Morphology and distribution of some marine diatoms of the genus *Rhizosolenia* in the lagoon of Nador (North East of Morroco).

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Abstract. This work aims to study the distribution and quantification of the genus Rhizosolenia known for its abundance and diversity among planktonic diatoms at 9 sampling stations in the Nador lagoon and during 2 seasons (spring and summer 2018). The diatoms collected in the 9 sampling stations were identified morphologically using an inverted optical microscope. A total of 10 species of the genus Rhizosolenia have been listed including: *Rhizosolenia bushsolei*, *R alata forma alata*, *R bergonii*, *R cochlea*, *R hyalina*, *R imbricata*, *R setigera*, *R bushsolei*, *Rhizosolenia sp* and *R styliformis*. The quantitative analysis of the species collected shows that the maximum cell density was recorded respectively at stations 9 and 7 located in the center of the Nador lagoon, with values of 21680 Cell/l and 15710 Cell/l. However, the minimum cell density was recorded at station 5 corresponding to Oued Bou Areg located at the edge of the lagoon with a value of 5120 Cell/l. *keywords*: Morphology, distribution, marine diatoms, genus Rhizosolenia, lagoon of Nador, North East of Morocco.

1 Introduction

Diatoms belonging to the class Diatomophyceae (Bacillariophyceae) are unicellular algae that constitute the most diverse group of eukaryotic phytoplankton, estimated at 12 000 species [1], Diatoms are photosynthetic unicellular microorganisms, they are the most widespread phytoplanktonic group in the marine environment. They can occur as single cells or clustered in colonies [2,3,4]. According to morphological criteria, diatoms are currently classified into penal diatoms and central diatoms. The central diatoms are diatoms having a radial symmetry, the valves are generally circular to elliptical, and present an ornamentation arranged around a central or eccentric point. Contrary to the penal diatoms which have a raphe that favors their movements, the central diatoms are devoid of movement [5]. Diatoms reproduce by vegetative propagation by cell division, which is the most common mode of multiplication [6]. They possess chlorophylls a and c, ßcarotene and xanthophylls [7]. Among the central diatoms are the genus of Rhizosoleinia. The marine diatom genus Rhizosolenia Brightwell (1858) is one of the most important diatom genera. Phytoplankton biomass can dominate in highly productive marine areas [8]. Traditionally, Rhizosolenia species usually show a girdle-segment structure on the pillars and specific shapes of the lateral processes, ear holes, brackets, and adjacent areas [9,10]. Ehrenberg (1843) established

Rhizosolenia using *R*. americana as the type species. Later he worked with Hustedt (1930), Hasle (1975), Sundstrom (1986), Round and others. (1990) and Hernández-Becerril and Meave del Castillo (1996, 1997) reviewed the genus. The genus *Rhizosolenia* includes many genera with specific morphologies, such as *Neocalyptera hernandez* beceril and mieb, *Pseudosolenia sandstrom*, *Probosia sandstrom*, Urosolenia round and Crawford. All of these genera are found exclusively in marine environments, with the exception of Urosolenia, which is confined to freshwater [11].

Shim (1994) reported the discovery of several species of the *Rhizosoleniaceae* family *Rhizosolenia* and Guinardia H. Peragallo and Dactyliosolen Castracane in coastal waters of Korea [12]. Added two genera to his checklist of Korean coastal waters: Proboscia and Pseudosolenia. Since then, further studies on *Rhizosolenia* and other related taxa have been conducted in South Korea. However, species identification, synonyms, and phylogenetic systems for the genus *Rhizosolenia* and other related taxa are still deficient.

This study has for main purpose morphological study and distribution of different species of Rhizosolenia at the level of the lagoon of Nador.

