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The genus *Ostreopsis* along the Algerian coastal waters (SW Mediterranean Sea) associated with a human respiratory intoxication episode

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Abstract – This work describes the first toxic event (human intoxications and sea urchin mortality) related to *Ostreopsis* bloom in Algerian coastal waters. The distribution of *Ostreopsis* is presented in three rocky beaches of Algiers Wilaya, where several cases of human intoxications were registered during July 2009. The maximum abundances attained by *Ostreopsis* in other stations of the network established by the Agency for the Protection and Promotion of Algiers Wilaya coasts (APPL) for monitoring HABs species are also presented. We discuss some of the factors that could explain this toxic episode.

***Ostreopsis* / toxic event / rocky beaches / sea urchin death**

INTRODUCTION

In the early 2000s, the first human intoxications attributed to aerosols from blooms of genus *Ostreopsis* were reported in several countries (Italy, France, and Spain) of the Northwestern Mediterranean Sea (Totti *et al*., 2007; Kermarek *et al*., 2008; Vila *et al.*, 2008). Since then, the detection of harmful *Ostreopsis* blooms has been documented in several places along the northern and southern Mediterranean countries (Illoul *et al*., 2009; Turki *et al.*, 2005 *in* Shears & Ross, 2009; Tichadou *et al*., 2010; Totti *et al*., 2010; Touahria & Séridji, comm. pers.). During July 2009, more than three hundred cases of human respiratory

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intoxications were registered simultaneously in several beaches of two close wilayas (counties), Algiers, Boumerdes and Aïn Temouchent (at 500 km west of Algiers), being published in the local media press (El Watan, 2009; Le Soir d'Algérie, 2009). 175 cases were registered in Boumerdes, 163 in Aïn-Temouchent and 100 in Algiers where 40 people had to be hospitalized based on Hospital sources. Fever, rhinitis, conjunctivitis, cough and skin irritations were the most common clinical symptoms observed. No phytoplankton analysis had been performed in Aïn-Temouchent or Boumerdes beaches. As a result, a monitoring program in Algiers area started a few days after the toxic event by the Agency for the Protection and Promotion of Algiers Wilaya coasts (APPL), highlighting the presence of dinoflagellates, in particular the genus *Ostreopsis*, as the most plausible causative agent of the intoxications. *Ostreopsis* was already reported in coastal waters of Algiers area in summers 2002 and 2003 and then at the end of last decade (2008-2010) (Illoul *et al*., 2008; Illoul *et al*., 2009; Touahria & Séridji, 2011). In this study, we present the temporal distribution during an inter-annual cycle of *Ostreopsis* in an area of Algiers Wilaya affected by the toxic event and their highest levels registered in all beaches of the monitoring network.

METHODS

Since July 2009, a study of potentially harmful microalgae is conducted as part of a monitoring program established by the Agency for the Protection and Promotion of Algiers Wilaya coasts (APPL) along fourteen beaches of Algiers area (36º 42' - 36º 49 N and 2º 49'-3º 21' E). To identify the causative agent of the human intoxications registered during July 2009 in five beaches of the western area of Algiers Wilaya (Ilot, Casino, Grand Rocher, Le Phare and Martin) (Fig. 1), a total of 9 samples were collected in these beaches between 19th and 22th of July, few days after the toxic event. Since August 2009, the monitoring program was focused in fourteen stations, including 3 from the previously affected area (Ilot, Grand Rocher and Martin) and 11 in enclosed beaches (rocky and sandy), which are more prone to promote HABs events (Fig. 1 and Tab. 1). At least one monthly sample was taken at each station since August 2009 to February 2011. However, the sampling frequency was increased to one sample per week during the summer period (August to September 2009 and June to August 2010). Temperature and salinity were measured by a conductivity-meter (WinLab Data Line equipped with RS 232). Water samples (250 ml) were collected at the surface and preserved in Lugol's solution for dinoflagellates identification and counting. Depending on the cell abundance, water sample aliquots of 50, 10 or 2.5 ml were settled in counting chambers for analysis on an inverted microscope (ZEISS, Axiovert 25C) following the Utermöhl method (Throndsen, 1995). Results were expressed as cells per liter (cells·L^{-1}). Macroalgae samples (red, brown and green algae) were occasionally taken during July 2009 in Ilot beach (IL), and between March and August 2010 in 4 stations (TH, IL, GR and OS). Thus, epiphytic *Ostreopsis* and associated species were quantified in a total of 24 macroalgae samples. To estimate the abundances of epiphytic *Ostreopsis* cells, a subsample (8 to 10 g) of the dominant macroalgae species (depending on the site) was delicately cut and placed in a glass bottle containing 10 ml of formaldehyde-filtered seawater (5% final concentration). In the laboratory, the bottles were

