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Distribution of dinoflagellate cyst assemblages in recent sediments from a southern Mediterranean lagoon (Mellah, Algeria) with emphasis on toxic species

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Abstract :

This is the first study on the dinoflagellate cysts inAlgerianwaters and in Mellah Lagoon (SouthWesternMediterranean), located within a protected reserve. In total, 42 species of dinocysts belonging to 7 orders, 12 families and 23 genera, were identified in the 26 superficial sediment samples from Mellah Lagoon. The distribution of dinocysts in the sediment of this lagoon is heteroge- neous. Indeed, their abundance oscillates between 1 and 315 cysts g^{-1} dry sediment (DS). Cyst morphotype assemblages were dominated by a few numbers of species: Alexandrium minutum (15.87%), Gonyaulax verior (9.81%), Protoperidinium spp. (7.74%), Alexandrium affine (7.05%), Scrippsiella trochoidea (6.67%), and Alexandrium pseudogonyaulax (6.19%). There is a positive correlation between the density of cysts and the depth ($r = 0.61$; $p < 0.05$), organic matter ($r = 0.70$; $p < 0.05$), water content ($r = 0.71$; $p < 0.05$), and the fine fraction of sediment ($r = 0.74$; $p < 0.05$). Surprisingly, although the Mellah Lagoon is almost semi-closed, it holds an important specific richness in dinocysts (42 species) higher than others observed in Mediterranean lagoons. However, cyst abundances are low compared to other lagoons in the Mediterranean Sea. Finally, the presence of dinocysts of Alexandrium catenella/tamarense, A. minutum, and Gymnodiniumcatenatum associated to paralytic shellfish toxins, A. pseudogonyaulax which produces goniodomin A, also Protoceratium reticulatum and Gonyaulax spinifera complex which produce yessotoxins, needs to implement a monitoring programto prevent a potential human intoxication due to the consumption of contaminated sea products by these potent neurotoxins.

Keywords : Dinoflagellate cysts, Environmental factors, Diversity, Toxic species, Spatial distribution, Mellah lagoon

Introduction

Phytoplankton blooms involving toxic dinoflagellates, emerging biological contaminants, have become a common problem in the Mediterranean Sea. Among the 4000 phytoplankton species recorded in the marine environment (Sournia et al. 1991), about 300 species are harmful to aquatic organisms and among them 80 species are known to produce phycotoxins (Granéli and Turner 2006). The Southern Mediterranean countries are experiencing important developments in toxic phytoplankton with periods of seafood intoxication resulting of economic repercussions. Among the studies carried out on these microalgae, we quote those conducted in Algeria (Ounissi and Frehi 1999; Frehi et al. 2007; Illoul et al. 2008, 2012), in Tunisia (Turki et al. 2014; Zmerli Triki et al. 2014, 2015a; Fertouna-Bellakhal et al. 2014, 2015; Ben Gharbia et al. 2017), on the Mediterranean coasts of Morocco (Taleb et al. 2001; EL Madani et al. 2011; Daoudi et al. 2012; Daghor et al. 2016). Coastal lagoons are concerned by expansion of toxic phytoplankton. These ecosystems are often important shellfish production areas, where the emergence of some potentially toxic dinoflagellate species could threat aquaculture activities and human health. The search for areas at risk of developing toxic dinoflagellates, the determination of the resting cysts belonging to toxic species in these ecosystems, can preserve aquaculture activities better.

Despite the ecological importance (contamination of the food web components), economic relevance (interdiction of exploitation of commercial species), and threat to human health, the monitoring of toxic species in the Algerian east coast is not implemented yet (Frehi et al. 2007; Hadjadji et al. 2014; Cheniti et al. 2018). In Mellah Lagoon, with the exception of an inventory of phytoplankton species made during the 1990s (Draredja 2007), there is no study dedicated to toxic dinoflagellates, particularly as regards to their dormancy forms or dinocysts. Mellah lagoon is relatively far (about 50 km) at east of the big industrial port of the city of Annaba which is classified as the industrial capital of Algeria and subject to spills of large quantities of ballast water from large vessels (Hadjadji et al. 2014; Cheniti et al. 2018). However, the currents from the prevailing north-westerly winds, as well as the Atlantic current along the southern coast of the western Mediterranean basin (Millot 1999, 2009), can carry long-distance water masses along the eastern shores, until the Tunisian coasts. The Mellah lagoon supports an important activity of fishing, breeding (mussels) and gathering (clams and hulls), and the proliferation of toxic microalgae could impact the human health via the consumption of the contaminated mollusks. The accumulation of dinoflagellate cysts in the sediment forms seed banks that can trigger phytoplankton blooms and species dispersal (Anderson et al. 1995). In fact, their germination ensures the proliferation of microalgae in the waters and the blooms initiation (McGillicuddy et al. 2003). Consequently, the accumulation of dormant cysts in the sediment is a source of the inoculation Q3 of the water column leading to harmful algal blooms (HABs) and their recurrence. Consequently, knowing the distribution of cysts in the surface sediment of lagoons is of great importance.

On the Algerian coasts including the Mellah lagoon, harmful algae events date from the early 2000s. Draredja (2007) was the first author to show the proliferation of *Alexandrium catenella* (6.4×10^3 cells L^{-1}) and *Prorocentrum minimum* (*P. cordatum*) (3.2×10^3 cells L^{-1}) in the Mellah Lagoon waters in April and July 2001, respectively. Frehi et al. (2007) reported the blooms in Annaba Bay of *A. catenella* (117×10^3 cells L^{-1}) and *Gymnodinium catenatum* (3.5×10^6 cells L^{-1}) in March and June 2002, respectively. Hadjadji et al. (2014), observed a proliferation of *A. catenella* (861×10^3 cells L^{-1}) in the same bay in May 2010. In the waters of the Algiers coast (two bays, harbor, and beach), Illoul et al. (2008) reported the occurrence of a bloom of *Dinophysis* cf. *acuminata* (1.2×10^3 cells L^{-1}) and *Dinophysis sacculus* (3.3×10^3 cells L^{-1}) in July–August 2002–2003. In the same region (in 5 beaches), Illoul et al. (2012) mentioned in July 2009 the appearance of a bloom of *Ostreopsis* spp. (7.9×10^4 cells L^{-1}) resulting of the intoxication and hospitalization of 300 persons.

