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RESEARCH ARTICLE

Impact of environmental disturbances on the dynamics of phytoplankton blooms.

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Abstract

From September 2012 to July 2013 the phytoplankton community was studied from five sampling stations located along the longitudinal axis of the Sidi Moussa lagoon, situatied in the atlantic Moroccan coast. Seasonal and spatial patterns of distribution in relation to some hydrological parameters (temperature, salinity and nutrients) are also reported.
The results obtained after quantification and identification of species of dinoflagellates during the study period showed a large variability in cell abundance and plankton blooms, the most important being those of the species to *peridinium quinquecorne* with 5.10^5 C/L and *kryptoperidinium foliaceum* arriving in concentrations above 10^7 C /l, this blooms were positively correlated physicochemical parameters.These two species, are identified for the first time in the Sidi moussa lagoon.

INTRODUCTION

Since Morocco is exposed to both the Atlantic Ocean and the Mediteranian sea and it has a great spatial, climatic, topographic and geological variability, it’s the richest Maghreb’s countries in wetlands (Dakki et El Hamzaoui, 1998).
A big part of the wetlands are mainly lagoons and they represent an original category due to their situation in the border: continents – oceans. Biologically, it’s a rich environment but ecologically and physically it’s very delicate. They are very sensitive to the human intervention that may upset the balance of this coastal ecosystem.
During the past century, the intensification of the agricultural practices has generated a considerable increase in the concentration of nitrogen and phosphorus in the fields near the coastline, which lead to unbalanced inputs of nutrients in the waters of the coastal zone and especially in confined environments such as lagoons and estuaries. The increase of the inputs (eutrophication) leads to an increase in phytoplankton biomass (Diaz and al. 2008) and causes a change in the structure of the phytoplankton community.
The importance of its biological productivity, the fragility of its ecological balance, toxic phytoplankton blooms and the strong anthropic action it undergoes, are all reasons that justify the study of the lagoon environment.
Phytoplankton blooms or Hamful Algae Bloom (HAB), can have strong impacts on marine life and human health (Zingone and al., 2000; MEA, 2005). There are about 2,000 cases of poisoning by toxic algae each year worldwide, 15% are fatal. (Hallegraeff, 1993; Anderson, 1995). Many studies have focused on this phenomenon: Yasumoto, 1979; Anderson 1989, 2009; Smayda 1990; Hallegraeff, 1993; Sournia 1985; Philips and al. 2002; Zingone and al.,

2000; Heisler and al, 2008; Anderson, 2000; harrhd et al, 2006, Belin Cet al , 2004. This study aims at the quantitative and qualitative identification of different taxa of dinoflagellates (2012-2013), in parallel measurements of nutrients, to try to understand the environmental factors that impact the dynamics and proliferation of this population dinoflagellates.

Study area

Sidi Moussa lagoon is part of the coastal resort of Sidi Moussa-Oualidia, one of the leading Moroccan wetlands. Located on the Atlantic coast of Morocco between the towns of El Jadida and Safi about 15 km south of JorfLasfer Industrial Park, and 41 km south of El Jadida. Its geographical coordinates are between 32 ° 57 'and 32 ° 59' north latitude and between 8 ° 45 '8 ° 47' in the west longitude (El khalidi et al, 2011).

Morphologically, we distinguish the presence of three areas:

- A narrow mouth providing a permanent connection with the Atlantic Ocean;
- A substantial sand deposit just inland of the mouth, and
- The main channel (maximum depth is 5 m), which runs across a shore with numerous secondary channels (maximum depth is 2 m), decreasing in depth upstream where it is limited by salt marsh.

Fig.1: The study area and location of the sampling sites.

Materials and methods

For this study, the sample was according to five sampling stations (Fig 1) during 2012-2013
They were chosen along the lagoon, from the pass to the bottom to better representation of the spatial distribution of phytoplankton populations in the water column. The frequency of sampling was bimonthly for analysis of phytoplankton, and monthly for physico-chemical parameters. Water samples were performed at high tide surface (photic zone) using a zodiac, and are collected by a 1L bottle, then they are fixed directly with Lugol 5ml / l.

Identification of Dinoflagellate species

Samples for different dosages and measures were taken from small, large and clean bottles previously washed with distilled water. The samples are kept cool and obscurity. Samples for phytoplankton analysis are homogenized, poured into sedimentation tanks (25ml) and placed on a flat surface without vibration in the dark at an ambient temperature for 6 to 8 hours.
After sedimentation, the identification and enumeration of species are using a type of inverted microscope (Nikon) according to the sedimentation method Utermöhl after fixation acid iodine (Diavert Leitz), (1958).
The systematic identification of different taxa of dinoflagellates present in samples of Sidi Moussa lagoon between 2012 and 2013, was carried out by two methods:

a) Calcofluor method:

It's a simple, fast and useful. It reveals the structure and arrangement of the plates. It's taking of a drop of the sample, and deposit it in the middle of the blade with a drop of Calco Fluoride 10 ug/ml. The preparation was covered with a coverslip and then observed in an epifluorescence microscope.

b) The method of scanning electron microscopy (SEM)

This technique make us able to see the sample in a three dimensional space at magnifications between 25 and 25 000.

