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# OCCURANCE AND SURVIVAL OF *Vibrio alginolyticusin* TAMOUDA BAY (MOROCCO)

M. SABIR[1, 2], N. COHEN[2*], A. BOUKHANJER[2] AND M.M. ENNAJI[1*]

1 Laboratoire de Virologie et Hygiène & Microbiologie, Faculté des Sciences et Techniques de Mohammedia-Université Hassan II, Mohammedia – Maroc.
2 Laboratoire de Microbiologie et d'hygiène des Aliments et de l'Environnement, Institut Pasteur du Maroc, Casablanca- Maroc.

**Abstract**

The objectives of this study were to investigate the spatial and seasonal fluctuations of *Vibrio alginolyticus* in marine environment of the Tamouda Bay on the Mediterranean coast of Morocco and to determine the dominant factors of the environment that govern these fluctuations. The samples (sea water, plankton, shellfish and sediment) were collected fortnightly for two years from three study sites on the coast Tamouda Bay in northern Morocco. The charge of *Vibrio* alginolyticus is determined by MPN method. The physicochemical parameters including temperature of sea water, pH, salinity, turbidity and chlorophyll a concentration were determined. Analysis of variance of specific variables and several principal component analyses showed that the temperature of seawater is the major determinant of seasonal distribution of *Vibrio alginolyticus*. The results showed a positive linear correlation between *Vibrio alginolyticus* and the water temperature, pH, turbidity and chlorophyll a. Similarly, there are seasonal variations and spatial of *Vibrio alginolyticus* in marine environment of the Tamouda bay and the highest concentrations were recorded in both years of study during the warm season whereas it was minimal during the cold season. Linear positive correlation was recorded between *Vibrio alginolyticus* populations in all ecological types of samples studied.

***Key words:*** Environmental parameters, Mediterranean sea, Morocco, Physicochemical parameters, seasonal variation, *Vibrio alginolyticus*.

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**Corresponding author**

Pr Ennaji Moulay Mustapha
Faculté des Sciences et Techniques de Mohammedia-Université Hassan II, Mohammedia – Maroc, B.P. 146 – Mohammedia 20650 – Morocco.
Tel: +212 661 748862
Fax: +212 5 23 31 53 53
E-mail: m.ennaji@yahoo.fr

* Senior authors with equal contribution

**Abbreviations:** **APW**: Alkaline Peptone Water; **°C**: Celsius; **Chlo. a**: chlorophyll *a*; **Fig**: figure; **G:** gram; **H**: hour; **L**: liter; **mL**: milliliter; **µm**: micrometer; **MPN**: Probable Number Method; **PCA**: Principal Component Analysis; $r_s$: Spearman coefficient; **SST**: surface seawater temperature; **TCBS**: Thiosulfate Citrate Bilesalt Sucrose; **V.**: *vibrio;* **%**: percent.

## INTRODUCTION

*Vibrio* (*V.*) is a genus of bacteria indigenous to the aquatic environment. Some bacterial species of the genus *Vibrio* now considered emerging pathogens have been implicated in foodborne infections in Humans (12), posing a public health problem. The four species most frequently isolated in clinical microbiology laboratories are *V. cholerae, V. parahaemolyticus, V. vulnificus and V. alginolyticus* (20) which is the subject of our study. Other studies in Morocco have shown variable prevalence depending on the species of *Vibrio*, with a frequency of isolation especially in larger crustaceans and bivalves. (9, 13) and showed that 72% of the isolates belonged to the species *Vibrio alginolyticus* (14).

*V. alginolyticus* is a halophilic *Vibrio*, normal host mussels and was isolated as fish and seafood variety. In Humans, it was isolated from pus from ear infections and conjunctivitis,

 

especially after swimming in seawater. Similarly, it was shown that *Vibrio alginolyticus* should be included in the list of pathogens causing skin infections, especially if patients have been in contact with seawater in warm climate regions or with marine animals (10).