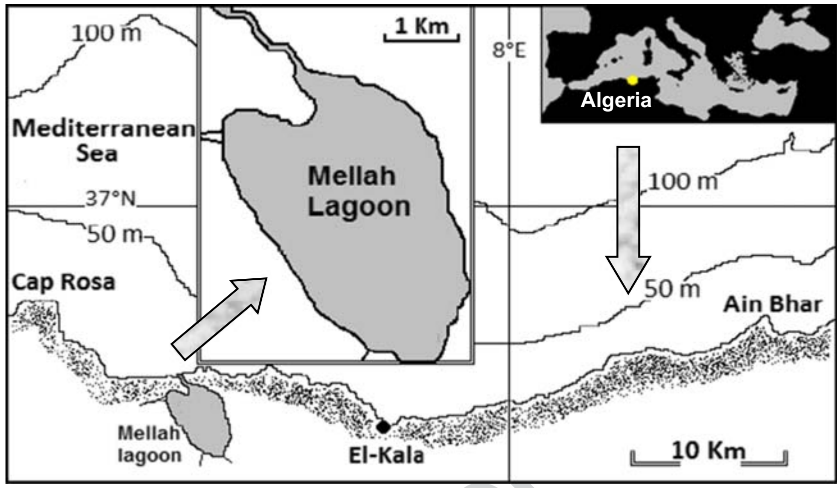
This study aims to (1) provide detailed description of dinoflagellate cysts, (2) provide information on the distribution and abundance of dinocysts, (3) assess seedbeds in potential risk areas, and (4) identify the potential environmental driving factors of cyst distribution. Results could help aquaculture farm managers and to serve as a basis for future studies of dinoflagellate dynamics in the lagoon.

Material and methods

Study area and sampling strategy

The Mellah Lagoon is located in the extreme eastern side of Algeria (8° 20′ E–36° 54′ N), on the southern shore of the western basin of the Mediterranean Sea (Fig. 1). The Mellah Lagoon is the unique lagoon in Algeria; it covers about 865 ha, with a length of 4.50 km and a width of 2.50 km. It's formed of a central depression, with a maximum depth not exceeding 5 m. This brackish water ecosystem is an integral part of the wetland complex of El-Kala national park, where there are other freshwater ecosystems: Oubéira and Tonga lakes. Guelorget et al. (1989) described the Mellah Lagoon as an environment that corresponds to a würmian lake endorheic depression invaded by the sea during the Flemish eustatic rise. According to the story this site was a freshwater lake and for aquaculture purposes, a channel was dug by the Maltese Pisani at the end of the eighteenth century for connecting it to the adjacent coast.

Sediment sampling was carried out during April 4 and 5, 2016 on 26 stations covering the entire lagoon (Fig. 2), using cylindrical cores (80 cm long, 8 cm diameter) operated by a professional diver. The majority of stations are located within the expanse (depth > 2 m), where the fine fraction is important; it is the sedimentary typology most favorable to the distribution of dinocysts. Three replicates at each station were adopted. The top 3 cm of undisturbed surface sediment from the three replicates samples for each sampling station were mixed together and then stored in total darkness at 4 °C until the analyses. Indeed, cysts are distributed mostly in the first three centimeters of the sediment surface (Erard-Le Denn et al. 1993). Dinocysts were separated from the sediments according to the modified density gradient method using Ludox CLX described by Yamaguchi et al. (1995); Erard-Le Denn and Boulay (1995) and Genovesi et al. (2007). Once the extraction operation is complete, the dinocysts are stored in



Q4 **Fig. 1** Geographical position of Mellah Lagoon

tubes covered with aluminum, then placed at 4 °C while waiting for the counting and identification phase.

A fraction of the sampled sediment was used for the analysis of the water content and the proportion of the fine fraction in the sediment. The water content in the sediment is calculated by drying the sediment at 60 °C until the substrate is completely dehydrated. The separation of the fine fraction from the coarse fraction is set at the limit of 63 μm. Sediment samples with a particle size greater than 63 μm were dried at 60 °C, then sieved through a series of the AFNOR type for 15 min, with a mesh size varying from 2000 at 63 μm (2000, 1600, 1400, 1250, 1000, 710, 500, 355, 280, 250, 180, 140, 125, 90, 80 and 63 μm). The separation between the different meshes was conducted using an automatic vibrator "Retch VS 1000." The contents of the sieves were calculated (in g and %) to determine the median which represents the mean diameters of the grains, it allows us to define the nature of the sediment for each station. The particle size classification of the sediments is referred to ISO 14 688-1. The sediment organic matter is obtained from the difference in weight between the dry sediment and the sediment incinerated at 450 °C for 12 h so to evaporate organics in the form of carbon dioxide. According to Guelorget et al. (1989), this method is justified because of the low sediment content of phyllitic minerals, which alone can lead to errors in this measure.

In parallel with sediment sampling, bathymetric surveys, diving with a depth meter and physicochemical parameters (temperature, salinity, pH and dissolved oxygen), were measured at 26 stations spread over the entire lagoon (Fig. 2), using a multiparameter "HANNA HI9828." Water transparency was measured using a Secchi disk with a standard diameter of 30 cm.

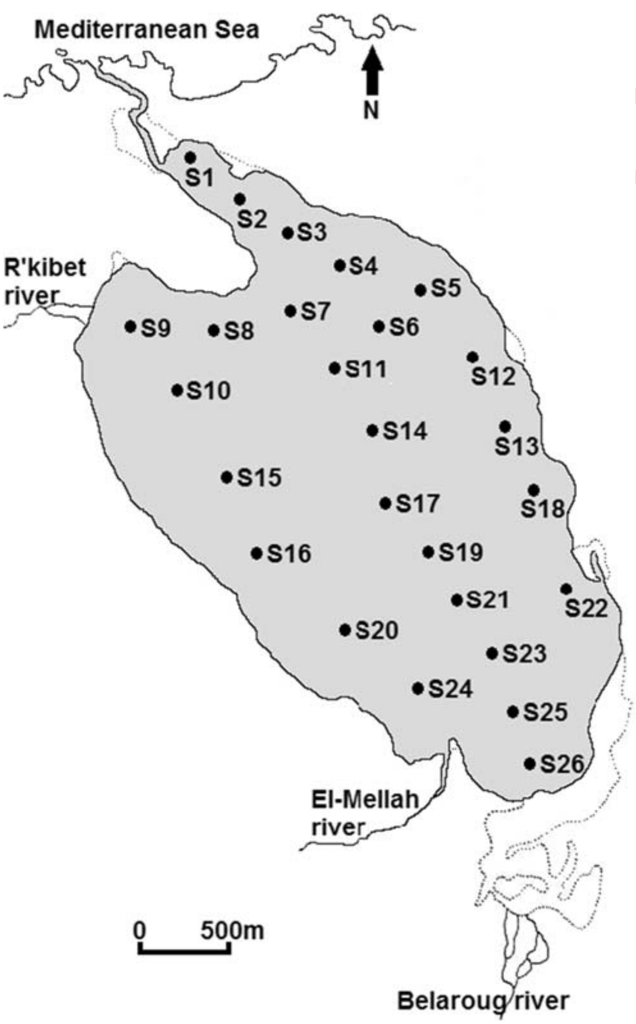


Fig. 2 Location of sampling stations in Mellah Lagoon (S station)

