Systematic and Dynamic Studies of Phytoplankton in the Bouregreg Site, Morocco

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Abstract

The site at the mouth of the Bouregreg wadi (Rabat) represents a preferred environmental model for the proliferation of phytoplankton, the presence of necessary nutrients such as nitrogen and phosphate. The goal of this work is to put a systematic classification of phytoplankton species and study their temporal dynamics during the years 2019/2020. Qualitative and quantitative analyzes of phytoplankton populations reveal the existence of 6 taxa (Alexandrium; Dinophisis; Gymnodinum; PseudoNitschia; Lingulodium and Prorocentrium) known to produce toxins. Principal component analysis (PCA) shows that Gymnodinum and Lingulodium evolve in the same direction of the temperature variation with correlation coefficients respectively of ($r = 0.489$; $p < 0.000$) and ($r = 0.399$; $p < 0.006$), and conversely to the variation of nitrates ($r = -0.516$; $p < 0.000$) and phosphates ($r = -0.391$; $p < 0.007$). However, Alexandrium and PseudoNitschia evolve in the opposite direction of the variation in salinity with correlation coefficients respectively of ($r = -0.372$; $p < 0.011$) and ($r = -0.391$; $p < 0.007$). The appropriate period for uttering corresponds to the seasons of summer and April. It is essential to set up an effective monitoring system because the environmental risks linked to the proliferation of microalgae are major.

Keywords: Phytoplankton; Physicochemical parameters; Toxic; Temporal; Morocco.

1. Introduction

Dinoflagellates are important components of plankton in terms of interaction with the trophic connection, and various taxa can be categorized as both phytoplankton and / or microzooplankton. The evolution of algae is governed by chemical, biological, physical [1, 2] and eutrophication [3] factors. Numerous studies around the world show that several marine algae produce powerful toxins due to high concentrations [4, 5]. Recognized human poisoning syndromes resulting from algal toxins (paralytic, neurotoxic, amnesic, diarrhetic shellfish poisonings, ciguatera fish poisoning [6], and putative estuary associated syndrome) impact human health through consumption of contaminated seafood, direct contact with bloom water, or inhalation of aerosolized toxin [7]. Thorough health risk assessment for the variety of algal toxins is hampered to varying degrees because either the toxin has not been identified or indicators for exposure and effects remain poorly defined. Predicting the occurrence and determining the impacts of harmful algal blooms in coastal ecosystems are the two major ecological risk assessment needs [8]. The significant negative impacts of harmful taxa on public health, the economy and natural resources have led to intensive monitoring programs to detect the presence of these species. Most phycotoxins are produced by dinoflagellates although cyanobacteria also produce saxitoxin (STX) and domoic acid (DA) which is produced by diatoms [4]. In Morocco, studies carried out along the Atlantic coast have revealed phenomena of colored waters [9] and biotoxins synthesized by certain phytoplankton species [10].

The estuary environment of Bouregreg Rabat is an extremely variable environment in terms of hydrological and physicochemical parameters [11]. These factors directly influence the biocenosis.

Phytoplankton are particularly sensitive to these variations [12], and therefore can be used to predict the effect of change in the aquatic ecosystem.

The purpose of this work is to assess the biodiversity of phytoplankton and the quality of the water, in the mouth connecting the Bouregreg wadi, to the Atlantic Ocean during the period 2019/2020.

2. Methodology

2.1 Study zones

The sampling site is located in the mouth of Bouregreg. The latter is a Moroccan river, 240 kilometers long, its average flow rate is 23 m^3/s but, in times of flooding, it can reach 1500 m^3/s. It originates in the Middle Atlas massif at an altitude of 1627 m at the level of Jebel Mtourzgane (province of Khemisset) and Grou (province of Khenifra) and flows into the Atlantic Ocean between the towns of Salé to the north and Rabat to the south.

Coordinates: 34 ° 02 ′ 09 ″ N, 6 ° 50 ′ 07 ″ W

2.2 Qualitative and quantitative study

After harvesting the plankton, the sample went to taxonomic study and identification using an optical microscope (type Olympus BX 50 F4). The quantitative study involves the enumeration of species using an inverted microscope. The calculation of the density (N) of the different algal groups encountered was determined using the formula:

$$N = n * S / s * v$$

n: the number of cells counted, S: the surface of the tank to be sedimented, s: the observed surface, v: the sedimented volume.

The density is expressed, for each taxon, in number of algae and cells per ml.