In Humans, it was isolated from pus from ear infections and conjunctivitis, skin lesions patients have been in contact with seawater, especially after bathing in seawater such was exceptionally isolated from sputum may sometimes be responsible suppuration. Similarly, *V. alginolyticus* can also cause soft tissue infections such as cellulitis and necrotizing infection of the tissue and opacification of the sphenoidal sinuses (27). Moreover, this genre has been shown to be responsible for gastroenteritis in humans (17, 32). *V. alginolyticus* is a species with wide geographic distribution in marine and estuarine waters, particularly in bathing areas (2, 5). She has been associated with several diseases of marine animals, including fish and shellfish (4, 7 21).

Reservoirs of *V. alginolyticus* are particularly copepod zooplankton, which show the importance of research to include bacterial plankton (29). Moreover, *V. alginolyticus* is considered the most frequent species living freely in water and sediment (23) and can survive in seawater even under starvation conditions while maintaining their virulence (7).

The objectives of this study are (i) assess the hygienic quality of fish products and their environment from *Vibrio alginolyticus*, (ii) to study the seasonal effects on the burden of *Vibrio alginolyticus* in marine environment and (iii) assess the relationships between physicochemical parameters and load *Vibrio alginolyticus* in different samples.

## MATERIALS AND METHODS

### *Description of the study area*

Tamouda Bay is located in the Mediterranean coast of Morocco, between Sebta at the North (35°54'N, 5°17'10"W) and Cap Negron at the South (35°40'N, 5°16'40"W) (Fig. 1) where the climate is typically Mediterranean. The average annual temperature is about 18°C, while the annual rainfall average ranges between 800 and 1000 mm.

### *Environmental sampling*

A total of six hundred forty-one (641) (236 water, 198 plankton, 67 bivalves and 130 sediment) samples were collected in three sampling sites of Tamouda Bay and analyzed for the monitoring of physicochemical parameters of seawater taken from the study area and research and counts of *V. alginolyticus* in water samples of planktons, bivalves and sediment. Twice per month, samples were made over a period of two years (January 2007-December 2008).

One of the three coastal sites, located at the mount of Smir's river (site 2), is described as at a high risk while the two others (sites 1 and 3) are considered as at low risk. The locations of the sites were determined by using of the global positioning system (Fig. 1). For each field visit, four ecological types of samples were collected, namely, seawater, planktons, shellfishes and sediments.

Using boat, seawater samples (2L) were collected at a depth of 1m from the surface in a sterilized plastic bottles. Planktons were collected by dragging the water horizontally, at a depth of about 1m, with 200-µm-mesh plankton net (33). Shellfishes samples were purchased from local fishermen, while the sediments were collected from the surface of the coast using sterile plastic pots. After collection, samples were transported immediately to the laboratory in insulated coolers with frozen gel-packs to maintain the temperature at around 4°C. The surface seawater temperature (SST) and pH of each study site were assessed using portable instruments simultaneously to each sample collection. At the sampling sites, salinity of seawater was measured by titrimetry (6). Chlorophyll *a* concentration was determined from 10 or 15 ml of seawater filtered onto 0.20-µm-pore-size nitrocellulose filters (Millipore Corp., Bedford, MA, USA). The filters were frozen until extraction with acetone (25). The pigment concentration was measured with a TD700 fluorometer (Turner Designs, Sunnyvale, Calif.).

### *Vibrio enumeration procedure*

The enumeration of *V. alginolyticus* our samples was carried out using the three tubes Most Probable Number Method (MPN) as described by Elliot (19) with some modifications. Briefly, 25 grams of shellfishes or sediments was inoculated into 25 mL of Alkaline Peptone Water (APW) and mixed using a stomacher for 30 seconds. Regarding seawater samples, 2L were concentrated using 0.20µm membrane filters (Millipore Corp., Bedford, MA, USA). In order to obtain a final concentrated volume corresponding to 100x the filters were washed with 20 ml APW. Planktons were used directly for *V. alginolyticus* enumeration. From these solutions, series of decimal dilutions were prepared in the tubes containing 9mL of APW. Each dilution was prepared in triplicate to determine the MPN. Inoculated APW was incubated at 37°C for 16-18h. The enrichment broth was then sub-cultured onto Thiosulfate Citrate Bilesalt Sucrose (TCBS) agar and incubated at 37°C for 18-24h. From each TCBS, suspected colony types yellow were picked out, streaked onto nutrient agar plus 2% NaCl to obtain pure cultures, screened for cytochrome oxidase and examined for NaCl requirement (0%, 3%, 6%, 8% and 10%). Other morphological, biochemical and cultural tests were conducted such as Gram staining, catalase reaction, and aminoacids decarboxylase reaction. Then, suspected colonies were submitted to phenotypic characterization by API 20E (bioMérieux, Marcy l'Etoile, France) employing 1% sterile saline as inoculum diluent.

