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Qualitative and Quantitative Study of Dinoflagellate Cysts in Surface Sediments of the Nador Lagoon (North-East Morocco)

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ABSTRACT

The qualitative and quantitative study of dinoflagellates (cysts and vegetative forms) was carried out on samples of surface sediments and water of the Nador lagoon (North-East Morocco), collected during winter in February 2019. Therefore, it was necessary to undertake a qualitative and quantitative study on dinoflagellate cysts and vegetative forms in parallel with the aim of specifying the factors that control the concentration of cysts in the sediments, as well as their specific diversities and blooms. Notably, dinoflagellates are of undeniable ecological interest, and are considered as sentinel species of the quantity of water because they respond quickly to environmental changes dueto their very fast life cycle. Moreover, their cysts, due to their extreme resistance and abundance in sediments, constitute an extremely important tool in the evaluation of current environments. Results showed that the total abundances of dinoflagellate cysts vary between a concentration of 720 cysts/g dry sediment (DS) and 7215 cysts/g DS. This work was mainly conducted to study the abiotic parameters of the Nador lagoon that control the concentration of cysts in the sediments, and address their specific diversity and the dominance of one group over the other.

INTRODUCTION

The microalgae account for 45% of the primary production on earth **(Field *et al.*, 1998)**, and play an important role in regulating the concentration of atmospheric CO_2 by sequestering and transporting it to the deep sea. Phytoplankton have both eukaryotic and prokaryotic representatives in the tree of life. Less than 5000 species of marine phytoplankton were formally described in the late 1980s **(Sournia *et al.*, 1991; Simon *et al.*, 2009),** and only 80 toxic species and about 200 species harmful to humans and aquatic fauna **(Sournia, 1995)** were determined. Toxic microalgal blooms are phenomena that have been known since time immemorial and correspond to large and sporadic blooms of toxin-producing microalgae **(Belin *et al.*, 2013)**. Over the past 30 years, the frequency of toxic microalgal blooms appeared to have increased in frequency, intensity, and geographic extent, posing risks to human health and ecosystem components **(Zingone & Enevoldsen, 2000; Hallegraeff, 2003)**.

Among the 4000 species of phytoplankton identified in the marine environment **(Sournia *et al.*, 1991)**, approximately300 species are harmful to aquatic organisms while80 species of them are known to produce phycotoxins **(Granéli &Turner, 2006)**. The dinoflagellates are a group of unicellular eukaryotic phytoplankton with flagella. The most toxic species belong to this group. The toxins produced by these organisms cause different syndromes in humans, such as paralytic shellfish poisoning (PSP), diarrhoeic shellfish poisoning (DSP), diarrhoeic shellfish poisoning (NSP) and amnesic shellfish poisoning (ASP) **(Zingone & Enevoldsen, 2000)**. The oligotrophic conditions of the Mediterranean Sea favour the proliferation of dinoflagellates; which are typical organisms of nutrient poor waters **(Maso & Garcés, 2006)**. Some species of dinoflagellates develop forms of resistance (cysts) during a phase of the life cycle **(Matantseva *et al.*, 2020)**.Derived from a zygote produced during sexual reproduction by gamete fusion **(Matsuoka & Fukuyo, 2000)**. The coastal lagoons are concerned by the expansion of toxic phytoplankton. These ecosystems are often important shellfish production areas, where the emergence of some potentially toxic dinoflagellate species could threaten aquaculture activities and human health **(Azavedo & Carmouze, 1994)**.The search for areas at risk of developing toxic dinoflagellates, and the determination of resting cysts belonging to toxic species in these ecosystems allow for better preservation of aquaculture activities. **Fartouna and Bellakhal (2016)** reported that, the Southern Mediterranean lagoons, such as the lagoons of Nador (Morocco), Bizerte (Tunisia) and Mellah (Algeria) are concerned by recurrent episodes of proliferation of toxic phytoplankton species, particularly dinoflagellates, *Alexandrium catenella*, *Alexandrium minutum*, *Dinophysis* sp., *Gymnodinium catenatum* and *Ostreopsis ovata*. These species are responsible for intoxications that can sometimes lead to death and cause serious socio-economic consequences. In Morocco, many studies wereconducted on these dinoflagellate cysts **(Hssaida, 1990; Hssaïda & Morzadec-Kerfourn, 1993; Hssaida *et al.*, 2014; Chekar *et al.*, 2016; Daghor *et al.*, 2016)**. Thus, the present work was organized to carry out a quantitative and qualitative study of dinoflagellates in the water and surface sediment of the Nador lagoon.

