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RESEARCH ARTICLE

Phytoplankton distribution in the Nador lagoon (Morocco) and possible risks for harmful algal blooms

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Abstract

1 - Phytoplankton populations and environmental parameters were analysed seasonally in the lagoon of Nador (Morocco) over an annual cycle (2006-2007) at six stations. Seven phytoplankton Classes were observed: Diatomophyceae, Dinophyceae, Raphidophyceae, Prasinophyceae, Euglenophyceae, Dictyochophyceae and Cyanophyceae. Seven taxa were identified that may present a risk of inducing harmful blooms i.e. *Alexandrium minutum*, *Pseudonitzschia delicatissima* and *P. fraudulenta*, *Prorocentrum minimum*, *Prorocentrum rhatymum*, *Dinophysis sacculus*, *Gymnodinium catenatum* and *Karenia mikimotoi*. Physico-chemical analyses were carried out on water samples: Dissolved Organic Carbon (DOC) and nutrients (NH_4^+, NO^{3-}, PO_4^{3-} and SiO_2).

2 - Favourable and unfavourable factors for bloom development were identified. The possible relations between environmental parameters and the occurrence of potentially harmful phytoplankton blooms were investigated through a Factorial Discriminant Analysis. The proliferation of potentially harmful phytoplankton was linked to the availability of various nutrients (N, P, Si). *Prorocentrum minimum* tolerated waters with high nitrate contents, while *Alexandrium minutum* tolerated nutrient-poor waters. The most frequently observed taxa, *Pseudonitzschia* species and *Dinophysis sacculus*, were adapted to variations of water quality. Excessive levels of nutrients appeared as inhibiting for proliferation of potentially harmful phytoplankton which disappeared for the benefit of a very dense bloom of *Tetraselmis* sp. (Prasinophyceae).

3 - A risk of toxicity was observed during all samplings throughout the lagoon, it was particularly pronounced during the summer sampling in the northern continental part of the lagoon with proliferation of *Pseudonitzschia* species.

Keywords: Nador lagoon, Phytoplankton, Environmental parameters, Anthropogenic pressure, Potentially Harmful Algal Blooms, Risks of toxicity.

Introduction

Lagoonal environments are vulnerable to environmental changes (Lloret *et al.*, 2008), particularly to enhanced anthropogenic pressure, which can lead to eutrophication (Viaroli *et al.*, 2004). Nutrient increases, particularly high levels of dissolved nitrogen and phosphorus are often related to growing human activities: i) agriculture because of the excessive use of fertilizers and their leaching during rainy events. ii) delivery of urban wastewaters directly into the lagoon, especially in summer due to the increased tourist population, iii) Fish farming, due to faecal products and unconsumed food. Water pollution induces macroalgal and phytoplankton development (De Casabianca *et al.*, 1997, Munkes, 2005, García-Pintado *et al.*, 2007).

The number of reports on harmful Algal Blooms (HABs) has significantly increased in the last two decades (Hallegraeff and Fraga, 1998, Hallegraeff *et al.*, 2003). Four explanations were proposed for this increase: i) growing scientific awareness for toxic algal species; ii) raising utilization of coastal waters for aquaculture; iii) increasing extent of eutrophized areas and /or unusual climatic conditions that imply stimulation of plankton blooms and iv) scattering of harmful algae all over new areas due to transportation of algal cysts (Dinoflagellate) through ship ballast waters or translocation of shellfish stocks (Hallegraeff *et al.*, 2003). HABs events lead to many deleterious effects such as contamination of seafood with toxins, fish mortality and alteration of water quality. In lagoons, such phenomena cause significant economic loss due to the ban of marine product sale (shellfish, fish) and impacts on tourism activities (swimming). Moreover, production of toxins from toxic algae could lead to serious consequences on human health. Phycotoxins affect human health through consumption of vector organisms (shellfish, fish) inducing neurological and/or gastroenteric diseases that can be lethal for humans.

Nador lagoon (NL) is one of the most important Mediterranean lagoons by its surface and biodiversity (Ramsar site since 2005 and 'Sites d'intérêt Biologique et Ecologique' (SIBE) status since 1996). Its shore is highly urbanized and the economy of the Nador area depends on it. The lagoon has been exposed to nutrient over-enrichment. In NL, comparison between the first evaluation of nitrogen level (Brethes, 1977) and studies performed two decades later (Berraho *et al.*, 1995 and Inani, 1995), showed a general increase in nitrogen contents from 1977 to 1995, whilst phosphorus concentrations decreased from 1995 (Berraho *et al.*, 1995, Inani, 1995) to 2006 (Ruiz *et al.*, 2006). As a result, important changes in phytoplankton population took place with increasingly frequent monospecific phytoplankton blooms (harmful or not).

The first monitoring of phytoplankton populations of Nador lagoon was performed weekly from 1998 to 2005, evidencing that the phytoplankton community was composed by seven classes: Cyanophyceae, Euglenophyceae, Chlorophyceae, Dictyochophyceae,Prymnesiophyceae, Diatomophyceae and Dinophyceae, with a dominance of Diatomophyceae and Dinophyceae (El Madani, 2005). Among the potentially HAB forming genera, *Alexandrium* spp. and *Pseudonitzschia* spp. were the most abundant, especially *Alexandrium minutum* which was detected every year during winter and spring (El Madani, 2005). At the same time, contamination of shellfish species by Paralytic Shellfish Poisoning (PSP) toxins were confirmed by chemical analysis (El Madani, 2005) and shellfish harvesting was forbidden during the period of risk. Other genera were also monitored, among which *Dinophysis* spp. was observed every year mainly from spring to autumn, while *Pseudonitzschia* spp. and *Prorocentrum* spp. were frequently observed throughout the year. All of these species formed blooms but none of them has been implied in shellfish contaminations by toxins during the period of the study.

Generally, the studies of phytoplankton populations available in the literature were restricted to spring and/or summer, the most favourable periods to phytoplankton development. The aims of the present paper were: i) to investigate the spatio-temporal distribution of the phytoplankton blooms in relation to environmental parameters and anthropogenic pressure, ii) to evaluate the possible risks of HABs in the lagoon and their possible relations with environmental parameters.

