Summary of the HANA Fourth Workshop on Harmful Algal Blooms in North Africa

Virtual meeting, 15- 16 March 2021

The North African Network for Harmful Algal Blooms (HANA) converted its fourth workshop virtually, 15-16 March 2021 after 10 years of the last meeting in December 2011. Agenda in Annex 1 hereto. It was attended by nineteen participants from Morocco, Tunisia and Egypt. Mauritania, Algeria and Libya were not represented.
The workshop platform is hosted by IOC and organized by Faculty of Science, Alexandria University. List of participants in Annex 2 hereto.

The meeting was multipurpose, it dealt with scientific presentations and reviews, including summary report of HANA situation during the last 10 years with one session for discussing HANA priorities and recommendations. The collected abstracts were made and sent to the participants (Annex 3 hereto).

The reviews and the scientific presentations evidence the quality and the diversity of the current activities in the region regarding HABs. In Morocco, priority is given to benthic dinoflagellates, analysis of PSP using MBA method and cysts studying their growth and impacts on shellfish production. In Tunisia junior scientists were presented topics related to the distribution of HAB species especially *Alexandrium pacificum* and *Pseudonitzschia bizertensis*, *Gymnodinium* . Monitoring of *Karenia selliformis* using flow cytometry was also presented. Association of bacteria in blooms and their role in the maintenance and disappearance of HAB is a new research topic in Tunisia. The economic losses generated by *Gymnodinium impudicum, Prorocentrum mexicanum, Alexandrium minutum* and *Karenia selliformis* were discussed. In Egypt, monitoring program related to cyanobacteria and benthic dinoflagellates was established only for scientific purpose. The research of HAB in Egyptian water focused on three main topics. The role of invasive macroalgal species for increasing harmful algal species both in the Mediterranean and Red Seas. Monitoring the epiphytic cyanobacteria along Alexandria water with molecular identification. Finally, studying the impact of climate change (ocean acidification and temperature) on the distribution of harmful algae. The participants agreed that There is a serious problem related to HABs along HANA region, because of economic loss due to fish mortality, invertebrate mortality, shellfish contamination and affecting tourism sector. The data concerning HAB events still insufficient (only 67 events were recorded) so more information needs to be exchanged within HANA countries through HANA website.

Recommendations:

1. Updated HAB data (events and species) continuously for HAEDAT and updating HANA website.
2. Maintain the regular meetings every year virtual or physically (two years)
3. Upgrading the expertise by asking IOC for organizing training courses at two levels:
    - Capacity Building and training for junior scientists in the field of harmful algal ecology, toxins and management with new techniques.
    - more advanced courses on molecular techniques of phytoplankton
    - training courses on determination of emerging toxins, mainly CTX and palytoxins.
4. Perform intra-regional intercalibration of the methods used for HAB studies for all the laboratories of HANA region.
5. Effective knowledge exchange among the different partners of HANA through the website, training courses.
6. Establish Data base for molecular identification of HABs in HANA region (INSTEM, Salammbo, Tunisia)

HANA Bureau

During the last session, the participants elected the bureau of HANA for the next two years as follows:

HANA Chair: Amany Ismael

HANA Vice Chair: Asma Hamza

National Coordinators:

Fathy El-Zayat for Egypt

Malika Bel Hassan for Tunisia

Reqia Sagou for Morocco

The participants agreed that the Chair of HANA can be elected twice only.

At the end of the meeting the HANA community agreed unanimously that the workshop had been both successful and stimulating.

ANNEX 1

IOC / HANA

FOURTH WORKSHOP ON HARMFUL ALGAL BLOOMS

IN NORTH AFRICA

Virtual Meeting 15- 16 MARCH 2021

AGENDA

10.00 –-10.15 Opening: Welcome

10.15 – 11.00 Session I Chair: Prof. Amany Ismael

- Summary Report of HANA situation
  Prof. Amany Ismael

- Country report Tunisia
  Asma Hamza

-Country report Egypt
  Amany Ismael

Session II. Chair: Prof. Asma Hamza

11.00- 11.20 Preliminary investigation of the microalgal diversity of the plastisphere collected from the floating plastic debris of Monastir Bay.
Tarchi M., Mahfoudi M., Hamza A., Zaaboub N. and Trabelsi L

11.20 - 11.40 Harmful marine microalgae and contamination of bivalve molluscs from shellfish production areas in the Dakhla Region of south of Marocco by ASP, PSP and LSP toxins during the period 2003-2019
Mina Dellal, Mohamed Malki, Samir Benbrahim , El Mustafa Ait Chatou A.Fahd, Med Bekkali , A.Errhaif, K.Chaira, R.Aboabdellah, R. Sagou, H.El Mortaji,M.Gma, L.Chafiq, M.S.Faaras, F.El Hassani Alaoui, A.Haidoud, H.Goungra, B.Ennafah, A.Bernoussi et A.Chafik.