MATERIAL AND METHODS

1. Study area

The lagoon of Nador, also called Sebkha Bou Areg or Mar Chica, is located in the north-east of Morocco on the Mediterranean coast at the level of meridians 2°45'- 2°55' W and parallel 35°10'N. The lagoon ecosystem of Nador developed in a continental depression oriented NW-SE during the rise of the sea level in the Holocene period **(Guillemin & Houzay, 1982).** It represents the second largest lagoon complex in North Africa (115 km^2 with 25 km length and 7.5 km width) and the largest in Morocco. The lagoon communicates with the Mediterranean Sea by a new artificial channel of 300 min width and 6.5 min depth. The hydrodynamic circulation in the lagoon is mainly Aeolian **(Guelorget *et al.*, 1987; Hilmi, 2005)** and the time of water renewal before the construction of the new inlet was estimated to be about 80 days **(Hilmi *et al.*, 2005).**

The sampling was carried out in the Nador lagoon in February 2019 at nine stations distributed in a way to ensure a wide spatial coverage of the lagoon (Fig.1).

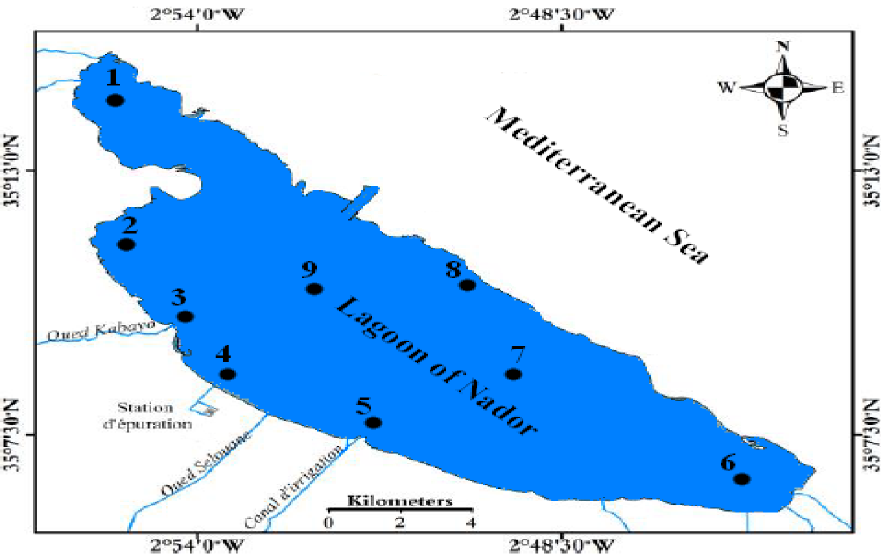

Fig. 1: The distribution of sampling stations

Station 1 corresponds to Beniansar (W 002°55,460 ; N 35°15,148), Station 2 corresponds to Tirakae (W002°55,522 ; N35°11,772), Station 3 corresponds to Oued caballo (W002°54,325 ; N35°09,820), Station 4 corresponds to the sewage treatment plant (W002°53,383 ; N35°08,299), Station 5 corresponds to Oued Bou Areg (W002°51,443 ; N35°07,549), Station 6 corresponds to Kariat Arakman (W002°45,167; N35°06,401), Station 7 is at the center lagoon side Arekmane (W002°48,884; N35°08,236), Station 8 is between the old pass and Mohandis (W002°49,802; N35°10,465), Station 9 is the lagoon center (W002°52,517; N35°11,012) (Fig.1).