Resting cyst identification and quantification

The taxonomic identification of cysts was performed according to the Matsuoka and Fukuyo (2000) methodology based on the microscopic observation of their morphological characteristics. Indeed, for each prepared Sedgewick plate, all the morphotypes of the cysts observed are photographed. The identification is carried out according to the morphological characteristics of the cysts under inverted light microscope. This direct method is based on morphological features and characters of cysts using identification keys and from plates illustrated in articles and publications dealing with dinocysts (Head 1996; Zonneveld 1997a, b; Zonneveld and Jurkschat 1999; Rochon et al. 1999; Head et al. 2001; Pospelova and Head 2002; Kim et al. 2007; Matsuoka et al. 2009). Moreover, the observations of the various species of cysts of the lagoon are carried out under the microscope "Olympus IX53," with photographs taken at × 40 magnification. Part of the final sample was used to quantify dinocysts using a sedimentation chamber and an inverted microscope. Calculation of the abundance of dinocysts in each sample allows to determine the number of resting cysts per gram of wet sediment. For this, solutions (from 5 to 20 ml) of seawater cyst extracts are used. For each, 1 ml is taken and distributed on a Sedgewick count plate allowing the counting of dinocysts under a "Leica DM750" microscope, at × 20 magnification. Expression of cyst densities was first evaluated per gram of wet sediment (Eq. 1). To reduce the variability due to the sediment water content, we expressed cyst densities per gram of dry sediment (Eq. 2).

$$N_{\text{cysts g}^{-1}\text{ wet sediment}} = \frac{\left(N_{\text{cysts counted in 1ml}}\right) \times 5}{g_{\text{wet sediment weigted for extraction}}} \quad (1)$$

$$N_{\text{cysts g}^{-1}\text{ dry sediment}} = \frac{\left(N_{\text{cysts counted in 1ml}}\right) \times 5}{\left(g_{\text{wet sediment weigted for extraction}}\right) \times (\text{Dry matter content of samples})} \quad (2)$$

N: number.

Accordingly, resting cyst quantifications were performed with a single extraction step, and cyst densities in sediments were estimated by applying a twofold correction factor (Genovesi et al. 2013).

Statistical analyses

A principal component analysis (PCA) is performed using R, version 3.4.2 (R Core Team 2017; Ihaka and Gentleman 1996) for Windows, whose objective is to relate the resting cyst distribution pattern to environmental variables. Additionally, a Spearman's correlation analysis is also used at $p < 0.05$ to determine the interaction between the resting cyst abundance in the lagoon and the environmental variables.

Results

Physicochemical parameters and sedimentological characteristics

The average water temperature calculated from the 26 stations surveyed during the study period (April 2016) was 19.09 ± 0.48 °C, with a maximum of 19.93 °C, reported at station 13 located in the east shore of the lagoon (depth = 1.90 m), and a minimum of 17.86 °C recorded in station 4 in the throttling zone of the lagoon (depth = 3.30 m) (Table 1). The fluctuations of salinity in the lagoon were directly related to sea-lagoon exchanges and to the inflow of freshwater through the three seasonal rivers. The average salinity of the water was 26.69 ± 0.48. The maximum salinity of 28.29 was recorded at station 1 in the north of the lagoon in front of marine inputs, while the minimum salinity of 26.06 was detected at station 26 at the extreme south of the lagoon far from marine influences (Table 1). The pH of the waters of the lagoon was slightly alkaline and oscillated between 8.15 and 8.31. In addition, the waters of the Mellah lagoon were well oxygenated, particularly with regard to the peripheral stations and the contents vary between 6.25 and 8.88 mg L^{-1}. During the spring season, the water transparency corresponding to the depth of disappearance of the Secchi disk oscillated between 2.10 and 3.10 m for the depth > 3.50 m (Table 1).

The particle size analysis of the prospected stations, in particular those with a depth greater than 2 m near the center of the lagoon, was characterized by an important rate in silt (< 63 μm), with contents that exceeded 50% (Fig. 3; Table 1). Almost all of the sites surveyed are characterized by medium sand. The water content of the sediments shows that the highest levels are found in the muddy bottoms, where the fine fraction (< 63 μm) dominates (Table 1). Extremes in water retention of sediments are recorded in station 13 (19.05%), where the fine fraction represents only 1.68%, and in station 23 (80.58%), with a rate in fine fraction of 95.55% (Table 1). The obtained results show that the content of fine fraction is increasing from the shore to the center of the lagoon. The highest rates (> 90%) are recorded in the deep zones of the lagoon (depth > 3.20 m). Station 17 (depth = 4.90 m), located in the west of Mellah, contains the highest rate (96.84%). The lowest levels of organic matter in sediments are observed in the periphery of the lagoon, with the depth not exceeding 1 m (Table 1). The lowest value (0.83%) is recorded in station 26

Q5 **Table 1** Geographic coordinates of the sampling stations in Mellah Lagoon with physicochemical data, total resting cyst counts (total RC counts), specific richness (SR), organic matter content (OM), water content (WC), coarse fraction (CF), and silt fraction (SF) in surface sediment. (Z: depth; Transp.: transparency; =Z: the bottom is visible; T: temperature; Sal.: salinity; DO: dissolved oxygen)