### *Statistical Analysis*

In order to compare the average values, an analysis of the variance was applied. A probability of 5% was fixed to accept or reject the null hypothesis of equality. The correlation between *V. alginolyticus* abundance and physicochemical parameters and between different environmental types of samples were carried out using

 

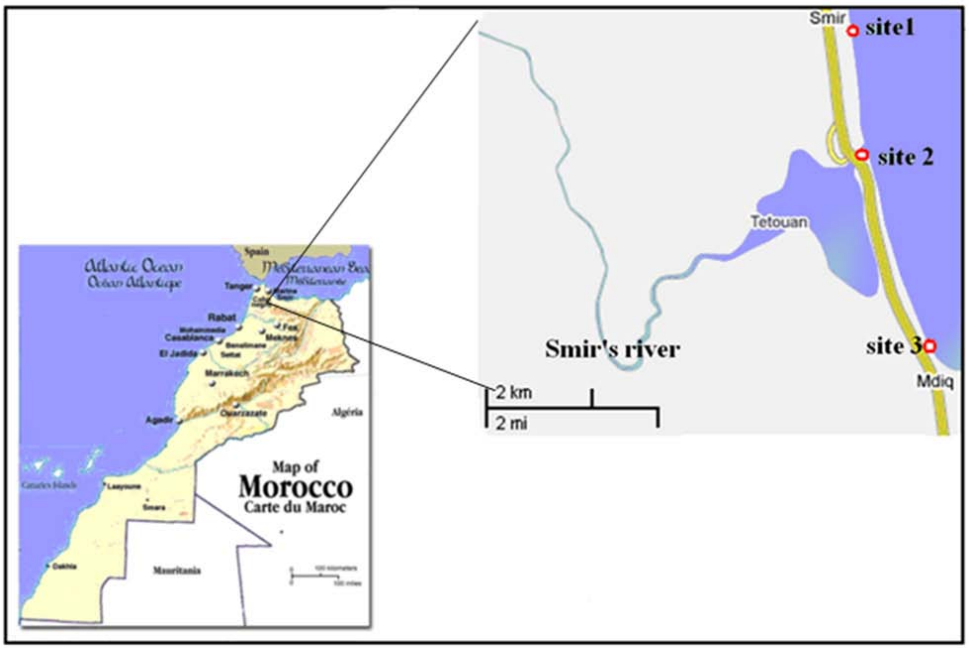


**Figure 1.** Map showing the geographical location of three sampling sites

**Table 1.** Correlation between *Vibrio alginolyticus* abundance in seawater, planktons, sediments, shellfishes and seawater temperature, seawater pH, salinity, turbidity, conductivity and chlorophyll *a*, over a 24-month period as determined by using Pearson's rank correlation coefficient ($r_s$).

| Variables | SST(°C) | pH | Sal. | Turb. | Cond. | Chlo.A | water | planktons | sediments | shellfishes |
|---|---|---|---|---|---|---|---|---|---|---|
| SST(°C) | **1** | | | | | | | | | |
| pH | **0,503** | **1** | | | | | | | | |
| Salinity. | **0,431** | -0,097 | **1** | | | | | | | |
| Turbidity. | -0,144 | 0,205 | -0,383 | **1** | | | | | | |
| Conductivity | 0,280 | -0,217 | **0,926** | -0,375 | **1** | | | | | |
| Chlo.A | 0,396 | 0,276 | 0,333 | -0,173 | 0,262 | **1** | | | | |
| water | **0,718** | 0,299 | 0,141 | -0,028 | -0,024 | 0,022 | **1** | | | |
| planktons | **0,908** | **0,447** | 0,377 | -0,284 | 0,248 | 0,402 | **0,778** | **1** | | |
| sediments | **0,866** | 0,363 | 0,319 | -0,126 | 0,254 | **0,436** | **0,703** | **0,909** | **1** | |
| shellfishes | **0,834** | **0,414** | 0,220 | -0,063 | 0,176 | 0,273 | **0,715** | **0,886** | **0,932** | **1** |