11.40- 12.00 Effect of Temperature on The Growth of Two Potentially Harmful Dinoflagellates *Coolia* And *Amphidinium* From the Agadir Coast.
Moussavou-Mouity Cyrielle Amour, **Bennouna Asmae**, **Abouabdellah Rachid**, **Alkhatib Houda**, **Alhayane Mohamed**, **Ababou Bouchra**

12.00- 12.20 Heavy metals accumulation in environmental matrices and their influence on benthic diatoms development in the tidal coast of Gulf of Gabes (Tunisia).

Lamia Dammak Walha, Fatma Abdmouleh Keskes, Asma Hamza & Chérif Sammari

12.20- 12.40 The impacts of metallic pollution and eutrophication on dinoflagellates cysts assemblages in the coast of Sfax.
Fatma Abdmouleh Keskes, Lamia Dammak Walha, Asma Hamza , Malika Bel Hassen

12.40- 13.00 Distribution of harmful dinoflagellate cysts in the surface sediments of Dakhla Bay of the South Morocco Atlantic Ocean and theirrelationship to environmental factors and primary productivity.
Karima chaira, Hassan Rhinane, Btissam Ennaffah, Mina Dellal, Fatima zohra Bouthir, Reqia Sagou, Hinde Abdelouahab, Abdelouahed Ben Mhamed, Sanae Ammari, and Mohamed Laabir

13.00- 13.20 Trophic behavior of shellfish *Ruditapes decussatus* to microalgae diversity in Sfax coasts (southern of Tunisia)
Amira Loukil-Baklouti, Asma Hamza, Wafa Feki-Sahnoun, Mabrouka Mahfoudhi and Othman Jarboui

Day II

Session III Chair: Dr Asmae Bennouna

10.00 – 10.20 New approach to prevent presence of biotoxin in shellfish before collection along Tunisian coasts.
Feki-Sahnoun Wafa, Barraj Nouha, Hamza Asma, Bel Hassen Malika

10.20 – 10.40 Comparative Studies Of Epiphytic Harmful Microalgae Between Selected Sites In The Mediterranean And Red Seas (Egypt).
Fathy El-Zayat, Kalil A., Halim Y. and Ismael, A.

10.40 – 11.00 The Influence of the Toxin Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Feeding and Survival of the Marine Copepod, *Acartia tonsa*
Ali H. Abdulhussain, Kathryn B. Cook , Andrew D. Turner, Adam M. Lewis, Mohamed A. Elsafi, Daniel J. Mayor

11.20 – 11.40 High resolution monitoring of the *Karenia selliformis* harmful algal bloom in the southern coast of Tunisia
Mohamed Ismail Boudriga, Amel Belaaj Zouari, Asma Hamza, Nader Ben hajd Hmida, Malika Bel Hassen

11.40 – 12.00 Home validation of MBA (AOAC 959.08) method used in analysis PSP toxins in Agadir's mussels and first application HPLC (AOAC2005.06)

ALKHATIB Houda, ABOUABDELL Rachid, BENNOUNA Asmae, ALAHYANE Mohamed, CHAFI Safaa, MOUSSAVOU-MOUITY Cyrielle Amour, FAHDE Abdelilah

12.00 – 13.30 **Open discussion, Recommendations and Elections**

Chair: Prof. Amany Ismael

Yun Sun

ANNEX 2

List of participants

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ANNEX 3

Abstracts

Preliminary investigation of the microalgal diversity of the plastisphere collected from the floating plastic debris of Monastir Bay.

Tarchi M[a]., Mahfoudi M[b]., Hamza A[b]., Zaaboub N[b]. and Trabelsi L.[a]

[a] Laboratory of Marine Biodiversity. National Institute of Marine Sciences and Technologies. University of Carthage. Tunisia

[b] Laboratory of Marine Science. National Institute of Marine Sciences and Technologies, University of Carthage. Tunisia.

Since 1950, the world has seen a massive industrialization of plastic production. Gradually, these new light, resistant and petrochemical objects have been invading our environment and threatening the balance of our aquatic ecosystem. In addition to the financial damage caused by plastic on ecosystems, the impact of this form of pollution on aquatic biodiversity and human health is still poorly understood.

Today, between 1,000 and 3,000 tons of plastic fragments are floating on the surface of the Mediterranean Sea. This floating debris, of different sizes, provides a habitat for biofilms and marine microorganisms (bacteria, viruses, microalgae, *etc*...) (Wright et al. 2020; Zettler et al. 2013). The work of Amaral-Zettler and collaborators (2020) has provided evidence that these substrates, bind pathogens, micropollutants, invasive species and harmful microalgae. This association of living organisms/plastic debris creates a “man-made” ecosystem called "plastisphere". So far, the consequences of this new ecosystem are not well known. In fact, the issue of transporting this ecosystem has become crucial in terms of its impact on aquatic and benthic biodiversity, the food chain and human health.

