coliforms during the rest of the seasons can be explained by strong agitation which is generated by a high wind speed, despite high rainfall and low temperature.

So, we can consider in this case that there is a threshold where the wind speed has a negative influence on the proliferation of germs although the other factors are favorable for the survival of fecal coliforms (temperature and high rainfall) (Benderbouz & Felahi 2018).

Streptococci are present at two Zenata and Paloma stations with maximum rates of 25.10^{4} CFU mL^{-1} during the summer at Zenata, the rest of the stations record the absence of these germs during the same season (Figure 14). Low values are recorded at Sidi Abd Rahman station during all seasons.

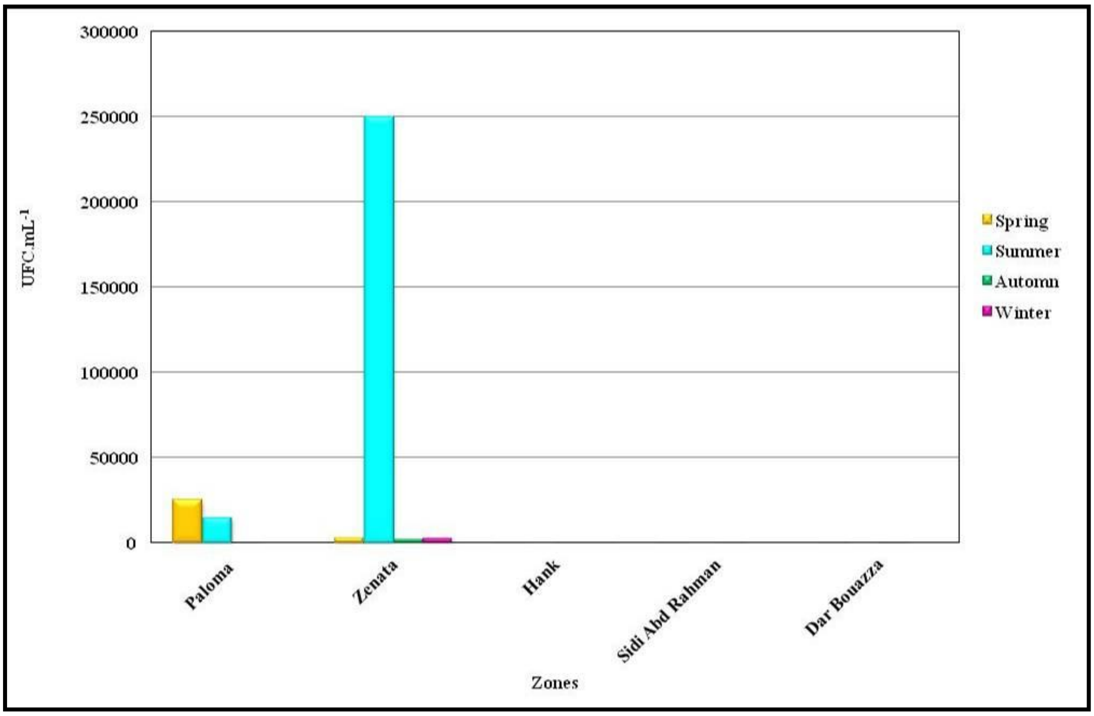

Figure 14. Streptococci rates in 2017.

The standards (Benderbouz & Felahi 2018) for the required quality of bathing water fix the guide value for streptococci at 100 CFU.100 mL^{-1} and in comparison with our results the majority of stations far exceed the standards.

Fecal streptococci are responsible for gastroenteritis, and are specific indicators of ancient faecal-human pollution; they are more resistant and persist for a long time in seawater (unlike *E. coli* (coliform)) given their resistance to a concentration of 6.5% NaCl and at a temperature of 10 to 45°C. This rise can be due to a high rainfall and a negligible wind speed which allowed the faecal Streptococci to survive (Benderbouz & Felahi 2018).

The presence of sulphite-reducing bacteria indicates old or intermittent faecal contamination and persistence in the environment (Hebbar 2005). Clostridiums are present in all areas in winter except in Dar Bouazza with maximum rates of 12.104 CFU mL^{-1} at the Hank station (Figure 15). Low values are recorded at Dar Bouazza station during all seasons except summer. In the autumn all stations have low levels of clostridia.