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2 Materials and methods

2.1. Study area and location of sampling stations

Lagoon ecosystems play a very important role in regulating freshwater inflows to the marine environment [13] and are considered biogeochemical "reactors" of nutrients and pollutants brought in from continental waters [14]. The Lagoon of Nador, also known as Sebkha Bou Areg or Mar Chica, is located in northeastern Morocco on the Mediterranean coast between 2°45 - 2°55'W and 35°10'N latitude. The Nador Lagoon ecosystem evolved in a northwest-southeast trending continental cyclone during Holocene Sea level rise [15]. In North Africa he is the second largest lagoon

complex (115 km², 25 km long and 7.5 km wide) and the largest in Morocco. The lagoon of Nador is located downstream of a gutter oriented SW-NW, consisting of two successive plains: the Gareb and Bou Areg plains. By its semi-elliptical shape, elongated parallel to the coast, the lagoon of Nador shows a morphological integration in the general continental geometry of the region [16]. The lagoon is connected to the Mediterranean Sea through a new artificial channel 300 m wide and 6.5m deep. Hydrodynamic circulation within the lagoon is predominantly aeolian [17,18]. With estimated water renewal time of about 80 days before construction of a new inlet [18]. The sampling was carried out in the lagoon of Nador and during two seasons (spring and summer 2018) at nine stations distributed to ensure a wide spatial coverage of the lagoon (Figure 1).



Fig. 1. Area of study and sampling stations

Table 1. GPS coordinates of sampling stations.

	Names	Locations
S1	Beni Ensar	W002°55,460; N35°15,148
S2	Tirakae	W002°55,522; N35°11,772
S3	Oued caballo	W002°54,325; N35°09,820
S4	Sewage treatment plant	W002°53,383; N35°08,299
S5	Oued Bou Areg	W002°51,443; N35°07,549
S6	Kariat Arakmane	W002°45,167; N35°06,401
S7	Center lagoon side Arekmane	W002°48,884; N35°08,236
S8	Between the old pass and Mohandis	W002°49,802; N35°10,465
S9	Lagoon center	W002°52,517; N35°11,012

2.2. Physico-chemical analyses

The physico-chemical parameters of water are measured with the use of specific sandes such as the parameters of (temperature, salinity) were measured in situ using a specific type of probe (ORION STAR A122) and concerning the parameter of (hydrogen potential) was measured by a pH-meter (IONOMETER-EUTECH-INSTRUMENTS-CYBERSCAN-PH-510)

2.3. Morphological identification

Phytoplankton sampling was carried out once a month at each station using a plankton net. In order to carry out a qualitative study, once the samples were collected, a part was left in a living state, and another part of the samples was fixed immediately with lugol or neutral formaldehyde (4%). The identification was done using an optical microscope, in the laboratory of INRH

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(Institut National de Recherche Halieutique de Nador). The quantitative study was carried out according to the Utermöhl sedimentation chamber method (Utermöhl, 1958); the filtered samples were sedimented for 24 hours in the sedimentation chambers and then quantified directly by the Leica "DM-IRB" inverted optical microscope.

2.4. Statistical analysis.

Results using XLStat-Pro® software yielded graphs similar to PCA. The correlation circle represents the factorial plane containing the initial observations, while representing the projection of the classes in the system discriminating the factorial axis. This allows you to visualize the quality of discrimination. This analysis was used to detect similarities or differences between *Rhizosolenia* data and environmental parameters.

3 Results and Discussion

3.1 Physico-chemical analyses

Temperature is a primary ecological factor that has a large influence on the physicochemical properties of aquatic ecosystems [19,20,21,22].

This parameter has a direct impact on other physical parameters such as salinity and dissolved oxygen, but it also has an effect on biological parameters such as species distribution and biological activity [23,24]. The temperature of the Mediterranean seawater is mainly determined by two main factors: the seasons and the depth. The temperature of the waters of the lagoon of Nador, recorded at the 9 stations sampled, during the spring period, oscillated between a minimum of 18 °C observed at station 9 which is in the center of the lagoon, with a depth of 7.27m and a maximum of 20.81 °C at station 6, which corresponds to Kariat Arekmane with a depth of 1.48m. The average recorded was about 19.4 °C. During the summer period, the minimum and maximum temperatures recorded at the 9 stations sampled in the lagoon of Nador were respectively 24.9 °C at station 5, which corresponds to Oued Bou Areg, with a depth of 0.70m and 26.9 °C at station 4, which corresponds to the treatment plant, with a depth of 0.60m. The average was about 25.9 °C (Figure 2).



