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Qualitative and quantitative study of the spring phytoplankton community in the Naïla lagoon (Moroccan Atlantic coast)

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Abstract
In order to contribute to the qualitative and quantitative assessment of phytoplankton community at Naïla lagoon (Khnifiss area: Southern Morocco), three sampling points were chosen along the lagoon. The sampling was carried out during the period April-May 2019 and was carried out in parallel with measurements of physico-chemical parameters (temperature, pH and dissolved oxygen). The phytoplankton recorded show an almost total dominance of diatoms (90%), while dinoflagellates represent only 10%. The most representative genera of diatoms are: *Chaetoceros*, *Navicula*, *Nitzschia*, *Pseudo-nitzschia*, *Pleurosigma*, *Coscinodiscus*, *Licmophora*, *Amphora* and *Diploneis*. The dinoflagellate group is represented by the genus *Alexandrium*. The majority of the phytoplankton identified in the Naïla lagoon is potentially of aquaculture interest. The genera *Chaetoceros*, *Amphora*, *Navicula and Nitzschia* are the most frequently used in aquaculture. However, *Pseudo-nitzschia* and *Alexandrium* inventoried during our work do not present any risk since their low cell densities, 1300 cells per liter and 200 cells per liter respectively, do not exceed alert thresholds. However, their presence requires rigorous monitoring, because these potentially toxic phytoplankton species, under other conditions or at highest concentrations, can have serious consequences on wildlife and consumer health.

1. Introduction

Most microalgae species are microscopic, unicellular aquatic organisms, which are usually known as "phytoplankton" [1,2]. The microorganisms constituting phytoplankton are endowed with performance and plasticity allowing them to colonize any type of environment such as: freshwater, oceans, sebkhas and lagoons. Microalgae are currently attracting considerable interest due to their metabolic power [2]. The interesting biological and biochemical properties of microalgae give them a large number of applications in the scientific and industrial worlds, including aquaculture [3-6]. Phytoplankton occupies a primordial trophic position and constitutes the first link in the food chains of aquatic ecosystems [7]. The rate of development of these populations determines that of primary consumers and the latter in turn regulate that of secondary consumers [8]. Approximately 90% of global aquaculture production uses phytoplankton as a food source during one or more stages of farming [9]. However, among the thousands

of marine phytoplankton species recorded, some of them can cause nuisance by producing toxic substances or phycotoxins [10]. These phycotoxins can transit along aquatic food chains and reach dangerous concentrations, even fatal, for humans at certain trophic levels [10-11]. The proliferation of these harmful and toxic microalgae species represents a real danger to consumer health and to the commercial and recreational exploitation of certain marine species [12].
To protect the health consumer against poisoning, the INRH (Institut National de Recherche Halieutique) initiated in 1995 a program for monitoring the toxicity of molluscs along the Moroccan coast [13]. The Naïla lagoon (Khnifiss area) located on the Atlantic coast of Morocco is a favorable environment for shellfish farming [14].
Given the lack of data on the composition of phytoplankton populations in the Naïla lagoon, this study is devoted to contribute in elaborating of an inventory of phytoplankton species and particularly microalgae of aquaculture interest and those that are potentially harmful. This work also aimed to launch a long-term follow-up program.

2. Material and methods

2.1. Study Site: Naïla Lagoon (Khnifiss area)

The lagoon of Naïla (28°02’54”°N, 12°13’66”°W) is located on the south Atlantic coast of Morocco in the rural commune of Akhfennir, Tarfaya circle and oriented along a NE-SW axis. This lagoon (Figures 1 and 2) is located 120 kilometers south of Tan-Tan and 70 kilometers north of Tarfaya. Its length is 20 km and its surface area is 65 km^2. The depths of the main channel are on average between 5 and 6 meters in the pass and then decrease in the upstream direction [14]. It is characterized by a climate which is located in the Saharan bioclimatic stage where the dominant winds (trade winds driving elements of the upwelling phenomenon) are from the North-North-West to North-East direction [15].

