**Regional Studies in Marine Science**
November 2022, Volume 56, Pages 102713 (11p.)
https://doi.org/10.1016/j.rsma.2022.102713
https://archimer.ifremer.fr/doc/00800/91213/



# *Ostreopsis* spp.: Morphology, proliferation and toxic profile in the North-West of Agadir (North Atlantic Ocean).

Alkhatib Houda [1, 2, *], Bennouna Asmae [2], Abouabdellah Rachid [2], Amzil Zouher [3], Herve Fabienne [3], Moussavou-Mouity Cyrielle Amour [4], Elayoubi Salaheddine [2], Bekkali Mohammed [1], Fahde Abdelilah [1]

[1] Hassan II University, Faculty of Sciences Ain Chock, Casablanca, Morocco
[2] National Institute of Fisheries Research (INRH), Agadir-LSSMM regional center, Morocco
[3] IFREMER, Unit PHYTOX, F-44311 Nantes Cedex 3, France
[4] Hassan First University of Settat, Morocco

* Corresponding author : Houda Alkhatib, email address : houdaalkhatib95@gmail.com

**Abstract :**

The distribution of benthic Ostreopsis species is poorly known in the Moroccan coasts. The current study is aiming to investigate: i) the distribution of Ostreopsis spp. cells on macroalgae and in the seawater column of the Cape Ghir area (North-West of Agadir) during the summer season of Ostreopsis from July 15 to November 17, 2020; ii) the toxic profile at the level of the three matrices (benthic Ostreopsis attached to macroalgae, Ostreopsis in the water column and mussels) via chemical analysis using mass spectrometry. A taxonomic study of Ostreopsis spp. was carried out using the light and inverted optical microscopes. Chemical analysis showed the presence of ovatoxins (OvTX-A and OvTX-B) in Ostreopsis samples in low quantities, in the order of fg.cell −1 and at levels below the limit of quantification (0.04 g.mL −1) in mussels. Morphological observations showed that the cells were ovoid and flattened, large, and ventrally pointed with a dorsoventral diameter (DV) and width (W) of 57- and 33-, respectively, and a dorsoventral/anteroposterior diameter (DV/AP) of about 3.23.

**Keywords** : Ostreopsis cf. siamensis, Ostreopsis cf. ovata, Ovatoxin, Microscopy, LC MS/MS.

AP: Anteroposterior diameter
DV: Dorsoventral diameter
Fg: Femtogram
FW: Fresh Weight
HAB: Harmful Algae Bloom
LOD: Limit Of Detection
LOQ: Limit Of Quantification
McTXs: Mascarenotoxins
OSTs: Ostreocins
OsTXs: Ostreotoxins
OvTX: Ovatoxins
PlTX: Palytoxin
Po: Apical pore
W: Wide

## 1. INTRODUCTION

Primarily considered as a tropical dinoflagellate genus described from the Gulf of Thailand (Siam), the genus *Ostreopsis* was described as *Ostreopsis siamensis* by Schmidt (1901). This genus did not receive major attention until the taxonomic study by Fukuyo (1981), who redefined the type species with the description of two new species, *Ostreopsis ovata*, and *Ostreopsis lenticularis*. Since then, eight new species of Ostreopsis have been described: *O. heptagona* D.R. Norris, J.W. Bomber & Balech (Norris et al., 1985), *O. mascarenensis* J.P. Quod (Quod, 1994), *O. labens* M.A. Faust & S.L. Morton (Faust and Morton, 1995), *O. belizeana* M.A. Faust, *O. caribbeana* M.A. Faust, *O. marina* M.A. Faust (Faust, 1999), *O. fattorussoi* Accoroni, Romagnoli & Totti (Accoroni et al., 2016) and *O. rhodesiae* Verma, Hoppenrath & Murray (Verma et al., 2016). The taxonomic identification of these species has been controversial due to the variability found within the different species and original descriptions using mainly morphological characters such as thecal plate pattern, cell shape, and size for identification (Chomérat et al., 2019).

To date, 20 analogs of PLTX-like compounds have been discovered, including, Ostreocins, Ovatoxins, and Isobaric Palytoxins (Ajani et al., 2017; Gémin et al, 2020).

Massive blooms of *Ostreopsis* threaten human health in some urbanized temperate areas and are responsible for health problems and beach closures (Tester et al., 2020). Indeed, since 2005, in the Mediterranean Sea, *Ostreopsis* has bloomed frequently which caused detrimental effects on marine benthic communities (Neves et al., 2018; Pavaux et al., 2019a) and on human health through skin contact (Tichadou et al., 2010; Tubaro et al., 2011), toxic aerosols (Durando et al., 2007; Villa et al., 2016; Berdalet et al., 2017) and contaminated seafood (Aligizaki et al., 2008; Amzil et al., 2012). These effects have been associated to Ovatoxins, which are analogs of palytoxin (PLTX), a highly toxic, water-soluble molecule originally described from the zoanthid *Palythoa toxica* (Moore and Scheuer, 1971). In the last 10 years, *O.* cf. *ovata* and *O.* cf. *siamensis* have been found in abundance in the Atlantic Ocean (Penna et al., 2010; Rodriguez et al., 2010; Silva et al., 2010; Nascimento et al., 2012; David et al., 2012; 2013; Gomez et al., 2017; Mendes et al., 2017; Machado et al., 2018; Santos et al., 2019; Solino et al., 2020; Drouet et al., 2021; Chomerat et al., 2022). *Ostreopsis* spp. has been detected in the Northeast Atlantic, on macroalgal samples from Madeira and Canary Islands (Penna et al., 2010). In Portugals mainland, *Ostreopsis* cf. *siamensis* was identified for the first time in the southwestern upwelling coast of Sines in seawater and on macroalgae in June 2008, October 2008, and September 2009 (Amorim et al., 2010). In the same year (2008) , these species were detected in the Portuguese mid-Atlantic archipelago of the Azores, along with *O. heptagona* and *O. ovata* (Silva et al., 2010). In 2019, *O.* cf. *siamensis* and *O.* cf. *ovata* were detected in Lagos and Lisbon (Santos et al., 2019).

On the Moroccan coasts, in particular in the south Atlantic coast of Cape Ghir (North of Agadir), specifically in 2004, the species of toxic microalgae in the marine environment were monitored within the framework of the HABs monitoring network set up by the National Institute of Fisheries Research (INRH). *Ostreopsis* species have become more frequent in the recent years and appears with densities that sometimes were alarming, (ranging from 3.7 x $10^3$ cells.$L^{-1}$ in 2004 to 9.8 x $10^3$ cells.$L^{-1}$ in 2007 and 1.2 x $10^4$ cells.$L^{-1}$ in 2008 to reach densities of about $10^5$ cells.$L^{-1}$ in 2009), which led to their inclusion since 2010 in the list of potentially toxic species monitored along the Moroccan coast (Bennouna et al., 2010). Apart from its identification by molecular biology (Bennouna et al., 2010; 2013), no study in Morocco has focused in the' distribution of *Ostreopsis* cf. *siamensis* in the different compartments of the marine environment and on the toxin profile as well as on the possible presence of other accompanying species in the environment.

The objective of this study is to: i) understand the occurrence of *Ostreopsis* in benthic and pelagic forms in the Cape Ghir area during the July 2020 bloom; ii) determines the toxin in the different biological matrices that were collected in the same area (*Ostreopsis* present in benthic and pelagic forms, mussels).

## 2. MATERIALS AND METHODS

### 2.1. Sampling of seawater, macroalgae, and mussels

The study was carried out in the Cape Ghir site (30°39',024N09°53',676W) that was located in the north of Agadir (Morocco) (Figure 1). It was selected through the Moroccan HAB and phycotoxins national monitoring program. This site contains natural mussel *Mytilus galloprovincialis* deposits and is a major tourist attraction. Temperature, salinity, and pH of the area range from 15.18 to 22.53°C, 36.1 to 38.08 psu, 7.83 to 8.75 respectively based on 2020 monitoring results. The geographical position of Morocco near the Canary Islands gives it the privilege of being influenced by one of the five global upwelling currents: The California Current, the Peru Current, the Canary Current, the Benguela Current, and the Somali Current (Makaoui et al., 2005). Upwelling activity is greater in summer than in winter when deep water reaches the surface (Salah et al., 2012; Bessa et al., 2018 et 2019).

