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Efflorescence of the Potentially Harmful Dinoflagellate *Prorocentrum micans* in the Oum Er Rbia Estuary – North Atlantic Moroccan Coasts

Rachida Bengriche[1*], El Mostafa Bouchafra[1], Jamila Chaouite[1], Mohammed Moncef[1]

[1] Laboratory of Environmental Study and Analysis, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, BP 20, 24000 El Jadida, Morocco

* Corresponding author's e-mail: rachida.bengriche94@gmail.com

ABSTRACT

The present work addressed the study an episode of red waters of great extent, which was observed off the Oum Er Rbia estuary in spring 2019. The responsible species was *Prorocentrum micans*. The study was carried out on three stations with different characteristics, distributed in the estuary upstream and downstream of an urban wastewater discharge. The cell densities observed were maximum in March 2019. Some environmental parameters (temperature, salinity, dissolved oxygen, chlorophyll (*a*) and pheopigments) were simultaneously recorded. The *P. micans* densities increased from downstream to upstream with cell concentrations of 1.03×10^5 cell/l in S3, 9.1×10^6 cell/l in S2 and 14.11×10^7 cell/l in S1. The contribution of *P. micans* to the total dinoflagellates density was higher and reached 99.54% in station S1. These blooms would be associated with high concentrations of chlorophyll (*a*) and pheopigments, which equaled 40 µg/l and 909 µg/l, respectively, and dissolved oxygen supersaturations (17.05 mg/l). This phenomenon also corresponded to a marked decrease in salinity. According to the application of the Kruskal-Wallis test, the difference was found to be significant between the three stations ($p = 0.0034$). In addition, densities were higher in surface waters than in bottom waters at all three stations. However, this difference did not appear to be statistically significant ($p > 0.05$).

Keywords: efflorescence, *Prorocentrum micans*, dinoflagellates, Oum Er Rbia estuary.

INTRODUCTION

Paralic environments are humid ecosystems of great ecological and biological importance. Given their importance, they have been the subject of several biological studies, especially planctology, as it has been demonstrated in different estuarine environments (Benabdellouahad, 2006; Basdi et al., 2012) and lagoons (Bennouna et al., 2000; Bennouna et al., 2002; El Madani et al., 2011; Natij et al., 2016). Due to certain hydrological, physical and metrological factors, certain phytoplankton species proliferate intensely and cause the 'algal blooms' phenomenon. These blooms can be caused by one or a few species dominating the phytoplankton assemblage up to millions of cells per liter (Hallegraeff, 2003). These blooms sometimes color the water and make the environment in which they grow hypoxic or anoxic (Hallegraeff et al., 1995). *Prorocentrum micans* (Ehrenberg, 1834) is a planktonic, neritic dinoflagellate with worldwide distributions (Gómez, 2005). It is commonly recognized as the origin of many red water phenomena, it has been the subject of several works which have been devoted to its flowering (Wang et al., 2001; Hesham M. et al., 2022). In the present study, the authors were interested in the bloom of the dinoflagellate *P. micans* in three different stations of the Oum Er Rbia estuary and highlighted its dominance over other phytoplankton species, especially of the class Dinophyceae.

MATERIALS AND METHODS

Study area

The Oum Er Rbia estuary (33°16' N, -8°20' W) is located on the North Atlantic Moroccan coast, at 17 km North of El Jadida. The sampling was carried out on March 28th, 2019, at three stations from the estuary (Figure 1):

- Station 1 – located upstream of the estuary, (S1: 33°17’0.3″ N; -8°20’6.11″ W), this station would be the most subject to the influence of fresh water. It has a depth of 5 meters.
- Station 2 – in the immediate vicinity of the outfall (S2: 33°17’44.97″N; -8°20’22.45″W), it is subject to a strong discharge of urban wastewater. This station is additionally exposed to an adjacent anthropogenic action. Its depth can reach 2.2 meters.
- Station 3 – located downstream of the estuary, (S3: 33°18’10.96″N, -8°20’23.54″W), it is most directly subject to the influence of marine waters. Its depth is 3.4 meters.