Physico-chemical analysis

For physico-chemical measurements (pH, salinity, temperature), they are measured directly in situ, using respectively, for a pH meter type WTW 597, a salinometer type WTW LF 197, also used as a thermometer.
The contents of nitrites, nitrates and orthophosphates were determined by chemical dosage according to the method of seawater analysis, reported in A.Amino & M.Chaussepieds 1983.

Results and discussion

Temperature

The values recorded between 2012 and 2013, for the temperature of the surface water in the lagoon of Sidi Moussa (Fig 2) showed that the curve of this parameter is slightly different between seasons. However it varies in the same way from different stations. The minimum value recorded during June month is 16 ° C (station P0), the maximum value is recorded during the January 2013 at (The same Station).

Salinity

The recorded values for salinity in the different stations of the lagoon of Sidi Moussa (Fig 2) follow a freshening gradient from downstream to upstream. The highest concentrations (36 ‰) are recorded at the station P0. Station P4 records the lowest values (23.9 ‰). This was mentioned in earlier work on the lagoon of Sidi Moussa (Bennoua, 1999)

pH

In the lagoon of Sidi Moussa the pH values fluctuate between 7.6 and 8.09, with the exception of the station P4 (pH 6.98) in November 2012. Generally, the pH of seawater is usually quite stable (varying between 7.5 and 8.5) (Wetzel, 1983).

Turbidity

The maximum turbidity value (8,57µTN) was recorded in April at station P0 while the minimum (0.124 NTU) was observed in December from the same point.

Fig. 2: Measurements relating to physico-chemical parameters of the water of Sidi Moussa lagoon during the study period.

The nutrient loads

Chemical analyses of the different parameters were performed in the laboratory chemistry at the National Fisheries Research Institute.

These analyzes are performed on water samples (not fixed) taken in parallel with those destined for quantitative analysis of the phytoplankton population

Ammoniacal Nitrogen

The measurements of ammoniacal nitrogen at 5 stations between September and July (Fig 3) show that the concentrations of this parameter vary in the same way for the three stations (P0, P1 & P2); the concentrations vary between 1.12 and 36.4 g/l. However, we record some higher fluctuations in the stations P3 and P4, where the highest concentrations were 130.48 mcg/l at the station P3 (December).

Orthophosphate

The phosphorus present in the sea water exists in a huge variety of shapes, grouped arbitrarily into two classes "insoluble" and "soluble"; the second class is named orthophosphate and is the form that can be directly assimilated by the phytoplankton (Oudot, 1983).The measured values of orthophosphate in different sampling stations (Fig 3) between September 2012 and 2013, show small fluctuations of concentrations. (The values vary between 39.425 and 749.55 µg/l). However, these concentrations increase progressively in the month of May, reaching, in June, the highest levels close to 4177.19 µg/l at the station P4. These concentrations remain important during the summer; this is particularly remarkable in the bottom of the lagoon.

Nitrite

Nitrite levels are low between September 2012 and April 2013 (Fig. 3), with concentrations that do not exceed 211.14 µg/l at the station P4.
From April, nitrites gradually increase up to in July, maximum values of 770.86 µg/l. This process is particularly remarkable at the station P4, these concentrations increase successively going towards the coast stations.

Nitrate

The concentrations of nitrates vary between a minimum value of 388.13 µg/l in February 2013 at station P1 and 8944.6 µg/l in June at station P2. During the whole study period, concentrations remain low at station 0 not exceeding 4216 mg/l. From the month of June, a considerable rise in nitrates at stations is observed at the stations (P2, P3 and P4) (Fig 3). This increase does not occur at station P0.

Fig.3: Measurements of chemical parameters of water of Sidi Moussa lagoon (Ammonia Nitrogen, orthophosphate, nitrite and nitrate) during the study period.

Phytoplankton populations

The qualitative analysis of phytoplankton samples (2012-2013), revealed the abundance of dinoflagellates and diatoms, and the Silicoflagellates group are episodic (tab. 1).
Taxonomically, 59 taxa were identified 36 diatoms are represented mainly by *Nitzghia sp Thalassiosiera sp., Lauderia sp., Chaetoceros sp,* and *Navicula sp, Grammatophora marina*. The 23 taxa of dinoflagellates listed in our samples are represented by the genera *Dinophysis sp., Alexandrium sp.,* Gymnodinium *sp.*, *Ceratium sp* and *Protoperidium sp*.
The species of this genus of dinoflagellates dominate the community with two massive blooms of two species: *Peridinium quinquecorne* and *Kryptoperidinium foliaceum.*
These phytoplankton blooms appeared alternately in the Sidi Moussa lagoon during the period (May-July 2013).

Tab.1 : Inventory of phytoplankton species occurring in the lagoon of Sidi Moussa.

- ✓ **Bloom of *Perdinium quinquecorne* :**

Quantitative analyses of dinoflagellate during (2012-2013), showed a high variability in cell abundance with blooms of *Peridinium quinquecorne* species; it's almost permanent in the Sidi Moussa lagoon. We note the most important cellular abundance on May 2013 at station P4 (42 320 cells / liter). The minimum value (1.26 10^5c / l) was recorded near the pass at the station P0. A second proliferation of this species was recorded in July, with a maximum value of 3.2 10^5 C / l, always at the bottom of the lagoon, at the station P3.

