Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.117, 2022

Determination of Marine Biotoxins Contamination Level of Mussel (Perna Perna) (Linne, 1758) from the Mamelles Bay, Dakar

Abdoulaye DIOUF [1,2], Jean FALL[2], Malick DIOUF[1], Diegane NDONG[3], Abdou Salam AM[4], Safietou MBAYE[1]
1.Faculté des Sciences et Techniques, ED-SEV, UCAD, BP: 5005
2.Institut Universitaire de Pêche et d'Aquaculture, UCADII Bâtiment Pédagogique, Rez de chaussée, BP: 5005
3.Direction des Ressources Animales et Halieutiques, Département de l'Agriculture, des Ressources en Eau et de l'Environnement, Commission de l'UEMOA, 380 Av. Pr. Joseph KI-ZERBO, 01 BP 543 Ouagadougou 01-Burkina Faso.
4.Faculté des Sciences et Techniques, Biologie Végétale, UCAD, BP: 5005
*Corresponding author. Jean FALL, E-mail: jean.fall@ucad,edu.sn

Abstract
This study evaluating marine biotoxins contamination level of mussel (*Perna Perna*) in the Mamelles Bay (Ouakam) of Dakar is the first report in Senegal. It took place over one year (from March 2018 to February 2019). Liquid chromatography with mass spectrometry (LC-MS-MS) reference method (Regulation (EC) 15/2011 of 10 January 2011) was used to quantify marine biotoxins. Okadaic acid (OA) and its esters are the most problematic of all the researched biotoxins. They are present throughout most of the year, except September and November, with an average monthly concentration of 163 µg/kg, slightly above the regulatory limit (160 µg/kg). The sanitary status of the mussel shows two peaks of contamination, the first of which occurs in the rainy season (August) and the second in the cold season (December), with values equal to 654 and 802 µg/kg respectively 4 to 5 times higher than the regulatory threshold set by Regulation (EC) No 853/2004 of 29 April 2004 supplemented by Regulation (EU) No 786/2013 amending its Annex III and Senegalese Order No 07951 of 12 May 2017. These two peaks coincide with (i) the wintering period, marked by continental land-based inputs due to rainwater runoff, but also with the temperature rise, or (ii) the cold period with the appearance of upwelling (upwelling of cold water, rich in nutrient salts). In addition to the chemical analyses carried out on the mussel, the research work on phytoplankton and the surveys of physicochemical parameters carried out on the water at the collection site show (i) the existence of toxic algae of the *Dinophysis* genus and *Gambierdiscus*, in June, i.e., one to two months before the first peak of contamination of the *Perna perna* by AO; (ii) also a coincidence between the appearance of these phenomena and variations in surface water temperature and salinity, which are determining factors in the appearance and development of harmful algal blooms. Concerning the other biotoxins investigated, in particular AZA and yessotoxins, it was noted that AZA was absent throughout the year, unlike yessotoxins, which were found in the flesh of *Perna perna* at concentrations below the regulatory limit set at 3750 µg/kg. The maximum being 51 µg/kg obtained in June, i.e., 73 times lower than the regulatory threshold. In the light of the results obtained, it appears that the periods of high contamination are the winter period and the upwelling period, which could lead to closure measures to guarantee the safety of the mussels. It would be interesting to extend the study to all other bivalve mollusks and gastropods, to carry out an inventory and quantify the toxic microalgae present in the water. To this end, the effectiveness of purification techniques would be tested.
Keywords: Mussel (*Perna perna*), Marine biotoxins, Toxic microalgae, Mamelles, Dakar.
DOI: 10.7176/FSQM/117-04
Publication date: July 31st 2022

INTRODUCTION

More than 60% of the world's population lives along coastal areas, and depends on the marine environment for their survival (National Research, 1999). The marine environment thus offers economic and nutritional benefits, including high nutritional value products such as fatty acids and proteins in the diet of many peoples.

However, it is also recognized that many intoxications related to products of marine origin are contracted either by simple contact with seawater, by inhalation of airborne substances during recreational activities in coastal areas, or by consumption of marine products (Haile *et al*., 1999).

Micro-algae blooms, which were rare during the 1980s, have become an increasingly widespread and worrying phenomenon due to their growing socio-economic impacts (Turki, 2004). The presence of poisonous species can lead to a ban on water use due to toxin release.

Over the last three decades, harmful or toxic algal incidents has increased in many parts of the world, both in terms of frequency and geographical distribution. Several studies have suggested possible relationships between climate and the magnitude, frequency, and duration of red tides commonly referred to as Harmful Algal

Bloom (HAB). Coastal blooms are considered an emerging problem linked to nutrient enrichment of coastal waters. Nutrient-rich coastal environments, especially semi-enclosed areas with low turbulence levels, constitute a new and unique environment in which several phytoplankton species with harmful effects can become dominant. In contrast to large-scale blooms dominated by mesoscale circulation, HABs are a more localized phenomenon, usually linked to areas of limited dynamism, such as bays, lagoons, harbors, beaches, and estuaries (Ferrante *et al*., 2013). HABs can be harmful to human health in three primary ways: skin contact, inhalation of airborne toxins, or consumption of affected marine food resources. Among foodborne diseases, five human syndromes are currently recognized as being caused by HABs (i) amnesic shellfish poisoning (ASP), (ii) Ciguatera Fish Poisoning (CFP), (iii) Diarrheic Shellfish Poisoning (DSP), (iv) Neurotoxic Shellfish Poisoning (NSP) and (v) Paralytic Shellfish Poisoning (PSP).

Amnesic shellfish poisoning is caused by eating shellfish that have accumulated domoic acid, a neurotoxin produced by certain phytoplankton strains. Diarrheic shellfish poisoning is a common and prevalent type of shellfish poisoning that makes gastrointestinal symptoms. Potent natural neurotoxins cause paralytic shellfish poisoning synthesized by microscopic dinoflagellates of the genera *Alexandrium, Gymnodinium, and Pyrodinium* in marine and freshwater environments. Neurotoxic shellfish poisoning is a disease caused by the consumption of shellfish contaminated with brevetoxins. Diarrhoeal shellfish poisoning (DSP) is characterized by severe gastrointestinal illness associated with the consumption of filter-feeding bivalves (Reguera *et al*., 2012).