SST: surface seawater temperature

*Values in bold are significantly different from 0 with a significance level alpha=0,05*

**Table 2.** Correlation between *Vibrio alginolyticus* occurrence and seawater temperature, salinity, turbidity and conductivity.

| | SST(°C) | Salinity | Turbidity | Conductivity |
|---|---|---|---|---|
| **N** | 16,5141 | 38,6969 | 1,7296 | 74,3212 |
| **Y** | 19,7170 | 38,7945 | 2,5442 | 74,6088 |
| two tailed test for difference | <0,0001 | <0,001 | <0,0001 | <0,0001 |
| | ** | ** | ** | ** |

SST: surface seawater temperature

**Table 3.** Comparison of $Log_{10}$ mean loads *Vibrio alginolyticus* recorded during the warm season and cold season.

| | Seawater | Plankton | Sediment | Shellfishes |
|---|---|---|---|---|
| warm season | 3,30011531 | 7,12942837 | 9,3423542 | 9,49634585 |
| cold season | 2,2084029 | 5,14277092 | 6,75424813 | 6,82136376 |
| p-value | 0,07471534 | 0,20256714 | 0,04411709 | 0,04374248 |
| | NS | NS | * | * |

 

Spearman coefficient ($r_s$). To determine the seasonal distribution of cultivable *V. alginolyticus* and to evaluate the effect of physicochemical parameters of seawater on the distribution of this bacterium, Principal Component Analysis (PCA) was conducted for the monthly averages of the values of *V. alginolyticus* abundance and physicochemical parameters recoded in the two sampling years. All statistical analysis methods were done using specialized statistical software.

## RESULTS

### *Environmental parameters*

The highest seawater temperature was recorded in June 2008 (23 °C) and the lowest in Decembre 2008 (15°C). Significant differences between the hot season (Mai to October) and the cold season (November to April) were found ($P<$ 0.01). Although high variability in seawater pH was observed, significant differences were found in the pH values between hot and cold seasons ($P<$ 0.0001). There were similar fluctuations in the salinity at sites 1 and 3 during the two years, but the salinity at site 2 was consistently lower and its fluctuation was more important. Salinity was lower in site 2 during cold and rain season, associated with hurricanes, brought considerable freshwater runoff from the river. Thus, significant difference in salinity between the two seasons was found ($P<$ 0.0001). High variability was also observed in chlorophyll *a* concentration and turbidity in the three sampling sites with a significant difference between hot and cold seasons ($P<$ 0.0001 for both). The highest values of turbidity were always recorded in site 2 because also the water runoff from Smir's river, especially in cold season (Fig. 2).

### *Temporal and spatial variations of Vibrio alginolyticus abundance*

The abundance of *V. alginolyticus* in Tamouda Bay fluctuated seasonally and ranged from undetectable to 9.9 $Log_{10}$ MPN/g with an average abundance of 5 $Log_{10}$ MPN/g.

*V. alginolyticus* showed very similar temporal patterns of abundance at the three sites but its abundance at site 2 was consistently higher. Consequently, sampling site 2 showed the significantly highest prevalence and abundance among all sites investigated, with *V. alginolyticus* present in up to 71% of the collected samples and a mean density of 5.2 $Log_{10}$ MPN/g against 3.8 $Log_{10}$ MPN/g and 3 $Log_{10}$ MPN/g in sites 1 and 3 respectively (Fig. 3a). *Vibrio alginolyticus* population dynamic followed similar seasonal trend during the 2 years (Fig. 3b). The maximum abundance was observed during the hot season