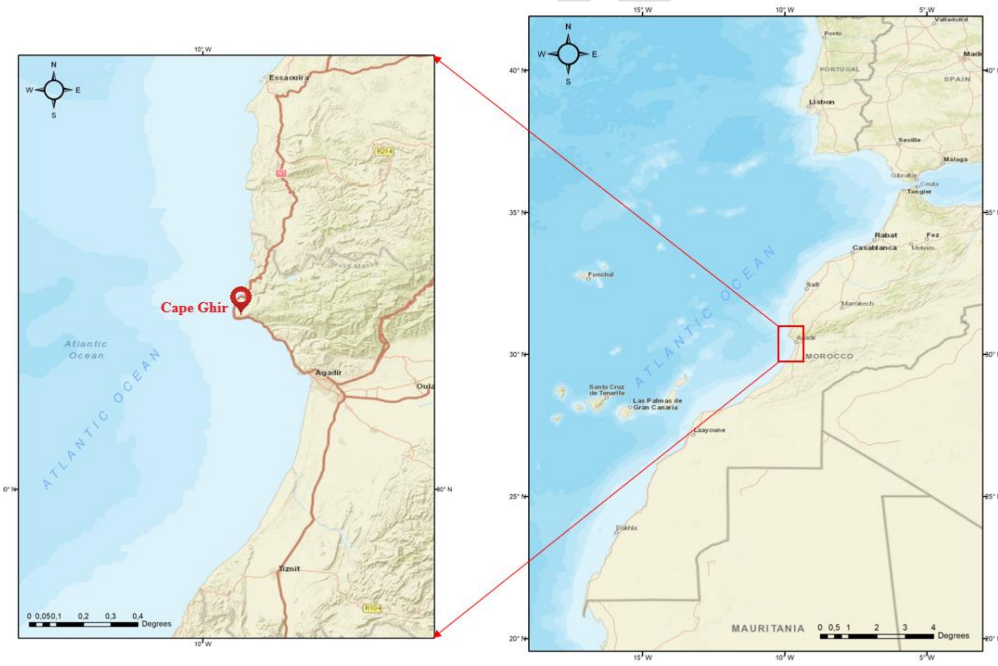



**Figure 1:** Map of Morocco (North Atlantic Ocean) and Agadir, showing the location of the Cape Ghir area where *Ostreopsis* spp were observed and collected as well as the mussels.

Seawater sampling was conducted at high tide (± 2 hours from the peak of high tide) at a depth of 0.5 to 1 meter between July 15 and November 17, 2020, using a sampling bottle. A volume of 25 mL of seawater sample was fixed to Lugol's for observation and enumeration. In addition, seawater samples were filtered through a GF/C filter and stored at -80°C for toxin analysis in pelagic *Ostreopsis* cells.

To detach the epiphytic community, the sampling, collection, and cell counting method was described by (Jauzein et al., 2018). For benthic *Ostreopsis* cells, the macroalgae *Jania* sp. were carefully collected at 1 meter depth in 250 mL plastic bottles.

Briefly, macroalgae were shaken vigorously in the sample bottle for one minute and the resulting water was sieved through 100 and 250 µm mesh to remove large particles. The filtrate was collected, placed in plastic bottles, filtered through GF/C filters and stored at -80 °C for toxin analysis in benthic *Ostreopsis* cells. Macroalgae were placed in plastic bags for fresh weight (FW) determination (Moreira and Tester, 2016). The FW of macroalgae varied from 53 to 230 g.

In parallel to the sampling of *Ostreopsis*, mussels *Mytilus galloprovincialis* were collected from the same study area. These mussels were washed, shelled, the digestive glands were collected, crushed, and stored also at -80 °C for toxin analysis.

### 2.2. Cell abundance

The abundance of *Ostreopsis* spp. cells was carried out at the phytoplankton laboratory of INRH in Agadir by decanting 25 mL of planktonic samples and 5 mL of benthic samples using the Utermöhl method (1958). The samples were analyzed with an inverted microscope equipped with phase-contrast (Leica microsystems, DMi8).

Epiphytic *Ostreopsis* abundance was expressed as cells.$g^{-1}$ FW of macroalgae and planktonic concentrations as cells.$L^{-1}$.

In order to make a statistical comparison between benthic and planktonic species, an analysis was made by ANACONDA an open source tool for Python, PANDAS and SciPy a Python library dedicated to correlation and p-value data analysis and Excel a spreadsheet program available in the MS Office stack.

### 2.3. Analysis of *Ostreopsis* toxins by liquid chromatography coupled to a tandem mass spectrum (LC-MS/MS)

Pelagic, benthic and mussel samples were examined for the presence of palytoxin (PLTX) (isobaric PLTX) and its known analogs Ovatoxins (OVTXs), Ostreocins (OSTs), Mascarenotoxins (McTXs), Ostreotoxins (OsTXs) at the IFREMER PHYTOX Unit (Nantes, France). Samples were analyzed according to the method described by Chomérat et al., (2019). *Ostreopsis* and mussel digestive gland samples were subjected to an extraction step with methanol 100% (quality for LC/MS-MS with a purity > to 99,9%). For the *Ostreopsis* samples, 5mL of methanol was added to the GF/C filters, then after homogenization, passed to the ultrasonic bath for 5 minutes. Four successive extractions were performed with methanol 100%. After centrifugation at 4300G for 5 minutes at 4°C, the supernatants obtained were pooled, evaporated to dryness under nitrogen flow, and finally recovered in 2 mL of methanol 100%. For the mussel samples, 1 g of crushed digestive glands were extracted with three times 3 mL of methanol 100%, homogenized, centrifuged at 4300G for 5 minutes at 4°C. The collected supernatants were evaporated to dryness under nitrogen flow and then resumed in 3 mL of methanol. Before analysis, the methanolic extracts were ultrafiltered on 0.2 µm (Nanosep MF, Pall). Two LC-MS/MS analysis methods using MRM (Multiple Reaction Monitoring) acquisition mode and one LC-UV-MS/MS method were performed to detect isobaric PLTX, 42-OH-PLTX, 12 OVTXs (-a to -k), 4 OSTs (OST-A, -B, -D and -E1), 3 McTXs (-A to -C) and OTX-1 and -3. The analyses were performed on a system using a Nexera UFLC chromatographic chain (Prominence UFLC-XR, Shimadzu, France), coupled to a API 4000QTRAP tandem mass spectrometer (AB Sciex, France) equipped with an electrospray source. The applied parameters are described by Chomérat et al, (2019). Toxins were separated on a Poroshell 120 EC-C18 column (100 x 2.1 mm, 2.7 µm, Agilent) equipped with a pre-column at 25°C. The mobile phases consisted of (A) water and 0.2% acetic acid and (B) 95% acetonitrile and 0.2% acetic acid. The injection volume was 5 µL. Calibration was performed using a Palytoxin standard (Wako Chemicals GmbH, Germany) with a 9-level concentration range. The limit of detection (LOD) and limit of quantification (LOQ) of the system was 0.02 and 0.04µg PLTX /mL, respectively, which is 0.1 and 0.2ng of PLTX injected on the column.

# 3. RESULTS

## 3.1. Morphology of *Ostreopsis* spp.

Cells were ovoid and flattened (Fig. 2-A), pointed toward the ventral region in apical view, with numerous golden to brown peridinin-chloroplasts. The epitheca and the hypotheca were about equal in size. The apical pore (Po) is long and narrow, curved parallel to the cell outline (Fig. 2-A). Plate 1' large and hexagonal, located in the center of the epitheca. Live cells swim with a geotropic orientation around the dorsoventral axis, remaining attached to a substrate most of the time.

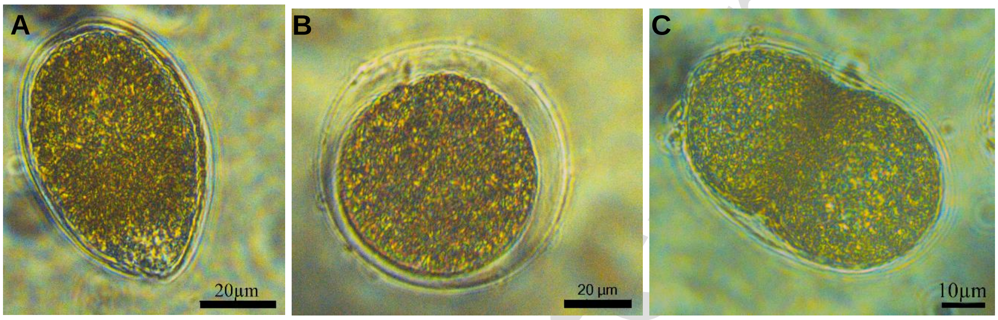



**Figure 2:** Field samples of *Ostreopsis* spp. A: Cell with an oval shape (vegetative cell). B: Cyst with a double membrane. C: Cyst of pairs of round cells covered by a membrane. Scale bars represent: A and B, 20µm; C, 10 µm.

The dorsoventral diameter (DV) of *Ostreopsis* spp ranged from 57.24-83.70 µm (72.44 ± 6.21; n = 60), the width (W) ranged from 33.56-51.61 µm (46.65 ± 6.20; n = 60), the DV/W ratio ranged from 1.10 - 1.91 (1.57 ± 0.17; n = 60). The DV/AP ratio between 1.5-3.95 (3.23 ± 0.78; n = 17). The apical pore (Po) appeared relatively curved on the left dorsal side of the epitheca (Fig. 3-A) with a length between 10.02 and 14.3 (12.18 ± 1.35; n = 15). Plate 1' was hexagonal, elongated, and relatively narrow, contacting plates 2', 3', 5’’, 1", 2", 6", and 7" (Fig. 3-A). The 3' was pentagonal and located toward the left dorsal side of the epithelium (Fig. 3-A). The eight (8) precision plates differed in size and shape. The 2" and 6" were dorsoventrally elongated. The 6" was the largest of the pre-cingulate. The 2" was the smallest. The 2’’’’ was dorso-ventrally elongated. The 2"' plate and 5"' plate are nearly parallel, the latter being smaller. The 3"' and 4"' were the largest post-cingular and quadrangular-shaped plates (Fig. 3-B). The 1"' is the smallest post-cingular while the 1"" was quadrangular (Fig. 3-B).