Historically, three different groups of toxins were associated with PSD: okadaic acid (OA) and dinophysistoxins (DTX), pectenotoxins (PTX), and yessotoxins (YTX) due to their co-extraction and unique response to conventional mouse bioassay. Although no longer associated with PSD, YTX, produced by the dinoflagellates *Protoceratium reticulatum, Lingulodinium polyedrum, and Gonyaulax spinifera,* are lethal to mice after intraperitoneal injection (Tubaro *et al*., 2010). Okadaic acid is a toxin produced by dinoflagellates of the genus *Dinophysis spp*. and *Prorocentrum spp. Prorocentrum lima* is the best known in Canada. It is a cosmopolitan dinoflagellate with a distribution from temperate waters to warm coral reefs. It affects living organisms when its concentration reaches 200-500 micrograms per body weight (Baden *et al*., 1995). Azaspiracids (AZAs) are the most recently discovered biotoxins produced by the dinoflagellate *Azadinium spinosum*. Although never associated with DSP toxins, AZAs cause diarrhea in humans (Furey *et al*., 2010). Amnesic shellfish poisoning (ASP) is caused by the neurotoxin domoic acid (DA) produced by certain species of the diatom genus *Pseudo-nitzschia* distributed worldwide (Lelong *et al*., 2012). DA was first detected in Galician mussels in 1994, *P. australis* dominated the plankton, and significant amounts of DA were found in concentrated plankton samples (Míguez *et al*., 1996). It is an excitatory dicarboxylic amino acid substance that competes with glutamate receptors in the central nervous system (Baden *et al*., 1995).

Many environmental factors, such as monsoon winds, weather, ocean circulation, upwelling, and human activities, play a role in forming HABs (Tang *et al*., 2004a). Upwelling brings nutrients from the bottom to the surface and promotes phytoplankton blooms. The diversity of harmful algae recorded in the region is similar to that found in other eastern frontier upwelling system. It includes species responsible for paralytic shellfish poisoning, diarrheic shellfish poisoning, amnesic shellfish poisoning, and azaspiracid poisoning. The major eastern boundary upwelling systems (EBUS) of the world's oceans are susceptible to harmful algal blooms (HABs, as they are highly productive, nutrient-rich environments prone to high biomass blooms (Pitcher *et al*., 2010; Trainer *et al*., 2010).

Senegal, with its 718 km of coastline and dense hydrographic network (rivers, lakes, ponds, etc.) and highly diversified marine ecosystems, is not spared from these phenomena. Moreover, the appearance of skin diseases among the fishers of Thiaroye, caused by their contact with seawater from fixed or drifting gillnets at the time of the recovery of their catches in 2020, is indicative of the existence of Harmful Algae Bloom in maritime waters.

Although there are some studies on marine algae (macro and micro), no study has yet been conducted on the toxicity level of bivalve mollusks in Senegal. Moreover, this is the reason for the restriction of national export approval at the EU level for bivalve mollusks, gastropods, tunicates, and echinoderms.

In this context, this study on the level of contamination of the mussel (*Perna perna*) (Linne, 1758) from the Mamelles de Dakar was conducted.

I- OBJECTIVES OF THE STUDY

1-1 OVERALL OBJECTIVE

This study aims to determine the level of contamination of the mussel (*Perna perna*) in the Mamelles de Dakar bay by marine biotoxins.

1-2 SPECIFIC OBJECTIVES

Specifically, the aim is to:

OS1: draw up an initial taxonomic inventory of potentially toxic phytoplankton species on the site;

OS2: report the presence of potentially toxic phytoplankton species that may be capable, under favorable conditions, of causing blooms;
OS3: determine the periods of health risks for the water and mussels, given that the site is not very far from the bathing area.

II- STUDY METHODOLOGY

2-1 FRAMEWORK OF THE STUDY

The study focused on the Mamelles site, located on the Cape Verde peninsula. It was carried out over one year (from March 2018 to February 2019). This oceanic area is marked by a rocky bottom, an attachment point for mussels. The proximity of the site to housing and the mussel's feeding method (filtration) suggest that they are contaminated with microalgae and, in turn, marine bio-toxins.

Figure 1: Location of the study site

2-2 MATERIAL AND METHODS

Each month, about 50 mussels, *Perna perna* (Linne, 1758), were collected by hand, by snorkeling, from the rocks. A sample of 30 individuals is taken at random to determine morpho-metric parameters (total shell length, empty shell mass, meat mass, and sex of the mussel) and then frozen for marine bio-toxins.

The water's environmental parameters (salinity and temperature) in which the mussels live were recorded. In addition, two 150 ml samples of seawater were also taken for microalgae research.

2-2-1 Methods of sampling and conservation of samples

Initially, two (02) sites on the Cape Verde Peninsula (Mamelles, Yoff) were selected for the study, which was held over 12 months (from March 2018 to February 2019). About 50 Mussels (*Perna perna*) were collected by hands and by snorkeling at rock level each month. Once at the Ouakam marine station, a random selection of 30 individuals was made so that all individuals had the same probability of being included in the batch to be studied. After a series of studies relating to the biological parameters of the mussel, 200 g of flesh were put in a plastic bag and kept cold in a cooler with the help of carbo-ice, and sent to the laboratory of the Institut Universitaire de

Pêche et d'Aquaculture (IUPA) of the Université Cheikh Anta DIOP de Dakar (UCAD). All the samples were kept in a freezer at -18°C until the 12-month sampling period. At the end of the collection period (12 months), 15 samples were obtained, 12 from Mamelles and three from Yoff. The reduced number of samples from Yoff is because from May 2018, this site was not accessible due to weather warnings related to the presence of swell.

Thus, the 15 samples obtained were sent to the Phytocontrol AGRIFOOD laboratory in France in November 2019 for marine bio-toxin analysis. As for the water samples were collected and stored in the refrigerator at the IUPA Laboratory before being analyzed at the Plant Biology Laboratory of the Faculty of Science and Technology (FST) of UCAD.

A multifunction environmental sensor (PCE-PHD1 brand.) was used to measure in situ the temperature (◦C), and a Refractometer-Salinometer was used to measure salinity (ppt). Since the first sampling, the picking site has been geolocated using a Garmin GPS.

2-2-2 Analysis methods

A- Research and quantification of biotoxins

Liquid chromatography with tandem mass spectrometry (LC-MS-MS) is a powerful analytical technique that combines the separation power of liquid chromatography with the highly sensitive and selective mass analysis capability of triple quadrupole mass spectrometry.