Figure 1: Naïla Lagoon.

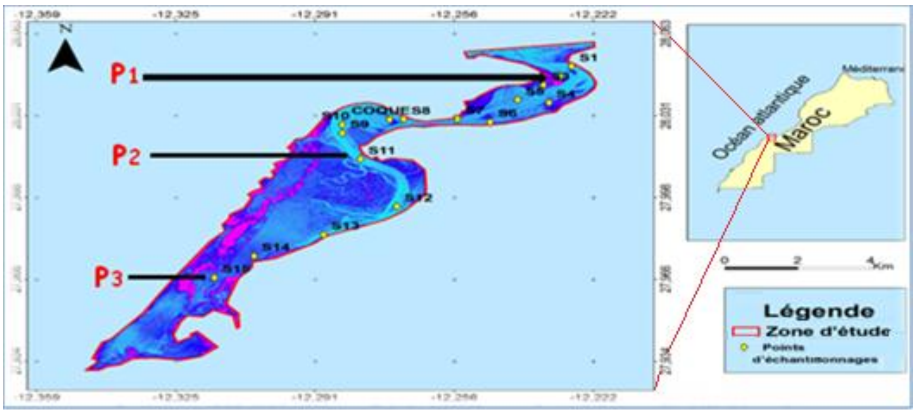

Figure 2: Map of geographic location of sampling points at Naïla Lagoon.

Fresh water inflows are almost absent and the only continental inflows into the lagoon come from the Oued Awdri, generally dry, which leads to the Sebkha Tazra saline [14]. It presents an ecological and scientific interest and it is recognized as a RAMSAR area (Convention on Wetlands of International Importance) since 1983 [16] and identified as a Sites of Biological and Ecological Interest (SIBE) area since 1996.

2.2. Sampling and methodology

The collection of a water sample is a delicate operation to which the greatest care must be taken; it conditions the analytical results and the interpretation that will be given [17]. Figure 2 shows the geographical location of the three sampling points (P1: downstream of the lagoon, P2: middle of the lagoon and P3: upstream of the lagoon) at Naïla lagoon. Two samples were taken each month during the period April-May 2019. For the qualitative study, a standard plankton net (24 cm in diameter and 50 cm long with a mesh of 20 µm) was used and it was trained horizontally. For the quantitative study, samples were taken directly from the sea surface using 1L bottles. Immediately after collection, the samples are stored, using a 2 % formalin and a 1% Lugol acid solution respectively for the qualitative and quantitative studies [11]. Sampling is always done in the morning at high tide because phytoplankton moves vertically in response to light. For each sample, the physico-chemical parameters (temperature, dissolved oxygen and pH) were measured using a multi-parameter kit (Handheld Multi-Parameter Instruments: WTW 350i).

2.3. Sample preparation

To resuspend the sample after a longer or shorter storage periods, it is necessary to shake the bottle containing the sample to be treated. Agitation is done manually for about 2 minutes and includes rotational movements. This agitation should not be too vigorous so as not to destroy the filaments and cenobes of the phytoplankton cells. After samples homogenization, 10 ml were taken to fill the sedimentation vats. Then, the vats were placed on a flat surface without vibration and covered with a round glass plate to prevent the deposit of dust and convection currents which prevent uniform distribution of the particles. This operation must be performed avoiding trapping air bubbles. Sedimentation takes place in the dark, at a constant ambient temperature and for 8 hours [18].

2.4. Determination and enumeration of phytoplankton species

The enumeration of phytoplankton is made according to the Utermöhl method [19] under a Leica DM IRB inverted microscope. This technique is the most widely used method in monitoring systems [20]. The phytoplankton composition is enumerated by performing a visual scan to observe and identify the phytoplankton found in the tanks.

This enumeration aims to describe the total microflora (the most represented species) or partial (number of toxic species). This procedure is repeated at various magnifications depending on the size of the species present, the specific determination scale and the ease of counting. Phytoplankton is determined using appropriate systematic identification keys [21, 22]. The total number of phytoplankton cells (N) contained in a liter is: N= (n x 1000)/V with (n= number of cells and V = volume of the sedimentation tank (ml)).