Sample collection and data analysis

The water samples were collected at the surface and at the bottom of each station, from a boat and using a “Niskin, type 1 GO” bottle with capacity of 6 liters.

Temperature and salinity measurements were taken directly *in situ* by a multiparameter analyzer (Multi 340i model WTW). The determination of chlorophyll (*a*) and pheopigment concentrations was done by spectrophotometry using Lorenzen’s equation (1967). Dissolved oxygen was fixed on site in lapped vials and then titrated in the laboratory using Winkler’s method (1888). Primary production was estimated using Winkler’s method. The sampling of biological material was carried out using plankton net for the qualitative census and using a Niskin bottle for the quantitative analysis. The samples designed for quantitative analysis were fixed *in situ* with lugol, Rhode’s solution and formalin. The microscopic identification of phytoplanktonic organisms was done according to several morphological criteria, cell counts were performed according to the standard technique of Utermöhl (1958) and in triplicate.

The Kruskal-Wallis test was applied to identify the spatial effect between the three stations. The Wilcoxon test was applied to identify the difference between surface and bottom. Data processing was done by the free software R Statistical v 4.1.0 (R Core Team 2020) as well as ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2023), Scales (Campitelli, 2020), corrplot (Wei et al., 2021), FactoMineR (Lê et al., 2008) and factoextra packages (Kassambara, 2020).

RESULTS AND DISCUSSION

Environmental parameters

The results relating to the environmental parameters recorded *in-situ* and measured in the

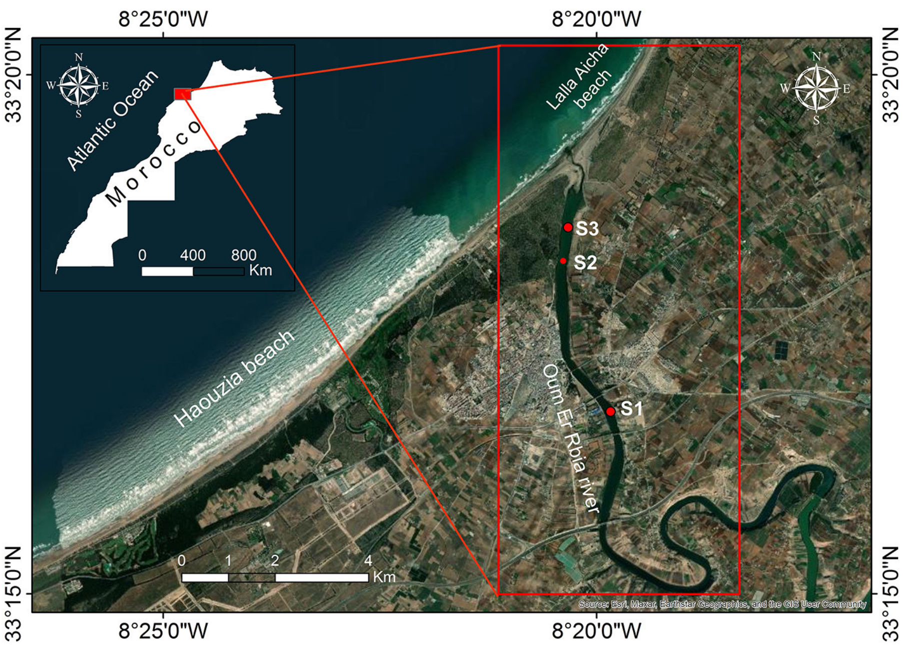

Figure 1. Geographic location of sampling stations (S1, S2 and S3) in the Oum Er Rbia estuary which flows into the Atlantic Ocean at the Azemmour commune

Table 1. Environmental parameters of the three stations (S1, S2 and S3) of the Oum Er Rbia estuary at the surface and at the bottom (March 2019)