A sample solution is pumped through a stationary phase (LC column) by a mobile phase flowing at high pressure. The chemical interaction between the sample components, the stationary and mobile phase involves different migration rates through the LC column, thus affecting separation. The wide variety of stationary and mobile phase combinations allow separation to be tailored to many complex solutions. After elution from the LC column, the effluent is directed to the mass spectrometer. The mass spectrometer for an LC/MS/MS system has an ionization source in which the effluent from the LC column is nebulized, desolvated, and ionized, creating charged particles. These charged particles then migrate in a high vacuum through a series of (quadrupole) mass analyzers applying electromagnetic fields. Extraction of lipophilic marine biotoxins with methanol from homogenized tissue were conducted:

- To determine the content of free AO, free DTX1, free DTX2, PTX1, PTX2, AZA1, AZA2, AZA3, YTX, homo-YTX, 45-OH-YTX, 45-OH-homo-YTX, GYM A, 13-desMe-SPX C, PnTX A and PnTX G in the samples, the extracts are analyzed directly by reverse-phase liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).
- To determine the content of total AO, total DTX1, and total DTX2 in the samples, alkaline hydrolysis is carried out before the LC-MS/MS analysis. Indeed, AO, DTX1, and DTX2 can be acylated at the hydroxyl of carbon 7 of the molecule (7-O-AO/DTXs), leading to the formation of an ester. This acylation results in the attachment of saturated or unsaturated fatty acid chains, leading to the formation of a group of toxic derivatives: DTX3. To be able to detect and quantify DTX3, alkaline hydrolysis of the DTX3 ester bond is necessary. This step allows the release of AO, DTX1, and DTX2. The total contents of AO, DTX1, and DTX2 are then determined by LCMS/MS (sum of the free form and the ester form).

According to the European Commission Regulation 15/2011 (27), for detecting lipophilic toxins, the indicated method is liquid chromatography coupled with mass spectrometry. For the lipid extract, 100 g of mussels were mixed with 300 mL of acetone (VWR Chemicals, Fontenay-sous-Bois, France) in an Ultra-Turrax T25 basic (Sigma, Saint Louis, MO), filtered, and re-extracted with 200 mL of acetone. The acetone supernatant was mixed and evaporated with a Rotavapor (Büchi R-200/205, Labortechnik AG, Switzerland). The volume of the aqueous extract was adjusted to 100 mL with distilled water, transferred to a separating funnel. Then 100 mL of diethyl ether (Sigma) was added for liquid-liquid separation. The aqueous layer was collected in the evaporation flask, while the organic layer was collected in a clean glass vessel. The aqueous phase was transferred to a separating funnel. This step was repeated twice. The three organic fractions were combined in the evaporation flask and evaporated to dryness using a rotary evaporator. The solvent was evaporated, and the resulting residue was suspended in a 1% aqueous solution of Tween 60 (Sigma).

B- Identification of phytoplankton

For the inventory of potentially toxic phytoplanktonic species or micro-algae, the samples previously fixed with Lugol's were observed in their raw state under an inverted microscope. Measurements (length, width, and diameters) and images were taken from each cell followed. Species identification was made according to previous works on the subject (Wagne *et al*. (2011), Maurer *et al*. (2010); Compère *et al*. (2009), and Paulmier (1994)[14, 15, 16, 17].

III- RESULTS AND DISCUSSION

3-1 RESULTS

The present study results show two distinct periods: low temperature from November to May (temperature between 15 and 20°C) and a period of high heat from June to October (temperature which revolves around 24

and 28°C). The water temperature varied from 15.4 to 28.7°C with an average of 22.16°C. Temperature increases were observed from May with a maximum equal to 28.7°C during the winter period (August, September, October).

From January to May, the salinity was about 38 PSU, and a period of high salinity from June to December (41 PSU) was observed. During the rainy season, there is a slight decrease in salinity. The salinity varied from 38 to 41 ‰ during the experiment, with averages of 39.58 ‰. Salinity increased at the end of the cold season (May) and reached a maximum in June and July (41 ‰), then decreases consecutively to the continental contributions of runoff water.

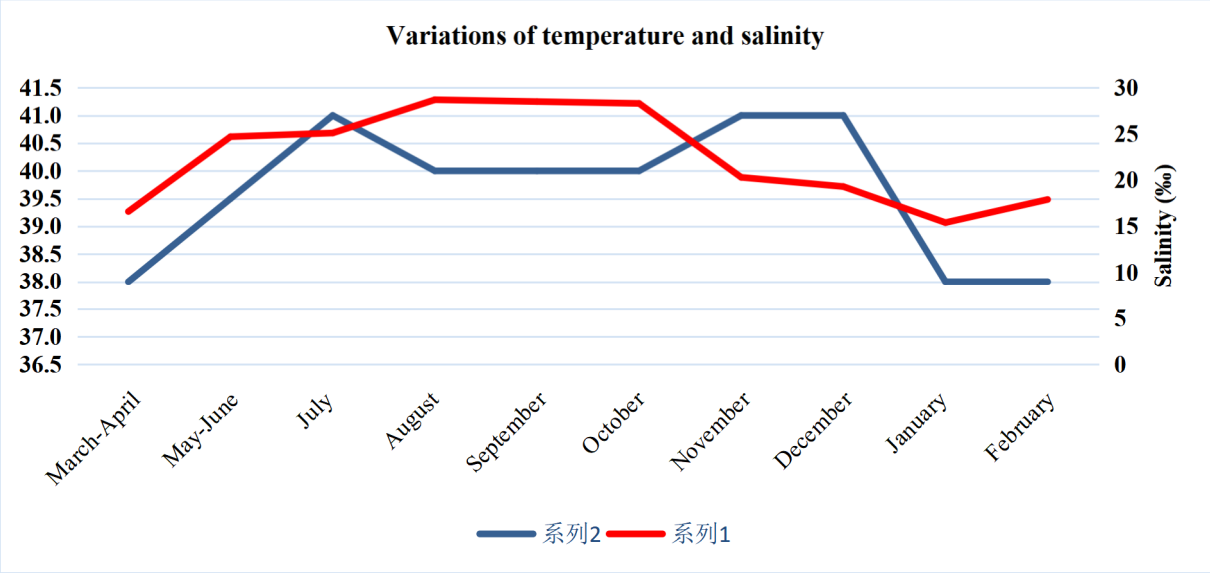

Figure 2: Variations of water's temperature and salinity

Free Okadaic Acid (OA) + esters are the most representative biotoxin in the samples analyzed. This biotoxin is found in mussels during most of the year, with contamination peaks in August and December.