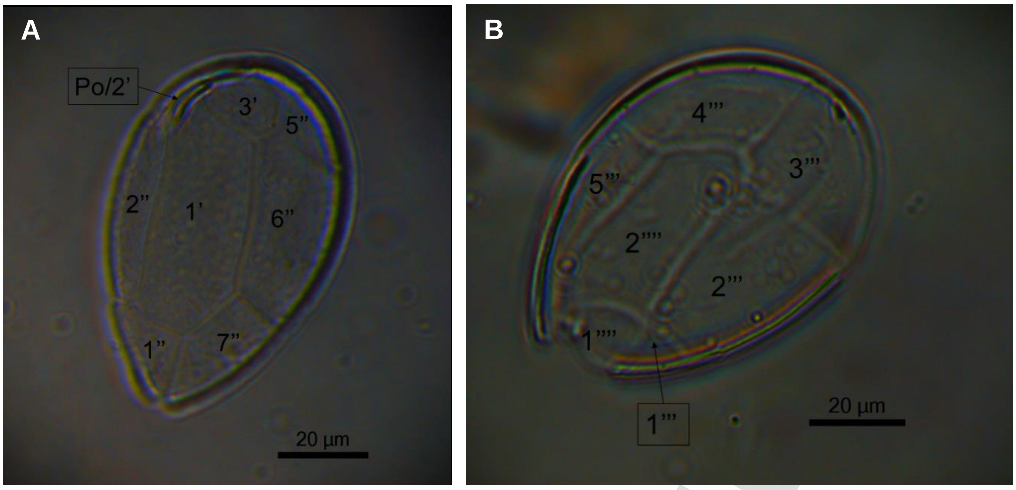



**Figure 3:** Thecae of field samples of *Ostreopsis* spp. collected from Cape Ghir fixed with Lugol's. (A) Epitheca with a curved apical pore (Po), (B) Hypotheca.

### 3.2. Monitoring of *Ostreopsis* spp.

During 2020, *Ostreopsis* spp occurrences started in mid-July until early November, with three peaks blooms for planktonic cells 6.2 x $10^4$ cells.$L^{-1}$; 4.3 x $10^4$ cells.$L^{-1}$ and 9.2 x $10^4$ cells.$L^{-1}$ in late July, early August and mid-August respectively (Figure 4). For benthic cells, three peaks are distinguished in early August, mid-August and late September with concentrations of 647 cells.$g^{-1}$ FW, 1536 cells.$g^{-1}$ FW and 1066 cells.$g^{-1}$ FW respectively (Figure 4). These bloom periods coincide with those generally observed at Cape Ghir each year.

The concentrations of pelagic and benthic *Ostreopsis* spp cells are shown in Figure 4. The maximum values (both concentration in water and number of cells on macrophytes) were recorded on August 19, 2020, as 9.2 x $10^4$ cells.$L^{-1}$ and 1.5 x $10^3$ cells.$g^{-1}$ FW respectively. After this peak, the abundance of *Ostreopsis* spp decreased with several fluctuations both in the water column and on macrophytes until it disappeared completely during the second week of November (Figure 4). During the last weeks of *Ostreopsis* occurrence, cysts were observed in the samples (Fig. 2-B-C). The correlation between benthic and planktonic cells during this study shows a positive correlation of r=0.68 and $p < 0.05$ (Figure 5), which explains that when benthic cells increase, planktonic cells also increase.

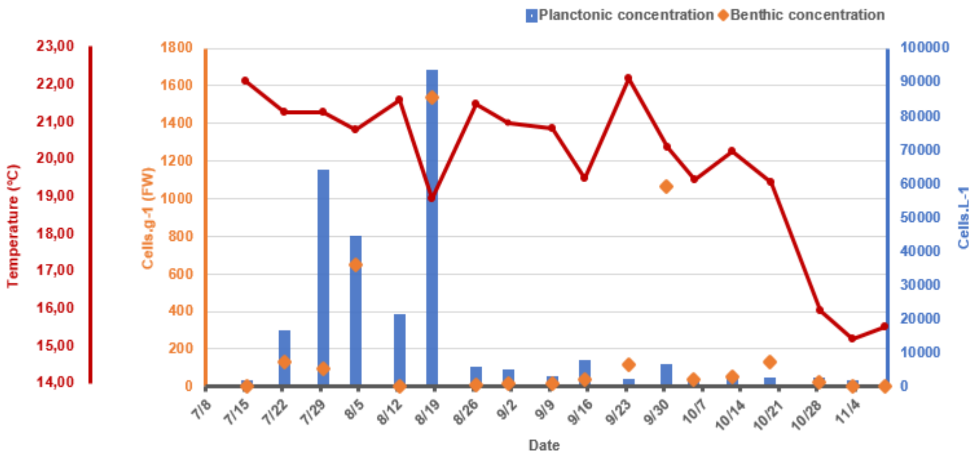
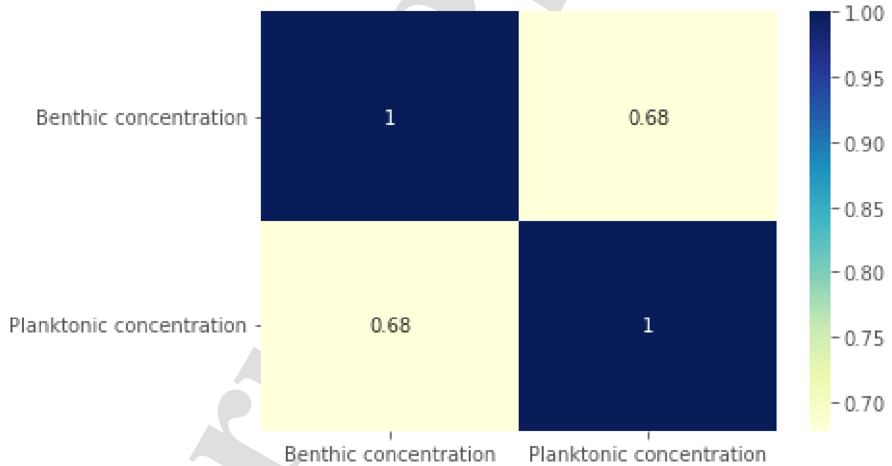



**Figure 4:** Cell concentrations of *Ostreopsis* on the macroalgae *Jania* sp. (Cells. $g^{-1}$ FW), in the water column (Cells.$L^{-1}$) and the temperature (°C) observed and measured at Cape Ghir during the period from 07/15/2020 to 11/17/2020.




| | Benthic concentration | Planktonic concentration |
|---|---|---|
| Benthic concentration | 0.00000 | 0.00198 |
| Planktonic concentration | 0.00198 | 0.00000 |

**Figure 5:** The correlation between benthic and planktonic cells (r=0,68; p=0,002)

### 3.3. Toxin analysis by LC-MS/MS

Among all metabolites targeted in the study, only two molecules, OvTX-A end –B were quantified in two matrices of *Ostreopsis* (pelagic and benthic forms) (Table 1). Total OvTXs (OvTX-A and OvTX-B) ranged from 4.1 to 60.4 fg.cell$^{-1}$ (Table 1). The toxin profile was dominated by OvTX-A (>90%), followed by OvTX-B in the analyzed samples (Figure 6). In mussels, the concentration of OvTX-A was below the limit of quantification (0.04 µg.mL$^{-1}$, or 0.2ng PLTX injected).

As no commercially standards for OvTXs are available, confirmation of the identity of OvTXs was done by mass spectrometry.

**Table 1:** Amount of Ovatoxins (OvTXs) present in the different matrices (LOD = 0.02 µg.mL$^{-1}$ and LOQ = 0.04 µg.mL$^{-1}$).

| Date | Matrix | OvTX-A (fg.cell$^{-1}$) | OvTX-B (fg.cell$^{-1}$) |
|---|---|---|---|
| **29/07/2020** | Pelagic *Ostreopsis* | 60.4 | <LOQ |
| **18/08/2020** | | <LOQ | <LOQ |
| **29/07/2020** | Benthic *Ostreopsis* | <LOQ | <LOD |
| **04/08/2020** | | 9.4 | 4.1 |
| **18/08/2020** | | 11.7 | <LOQ |
| **01/09/2020** | | <LOQ | <LOD |
| **12/08/2020** | Mussels (digestive glands ) | <LOQ | <LOD |