The Dinoflagellates and cysts in the surface sediments were studied qualitatively and quantitatively. The sampling of sediments and phytoplankton were carried out in parallel with the measurements of the (In-situ and Ex-situ) physicochemical parameters of the lagoon water at the nine sampling sites.

2. Sampling Strategy

At each station, the sampling of surface sediments of the lagoon of Nadoris was done using cylindrical cores (80 cm long, 8 cm in diameter) operated by a professional diver. Only the top 3 cm of surface sediments were taken, then stored in total darkness at 4°C until analysis.The cysts weremainly distributed in the first three centimetres of the sediment surface (**Erard *et al.*, 1993)**. The sampled sediment was used for analysis of water content, total organic carbon and total inorganic carbon. Cysts from the sediment were separated by the modified density gradient method using Ludox CLX described by **Erard *et al.*(1995)**, **Yamaguchi *et al.*(1995)** and **Genovesi *et al.*(2007)**. Once the extraction operation was completed, the dinocysts werestored in aluminum-covered tubes, and then placed at 4°C to wait the counting and identification phase, using an inverted light microscope.

The identification of dinocystsis based on morphological characteristics was determined using identification keys and plates illustrated in articles and publications dealing with dinocysts **(Head, 1996; Zonneveld 1997a, 1997b; Zonneveld & Jurkschat, 1999; Rochon*et al.*, 1999; Head *et al.*, 2001; Pospelova & Head, 2002; Kim *et al.*, 2007; Matsuoka *et al.*, 2009; Draredja *et al.*, 2020).**

The observation of the different species of cysts of the Nador lagoon was carried out with an inverted microscope "Leica DM750", with a magnification of 40x. For each sample, 1 ml was taken and distributed on a Sedgewick counting plate allowing the counting of dinocysts under the inverted microscope. The calculation of the abundance of dinocysts in each sample allows determining the number of cysts at rest per gram of wet sediment. In parallel with a study of the water content, organic and inorganic carbon of the sediment and a study of the organic matter content.

On the other hand, the analysis of physicochemical parameters of the water of the Nador lagoon for each sampling station was adjusted with in situ measurements (temperature, pH, salinity, conductivity, dissolved oxygen and turbidity) the help of the specific probe and the analysis of nutrients (nitrogen and phosphorus), as well as the analysis of biological parameter (Chl-a)

The physico-chemical analysis of the water was carried out at the INRH-Nador laboratory, whereas the analysis of the sediments was performed at the OLMAN- BGPE laboratory. Additionally, the study of cysts was achieved at IFREMER- France (Sète).

The temperature and salinity were obtained respectively using an ORION STAR A122. The pH measurements were recorded using a pH meter IONOMETER-EUTECH-INSTRUMENTS-CYBERSCAN-PH-510, and the turbidity of the seawater was measured in situ with a turbid meter (EUTECH TN-100 instrument). For the analysis of nutrients (nitrates, nitrites and phosphates), the methods used weredescribed in the manual of chemical analysis in the marine environment **(Aminot & Chaussepied, 1983; Aminot & Kérouel, 2004)**. The Chlorophyll (a) and the suspended solids were determined by the 0.45µm membrane filtration method.

On the other hand, the determination of the organic matter wasobtained by incineration of the samples (loss of weight in fire or loss by calcination) **(CEAEQ, 2003)**. The percentage of organic matter wascalculated following the successive equation:

%O.M. = dry sample weight (g) - incinerated sample weight (g)/dry sample weight (g)*100.

The total inorganic carbon (TIC) wasdeduced from the loss ofignition at 950°C according to the following relationship:

Law 950 = (mass B - mass C/mass B)*100, With CIT= Law 950*0.273.