Homo yessotoxin was also detected in mussel samples in February and March, coinciding with the cold period in Senegal.

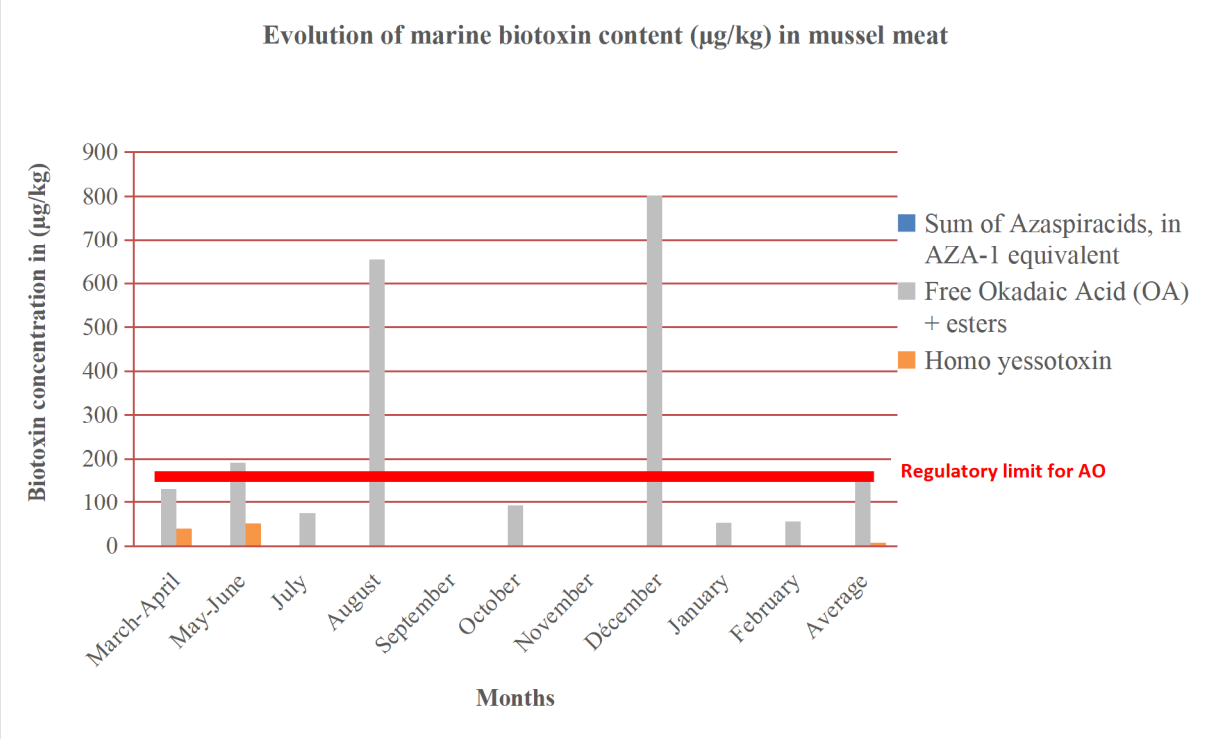

Figure 3: Comparative monthly evolution of the content of the different biotoxins found in mussel meat

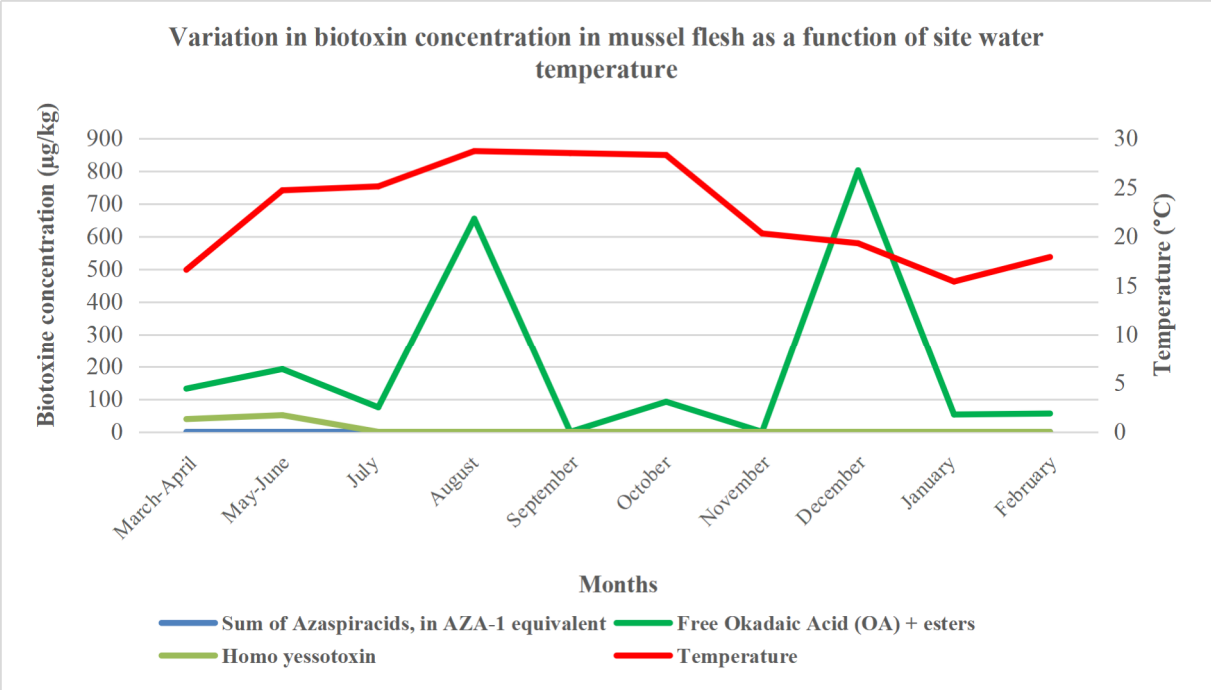

Figure 4: Evolution of biotoxin content in mussel flesh as a function of water temperature