- ✓ **Bloom of *kryptoperidinium foliaceum***

While the concentration of the species *Peridinium quinquecorne* decline in June, a massive proliferation of the species of dinoflagellates *kryptoperidinium foliaceum* was recorded at the lagoon, with thresholds exceeding 6.10^7C / l at the station P4. This proliferation was accompanied by a very good representation of genus *Dinophysis sp* with concentrations that exceed (4.10^3 C/l), *Alexandrium* sp (1.10^2 C / l), *Gymnodinium sp* with (1.10^2 C / l) and *Ceratium sp* (1.10^3 C / l).

Species diversity

Phytoplankton abundances, number of species (S), and Shannon index of diversity (H', bits) were used as univariate descriptors. The relationship among total phytoplankton, dinoflagellate and diatom abundances, number of species, Shannon index of diversity and environmental parameters were analyzed.

Index of SHANNON – WIENER

The index of Shannon - Wiener (1963) is recommended by several authors (Gray et al, 1992) to describe the diversity of phytoplankton and the situation of the ecosystem. This index is used in work relating to the global ocean (Irigoienet al.2004), different regions like the North Sea (Kabuta & Duijts 2000); the Mediterranean (Ignatiades et al., 2009); Golf of Persian (Polikarpov et al., 2000) or experiments in a controlled environment (Estrada et al. 2003).

Tab 2. : The Shannon index (H ') at the five sampling points.

The index of species diversity H 'varies between 1 and 2.5 bits / cell in coastal waters (sometimes lower in estuarine area) and between 3.5 and 4.5 in ocean waters (Margalef ,1978). Therefore, our results for the lagoon of Sidi Moussa clearly illustrate the fall of diversity characterizing the phytoplankton blooms during the study period. In January and February, H 'is almost absent at all stations (0 and 0.50 bits / cell). Adesalu and Nwankwo, (2008) and Rajagopal, (2010) reported that the low value of Shannon's index of phytoplankton population in rainy season is due to dilution of area.

And we see that during this period it has low species diversity. After a significant increase on H ' in May characterizing a multi-species population, index fall again in June particularly at the station P4. So during this period, H ' indicates the development of a new dominant phytoplankton species. Again in July, we observe an increase in diversity, reaching values between 1.27 and 3.03 bits / cell.

The increase of H 'reflects dominance of a small number of species relative to the general population. We can see that this index moves inversely compared to cell concentrations confirming blooms training. in our case, it is the bloom of the species (*Kryptoperidinium foliaceum).*

Bloom reported during the study period

***Protoperidinium quinquecorne* (Gran) Balech1974 .**

Synonymie: *Peridinium quinquecorne* Abé 1927

P.quinquecorne is an armored dinoflagellate, characterized by four prominent antapical spines and a more-or-less angular shape (Abé, 1981). It s a small dinoflagellate, 17.5-42.5 µm long, 15-35 µm wide.

All species of the genus are Peridinium freshwater species except *Peridinium quinquecorne*, which also tolerates brackish freshwater (José Antolín Aké-Castill, 2011).

P.quinquecorne is a common dinoflagellate found around the world. it is a cosmopolitan species, and has proliferated in many parts of the world: in China (Shen and al, 2001), in Island (Stanca, E.,2013), the Gulf of California (Ismael Garate and al, 2008), the northern Spain (Madariaga and al., 1989), the Gulf of Mexico (Barón-Campis and al., 2005; Okolodkov and al., 2007), Tampa Bay, Florida (Gardiner and Dawes, 1987), inMediterranean Sea (Halim, 1965; Spatharis et al., 2009), Maribago Bay, Philippinnes (Horstmann, 1980), Northern Spain (Madariaga et al., 1989), South Africa (Horiguchi & Pienaar, 1991), the Mexican Pacific (Cortés-Altamirano, 2002; Okolodkov & Gárate-Lizárraga, 2006), and the Caribbean Sea (Margalef, 1961; Faust et al., 2005). Some author (Ismael Garate-Lizárraga, 2008) cited that the bloom of *Peridinium quinquecorne* always occurs in the marine environment near the coast.

P.quinquecorne has a wide geographical distribution (estuarine to neritic environments) with a high tolerance to temperature fluctuations (Horstman, 1980), although germination of cysts from Finland sediments under experimental conditions, suggests that this species is able to grow under salinities of 6 ‰ and temperatures between 10 and 20 °C (Pertola et al., 2006).

According to Paulmier, 1971 and Gàrate-Lizárraga et al., 2008; the presence of *P.quinquecorne* is recorded between the months of April and July.

In Morocco (present work), this is the first time that this species proliferates in the lagoon of Sidi Moussa. This bloom has not been identified at stations near the pass (P0, P1 and P2), but it occurred at the bottom of the lagoon (P3 and P4), so it's could be an autochton bloom due to local cause.

The analysis of the curves of nutrient concentration (nitrites, nitrates and orthophosphate) (Fig 3) and turbidity (Fig 2) shows very high values registered between the months of May and July at stations P3 and P4, where the species *P.quinquecorne* proliferated. This proliferation may be related to environmental enrichment with nutrient, this is confirmed by statistical analysis (PCA) (Fig 4); that show a very good correlation between the rising of the concentrations of nitrites and orthophosphates with the increase of the cell concentration of the species of *Peridinium quinquecorne.* The same synthesis was developed by Shamsudin and al., (1996), who noted that *Peridinium quinquecorne* species prefers and proliferates in nutrient rich environments; (Horstmann 1980) confirmed in his work that *P.quinquecorne* seems to prefer shallow waters where it can tolerate a wide temperature range (Horstman, 1980).