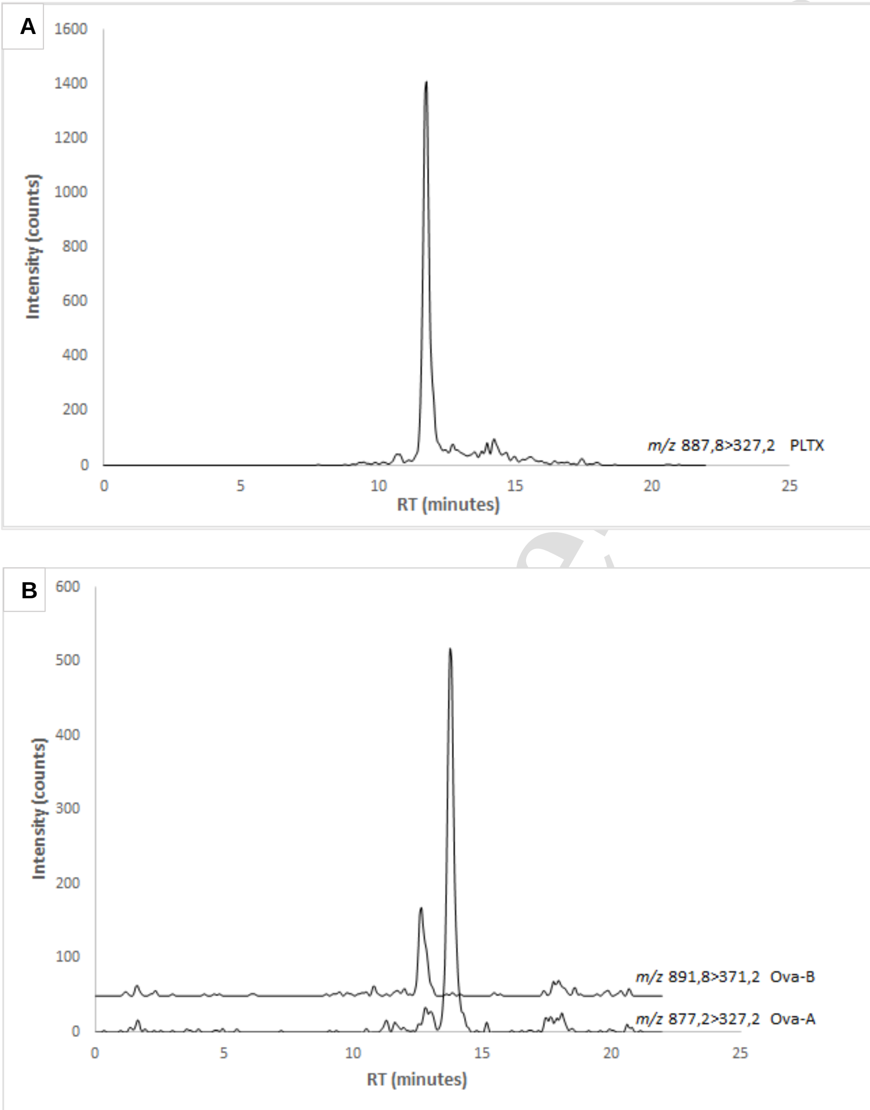



**Figure 6:** Chromatogram of a LC/MS-MS analysis representing the different compounds identified. A: Standard Palytoxin (PlTX) at 0.3 µg.mL$^{-1}$ (i.e. 1.5 ng PLTX on column); B: Ovatoxins (OvTX-A and OvTX-B) in the benthic *Ostreopsis* sample of 08/18/2020.

# 4. DISCUSSION

## 4.1. Morphology of *Ostreopsis* spp. cells

The taxonomy of *Ostreopsis* species is based mainly in cell morphology and thecal plate pattern. However, morphological descriptions of these dinoflagellates are sometimes contradictory, as reported by several authors (Penna et al., 2005 ; Aligizaki and Nikolaidis, 2006). Currently, to distinguish these two species, cell size and especially cell shape expressed as dorsoventral/transdiameter (DV:W) and dorsoventral/anteroposterior (DV:AP) diameter. Ratios have been proposed as distinguishing characteristics for morphological identification supported also by molecular analysis (Penna et al., 2005 ; Aligizaki and Nikolaidis, 2006). In particular, cells of *O.* cf. *siamensis* appear more flattened than those of *O.* cf. *ovata*, showing DV:AP > 4 (Penna et al., 2005) whereas for *O.* cf. *ovata* DV:AP < 2 or ~2 (Penna et al., 2005 ; Aligizaki and Nikolaidis, 2006 ; Selina and Orlova, 2010).

In this study, *Ostreopsis* spp exhibited sizes (DV: 57 - 84 µm, W: 33 - 52 µm, DV:W: 1.1 - 1.9) roughly similar to the *Ostreopsis siamensis* (including *Ostreopsis* cf. *siamensis*) presented elsewhere (Chang et al., 2000 ; Penna et al., 2005 ; Selina and Orlova, 2010 ; David et al., 2013) as well as field *Ostreopsis* cf. *ovata* measured by David et al. (2013) and *ostreopsis* cf.*ovata* in culture measured by Abdennadher et al. (2017) (Table 2). Our cells are shorter than field *O.* cf. *siamensis* measured by Fukuyo (1981) and Faust et al. (1996) and longer than *Ostreopsis* cf. *ovata* measured by Fukuyo (1981) ; Tognetto et al. (1995) ; Chang et al. (2000) ; Aligizaki and Nikolaidis, (2006) ; Monti et al. (2007) and, Selina and Orlova (2010) (Table 2). The DV:AP ratio: 3.2 higher than *O. ovata* and *O.* cf. *ovata* mentioned in Table 2 and close to the ratios of *O.* cf. *siamensis* calculated by Penna et al. (2005) ; Aligizaki and Nikolaidis (2006) and, Selina and Orlova (2010). Moroccan *O.* cf. *siamensis* was previously identified in the coastal waters of Cape Ghir by epifluorescence microscopy and confirmed by molecular biology based on the amplification of the 5,8S-ITS and LSU ribosomal genes (Bennouna et al., 2013). The “Po” was close to that measured by David et al. (2013) in *O.* cf. *siamensis* and *O.* cf. *ovata* from a field found northeast of the Atlantic Ocean (Table 2) as well as that measured by Abdennadher et al. (2017) in the *Ostreopsis* cf. *ovata* in culture collected from the South-Eastern Mediterranean Sea, and smaller than that found in *O.* cf. *siamensis* in eastern China by Faust et al. (1996) (Table 2).

**Table 2:** Morphological data and potential toxicity of *Ostreopsis siamensis*, *O.* cf. *siamensis*, *Ostreopsis ovata* in the world

| Species | DV length (µm) | Width (µm) | DV:W | DV:AP | Po plate (µm) | Potential Toxicity | Compound name | Method of analysis | Ocean/Sea | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| ***O. siamensis*** | 90 F | - | - | - | - | - | - | - | Gulf of Thailand | **Schmidt (1901)** |
| | 60-100 F | 45-90 F | 0,9-1,6 | - | - | - | - | - | Pacific Ocean | Fukuyo (1981) |
| | 60-85 F | 38-45 F | - | - | - | - | - | - | Pacific Ocean | Chang et al. (2000) |
| ***O.* cf. *siamensis*** | 108-123 F | 76-86 F | 1,4* | - | 27 | - | - | - | East China Sea | Faust et al. (1996) |
| | 63-90 F | 34-56 F | - | > 4 | - | + | Putative Palytoxin | Hemolytic test | Mediterranean sea | Penna et al. (2005) |
| | 50-75 C | 38-62 C | | | 7.4-9.7 | | | | | |
| | 36-66 F | 24-50 F | - | 2,8[a] | ≈ 11 | - | - | - | North Aegean Sea | Aligizaki et Nikolaidis (2006) |
| | 63-78 F | 36-54 F | 1,3-1,9 | 2,9[a] | 11-13 | - | - | - | Sea of Japan, Russia | Selina and Orlova (2010) |
| | 50-62 C | 41-50 C | 1,1-1,3 | - | - | Not toxic | - | LC-MS/MS | Atlantic Ocean | Ciminiello et al. (2013) |
| | 52-74 F | 27-57 F | 1,1-2,1 | - | 10,3-11,9 | - | - | - | Atlantic Ocean | David et al. (2013) |
| | 32,3-46,9 C | 27-57 C | 1,2-1,4 | - | - | Not toxic | - | LC-MS/MS | Coral Sea | Verma et al. (2016) |

| Species | DV length (µm) | Width (µm) | DV:W | DV:AP | Po plate (µm) | Potential Toxicity | Compound name | Method of analysis | Ocean/Sea | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| ***O. ovata* (Fukuyo, 1981)** | 50-56 F | 25-35 F | - | - | - | - | - | - | Pacific Ocean | **Fukuyo (1981)** |
| | 34-67 F | 25-40 F | - | - | - | - | - | - | Tyrrhenian Sea | Tognetto et al. (1995) |
| | 38-50 F | 25-35 F | - | - | - | - | - | - | Pacific Ocean | Chang et al. (2000) |
| ***O.* cf. *ovata*** | 27-65 C | 19-57 C | - | < 2 | 6.9-9.6 | + | Putative Palytoxin | Hemolytic test | Mediterranean sea | Penna et al. (2005) |
| | 45-62 F | 28-48 F | 1,77 | 2,07 | - | - | - | - | North Aegean Sea | Aligizaki et Nikolaidis (2006) |
| | 41-59 F | 26-41 F | - | 1,5-2,8 | 6,6-9 | - | - | - | Adriatic Sea | Monti et al. (2007) |
| | 36-60 F | 24-45 F | 1,2-2,3 | 2,1[a] | 6,3-8,3 | - | - | - | Sea of Japan, Russia | Selina and Orlova (2010) |
| | 55-84 F | 30-62 F | 1,2-1,9 | - | 9,6-13,5 | - | - | - | Atlantic Ocean | David et al. (2013) |
| | 30.2-48.3 C | 28.7-3.7 C | 1,1-1,8 | - | - | + | Palytoxin-like compounds , OvTXs, and OSTs | LC-MS/MS | Coral Sea | Verma et al. (2016) |
| | 27-65 C | 19-57 C | 1,11-2,2 | 1,21-2 | 8;82-12,14 | + | - | Mouse bioassay | South-Eastern Mediterranean Sea | Abdennadher et al. (2017) |
| ***Ostreopsis* spp (*siamensis* and *ovata*)** | 57-84 F | 33-52 F | 1,1-1,9 | 3,23 | 10-14,3 | + | Ovatoxins (A and B) | LC-MS/MS | Atlantic Ocean | In this study |

F: Field cell; C: Cells in culture; -: No data available;*: Measured from illustrations; [a]: Mean value; +: Toxic species.