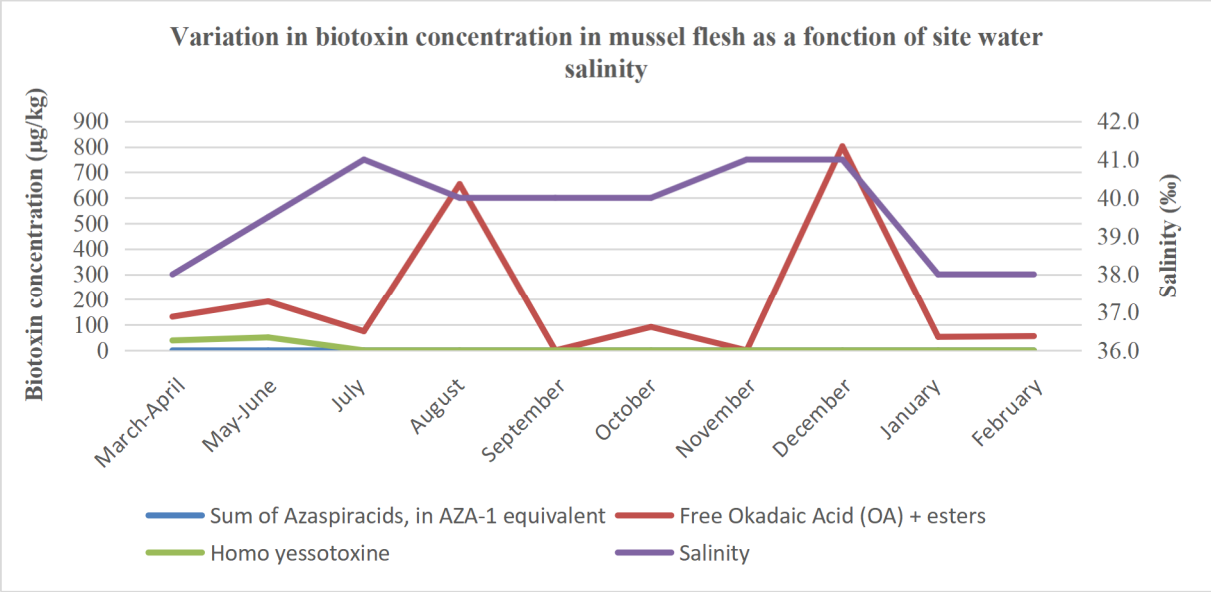

Figure 5: Evolution of the biotoxin content in mussel flesh as a function of water salinity

The general observation is that following substantial variation in temperature or salinity, the AO content increases considerably. This is the case of the peaks observed in August and December, respectively, following an increase in temperature (decrease in salinity) one month before and a drop in temperature (increase in salinity) for the same time lag i.e., one month.

These phenomena could be because the July-August period corresponds to strong winter heatwaves, generally coinciding with heavy rainfall; rainwater carries a multitude of nutrients through runoff, while the November-December period is that of upwellings along the senegalese coast, with upwelling of cold water rich in nutrient salts. These results are confirmed by the variations in biotoxin content according to the condition factor, characterizing the richness of the mussel living environment (see figure below). As for the one-month lag observed between the temperature increases and the increase in the concentration of biotoxins in the mussel flesh could be justified by the existence of a latency period between the primary development generated by the phenomena and the feeding of the mussels.

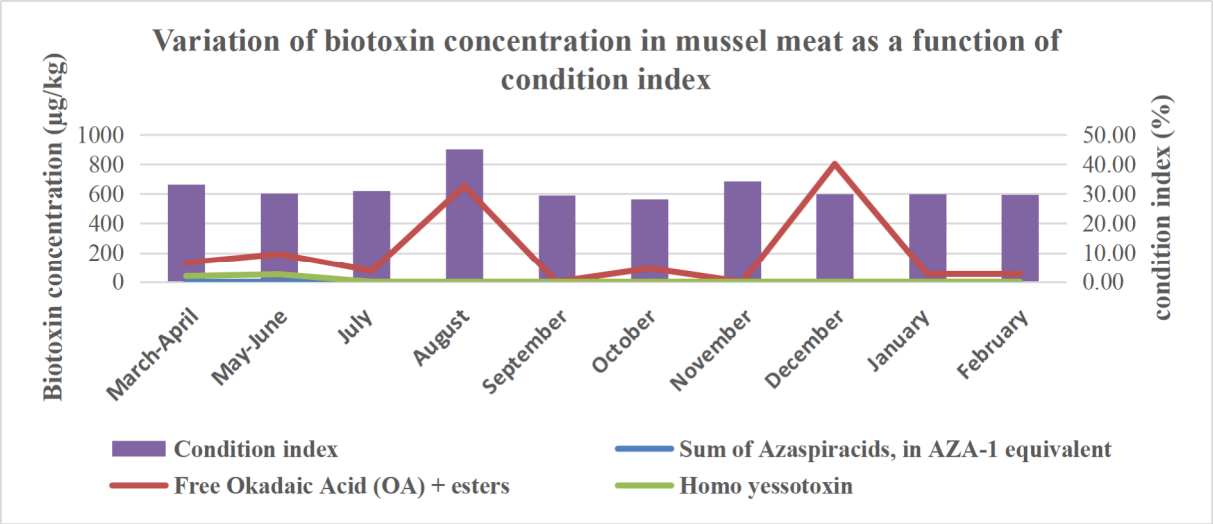

Figure 6: Evolution of the biotoxin content in mussel flesh as a function of the condition index of their living environment

Table I: Results of biotoxins in mussels and toxic microalgae in the water of the sampling site according to the months.

	March-April	May-June	July	August	September	October	November	Décember	January	February	Average
Sum of Azaspiracids, in AZA-1 equivalent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Free Okadaic Acid (OA) + esters	132	192	75	654	ND	92	ND	802	53	56	163
Homo yessotoxin	39	51	ND	ND	ND	ND	ND	ND	ND	ND	7,5
Status of toxic microalgae (number of poisonous species)	0	12	0	0	0	0	0	0	0	0	-

The general observation is that biotoxins (AO and Homo yessotoxin) are present in mussel meat from December to August, with peaks encountered in the winter period (May to August) and the cold period (December-February). Thus, the two (02) peaks observed for AO took place in August and December, with values equal to 654 and 802 µg/kg, respectively, mainly exceeding the regulatory value set at 160 µg/kg, i.e., five times greater than the said regulatory value for the August peak. Concerning Homo yessotoxin, the mountain is located between May and June with a weight of 51 µg/kg, well below the regulatory limit set at 3750 µg/kg; i.e., 73 times lower than the latter.

The sum of Azaspiracides in AZA-1 equivalent was not detected during the analyses. This could be justified because either the Azaspiracids are absent or our analysis equipment does not have a detection threshold that can detect and quantify them.