Station	Geographic coordinates	Z (m)	Transp. (m)	T (°C)	Sal.	pH	DO (mg L^{-1})	WC (%)	SF (%)	CF (%)	OM (%)	SR	Total RCs counts (cysts g^{-1} DS)
1	36° 54′ 44″ N–8° 18′ 47″ E	1.70	=Z	19.15	28.29	8.25	7.31	46.62	20.10	79.90	6.27	11	38.25
2	36° 54′ 38″ N–8° 18′ 57″ E	1.90	=Z	18.73	27.48	8.20	6.36	25.91	6.19	93.81	2.97	10	21
3	36° 54′ 33″ N–8° 19′ 08″ E	2.90	=Z	18.01	27.70	8.15	7.50	74.87	86.73	13.27	18.53	10	170.30
4	36° 54′ 23″ N–8° 19′ 21″ E	3.30	=Z	17.86	27.15	8.23	8.45	69.46	75.55	24.45	17.46	10	71.25
5	36° 54′ 22″ N–8° 19′ 31″ E	4.00	=Z	19.30	26.49	8.22	7.88	72.87	81.82	18.18	15.95	10	178.50
6	36° 54′ 14″ N–8° 19′ 25″ E	3.80	=Z	19.29	26.55	8.30	7.42	71.90	71.45	28.55	19.40	5	103.50
7	36° 54′ 07″ N–8° 19′ 12″ E	3.30	=Z	18.58	26.40	8.20	7.95	73.42	89.58	10.42	21.21	7	121.50
8	36° 54′ 06″ N–8° 18′ 57″ E	2.90	=Z	18.89	26.54	8.21	8.88	73.39	93.34	6.66	23.54	10	249
9	36° 54′ 06″ N–8° 18′ 37″ E	2.20	=Z	19.21	26.20	8.21	6.22	61.42	59.34	40.66	17.23	7	190.50
10	36° 53′ 49″ N–8° 18′ 45″ E	3.10	=Z	18.93	26.40	8.30	8.37	66.91	79.17	20.83	11,48	12	141
11	36° 53′ 50″ N–8° 19′ 18″ E	4.10	2.10	18.86	26.79	8.27	8.30	75.71	95.66	4.34	22.51	12	177
12	36° 53′ 54″ N–8° 19′ 58″ E	3.80	=Z	19.54	26.34	8.30	6.95	25.62	7.76	92.24	2.16	8	55.50
13	36° 53′ 38″ N–8° 20′ 04″ E	1.90	=Z	19.93	26.36	8.31	7.99	19.05	1.68	98.32	1.11	3	4.50
14	36° 53′ 37″ N–8° 19′ 34″ E	4.70	2.50	18.60	28.12	8.25	6.80	70.48	84.75	15.25	19.89	8	180
15	36° 53′ 31″ N–8° 18′ 53″ E	3.90	2.40	19.11	26.33	8.30	8.25	74.13	95.52	4.48	23.32	14	315
16	36° 53′ 17″ N–8° 18′ 49″ E	2.80	2.30	19.27	26.34	8.20	7.21	47.74	24.27	75.73	8.78	13	84
17	36° 53′ 19″ N–8° 19′ 22″ E	4.90	3.10	19.22	26.47	8.26	6.90	78.82	96.84	3.16	22.76	14	303
18	36° 53′ 31″ N–8° 20′ 09″ E	1.90	=Z	19.24	26.32	8.24	6.77	23.84	2.39	97.61	1.15	2	3
19	36° 53′ 16″ N–8° 19′ 42″ E	4.80	3.00	19.50	26.45	8.26	6.89	78.04	96.36	3.64	21.99	17	253.50
20	36° 53′ 04″ N–8° 19′ 20″ E	4.60	2.40	19.40	26.52	8.25	6.91	76.69	96.05	3.95	24.65	8	43
21	36° 53′ 02″ N–8° 19′ 52″ E	4.90	2.50	19.48	26.49	8.19	6.25	77.34	94.94	5.06	21.71	16	144
22	36° 53′ 05″ N–8° 20′ 18″ E	1.80	=Z	19.02	26.27	8.22	6.80	21.59	25.51	74.49	4.30	2	1.50
23	36° 52′ 57″ N–8° 19′ 54″ E	4.10	3.00	19.39	26.47	8.30	6.75	80.58	95.55	4.45	24.26	7	66
24	36° 52′ 49″ N–8° 19′ 32″ E	3.00	=Z	19.71	26.20	8.30	6.89	28.84	4.48	95.52	1.82	7	6
25	36° 52′ 46″ N–8° 20′ 07″ E	3.70	=Z	19.92	26.41	8.22	6.69	62.04	41.17	58.83	7.81	13	46.50
26	36° 52′ 33″ N–8° 20′ 14″ E	0.70	=Z	19.93	26.07	8.29	6.08	22.53	1.62	98.38	0.83	4	1.50

(depth = 0.70 m), with a substrate composed with a pure sand. The highest rates are found inside the lagoon, where the fine fraction is dominating (Table 1). Thus, the maximum content of 24.65% is detected in station 20 located in the center of the lagoon (depth = 4.60 m), where the fine fraction is clearly dominant (96.05%). Overall, the distribution of organic matter in the lagoon is very heterogeneous, with an average of (13.96 ± 8.88) %.

Dinocyst distribution and abundance

A total of 42 species of dinocysts belonging to 7 orders, 12 families and 23 genera, were identified in the 26 superficial sediment samples from Mellah Lagoon (Table 2). The distribution of taxa in dinocysts is organized into the following: Peridiniales (3 families, 7 genera, and 14 taxa), Gonyaulacales (3 families, 7 genera, and 15 taxa), Gymnodiniales (2 families, 5 genera, and 9 taxa) (Plate 1), Suessuales, Prorocentrales, Thoracosphaerales, and Tovelliales (1 family, 1 genera, and 1 taxon for each order). The biological, paleontological names, the harmful effects, and relative abundance of dinoflagellate cysts identified in the present study Mellah Lagoon are showed in Table 2. The distribution of dinocysts in the surface sediments of the Mellah Lagoon is very uneven. Indeed, their abundance oscillates between 1.50 cysts g^{-1} DS in both station 22 (south east of the lagoon) and station 26 (south of the lagoon) and 303 and 315 cysts g^{-1} DS in station 17 (center of the lagoon) and station 15 (West center of the lagoon), respectively (Fig. 5). The average of cyst abundance in the whole lagoon is 114 cysts g^{-1} DS. The highest density of dinocysts is found in station 15 characterized by a bottom of sandy silt, where the hydrodynamic intensity is relatively low and in station 17 in the center of the lagoon the deepest zone (4.90 m) of the lagoon, than characterized by bathymetric confinement (Fig. 4), while the lowest concentration of these cysts was detected at both stations 22 and 26 (depth < 1.80 m) near the coast so with a high agitation and characterized by a substrate dominated by pure sands. The specific richness extremes vary between 2 in stations 18 (depth = 1.90 m) and 22 (depth = 1.80 m), near the coast south east of the lagoon and 17 in station 19 (depth = 4.80 m) in the center of the lagoon. Generally, the silt fraction and the deepest sites are the richest in cysts than the sandy fraction near the coast (Fig. 5). The Mellah Lagoon dinocysts are represented mainly by three orders: Gonyaulacales (54.56%), Peridiniales (35.01%), and Gymnodiniales (8.85%) (Fig. 6). The other groups are very poorly represented (1.85%). In Mellah Lagoon, only one species is classified as common or constant (F > 75%): *Alexandrium minutum*, while the regular species 50% < F <

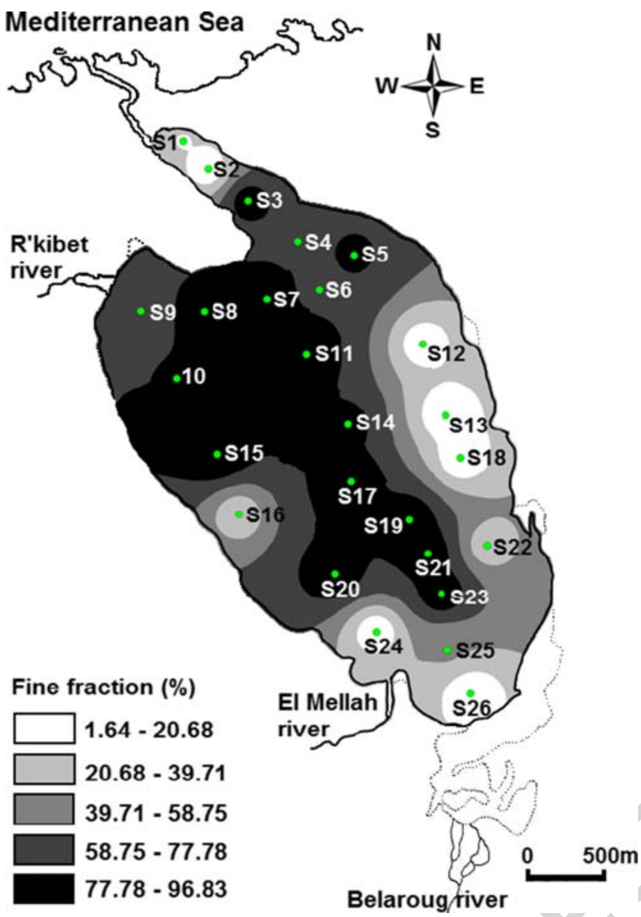


Fig. 3 Spatial distribution of fine fraction (%) in the superficial sediment of Mellah Lagoon