Given this "devastation" caused by plastic and this new "man-made" ecosystem that is emerging in aquatic ecosystems, several questions are being asked:

- What are the colonizing microalgae of this new ecosystem called "the plastisphere"?
- Is there a difference between microalgae growing on the plastisphere and microalgae in the environment surrounding the plastisphere?
- Are there harmful microalgae growing on the plastisphere?

Our main objective is to identify and quantify the various microalgae populations present in the plastisphere found in the Monastir Bay.

We also aim to compare the characteristics of microalgae that live in their natural habitat and those of microalgae present in the plastisphere.

Harmful marine microalgae and contamination of bivalve molluscs from shellfish production areas in the Dakhla Region of south of marocco by ASP, PSP and LSP toxins during the period 2003-2019

By

Mina Dellal, Mohamed Malki, Samir Benbrahim , El Mustafa Ait Chatou A.Fahd, Med Bekkali , A.Errhaif, K.Chaira, R.Aboabdellah, R. Sagou, H.El Mortaji,M.Gma, L.Chafiq, M.S.Faaras, F.El Hassani Alaoui, A.Haidoud, H.Goungra, B.Ennafah, A.Bernoussi et A.Chafik.

The Dakhla Region is a paralic environment that is distinguished by various potentialities, including fishing, aquaculture, tourism, etc... However, the development of these human activities

can lead to negative impacts on this environment, which can constitute a threat to human health. This requires special attention to this environment and the implementation of programs to protect and preserve this ecosystem. To this end, programs for the monitoring of
phycotoxins and toxic phytoplankton in the classified shellfish production areas of this Region are launched from May 2003.
Monitoring results of toxic micro-algae and toxins in bivalve molluscs from shellfish production areas in the Dakhla region, during the period 2003-2019, the following results were reported:
- The most common harmful phytoplanktonic species found in the seawater of the production areas of the Dakhla Region and responsible for the contamination of shellfish by toxins are the toxic diatoms of the Genus: Pseudo-nitzchia, Dinophysis, *Prorocentrum lima*, *Gymnodinium Catenatum* and Alexandrium.
- The proliferation of *Pseudonitzschia spp*. is frequently noted throughout this monitoring period, however the limit thresholds for this species have been very rarely reached, the toxicity of this species is rarely confirmed by contamination of bivalve molluscs by negligible traces of ASP toxins.
- On the other hand, the proliferation of species producing PSP toxins, in shellfish especially Alexandrium, is rare or often encountered with low concentrations, except during the year 2006-2007, especially from October 2006 to March 2007, where proliferation has been very abundant in all areas of production of the Region and having induced the contamination of the different types of filter MBV and burners by the PSP toxins whose highest concentration was noted during the month of December 2007. However, during this whole period proliferation of the *Gymnodinium catenatum* is never recorded concentrations rarely recorded, for this species do not exceed 200 cells per liter at most.
-Different periods of contamination of shellfish by LSP toxins were often noted during the period (2003-2019) and the species responsible is often the complex Dinophysis. Indeed, episodes of contamination of the digger and filter shells have been recorded from December 2005 to January 2006, from April to May 2007, March 2011, December 2012, and January and December 2016, November and December 2017 and finally March 2019. The duration of the episodes of contamination of MBV by the LSP, depends on the species of shellfish in question, generally the contamination of the diggers is most quickly reached, however the decontamination of these species sometimes requires long periods.

Effect of Temperature on The Growth of Two Potentially Harmful Dinoflagellates *Coolia* And *Amphidinium* From the Agadir Coast.

MOUSSAVOU-MOUITY Cyrielle Amour[1,2], BENNOUNA Asmae[2], ABOUABDELLAH Rachid[2], ALKHATIB Houda [2,3], ALHAYANE Mohamed[2], ABABOU Bouchra[1]

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The bloom of toxic/harmful phytoplankton is favored by several environmental factors, the most influential of which is the climatic factor. The most climatic factor studied by researchers is the temperature factor because as several studies have shown it favors the growth of these species.

However, in Morocco, cultures of potentially harmful or toxic species and the study of the impact of temperature on their growth are rare.
The effect of 15°C and 20°C temperatures on the growth of two potentially harmful benthic dinoflagellates (*Coolia* sp *and Amphidinium* sp) isolated from the Moroccan North Atlantic (Agadir coast) has been studied . The strains were grown in a silica-free L1 medium at a light intensity of 1300 lux and under a 12h/12h light/dark photoperiod and the growth rate was determined according to the formula (LnN1-LnN2)/(t2-t1). The results show that the temperature 20°C compared to 15°C leads to an increase in the growth rate of both species.