Materials and Methods

Study area

The Nador lagoon is among the largest lagoons in North Africa (115 km^2, 27 km long and 7.5 km wide). It is located in the southern shore of the Mediterranean Sea (NE of Morocco: 35°10'N, 2°45'-2°47'E) in a semiarid region (Figure 1). A maximum depth of 8 m is reported in the center of the lagoon. The lagoon connects to the sea via one tidal inlet, Bokhana, through which exchanges with the sea occur. Continental water inputs arrive to the lagoon by different temporary and two perennial streams: Oued Kabayo and Oued Selouane.

The maximum salinity range recorded for NL (Winter: 35.1-36.9, Summer: 37.7-40.9) are characteristic of marine water. The temperature range was from 13.6°C in Winter to 30.5°C in Summer (Bloundi, 2005). The wind direction is West/South-West dominant from November to May and East/North-East dominant from May to October (Tesson, 1977; Guélorget *et al.*, 1987; Hilmi, 2005).

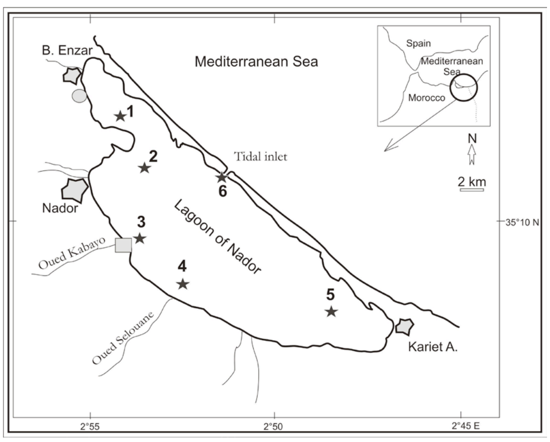

Figure 1. Presentation of Nador lagoon (Morocco). Location of sample stations (stars), stabilization ponds (circle) and waste water treatment plant (square). The main towns around the lagoon are Nador, Beni Enzar (B.Enzar) and Kariet Arekmene (Kariet A).

Six sampling stations were chosen according to the main pollution sources and contact with the sea: S1 is located in a confined area, close to urbanization (Beni Enzar) in front of stabilization ponds; S2 situated near a fish farm (bass, sea bream) which closed down at the end of 2006 for economical reasons; S3 is situated in front of the outflow of a malfunctioning Waste Water Treatment Plant (WWTP); S4 is near Oued Selouane which brings urban, agricultural and industrial wastes of Selouane town; S5 is in a confined area, where urban impacts are important from Kariet Arekmene town, with discharges of nontreated waste waters. The highest marine influence is noticeable at S6 located near the tidal inlet.

Sampling and preparation of samples

In order to study the spatio-temporal variations of phytoplankton composition in relation to physico-chemical parameters, phytoplankton and water samples were collected, at 0-1 m depth, at each sampling stations (S1 to S6) during four sampling campaigns: 3 November 2006, 24 January 2007, 12 April 2007 and 3 September 2007.

Phytoplankton samples

Two types of phytoplankton samples were collected, immediately preserved by neutral formalin (0.4%) and kept in dark at 5°C before analysis. The first sampling was done for the taxonomic study, using a 20 µm mesh phytoplankton net and was sampled once from each station at every season, for a total of 24 net phytoplankton samples. Every samples were settled in 5 and 10 ml chambers for 4 to 6 hours and phytoplankton species were identified after removal of the supernatant following the Utermöhl method (1958) and scanned at 100x, 200x, 400x linear magnification (depending on density of species) using a Leica-DMLII inverted microscope.

Armoured Dinoflagellates were determined according to the thecal plate configuration (Balech, 1995 and 2002). The separated thecal plates were examined by phase-contrast microscopy (Tomas, 1997). The identification of *Alexandrium minutum* was confirmed by Professor Halim, who discovered this species in Alexandria, Egypt (Halim, 1960 and personal communication). Identification of *Pseudonitzschia* species were realized under Scanning Electron Microscope (SEM) after HCl 10% treatment following Simonsen method described in Tomas (1997).

The taxonomical identification was in agreement with Lecointre and Leguyader (2001) as recommended by Reviers (2003) and the nomenclature complied with the rules of the International Code of botanical Nomenclature (Greuter *et al.*, 1988). Phytoplankton identification was made according to group systematic using classical reference books and illustrations: Trégouboff and Rose, 1978, Sournia, 1986, Hallegraeff *et al.*, 2003. In this study, phytoplankton biodiversity was assessed as the number of taxa recovered from net samples.

The second sampling was realized for the quantitative study (counting expressed in cell number.l^{-1}), performed with Niskin bottle once from each station at every season, for a total of 24 water phytoplankton samples. Every sample was allowed to settle in 25 ml counting chamber for one night following the Utermöhl method (1958) and scanned at 200x, 400x linear magnifications using Leica-DMLII inverted microscope. Enumeration was performed for the whole chamber following the Hasle method described in Sournia (1978). This quantitative study led to the determination of specific density which is represented by the number of individuals belonging to each taxon determined in the water phytoplankton sample. For *Pseudonitzschia*, enumeration includes the two species *delicatissima* and *fraudulenta* that can only be identified by SEM.

Water samples

Water samples were manually collected in 500 ml polyethylene bottles, kept in a dark cooler and transported to the laboratory. From each station at every season, triplicates were sampled for environmental analyses with a total of 72 water samples. All water samples were filtered through glass fiber filters (GF/F, Whatman), previously combusted at 450°C during 4 hours and filtrates were transferred in glass bottles. For silicate analysis, a second filtration was carried out through cellulose nitrate filters and filtrates were transferred in polycarbonate tubes. All filtrates were stored in the dark in a refrigerated room (4°C) before analysis. Environmental analyses were conducted within 48 hours after sampling.

Physico-chemical analyses of environmental parameters

Physico-chemical analyses were performed on filtrates.