RESULTS AND DISCUSSION

1. Pysicochemical parameters

The average water temperature of the Nador lagoon in the nine stations (February 2019) was 17.25°C (Table 1), with a maximum of 18.4°C recorded at station 4 of a depth of 0.6 m. While, a minimum of 16°C was recorded in station 3 which corresponds to Oued Caballo with a depth of 0.70m. The average pH value was7.8 with a maximum value of 8 recorded in station 1, which corresponds to Beni Ansar with a depth of 8m. A minimum value of 7.6 was recorded in station 3 that corresponds to Oued Caballo of 0.70m depth (Table 1). In relation to salinity, the highest value was 38.8‰ detected in station 1, whilethe lowest was in station 3 (33.1‰) (Table 1). Electrical conductivity (EC) is another parameter used for water quality monitoring **(Pal *et al.*, 2015)**. The lowest and the highest values of electrical conductivity were recorded respectively at the sites 3 (51.8 mS/cm) and1 (57.9 mS/cm) (Table 1). Concerning the average turbidity of the water of the lagoon, it was 0.86 NTU oscillated between a null value observed at station 1 and a maximum of 2.34 NTU recorded at station 3(Table 1). While the minimum and the maximum dissolved oxygen measured at the lagoon of Nador were respectively 9.37mg/l at station 4 which corresponds to the treatment plant with depth of 0.60 m, and of 12.6mg/l at station 2 which corresponds to Tirakae with a depth of 0.40m. These values are comparable with the one found by **El Madani (2012)** and **Mostareh (2018).**

Table 1: The in-situ physicochemical parameters of the Nador lagoon (February 2019)

Stations	Temperature (°C)	Salinity	pH	Conductivity (mS/cm)	Turbidity (NTU)	Dissolved Oxygen (mg/l)
St1	17.30	38.80	8.00	57.90	0.00	10.37
St2	17.50	37.80	7.94	56.30	0.31	12.60
St3	16.00	33.10	7.60	51.80	2.34	7.99
St4	18.40	37.40	7.70	56.80	1.71	7.34
St5	17.60	37.30	7.71	56.60	1.01	7.50
St6	17.00	37.10	7.92	56.40	0.69	9.47
St7	17.00	37.50	7.83	56.80	0.58	9.50
St8	16.60	35.90	7.86	57.30	0.63	9.46
St9	17.85	37.40	7.94	56.80	0.46	10.10

The distribution of the suspended solid contents during the period of this study (Table 2) shows that the highest value of 0.036mg/l was recorded in the site 6 which corresponds to Kariat Arakman with a depth of 1.48m. However, the minimum value was recorded at station 7 (0.022 mg/l). Chlorophyll-a contents varied between a zero value at station 1 (Table 2), and a maximum value of 0.267µg/l at station 9, with an average of 0.13 µg/l. The concentrations of orthophosphates varied between a minimum value of 0.090 mg/l recorded at the station 9 (Table 2), and a maximum of 0.235 mg/l recorded at the station 4, which is near the wastewater treatment plant (WWTP). This WWTP rejected its effluents after treatment which is characterized by their high phosphate content. Regarding the nitrate content in the water of the Nador (Table 2), results showed that the average value of this nutrient during February 2019 was 0.375mg/l, with a maximum of 0.743mg/l recorded at station 1, while the minimum was found at station 8 (0.007 mg/l), which is characterized by a depth of 7.86m. The maximum value of nitrite was 0.150mg/l recorded at station 3, while the minimum value was 0.009mg/l detected at station 8, with an average of 0.079mg/l (Table 2).