***Peridinium foliaceum* (Stein) Biecheler**

[Synonyms*: Glenodinium foliaceum* Stein, *Kryptoperidinium foliaceum* Lindemann*]*

Kryptoperidinium foliaceum cells are spherical with a lateral compression. Their size ranges from 29.3 to 52.0 µm. The thecae are very fragile, which renders observation of plates difficult .A highly flattened, motile marine dinoflagellate. This is also a well known species as a diatom-harboring dinoflagellate and extensively studied (Horiguchi T., 2004).

Similar to *P.quinquecorne*, *K. foliaceum* is also a dinoflagellates which has been widely studied by several authors (Dodge & Crawford 1969; Withers & Haxo, 1978; J. M. Trigueros 2000 ; Kempton et al., 2002; Horiguchi, 2004; Wolny et al., 2004; Figueroa et al., 2009 ; S. Turki, 2007 ; Saburova et al., 2012). It is well known that *Peridinium foliaceum* is a eurohaline species, it s can tolerate great range of salinities from fresh and brackish waters up to true oceanic salinity (Johnston & Gilliland, 2000; Kempton et al., 2002; Lo´pez-Flores, 2005; Figueroa et al., 2009; Hallegraeff et al., 2010; Konovalova & Selina, 2010; Domingues et al., 2011), between 2.16‰ (Pybus *et al.* 1984, Jenkinson 1990) until more than 100‰ (saburova, 2012).

On another side, *K.foliaceum* tolerate temperatures between 10°C (Sousa & Silva, 1961) until 29,5°C (Turki, 2007)

In Kuwait, in hypersaline environment K.foliaceum proliferated in temperature which does not exceed 18 °C (Saburova, 2012); On the sidi moussa lagoon (Atlantic moroccan coast), the highest concentrations 1.04 10^7 C/l of this species are observed at a temperature of 19, 8°C and a salinity of 30,7‰.

K. foliaceum is included in the list of bloom-forming species, very restricted in space and time, that occur every year in Northern Catalonia (Western Mediterranean, Spain) (MAST-IOC, 1995).

According to Takeo Horiguchi, *P.quinquecorne* and *K.foliaceum* are part of small group of dinoflagellates which is known to possess an endosymbiotic alga of diatom origin. This could explain their sequentially proliferation in the same environmental conditions.

Fig 4.: Principal Component Analysis (ACP).

Both of them has never been reported previously from Sidi moussa lagoon, and their tolerance to wide ranges of temperature and salinity makes us understand that their blooms could be caused by a remarkable elevation of nutrients, precisely at the stations P3 and P4. These results confirm once again the relationship between HABs and eutrophication phenomena.

P.quinquecorne and *Kryptoperidinium foliaceum* are not a toxic species but can be associated with low dissolved oxygen levels in the water and cause fish kills in confined areas.

Photo 1: A. Presentation of the first apical plate of the species K.foliaceum, **B.** K .foliaceum under epifluorescence light, C: K.foliaceum x40, D: Bloom of the species K.foliaceum in the waters of lagoon.

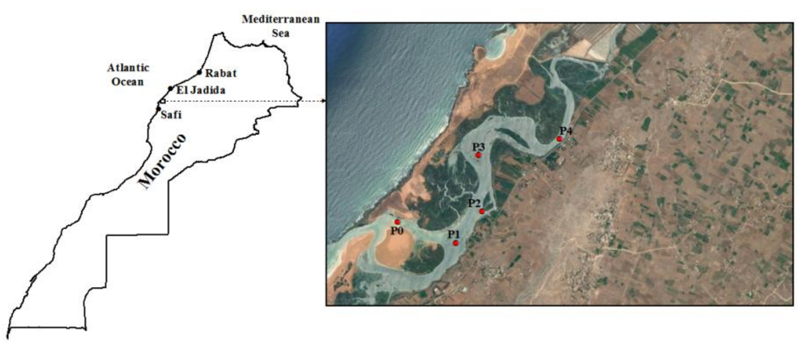


Fig.1: The study area and location of the sampling sites.