Dissolved Organic Carbon (DOC) was analysed in order to evaluate the pool of organic matter either of allochthonous or autochthonous origin. DOC, expressed in mgl^{-1}, was analyzed in a V*CSN* Shimadzu TOC/TN Analyzer by oxidative combustion (720°C) with a platinized alumina catalyst under ultra-pure oxygen flow and infrared detection method (NDIR). The mean of two to three injections of 50 μL is reported for every sample and precision, described as a coefficient of variance (C.V.), was < 2% for each replicate injection. Detection limit for DOC was 0.1 mgl^{-1}.

Chemical analyses concerning nutrients were performed using colorimetric methods with

a Hach DR/890 Colorimeter (Hach, 2004).
Nitrates (NO_3^-) (µM) were analysed by chromotropic acid method: 1 ml of water sample is mixed with sulphuric acid before addition of chromotropic acid. Detection limit for nitrates was 14.5 µM
Ammonium (NH_4^+) was analysed by salicylate method with 2 ml of water sample. Ammonia compounds are initially combined with hypochlorite before reaction with salicylate and oxidation with a catalyst "nitroprusside". Detection limit for ammonium was 1.7 µM.
Phosphates (PO_4^{3-}) were analysed by ascorbic acid method with 10 ml of water sample. Phosphates react with molybdate in acid solution before reduction by ascorbic acid. Detection limit for phosphates was 0.2 µM.
Silicates (SiO_2) were analysed with 10 ml of water sample by heteropoly blue method which is an extension of the silicomolybdate method to increase sensitivity. Reaction of molybdate ion with silicates and phosphates occurs under acid conditions before addition of citric acid and reducing agent. Detection limit for silicates was 0.2 µM.

Factorial Discriminant Analysis
The Factorial Discriminant Analysis (FDA) is a linear analysis which leads to a generalized Principal Component Analysis (PCA) concerning the barycenters of the classes. FDA runs into two steps: the first one is a PCA applied to the initial variables or parameters (quantitative data) and the second one discriminates the classes (qualitative data) on the basis of their barycenters. Factorial Discriminant Analysis was achieved using Addinsoft® XLStat-Pro® software which leads to a twofold graphical representation: correlation circle based on the factorial plane containing initial observations and on the other hand the projection of the classes in the discriminating factorial axes system which allows to display the discrimination quality. Our data set is composed of 336 observations obtained for 14 variables (Salinity, Temperature, DOC, NH_4^+, NO_3^-, PO_4^{3-}, SiO_2 and specific densities of seven potentially harmful taxa: *Alexandrium minutum*, *Pseudonitzschia delicatissima*, *P. fraudulenta*, *Prorocentrum minimum*, *Prorocentrum rhatymum*, *Dinophysis sacculus*, *Gymnodinium catenatum* and *Karenia mikimotoi*) measured from six stations (1st class) at the four seasons (2nd class). Our goal was to test if the occurrence of blooms of potentially harmful algae could be related to environmental parameters according to discrimination of the stations and the seasons.

Results
Total Phytoplankton
In this study, 124 species were identified, belonging to seven different Classes, i.e. Diatomophyceae, Dinophyceae, Raphidophyceae, Prasinophyceae, Euglenophyceae, Dictyochophyceae and Cyanophyceae (cyanobacteria). The annual averages of taxa numbers were calculated for each class. These results were expressed in percentage to compare the specific diversity between stations (Figure 2). Overall, Diatomophyceae and Dinophyceae were the two dominant classes with respectively 40% and 51% of average specific diversity. In the other classes, average specific diversity varied from 1% to 4%. Within the Dinophyceae, armoured species were widely represented (84.4%). The specific diversity was maximum at S1 with six classes while it was minimum at S4 with three Classes.
The Total Phytoplankton Density (TPD) corresponding to the number of cells per litre, showed spatio-temporal variations (Figure 3). During the summer sampling, the highest cell concentrations were measured at three stations: S3 (100.0 10^5 cell.l^{-1}), S2 (3.0 10^5 cell.l^{-1}) and S1 (2.3 105cell.l^{-1}), while at S4, S5 and S6 stations, the TPD was less than 0.5 10^5 cell.l^{-1}. In Spring, a high TPD was reached at S3 (1.6 10^5 cell.l^{-1}) while TPD

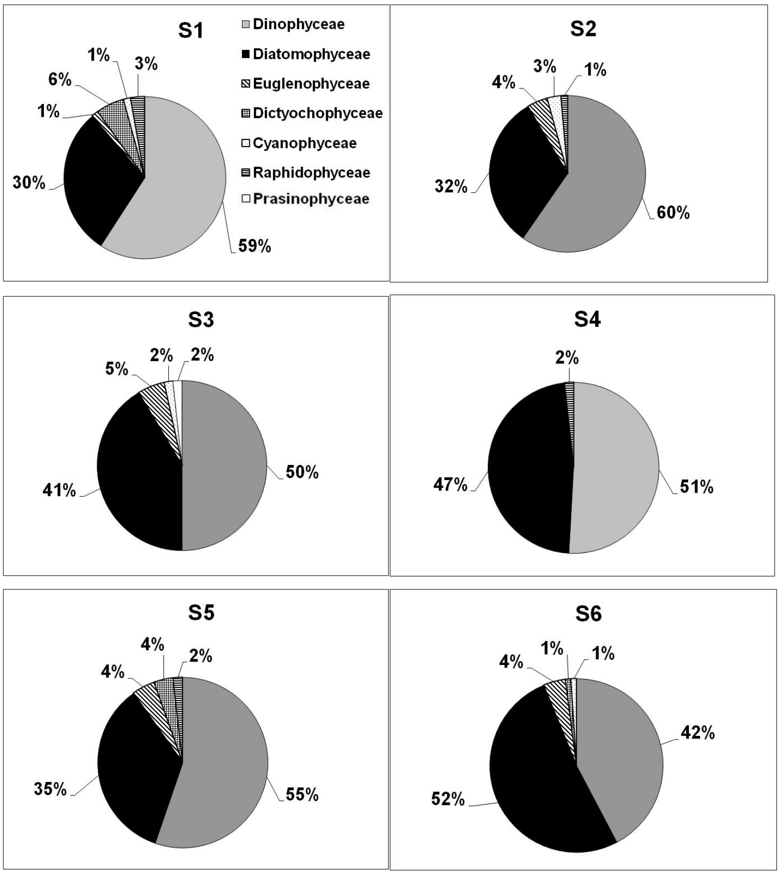

Figure 2. Percentages of different Classes of the phytoplanktonic populations at each station in Nador lagoon.