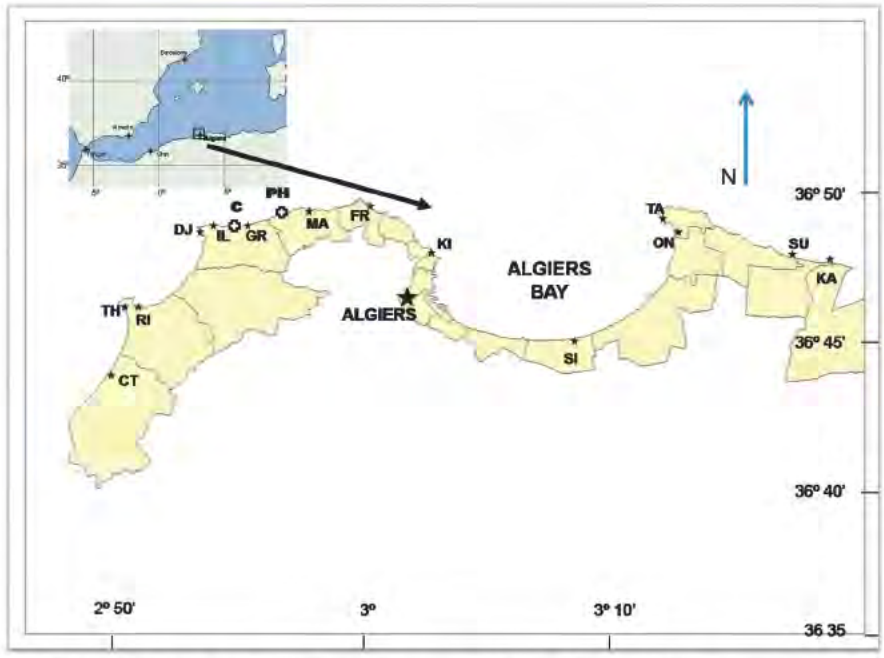


Fig. 1. Monitoring area and sampling stations of Algiers Wilaya (APPL agency). See details about sampling stations in Table 1.

Table 1. *Ostreopsis* highest abundances in the water column along the 16 sampled stations in summer 2009 and 2010. The station name, code and bottom type are indicated. Toxic episodes are marked with an asterisk

Station	*Code*	*Type*	*Maximal abundances (cells·L^{-1})*					
			Years					
			2009			*2010*		
			July	*Aug*	*Sept*	*June*	*July*	*Aug*
Centre Touristique Zéralda	CT	sandy	/	n.d.	40	n.d.	40	n.d.
Thalassothérapie	TH	rocky	/	80	160	5000	2240	5200
El Riadh	RI	sandy	/	80	340	n.d.	160	1320
Djamila Beach	DJ	rocky	160	240	160	80	80	280
Ilot	IL	rocky	4800*	4840	280	n.d.	1480	21680
Casino	C	rocky	2000*	/	/	/	/	/
Grand rocher	GR	rocky	2240*	920	160	3440	1000	2240
Le Phare	Ph	rocky	3840*	/	/	/	/	/
Martin	MA	rocky	5920*	2400	480	8600	400	640
Franco	FR	sandy	/	360	400	80	440	640
El kettani	KI	rocky	/	320	220	120	300	40
Sirene	SI	sandy	/	n.d.	n.d.	n.d.	n.d.	240
Ondine Sud	ON	sandy	40	20	2600	n.d.	1200	640
Tamentfoust	TA	rocky	/	n.d.	n.d.	280	3240	80
Surcouf	SU	sandy	140	4400	160	n.d.	40	2240
El kaddous	KA	sandy	/	/	/	n.d.	120	n.d.

*: Toxic episodes.

n.d.: not detected (abundances below 20 cells·L^{-1}).