75%) are as follows: *Alexandrium verior*, *A affine*, *Scrippsiella trochoidea* and *Protoperidinium* spp. (Table 2). The dinoflagellate cysts were dominated by a few species (Fig. 7): *Alexandrium minutum* (15.87%), *Gonyaulax verior* (9.81%), *Protoperidinium* spp. (7.74%), *Alexandrium affine* (7.05%), *Scrippsiella trochoidea* (6.67%), and *Alexandrium pseudogonyaulax* (6.19%). Among the 42 dinocysts detected in surface sediment of lagoon, 8 are considered to be potentially noxious/toxic as *Alexandrium catenella/tamarense*, *Alexandrium margalefi*, *Alexandrium minutum*, *Alexandrium pseudogonyaulax*, *Gonyaulax spinifera* complex, *Protoceratium reticulatum*, *Gymnodinium catenatum*, and *Prorocentrum minimum* (Table 2).

Relationship between environmental factors and resting cyst abundance

Spearman's correlation analyses show that the correlations between the abundance of dinocysts and the environmental factors of the surface of sediment such as silt fraction ($r = 0.74$; $p < 0.05$), water content ($r = 0.71$; $p < 0.05$), organic matter ($r = 0.70$; $p < 0.05$), and the depth ($r = 0.61$; $p < 0.05$) are positive and significant. The multivariate analysis (PCA) indicates that the density of dinocysts is significantly correlated with the environmental factors mentioned above (Fig. 8). This PCA shows that the first two factorial axes yielded nearly 74.35% of the information. Axis F1 explains 54.26% of the total variation; it is built mainly by the positive correlation of the variables silt fraction ($r = 0.98$), water content ($r = 0.96$), organic matter ($r = 0.96$), depth ($r = 0.76$), and total RC counting ($r = 0.74$) and which also contribute significantly to its construction ($\cos^2 = 0.96$, $\cos^2 = 0.93$, $\cos^2 = 0.92$, $\cos^2 = 0.58$ and $\cos^2 = 0.54$, respectively) and negatively with the variable coarse fraction ($r = -0.98$), which strongly contribute to the construction of this axis ($\cos^2 = 0.96$). In addition, axis F2 explains 20.09% of the total variation; it is built mainly by the positive correlations of the variable temperature ($r = 0.77$) and pH ($r = 0.65$) which remarkably contribute to the construction of this axis ($\cos^2 = 0.59$ and $\cos^2 = 0.43$, respectively) and the negative correlation of variables salinity ($r = -0.74$) and dissolved oxygen ($r = -0.15$) with a difference in contribution ($\cos^2 = 0.55$ and $\cos^2 = 0.02$, respectively).

Discussion

This is the first study reporting the distribution, abundance and diversity of cyst assemblages in the superficial sediment of Algerian Mediterranean waters (Mellah Lagoon). Dinoflagellate cysts were found in the 26 sampled stations, but with heterogeneous abundances and patchy distribution.

There are many species of microalgae with a benthonic phase in their life cycle during which they are deposited on the seabed where they usually remain dormant. The dinoflagellate species producing the most important HABs are often characterized by cysts production. These cysts can remain in the sediment for months or even years before they germinate when environmental conditions become favorable. Then, the produced vegetative cells multiply exponentially to form blooms. Despite being a lesser known aspect of life cycle of HAB species, the dormancy phase of dinoflagellates (cyst) is often a key factor to understand HAB development. Many studies have been performed on the dinocyst assemblages in recently deposited sediment of the coastal ecosystems of Western Mediterranean basin (Montresor et al. 1998; Bravo et al. 2006, 2008; Satta et al. 2010, 2013; Rubino et al. 2010; Feki et al. 2013). Mediterranean lagoons were also investigated (Genovesi et al. 2009, 2013; Bouchouicha Smida et al. 2012; Satta et al. 2014; Fertouna-Bellakhal et al. 2014, 2015; Zmerli Triki et al. 2014; Zmerli Triki et al. 2015b; Zmerli Triki et al. 2016; Daghor et al. 2016; Dhib et al. 2016; Zmerli Triki et al. 2017). Unfortunately, no such studies have been conducted to date in Algerian waters.

A detailed spatial distribution of resting cysts present in superficial sediment (< 5 cm) is reported for the first time in Mellah Lagoon. Our results show that cyst densities in this

Table 2 Biological, paleontological names, and harmful effects of dinoflagellate cysts identified in the present study Mellah Lagoon. D: dominance (%), F: frequency (%): 75–100% (common or constant species), 50–75% (regular species), 25–50% (accessory species), < 25% (accidental or occasionally species). PSP: paralytic shellfish poisoning, PTP: potentially toxin producer, HBP: high biomass proliferation, GDA: goniodomin A, YTX: yessotoxins, TTX: tetrodotoxin. (1): Figueroa et al. (2009); (2): Laabir et al. (2013); (3): Hallegraeff et al. (1991); (4): Bravo et al. (2006); (5): Klein et al. (2010); (6): Rhodes et al. (2006); (7): Paz et al. (2004); (8): Tang and Gobler (2012); (9): Anderson et al. (1989); (10): Reñé et al. (2011); (11): Vlamis et al. (2015)