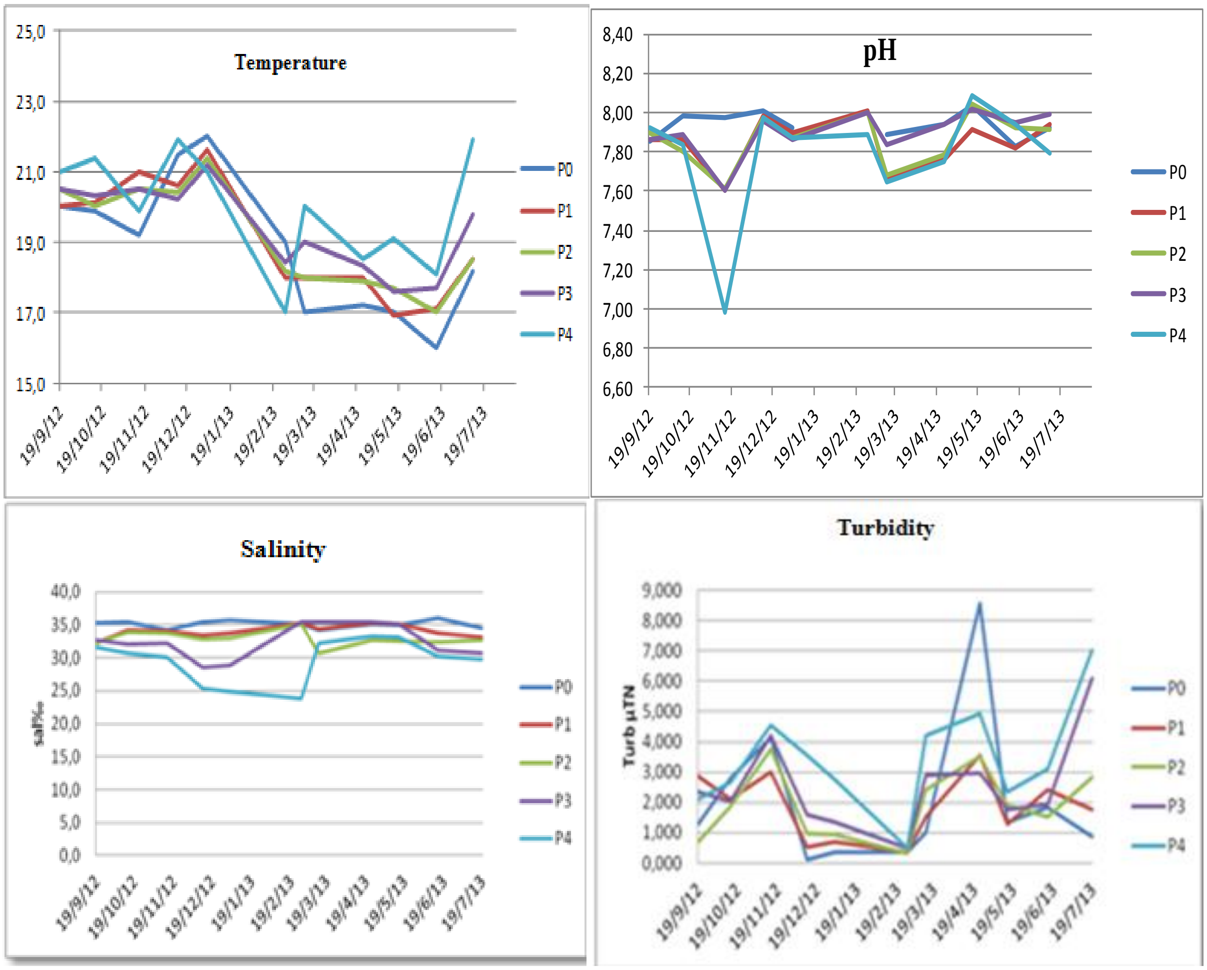
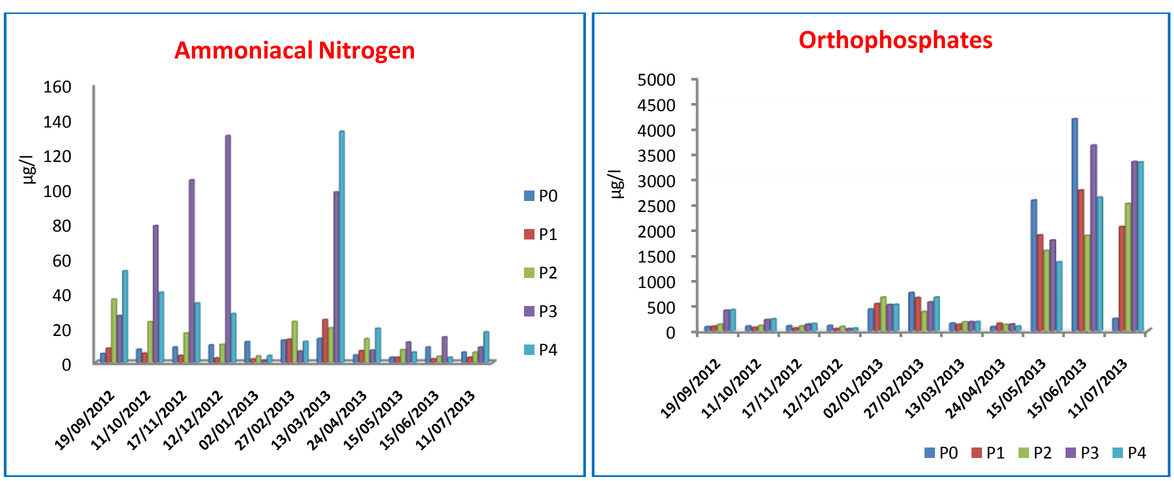


Fig. 2 : Measurements relating to physico-chemical parameters of the water of Sidi Moussa lagoon during the study period.