In bold: Casino and Le Phare stations sampled once during the toxic event.

strongly shaken and the macroalgae removed from the sample containing the dislodged epiphytic cells (Vila *et. al*., 2001). In the case of July 2009 sampling, aliquots (10 or 2 ml) of samples were settled in counting chambers and analyzed on an inverted microscope (ZEISS, IM 35) by the Utermöhl method. The samples recorded in 2010 were settled in a calibrated Sedgwick Rafter chamber (1 ml) and analyzed using a direct microscope (Leitz Dialux 22 EB). Results were expressed as cells per gram of macroalgae fresh weight (cells·g^{-1} fw).

In order to identify the *Ostreopsis* species, molecular analyses (PCR amplification of partial LSUrDNA gene and internal transcribed spacer regions, ITS-1 and ITS-2) using primers already published (Lenaers *et al*., 1989; Kotob *et al*., 2009) were attempted on different Lugol fixed samples collected during a former summer sampling (August 2008) and during the 2009 and 2010 blooms.

Meteorological information was obtained from the weather station of Dar-El-Beida at about 15 Km east Algiers (http://meteo.infospace.ru).

RESULTS AND DISCUSSION

Relatively high levels of *Ostreopsis* were detected in the first samples taken a few days after the toxic event (19^{th} and 22^{nd} of July 2009) in the five beaches (Ilot, Casino, Grand Rocher, Le Phare and Martin) (Tab. 1) with the maximum abundance ($5.9{\cdot}10^3$ cells·L^{-1}) reached in Martin beach, the 19^{th} of July. The presence of *Ostreopsis* was detected from July to December 2009 and from April 2010 to January 2011 in water samples (from 20 to $2.2{\cdot}10^4$ cells·L^{-1}). When macroalgae were sampled (July 2009, and from March to August 2010) *Ostreopsis* was detected (abundances ranging between 542 and $7.9{\cdot}10^4$ cells·g^{-1} fw). The highest epiphytic *Ostreopsis* abundance registered reached $7.9{\cdot}10^4$ cells·g^{-1} fw the 8^{th} July 2010 on *Corallina* sp. (red algae) in Ilot beach.

Temporal distribution of *Ostreopsis* in the water column of three of the five rocky beaches affected by the toxic event is shown in Fig. 2. Abundances registered during summer 2010 were generally higher than those recorded during summer 2009. During 2009, *Ostreopsis* was present until December while during 2010 it was detected until September. From September 2009 to February 2011, surface water temperature (Fig. 3) and salinity (Fig. 4) ranged from 15 to 26ºC and from 36.5 to 38.4, respectively. High levels of *Ostreopsis* generally match with highest temperatures and relatively high salinity values (Figs 2-4). The maximum abundances ($\geq 2{\cdot}10^3$ cells·L^{-1}) of *Ostreopsis* (Tab. 1) were mostly registered in rocky beaches of the western part of the study area at temperatures ranging from 19.8 to 25.8ºC and salinities from 36.5 to 37.9.

Concomitant macrophyta sampling conducted a few days after the toxic event showed relatively low epiphytic abundances of *Ostreopsis* on the red algae *Corallina* sp. in Ilot beach ($1.5{\cdot}10^3$ and $2{\cdot}10^4$ cells·g^{-1} fw, the 22^{nd} and 29^{th} July 2009, respectively). Otherwise, similar or higher levels of *Ostreopsis* than those observed after the toxic episode were found in 2008 (Illoul *et al*., 2009) and 2010 (present work) without human intoxication being registered. In August 2010, Ilot beach presented $2.2{\cdot}10^4$ cells·L^{-1}; in August 2008, Illoul *et al.* (2009) registered $3{\cdot}10^3$ cells·L^{-1} in Bou-Ismaïl Bay waters (western Algiers area). Epiphytic *Ostreopsis* abundances reached the highest values during summer 2010 in Ilot Beach on *Corallina* sp. ($7.9{\cdot}10^4$ cells·g^{-1} fw), *Sargassum* sp. ($3.5{\cdot}10^4$ cells·g^{-1} fw)

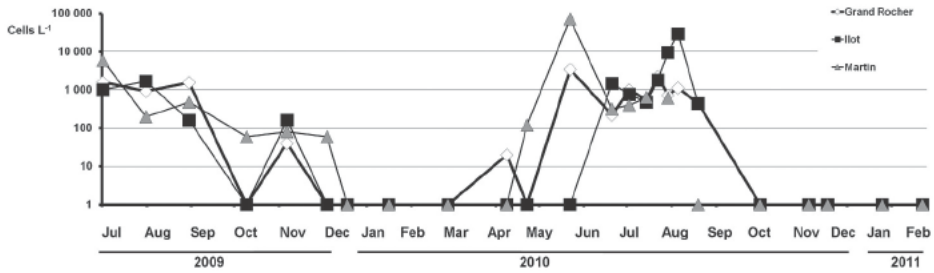


Fig. 2. Distribution of *Ostreopsis* spp from July 2009 to February 2011 in Grand Rocher, Ilot and Martin beaches.