Table 2: Ex-situ physicochemical parameters of the Nador lagoon (February 2019)

Stations	**Suspended solids (mg/l)**	**NO_2 (mg/l)**	**NO_3 (mg/l)**	**PO_4 (mg/l)**	**Chl (a) (µg/l)**
St1	0.026	0.024	0.743	0.145	0
St2	0.028	0.028	0.034	0.120	0.106
St3	0.028	0.150	0.399	0.220	0.160
St4	0.033	0.028	0.192	0.235	0.106
St5	0.029	0.027	0.054	0.190	0.053
St6	0.036	0.025	0.049	0.110	0.053
St7	0.022	0.020	0.064	0.135	0.053
St8	0.026	0.009	0.007	0.100	0.106
St9	0.027	0.015	0.019	0.090	0.267

2. Sedimentological analysis and distribution and abundance of dinoflagellate cysts

Table (3) shows that the maximum value of organic matter (OM) reached a value of 51.64% at station 9 and a minimum value of 23.87% at station 2. While, the maximum value of total organic carbon(TOC) reaches a value of 25.82% at station 9 and a minimum value of 11.93% at station 2. While, the maximum value of the total inorganic carbon (TIC) was6.91%(station 8), whereas the minimum value was1.34% (station 3). These results suggest that the high content of the total organic carbon in sediment is associated with the predominantly smectite clay faces of the deeper central zones **(El-Alami *et al.*, 1998)**. The valuesrecorded during the present work are relatively high compared to those previously found in Mar Chica (**El-Alami*et al.*,1998)**. Likewise, when compared to results other lagoon systems **(Tesson, 1982; Lakhdar, 1987)**. On the other hand, the currentresults showed high rate of organic matter in all samples, especially those collected from the centre of the lagoon. These results are in agreement with those of **Abouhala *et al.* (1995)** and **Lefebvre *et al.*(1997) .**Remarkably, the identification of dinoflagellate's cysts is done according to morphological characteristics with the use of an inverted light microscope. Many species of cysts such as cysts with a brown, black, or grey colour are very difficult to identify because of their similarity adding to their dark colour that makes it difficult to distinguish either the internal parts (red bodies ...) or theexternal parts (ornaments...).The lagoon of Nador presents a relatively important diversity of cysts with 64 different morphotypes.

In the present work, only 16 species were identified because it is very hard to identify the exact species from the cyst. In the present work and at 9 sampled stations, the main cysts dominated were *Alexandrium minutum, Scripsiella ramonii, Polykrikos schwartzii, Polykrikos kofoidii,* and *Operculodinium israelainum*, with heterogeneous abundances and uneven distribution. The maximum density of cysts in the lagoon of Nador reached 7215cysts/g at station 6, and a minimum density of 720cysts/g was detected at station3 (Table 3). The cyst with the highest cumulative density was *Alexandrium minutum* with a value of about 3045 cysts/g observed at station 6. This was followed by a density of 1710 cysts/g of *Scripsiella ramonii* observed at station 5. Eminently, the densities of cysts found varied between 720 and7215 cysts/g), which are higher than those found in the Mediterranean coastal waters such as those of the Mellah lagoon in Algeria recording a maximum density of 315 cysts/g **(Draredja *et al.*, 2020).** Moreover, the present recorded densities were also higher than those of Ghar El Melahin lagoon in Tunisia, determining a density of 229 cysts/g **(Dhib *et al.*, 2016)**. Compared to the results of Homa lagoon (Tukey) with a density of cysts reaching71 cysts/g **(Aydin *et al.*, 2014)**; and the density found in Cabras lagoon in Sardinia, Italy with a density of 287 cysts/g **(Satta *et al.*,2014)**, the current values are also higher.

Table3: Geographical coordinates of the sampling stations in the Nador lagoon, with total number of resting cysts (Total RCs counts), organic matter (OM), total organic carbon (TOC), total inorganic carbon (TIC), and water content (WC).