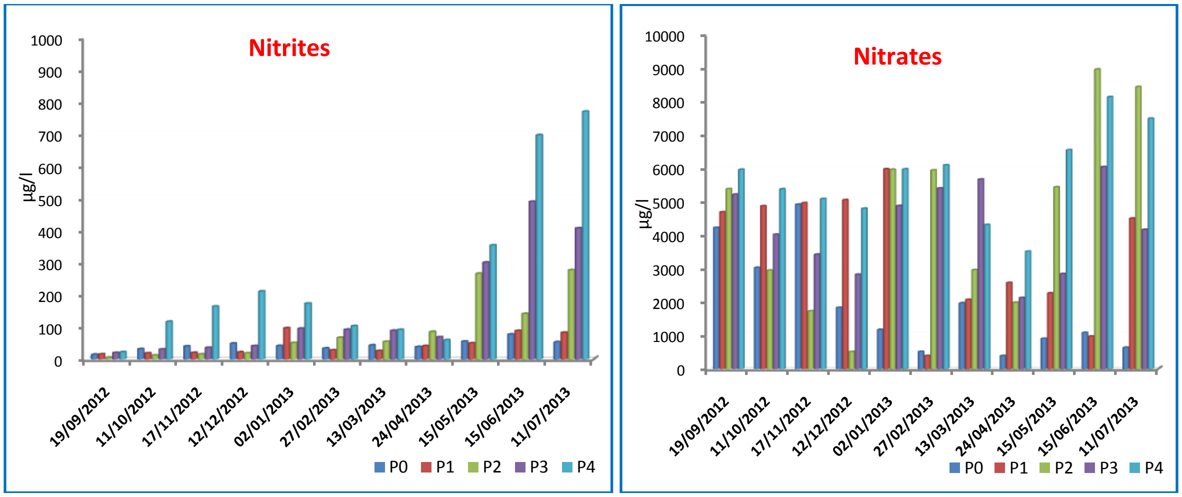


Fig.3: Measurements of chemical parameters of water of Sidi Moussa lagoon (Ammonia Nitrogen, Orthophosphate, nitrite and nitrate) during the study period.

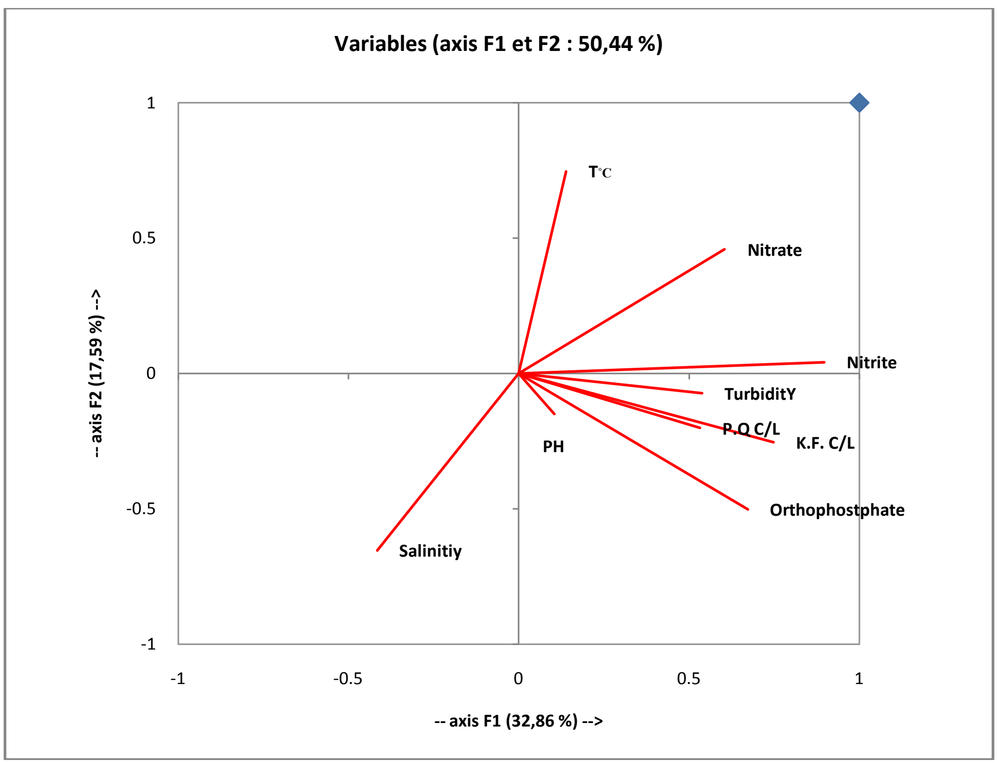
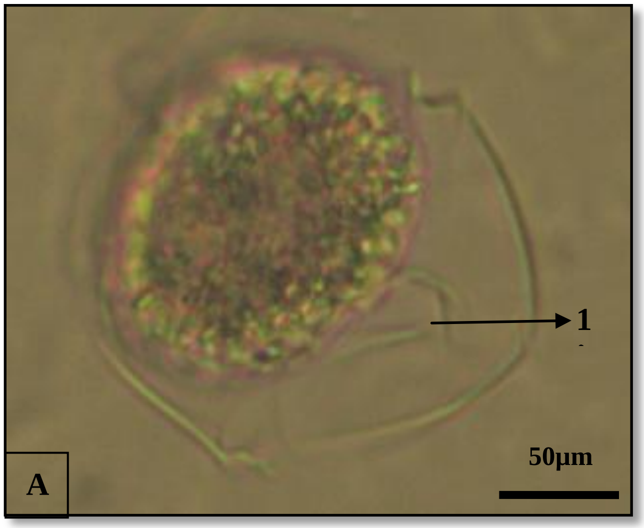