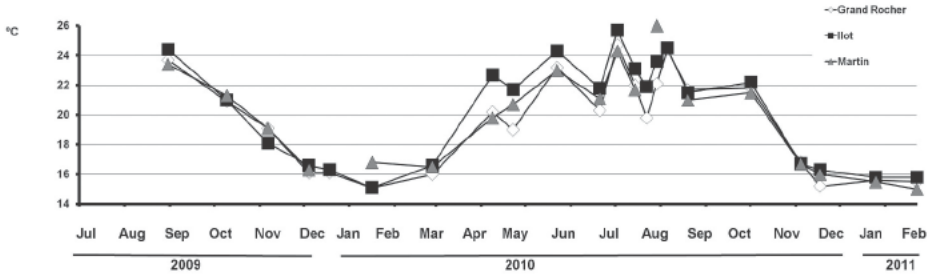


Fig. 3. Distribution of water temperature from July 2009 to February 2011 in Grand Rocher, Ilot and Martin beaches.

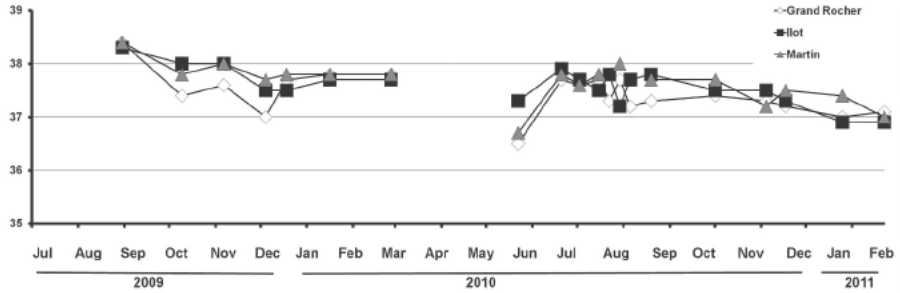


Fig. 4. Distribution of water salinity from July 2009 to February 2011 in Grand Rocher, Ilot and Martin beaches.

and *Codium* sp. ($3{\cdot}10^4$ cells·g^{-1} fw), the 8th July, 9th and 23rdAugust 2010, respectively. Comparable results were registered in French Mediterranean areas (Mangialajo *et al.*, 2011).

Only five beaches (Tab. 1) located in a restricted area in the western part of Algiers Wilaya have been affected by the intoxications. Despite the presence of *Ostreopsis* spp. in other stations (Tab. 1), no harmful events have been reported eastern or western of these five beaches. In this study, the affected area is characterized by shallow rocky beaches, suitable habitats for *Ostreopsis* growth. These beaches are directly exposed to the dominant winds whose direction varied

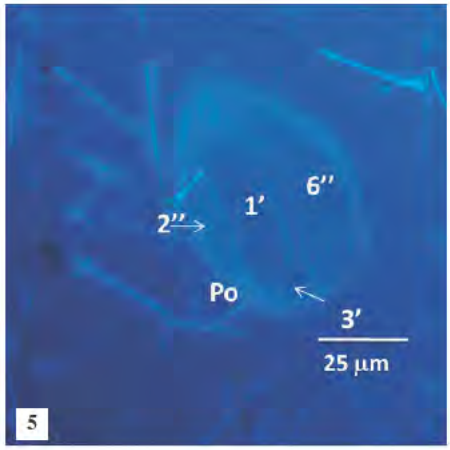
between north and northwest and whose velocity ranged between 3-5 $m \cdot s^{-1}$ during the toxic event (12-18th of July). These dominant and continuous winds probably led aerosols produced after the *Ostreopsis* bloom towards the coast. Their effect could have been enhanced by squalls without rain (NW, 9 $m \cdot s^{-1}$) registered on July 13th by the weather station of Dar-el-Beida. It is important to note that the beaches of the eastern neighbouring Wilaya (Boumerdes), affected by a similar toxic event during the same period, were also exposed to those prevailing winds. Furthermore, the beaches of Algiers Wilaya affected by the outbreak are among the most important recreational areas of this highly urbanized region with houses often very close to the sea. This is probably why both residents and swimmers presented equivalent symptoms (respiratory irritations and conjunctivitis) as observed in Spanish or Italian beaches (Vila *et al*., 2008; Mangialajo *et al.*, 2011). The fact that the adverse effects on human health of *Ostreopsis* aerosols did not affect some beaches of the study area was probably related to the low number or the absence of houses nearby. Moreover, these beaches are protected from the north-western dominant winds that prevailed during the toxic episode. Indeed, some stations as Ondine and Djamila Beaches are located inside two protected bays, Algiers and El Djamila.