Order	Family	Biological names	Paleontological names	Toxicity	D (%)	F (%)
Peridiniales	Dinophysiacea	*Diplopsalis lenticula* (Bergh, 1881)	-	-	3.41	30.76
	Protoperidiniaceae	*Kryptoperidinium foliaceum* (F.Stein) Lindemann, 1924	-	HBP (1)	1.25	15.38
		Pentapharsodinium dalei Indelicato & Loeblich III, 1986	-	-	1.01	11.53
		Pentapharsodinium tyrrhenicum (Balech) Montressor, Zingone & Marino, 1993	-	-	0.75	7.69
		Preperidinium sp.	*Dubridinium* sp.	-	1.25	23.07
		Protoperidinium avellana (Meunier, 1919) Balech, 1974	*Brigantidinium cariacoense* (Wall) Reid	-	1.10	15.38
		Protoperidinium claudicans (Paulsen, 1907) Balech, 1974	*Votadinium spinosum*	-	0.91	15.38
		Protoperidinium conicoides (Paulsen, 1905) Balech, 1974	*Brigantidinium simplex*	-	4.72	30.76
		Protoperidinium conicum (Gran) Balech, 1974	*Selenopemphix quanta*	-	0.25	7.69
		Protoperidinium minutum (Kofoid) Loeblich III, 1970	Cyst of *Archaeperidinium minutum*	-	1.91	15.38
		Protoperidinium spp.	*Brigantedinium* sp.	-		
			Islandinium brevispinosum	-	6.53	50
			Echinidinium sp.	-		
		Scrippsiella trochoidea (Stein) Loeblich III, 1976	-	-	6.63	50
		Scrippsiella sp.	-	-	2.06	26.92
	Kolkwitziellaceae	*Zygabikodinium lenticulatum* Loeblich Jr. & Loeblich III, 1970	*Dubridinium caperatum*	-	1.05	11.53
Gonyaulacales	Gonyaulacaceae	*Alexandrium affine* (H. Inoue & Y. Fukuyo) Balech, 1995	-	-	7.01	53.84
		Alexandrium catenella/tamarense (Lebour, 1925) Balech, 1995	-	PSP (2)	2.27	34.61
		Alexandrium margalefi (Balech, 1994)	-	PTP (3)	1.55	23.07
		Alexandrium minutum (Halim, 1960)	-	PSP (4)	15.77	88.46
		Alexandrium pseudogonyaulax (Biecheler) Horiguchi ex Kita & Fukuyo 1992	-	GDA (5)	6.16	46.15
		Alexandrium sp.	-	-	1.65	19.23
		Bitectatodinium spongium (Zonneveld) Zonneveld & Jurkschat 1999	-	-	0.90	3.84
		Gonyaulax spinifera complex (Claparède & Lachmann) Diesing, 1866	*Spiniferites bentorii*	YTX (6)		
			Spiniferites delicates	YTX (6)	2.97	19.23
			Spiniferites ramosus	YTX (6)		
		Gonyaulax verior Sournia, 1973	-	-	9.75	69.23
	Pyrophacaceae	*Fragilidium mexicanum* Balech, 1988	-	-	0.50	7.69
		Fragilidium sp.	-	-	0.30	7.69
		Protoceratium reticulatum (Claparède & Lachmann) Bütschli, 1885	*Operculodinium centrocarpum*	YTX (7)	3.16	34.61
		Protoceratium sp.	-	-	0.35	7.69
		Pyrophacus steinii (Schiller) Wall & Dale, 1971	*Tuberculodinium vancampoae*	-	0.91	11.53
	Hystricho-sphaeridiaceae	*Trichodinium castanea* (Deflandre) Clarke & Verdier 1967	-	-	0.60	7.69
Gymnodiniales	Gymnodiniaceae	*Cochlodinium polykrikoides* Margalef, 1961	Cyst type Cp[a]	HBP (8)	3.02	34.61
		Gymnodinium catenatum H.W.Graham, 1943	-	PSP (9)	0.85	3.84
		Gymnodinium litoralis A.Reñé, 2011	-	HBP (10)	0.40	7.69
		Gymnodinium sp.	-	-	1.61	11.53
		Gyrodinium impudicum S.Fraga & I.Bravo, 1995	-	-	0.37	7.69
		Gyrodinium instriatum Freudenthal & J.J.Lee, 1963	-	-	0.58	7.69
	Polykrikaceae	*Pheopolykrikos hartmannii* (Zimmerman) Matsuoka & Fukuyo, 1986	-	-	0.07	7.69
		Polykrikos kofoidii Chatton, 1914	-	-	0.30	3.84
		Polykrikos schwartzii Bütschli, 1873	-	-	0.90	11.53
Prorocentrales	Prorocentraceae	*Prorocentrum minimum* (Pavillard) J.Schiller, 1933	-	TTX (11)	0.45	3.84
Suessuales	Biecheleriaceae	*Biecheleria cincta* (Siano, Montresor & Zingone) Siano, 2012	-	-	0.81	23.07
Thoracosphaerales	Thoracosphaeraceae	*Posoniella tricarinelloides* (G.Versteegh) Streng, Banasová, D.Reháková & H.Willems (2009)	-	-	0.15	3.84
Tovelliales	Tovelliaceae	*Tovellia paldangensis* Z.Li, M.S.Han & H.H.Shin 201	-	-	0.15	3.84

unique preserved ecosystem are characterized by moderate values (up to 315 cysts g^{-1} DS) compared to some Mediterranean coastal waters. Indeed, the found cysts densities are lower than those in Bizerte lagoon (Tunisia) (20,126 cysts g^{-1} DS and 2742 cysts g^{-1} DS reported by Fertouna-Bellakhal et al. 2014 and Zmerli Triki et al. 2017, respectively) and in Izmir Bay (Turkey) (3292 cysts g^{-1} DW reported by Aydin et al. 2011). However, the cyst abundances of Mellah lagoon are similar to those found in Ghar El Melh lagoon (Tunisia) with up to 229 cysts g^{-1} DS (Dhib et al. 2016) and Cabras in Sardinia (Italy) with up to 287 cysts g^{-1} DW (Satta et al. 2014), but higher than that found in Homa Lagoon (Turkey) with up to 71 cysts g^{-1} DW (Aydin et al., 2014). Regarding to species composition, interestingly, despite the restricted surface (865 ha) and the relatively recent age of the Mellah, this lagoon contains a relatively high species richness (42 species) when compared to all the Mediterranean lagoons mentioned above except the lagoon of Bizerte which contains the same number of species as the Mellah Lagoon. Our results show that six species dominate: *Alexandrium minutum, A. affine, A. pseudogonyaulax, Protoperidinium* spp., *Gonyaulax verior*, and *Scrippsiella trochoidea*. Among these species, two species are associated