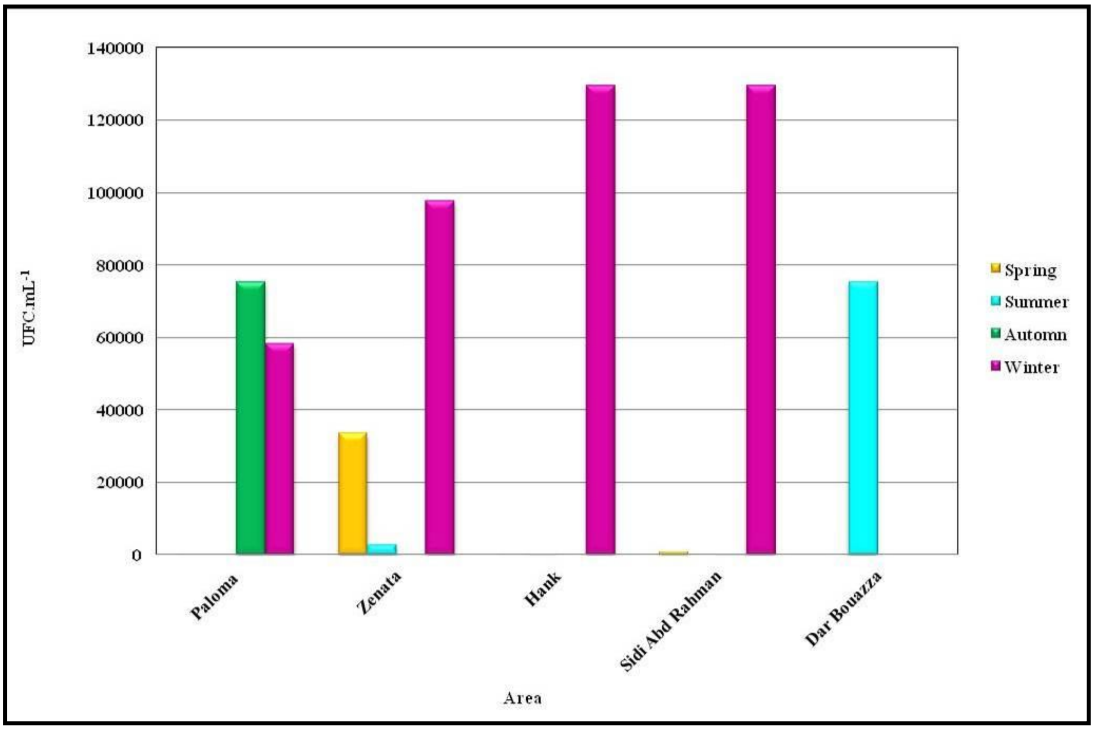

Figure 15. Clostridium rates in 2017.

Clostridium perfringens is ubiquitous, aerotolerant, sporulating anaerobic bacterium. The main reservoirs are generally considered to be the soil and the intestinal tract of humans (including healthy ones) and animals (poultry, cattle, pigs, fish) (ANSES 2012).

According to French and European standards (Vincent 2017) which fix the rates of sulphite-reducing spores at 0.100 mL^{-1} our results show that these standards have been exceeded, except for the Dar Bouazza station in winter, autumn and spring and at the Hank station in autumn.

Strains isolated from water samples have highlighted 8 species which are: *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Citrobacter* sp., *Enterobacter cloacae*, *Providencia stuartii*, Morganella sp., *Salmonella typhi*, and *Proteus mirabilis*.

Discussion*.* According to the regulations EC: 1881/2006, and EC: 629/2008 (Kyprianou 2006; Vassiliou 2008), the maximum values for lead and cadmium should be 0.5 $mg.kg^{-1}$ for crustaceans. The lead and cadmium contents do not show alarming doses in the majority of stations, except for Hank and Dar Bouazza with grades of 0.90 and 0.83 respectively in 2017.

These values which slightly exceed the standards can be explained by the sedentary nature as well as by the mode of nutrition of *M. edulis* which is done by filtration (Drif 2012) and which induces a bioaccumulation over time of these metals within the species or by direct infiltration and accidental release into the environment of these metals, followed by elimination of these contaminants by proteins and enzymes such as metallothionein which play an important role in the cellular protection processes against the actions of dangerous agents and for their function regulating internal contents of essential metals (Cu, Zn) and detoxifying non-essential metals (Ag, Cd, Hg) (Ben Cheikh 2017).

From what we obtained in our results for zinc we see that our values are much lower than the French national median which fixes the maximum content of zinc at 110 mg kg^{-1} (Belabed et al 2008) by dry weight in mussels.

Zinc contents are higher than lead and cadmium metals, since the molds are capable of accumulating the different concentrations of this metal due to accomplishing its metabolic effect using copper by participating in various metalloenzyme structures and in the transport of oxygen, hence the active site of the molecules of hemocyanin (respiratory pigment of molluscs) which gives the hemolymph analogous to blood in vertebrates the blue-green color (Karima 2014).

According to the limit values "International Standards accepted by (IAEA 2005)" (Bouguenoune & Amirat 2018) which are 0.574 mg kg^{-1} for lead, 0.0173 mg kg^{-1} for cadmium and 128 mg kg^{-1} for zinc and in comparison with our results obtained we note that they do not exceed the maximum admissible doses of heavy metals in algae, except the concentrations obtained for cadmium which largely exceed the threshold.