Heavy metals accumulation in environmental matrices and their influence on benthic diatoms development in the tidal coast of Gulf of Gabes (Tunisia)

Lamia Dammak Walha[1, 2], Fatma Abdmouleh Keskes [1, 2], Asma Hamza[1] & Chérif Sammari[1]
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2: Faculty of Science of Sfax

Distribution and abundance of benthic diatoms were assessed in/on different substrates in relation with heavy metals concentrations in the Gulf of Gabes. Tida Flat Waters (TFW), sediments and macroalgae (*Ulva rigida*) were collected for one year (from March 2015 to February 2016) in five stations (El Aouabed, Tabia, Skhira, Gabes Harbor and Zarrat) within the monitoring sites implanted along the Gulf of Gabes coastline. This study's purpose is to investigate the effects of heavy metal pollution to benthic diatom in tidal area in coasts of Gulf of Gabes. We evaluated the variability of benthic diatom community and assemblage in different substrate (TFW, sediment and *Ulva rigida*). Our results showed that diatom diversity and abundance was influenced by metals concentrations, stations and substrata. Gabes harbor presents a positive correlation with metal contamination Cd ($r= 0,62$; $p<0,05$) and the decrease of species diversity.
Despite the extremely high enrichment of some heavy metals, diatoms were also consistently found in the most polluted area, revealing a high adaptation to these major contamination levels.

The impacts of metallic pollution and eutrophication on dinoflagellates cysts assemblages in the coast of Sfax

Fatma Abdmouleh Keskes[1,2], Lamia Dammak Walha [1, 2], Asma Hamza [2], Malika Bel Hassen [2]
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The spatial distribution of dinoflagellates cysts was studied to understand the impact of pollution and eutrophication on the surface sediment of the coast of Sfax. Trace metals, nutrients, vegetative forms and dinoflagellates cysts were analysed. Sixteen dinoflagellates cysts morphotypes were identified and qualified at 15 sampling stations monthly in the period Mars 2015 to February 2016. The mixo-heterotrophic cyst of *Alexandrium minutum* dominated the study area and had the

highest abundance in most of the stations, following *Gonyaulax* sp and *Prorocentrum lima*. The highest cyst concentration was recorded in the coastal part of study area. Eutrophication enhanced the productivity of mixo- heterotrophic cysts. The statistical analyzes underlined the classification of the stations and the existing affinities in the different
biotopes studied. The study of the correlations of cysts and the environmental parameters has made it possible to identify the metal pollutants that may be involved in this process.
The vegetative forms are more frequent during the spring period and most abundance of dinoflagellates cysts is recorded during the winter period which would be a consequence of the blocking germination of the cyst banks following the decrease in temperatures during this season.

Distribution of harmful dinoflagellate cysts in the surface sediments of Dakhla Bay of the South Morocco atlantic ocean and their relationship to environmental factors and primary productivity

Karima chaira[1-2-7], Hassan Rhinane[2] , Btissam Ennaffah[3], Mina Dellal[4] , Fatima zohra Bouthir[1], Reqia Sagou[1], Hinde Abdelouahab[1] , Abdelouahed Ben Mhamed[5] , Sanae Ammari[6], and Mohamed Laabir[7]

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The Dakhla bay, located along the south Atlantic Coastline of Morocco, is the longest and the most important site in Morocco due to its halieutic richness.it holds important shell-farming companies. This bay is an ecosystem with great potential in terms of aquaculture, mainly shellfish farming, and shelters favorable zones for tapiculture, mytiliculture and ostreiculture. This bay is subject to sporadic HABs species. This study of dinoflagellate cyst mapping is the first of its kind from the Dakhla bay. the objectives of the present survey were (i) to evaluate to the spatial variation of dinoflagellate cyst assemblages in the sediment along the Dakhla bay (ii) to assess the densities of its resting across Dakhla bay (iii) to compare planktonic dinoflagellate identifieds and dinocyst data (iv) to compare the cyst assemblages in this study with that of other sites. The data were collected in a scientific campaign by zodiac carried out from 10th to 16 April 2018 by core samplers (47 cm long,5 cm diameters) following a sampling network of 49 stations distributed randomly in the study area. The surface layer of the sediment cores (3 cm) was sliced and kept at 4°C until analysis. The highest total cyst abundance was 304 cysts. g^{-1} dry sediment (DS). By applying the Geographic Information System tool, one zone of preferential accumulation of cysts have been identified representing areas at risk. The Pearson statistical test

revealed a positive and significant correlation between cyst abundance and water content, organic matter and of fine sediment (<63µm) percentages. Our study showed the presence of morphotypes of potentially toxic species (*Alexandrium minutum, Alexandrium pacificum, L. polyedrum, Gymnodnium catenatum* and *Gonyaulax cf spinifera*). The cysts of these species present in the sediment of the Dakhla bay could germinate, when environmental conditions become favorable, and in turn could inoculate the water column with the subsequent bloom formation. This could explain the recurrence of the blooms observed in this ecosystem and the related intoxication of the mollusks measured by RSSL survey implemented by INRH (Institut National de Recherche Halieutique). This study confirms the usefulness of cyst analysis in the assessment of harmful bloom risk in this area important for oyster's culture.