DV: Dorsoventral diameter; W: Width; AP: Anteroposterior diameter; Po: Apical pore. Authors in bold: Original description.

### 4.2. Monitoring of *Ostreopsis* spp.

On the Moroccan Atlantic coast Cape Ghir, the species *O.* cf. *siamensis* was first observed in October 2004 and the species have increased in intensity and frequency since that date. The abundance of *Ostreopsis* in the coastal area of Cape Ghir has followed a marked seasonal trend, with the appearance of these cells generally observed in early summer to late autumn (Bennouna et al., 2010; 2013).

The distribution of *Ostreopsis* spp. fixed on macroalgae, was observed at depth of 1 meter, as well as in the water column. Pelagic and benthic forms of *Ostreopsis* spp. have already been reported by other authors in the Mediterranean Sea (Bomber et al., 1989; Vila et al., 2001; Totti et al., 2010; Accoroni et al., 2011; Amzil et al., 2012; Cohu and Lemée., 2012; Cohu et al., 2013; Brissard et al., 2014; Hachani et al., 2018; Gemin et al., 2020). In our study, positive correlation was observed between planktonic and benthic cells ($r = 0.68$ and $p<0,05$), which corroborates data in the literature (Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008, 2011; Cohu et al., 2013; Jauzein et al., 2018). During the first week of August and the first and third week of September 2020, the increase in benthic cells and the decrease in planktonic cells can be explained by the migration of planktonic cells to macroalgae as it was shown by Pavaux in her thesis in 2019 (Pavaux, 2019b), that benthic *Ostreopsis* cells are present in abundance in the morning at the beginning of the day between 8:00 am and 12:00 pm while maximum abundances are reached later in the water column and at the surface (most often at 4:00pm). This coincided with the date of sample collection between 8:35am and 1:15pm (based on the dates mentioned in the field tags). Except for the case observed at the end of July 2020, where epiphytic cells decreased while planktonic cells increased, this was due to the sample collection time being at 4:00pm (based on the dates mentioned in the field tags). As in previous summers in the South Atlantic of Morocco, the proliferation of *Ostreopsis* was therefore again noted in this area during the summer of 2020 (Bennouna et al., 2010 ; 2013). Noting that this has been recorded in several seas (Italy, Sines, Cascais, Iberian Peninsula Selvagens Islands), where maximum concentrations of *Ostreopsis* are recorded in summer-early autumn (Ciminiello et al., 2013 ; David et al., 2013 ; Santos et al., 2019 ; Solino et al., 2020).

The presence of cysts in macroalgal samples that were collected during the decline phase of *Ostreospsis* could be related to the stressful conditions of the environment, particularly the decrease in temperature in the late autumn-early winter period, as reported in the literature (Aligizaki and Nikolaidis 2006; Bravo et al., 2012; Accoroni et al., 2014). Indeed, these authors report that *Ostreopsis* cysts can only germinate if water temperatures reach 25°C.

### 4.3. Toxin analysis by LC-MS/MS

The results of physicochemical analyses by LC-UV-MS/MS from the collected *Ostreopsis* samples show the presence of OvTXs, toxins produced by *O.* cf. *ovata*, and *O. fattorussoi* species. Indeed, Ciminiello et al, (2010 and 2012a) showed by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) that the species *O. ovata* is the origin of the production of OVTXs. Tartaglione et al. (2017) showed that the species *O. fattorussoi* from Cyprus (Mediterranean Sea), produces OVTX- i, -j1, -j2, and -k. In contrast Accoroni et al. (2016) showed that Lebanese *O. fattorussoi* produces OVTX-a and its structural isomers OVTX-d and -e, but does not produce OvTX-i, -j1, -j2, and -k.

The presence of the toxic species *O.* cf. *ovata*, reported by Solino et al. (2020) in the waters of Selvagens Islands (located in the same latitude as Cape Ghir - Agadir) supports the probability of the presence of *O.* cf. *ovata* on these Atlantic coasts of Morocco. In addition, *O.* cf. *ovata* has already been recorded in the West Atlantic Sea, including the Caribbean and Brazil (Moreira, 2010 ; Nascimento et al., 2012 ; Gomez et al., 2017 ; Mendes et al., 2017 ; Machado et al., 2018). *O.* cf. *ovata* strains have been identified in temperate areas of the eastern Atlantic, such as Madeira (Penna et al., 2010), the Azores (Silva et al., 2010), and southern mainland Portugal (David et al., 2012 ; Santos et al., 2019).

The absence of known toxins (Ostreocins: OST-A, -B, -D, E1) produced by *O. siamensis* suggests that the Moroccan *O.* cf. *siamensis* strain is not toxic as shown by Amonim et al. (2010) and Ciminiello et al. (2013) in *O.* cf. *siamensis* strains isolated from Sines and Cascais in Portugal and by Verma et al. (2016) on *O.* cf. *siamensis* strains isolated in Australia. Guerrini et al. (2010) have been shown that species toxicity is highly dependent on geographical population differences and environmental conditions. On the other hand, Penna et al. (2005) showed that all samples isolated from *O.* cf. *siamensis* from the Mediterranean Sea were toxic by hemolytic test while Ciminiello et al. (2013) detected the presence of a low amount of palytoxin (in the order of fg.cell$^{-1}$) in samples from the Mediterranean Sea.

The presence of low concentrations of OvTX-A and OvTX-B detected by LC-MS/MS in benthic and pelagic *Ostreopsis* can probably be explained by the presence of *Ostreopsis ovata* with *O.* cf. *siamensis* in the Cape Ghir area. In the Northeast Atlantic, the known distribution of *O.* cf. *siamensis* suggests that this species has a wider distribution than *O.* cf. *ovata* (Amorim et al., 2010). Drouet el al. (2021), showed the colonization of the NE Atlantic coasts by *O.* cf. *siamensis* from the northern part of the Bay of Biscay to the entrance of the English Channel, as well as the formation of blooms of this species in the southern Bay of Biscay. Laza-Martinez et al. (2011) ; David et al. (2013) ; Seoane and Siano (2018) showed that only *O.* cf. *siamensis* has been observed in the northern part of the coast line of the Cantabrian Sea. David et al. (2013) suggest that *O.* cf. *siamensis* may be more adapted to colder waters than *O.* cf. *ovata*. During summer blooms of *Ostreopsis* on French Basque coast (Atlantic) in 2021, Chomérat et al (2022) report, for the first time, the presence of both *O.* cf. *siamensis* together with *O.* cf. *ovata*, for which French Basque coast appears as a new upper distribution limit. In addition, the presence of OvTXs -A, -B, in the field sample and in a cultivated strain in culture confirmed the toxic nature of the bloom and allowed to identify *O.* cf. *ovata* as the producer.

The most common strain contains OvTX-A as the main analog, followed by OvTX-B (Nascimento et al., 2012; Brissard et al., 2014; Ciminiello et al., 2012a; García Altares et al., 2015; Carnicer et al., 2016; Ninčević Gladan et al., 2019).

In the Cape Ghir area, the toxin profile with a dominance of OvTX-A followed by OvTX-B, has already been identified in strains of *O.* cf. *ovata* from the Mediterranean Sea and Brazil (Gémin et al., 2020 ; Nascimento, et al., 2020), The analysis of toxin profile of fifty-five *O.* cf. *ovata* strains isolated from the Mediterranean Sea shows, qualitative variability (eight different analogues of OvTXs) and intra-specific quantitative toxin content (Tartaglione et al., 2017).

In the Cape Ghir area, in the *Mytilus galloprovincialis*, only OvTX-A is detected at a level below the limit of quantification (< 0.04 µg.mL$^{-1}$, 0.2 ng PLTX injected on column of LC/MS-MS).

## 5. CONCLUSION

At the Cape Ghir, the screening for palytoxins like showed that only ovatoxins (OvTX-A, -B) were quantified in field samples of pelagic and benthic *Ostreopsis*, and were detected in mussels. The toxin profile with OVTX-A and -B as predominant compounds in the field sample corroborate the presence of *O.* cf. *ovata* since these molecules have only been find in that species and they were likely produced by this taxon in the bloom of *Ostreopsis* spp. . The toxins of *O. siamensis* (ostreocins) were not detected.in these samples. Taxonomic and chemical analyses of *Ostreopsis* spp. In field samples suggest that two of the morphotypes, *O.* cf. *ovata*, and *O.* cf. *siamensis*, coexist at the Cape Ghir area (Agadir, Morocco). Confirmation of these species will require a genetic approach on isolated field cells and from cultivated strains,

Further ecological studies aiming at understanding the population dynamics and ecological preferences of both *Ostreopsis* species at Cape Ghir should address this question in the future, as it is important for bloom and risk anticipation.

**AUTHOR CONTRIBUTIONS**

- Field sampling: HA, MA, AB.
- Microscopic observations and enumeration: HA, AB, CM.
- Acquisition and analysis of physicochemical data (including extraction steps): FH.
- Writing and preparation of the article: HA, AB.
- Writing, editing, and validation: HA, RA, AB, SE, ZA, FH, MB, AF.