This has not been precisely demonstrated concerning the influence of microalgae on the content of marine biotoxins in the flesh of mussels. Nevertheless, the genera containing the species known to be more toxic were identified in the water samples taken in June, notably the genera D*inophysis* and *Gambierdiscus*. Of the 17 species identified, 12 belong to the class Dinophyceae, i.e., a little more than 70% of the species identified.

Table II shows the 23 phytoplankton species identified in the Bay des mamelles, highlighting species considered toxic.

Table II: Phytoplankton species identified in the Bay des mamelles, with highlighting of species considered toxic.

Species	Genus	Family	Class
Ceratium furca	*Ceratium*	Cerratiaceae	Dinophyceae
Ceratium tripos			
Ceratium azoricum			
Dinophysis caudata	*Dinophysis*	Dinophysaceae	
Dinophysis accuminata			
Dinophysis sacculus			
Gambierdiscus toxicus	*Gambierdiscus*	Goniodomataceae	
Prorocentrium micans	*Prorocentrium*	Prorocentraceae	
Protoperidinium ovum	Protoperidinaceae	Protoperidinaceae	
Protoperidinium steinii			
Ostreopsis ovata	*Ostreopsis*	Ostreopsidaceae	
Alexandrium sp	*Alexandrium*	Peridinanceae	
Coscinodiscus occulis iridis	*Coscinidiscus*	Coscinodiscaceae	Bacillariophyceae
Coscinodiscus sp			
Chaetoceros curvisetus	*Chaetoceros*	Chaetocerataceae	
Chaetoceros danicus			
Chaetoceros sp			
Pleurosigma sp	*Pleurosigma*	Naviculaceae	
Pleurosigma sp 1			
Biddulphia bidulphiana	*Biddulphia*	Biddulphiaceae	
Melosira sp	Melosira	Melosiraceae	
Rhizosolania sp	*Rhizosolania*	Rhizosolanaceae	
Oscillatoria sp	*Oscillatoria*	Oscillatoriaceae	Cyanophyceae

3-2 DISCUSSION

The results of the present study show that the water temperature varied from 15.4 to 28.7 ° C with an average of 22.16 °C with two distinct periods: low temperature from November to May (temperature between 15 and 20°C) and a period of high heat from June to October (temperature in which revolves around 24 and 28°C).

The salinity varied from 38 to 41 ‰ during the experiment, with an average of 39.58 ‰. From January to May, the salinity was around 38 PSU, and a period of high salinity from June to December (41PSU) was observed. During the rainy season, there is a slight decrease in salinity. Salinity increased at the end of the cold season (May) and reached a maximum in June and July (41 ‰), then decreased consecutively to the continental contributions of runoff water.

Previous studies have shown that salinity and temperature are important environmental factors for initiating blooms of certain microalgae, and also modify the toxicity levels of e.g., *Heterosigma* (Haque and Onoue 2002). The formation of *Heterosigma* blooms has been associated with the freshwater flow and reduced salinity below 15‰ (Taylor and Haigh 1993). Aligosaki *et al*. (2006) showed the development of *Ostreopsi ovata* at Présace in France from July to November, with a temperature ranging from 13.9 to 29.7°C. Xu *et al*. (2016) report that the optimal growth range of *Gambierdiscuss spp* in the Canary Islands is between 24 and 31°C with minima between 15-21°C and maxima between 31-34°C. Amri *et al*. (2010) found that the temperature of some phytoplankton classes varied between 14 and 29°C with an average of 25°C.

In the present study, 23 phytoplanktonic species were identified in the Bay of Mamelles. Among these microalgae, the genera containing the most toxic species were placed in the water samples taken in June, notably the genera *Dinophysis* and *Gambierdiscus*. Of the 17 species identified during this period, 12 belong to the class Dinophyceae, i.e., a little over 70% of the species identified.

Three species of *Gambierdiscu*s were detected in the Cape Verde Islands in 2014. These are *Gambierdiscus australes*, *G. excentricus* and *G. silvae* (Fraga and Rodríguez, 2014). Rodríguez *et al*. (2017) reported that the apparent extension of the distribution of Gambierdiscus appears to be a direct consequence of increased temperatures derived from climate change. These same authors also reported that the two easternmost islands closest to the African coast (Lanzarote and Fuerte Ventura) showed the maximum cell concentrations and higher mean values of *Gambierdiscus spp*. Rodríguez *et al*. (2017) reported that massive blooms of *Ostreopsis spp*. were detected in several samples from Caleta Caballo (Lanzarote) with concentrations up to 28117 and 151499 cells g-1 alga. Rodríguez *et al*. (2017) reported that regardless of global warming, seawater temperatures in subtropical latitudes like the Canary Islands are adequate for the growth of warm benthic dinoflagellates found elsewhere in our study (*Ostreopsis, Prorocentrum,* etc.).

The results of the present study show that samples of *Perna perna* mussels collected at the Dakar teats in the Atlantic Ocean show OA values from March to August with peaks above the regulatory limit in May-June (192 µg/kg) and August (654 µg/kg) with Dec to Feb respectively with a peak in Dec (802 µg/kg). These results are not in line with the European Union (EU) regulation setting the maximum allowable level of DSP toxins in shellfish at 160 µg/kg AO equivalent (Regulation (EC) No 853/2004). These results align with those of Smienk *et al*. (2013). They reported that the average values attributed to OA-toxins for the test materials were 255.0 µg of total OA equivalents/kg *M. edulis*. Hossein *et al*. (2011) determined that the concentration of toxins in the incriminated batch was 1,261 µg OA eq/kg mussel meat. The maximum concentration was 8.4 mg kg-1 detected in mussels collected in October. Imène Kacem *et al*. (2010) showed that all mussel samples were contaminated with OA at levels of about 10.2 µg/100 g wet weight (PP2A test). However, the OA group in oysters was only detected from April to July at a maximum limit of 1.45 eq µg/100 g wet weight (PP2A test). Lindegarth *et al*. (2009) showed that *Mytilus edulis* rapidly accumulated group OA toxins to levels about ten times higher than the regulatory limit (160 µg OA kg-1 mussel), while concentrations never reached this limit in *Ostrea edulis* during field exposure. Gerssen *et al*. (2010) found concentrations ranging from 18.2 to 67.5 µg OA kg-1 equivalent in mussels (*M. edulis*) collected from shellfish harvesting areas in the Netherlands. Orellana *et al*. (2017) showed that OA/DTX2 were abundantly accumulated in analyzed mussels (*M. edulis*, *Crassostrea gigas,* and *Patella sp*.) from the Belgian part of the North Sea reached values of 25.4 and 169.2 µg wet kg-1 equivalent, respectively. AO levels, ranging from 40 to 611 µg of AO equivalent, kg-1 were also detected by Garibo *et al*. (2014) in samples of *M. galloprovincialis* obtained in the framework of the Catalan coastal shellfish monitoring program (NW Mediterranean) during a PSD event in 2012.