was very low (< 0.3 10^5 cell.l^{-1}) in the other stations. In autumn, the maximum TPD was 10^5 cell.l^{-1} at S2, 0.9 10^5 cell.l^{-1} at S5 and 0.6 10^5 cell.l^{-1} at S3. In the other stations, values of TPD varied between 0.04 10^5 cell.l^{-1} at S1, 0.2 10^5 cell.l^{-1} at S4 and 0.1 10^5 cell.l^{-1} at S6. During the winter sampling, the lowest values of TPD were recorded from 0.01 10^5 cell.l^{-1} at S4 to 0.3 10^5 cell.l^{-1} at S3.

Potentially Harmful Phytoplankton

Within total phytoplankton, some species are usually considered as potentially toxic. However, since in this study toxins were not analyzed, we use the term of "potentially harmful phytoplankton". Seven potentially harmful taxa were identified in the phytoplankton of Nador: *Alexandrium minutum*, *Gymnodinium catenatum*, the *Pseudonitzschia* group comprising the two species *P. delicatissima* and *P. fraudulenta*, *Prorocentrum minimum*, *Prorocentrum rhatymum*, *Dinophysis sacculus* and *Karenia mikimotoi*.

The spatio-temporal variations of Potentially Harmful Phytoplankton (PHP) related to Total Phytoplankton (TP), expressed in percentage, are shown in Figure 4. PHP was present in every station for each season with different proportions. During the autumn sampling, more than 80% of TP was composed of PHP at stations S5 (92%), S4 (86%), S2 (82%) and S3 (80%), while at S1 and S6, PHP represented 55 and 68% of TP respectively. In Winter, an important percentage of PHP was recorded at S5 (97%) and S4 (41%), while the percentage never reached 20% at the other stations. During the sampling in

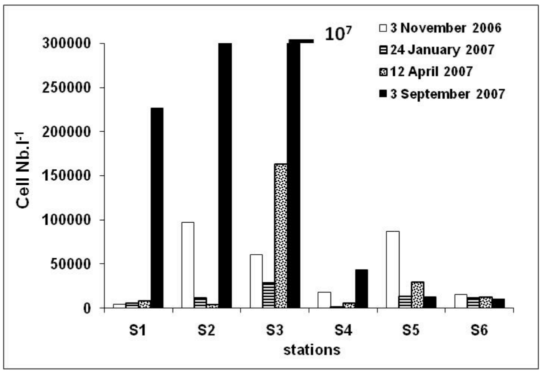

Figure 3. Spatio-temporal variations of Total Phytoplankton Density, expressed in cell number.l^{-1}.

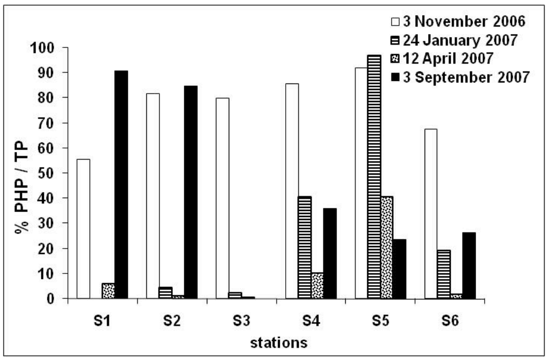

Figure 4. Spatio-temporal variations of the ratio: cell number of Potentially Harmful Phytoplankton (PHP) to cell number of Total Phytoplankton (TP) expressed in percentage (%).

spring, PHP was low (< 7%) except at S4 (10 %) and at S5 (41%). During the sampling in summer, highest percentages of PHP were detected at S1 (91%) and S2 (85%). At the other stations, percentages of PHP reached 36% at S4, 27% at S6 and 24% at S5. At S3, no PHP was observed during this sampling.
The potentially harmful taxa were counted at each station (S1 to S6) and, for the different samplings, the results are presented in Table 1. Blooms were apparently present throughout the year and the most frequently reported species were *Pseudonitzschia* species (20 blooms), *Dinophysis sacculus* (13 blooms) and *Alexandrium minutum* (12 blooms). Among the four seasons, during the sampling in summer, seven potentially harmful taxa were present at S1. They were forming six major blooms (blooms with highest densities) on different stations, excepted for *Gymnodinium catenatum* for which we observed the maximum density at S5 during the sampling in autumn. During the samplings in winter and spring, we only observed three PHP taxa, i.e., *Alexandrium minutum*, *Dinophysis sacculus* and *Pseudonitzschia* species and also *Dinophysis sacculus*, *Prorocentrum minimum*
and *Pseudonitzschia* species, respectively. These were not major blooms. Only during the sampling in summer, we observed the uncommon *Prorocentrum rhatymum* at S1 and S2, while the scarce *Karenia mikimotoi* was observed at S1. Exceptionally, S3 presented no potentially harmful phytoplankton during the sampling in summer. Station S6 near the tidal inlet was the poorest station with only two taxa (*Alexandrium minutum* and *Pseudonitzschia* species).

Physico-chemical parameters
The physico-chemical parameters were measured over the whole lagoon and showed spatial variations (Figure 5). The lowest values of all parameters were measured in winter. In autumn we observed high mean values of DOC (2.41 ± 0.49 mgl^{-1}) and relatively high mean values of nitrates (44.6 ± 16.6 µM or 2.8 ± 1.0 mgl^{-1}) and of silicates (8.3 ± 3.2 µM or 0.50 ± 0.19 mgl^{-1}). During the sampling in spring we observed the highest concentrations of nitrates with a maximum at S2 (129.5 ± 8.2 µM or 8.0 ± 0.5 mgl^{-1}). During the sampling in summer, S3 showed exceptional concentrations of ammonium (65.6 ± 7.1 µM or 1.18 ± 0.13 mgl^{-1}), phosphates (6.5 ± 0.4 µM or 0.62 ± 0.04 mgl^{-1}) and silicates (46.7 ± 0.4 µM or 2.80 ± 0.03 mgl^{-1}). Silicate contents presented higher values at S3 and S4 due

Table 1 - Determination and densities (cell number.l^{-1}) of potentially harmful algal blooms in Nador lagoon, according to the four sampling dates and stations (S1 to S6). Between brackets: PSP (Paralytic Shellfish Poisoning), DSP (Diarrheic Shellfish Poisoning), ASP (Amnesic Shellfish Poisoning). In bold characters: the highest density values.