Station	Geographic Coordinates	OM (%)	TOC (%)	TIC (%)	Total RCs counts (cysts/g)
St1	N35°15.14- W002°55.46	32.19	16.09	4.63	3750
St2	N35°11.77-W002°55.52	23.87	11.93	1.62	1530
St3	N35°09.82-W002°54.32	23.95	11.97	1.33	720
St4	N35°08.29-W002°53.38	28.63	14.31	4.33	2205
St5	N35°07.36-W002°51.44	24.94	12.47	2.81	6795
St6	N35°06.40-W002°45.16	44.46	22.23	4.50	7215
St7	N35°08.23-W002°48.88	48.88	24.44	4.51	6390
St8	N35°10.46-W002°49.80	32.65	16.32	6.91	3240
St9	N35°11.01-W002°52.51	51.64	25.82	4.45	2670

2.1. Abiotic parameters: Sediment water content

To determine the abundance of cysts per gram of dry sediment, the water content percentage of the sediment was measured. Moreover, the water content of the sediment sampled in the different stations of the Nador lagoon wasbetween 22.09% at station 2 which corresponds to Tirakae and 49.49% at station 9, with an average of35.79%. Meanwhile, stations 2, 3, 4 and 5 presented the lowest water content with contents between 22.09% and 27.40% in water. While, stations 1, 6, 7 and 9 recordedthe highest water content with values ranging from 32.50% to 49.50% andan average value of 33.33% (Fig.2).The sediments of the Nador lagoon studied hadquite high natural moisture content. Water contents were between 22.09 and 49.50%, these variations in natural water content may be related to the amount of fine elements in the analysed samples. Therefore, the values of water content are found significant in all samples, which explains the hygroscopic character and the high value of porosity (**Rollet & Bouaziz, 1972**).

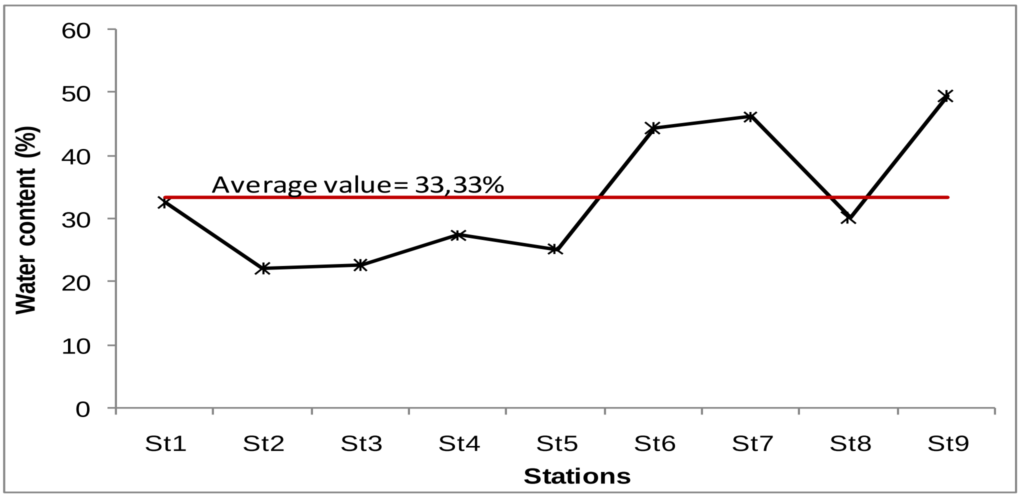

Fig.2: Water content of the sediments in Nador Lagoon

3. Statistical analysis

3.1 Relationship between environmental factors and resting cysts abundance

The multivariate analysis (PCA) shows that the first two factorial axes provide nearly 60.20% of the information (Fig. 3). The F1 axis explains 44.09% of the total variation, it is constructed mainly by the positive correlation between the variables; water content, total organic carbon(COT), total inorganic carbon (CIT), abundance, temperature, salinity, pH, dissolved oxygen (DO) and depth; and negatively with the variables Chl(a), turbidity, NO_2, MES, PO_4 and NO_3. On the other hand, the F2 axis explains 16.11% of the total variation, it is constructed mainly by the positive correlations of the variables; water content, total organic carbon (COT), total inorganic carbon (CIT), abundance, Chl(a), turbidity, NO_2 and MES; and the negative correlation of the variables temperature, salinity, pH, dissolved Oxygen (D.O), depth, NO_3 and PO_4.