In the present study, AZAs toxins were not detected in mussels during the period March 2018 to February 2019. These results align with the regulatory level of 160 µg/kg. Similarly, Taleb *et al*. (2006) reported that in Morocco in mid-July, levels of 0.9 µg/kg were obtained. In contrast, Blanco *et al*. (2017) reported AZA concentrations up to a maximum of 5.4mg AZA1 equivalent/kg in mussels (*M. galloprovincialis*) collected during official monitoring programs from production areas along the Atlantic and Cantabrian coasts of Spain.

In the present study, yessotoxins (YTX) were detected in *Perna perna* mussels at a level of 51µg/kg between March and June with a peak in May-June 2018. This value is below the European authorities' regulatory limit of 1 mg YTX equivalent/kg (Regulation 853/2004/EC). In contrast, Visciano *et al*. (2013) Samples of *Mytilus galloprovincialis* collected from shellfish growing areas located in the Adriatic Sea showed YTX values ranging from 1.30 mg to 1.63 mg YTX equivalent /kg; subsequently, the concentrations found by the LC-MS/MS method ranged from 1.22 mg to 1.97 mg YTX equivalent /kg. On the other hand, García-Mendoza *et al*. (2014) revealed that YTX was the second most abundant group in mussels collected in Todos Santos Bay. Only one sample had a concentration (1.08 mg/kg) above the action level for this toxin indicated in the Mexican regulation but below the current action limit of 3.75 mg kg-1 considered in the European regulation (EC 786/2013). YTX was the main fat-soluble toxin present in mussels with a concentration range from 164 to 326 mg/100 g of whole flesh. According to Schirone *et al*. (2013), YTX levels were found in *M. galloprovincialis* specimens collected in three Italian regions (Abruzzo, Molise, Emilia Romagna) along the Adriatic Sea coast, at concentrations ranging from 0.2 to 1.8 mg YTX equivalent kg-1.

CONCLUSION

This study shows that the level of contamination of mussels by biotoxins is globally unsatisfactory. Of all the samples analyzed, Okadoic acid (OA) and its esters are the most problematic biotoxins. Their concentration in the flesh exceeds the regulatory threshold in May, June, August, and December. This exceedance is much more pronounced in December when the concentration of AO is almost five times the established standard. Concerning algal blooms, toxic algae of the genera Dinophysis, Gambierdiscus, Ostreopsis on the sampling sites is a relevant indicator that corroborates the results obtained.

This study on biotoxins in mussels being a first in Senegal, yet on the level of toxicity of bivalve mollusks, deserves to be extended to all species and to all collection, harvesting, and purification areas, in order to determine their sanitary status and eventually classify them in zones A, B, C or D, as required by national and international regulations. This would undoubtedly lead to the extension of Senegal's approval to export these products, which have a significant commercial value.

Therefore, in perspective, this study should be extended to all other bivalves and gastropod mollusks, but also the inventory and quantification of toxic microalgae should be carried out.

In this way, the effectiveness of purification techniques can be tested. The results of all these experiments could help to confirm or invalidate the exploitation and biological rest periods established for local communities in an empirical manner.

Acknowledgment

The authors would like to thank M. Abdoulaye DIOP, member of the Comité Local de Pêche Artisanale (CLPA)

of Ouakam for their support by providing pirogue for transport and by collecting mussel used for this research.

BIBLIOGRAPHY

Aligizaki, K., Nikolaidis, G., Fraga, S. (2008). Is *Gambierdiscus* expanding to new areas? Harmful Algae News. 36 : 6–7.

AMRI, S., Zidane, B. and Brahim, O. (2010). Inventaire des cyanobacteries potentiellement toxiques dans la tourbiere du lac noir « parc national d'el-kala » (algerie). *Rev. Microbiol. Ind. San et Environn. 4* : *49-68.*

Baden, D., Fleming. L. (1995). Marines Toxins. Handbook of clinical neurology: intoxications of the nervous system. II Natural toxins and drugs. F. A. D. Wolff. Amsterdam., Elsevier, 21.

Blanco, J., Arévalo, F., Moroño, Á., Correa, J., Muñíz, S., Mariño, C. (2017). Presence of azaspiracids in bivalve molluscs from Northern Spain. Toxicon 137:135–143.

Compère, P., Riaux-Gobain, C. (2009). Diatomées de quelques biotopes marins, saumâtres et dulçaquicoles de Guinée (Afrique occidentale). Systematics and Geography of Plants. 79:33-66.

Ferrante, M., Oliveri, C. G., Fiore, M., Rapisarda, V., Ledda, C. (2013). Harmful algal blooms in the Mediterranean sea: effects on human health. Euromediterranean Biomedical Journal 8:25-34.

Fraga, S. and Rodríguez, F. (2014).Genus *Gambierdiscus* in the Canary Islands (NE Atlantic Ocean) with descri ption of *Gambierdiscus silvae sp*. nov., a new potentially toxic epiphytic benthic dinoflagellate. Protist 165:839-853.

Furey, A., O'Doherty, S., O'Callaghan, K., Lehane, M. and James, K. J. (2010).Azaspiracid poisoning (AZP) tox ins in shellfish: toxicological and health considerations. Toxicon. 56:173-190.

García, C., Contreras, H.R. (2014). Effects of both paralytic shellfish toxins and diarrhetic shellfish toxins in human poisoning: toxicity, distribution and biotransformation. In: Hay RM, editor. Shellfish human consumption, health implications and conservation concerns. New York: Nova Science Publishers. 345–384.

Garibo, D., Campbell, K., Casanova, A., de la Iglesia, P., Fernández-Tejedor, M., Diogène, J. (2014). SPR immunosensor for the detection of okadaic acid in mussels using magnetic particles as antibody carries. Sensors Actuat. B. Chem. 190: 822–828.

Gerssen, A., Van Olst, E. H. W., Mulder, P. P. J., de Boer, J. (2010). In-house validation of a liquid chromatography tandem mass spectrometry method for the analysis of lipophilic marine toxins in shellfish using matrix-matched calibration. Analytical and Bioanalytical Chemistry. 397:3079-3088.