2.3 Analysis of physicochemical parameters

The physicochemical parameters chosen in this study are: Salinity, temperature, dissolved oxygen, nitrate and phosphate

2.4 Statistical analyses

Qualitative characteristics are expressed in frequencies and quantitative characteristics are expressed as mean ± standard deviation. Joint analyses have been applied such as Correlation Analysis (CA), Multiple Correspondence Analysis (MCA) and One-Way Analysis of Variance (ANOVA I) as well as Principal Component Analysis (PCA).

3. Results and discussion

3.1 Systematic study

The inventory of taxa in the sampling site identified 6 taxa each belonging to a family. The species are the following Alexandrium sp.; Dinophisis sp. And PseudoNitschia sp, are known for their ability to produce toxins that can harm the surrounding fauna (Table 1).

3.2 Study of the plankton density in the Bouregreg site

12966 Cells per liter were collected during the two years of collection (2019/2020), with an average of 1071.68 Cells per ml. However, two groups of plankton were therefore reformulated, the first is composed of the less abundant species such as Alexandrium (91.87 cells / ml), Dinophisis (83.91 cells / ml) and Gymnodinum (264, 57 cells / ml) and the second is composed of PseudoNitschia species

(2285.65 cells / ml); Lingulodium (1251.30 cells / ml) and Prorocentrium (2452.83 cells / ml), which displayed fairly high densities.

Table 1. Toxic and / or harmful characteristics of the collected specie

Action	Species	Family	Toxin
Toxins accumulate in organisms (shellfish certain fish ...) and can contaminate humans via the food chain	Alexandrium sp.	Pyrophacaceae	Saxitoxin and its derivatives
	Dinophysis sp.	Dinophysiaceae	Okadaic acid, ynophysistoxins, pectenotoxins and yessotoxins
	PseudoNitschia sp.	Bacillariophyceae	Domoic acid
Ichthyotoxins are released into the water and are therefore directly toxic to marine, plant or animal	Lingulodium sp.	Gonyaulacaceae	Production of yessotoxins (YTX)
	Prorocentrium sp.	Prorocentraceae	Indirect action: massive efflorescence and anoxia (oxygen deprivation)
	Gymnodinum sp	Gymnodiniaceae	-

Table 2. Distribution of average densities of taxa identified in the Bouregreg site

Taxon	Number of outputs	Minimum Cells / liter	Maximum Cells/liter	Sum Cells/liter	Cells / liter Average	Cells / liter Average
Alexandrium	23	0	220	2113	91.87	13.451
Dinophisis	23	1	300	1930	83.91	17.377
Gymnodinum	23	0	1520	6085	264.57	81.667
PseudoNitschia	23	0	8000	52570	2285.65	470.794
Lingulodium	23	0	7650	28780	1251.30	364.545
Prorocentrium	23	0	11000	56415	2452.83	575.648

3.3 Monthly temporal distribution of taxa

Figures 2a and 2b present the results of the monthly evolution of the less frequent taxa (Alexandrium; Dinophisis and Gymnodinum) and the most abundant taxa (PseudoNitschia; Lingulodium and Prorocentrium) in the Bouregreg site (Rabat). Indeed,

• Figure (2a): the distribution of the monthly mean density of the three taxa of the first group shows that the Alexandrium and Dinophisis species show a stable dynamic evolution during the three seasons (spring, summer and autumn) with maximum densities which do not exceed 300 cells / ml. With regard to Gymnodinum, two peaks were considered, one during the month of August with an average density of 850 cells / liter and a second peak in the month of October with a density of 860 cells / liter which corresponds to summer and early autumn seasons. Fisher's test shows a significant effect of the "month" variation on the mean density distribution for the Alexandrium taxon (Fisher = 3.33; $p < 0.029$)

• Figure (2b): the temporal evolution of the three most abundant taxa in the site shows that Prorocentrium follows an increasing evolution from the month of May to reach a maximum average density of 7500 cells / ml in the month of November. Regarding Lingulodium taxa, it shows a single peak during the month of July with an average density of 5025 cells / ml. However, the dynamic evolution of the taxon Prorocentrium is characterized by two peaks, one in April and the other in August, September and October. Fisher's test shows a significant effect of the variation "months" on the distribution of the mean density for the Lingulodium and Prorocentrium taxa with respectively (Fisher = 2.47; $p < 0.05$) and (Fisher = 3.34; $p < 0.029$).