Station	T, °C	Salinity, g/l	Dissolved oxygen, mg/l	Chlorophyll (a), µg/l	Pheopigments, µg/l
S1 Surface	19.4	27.8	12.15	40.94	909.4
S1 bottom -5 m	18.1	34.4	2.61	9.61	247.35
S2 Surface	19.8	30.2	17.05	8.84	231.7
S2 Bottom -2.2 m	8.4	34.5	5.42	1.6	35.24
S3 Surface	18.6	31.7	9.03	0.56	15.51
S3 Bottom -3.4 m	17.7	35.3	8.43	0.56	3.32

laboratory are summarized in Table 1. Among all the parameters that constitute the physico-chemistry, very high quantities of dissolved oxygen were observed on the surface of the station S1 (12.15 mg/l) and station S2 (17.05 mg/l) in Mars 2019, while their depths approach hypoxia (2.61 mg/l at the bottom of S1 and 5.42 mg/l at the bottom of S2). The S3 station exposed to the tides showed a slight difference between the surface and the bottom during the same period.

Dinoflagellates

Microscopic observations have revealed, among the dinoflagellates encountered, the dominance of several species of *Prorocentrum*. It was possible to identify, in addition to *P. micans*, several other taxa including *P. minimum*, *P. triestinum* and *Prorocentrum spp, Gyrodinium spirale*, *Protoperidinium steinii*, *Polykrikos sp*, *Oxytoxum sp*, and *Dinophysis accuminata.*

Cell concentrations of dinoflagellates, all species combined, were detected in March 2019. They fluctuated remarkably between a maximum recorded at the surface of S1 having reached 14.18×10^7 cell/l and a minimum of 1.97×10^4 cell/l noted at the bottom of station S3. The cell density of dinoflagellate *P. micans* consequently decreased from upstream to downstream towards the mouth. Similarly, decreases in cell densities were clearly noticed passing successively from the surface to the bottom of each station, since they fell from the surface to the bottom of each station of 14.18×10^7 to 1.99×10^5 cell/l in S1; of 9.2×10^6 to 3.37×10^5 cell/l in S2 and of 1.43×10^5 to 1.97×10^4 cell/l in S3.

Prorocentrum micans

In the Oum Er Rbia estuary, *P. micans* was the most frequently encountered and dominant species among dinoflagellates at all three stations. It was generally denser in the waters originating from the surface than in those originating from the bottom (Figure 2).

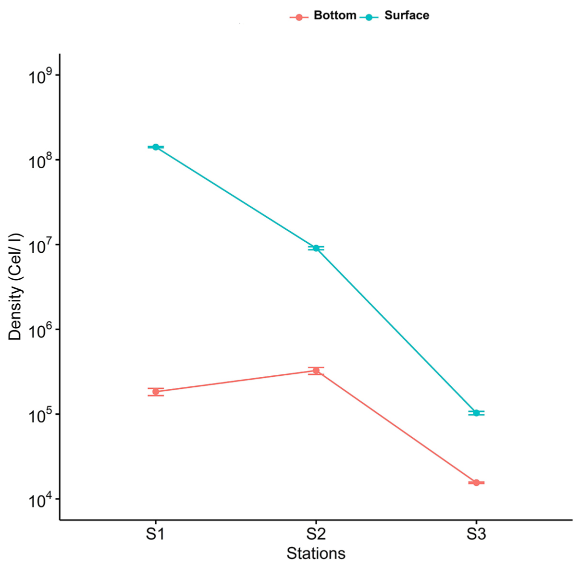

Figure 2. Spatial evolution of *P. micans* cell density at the surface and at the bottom by station (S1, S2 and S3) of the Oum Er Rbia estuary (in March 2019)

From a dominance perspective, *P. micans* presented itself with variable densities according to the three stations. Its quantitative variations globally dominated those of the other dinoflagellates. This taxon was a principal component of dinoflagellates at stations S1 and S2 in the Oum Er Rbia estuary in March 2019 (Figure 3). It represented 99.54% and 92.31% of the total dinoflagellates at the surface and bottom of station S1, respectively, and 98.71% and 96.57% at the surface and bottom of station S2. In contrast, at station S3, the percentage of its representation had fallen to 80.62% at the surface and 68.62% at the bottom, respectively, in relation to the total density of all dinoflagellates (Figure 3).