**FUNDING SOURCES**

This research did not receive any specific grant from funding agencies in the public, commercial, or nonprofit sectors.

**ACKNOWLEDGMENTS**

This work was supported by the National Institute of Fisheries Research (INRH), region center Agadir. Thanks to the University of Hassan II Casablanca and the Faculty of Sciences Aïn Chock that allowed this collaboration. The authors thank the Director-General of INRH Mr. Abdelmalek FARAJ and the Dean of the Faculty of Science Dr. Omar SADDIQI for their material support during the study. We also thank Mr. Mohamed ALAHYANE, Miss Safaa CHAFI, and Mr. ARBABE for their technical support during the study.

**REFERENCES**


Abdennadher, M., Zouari, A. B., Sahnoun, W. F., Alverca, E., Penna, A., Hamza, A. 2017. *Ostreopsis* cf. *ovata* in the Gulf of Gabès (south-eastern Mediterranean Sea): morphological, molecular and ecological characterization. *Harmful Algae, 63*, 56-67. *doi:* 10.1016/j.hal.2017.01.009

Accoroni, S., Romagnoli, T., Colombo, F., Pennesi, C., Di Camillo, C. G., Marini, M., Battocchi, C., Ciminiello, P., Dell'Aversano, C., Iacovo, E. D., Fattorusso, E., Tartaglione, L., Penna, Totti, C., 2011. *Ostreopsis* cf. *ovata* bloom in the northern Adriatic Sea during summer 2009: Ecology, molecular characterization and toxin profile. *Marine pollution bulletin, 62*(11), 2512-2519. *doi :* 10.1016/j.marpolbul.2011.08.003

Accoroni, S., Romagnoli, T., Pichierri, S., Totti, C., 2014. New insights on the life cycle stages of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*. *Harmful Algae 34*, 7-16. *doi:* 10.1016/j.hal.2014.02.003

Accoroni, S., Romagnoli, T., Penna, A., Capellacci, S., Ciminiello, P., Dell'Aversano, C., Tartaglione, L., Abboud-Abi Saab, M., Giussani, V., Asnaghi, V., Chiantore, M., Totti, C., 2016. *Ostreopsis fattorussoi* sp. nov. (Dinophyceae), a new benthic toxic *Ostreopsis* species from the eastern Mediterranean Sea. *J. Phycol. 52* (6), 1064–1084. *doi:* 10.1111/jpy.12464

Ajani, P., Harwood, D. T., Murray, S. A., 2017. Recent trends in marine phycotoxins from Australian coastal waters. *Marine drugs, 15*(2), 33. *doi:* 10.3390/md15020033

Aligizaki, K., Nikolaidis, G., 2006. The presence of the potentially toxic genera *Ostreopsis* and *Coolia* (Dinophyceae) in the North Aegean Sea, Greece. *Harmful Algae 5* (6), 717–730. *doi:* 10.1016/j.hal.2006.02.005

Amorim, A., Veloso, V., Battocchi, C., Penna, A., 2010. First detection of *Ostreopsis* cf. *siamensis* in Portuguese coastal waters. In *14th International Conference on Harmful Algal*, 133-133. UNESCO.

Amzil Z., Sibat M., Chomerat N., Grossel H., Marco-Miralles F., Lemee R., Nezan E., Sechet V., 2012. Ovatoxin-a and Palytoxin Accumulation in Seafood in Relation to

*Ostreopsis* cf. *ovata* Blooms on the French Mediterranean Coast. *Marine Drugs*, 10(2), 477-496. *doi*: 10.3390/md10020477

Bennouna, A., El Attar, J., Abouabdellah, R., Palma, S., Penna, A., Moita, M.T., 2010. First record of *Ostreopsis* cf. *siamensis* in Moroccan Atlantic upwelling waters. *Harmful Algae News* N°42, 1-3.

Bennouna, A., El Attar, J., Abouabdellah, R., Chafik, A., Penna, A., Oliveira, P. B., Palma, S., Moita, M. T., 2013. *Ostreopsis* cf. *siamensis* blooms in Moroccan Atlantic Upwelling waters (2004-2009). Proceeding of the *14th International Conference on Harmful Algae*, 19-22.

Bessa, I., Makaoui, A., Hilmi, K., & Afifi, M., 2018. Variability of the mixed layer depth and the ocean surface properties in the Cape Ghir region, Morocco for the period 2002–2014. *Modeling Earth Systems and Environment*, 4(1), 151-160. *doi:* 10.1007/s40808-018-0411-7

Bessa, I., Makaoui, A., Agouzouk, A., Idrissi, M., Hilmi, K., & Afifi, M., 2019. Seasonal variability of the ocean mixed layer depth depending on the cape Ghir filament and the upwelling in the Moroccan Atlantic coast. *Materials Today: Proceedings*, 13, 637-645. *doi;*10.1016/j.matpr.2019.04.023

Bomber, J.W., Rubio, M.G., Norris, D.R., 1989. Epiphytism of dinoflagellates associated with the disease ciguatera substrate specificity and nutrition. *Phycologia 28* (3), 360–368. *doi:* 10.2216/i0031-8884-28-3-360.1

Bravo, I., Vila, M., Casabianca, S., Rodriguez, F., Rial, P., Riobó, P., & Penna, A., 2012. Life cycle stages of the benthic palytoxin-producing dinoflagellate *Ostreopsis* cf. *ovata* (Dinophyceae). *Harmful algae 18*, 24-34. *doi:* 10.1016/j.hal.2012.04.001

Brissard, C., Herrenknecht, C., Séchet, V., Hervé, F., Pisapia, F., Harcouet, J., Lemée, R., Chomérat, N., Hess, P., Amzil, Z., 2014. Complex toxin profile of French Mediterranean *Ostreopsis* cf. *ovata* strains, seafood accumulation and ovatoxins prepurification. *Mar. Drugs 12* (5), 2851‑2876. *doi:* 10.3390/md12052851

Carnicer, O., García-Altares, M., Andree, K. B., Diogène, J., and Fernández-Tejedor, M., 2016. First evidence of *Ostreopsis* cf. *ovata* in the eastern tropical Pacific Ocean, Ecuadorian coast. *Botanica Marina, 59*(4), 267-274. *doi :* 10.1515/bot-2016-0022

Chang, F.H., Shimizu, Y., Hay, B., Stewart, R., Mackay, G., Tasker, R., 2000. Three recently recorded *Ostreopsis* spp. (Dinophyceae) in the New Zealand: temporal and regional distribution in the upper North Island from 1995 to 1997. *New Zealand Journal of Marine and Freshwater Research, 34,* 29–39. *doi :* 10.1080/00288330.2000.9516913

Chomérat, N., Bilien, G., Derrien, A., Henry, K., Ung, A., Viallon, J., Taiana, D.H., Mahana, I.G.C., Roué, M., Hervé, F., Réveillon, D., Amzil, Z., and Chinain, M., 2019. *Ostreopsis lenticularis* Y. Fukuyo (Dinophyceae, Gonyaulacales) from French Polynesia (South Pacific Ocean): A revisit of its morphology, molecular phylogeny and toxicity. *Harmful Algae, 84*, 95-111. *doi :* 10.1016/j.hal.2019.02.004

Chomérat N., Antajan E., Auby I., Bilien G., Carpentier L., De Casamajor M.N., Ganthy F., Hervé F., Labadie M., Méteigner C., Paradis C., Perrière-Rumèbe M., Sanchez F., Séchet V. and Amzil Z., 2022. First Characterization of *Ostreopsis* cf. *ovata* (Dinophyceae) and Detection of Ovatoxins during a Multispecific and Toxic *Ostreopsis* Bloom on French Atlantic Coast. Drugs 20, 461. *doi:*10.3390/md20070461

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, G.S., Tartaglione, L., Grillo, C., Melchiorre, N., 2006. The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. *Anal. Chem. 78*, 6153–6159. *doi:* 10.1021/ac060250j

Ciminiello, P., Dell'Aversano, C., Iacovo, E. D., Fattorusso, E., Forino, M., Grauso, L., Tartaglione, L., Guerrini, F., and Pistocchi, R., 2010. Complex palytoxin- like profile of *Ostreopsis ovata*. Identification of four new ovatoxins by high- resolution liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry, 24*(18), 2735-2744. *doi:* 10.1002/rcm.4696

Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Forino, M., Grauso, L., Tartaglione, L., Guerrini, F., Pezzolesi, L., Pistocchi, R., Vanucci, S., 2012a. Isolation and structure elucidation of ovatoxin-a, the major toxin produced by *Ostreopsis ovata*. *Journal of the American Chemical Society*. *134* (3), 1869–1875. *doi:* 10.1021/ja210784u

Ciminiello, P., Dell'Aversano, C., Iacovo, E.D., Fattorusso, E., Forino, M., Tartaglione, L., Battocchi, C., Crinelli, R., Carloni, E., Magnani, M., Penna, A., 2012b. Unique toxin profile of a Mediterranean *Ostreopsis* cf. *ovata* strain: HR LC-MS (n) characterization of ovatoxin-f, a new palytoxin congener. *Chemical Research in Toxicology*. *25* (6), 1243–1252. *doi:* 10.1021/tx300085e

Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., Yasumoto, T., Battocchi, C., Giacobbe, M., Amorim, A., Penna, A., 2013. Investigation of toxin profile of Mediterranean and Atlantic strains of *Ostreopsis* cf. *siamensis* (Dinophyceae) by liquid chromatography-high resolution mass spectrometry. *Harmful Algae 23,* 19–27. *doi:* 10.1016/j.hal.2012.12.002

Cohu, S., Lemée, R., 2012. Vertical distribution of the toxic epibenthic dinoflagellates *Ostreopsis* cf. *ovata*, *Prorocentrum lima* and *Coolia* monotis in the NW Mediterranean Sea. *Cahier de Biologie Marine. 53,* 373–380.