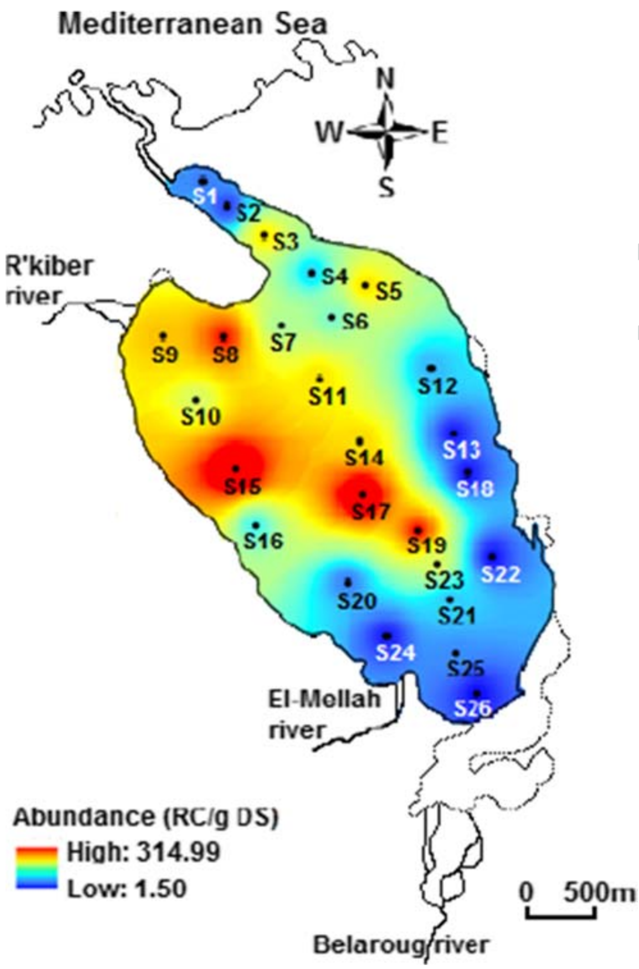


Plate 1 Light microscopy photographs of selected morphotype cysts isolated from surface sediments in Mellah Lagoon. I—Peridiniales: 1—*Diplopsalis lenticula;* 2—*Protoperidinium conicoides*; 3—*Preperidinium* sp.; 4—*Pentapharsodinium dalei*; 5—*Protoperidinium avellana*; 6—*Protoperidinium* sp.; 7—*Scrippsiella trochoidea*; 8—*Zygabikodinium lenticulatum*. II—Gonyaulacales: 9—*Alexandrium pseudogonyaulax*; 10—*Bitectatodinium spongium*; 11—*Gonyaulax spinifera* complex. III—Gymnodiniales: 12—*Gymnodinium catenatum*; 13—*Polykrikos kofoidii*; 14—*Polykrikos schwartzii*. Scale bar 10 μm

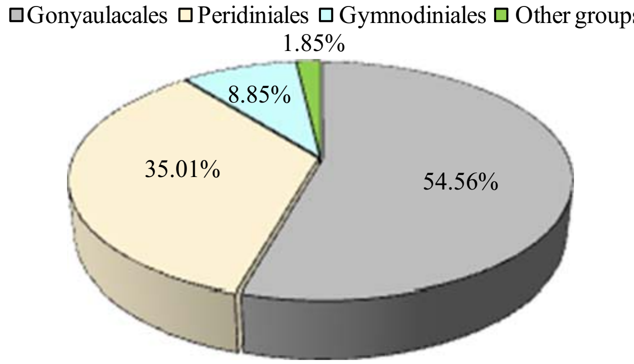


Fig. 5 Spatial distribution of resting cysts specific richness in the superficial sediment of Mellah Lagoon

to HABs, the first *A. minutum* (15.87%) producing paralytic shellfish toxins (PSTs) (Anderson et al. 2012) is the most dominant and the second one is *A. pseudogonyaulax* (6.19%) which was shown to produce goniodomin A, a potent toxin (Zmerli Triki et al. 2016). These species could in the near future form HABs and therefore impact negatively the

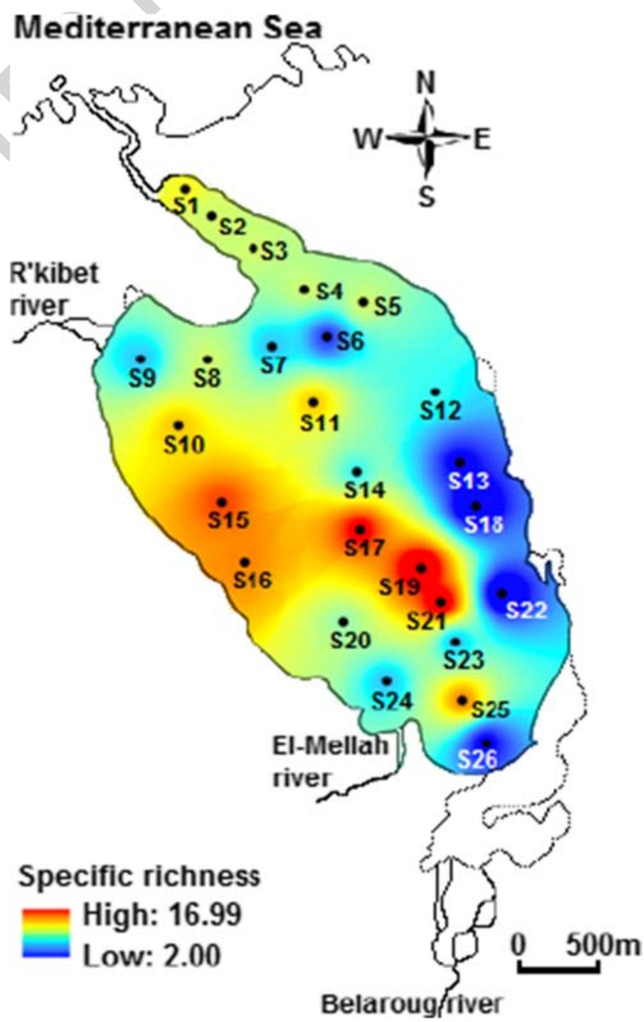


Fig. 4 Spatial distribution of resting cyst abundance (RC g^{-1} DS) in the superficial sediment of Mellah Lagoon

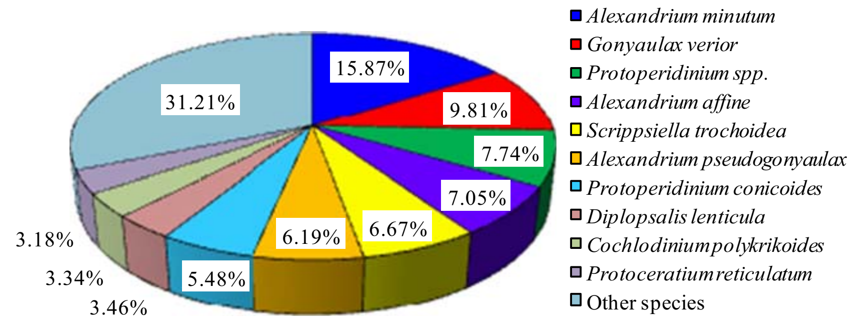


Fig. 6 Relative abundances (%) of different groups of dinocysts collected in the superficial sediment of the Mellah Lagoon (April 2016)

biological components of the Mellah lagoon with potential human intoxication.

The accumulation of dormant cysts in the sediment resulting of "cyst banks" could be a source of seeding allowing the initiation of blooms and the recurrence of these phenomena. The mapping of the different resistance cysts present in the superficial sediment of the Mellah Lagoon is important ecologically. Generally, dinocysts size is ranged between 20 and 100 μm, allowing them to behave like fine silt particles in the natural environment (Dale 1983; Lacasse et al. 2013). Fine grain sized sediments are characterized by higher cyst concentration when compared to sandy sediment (Matsuoka et al. 2003; Horner et al. 2011). In the Mellah lagoon, the highest abundances are found in the fine-rich bottoms by moving towards the center of the lagoon from 2.5 m. This also applies to the sheltered areas located in the North West and in the West center of the lagoon. Fertouna-Bellakhal et al. (2014) reported that the spatial distribution and cyst abundance are controlled by local currents in Bizerte Lagoon (Tunisia).