New approach to prevent presence of biotoxine in shellfish before collection along Tunisian coasts

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When the toxicity in shellfish exceeds the threshold, the Ministry of Agriculture initiates the alert procedure and faxes the laboratory to the decision-makers for the suspension of collection in the shellfish production area. This decision is communicated by fax to the shellfish collectors as soon as 48 hours after the analysis. We will lose physical and material effort. The batches of shellfish collected will be destroyed (up to 5-ton/year). The collection rate decreases by less than 50% of its potential. The total number of collectors and the total number of working days decrease (50%). Our added value is to deliver SMS that broadcast reliable information in real time faster to inform and alert collectors of the condition of the area immediately after having the final phytoplankton analysis result in order to suspend collection.

Comparative Studies Of Epiphytic Harmful Microalgae Between Selected Sites In The Mediterranean And Red Seas (Egypt)

Fathy El-Zayat, Kalil A., Halim Y. and Ismael,A.

Oceanography Dept., Faculty of Science, Alexandria University, Alexandria, Egypt.

The first attempt to study of epiphytic harmful dinoflagellate was made by Ismael and Halim (2006), with the first record of *Ostreopsis* spp in Egyptian waters.

The benthic potentially harmful species epiphytic on macroalgae in Alexandria coastal waters have been surveyed by Ismael and Halim (2006, 2007, and 2010), El-Zayat (2012), Ismael (2014) and the present study.

This article reviews species composition and relative abundance of benthic potentially harmful epiphytic assemblages on macroalgae at the two sites investigated. The present study represents a follow up of the seasonally dynamics of potentially harmful epiphytic microalgae along the coast of Abu-Qir and Ras Mohammed from summer 2016 to spring 2017.

In Abu-Qir (Mediterranean Sea), the epiphytic microalgae community during the present work comprised 79 species. Dinoflagellates was the dominant potentially harmful microalgal component.
In Ras Mohammed (Red sea), the epiphytic microalgae community during the present work comprised 111 species. The potentially harmful epiphytic microalgal species during the period of study comprise 7 species recorded from RM site Cyanobacteria was the dominant potentially harmful microalgal component.

The Influence of the Toxin Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Feeding and Survival of the Marine Copepod, *Acartia tonsa*

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Blooms of harmful algae are increasing globally, yet their impacts on copepods, an important link between primary producers and higher trophic levels, remain largely unknown. Algal toxins may have direct, negative effects on the survival of copepods. They may also indirectly affect copepod survival by deterring feeding and thus decreasing the availability of energy and nutritional resources. Here we present a series of short-term (24 h) experiments in which the cosmopolitan marine copepod, *Acartia tonsa*, was exposed to a range of concentrations of the toxic dinoflagellate, *Alexandrium catenella* (strain 1119/27, formerly *Alexandrium tamarense*), with and without the presence of alternative, non-toxic prey (Rhodomonas sp.). We also present the toxin profile concentrations for *A. catenella*. The survival and feeding of *A. tonsa* were not affected across the range of concentrations recorded for *A. catenella* in the field; increased mortality of *A. tonsa* was only discernible when *A. catenella* was present at concentrations that exceed their reported environmental concentrations by two orders of magnitude. The observed lethal median concentration (LC50) for *A. tonsa* exposed to *A. catenella* was 12.45 ng STX eq L−1. We demonstrate that *A. tonsa* is capable of simultaneously ingesting both toxic and non-toxic algae, but increases clearance rates towards non-toxic prey as the proportional abundance of toxic *A. catenella* increases. The ability to actively select non-toxic algae whilst also ingesting toxic algae suggests that consumption of the latter does not cause physical incapacitation and thus does not affect ingestion in *A. tonsa*. This work shows that short-term exposure to toxic *A. catenella* is unlikely to elicit major effects on the grazing or survival of *A. tonsa*. However, more work is needed to understand the longer-term and sub-lethal effects of toxic algae on marine copepods

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High resolution monitoring of the *Karenia selliformis* harmful algal bloom in the southern coast of Tunisia

Mohamed Ismail Boudriga, Amel Belaaj Zouari, Asma Hamza, Nader Ben hajd Hmida, Malika Bel Hassen