Cohu, S., Mangialajo, L., Thibaut, T., Blanfuné, A., Marro, S., Lemée, R., 2013. Proliferation of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation to depth, biotic substrate and environmental factors in the North West Mediterranean Sea. *Harmful Algae 24*, 32–44. *doi:* 10.1016/j.hal.2013.01.002

David, H., Moita, M. T., Laza-Martínez, A., Silva, A., Mateus, M., Pablo, H., Orive, E., 2012. First bloom of *Ostreopsis* cf. *ovata* in the continental Portuguese coast. *Harmful algae news*, 45, 12-13.

David, H., Laza-Martínez, A., Miguel, I., and Orive, E., 2013. *Ostreopsis* cf. *siamensis* and *Ostreopsis* cf. *ovata* from the Atlantic Iberian Peninsula: Morphological and phylogenetic characterization. *Harmful algae, 30,* 44-55. *doi:* 10.1016/j.hal.2013.08.006

Drouet, K., Jauzein, C., Herviot- Heath, D., Hariri, S., Laza- Martinez, A., Lecadet, C., Plus, M., Seoane, S., Sourisseau, M., Lemée, R., Siano, R., 2021. Current distribution and potential expansion of the harmful benthic dinoflagellate *Ostreopsis* cf. *siamensis* towards the warming waters of the Bay of Biscay, North- East Atlantic. *Environmental Microbiology*, 23(9), 4956-4979. *doi:* 10.1111/1462-2920.15406

Durando, P.; Ansaldi, F.; Oreste, P.; Moscatelli, P.; Marensi, L.; Grillo, C.; Gasparini, R.; Icardi, G.; Surveillance & Collaborative Group for the Ligurian Syndromic Algal Surveillance, 2007. *Ostreopsis ovata* and human health: epidemiological and clinical features of respiratory syndrome outbreaks from a two-year syndromic surveillance, 2005-06, in north-west Italy. Weekly releases (1997–2007), 12(23), 3212. *doi:* 10.2807/esw.12.23.03212-en

Faust, M. A. Morton, S. L., 1995. Morphology and ecology of the marine dinoflagellate *Ostreopsis labens* sp. nov. (Dinophyceae). *Journal of Phycology*. 31, 456-463. *doi:* 10.1111/j.0022-3646.1995.00456.x

Faust, M. A., 1999. Three new *Ostreopsis* species (Dinophyceae): *O. marinus* sp. nov., *O. belizeanus* sp. nov., and *O. carribeanus* sp. nov. *Phycologia* 38(2), 92–99. *doi:* 10.2216/i0031-8884-38-2-92.1

Fukuyo, Y., 1981. Taxonomical study on benthic dinoflagellates collected in Coral Reefs. *Bull. Japanese Society Scientific Fisheries, 47,* 967–978. *doi:* 10.2331/suisan.47.967

Gémin, M.-.P., Réveillon, D., Hervé, F., Pavaux, A. S., Tharaud, M., Séchet, V., Bertrand, S., Lemée, R., Amzil, Z., 2020. Toxin content of *Ostreopsis* cf. *ovata* depends on bloom phases, depth and macroalgal substrate in the NW Mediterranean Sea. *Harmful Algae;* 92-101727. *doi:* 10.1016/j.hal.2019.101727

Gladan, Ž.N., Arapov, J., Casabianca, S., Penna, A., Honsell, G., Brovedani, V., Pelin, M., Tartaglione, L., Sosa, S., Dell'Aversano, C., Tubaro, A., Žuljevic, A., Grbec, B., Cavar, M., Bužanˇci´, M., Bakra A., and Skejić, S., 2019. Massive occurrence of the harmful benthic dinoflagellate *Ostreopsis* cf. *ovata* in the Eastern Adriatic Sea. *Toxins, 11*(5), 300. *doi:* 10.3390/toxins11050300

Gomez, F., Qiu, D., Lopes, R. M., Lin, S., 2017. Morphological and molecular characterization of the toxic dinoflagellate *Ostreopsis* cf. *ovata* (Gonyaulacales:

Dinophyceae) from Brazil (South Atlantic Ocean). *Revista de Biología Tropical*, *65*(3), 1022-1032. *doi:* 10.15517/rbt.v65i3.26263

Guerrini, F., Pezzolesi, L., Feller, A., Riccardi, M., Ciminiello, P., Dell'Aversano, C., Tartaglione, L.,Dello Iacovo, E., Fattorusso, E., Forino, M., Pistocchi, R., 2010. Comparative growth and toxin profile of cultured *Ostreopsis ovata* from the Tyrrhenian and Adriatic Seas. *Toxicon*, *55*(2-3), 211-220. *doi:* 10.1016/j.toxicon.2009.07.019

Hachani, M. A., Dhib, A., Fathalli, A., Ziadi, B., Turki, S., Aleya, L., 2018. Harmful epiphytic dinoflagellate assemblages on macrophytes in the Gulf of Tunis. *Harmful algae*, *77*, 29-42. *doi:* 10.1016/j.hal.2018.06.006

Jauzein, C., Açaf, L., Accoroni, S., Asnaghi, V., Fricke, A., Hachani, M. A., Lemée, R. 2018. Optimization of sampling, cell collection and counting for the monitoring of benthic harmful algal blooms: application to *Ostreopsis* spp. blooms in the Mediterranean Sea. *Ecological Indicators*, 91, 116-127. *doi*: 10.1016/j.ecolind.2018.03.089

Laza-Martinez, A., Orive, E., Miguel, I., 2011. Morphological and genetic characterization of benthic dinoflagellates of the genera *Coolia*, *Ostreopsis* and *Prorocentrum* from the southeastern Bay of Biscay. *Eur J Phycol* 46: 45–65. *doi:* 10.1080/09670262.2010.550387

Machado, R. C. A., Feitosa, F. A., Koening, M. L., and Montes, M. F., 2018. Spatial and seasonal variation of the phytoplankton community structure in a reef ecosystem in North-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom*, 98(3), 557-566. *doi:* 10.1017/S0025315416001600

Makaoui, A., Orbi, A., Hilmi, K., Zizah, S., Larissi, J., Talbi, M., 2005. L'upwelling de la côte atlantique du Maroc entre 1994 et 1998. *Comptes Rendus Geoscience*, 337(16), 1518-1524. *doi:* 10.1016/j.crte.2005.08.013

Mangialajo, L., Bertolotto, R., Cattaneo-Vietti, R., Chiantore, M., Grillo, C., Lemee, R., Melchiorre, N., Moretto, P., Povero, P., Ruggieri, N., 2008. The toxic benthic dinoflagellate *Ostreopsis ovata*: Quantification of proliferation along the coastline of Genoa, Italy. *Marine pollution bulletin*, *56*(6), 1209-1214. *doi:* 10.1016/j.marpolbul.2008.02.028

Mangialajo, L., Ganzin, N., Accoroni, S., Asnaghi, V., Blanfuné, A., Cabrini, M., CattaneoVietti, R., Chavanon, F., Chiantore, M., Cohu, S., Costa, E., Fornasaro, D., Grossel, H., Marco-Miralles, F., Masó, M., Reñé, A., Rossi, A.M., Sala, M.M., Thibaut, T., Totti, C., Vila, M., Lemée, R., 2011. Trends in *Ostreopsis* proliferation along the Northern Mediterranean coasts. *Toxicon 57* (3), 408–420. *doi:* 10.1016/j.toxicon.2010.11.019

Mendes, M. C. D. Q., Nunes, J. M. C., Menezes, M., Fraga, S., Rodríguez, F., Vázquez, J. A., Blanco, J., Franco, J.M., and Riobó, P., 2017. Toxin production, growth kinetics and molecular characterization of *Ostreopsis* cf. *ovata* isolated from Todos os Santos Bay, tropical southwestern Atlantic. *Toxicon 138*, 18-30. *doi:* 10.1016/j.toxicon.2017.08.007

Moore, R. E., Scheuer, P. J., 1971. Palytoxin: a new marine toxin from a coelenterate. *Science, 172*(3982), 495-498. *doi:* 10.1126/science.172.3982.495

Moreira G. A. R., 2010. *Ostreopsis ovata* Fukuyo y *O. siamensis* Schmidt, dos nuevos registros de dinoflagelados bentónicos tóxicos para Cuba. http://hdl.handle.net/1834/4534

Moreira, A. and Tester, A. P., 2016. Methods for sampling benthic microalgae. Guide for designing and implementing a plan to monitor toxin-producing microalgae. 2nd Edition. Paris, France & Vienna, Austria, *UNESCO & IAEA*, 66pp. (IOC Manuals and Guides, 77). *doi:* 10.25607/OBP-1372

Nascimento, S. M., Corrêa, E. V., Menezes, M., Varela, D., Paredes, J., Morris, S., 2012. Growth and toxin profile of *Ostreopsis* cf. *ovata* (Dinophyta) from Rio de Janeiro, Brazil. *Harmful algae*, *13*, 1-9. *doi:* 10.1016/j.hal.2011.09.008

Nascimento, S. M, Neves, R. A. F., De'Carli, G. A. L., Borsato, G. T., da Silva, R. A. F., Melo, G. A., de Morais, A. M., Cockell, T. C., Fraga, S., Menezes-Salgueiro, A. D., 2020. *Ostreopsis* cf. *ovata* (Dinophyceae) Molecular Phylogeny, 825 Morphology, and Detection of Ovatoxins in Strains and Field Samples from Brazil. *Toxins* 12, 70, 826. *doi:* 10.3390/toxins12020070.