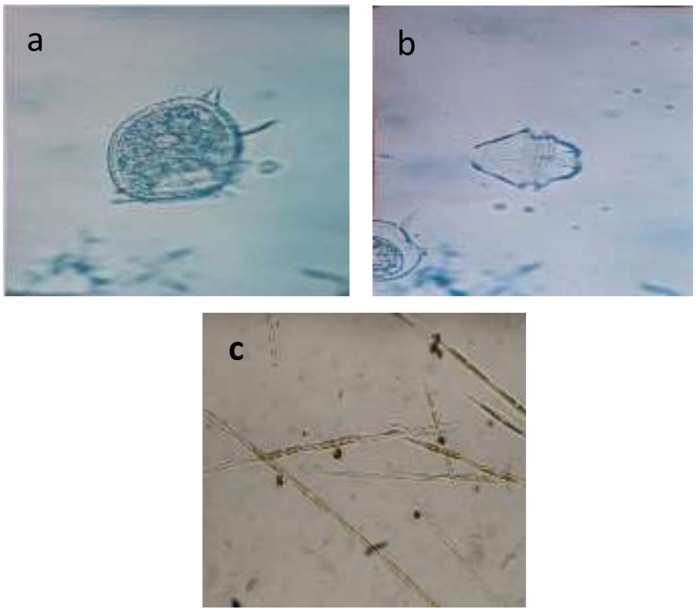

Figure 1. Microscopic observation of some taxa identified in the Bouregreg site
(a) Dinophysis sp. (b) Alexandrium sp. (c) PseudoNitschia sp.