The variability of phytoplankton populations has often been apprehended with fairly large time scales (monthly or even seasonal). However, these populations multiply at finer scales, one to two divisions per day. In addition, phytoplankton, and in particular nano- and pico-plankton organisms have the particularity of responding to certain hydrometeorological phenomena and their responses are often on the hour scale. It was therefore necessary to use approaches that meet these constraints to better understand the daily cell cycles of these planktonic populations. The in-situ study of the dynamics of microphytoplankton populations at high resolution and in particular the determination of their growth rates constitutes methodological challenges that flow cytometry is in the progress of overcoming. Indeed, high frequency cytometry is the reference tool to respond to these constraints and allowing automated in situ observation concerning the individual scale of cells (Sosik et al., 2003; Dugenne, 2017). This approach is particularly useful for the study of species responsible for blooms with a high toxic potential. Coupling meteorological data and nutrient availability in addition to the species observation makes it possible to study the impact of physicochemical and hydrometeorological phenomena on the dynamics of phytoplankton population, and quantify their impacts in terms of production, biomass, and selective responses to environmental changes.

The phytoplankton monitoring program (REPHY), established since 1995, reported the HABs events mainly occurring in the Gulf of Gabès (Hamza, 2003). The statistics of 10 years weekly observations reported over 176 HABs events in this area (Féki et al., 2018). The dinoflagellates *Karenia selliformis* accounted for 64% of toxic blooms (Féki et al., 2008). It becomes therefore essential to focus on the dynamics of this species by combining high-frequency measurements of the species as well as the acquisition of high-resolution physical data to understand the relationships between the physicochemical parameters and the dynamics of the species.

A sampling survey to monitor a bloom of the harmful dinoflagellate *Karenia selliformis* on an hour scale was conducted during 14 days in 2019 at the commercial harbor of Sfax. The analysis of the optical properties of the cells revealed that compared to other distinct populations of Picocyanobacteria, Picoeukaryotes and Nanoeukaryotes, the population of *Karenia selliformis* predominated the system in terms of abundance and biomass with a low diversity during the bloom. In addition, during the spring tides the highest concentrations of the species were recorded, reaching up to 8 10^6cells / l. This suggests that the harbor constitutes an accumulation area where the species is entering from the open sea and that the accumulation is more linked to the hydrodynamical variability rather than to a net growth of the species. The fluctuation of the species according to the tide with abundance maxima recorded during the high tide reinforces the hypothesis that the tide fluctuation would be a determining factor in the proliferation of *Karenia selliformis* in the harbor.

Trophic behavior of shellfish *Ruditapes decussatus* to microalgae diversity in Sfax coasts (southern of Tunisia)

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Harmful microalgae are becoming more frequent and problematic essentially in urbanized coastal zones. Coasts of Sfax contains substantial natural stocks of the grooved carpet shell *Ruditapes decussatus*. Unfortunately, exploitation of this stock has been frequent bunged after alerts of presence of toxic phytoplankton.

The aim of this work was to investigate the microalgae communities in different compartments (water column, sediment, biofilm and intervalvary liquid) coupled with environmental factors through the shellfish production along the southern coasts of Sfax (Gulf of Gabes). This study allowed us to highlight the population that will be put to the benefit of bivalves and especially their spatial and temporal variability.

The comparison between the species counted in the intervalvary liquid of the bivalve *Ruditapes decussatus* and those observed in the three analyzed compartments revealed that this shell chose a selective feeding mode to words these populations. Indeed, this bivalve seems to direct its diet towards cyanophyceae, cryptophyceae and diatoms while avoiding the toxic species.

Home validation of MBA (AOAC 959.08) method used in analysis PSP toxins in Agadir's mussels and first application HPLC (AOAC2005.06)

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The marine environment under very specific conditions knows the proliferation of certain toxic species of phytolankton, this phenomenon also affects the Moroccan coasts. Some species of these microalgae produce toxins, which can affect marine organisms and can be accumulated by bivalves. Subsequent consumption of contaminated seafood may induce human's intoxication. In order to protect the consumer, a regular monitoring of these microalgae and these toxins has been carried out by the National Institute of Fisheries Research (INRH) since 1992. The AOAC 959.08 method was used for detection and quantification of toxins in Agadir's mussels. In 2019 a home validation of MBA was conducted according to the Standard Operating Procedure by an internal certified sample (AOAC, Paralytic Shellfish Poison, Biological Method, final Action, Method 959.08 in Official Methods of Analysis (1995) of the AOAC, Natural Toxins, Chapter 49, page 46). 3 levels of the sanitary threshold (800 µg.eq STX/kg of shellfish meat) were studied: ½, 1 and 1.5 time, in this work we will présent the level of sanitary threshold. The internal certified