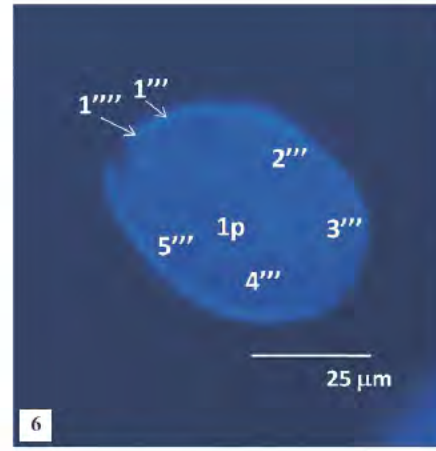
It appears that the *Ostreopsis* abundances responsible for the toxic event in our area are generally lower than some values registered in the northern Mediterranean, both in the water column and on macroalgae. Indeed, Tichadou *et al.* (2010) indicated $> 3 \cdot 10^4$ cells L^{-1} during French toxic episodes. Similarly, for epiphytic results, $2 \cdot 10^4$ cells g^{-1} fw were registered a few days after the Algerian toxic episode while Vila *et al*., (submitted in this volume) indicated values around 10^5 cells$\cdot g^{-1}$ fw macroalgae or $> 10^4$ cells$\cdot L^{-1}$ in seawater during toxic events in Llavaneres beach (Spain).

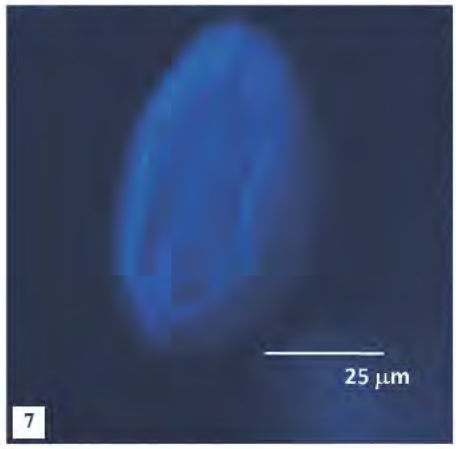
It is remarkable that coinciding with the *Ostreopsis* bloom, a mass mortality of sea urchins (over a hundred) was observed in Ilot beach. This mass mortality only occurred at this location, despite the *Ostreopsis* bloom in July 2009 also affected other important sea urchin (*Paracentrotus lividus*) population between Ilot and Martin (W. Refes, Pers. Comm.). This phenomenon remains unexplained and requires more investigation. To our knowledge, no previous reference of mass mortalities of sea urchins in the coastal Algiers region has been reported, but similar cases of sea urchins mortalities were already registered on Spanish (Vila *et al.*, 2008) and Italian (Ciminiello *et al*., 2006 *in* Shears & Ross, 2009) coasts.

The identification of *Ostreopsis* species by light microscopy (Figs 5 to 7) is difficult due to their similar thecal plate patterns and controversial morphological description (Penna *et al*., 2005). Molecular methods were applied in some preserved samples, but the analyses were unsuccessful probably due to fixative interferences (Lugol's iodine). New tests will be conducted next summer in fresh and/or ethanol-preserved samples.

Finally, as previously said, since August 2009, the APPL network monitors the harmful algal blooms occurrences (caused by *Ostreopsis* and other potentially harmful species) along the beaches of Algiers Wilaya. Due to the prominent epiphytic character of *Ostreopsis* the monitoring should be based both in plankton and benthos samples. As recommended by Mangialajo *et al.* (2011), a sampling of benthic *Ostreopsis* populations should be performed because the benthic abundance are more conservative than the planktonic ones and represent the stock of available biomass.







Figs 5-7. *Ostreopsis* sp. LM- epifluorescence. **5.** Epithecal view. **6.** Hypothecal view. **7.** Side view.

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