This multivariate analysis (PCA) indicates that the abundance of dinocysts is significantly correlated with the aforementioned environmental factors of the sediment surface (Fig. 3), such as water content, total organic carbon (COT), and total inorganic carbon (CIT).

Fig. (4)shows that a linear relationship between organic matter and cyst abundance is spotted. It is also observed that with increasing organic matter the abundance of cysts increases, except for the two stations of St5 and St9.

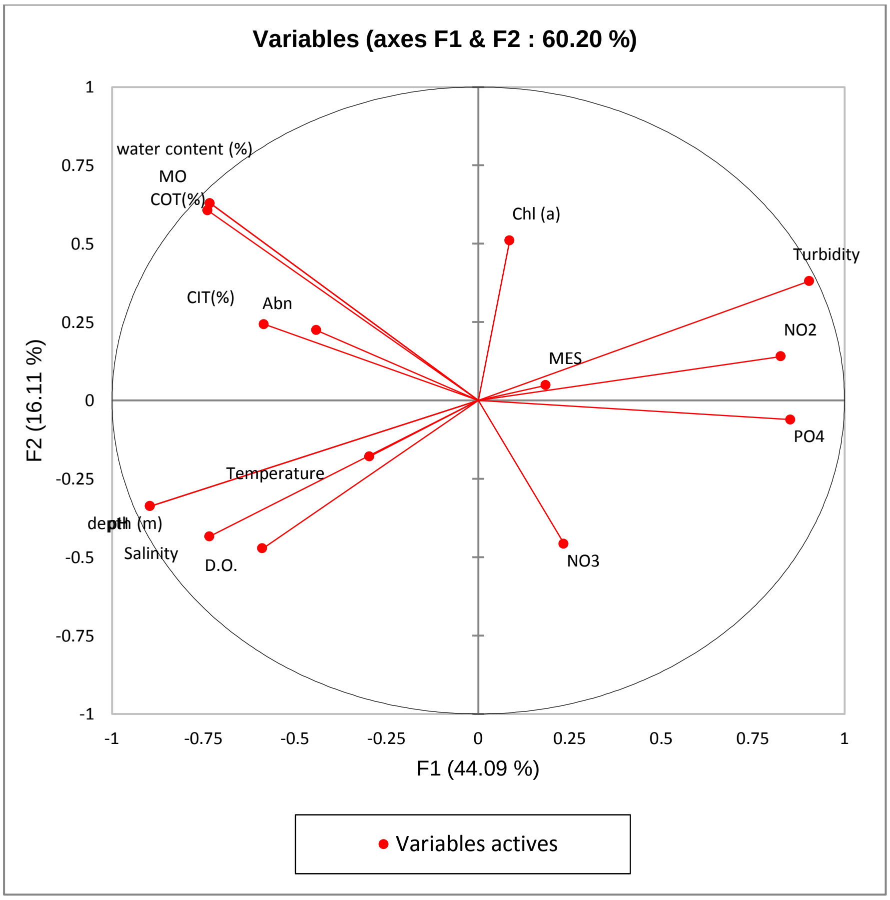

Fig.4. Principal component analysis (PCA) for dinocyst density (TRC count) related to environmental factors (T: temperature, Sal: salinity, DO: dissolved oxygen, pH, Cond: Conductivity, Turbidity, MES, NO_2, NO_3, Chl(a), PO_4, WC: water content, OM: organic matter, COT: total organic carbon, CIT: total inorganic carbon (axes F1 and F2 = 60.20%).