The most accumulated metal in algae is zinc which is confirmed by other studies, which have shown that zinc is more accumulated by red algae species (Karima 2014).

Metal concentrations vary within the same species from different sites. This is due to biotic or abiotic factors, anthropogenic factors and the heterogeneous distribution of metals in the ecosystem. These variations are related to tissue age, life cycle, ambient concentrations of metals and other environmental conditions that may influence it (Karima 2014).

The determination of heavy metals in our study showed that:

- the presence of cadmium at the two stations, Hank and Dar Bouazza with values of 0.903 and 0.835 mg kg^{-1} respectively and very low lead values in all stations;
- zinc remains the most accumulated metal in *M. edulis*;
- as for the results achieved by Bouthir et al (2004) the average cadmium contents are generally low, with the exception of an increase recorded at Sidi Abderhmane and Dar-Bouazza with respective averages of 1.21 and 0.98 µg g^{-1}. The lead content is generally low.

With regard to the good physicochemical state, the composition of the water measured at the various points must be in conformity to quality standards. The analysis of the physico-chemical quality of the water from these stations makes it possible to highlight that at these ranges, all the physico-chemical analyzes comply with standards except for BOD_5 which shows that there is a presence of the bacterial flora, which was further confirmed in the microbiological study.

The physico-chemical analysis showed that the contamination did not reach worrying levels because the contents of the parameters measured showed a decrease (nitrates: 290 µg L^{-1}, nitrites: 170 µg L^{-1}, orthophosphates: 4 µg L^{-1}) to Sidi Abd Rahman compared to the work of Bouthir et al (2004), which showed the presence of a high load of nitrogenous materials and orthophosphates (nitrates: 2058 µg L^{-1}, nitrites: 216 µg L^{-1}, orthophosphates: 96.8 µg L^{-1}) at the same station.

However, the contents of the various parameters return to normal at the level of the weakly anthropized Paloma and Dar-Bouazza sites due to the permanent mixing of coastal waters, mainly due to hydrodynamic agents ensuring the mixing of surface waters with those of the bottom and offshore, and thus favoring a dispersion of the polluting load.

Microbiological analysis showed the presence of coliforms and fecal streptococci with high levels during the dry season (summer), while the presence of anaerobic sulphite-reducing spores was recorded during the wet season (winter). This is due to several physicochemical factors, sewage discharges and urban activities.

Bacterial identification has enabled us to distinguish different bacterial species in seawater which belong to the genera: *Salmonella, Klebsiella, Citrobacter, Enterobacter, Providencia, Morganella* and *Proteus*. The majority of these species are coliforms, which confirms that the contamination of these waters is of faecal rather than environmental origin because these species are generally hosts of the human intestine and animals.

Conclusions. Marine ecosystems are one of the most precious resources on the planet, and their preservation is essential to maintaining biodiversity and natural balance, as well as life on earth. The marine and coastal environments are subject to perpetual changes of physical, chemical and bacteriological origin.

Swimming in natural waters can lead to more or less intense contact with chemical contaminants such as heavy metals as well as with pathogenic germs which can be present in water in more or less quantity. Transmission of microorganisms can be by faecal-oral route (ingestion of water) or by contact with the skin and mucous membranes.

Our results showed the presence of heavy metals in the species studied. Contamination by the three metals (lead, cadmium and zinc) does not reach worrying levels and seems to be much lower than the maximum doses admitted for the majority of stations even if zinc and cadmium proved to be the most concentrated metals in two species.

The results obtained for the physico-chemical analysis showed that the quality of the bathing water in the five zones studied complies with the standards and does not present any risk for the population except for the BOD_5 values which show increases in comparison with the standards and this can be explained by the excessive activity of microorganisms which has been demonstrated in the microbiological part.

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Authors:
Hanane Idali, Hassan 2 University, Faculty of Science Ain Chock, Laboratory of Health and Environment, Maarif, B.P. 5366, Casablanca, Morocco, e-mail: idali.hanane27@gmail.com
Abdelilah Fahde, Hassan 2 University, Faculty of Science Ain Chock, Laboratory of Health and Environment, Maarif, B.P. 5366, Casablanca, Morocco, e-mail: abdoufahde@hotmail.com
Yassine Schahrakane, Hassan 2 University, Faculty of Science Ain Chock, Laboratory of Immunology and Biodiversity, Maarif, B.P. 5366, Casablanca, Morocco, e-mail: y.cherakane@hotmail.com
Abderrazzak Rachidi, Laboratory of Chemistry and Bromatology, National Office of Food Sinity Security, Casablanca, Morocco, e-mail: abderrazzak57@gmail.com

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