Despite this appearance, no significant differences were displayed statistically between the bottom and surface of the three stations in terms of *P. micans* densities ($p > 0.05$). Densities were remarkably higher at S1 (station upstream

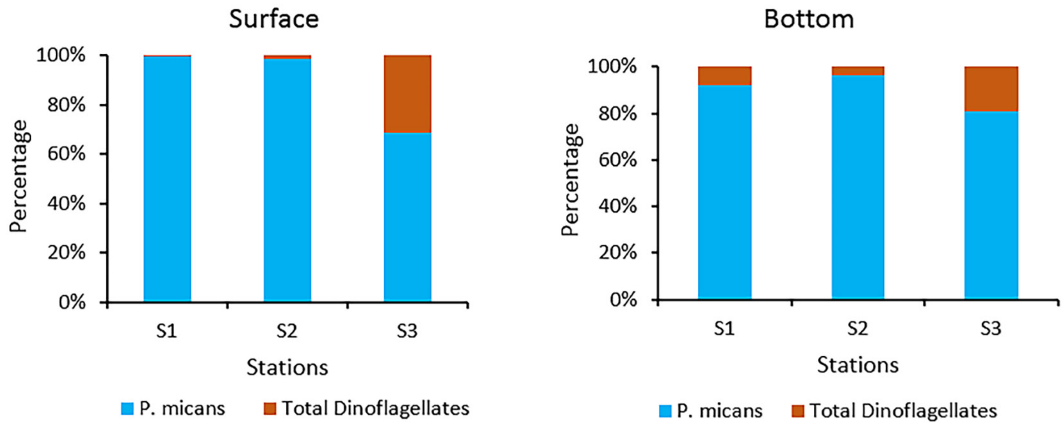

Figure 3. Stacked column plot showing the contribution of *P. micans* to total dinoflagellate density by surface and bottom station

of the estuary) with 14.11×10^7 on its surface and 1.84×10^5 cell/l at its bottom, followed by those recorded at the surface and at the bottom of the station S2 (9.1×10^6 and 3.26×10^5 cell/l). Minimum densities were recorded at station S3 jointly at the surface and at the bottom (1.03×10^5 – 1.55×10^4 cell/l). The distribution of *P. micans* in the three stations (S1, S2 and S3) proved to be statistically very significant ($p = 0.0034$), with a clear significant difference between S1 and S3 ($p = 0.0022$) on the one hand and between S2 and S3 on the other hand, thus showing identical significance (Figure 4). However, no significant difference was detected between S1 and S2, which are close to each other.

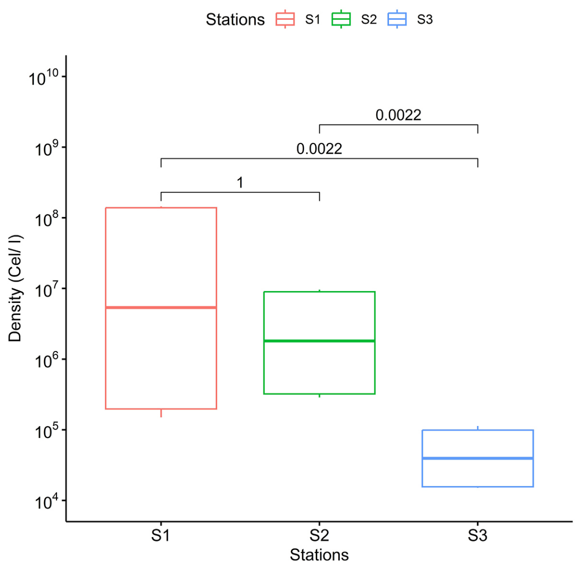

Figure 4. Comparison of the density of *P. micans* of the three stations (S1, S2 and S3) of the waters of the Oum Er Rbia estuary (March 2019)