concentrations used was 856 µg.eq STX/kg of shellfish meat. The conversion from Mouse Unity to equivalent µg saxitoxin was confirmed during study by the conformity of the factor of conversion FC=0.2 +- 0.04. The limit of quantification (LOQ) of the laboratory (FC=0.2) is 325.5 µg.eq STX/kg of shellfish meat this limit in comparison with that calculated in this level was inferior: 599 µg.eq STX/kg of shellfish meat (LQD: validated). The Study of parameters influencing the analysis of PSP: storage at -20 ° C and 5± 3, pH and heating, show a stability of these toxins after storage in the freezer for à long periode > 1 year and in fridge short period 1 week. An CV% obtained was <20%, (limit of compliance tolerated by the precision of AOAC method 959.08). Concerning pH and heating variations in the concentrations were noted during the variations in the acidity in the heating of the extract.This biological method will be replaced by chemical method for the detection of PSP (AOAC 2005.06) in several countries according to the new amendment of the European Commission Regulation (Amending Commission Implementing Regulation (EU) 2019/627). This method is based on high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) and determines quantity and profile of each toxin present in samples. The first analysis conducted in laboratory by HPLC-FLD was done on the internal control samples (used in home validation), the result of screening showed the presence of GTX1,4; dcNEO and NEO. For the 2021 Agadir region samples, no toxins were detected.

National report on HABs in Tunisia

Hamza Asma

HABs study in Tunisia can be approached at three levels

The research of HABs topic

Several scientists in Tunisia carry out significant research effort. The research effort has adopted in recent decades to promote the axis of study on HABs and solve the problems related to these organisms. Teaching of phytoplankton is currently being established in Tunisia and several universities and higher schools have included programs relating to this topic. Indeed, national and international research and publications have evolved considerably over the past decade. The HABs have treated in different ecosystems and in different environments. We have counted more than a hundred articles relating to these phenomena, bringing together the taxonomy, ecology and toxicity of proliferating organisms. Some focus were oriented to microepiphytic species and their distributions on different supports on different Tunisian coasts. Encystment of dinoflagellates has also very well treated and the impact of this process on the generation of blooms is reported . New species for the microplanktonic flora of the Tunisian coasts have described, namely *Alexandrium pacificum* or *Pseudonitshia bizertensis*. The impact of metal pollution on toxic phytoplankton species arises from the interest of continuing this section in order to better understand the phenomena of toxicity and this subject mainly concerned a few dinoflagellates and certains diatoms. New methods for the study of toxic species have defined such as the application of q-pcr or high frequency cytometry to identify the dynamics of organisms. The application of network modelling on the HABs appear also as new approach to understand the evolution of them and to predict their occurence. These works mainly concern *Karenia selliformis*, *Alexandrium minutum* and *Coolia monotis.* The trophic behavior of phytoplankton organisms and the demonstration of the relationships between the different microalgae groups and the environmental parameters are the subject of notable papers. There are also several studies (more than thirty in the last decade) deal with toxic cyanobacteria in freshwater. We also note the participation of Tunisia researchers

in some European programs focus to HABs such as the M3HAB (ENICBCmed project) and the participation in multidisciplinary research campaigns in the Mediterranean Sea like the Hypocampe, COZOMED, MERITE, with the IRD institute. Several approaches and methods for the study of phytoplankton among other toxic species have developed in these marine cruises. A new aspect of research is also currently under development and investigation, namely the association of bacteria in blooms and their role in the maintenance and disappearance of these episodes.

Monitoring and survey of toxic phytoplankton

The phytoplankton monitoring networks in the marine shellfish production areas in Tunisia began since 1995 and the data concerning this monitoring feed a database which records the toxic species frequenting our coasts, the threshold concentrations of toxicity and the toxic profiles. The monitoring of toxic phytoplankton is currently also being developed for freshwater environments and fish aquaculture infrastructures. The laboratories responsible for this monitoring (phytoplankton and phycotoxins) are currently setting up quality assurance programs and they are quite advanced in their planning. Biotoxin tests are entirely carried out by chemical protocols (HPLC and LCMS) and mouse tests are almost abandoned. The deployment of Solid Phase Adsorption Toxin Tracking (SPATT) as a complementary method to monitor toxic events in some region showed the detection of OA and DTX-1. The application of the RBA technique for the detection of paralytic and neuronic phycotoxins is actually under development.