		Station					
Taxa **(Potential Harmful effects)**	**Sampling date**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Alexandrium minutum **(PSP)**	3 November 2006		120	14 240	640	160	1 400
	24 January 2007					12 280	1 200
	3 September 2007	480	1 600		**15 120**	200	240
Dinophysis sacculus **(DSP)**	3 November 2006			640			
	24 January 2007		40		40	200	
	12 April 2007	520	40	400	280	400	
	3 September 2007	1 160	**2 720**		200	40	
Gymnodinium catenatum **(PSP)**	3 November 2006					**1 280**	
	3 September 2007	160	560				
Karenia mikimotoi **(Fish-killing)**	3 September 2007	**2 560**					
Prorocentrum minimum **(Haemolytic - cytotoxic)**	3 November 2006		280				
	12 April 2007			160	40		
	3 September 2007	80	**960**			40	
Prorocentrum rhatymum **(Haemolytic)**	3 September 2007	**1 240**	80				
Pseudonitzschia delicatissima **(ASP)** and ***Pseudonitzschia fraudulenta*** **(non-toxic)**	3 November 2006	2 440	78 960	33 880	14 680	78 880	8 880
	24 January 2007		480	680	480	680	1 040
	12 April 2007			280	280	11 600	240
	3 September 2007	200 000	**250 000**		320	2 880	2 560

to the freshwater inputs from Oued Kabayo and Oued Selouane respectively, bringing dissolved Silica from lithogenic dissolving in terrestrial environments. Elsewhere, values were quite constant.

The results of nutrient analyses highlighted the anthropogenic influence all over the Nador lagoon. Compared to previous studies in the NL, our nitrate results were lower than those found by Berraho *et al.* (1995) and higher than those reported by Brethes (1977) and Inani (1995). Ammonium values were more important than those found by Ruiz *et al.* (2006). Phosphate values were similar to those from Inani (1995) in Summer, they were higher than those of Ruiz *et al.* (2006) and they were lower than those of Berraho *et al.* (1995) and Inani (1995) in Winter.

Factorial Discriminant Analysis–potentially harmful algal blooms and environmental factors

The data set was partitioned into two classes (station and season) and was successively processed according to the two classes. Each data processing leads to a different spatial representation depending on station and season.

Factorial analysis of the data according to stations indicates that 69.54% of the variance is explained by the first two axes which are used for data interpretation (Figure 6). The first axis represents 44.91% of the total information while the second axis represents 24.64%.

According to stations, in the Correspondence Chart (Figure 6A), variables are correlated into three groups: 1) SiO_2, *Prorocentrum rhatymum*, *Karenia mikimotoi*, *Alexandrium minutum*, NH_4^+ and PO_4^{3-} along axis F1; 2) *Prorocentrum minimum*, NO_3^-

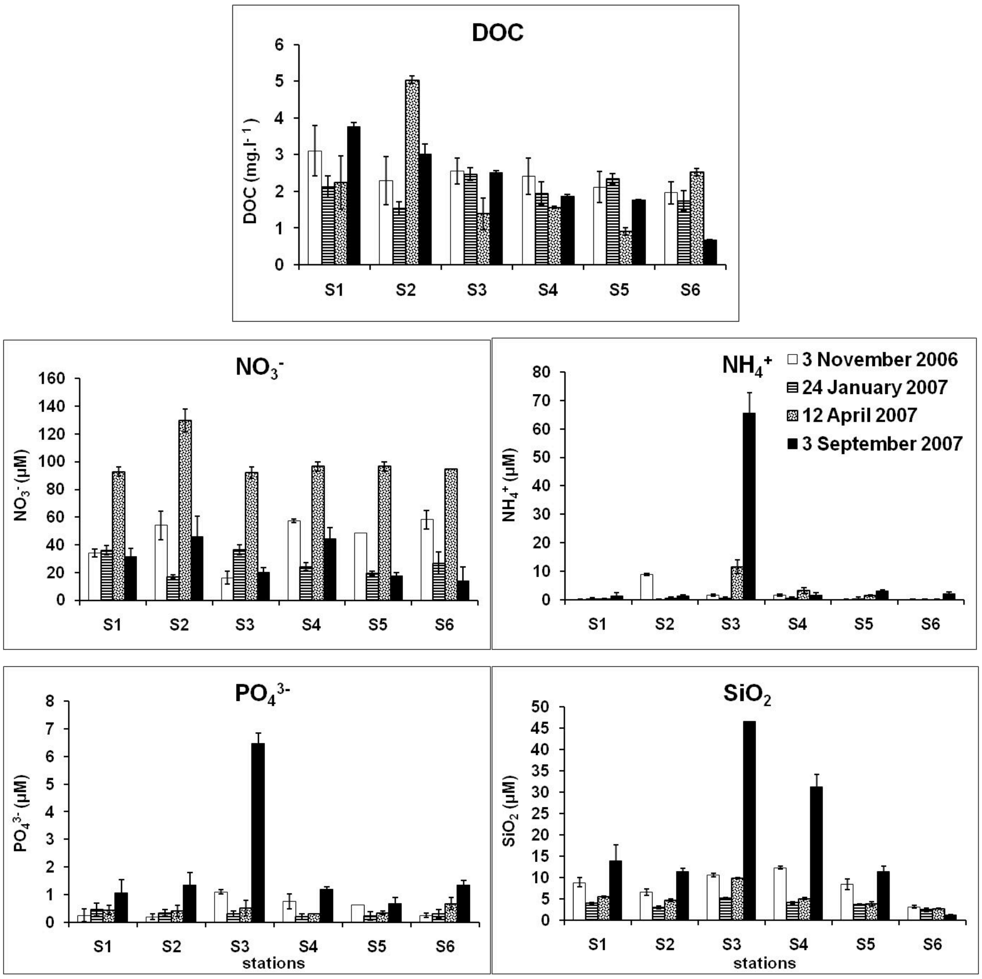

Figure 5. Spatio-temporal variations of environmental factors: Dissolved Organic Carbon (DOC), nitrates (NO_3^-), ammonium (NH_4^+), phosphates (PO_4^{3-}) and silicates (SiO_2). DOC is expressed in mgl^{-1} and nutrients are expressed in µM. The sampling dates are identical for each graph.