3. Results and discussion

3.1. Physico-chemical parameters

All physicochemical parameter values measured at the three sampling points during the period April-May are very similar for temperature, pH and dissolved oxygen (Figure 3). The water temperature values fluctuate between 19.5 °C (point 1) and 19.8 °C (points 2 and 3). The temperatures recorded at the three sampling points are within the normal range [23]. A similar study, carried out at the Oualidia lagoon (Moroccan Atlantic coast) on spring of 2011, has shown that the surface water temperature varied between 18.7°C in the downstream station and 20.6°C in the upstream station with an average value of 19.55°C [24]. At high tide, when our samples were taken, the waters were stirred by wave currents and the temperature differences are therefore insignificant between surface and bottom waters [23]. However, this difference is much more pronounced in areas at the very bottom of the lagoon, due to shallow depths.

The pH of the marine environment is generally basic; it is between 7.5 and 8.5 [25]. The pH values at the three points are almost the same with a slight difference at the third point (Figure 3). They varied between 8.3 at points 1 & 2 and 8.42 for the sampling point upstream of the lagoon.

Dissolved oxygen values are identical at sampling points 1 and 2, while at point 3 the value is slightly higher (Figure 3). The lagoon waters are more oxygenated and the values are moderately between 9.04 and 9.25 mg per liter. The low dissolved oxygen concentration gradient from downstream to upstream of the lagoon can be explained by the fact that during waves, the renewal of the waters improves oxygen levels inside the lagoon [14]. Similar results were recorded in spring of 2011 in the Oualidia lagoon surface water with an average oxygenation value of 9.29 mg/l [24]. The high oxygenation values recorded during this period can also be explained by the photosynthetic activity of phytoplankton.

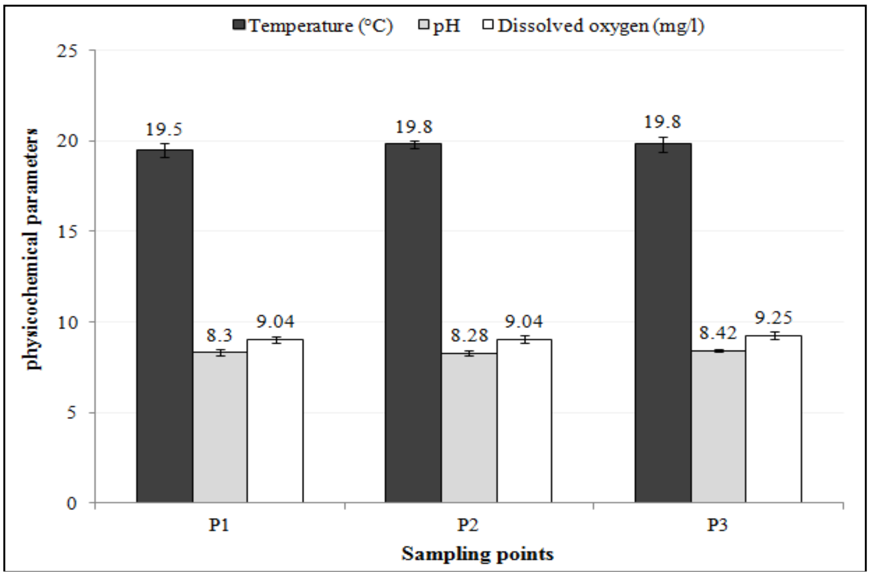

Figure 3: Naïla lagoon physicochemical parameters in the different sampling points