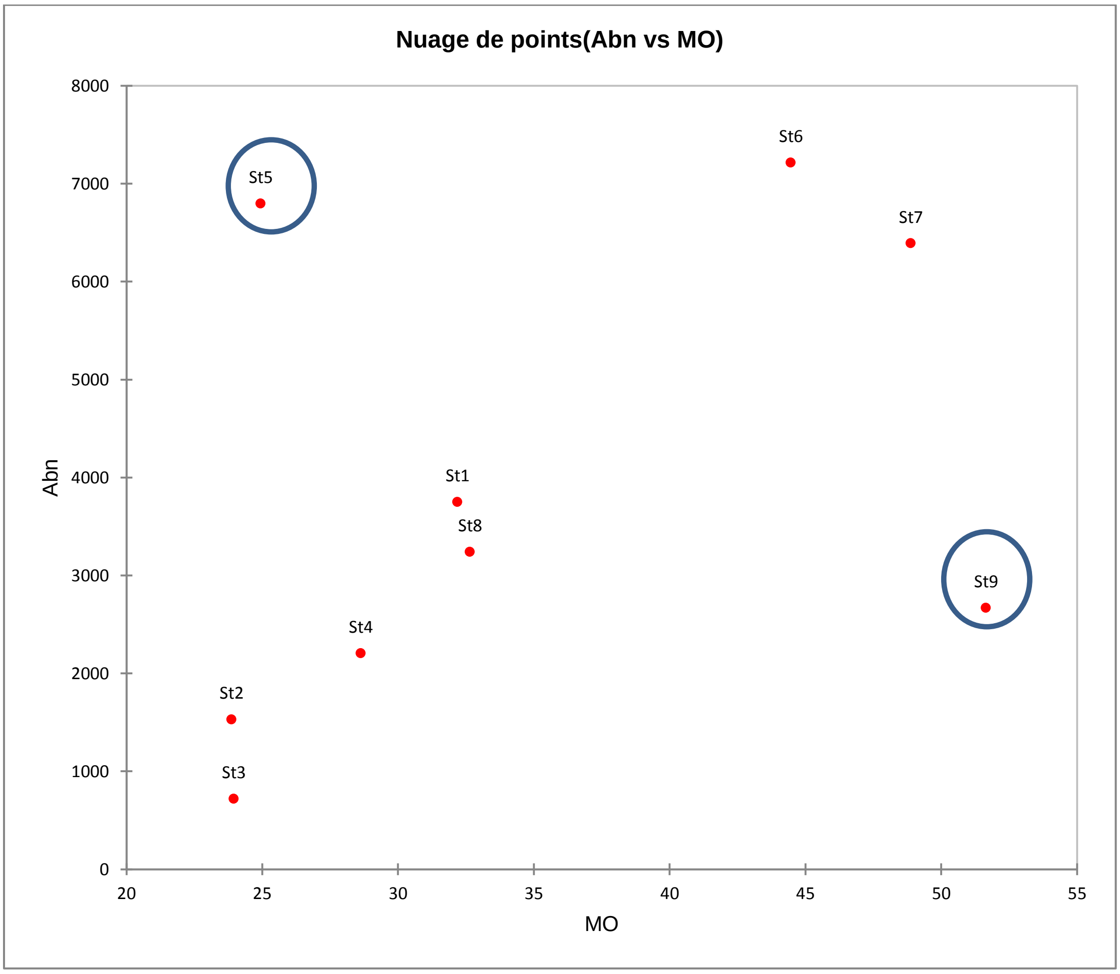

Fig.5. Scatter plot (Abandonment of dinocysts as a function of organic matter)

CONCLUSION

The qualitative and quantitative analysis of dinoflagellate cysts of the Nador lagoon was conducted at nine stations. Results revealed that the total abundance of dinoflagellate cysts varied between a concentration of 720 cysts/g dry sediment (DS) and 7215 cysts/g DS. Quantitatively, the total values of dinoflagellate cysts of the Nador lagoon observed in February 2019 showed that, the highest value was6795 cysts/g DS atOued BouArg site, 7215 cysts/g at Kariat Arakma and 6390 cysts/g DS in the centre of the lagoon of Nador, characterized by a depth of 7.83 meters. It is worth noting that, the main dominant cysts were*Alexandrium minutum*, *Scripsiella ramonii*, *Polykrikos schwartzii/kofoidii*, *Operculodinium israelainum*, with heterogeneous abundances and uneven distribution.

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