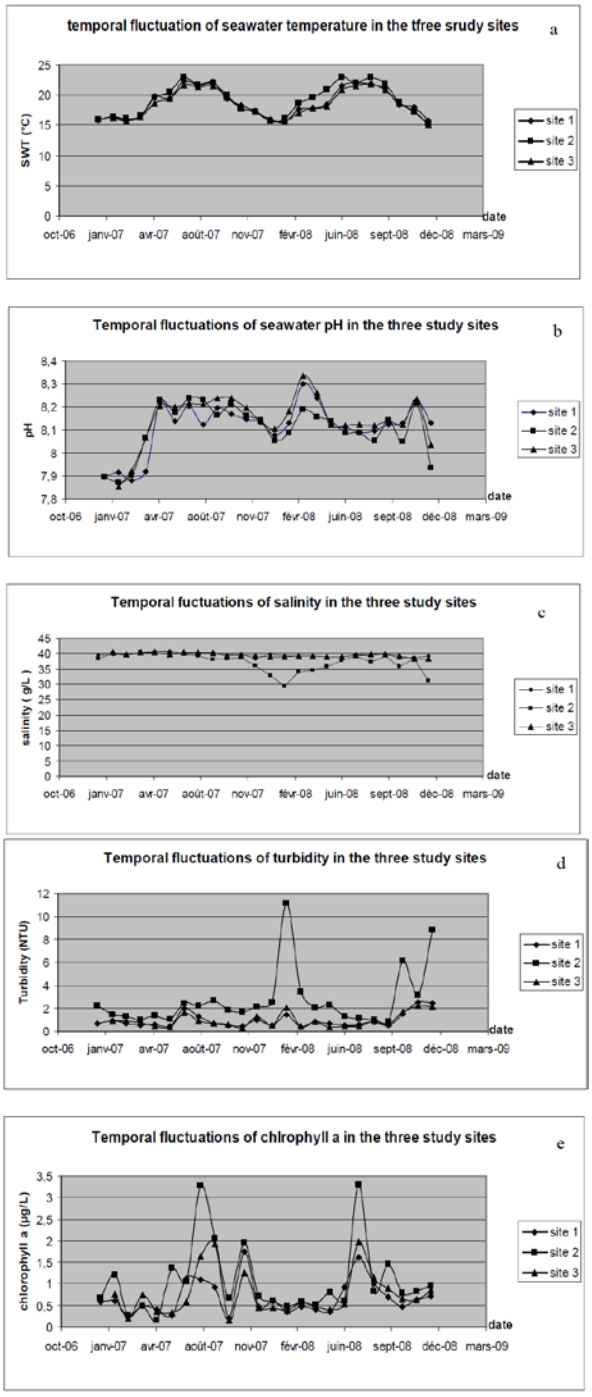


**Figure 2.** Temporal fluctuations of physicochemical parameters in the three study sites: (a) seawater temperature, (b) seawater pH, (c) salinity, (d) turbidity and (e) chlorophyll *a*

and the minimum in cold season with significant difference between the two seasons in sediment and shellfishes ($P<$ 0.04) (Table 3). This may be explained by the important flow of visitors in Tamouda Bay during summer period. *V. alginolyticus* showed also similar temporal patterns of abundance in different ecological types of simples. Nevertheless, the abundance in seawater was consistently lower. On the other

 

hand, a highly significant correlation of *V. alginolyticus* abundance with seawater temperature was found ($r_s$ = 0.718*; P*< 0.0001) as well as with seawater pH, ($r_s$ > 0.3*; P*< 0.0001. But no significant correlation was found between salinity and *Vibrio alginolyticus* abundance (P> 0.05). Chlorophyll *a* and pH have also shown high significant correlation with seawater temperature ($r_s$ = 0.40*; P*<0.0001 and $r_s$ = 0.5*; P*<0.0001 respectively). Moreover, a linear positive correlation was recorded between *Vibrio alginolyticus* populations in all ecological types of samples studied (table 1).