Fig 4.: Principal Component Analysis (ACP).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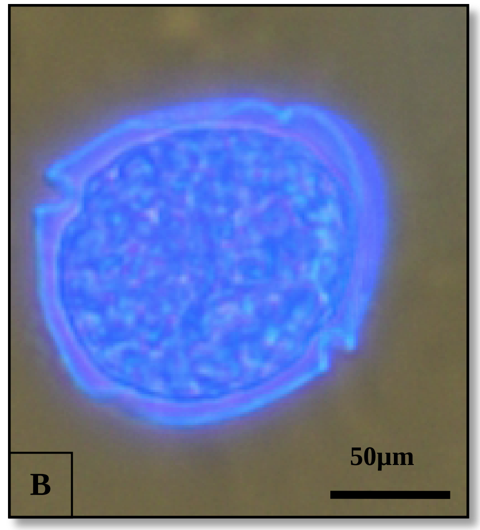
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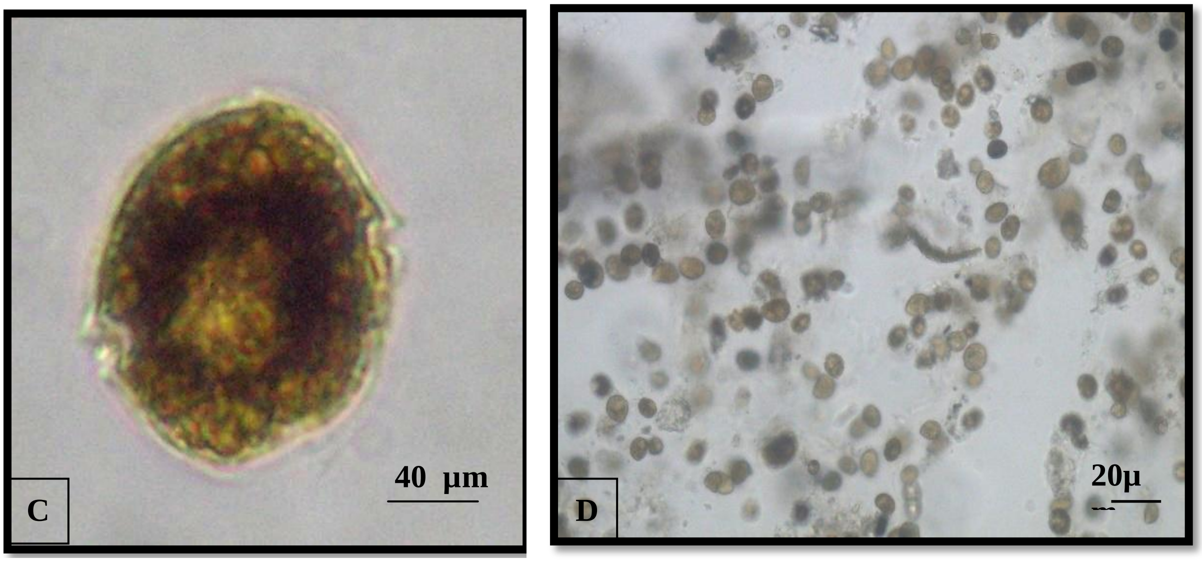

Photo 1: A. Presentation of the first apical plate of the species K.foliaceum, B: K .foliaceum under epifluorescence light, C: K.foliaceum x40, D: Bloom of the species K.foliaceum in the waters of lagoon.