Pseudonitzschia species, Temperature and *Dinophysis sacculus* along axis F2; 3) DOC, *Gymnodinium catenatum* and Salinity between the two axes.

The scatterplot highlights a spatial discrimination (Figure 6B). Along axis F1, Station S6 (negative values) under marine influence, is characterized by minimal contents in ammonium and silicates (Figure 5), associated with the lowest diversity (2 taxa) and the lowest density (Table 1). On the opposite Station S3 (positive values) influenced by urban pollution, is mostly distinguished during the sampling in summer by maximal values of ammonium, phosphates and silicates (Figure 5) linked with exceptional absence of potentially harmful phytoplankton. Axis F2 separates, on one hand, Station 2 (positive values) characterized by a great diversity (6 taxa), the highest density including three major blooms (*Dinophysis sacculus*, *Prorocentrum minimum* and *Pseudonitzschia* species) during the sampling in summer (Table 1), linked with the availability of organic matter and nutrients (Figure 5) and on the

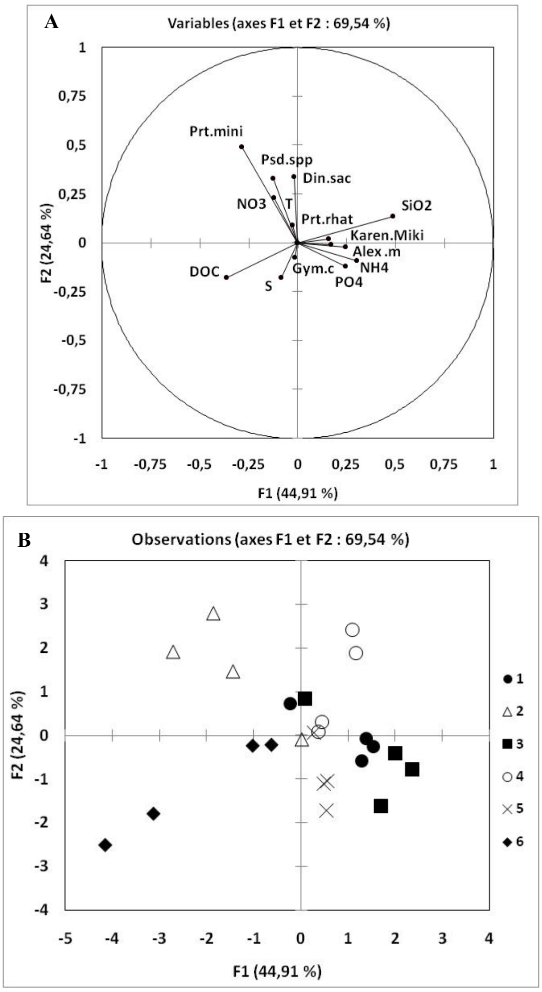

Figure 6. Results of Factorial Discriminant Analysis applied to spatial discrimination plotted according to axes 1-2.

A- Correspondence chart with distribution of the variables: Salinity (S), Temperature (T), Dissolved Organic Carbon (DOC), nitrates (NO3), ammonium (NH4), phosphates (PO4), silicates (SiO2), *Alexandrium minutum* (Alex.m), *Pseudonitzschia* species (Psd.spp), *Prorocentrum minimum* (Prt.mini), *Prorocentrum rhatymum* (Prt.rhat), *Dinophysis sacculus* (Din.sac), *Gymnodinium catenatum* (Gym.c) and *Karenia mikimotoi* (Karen.Miki).

B- Discrimination of the six stations with distribution of the observations. Stations are: S1 (1), S2 (2), S3 (3), S4 (4), S5 (5) and S6 (6).

other hand, Station S6 (negative values), the poorest station.

Factorial analysis of the data according to seasons indicates that 94.04% of the variance is explained by the first two axes (Figure 7) which are used for data interpretation. Axis F1 represents 52.70% of the total information while axis F2 brings 41.34 %. Because, during the seasons we did not replicate our samplings we cannot conclude how blooming of species is determined by the seasons. Nevertheless, the four different samplings realised during the different seasons gives us a first idea of the variability within the year. According the different samplings, the Correspondence Chart (Figure 7A) shows that variables are correlated into two groups: 1) NO_3^-, DOC and Salinity along axis F1; 2) *Alexandrium minutum*, *Karenia mikimotoi*, *Prorocentrum rhatymum*, *Pseudonitzschia* species, PO_4^{3-}, SiO_2, Temperature, *Prorocentrum minimum*, NH_4^+, *Dinophysis sacculus* and *Gymnodinium catenatum* along axis F2.

The scatterplot highlights a discrimination between samplings (Figure 7B). Along axis F1, the sampling in spring (positive values) is characterized by maximum contents in nitrates (Figure 5) and by the presence of three taxa (*Pseudonitzschia* species, *Dinophysis sacculus* and *Prorocentrum minimum*) (Table 1). On the opposite, during the sampling in winter (negative values) presents minimal values of nitrates (Figure 5) and presence of three taxa (*Pseudonitzschia* species, *Dinophysis sacculus* and *Alexandrium minutum*) (Table 1). Axis F2 separates the sampling in spring (negative values) differentiated from the sampling in summer (positive values) with maximal contents in phosphates and silicates (Figure 5), the highest diversity (7 taxa) and the highest density including six major blooms (Table 1).