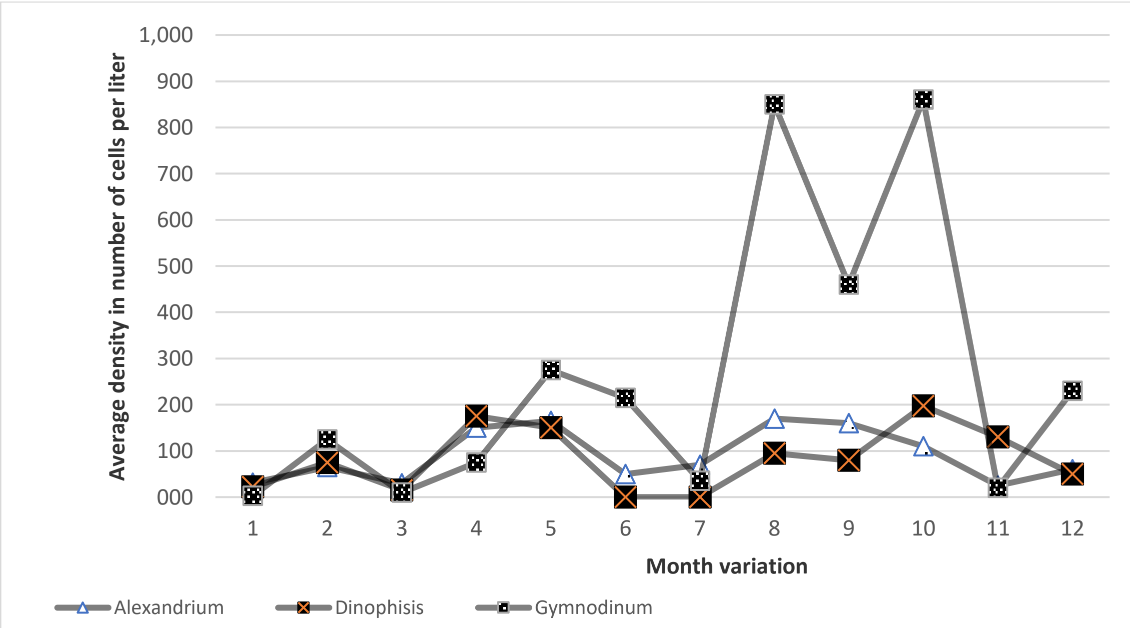

Figure 2 a. Monthly evolution of the least abundant taxa

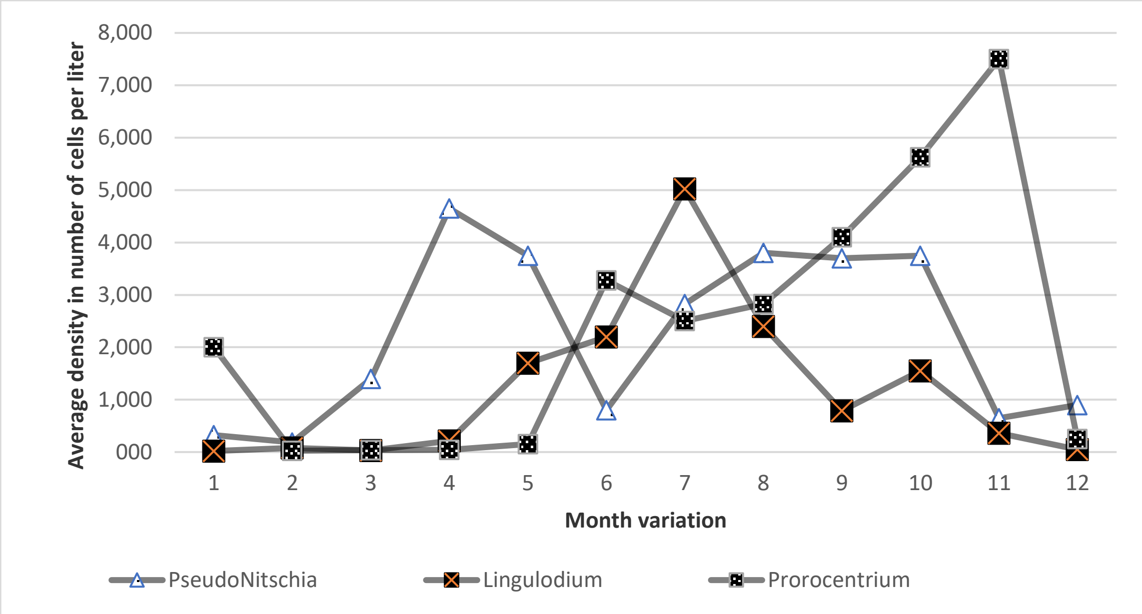

Figure 2 b. Monthly evolution of the most abundant taxa

3.4 Physicochemical parameters in the Bouregreg site

Table (3) shows the results of the physicochemical parameters in the Bouregreg site. In fact, the average temperature, salinity and nitrate showed annual average values of 16 °C, 33.5 ‰ and 38 µmol / l. The multiple correlation between the parameters shows that the temperature is negatively correlated with nitrate and phosphate with correlation coefficients respectively of r = -0.730 and r = -0.638. Similarly, a positive correlation associates' nitrate and phosphate with a correlation coefficient of r = 0.612.

3.5 Global analysis

For a global analysis, a PCA was used.Indeed, the two components alone absorb 58.88% of the total inertia. In addition, the projection of the 6 taxa in the space delimited by components 1 and 2 made it possible to distinguish two groups (figure 3):

Table 3: Physicochemical parameters at the Bouregreg site

Setting	Average	Ecart type	Min.	Max.
Temperature ° C	16.0	24.0	19.609	2.3595
Salinity ‰	33.5	36.0	34.691	0.8898
Oxygen content mg / l	8.2	10.5	9.339	0.6192
Nitrate µmol / l	38.0	70.0	50.826	9.5568
Phosphate µmol / l	0.70	2.90	1.8204	0.64967

• The first group is located on the positive side of component 1, it gathers the taxa Gymnodinum and Lingulodium which evolve in the same direction of the temperature variation with correlation coefficients respectively of (r = 0.489; p <0.000) and (r = 0.399; p <0.006), and conversely to the variation of nitrates (r = - 0.516; p <0.000) and phosphates (r = -0.391; p <0.007). The period suitable for proliferation corresponds to the summer and early spring seasons.

• A second group located on the positive side of component 2 gathers the taxa. Alexandrium and PseudoNitschisa. These two taxa evolve in the opposite direction to the variation in salinity with correlation coefficients of (r = -0.72; p <0.011) and (r = -0.91; p <0.007) respectively. the period suitable for uttering corresponds to the seasons of summer and the month of April.