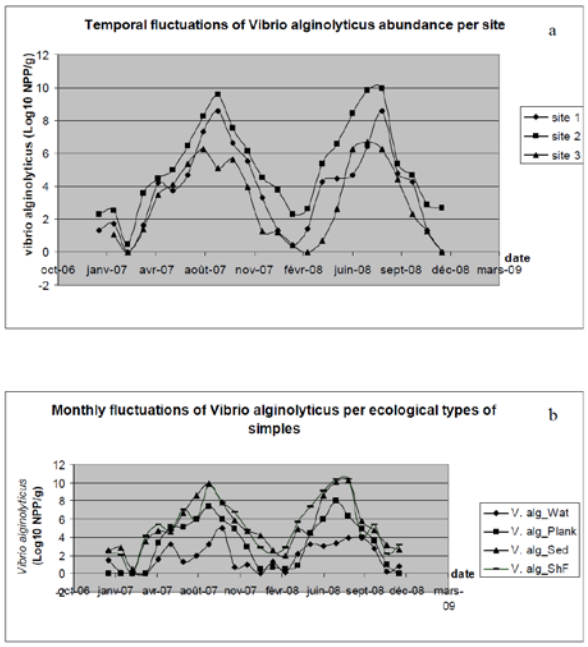


**Figure 3.** Temporal and spatial fluctuations of *Vibrio alginolyticus* (a) Temporal fluctuations per site, (b) spatial seasonal variation per ecological type of simples.

## *Seasonal distribution of environmental parameters*

Ranking environmental variables of seawater by PCA showed monthly data divided in two groups related to seawater temperature (Fig. 4). Obtained results have clearly differentiated between cold season (November to April) and the hot one (Mai to October). Seawater temperature is the main factor determining the distribution for PCA 1 (87.1%), turbidity the main factor for PCA 2 (80.5%) and salinity the main factor for PCA 3 (50.7%). Component 1 accounted for 90.8% of the variance in the data, Component 2 for 6.2%, and Component 3 for 2.3%. This distribution in PCA variables showed gradient patterns related to seawater temperature and turbidity during the year, which indirectly explains the variation of *V. alginolyticus* abundance.

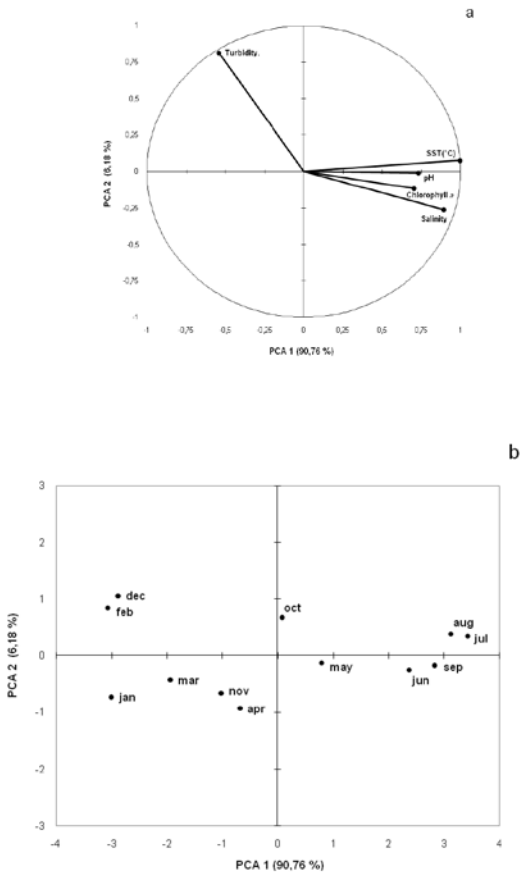


**Figure 4.** (a) Principal component plot of the environmental parameters (seawater temperature, pH, salinity, turbidity and chlorophyll *a* concentration). (b) Temporal distribution of environmental parameters

## *Seasonal distribution of Vibrio alginolyticus abundance*

Ranking of months according to the abundance of *Vibrio alginolyticus* in different ecological types of samples showed similar seasonal distribution than environmental parameters supported by the first principal component, which accounted for 90.9 % of the variance in the data (Fig. 5).

# DISCUSSION

Globalization is a source of risks because it led to the standardization of products and promotes the dominant players in the market at the expense of the weakest.

The environmental impacts of tourism are compounded by their seasonal and spatial concentration (coast). This encourages bacterial abundance with the risk of diseases that it may impose.