Fig. 2. Temperature variation during the two study periods (Spring and Summer) at the Nador lagoon.

Our results for the period studied were comparable to those of previous studies [25,26,27,28,29,30,31,32] conducted before the opening of the new pass. This allowed us to conclude that this development did not affect the thermal homogeneity of the ecosystem as a whole since it did not induce a perceptible thermal zonation.

Salinity is a parameter that can be variable in coastal environments. It is a basic descriptor of water bodies. In particular, it reflects the influence of rivers [33].

In coastal environments, salinity is mainly used to monitor inland water inputs or anthropogenic inputs. Fauna and flora in aquatic ecosystems are dependent on salinity. In general, lagoon environments often have higher salinity values than the marine environment [34].



Fig. 3. Salinity variation during the two study periods (Spring and Summer) at the Nador lagoon.

In the present study and during the spring period, the average salinity of the waters of the lagoon of Nador, recorded in the 9 sampled stations, oscillated between a minimum of 34.6ppm observed at station 4 which corresponds to the treatment plant and a maximum of 37 ppm. noted at station 8, which is between the old pass and Mohandis. The average recorded was about 35.8 ppm. Our results for the spring period were comparable to those of previous studies, [26,27]. During the summer period, the average minimum and maximum salinity recorded in the 9 sampled stations were respectively 35 ppm at station 3, which corresponds to Oued caballo and 38.7 ppm noted at station 1, which corresponds to Beni Ensar, with a depth of 0.70m. The average was about 36.85 ppm (Figue 3). The value of the average salinity found is comparable to that found by [27,28,29,30,35].

The pH, or hydrogen potential, is an indicator of the quality and nature of mineral ions in solution in water [36,37]. However, variations in ocean surface pH are also caused by atmospheric CO_2 [38]. The increase in atmospheric CO_2 (mainly of anthropogenic origin) induces ocean acidification (progressive decrease in pH) [39]. During the study period, the pH of the waters of the Nador lagoon, measured in the 9 sampled stations, during the spring period, oscillated between a minimum value of 7.7 observed in station 3 and a maximum value of 8 noted in station 1. The average recorded was approximately 7.85. In the summer period, the minimum and maximum pH values recorded in the lagoon of Nador were respectively 7.89 in station 4, which

corresponds to the treatment plant and 8.3 measured in station 6, which corresponds to Kariate Arekmane. The average was about 8.09 (Figure 4).





Our results are similar to those found by [25,27,28,40].

3.2 Distribution and abundance of Rhizosolenia

In the study period, 10 species of Rhizosolenia were listed in the Nador Lagoon (Table 2); namely:

Rhizosolenia shrubsolei Cleve 1881, Rhizosolenia alata forma alata T.Brightwell, 1858, Rhizosolenia bergonii H.Peragollo,1892, Rhizosolenia cochlea Brun,1891, Rhizosolenia hyalina Ostenfeld,1901, Rhizosolenia imbricata T.Brightwell, 1858, Rhizosolenia setigera T.Brightwell, 1858, Rhizosolenia shrubsolei Cleve, 1881, Rhizosolenia sp and Rhizosolenia styliformis T.Brightwell, 1858.

	S 1	S2	S3	S4	S5	S 6	S7	S 8	S9	Freq
Rhizosolenia shrubsolei Cleve 1881		-	-	-	-	-	-	+	-	1
Rhizosolenia alata forma alata T.Brightwell, 1858	+	+	-	-	-	-	+	-	-	3
Rhizosolenia bergonii H. Peragollo,1892	-	+	+	+	+	+	+	+	+	8
Rhizosolenia cochlea Brun,1891		-	-	-	-	-	+	-	+	2
Rhizosolenia hyalina Ostenfeld,1901	-	-	+	-	-	-	-	-	+	2
Rhizosolenia imbricata T.Brightwell, 1858		+	-	-	-	-	-	-	+	2
Rhizosolenia setigera T.Brightwell, 1858	+	+	+	+	+	+	+	+	+	9
Rhizosolenia shrubsolei Cleve, 1881	-	-	+	-	-	+	+	-	+	4
Rhizosolenia sp		+	+	+	+	+	+	+	+	9
Rhizosolenia styliformis T.Brightwell, 1858		-	-	-	-	-	+	+	-	3

Table 2. Characteristics of different Rhizosolenia species found in Nador Lagoon

♥ Note : + Present, - Absent.