3.2. Qualitative study of Naïla lagoon phytoplankton

During the period April-May, the microscopic study of phytoplankton communities in Naïla lagoon resulted in the identification of several taxa (Table 1). The classification and some characteristics of the phytoplankton inventoried are summarized in Table 1. The identified species represent 9 families and 4 classes (Bacillariophyceae, Mediophyceae, Dinophyceae and Coscinodiscophyceae). The type of phytoplankton community is essentially composed of diatoms (90%) and dinoflagellates (10%); the same distribution was found in similar lagoons, notably Oualidia lagoon (Morocco) [13, 26]. The dominance of diatoms is mainly due to their preferential proliferation in nutrient-rich environments, i.e. nitrogen and phosphate products [18]. The Naïla lagoon is characterized by a water circulation mode according to an alternating (ebb and flow) and bidirectional rhythm [14]. This hydrological feature contributes to the site's richness in phosphate and nitrogen products, particularly during the spring period (period of our study), which favors the dominance of diatoms compared to other taxonomic groups.

Diatoms are represented by 9 genera (*Chaetoceros*, *Navicula*, *Nitzschia*, *Pseudo-nitzschia*, *Pleurosigma*, *Coscinodiscus*, *Licmophora*, *Amphora* and *Diploneis*) while only the genus *Alexandrium* represents dinoflagellates (Table 1).

Table 1: List of phytoplankton identified in the Naïla lagoon with their classification and main characteristics.

Species list	Classification [27]	Main characteristics [21, 22, 27]
Navicula sp.	**Class** : Bacillariophyceae **Order**: Naviculales **Family** : Naviculaceae **Genus** : *Navicula*	**Shape:** solitary, usually in the form of a "shuttle". **Size:** width 7– 21 µm, length 32–130 µm, diameter 6-42 µm
Chaetoceros sp.	**Class** : Mediophyceae **Order**: Chaetocerotales **Family** : Chaetocerotaceae **Genus** : *Chaetoceros*	**Shape:** chain-shaped; more or less rectangular for the belt. **Size:** diameter 2–85 µm, length 2 – 45 µm
Nitzschia sp.	**Class** : Bacillariophyceae **Order**: Bacillariales **Family** : Bacillariaceae **Genus**: *Nitzschia*	**Shape:** solitary and very weakly silicified with elongated extremities. **Size:** width 2 – 14 µm, length 6 – 375 µm.
Pseudo-nitzschia sp.	**Class** : Bacillariophyceae **Order**: Bacillariales **Family** : Bacillariaceae **Genus** : *Pseudo-nitzschia*	**Shape:** elongated, fusiform **Size:** width 2-8 µm, length 40-175 µm
Licmophora sp.	**Class** : Bacillariophyceae **Order** : Licmophorales **Family** : Licmophoraceae **Genus** : *Licmophora*	**Shape:** fan-shaped triangular, striking around a central rod **Size:** width 2.4 – 3.6 µm, length 25 – 43 µm
Pleurosigma sp.	**Class** : Bacillariophyceae **Order**: Naviculales **Family** : Pleurosigmataceae **Genus** : *Pleurosigma*	**Shape:** solitary and the contour of the valves is lanceolate **Size:** 90 – 600 µm
Alexandrium sp.	**Class** : Dinophyceae **Ordre**: Gonyaulacales **Family**: Pyrophacaceae **Genus** : *Alexandrium*	**Shape:** Spherical **Size:** width 17 – 44 µm, length 22 – 51 µm
Diploneis sp.	**Class** : Bacillariophyceae **Order** : Naviculales **Family**: Diploneidaceae **Genus** : *Diploneis*	**Shape:** Panduriform valve, with a clear constriction in the centre. **Size:** width 20 µm, length 40-45 µm, diameter 20-42 µm
Coscinodiscus sp.	**Class** : Coscinodiscophyceae **Order** : Coscinodiscales **Family**: Coscinodiscaceae **Genus** : *Coscinodiscus*	**Shape:** Wedged cylindrical disc and solitary. **Size:** Diameter 35 - 200 µm, height 30 - 180 µm
Amphora sp.	**Class** : Bacillariophyceae **Order** : Thalassiophysales **Family** : Catenulaceae **Genus** : *Amphora*	**Shape:** Recognized by their strongly dorsiventric frustules. **Size:** Length 63 µm by 32.4 µm wide.