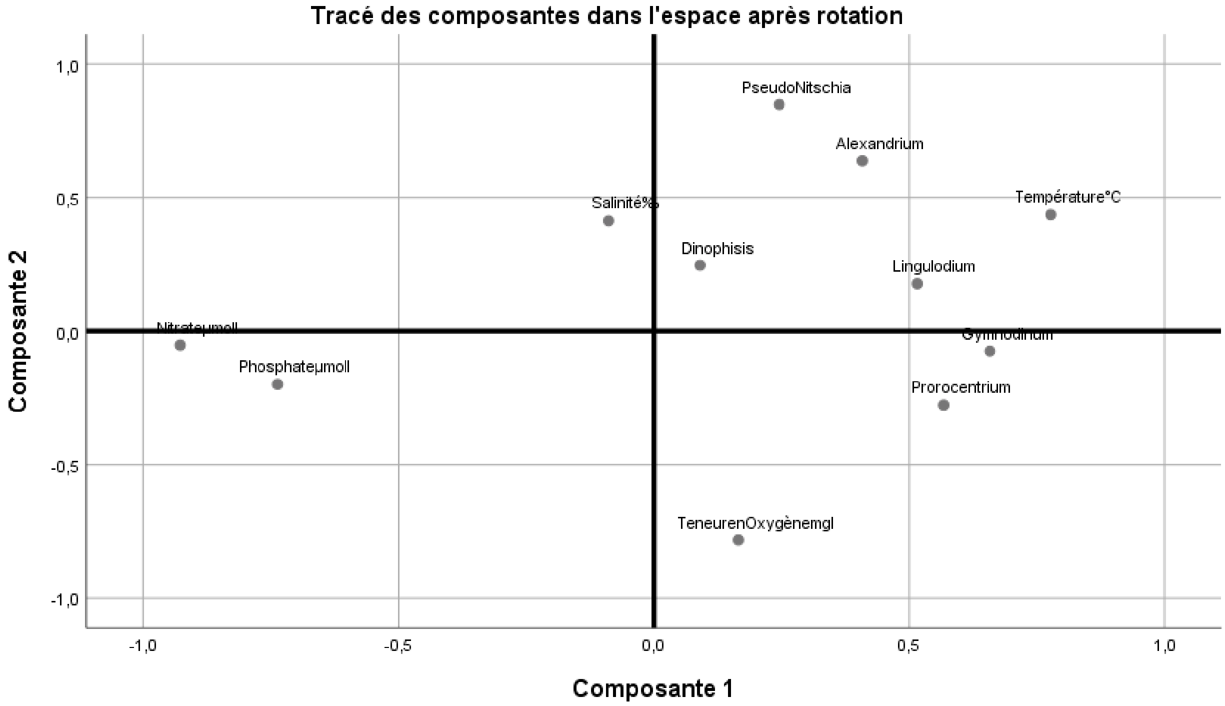

Figure 3. PCA presentation of taxa and physicochemical parameters in the Bouregreg site

The study of phytoplankton biodiversity in Morocco remains limited, it is in this perspective that we have proposed to contribute to the inventory of planktonic taxa in the site of the mouth of the Wadi Bouregreg. This site of geomorphic estuaries is experiencing productivity and abundance of phytoplankton as a result of seasonal fluctuations in water temperature. The identification of taxa in this site has identified 6 species such as Alexandrium; Dinophisis; Gymnodinum; PseudoNitschia; Lingulodium and Prorocentrium. However, most of these species have a power of intoxication which varies from low to high and this has been confirmed by the work of **Dalefield (2017)** [13] in Australia and New Zealand, **Van Dolah (2000)** [14]; **Wright and Cembella (1998)** [15], who show that the genera Alexandrium (Gonyaulax), and Gymnodinium can synthesize saxitoxins which cause paralytic mollusc poisoning. Studies carried out in Mauritania by **Wagne (2011)** on the bay of Greyhound show the toxic power of Dinophysis [16].

The study of the temporal and spatial dynamics shows that the maximum cell densities are observed in warm periods and during the inter-seasons. The development of photosynthetic biomass is limited by the increased nutrient load, which is observed for most of the species collected [17, 18, 3]. The nutrient load especially nitrogen and phosphate are limiting factors for the growth of these taxa. Indeed, winters or early spring reduced the availability of nutrients for subsequent summer regeneration, and high flow conditions in summer eliminated flowers [19, 20]. The alternating seasonal presence of taxa can be explained by competition, considered to be an important factor in determining the composition of the phytoplankton community [21, 1, 22]. The situation in this area is becoming a major ecological and economic concern for the coast, given the abundance of the most toxic plankton taxa.

On the other hand, the high salt content does not influence the presence of these taxa in sufficient quantity.

Conclusion

It is essential to set up an effective surveillance system because the environmental risks linked to the proliferation of microalgae represent a growing threat, both for public health and for the Moroccan economy. Now, in Morocco, the dynamic monitoring of phytoplankton and the appearance of harmful, even toxic events, is at the very heart of important health and environmental issues

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

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