A total of 10 taxa of Rhizosolenia, which are stronger than other studies conducted in the same lagoon of Nador, showing that the genus of *Rhizosolenia* represented a total of 7 taxa according to a study conducted by El Madani (2012) [40]. And 4 taxa according to a study conducted by Mostareh (2017) [25]. On the other hand, and according to a study conducted by Yun and Lee (2011) [41]. At the Korean coast noted a total of 5 taxa of *Rhizosolenia*.

In this study, and according to a quantitative analysis of *Rhizosolenia* species (Figure 5), our results show that the most abundant total cell density was recorded at station 9 which is in the center of the Nador lagoon, with a depth of 7.27m and with a value of 21680 Cell/l. Followed by an important density of the order of 15710 Cell/l, recorded at station 7 which is in the Center lagoon side Arekmane, with a depth of 7.10m. In this case we can see that the genus Rhizosolenia is more dominant in the center of the lagoon. So, the dominance is under marine influence. However, the minimal cell density is recorded at station 5 corresponding to Oued Bou Areg with a depth of 0.70m, located on the edge of the lagoon with 5120 Cell/l. In this case we note that the weak genus of *Rhizosoleina* of the lagoon is under the

continental influence. We can see that the depth of the lagoon introduces as a parameter influencing the distribution of Rhizosolenia species.



Fig. 5. Total number of *Rhizosolenia* (Cell/l) in the sampling stations.

From the Figure 6, it can be seen that during the sampling campaigns, the *Rhizosolenia sp* complex presented the highest cumulative density, oscillating around 16300 Cell/L, followed by the *Rhizosolenia setigera* (T.Brightwell, 1858) with an abundance of 14110 Cell/I. However, the *Rhizosolenia shrubsolei* (Cleve 1881), presented the lowest cumulative density and was around 1450 Cell/L (Figure. 6).



Fig. 6. Cellular abundance of each *Rhizosoleinia* species, at the 9 stations during the sampling campaigns.

3.3 Statistical analysis

The multivariate analysis (PCA) shows that the first two factorial axes provide nearly 72.24% of the information (Figure 6).



Fig. 7. Principal component analysis (PCA) of the average Rhizosolenia as a function of environmental factors.

According to the F1 axis, which alone explains 41.82% of the total variation, it is shown mainly by a positive correlation between the average of Rhizosolenia and the average values of the environmental factors such as: (temperature, salinity and pH). As well as according to the F2 axis, we notice that the average values of the environmental parameters (temperature, salinity and pH) are positively correlated with the average value of the Rhizosolenia taxon.

4 Conclusion

During the present study, the morphology, taxonomy and distribution of the diatom genus Rhizosolenia at the level of the Nador lagoon were described. It is clearly noted the presence of 10 species of Rhizosolenia as well as the maximum abundance was found in the center of the lagoon on the two stations 9 and 7 which are impacted by marine influence whereas minimum abundance was found in the edge of the lagoon on the station 5 which is subject of continental influence. On the other hand, the *Rhizosolenia sp* complex presented the highest cumulative density, oscillating around 16300 Cell/L, followed by *Rhizosolenia setigera* (Brightwell, 1858) with an abundance of 14110 Cell/l. However, *Rhizosolenia shrubsolei* (Cleve 1881), presented the lowest cumulative density and was around 1450 Cell/L. Statistically, Rhizosolenia species distribution is positively correlated with the environmental parameters studied namely temperature, salinity and pH.

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