Distrophic crises and economic losses generated by HABS

The years 2018 and 2020 were spectacular in blooming toxic phytoplankton species in many Tunisian coasts. We noted summer blooms *for Gymnodinium impudicum* in Carthage coasts and *Alexandrium minutum* in Sfax. The bloom of *Karenia selliformis* appears in fall and ravaged fisheries especially in the Gulf of Gabès; significant losses of fish have reported, and a social crisis has even set in. Bloom of *Prorocentrum mexicanum* were also declared on the coasts of Monastir in summer 2020 and molecular identification of the species is in progress. Our approach for the monitoring of these episodes is formulated in daily sampling campaigns, satellite monitoring of the progress of red tides and especially discussion forums with fishermen and authorities to maintain a dialogue and to instore participatory approach to resolve problems.

Problems encountered and recommendations for the future

- Lack of a real synergy between the different research groups and sometimes we notice a redundancy in the treated subjects
- Absence of a national reference laboratory for toxic phytoplankton and biotoxins and we suggest a HANA reference laboratory
- Budget restrictions for research in some of our labs do not allow us to participate in international workshops and symposium

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Harmful Algal Blooms in the Egyptian waters

Amany A. Ismael

The long-term records of harmful algae in the Egyptian waters showed that the blooms went through three phases with overall, nearly 39 harmful species (Egyptian Mediterranean

and Red Sea Coasts) which are known or suspected to produce toxins occur in Egyptian waters which pose serious hazards to human health and marine resources (Ismael 2021). Since the last HANA meeting in 2011, seven potentially harmful species were added namely: *Microcystis aeurginosa*, *Lyngybya majuscule*, *Oscillatoria limosa*, *Planktothrix agardhii*, *Chaetoceros socialis*, *Nitzschia longissma* and *Prorocentrum concavum*.

The numerous HAB species recorded in the Egyptian waters has the potential to threaten seafood resources and cause severe adverse economic consequences. Of particular concern are the negative impacts on aquaculture, which has been a key component in the Egyptian national food supply for centuries. HABs have caused massive mortalities of marine fish and shellfish due to anoxia or the production of toxins. *Donax trunculus*, one of the most important shellfish harvested in Egypt showed massive mortality due to oxygen depletion due to the proliferation of *Noctiluca scintillans* off Nile Delta, 2011.

Studies of HAB in the Egyptian waters went through three main topics:

1- **Monitoring of harmful cyanobacteria and Role of invasive macroalgae for increasing HAB species:**
   - The role of invasive macroalgal species for increasing harmful algal species was studied during the period 2016-2017 (El-Zayat, 2019).
   - Eleven epiphytic harmful species were recorded from the Mediterranean site and seven HAB species were recorded from Red Sea Site.
   - *Prorocentrum concavum* was recorded for the first time from the Egyptian waters.
   - The invasion of green algae *Caulerpa racemosa* via Suez Canal led to the increase of the epiphytic harmful cyanobacteria
   - *Ostreopsis* species was the most dominant represented 81% of the total HAB species in the Mediterranean site, While *Lyngybya majuscula* represented 50% of the total HAB species in the Red Sea site.

2- **Monitoring of epiphytic harmful Cyanobacteria along Alexandria coast:**
   - The objective of the study was to identify the potentially harmful epiphytic cyanobacteria and follow up their distribution along Alexandria Coast.
   - Morphological and molecular analysis were used for identification of dominant species.

3- **Impact of Ocean Acidification in Egyptian Coastal Areas:**
   - The impact of Ocean acidification on the distribution of macroalgae and its epiphytic harmful Algae in Alexandria coastal waters was studied since 2006 as a part of the monitoring programme of the Oceanography Department.In the Egyptian coastal waters, there are uncertainty about how macroalgae community and their epiphytic harmful microagae will be affected by ocean acidification. More studies are needed concerning this subject.

- All the data of harmful events and records in the Egyptian waters are linked to the Harmful Algal Information System (HAIS) and HAEADAT and will be updated regularly.
- Through the monitoring programe in the Oceanography Department a guideline (monograph) of Harmful Algal species in the Egyptian Mediterranean waters with SEM is prepared which will help the researcher and stockholder.

- Faculty of Science, Alexandria University are participating in two projects related to HABs namely, RAF7014 Applying Nuclear Analytical Techniques to Support Harmful Algal Bloom Management in the Context of Climate and Environmental Change, Phase II (IAEA) and Climate change Management through Mitigation and Adaptation (AdapTM). Co-funded by the Erasmus+ Programme of the European Union.

Work plan:

- Through the monitoring program in the Oceanography Department a guideline (monograph) of Harmful Algal species in the Egyptian Mediterranean waters with SEM will be prepared which will help the researcher and stockholder.
- There is uncertainty about how macroalgae community and their epiphytic harmful microagae will be affected by ocean acidification. More studies are needed concerning this subject**.**
- Sediment samples were collected for studying HABs along the Nile Delta and Eastern Egyptian Coast.
- Capacity Building and training for junior scientists in the field of harmful algal ecology, toxins and management with new techniques