Generally, P. micans required suitable environment like temperature, salinity, irradiance, pH (Wang et al., 2001; Pei et al., 2022). In the present study, a potential impact of the urban discharge of the city of Azemmour would have an impact on the distribution of the algal bloom caused by *P. micans* at the water level of the Oum Er Rbia estuary, since the flowering of *P. micans* was very accentuated in station S2, located in the immediate vicinity of the urban discharge of the city of Azemmour. It is also at station S1 which is affected in the same way by the waters of the discharge, particularly at rising tide. High levels of iron would support phytoplankton blooms (Schine et al., 2021). The bloom observed could be fed by iron, potentially present in urban wastewater. Minimum densities of *P. micans* were recorded at station S3. This observation could be explained by the alleviation of the rejection impact by the contributions of marine waters.

The high densities of *P. micans*, recorded during this study, were jointly accompanied by high concentrations of chlorophyll (*a*) ($r = 0.97$) and pheopigments ($r = 0.95$), in particular in the station S1, which reached 40 µg/l and 909 µg/l, respectively, and which would be induced, consequently, by a strong primary production **(**0.11 g/m^3/h). A drop in salinity (r = - 0.78) accompanied, at the same time, the high densities of *P. micans* (Figure 5). The principal component analysis (PCA) was approached to analyze the existing correlations within our data having considered six variables: the cell density of *P. micans*, temperature, salinity, dissolved oxygen, chlorophyll (*a* and pheopigments. Table 2 shows which variables contribute most to the construction of each component.

Once the axes are created, the PCA has made it possible to reorganize all the information by creating a first axis that synthesizes the most information possible with a value of 74.8%. Then, a second axis with a value of 20.4% was created. The F1 axis and the F2 axis contribute to 95.6 % of the total variance (Figure 6). The variables that most influenced the construction of the axes are those with the highest contributions. The most contributing variables were highlighted on the correlation graph (Figure 6). The density of *P. micans*, chlorophyll (*a*) and pheopigments thus contribute equally to the construction of the first axis. It was deduced that these variables are positively correlated with each other. The salinity variable also contributes, more strongly, to the construction of the first axis but is negatively correlated with these last three variables. For the second axis, the dissolved oxygen variable contributes essentially to its construction. From this analysis, it could be identified that factor 1 causes the most variance. This first dimension corresponds

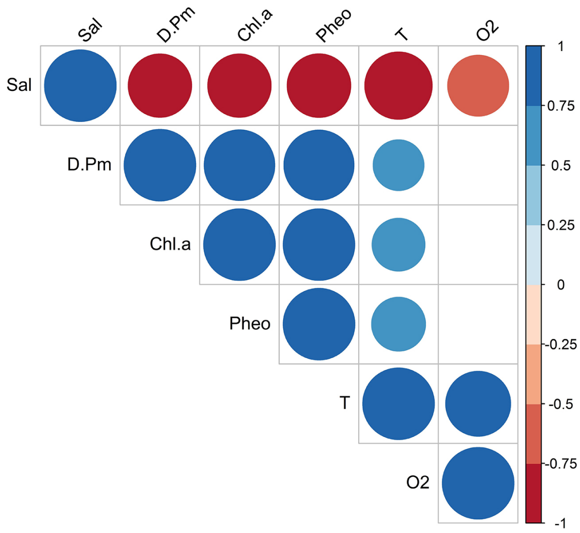

Figure 5. Correlations between *P. micans* density (D.Pm) and environmental parameters (March 2019) with consideration of p-values of calculated correlations. The size of the circles is proportional to the correlation coefficient; empty boxes correspond to non-significant correlations

Table 2. Contribution of variables to the factorial axes (Oum Er Rbia Estuary-March 2019)

Parmeter	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
D. Pm	18.12	12.02	11.94	8.43	47.52
T	14.81	20.80	38.45	8.02	17.91
Sal	20.33	3.07	4.28	56.29	16.01
Chl. a	18.61	12.65	0.03	7.87	3.30
Pheo	18.75	11.53	0.42	14.96	13.85
O_2	9.38	39.93	44.87	4.41	1.41