Phytoplankton can play a very important role in aquaculture due to its good nutritional quality. Different microalgae in aquaculture have been studied and analyzed for their contribution to the growth of various invertebrate species and species frequently used in mariculture [28]. Microalgae have been used as live natural feeds for larvae and juveniles of many finfish and crustaceans, for all bivalve molluscs (clams, oysters, scallops) and as food for zooplankton in aquaculture food chains [29]. In terms of microalgal communities, diatoms are distinguished by their high nutritional content and can contribute with essential amino acids and highly unsaturated fatty acids [29]. Most of the diatom genera identified in the Naïla lagoon have the potential to be used in aquaculture.

Chaetoceros is a genus of solitary diatom algae mainly in chains. The cells have a rectangular shape characterized by the presence of one or more chloroplasts inside the cell. It is used in particular for feeding larval penaeid shrimp, feeding the larval, post larval and juvenile stages of bivalves, as well as for feeding *Artemia* [30, 31]. Becker [32] has shown that *Chaetoceros gracilis* is among the most frequently used species in commercial mariculture.

Navicula is a genus of algae that have a rectangular boat shape with a rounded end; it has two flattened chloroplasts, each elongated on each side of the cell, the presence of a raphe in both valves and a central nodule. *Navicula* is also used for abalone, shrimp and sea urchin farming. In addition, the highest levels of fatty acids were observed in treatments with diatoms, indicating the benefits of *Navicula* sp. on increasing the growth and fatty acid content of post-larvae of white shrimp (*Litopenaeus vannamei*) cultivated in bioflock systems [33].

Nitzschia is a genus of solitary diatom algae but they can also be in colonies. The cells have a spindle shape characterized by the presence of chloroplasts at each cell end. *Nitzschia* is a microalgae recommended for abalone farming, nutrition of larval penaeid shrimp [30, 34, 35] and also in sea urchin nutrition [35]. The species *Navicula lenzi*, *Navicula vemnuede*, *Nitzschia laveas* showed the best results for the survival and growth tests of abalone juveniles [36].

The genus *Amphora* has been described as an excellent food for post-larvae abalone, while a mixture of feeds containing *Amphora* and *Nitzschia laevis* or *Navicula lenzi* ensures very high survival and growth rates [37, 38]. The results showed that *Amphora*, *Navicula* could be used as a food supplement to improve the growth and survival of post-larvae of *Penaeus monodon* [39].

Hua [40] analyzed the gut content of the microbiota of Opihi (marine gastropod mollusc) and found the presence of *Chaetoceros* sp., *Navicula* sp., *Amphora* sp., *Licmophora* sp., *Nitzschia* sp., *Pleurosigma* sp., *Diploneis* sp. and other species of microalgae and bacteria.

However, toxic phytoplankton algae are present in all marine environments around the world. The INRH Laâyoune is particularly interested in the search for species belonging to the genera *Pseudo-nitzschia*, *Dinophysis* and *Alexandrium*. These three species are the most dangerous and can be the cause of fatal intoxications. The species *Dinophysis* sp. is known worldwide as a producer of diarrhoeal toxins (DSP: Diarrheic Shellfish Poison) [41]. It was not observed during this study.