Discussion

Blooms and environmental conditions

For the first time in Nador lagoon, this

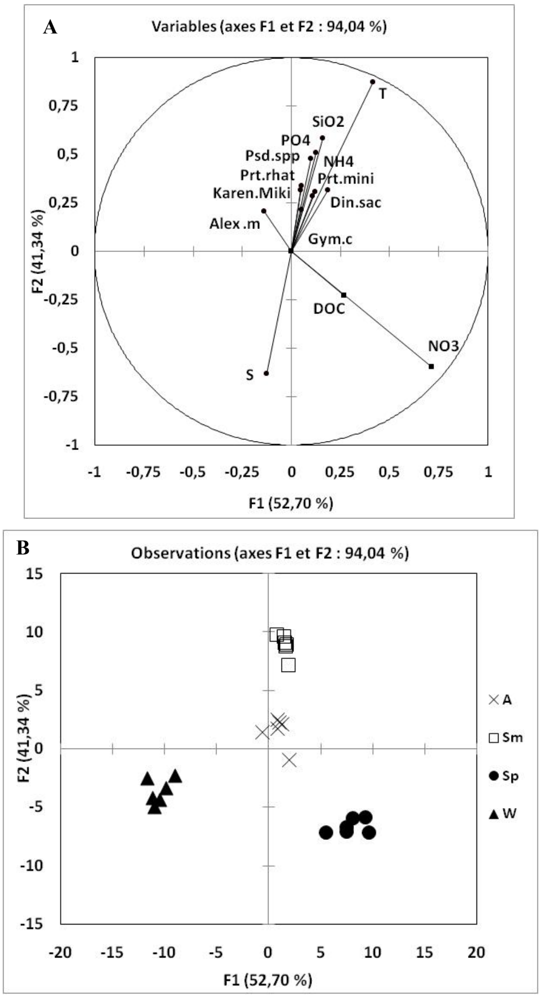

Figure 7. Results of Factorial Discriminant Analysis applied to the four different sampling dates plotted according to axes 1-2.

A- Correspondence chart with distribution of the variables: Salinity (S), Temperature (T), Dissolved Organic Carbon (DOC), nitrates (NO3), ammonium (NH4), phosphates (PO4), silicates (SiO2), *Alexandrium minutum* (Alex.m), *Pseudonitzschia* species (Psd.spp), *Prorocentrum minimum* (Prt.mini), *Prorocentrum rhatymum* (Prt.rhat), *Dinophysis sacculus* (Din.sac), *Gymnodinium catenatum* (Gym.c) and *Karenia mikimotoi* (Karen.Miki).

B- Discrimination of the four sampling dates with distribution of the observations: 3 November 2006 (A), 24 January 2007 (W), 12 April 2007 (Sp) and 3 September 2007 (Sm).

study of phytoplankton was carried out with four samplings throughout one year. We investigated the total phytoplankton populations, among which potential harmful species were identified and we analysed the environmental parameters at the same time. The total phytoplankton populations compared between Nador lagoon and other western Mediterranean lagoons revealed a similar phytoplanktonic composition from Mediterranean Sea with the two dominant Classes (Diatomophyceae and Dinophyceae) and the frequent presence of potentially harmful species (Armi *et al.*, 2010, Konsowa, 2007 and Paulmier, 1984).

The bloom dynamic of total phytoplankton appeared to be conditioned by several factors, favourable and unfavourable.

Among the favourable factors, the dissolved organic matter was available throughout the year (DOC, Figure 5). Mineralization processes of this dissolved organic matter provided a continuous source of nutrients directly in the water column for phytoplankton (Capblancq, 1995, Aminot and Kérouel, 2004) in addition to the sediment water fluxes resulting from decomposition processes in sediment, which is rich in organic matter (Bloundi *et al.*, 2008), after resuspension processes. During the sampling in summer, nutrients were available in abundance (Figure 5) but the combination of high diversity and high density of TP was localised at S1 and S2 (Figures 2 and 3). These areas sheltered from wind, promoted development of phytoplankton because of stratification and relative stability of water column, (Margalef *et al.*, 1979, Gailhard, 2003 and Razinkovas *et al.*, 2008). Stations with high pollution level such as fish farm (S2), the WWTP inputs (S3) and the untreated discharges from Kariet Arekmene town (S5), led to the development of saprobiontic Euglenophyceae Class which assimilate lots of organic matter (Barrera *et al.*, 2008) and the presence of Cyanophyceae Class (Cyanobacteria) which is characteristic of the first step in waste water treatment (Barrera *et al.*, 2008). Exceptional high values of ammonium, phosphates and silicates measured in Summer near the leaking of the malfunctioning WWTP of Nador (S3) (Figure 5), induced the proliferation of a mono-specific bloom of *Tetraselmis* sp. (Prasinophyceae Class) (Figure 3) which was reported in heavily eutrophicated waters and indicated a high level of pollution (Wehr and Sheath, 2003; Barrera *et al.*, 2008). The marine influence (S6) permitted the dominance of Diatomophyceae Class which was reported to be favoured by vertical mixing of water column (Margalef *et al.*, 1979; Gailhard, 2003).

Unfavourable factors may limit or inhibit development and proliferations of blooms. The minimum diversity and the weak density were measured at S4, characterized by high turbidity of water from Oued Selouane. The development of phytoplankton was limited because of reduction of light penetration in water column. In some cases, the competition for nutrients could limit the development of one Class with respect to another. For example, at S1 and S5 the Dictyochophyceae Class was unfavoured compared to Diatomophyceae Class, which responds more rapidly to nutrient inputs (silica) (Cloern and Dufford, 2005).

Within the three decades from 1977 to 2007, there was generally observed in NL an increase of nitrogen contents (ammonium and nitrates) and phosphate contents (Brethes, 1977, Berraho *et al.*, 1995; Inani, 1995, Ruiz *et al.*; 2006, this study). In the last decade, the monitoring of HABs by INRH showed an increase of development of potentially HABs in NL (El Madani, 2005). To study if the occurrence of blooms of potentially harmful algae could be related to environmental parameters, one FDA was applied to our data and it provided trends according to discrimination of the stations and the seasons.