 

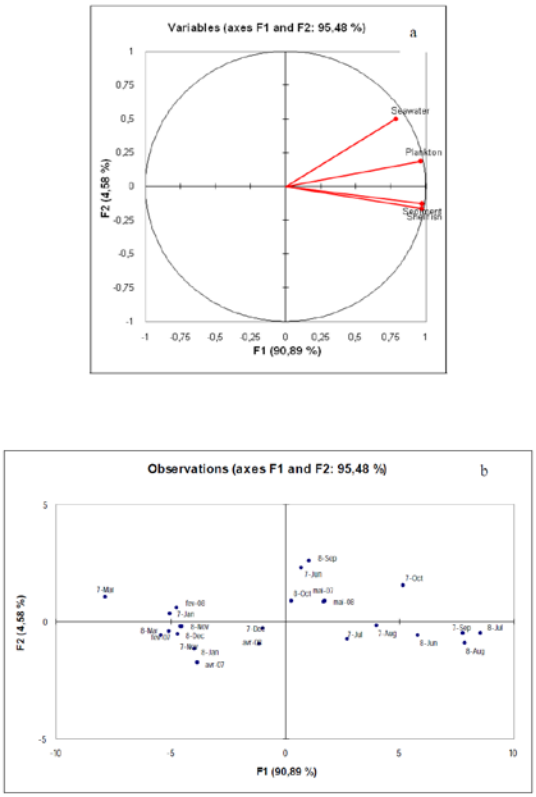


**Figure 5.** (a) Principal component plot of *Vibrio alginolyticus* abundance in all ecological types of samples (seawater, planktons, shellfishes and sediments). (b) Temporal distribution of *Vibrio alginolyticus* abundance.

Therefore, in this study, we have tried to contribute to the clarification of the ecological relationships between environmental parameters and *V. alginolyticus* in a Mediterranean region of the Morocco.

The results showed that the temperature of seawater is positively correlated with *V. alginolyticus* and accounted for 0.72 in seawater, 0.91 in plankton, 0.87 in sediment and 0.83 in shellfisc. This is consistent with other studies where a significant positive correlation was observed between the temperature of the seawater and the total *Vibrio* abundance (16, 22) Furthermore it there were seasonal fluctuations in abundance with winter minimum and a peak in August, which corresponds with the highest recorded temperatures (22°C) (26, 36 ).

Although pH and chlorophyll are correlated with *V. alginolyticus*, they are not directly responsible for its variability. While this study showed no correlation between the charges in *V. alginolyticus* and salinity due to the lack of variation of these parameters coastal geography where the average salinity was 35 g / L, between 30 and 41 g / L. which is consistent with the results shown in the coastal waters of the Adriatic Sea (Mediterranean) (3). However calculating correlation coefficient between *Vibrio* abundance and salinity in site 2 have shown significant correlation (rs = -0.24; P<0.05) because in this sampling site there are more fluctuations of salinity because freshwater runoff from Smir's river. The high variability of turbidity in the three sampling sites showed a significant difference between warm and cold seasons (P <0.0001 for both seasons), which is consistent with other studies that reported a positive correlation between the presence of *Vibrio spp*. and turbidity (15, 37).

This study also showed a positive linear correlation between the presence of *V. alginolyticus* abundance in sea water, plankton, shellfish and sediment. Moreover, our results showed that during the hot season, the load *V. alginolyticus* in sediments and shellfish is three times higher than in seawater. This is in total agreement with other reports where it has been demonstrated that plankton organisms are a rich source of nutrients that can enhance species of *Vibrio* at densities higher than the surrounding water (1, 29, 35). Other reports have shown that sediment resuspension is a major source of bacteria in water (34). Similarly, our study showed the positive association of *V. alginolyticus* in shellfish, seawater and plankton that can be explained by the ability of shellfish to filter sea water and concentrate the bacteria content in their liquor (12).

In summary, our analysis showed seasonal and spatial variations of *V. alginolyticus* in marine environment Tamouda bay. The highest concentrations were recovered during the warm seasons. The temperature was the main factor influencing the concentration of *V. alginolyticus*. These results are very important for assessing risk of infection after bathing tourists especially since Tamouda Bay is becoming a major resort center on the Mediterranean coast.

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