3.3. Quantitative study of Naïla lagoon phytoplankton

Among the diatoms, *Chaetoceros* (88.5%) and *Amphora* (6.7%), were recorded as the dominant genera in the phytoplankton assemblage of the Naïla lagoon and in third order come the genus *Diploneis* (1.5%) followed by *Navicula* (1.2%) (Figure 4). However, this abundance of observed phytoplankton also varies according to the sampling point (Figure 5). In the downstream of the lagoon (P1), a very high abundance of *Chaetoceros* was observed with a number of cells exceeding 10^5 per liter, followed by *Amphora* (7500 cells/l) then *Navicula* (1100 cells/l), while *Pleurosigma* was absent at this point. The phytoplankton found at sampling point 2 (center of the lagoon) are dominated by *Chaetoceros* (151500 cells/l) followed by *Amphora* (8000 cells/l) in second order then *Diploneis* (1900 cells/l), however the presence of *Pleurosigma* (100 cells/l) is noted. In the upstream of the lagoon (P3), *Chaetoceros* (152500 cells/l) is still very abundant, followed by *Amphora* (7300 cells/l), but the absence of *Pseudo-nitzschia*, *Pleurosigma* and *Alexandrium* was noted. The cell density of *Alexandrium* was not exceeding 200 cells per liter at this lagoon; it respectively 180 and 190 cells / l for sampling points 1 and 2.

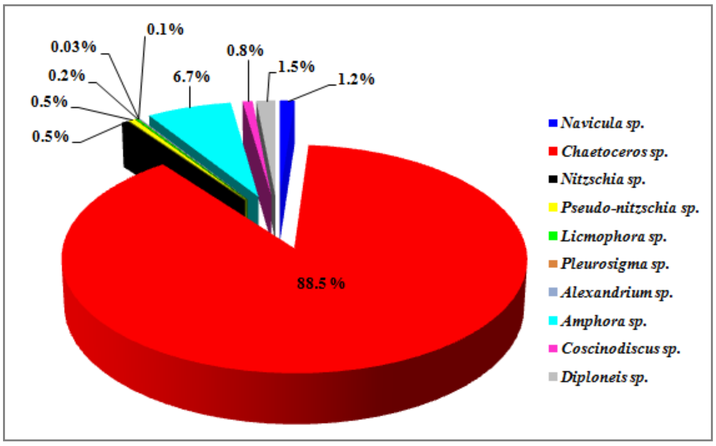

Figure 4: Abundance (%) of phytoplankton found in Naïla Lagoon

The difference in distribution of the different phytoplankton taxa inventoried is due to the fact that they have strict requirements and tolerances with regard to the luminosity and the physico-chemical parameters of the environment and nutritional needs [42-46]. Temperature, salinity, depth, pH, dissolved oxygen and suspended sediment concentration play an important role in determining diversity, promoting or limiting the growth of different phytoplankton groups [42]. Nutrient availability is the main factor controlling phytoplankton under the adequate light and temperature conditions [43]. Indeed, it can affect its growth rate [44], its biomass and its specific composition [45]. In addition, disturbance of the nitrogen/phosphorus ratio results in the selection of some microalgae over others [46]. Depth can also play a determining role in the distribution of taxa [42]. Depths in the main channel of Naïla Lagoon average between 5 m and 6 m in the pass and then decrease upstream direction [14].

Our study of phytoplankton shows the presence of two toxic species, *Pseudo-nitzschia* sp. and *Alexandrium* sp., which are found downstream and in the center of the lagoon studied (Figure 5).