According to stations (Figure 6), the statistical analysis revealed that along axis F1 waters with excessive abundance in nutrients (urban pollution) are characterized by virtually no potentially harmful phytoplankton as we observed during the summer sampling at S3, while waters with lower contents in nutrients (marine influence at S6) showed a development of potentially harmful phytoplankton that was limited to coastal marine species (*Alexandrium minutum* and *Pseudonitzschia* species) less nutrient demanding. The axis F2 illustrated the proliferation of potentially harmful phytoplankton linked to the availability of organic matter and nutrients (S2).

As highlighted before, since we did not replicate our samplings we cannot conclude how blooming of species is determined by the seasons. Nevertheless, the differences (Figure 7), along axis F1 can be related to intra-annual variations. Thus, the presence of *Prorocentrum minimum* during the sampling in spring can be explained by its preference for high nitrate concentrations. In contrast, it was not detected during the sampling in winter, when nutrient concentrations were low. Among the three most frequent taxa, *Alexandrium minutum* was able to proliferate in nutrient-poor waters as observed during the sampling in winter, but high levels in nitrates appeared as a limiting factor for its development since it was not detected during the sampling in spring. Summer appeared to be the most favourable season for proliferations of potentially harmful phytoplankton when various nutrients (N, P, Si) were probably available, whereas this proliferation was limited in during the sampling in spring when only one nutrient (nitrates) was abundant. The occurrence of main taxa, *Pseudonitzschia* species and *Dinophysis sacculus*, throughout the year displayed their adaptation ability to the variations of water quality.

Risks of toxicity

In this work, the risk of toxicity in NL was evaluated from the presence and densities of potentially harmful taxa (Table 1) because toxins were not analysed.

This spatio-temporal study showed that seven potentially harmful taxa (*Alexandrium minutum*, *Pseudonitzschia* species, *Prorocentrum minimum*, *Prorocentrum rhatymum*, *Dinophysis sacculus*, *Gymnodinium catenatum* and *Karenia mikimotoi*) were present in the NL throughout the year and formed numerous blooms distributed all over the lagoon (Table 1). Many times this potentially harmful phytoplankton exceeded 80% of the total phytoplankton (Figure 4).

Our four samplings seem to indicate that Summer was the season associated with the greatest risk of toxicity with 21 blooms often of maximum density being observed during the summer sampling. However, to confirm this trend, sampling needs to be replicated during the different seasons. Among the seven potentially harmful taxa, six taxa formed their major blooms (highest densities) in the continental part of the lagoon. The second highest risk of toxicity was observed during the sampling in autumn with 14 PHP blooms from five potentially harmful taxa. Only for *Gymnodinium catenatum* we observed the major bloom during this sampling (S5). During our sampling period, one human intoxication was reported following ingestion of mussels and *Dinophysis sacculus* was suspected as the causative agent because of diarrhoea-symptoms. During winter and spring, the risk of toxicity seems limited as we observed only low densities of three potentially harmful taxa, representing 10 and 11 blooms during the samplings in winter and spring, respectively. During the sampling in winter, the risk of toxicity was concentrated on the marine side of the lagoon.

The spatial distribution of potentially harmful taxa displayed that the greatest risk of toxicity was located in the north continental part of the lagoon. This risk of toxicity was maximum at station S2 with the presence of six taxa and the maximal density including three major blooms. The risk of toxicity remained high at station S1 with seven taxa and high densities of whom two major blooms. At the marine station S6, the risk of toxicity was limited with the minimal density and the presence of only two potentially harmful taxa.

Exceptionally, no risk of toxicity was detected at the most polluted station (urban pollution) during our sampling in summer.

Among the seven potentially harmful taxa, the greatest risk of toxicity was represented by *Pseudonitzschia* species with 20 blooms. Abundance of silicates combined with waters enriched in organic matter (DOC contents, Figure 5) have been described as favourable factors for the proliferation of these species (Loureiro *et al.*, 2009) in NL throughout the year. The common *Dinophysis sacculus*, associated with freshwater inputs, represented also a high risk of toxicity with 13 blooms during the year all over the lagoon, except at S6. The frequent risk of toxicity due to *Alexandrium minutum* (12 blooms) could be explained by the presence of cyst reserve in the sediment, which can develop when conditions become favourable (El Madani, 2005). Some cysts of this species were observed in our water samples (data not shown). With the other potentially harmful taxa, the risk of toxicity was more limited. *Prorocentrum minimum* (planktonic species) presented six blooms, while *Prorocentrum rhatymum* (benthic-epiphytic species) presented only two blooms corresponding to the period of macroalgae development.

Gymnodinium catenatum, a rare species (3 blooms) should be transported into NL by tidal currents after a windy period because it is known to produce blooms around the Mediterranean sea (Gailhard, 2003; Gómez, 2008; Frehi *et al.*, 2007; Iloul *et al.*, 2008 and according to INRH monitoring program). The exceptional presence of

Karenia mikimotoi (1 bloom) seemed to be favoured by intensive rainfall recorded before our sampling campaign and warm temperature conditions (Silke *et al.*, 2005) but it did not cause any mortality of fishes.

Conclusion

This yearly survey of phytoplankton blooms in Nador lagoon is a first step to which further studies will be compared.

The occurence of potentially harmful phytoplankton throughout the year and over the whole lagoon, puts the Nador lagoon in a permanent alarming state.

Exceptionally we observed during our sampling in summer that the most polluted situation (excessive N, P, Si) corresponded to a local disappearance of potentially harmful phytoplankton in favour of the proliferation of *Tetraselmis* sp. (Prasinophyceae). This observation could suggest the existence of an upper threshold of tolerance for potentially harmful phytoplankton concerning nutrients beyond the limit of which they cannot develop. On the contrary, *Tetraselmis* sp. was able to proliferate in these waters over-enriched in nutrients. For the first time, *Karenia mikimotoi* was identified in Nador lagoon during this study.

This spatio-temporal study highlighted the importance of blooms of total phytoplankton and specifically those of potentially harmful phytoplankton. Differences in the spatial distribution of the potentially harmful phytoplankton have also appeared between the continental stations versus the marine ones. To improve our description of blooms, samplings should be replicated within seasons ideally by reducing the time interval between successive samplings.

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