Cheniti et al. (2018) demonstrated the potential introduction of several HAB species by ballast water in the Annaba harbor, the second most important industrial and commercial port in Algeria. Also, Annaba bay holds an important HAB species diversity (Frehi et al. 2007; Hadjadji et al. 2014). Mellah Lagoon is located only 50 km from Annaba bay and harbor. One can suppose the transfer of HAB species present in Annaba waters thanks to the currents along the coast and to permanent water exchange between the open Mediterranean Sea and Mellah Lagoon (Millot 1999, 2009). However, this hypothesis needs to be verified by further investigations. Results from a survey of an annual spatio-temporal variation of phytoplankton in three stations distributed along a longitudinal axis (North-South) in Mellah Lagoon (Draredja et al. 2019) show a high correlation ($r = 0.99$) between the abundance of total phytoplankton cells in the column of water and the density of cyst assemblages in the superficial sediment. In addition, a positive correlation ($r = 0.66$) was observed between the abundance of the *Alexandrium minutum* cysts and its vegetative form in the water column.

Several studies have reported that high cyst accumulations are recorded in fine grained rather than in sandy sediments in various coastal marine systems (Yamaguchi et al. 1996; Kremp 2000; Matsuoka et al. 2003; Anglés et al. 2010). In

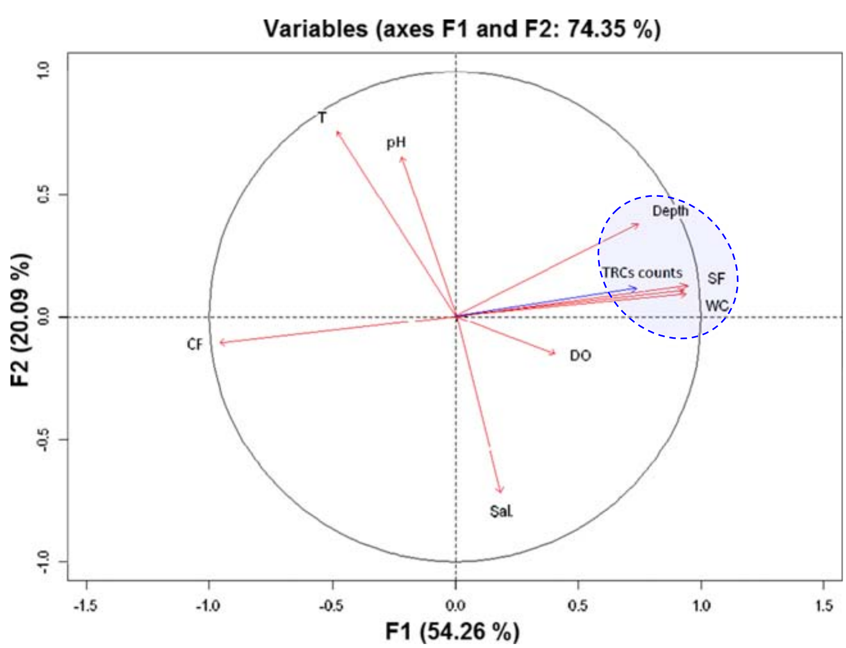


Fig. 7 Relative abundances (%) of main species of dinocysts collected in the superficial sediment of the Mellah Lagoon (April 2016)

Q6 **Fig. 8** Principal component analysis (PCA) for the dinocyst density (TRC counts) related to the environmental factors (T temperature, Sal. salinity, DO dissolved oxygen, pH, WC water content, SF silt fraction, OM organic matter, CF coarse fraction, and depth) (axes F1 and F2 = 74.35%)

addition, the highest level of sedimentary organic matter in which dinocysts is usually observed in the stations with the highest proportions of fine particles (< 63 μm). Sedimentary organic matter and dinocysts have a strong affinity for fine sediment particles because they adsorb on mineral surfaces. This adsorption process contribute to a better conservation of the organic matter in a general way and thus lead to a correlation between the sedimentary organic matter and the proportion of fine particles (< 63 μm) ($r = 0.90$) (Draredja 2007; Magni et al. 2008; Yu et al. 2009). Our study corroborates the previous work as in the Mellah Lagoon; the highest concentrations of sedimentary organic matter are observed in the stations with the highest proportion of fine particles (Draredja et al. 2013).

The highest abundances (between 121 and 303 cysts g^{-1} DS) were registered in the sediment characterized by > 85% of the fine fraction, 70–78% of the water content, and 19.89–22.75% of the organic matter content. Interestingly, bathymetry positively affects the abundance of dinocysts in the superficial sediments of Mellah Lagoon. The deepest central stations (between 3.10 and 4.90 m) are the richest in cysts. The low hydrodynamic, especially in the center of the Mellah, the deepest zone of the lagoon (between 4 and 4.90 m), and the sheltered zone in the center and northwest of the lagoon (Guelorget et al. 1989), facilitates sedimentation of the cysts. The stations of these mentioned zones (stations 10, 11, 14, 15, 17, 19, and 21) show important abundances in cysts (between 141 and 315 cysts g^{-1} DS). It is contrary to the sites located in the banks (depth < 1.50 m) and exposed to the prevailing north–west winds where the densities in cysts are relatively low (between 1 and 70) with the exception of the station 5 located in the north–east of the lagoon (178 cysts g^{-1} DS) having a depth of 4 m.

The present study shows the following: (1) Although the Mellah Lagoon is almost semi-closed, because of its remoteness from the adjacent coast, where it is connected to it by a long (900 m), narrow (5–10 m), and winding channel, it encloses a relatively high specific richness in cysts (48 species) in comparison to other Mediterranean lagoons; (2) a heterogeneous and patchy cyst distribution and a relatively dinoflagellate cyst abundances when compared with some southern Mediterranean ecosystems as Bizerte lagoon in Tunisia; (3) as in the majority of marine and lagoon environments, soils rich in fine fractions, organic matter and water (soft vases), so deep or sheltered contain a higher number of cysts compared to the banks of the banks with hydrodynamic ford, with a dominant coarse fraction poor in organic matter. To conclude, the presence of some dinocysts belonging to HAB species related to PSTs such as *Alexandrium catenella/tamarense*, *Alexandrium minutum*, and *Gymnodinium catenatum*; PTP (potentially toxin producer) such as *Alexandrium margalefi*; Goniodomin A such as *Alexandrium pseudogonyaulax*; and Yessotoxin such as *Gonyaulax spinifera* complex and *Protoceratium reticulatum*, needs to implement a monitoring program to detect toxic species in order to prevent potential human intoxication due to the consumption of contaminated shells or/and fishes. A parallel study on the distribution of phytoplankton in the Mellah shows a positive correlation between the density of total microalgae in the water column and that of cyst assemblages in sediments from north to south of the lagoon (Draredja et al. 2019). However, to understand better the potential impact of the highlighted HAB species present in Mellah lagoon by their vegetative and benthic forms, additional studies should be conducted including the isolation of cells and establishment of clonal cultures, genetic and toxin characterization of the HAB species. We also have to investigate mollusks intoxications by LC-MS/MS as Mellah Lagoon holds since several years the exploitation of shells and fishes. In addition to HAB species proliferation monitoring program, we should also include cyst community studies for early warning of HAB development and also as an important precautionary management policy to prevent any transfer of HAB species by sediment dredging.

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