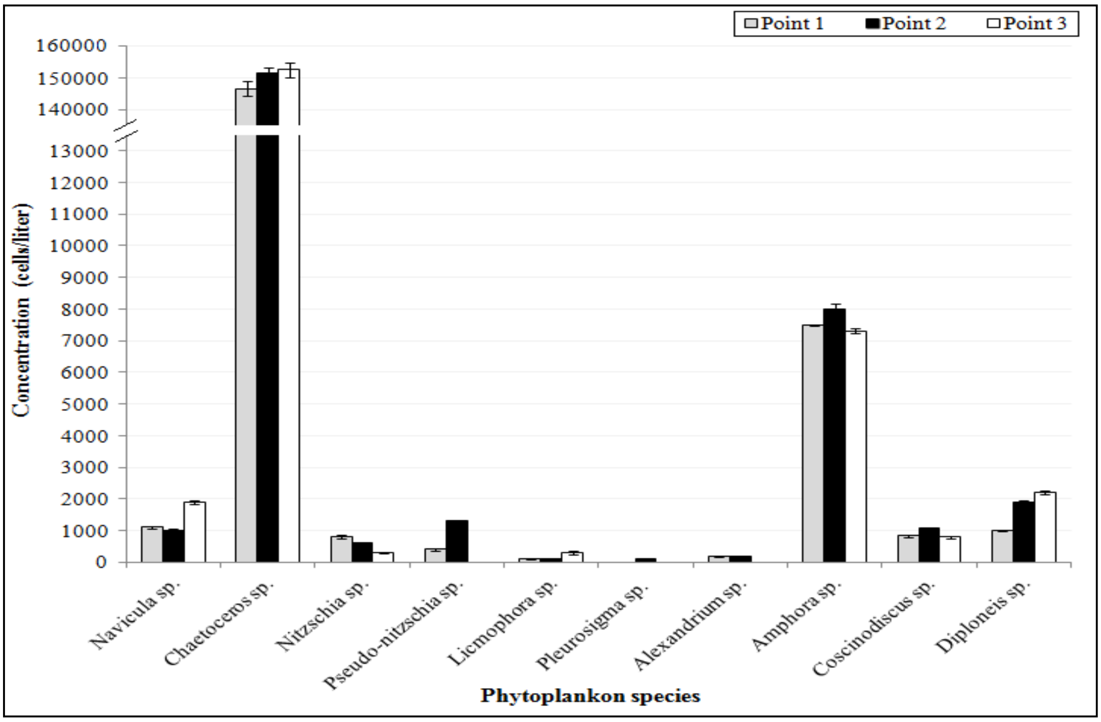

Figure 5: Mean concentrations of the different phytoplankton inventoried in the three sampling points carried out in the Naïla lagoon.

Pseudo-nitzschia is a well-marked species in the study site, its density varied from 400 cells per liter in downstream to 1300 cells per liter in the center of the lagoon. Some species of *Pseudo-nitzschia* produce

Amnesic Shellfish Poisoning (ASP) toxins [47], which can cause neurological disorders, including memory loss, in consumers of contaminated shellfish. ASP toxins are sought when *Pseudo-nitzschia* is detected at concentrations greater than 10^5 cells per liter [48]. This genus has not caused toxicity; it is still below the alert threshold.

The species *Alexandrium* sp. was encountered during the month of April and was present until the end of May. It is a nuisance species found in our samples, but with concentrations not exceeding 200 cells per liter. The genus *Alexandrium* is associated with the production of PSP (Paralytic Shellfish Poisoning) toxins [49-52]. The proliferation of these microalgae, especially in aquaculture sites, can affect shellfish production and can also be fatal to human health. However, the search for PSP toxin is performed when an increase in the concentrations of the *Alexandrium* alga is observed (>10^3 cells per liter) [52]. During the period of our study, none exceeded of the alert threshold is recorded.

Conclusion

This present work allowed us to study the phytoplankton community of the Naïla lagoon and to verify the presence of toxic microalgae harmful for the fauna which feeds on it and for the consumer. In this lagoon, and during the spring period of 2019, the microalgae species inventoried were composed of two groups dominated by diatoms. Most of the diatoms found (*Chaetoceros*, *Amphora*, *Navicula*, *Diploneis*, *Nitzschia*, *Pleurosigma* and *Licmophora*) are potentially usable in mariculture. Two toxic species (*Alexandrium* sp. and *Pseudo-nitzschia* sp.) were repertoried but with concentrations not exceeding alert thresholds.

The presence of these two harmful or toxic phytoplankton, even at low densities, requires the establishment of an effective and rigorous monitoring system in Naïla lagoon. The proliferation of these toxic microalgae requires the measurements of toxin levels in wildlife of economic interest, including oysters, mussels and clams harvested from the lagoon.

This study is only an initiation of research towards more in-depth work in order to investigate phytoplankton biodiversity in this particular lagoon. It is very important to determine the annual availability of useful phytoplankton and also harmful phytoplankton charge in this lagoon as well as the determination of the environmental factors responsible for the proliferation of each species.

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