Haile, R. W., Witte, J. S., Gold, M., Cressey, R., McGee, C., Millikan, R.C, Glasser ,A., Harawa, N., Ervin, C., Harmon, P., Harper, J., Dermand, J., Alamillo, J., Barrett, K., Nides, M., Wang, G. (1999). The health effects of swimming in ocean water contaminated by storm drain runoff." Epidemiology. 10: 355-363.

Haque, S.M., Onone, Y. (2002). Effects of salinity of growth and toxin production of a noxious phytoflagellate, *Heterosigma akashiwo* (Raphidophyceae). Bot Mar. 45:356–363

Hossen, V., Jourdan-da Silva, N., Guillois-Bécel, Y., Marchal, J., Krys, S. (2011). Food poisoning outbreaks linked to mussels contaminated with okadaic acid and ester dinophysistoxin-3 in France, June 2009.SEP Euro Surveill.16: 46.

Imène, K., Noureddine, B., Béchir, H. (2010). Comparison of Okadaic Acid Profiles in Mussels and Oysters Collected in Mediterranean Lagoon, Tunisia, International Journal of Biology. 2:2.

Lelong, A., Hégaret, H., Soudant, P. and Bates, S. S. (2012).Pseudonitzschia(Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia. 51:168-216.

Lindegarth, S., Torgersen, T., Lundve, B., Sandvik, M. (2009). Differential retention of okadaic acid (OA) group toxins and pectenotoxins (PTX) in the blue mussel, *Mytilus edulis* (L.), and european flat oyster, *Ostrea edulis* (L.). J Shellfish Res. 28:313–323.

Maurer, D., Bec, B., Neaud-masson, N., Rumebe, M., Auby, I., Grémare, A. (2010). Etude des relations entre le phytoplancton et les phénomènes de toxicité d'origine inconnue dans le Bassin d'Arcachon. Rapport IFREMER. Pages 85.

Míguez, A., Fernández, M. L. and Fraga, S.(1996). First detection of domoic acid in Galicia (NW of Spain). In: Harmful and Toxic Algal Blooms. Yasumoto, T., Oshima Y. and Fukuyo, Y. (eds). IOC-UNESCO. 143-145.

National Research. (1999). Monsooms to microbes: understanding the ocean's role in human health. Washington D.C., National Academic press. pages 160.

Orellana, G., Van Meulebroek, L., De Rijcke, M., Janssen, C. R., and Vanhaecke, L. (2017). High resolution mass spectrometry-based screening reveals lipophilic toxins in multiple trophic levels from the North Sea. Harmful Algae. 64 : 30–41.

Paulmier G. (1994). Les dinophycées pélagiques et benthiques du Golfe de Gascogne sud de la Bretagne à Arcachon. Annales de la Société des Sciences Naturelles de la Charente-Maritime. 8 :289-357.

Pitcher, G. C., Figueiras, F. G., Hickey, B. M. and Moita, M. T. (2010). The physical oceanography of upwelling systems and the development of harmful algal blooms. Progress in Oceanography. 85:5-32.

Reguera,B., VeloSuárez, L., Raine, R. and Park, M.G. (2012). Harmful Dinophysis species: a review. Harmful Algae. 14 : 87-106.

Rodríguez F, Fraga S, Ramilo I, Rial P, Figueroa RI, RiobóP, Bravo I,. Canary Islands (NE Atlantic) as a biodiversity 'hotspot' of Gambierdiscus: Implications for future trends of ciguatera in the area" Harmful Algae 67, 2017 pages131-143

Schirone, M., Visciano, P., Luciani, M., Ciarelli, A., Berti, M., Tofalo, R. (2013). Yessotoxin determination in *Mytilus galloprovincialis* revealed by an in vitro functional assay. Environ. Sci. Pollut. Res. 20:1189-1192.

Smienk, H., Domínguez, E., María, L., Rodríguez-VelaSco, DaViD, C., Katrin, K., Panagiota, K., Ana, G., Gaba, D., Alberto, O., Juan, M., Vieites, Pedro, R. and Luis, M. (2013). Quantitative Determination of the Okadaic Acid Toxins Group by a Colorimetric Phosphatase Inhibition Assay: Interlaboratory Study Journal of AOAC international. 96: no. 1.

Taleb, H., Vale, P., Amanhir, R., Benhadouch, A., Sagou, R., Chafik, A. (2006). First detection of azaspiracids in mussels in north west Africa. Journal of Shellfish Research 25:1067–1070.

Tang, D. L., H. Kawamura, T. V. Dien and Lee, M. A. (2004a) Offshore phytoplankton biomass increase and its oceanographic causes in the South China Sea. Marine Ecology Progress Series. 268:31–41.

Taylor, F. J. R. and Haigh, R. (1993). The ecology of fish-killing blooms of the chloromonad flagellate Heterosigma in the Strait of Georgia and adjacent waters. In: Smayda, T. J. & Y. Shimizu (eds), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam. Pages 705–710.

Trainer, V. L., Pitcher, G. C., Reguera, B. and Smayda, T. J. (2010). The distribution and impacts of harmful alg al bloom species in easternboundary upwelling systems. Progress in Oceanography. 85:33-52.

Tubaro, A., Dell'Ovo, V., Sosa, S. and Florio, C.(2010). Yessotoxins: a toxicological overview Toxicon. 56:163-172.

Turki, S. (2004). Suivi des micro-algues planctoniques toxiques dans les zones de production, d'élevage des moules bivalves et d'exploitation dans les oursins du nord de la Tunisie. Bull. Inst. Nat. Scie. Tech. Mer de Salammbô. 31: 83- 96.

Visciano, P., Schirone, M., Tofalo, R., Berti, M., Luciani, M., Ferri, N. (2013). Detection of yessotoxin by three different methods in *Mytilus galloprovincialis* of Adriatic Sea. Chemosphere. 90 :1077-1082.

Wagne, M. M., Ould Brahim, H., Dartige, A., and Séfrioui, S. (2011). Contribution à l'étude du phytoplancton potentiellement nuisibles de la baie de Lèvrier. Bulletin de l'Institut Scientifique, Rabat, section Sciences de la Vie. 33 : 31-41.

Xu, Y., Richlen, M.L., Liefer, J.D., Robertson, A., Kulis, D., Smith, T.B. (2016) Influence of Environmental Variables on Gambierdiscus spp. (Dinophyceae) Growth and Distribution. 11:153-197.