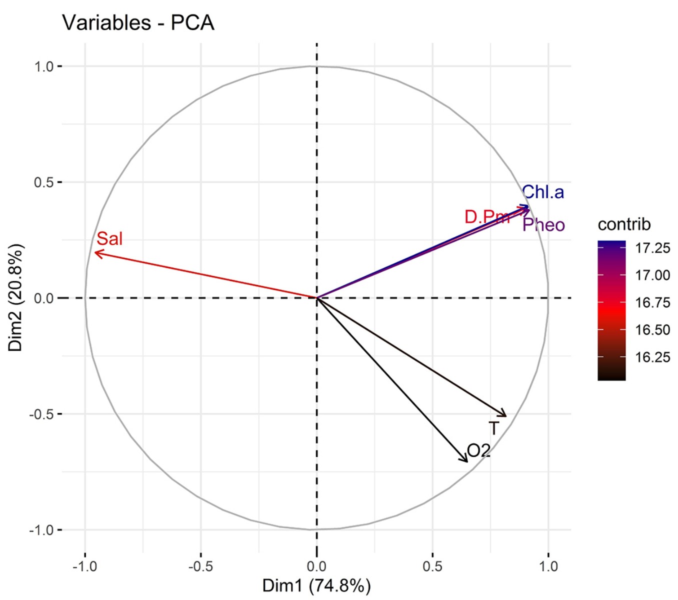

Figure 6. Correlation plot of contributing variables to the principal factorial axes (Oum Er Rbia estuary – March 2019 with D.Pm : density of *P. micans*)

rather to primary production and salinity, which is negatively correlated. Factor 2 would be rather related to the oxygenation of the waters.

CONCLUSIONS

At the end of this preliminary note, the estuary of Oum Er Rbia has known, during the month of March 2019, a bloom that would be recorded for the first time in the stations surveyed. It turns out that the species *P. micans* was the principal phytoplanktonic component responsible for this red water episode following its blooms. The proliferation of *P. micans* was clearly more pronounced in both stations S1 and S2, accompanied by an impoverishment of phytoplanktonic biodiversity, particularly within the dinophyceae.

The salinity of the waters of these two stations was lower compared to that of the station S3 which, conversely, presented lower densities of *P. micans* compared to the total density of all dinophyte species. This observation would be due to the tidal currents which would reduce the impact of the discharge waters on biodiversity and would greatly influence the composition of the dinophyceae communities which would eventually find a restored biodiversity. The episodes of hypoxia occurring in depth of S1 and S2 would be the consequence of the strong blooms of *P. micans* which would have induced an excessive supply of organic matter during these processes which would sediment and degrade in depth by the bacteria in the environment by consumption of dissolved oxygen. Conversely, the mixing by the tides, at the level of the waters of the S3 station, would restore the balance of the oxygenation of the waters between the surface and the bottom.

Finally, it would be necessary to regularly detect the occurrence of red water episodes in this estuarine environment, which would require much closer monitoring, and to consider detecting the presence of probable toxins of phytoplanktonic origin at different trophic levels, particularly in certain organisms of economic interest.