Neves, R.A.F., Contins, M., Nascimento, S.M., 2018. Effects of the Toxic Benthic Dinoflagellate *Ostreopsis* cf. *ovata* on Fertilization and Early Development of the Sea

Urchin Lytechinus Variegatus. *Marine Environmental. Research.* 135, 11–17, *doi:* 10.1016/j.marenvres.2018.01.014.

Norris, D. R., Bomber, J. W. Balech, E., 1985. Benthic dinoflagellates associated with ciguatera from the Florida Keys. I. *Ostreopsis heptagona* sp. nov. In: (D. M. Anderson, A. W. White and D. G. Baden, eds) Toxic Dinoglagellates. *Elsevier Science Publ. Co., New York*, pp. 39-44.

Pavaux, A. S., Rostan, J., Guidi-Guilvard, L., Marro, S., Ternon, E., Thomas, O.P., Lemée, R., Gasparini, S., 2019a. Effects of the Toxic Dinoflagellate *Ostreopsis* cf. *ovata* on Survival, Feeding and Reproduction of a Phytal Harpacticoid Copepod. *J. Exp. Mar. Biol. Ecol.* 516, 103–113. *doi:* 10.1016/j.jembe.2019.05.004.

Pavaux A.S., 2019b. Ecologie chimique du dinoflagellé toxique *Ostreopsis* cf. *ovata* en Méditerranée Nord Occidentale. Laboratoire d'Océanographie de Villefranche. Sorbonne Université.

Penna, A., Vila, M., Fraga, S., Giacobbe, M.G., Andreoni, F., Riobó, P., Vernesi, C., 2005. Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the Western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8S rDNA sequences. *Journal of Phycology*, 41(1), 212-225. *doi:* 10.1111/J.1529-8817.2005.04011.x

Penna, A., Fraga, S., Battocchi, C., Casabianca, S., Giacobbe, M. G., Riobó, P., Vernesi, C., 2010. A phylogeographical study of the toxic benthic dinoflagellate genus *Ostreopsis* Schmidt. *Journal of biogeography*, 37(5), 830-841. *doi:* 10.1111/j.1365-2699.2009.02265.x

Quod, J. P., 1994. *Ostreopsis mascarenensis* sp. nov (Dinophyceae) dinoflagellé toxique associé à la ciguatéra dans l'Océan Indien. *Cryptogamie Algologie*. 15:243–251.

Rhodes, L., Towers, N., Briggs, L., Munday, R., Adamson, J., 2002. Uptake of palytoxin-like compounds by shellfish fed *Ostreopsis siamensis* (Dinophyceae). *New Zealand Journal of Marine and Freshwater Research*, *36*(3), 631-636. *doi:* 10.1080/00288330.2002.9517118

Salah, S., Ettahiri, O., Berraho, A., Benazzouz, A., Elkalay, K., & Errhif, A., 2012. Distribution des copépodes en relation avec la dynamique du filament de Cap Ghir, (Côte atlantique du Maroc). Comptes Rendus Biologies, 335(2), 155-167. *doi :* 10.1016/j.crvi.2011.11.008

Santos, M., Oliveira, P.B., Moita, M.T., David, H., Caeiro, M.F., Zingone, A., Amorim, A., Silva, A., 2019. Ocurrence of *Ostreopsis* in two temperate coastal bays (SW Iberia): insights from the plankton. *Harmful Algae 86,* 20–36. *doi:* 10.1016/j.hal.2019.03.003

Schmidt, J., 1901. Preliminary report of the botanical results of the Danish Expedition to Siam (1899-1900). Pt. IV, Peridinales. Bot. Pidsslerift. 24, 212–221.

Selina, M.S., Orlova, T.Y., 2010. First occurrence of the genus *Ostreopsis* (Dinophyceae) in the Sea of Japan. *Botanica Marina,* 53, 243–249. *doi:* 10.1515/BOT.2010.033

Seoane, S. and Siano, R., 2018. New limits of *Ostreopsis* distribution in the Bay of Biscay: a first report of *Ostreopsis* in Santander Bay, Cantabria (Northern Spain). *Harmful Algae News* 60: 10.

Silva, A., Brotas, V., Orive, E., and Neto, A. I., 2010. First records of *Ostreopsis heptagona*, *O.* cf. *siamensis* and *O.* cf. *ovata*–in the Azores archipelago, Portugal. *Harmful algae news*, 42, 1-2. http://hdl.handle.net/10400.3/1200

Solino, L., García-Altares, M., Godinho, L., & Costa, P. R., 2020. Toxin profile of *Ostreopsis* cf. *ovata* from Portuguese continental coast and Selvagens Islands (Madeira, Portugal). *Toxicon*, 181, 91-101. *doi:* 10.1016/j.toxicon.2020.04.102

Tartaglione, L., Dello Iacovo, E., Mazzeo, A., Casabianca, S., Ciminiello, P., Penna, A., and Dell'Aversano, C., 2017. Variability in Toxin Profiles of the Mediterranean *Ostreopsis* cf. *ovata* and in Structural Features of the Produced Ovatoxins. *Environmental science & technology*, *51*(23), 13920-13928. *doi:* 10.1021/acs.est.7b03827

Tester, P. A., Litaker, R. W., Berdalet, E., 2020. Climate change and harmful benthic microalgae. *Harmful Algae*, 91, 101-655. *doi:* 10.1016/j.hal.2019.101655

Tichadou, L., Glaizal, M., Armengaud, A., Grossel, H., Lemée, R., Kantin, R., Lasalle, J., Drouet, G., Rambaud, L., Malfait, P., & De Haro, L., 2010. Health impact of unicellular algae of the *Ostreopsis* genus blooms in the Mediterranean Sea: experience of the French Mediterranean coast surveillance network from 2006 to 2009. *Clinical toxicology*, 48(8), 839-844. *doi:* 10.3109/15563650.2010.513687

Tognetto, L., Bellato, S., Moro, I., Andreoli, C., 1995. Occurrence of *Ostreopsis ovata* (Dinophyceae) in the Tyrrhenian Sea during summer 1994. *Botanica Marina*, 38(1-6), 291-296. *doi:* 10.1515/botm.1995.38.1-6.291

Totti, C., Accoroni, S., Cerino, F., Cucchiari, E., Romagnoli, T., 2010. *Ostreopsis ovata* bloom along the Conero Riviera (northern Adriatic Sea): relationships with environmental conditions and substrata. *Harmful Algae*, 9 (2), 233–239. *doi:* 10.1016/j.hal.2009.10.006

Tubaro, A., Durando, P., Del Favero, G., Ansaldi, F., Icardi, G., Deeds, J.R., Sosa, S., 2011. Case definitions for human poisonings postulated to palytoxins exposure. *Toxicon* 57 (3), 478–495. *doi:* 10.1016/j.toxicon.2011.01.005

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. *doi:* 10.1080/05384680.1958.11904091

Verma, A., Hoppenrath, M., Dorantes-Aranda, J.J., Harwood, D.T., Murray, S.A., 2016. Molecular and phylogenetic characterization of *Ostreopsis* (Dinophyceae) and the description of a new species, *Ostreopsis rhodesae* sp. nov., from a subtropical Australian lagoon. *Harmful Algae* 60, 116–130. *doi:* 10.1016/j.hal.2016.11.004

Vila, M., Garcés, E., and Masó, M., 2001. Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean. *Aquatic microbial ecology*, 26(1), 51-60. *doi:* 10.3354/ame026051

Vila, M., Abós-Herràndiz, R., Isern-Fontanet, J., Àlvarez, J., Berdalet, E., 2016. Establishing the Link between *Ostreopsis* cf. *ovata* Blooms and Human Health Impacts Using Ecology and Epidemiology. *Sci. Mar.* 80, 107–115. *doi:*10.3989/scimar.04395.08A

**AUTHOR CONTRIBUTIONS**

**ALKHATIB Houda:** Conceptualization, Methodology, Formal Analysis, Investigation, Writing – Original Draft

**Bennouna Asmae:** Conceptualization, Methodology, Validation, Writing – Review and Editing

**ABOUABDELLAH Rachid:** Conceptualization, Validation, Writing – Review and Editing

**AMZIL Zouher:** Conceptualization, Validation, Writing – Review and Editing

**HERVE Fabienne:** Formal analysis, Writing – Review and Editing

**MOUSSAVOU-MOUITY Cyrielle Amour :** Investigation, Writing – Review and Editing

**SALAHEDDINE ELAYOUBI:** Validation, Writing – Review and Editing

**MOHAMMED BEKKALI:** Validation, Project administration

**ABDELILAH FAHDE:** Validation, Project administration

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.