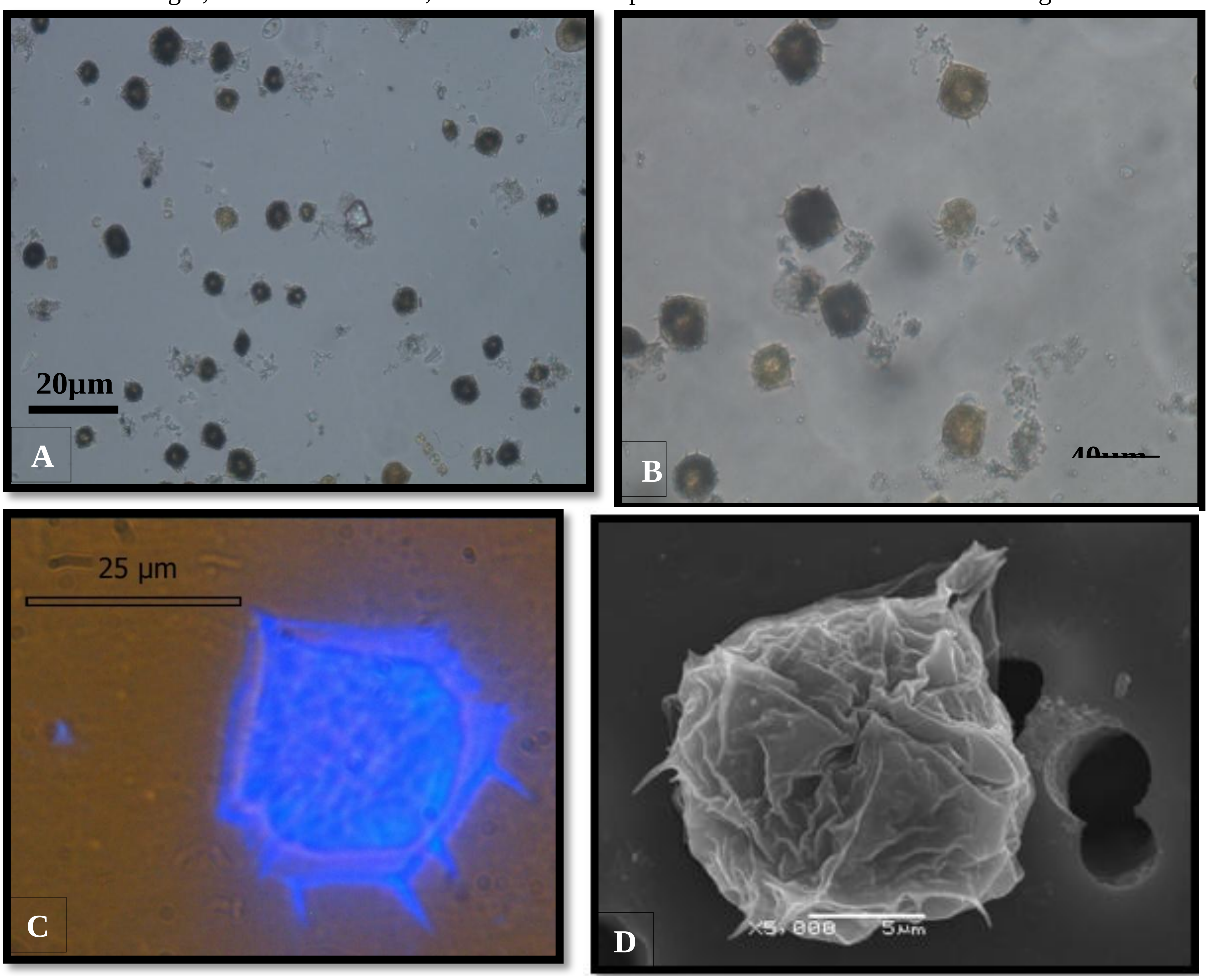

Photo 2: A, B: Bloom of the species *P.quinquecorne*, C: *P.quinquecorne* under epifluorescence light, D: photo of *K.foliaceum* taken under SEM

Dinoflagellates	**Diatoms**
Alexandriumsp	*Pseudonitzshiasp*
Ceratiumsp	*Nitzshiasp*
Gonyaulaxsp	*Surirellasp*
Scripsiellasp	*Rhizosoleniasp*
Dinophysis fortii	*Grammatophora marina*
Dinophysis sacculus	*Achnantessp*
Gymnodiniumsp	*Hermesiumsp*
Dinophysis sp	*Leptocylindrusminimus*
Kryptoperidiniumfoliaceum	*Amphora*
Prorocentrumsp	*Leptocylindrusdanicus*
Prorocentrumsucltellum	*Ostrupiasp*
Prorocentrummicans	*Dictyocha*
Pentapharsodiniumsp	*Chaetocerossp*
Protoperidiniumsp	*Navicula sp*
Peridinium quinquecorne	*Thalassiosirasp*
Ceratiumfusus	*Melosirasp*
Oxyphisissp	*Druridgeasp*
Lingulodiniumpolyedrum	*Cocconeissp*
Protoceratiumreticulatum	*Lauderiaborealis*
Polykrikossp	*Pleurosigmasp*
Heterocapsasp	*Gyrosigmasp*
Katodiniumsp	*Coscinodiscussp*
Mesodiniumsp	*Licmophorasp*
Gyrodiniumsp	*Lauderiaannulata*
Diplopsalissp	*Biddulphiasp*
Oxytoxumsp	*Paraliasulccata*
	Entomoneissp
	Guinardiastolterfothii
	Asterionellaglacialis
	Falculasp
	Fragilariasp
	Diploneissp
	Thalassionemanitzshoides
	Cerataulinapelagica
	Odentellamobiliensis
	Raphoneissp
	Tabullariasp
	Helicothecaricard
	Mesodiniumsp

Tab.1 : Inventory of phytoplankton species occurring in the lagoon of Sidi Moussa.

Station	sept-2012	oct-2012	nov-2012	dec-2012	jan-2013	febr-2013	mars-2013	apr-2013	mai-2013	jun-2013	jul-2013	august-2013
P0	1,69	1,79	1,66	1,37	_	_	_	1,74	0,61	0,10	1,28	1,30
P1	2,15	2,45	0,92	0,92	_	0,59	0,81	1	0,22	2,28	1,12	1,81
P2	1,35	2	1,92	_	_	_	1,22	1,61	0,60	0,10	3,03	1,37
P3	1,38	1,63	0,72	1	_	0,50	0,00	2,15	0,75	1,25	1,24	1,72
P4	2,31	0,95	0,35	0,35	_	_	1,70	1,38	0,25	0,01	1,40	1,90

Tab 2: The Shannon index (H ') at the five sampling points.

Conclusion

The present study demonstrated the relationship between the bloom and the nutrient enrichment. During the study period (2012-2013), two blooms were recorded at the bottom of the lagoon at stations P3 and P4, and the incriminated species were *Peridinium quinquecorne* and *kryptoperidinium foliaceum*. It's the first time that these two species proliferate. These blooms coincide with an especially notable elevated nutrient loads in the lagoon (Nitrates, Nitrites and Orthophosphates), and the source of the contamination may be the industrial installations from Jorf Al Asfar of the phosphate, located 10 Km away from the lagoon.

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