REFERENCES

1. Badsi, H., Ali, H. O., Loudiki, M., Aamiri, A. 2012. Phytoplankton diversity and community composition along the salinity gradient of the Massa estuary. American Journal of Human Ecology, 1(2), 58–64.
2. Benabdellouahad S. 2006. Structure, dynamique et typologies physico-chimiques et phytoplanctoniques de l'estuaire du Bou Regreg (Côte Atlantique Marocaine.) Thèse de doctorat, Université Mohammed V Faculté des Sciences Agdal, Rabat, Maroc.
3. Bennouna, A., Assobhei, O., Berland, B., El Attar, J. 2000. Étude des populations phytoplanctoniques de la lagune de Oualidia (Maroc) ; dinoflagellés potentiellement nuisibles. Marine life, 10(1–2), 3–18.
4. Bennouna, A., Berland, B., El Attar, J., Assobhei, O. 2002. Eau colorée à *Lingulodinium polyedrum* (Stein) Dodge, dans une zone aquacole du littoral du Doukkala (Atlantique marocain). Oceanologica acta, 25(3–4), 159–170.
5. Campitelli, E. 2020. ggnewscale: Multiple Fill and Colour Scales in'ggplot2'. R package version 0.4, 1.
6. El Madani, F., Chiaar, A., Chafi, A. 2011. Phytoplankton composition and abundance assessment in the Nador lagoon (Mediterranean coast of Morocco). Acta Botanica Croatica, 70(2), 269–288. https://doi.org/10.2478/v10184-010-0016-3.
7. Gómez, F. 2005. A list of free-living dinoflagellate species in the world's oceans. Acta Botanica Croatica, 64(1), 129–212.
8. Hallegraeff, G. 2003. Harmful algal blooms : A global overview. Manual on harmful marine microalgae, 33, 1–22.
9. Hallegraeff, G., McCausland, M., Brown, R. 1995. Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters. Journal of plankton research, 17(6), 1163–1176.
10. Hesham, M.A.E.F., Dina, M.A., Mohamed, I. 2022. Seasonal dynamics and ecological drivers of *Prorocentrum micans* Ehrenberg dinoflagellate blooms in Qarun Lake, Egypt. The Egyptian Journal of Aquatic Research, 48(4), 375–382. https://doi.org/10.1016/j.ejar.2022.07.001.
11. Kassambara, A. 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses. http://www.sthda.com/english/rpkgs/factoextra.
12. Kassambara, A. 2023. ggpubr : « ggplot2 » Based Publication Ready Plots (0.6.0). https://CRAN.R-project.org/package=ggpubr.
13. Lê, S., Josse, J., Husson, F. 2008. FactoMineR : un package R pour l'analyse multivariée. Journal of Statistical Software, 25(1), 1–18.
14. Lorenzen, C.J. 1967. Determination of chlorophyll and pheo-pigments : Spectrophotometric equations. Limnol. Oceanogr., 12, 343–346.
15. Natij, L., Damsiri, Z., Chaouti, A., Loudiki, M., Khalil, K., Elkalay, K. 2016. Spatio-temporal patterns of the Microphytoplankton community structure and distribution in a North African lagoon. Journal of Materials and Environmental Science, 7(12), 4419–4434.

16. Pei, L., Hu, W., Wang, P., Kang, J., Mohamed, H.F., Wang, C., Liu, L., Luo, Z. 2022. Morphologic and phylogenic characterization of two bloom-forming planktonic Prorocentrum (Dinophyceae) species and their potential distribution in the China Sea. Algal Research, 66(102788). https://doi.org/10.1016/j.algal.2022.102788
17. R Core Team 2020. European Environment Agency. (s. d.)., https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006.
18. Schine, C.M.S., Alderkamp, AC., van Dijken, G., Gerringa, L.J.A.,Sergi, S., Laan, P., Van Haren, H., Van de Poll, W.H., Arrigo, K.R. 2021. Massive Southern Ocean phytoplankton bloom fed by iron of possible hydrothermal origin. Nature Communication, 12(1211). https://doi.org/10.1038/s41467-021-21339-5.
19. Utermöhl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik : Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen, 9(1), 1–38.
20. Wang, Z., Zhang, Q., Lu, H. 2001. Effects of temperature, salinity, light and pH on the growth of red tide organisms *Prorocentrum mican*. Oceanologia et Limnologia Sinica, 1(32), 15–18.
21. Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., Zemla, J. 2021. Package "corrplot" : Visualization of a Correlation Matrix. 2017. Version 0.84.
22. Wickham, H. 2016. Amazon.fr-Ggplot2: Elegant Graphics for Data Analysis-Wickham, Hadley-Livres, https://www.amazon.fr/ggplot2-Elegant-Graphics-Data-Analysis/dp/331924275X.
23. Winkler, L.W. 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes. Berichte der deutschen chemischen Gesellschaft, 21(2), 2843–2854. https://doi.org/10.1002/cber.188802102122.