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Analysis of Toxin Amnesiac by High Performance Chromatography Liquid in Bivalves of the Dakhla Region

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ABSTRACT

In this study we performed an ASP analysis in bivalve molluscs of the Dakhla region, especially mussels and oysters. This work was performed between March and June 2015. The sample was conducted in four sites in the Dakhla region, namely Boutalha, Duna Blanca, Puertitto and Oum Labouir. After analysis by HPLC / UV it was shown that the greatest percentage of toxicity of domoic acid (DA) was recorded in June, followed by the months of April and May, while the month of Mars was marked by a low DA toxicity.

Key word: ASP, Domoic Acid, toxicity, HPLC/UV, *Pseudo-Nitzshia*, efflorescence, phytoplankton.

I. INTRODUCTION

There are indications that toxic algal blooms are increasing because of pollution of coastal waters and worldwide shipping. This article deals with the marine biotoxin domoic acid, also known as amnesic shellfish poison (ASP), and its main producing pinnate diatom genus Pseudo-nitzschia (Bacillariophyceae) (Mos, 2000). The Domoic acid, belongs to a group of amino acids, called the kainoîds, which are classed as neuroexcitants or excitoxins that interfere with the neurotransmission mechanisms in the brain. The toxin can be accumulated in shellfish feeding on a number of toxic Pseudo-nitzschia species (Bates *et,al.,* 1998). Ingestion of seafood contaminated with domoic acid can lead to an intoxication which symptoms include (among others) abdominal cramps, vomiting, disorientation and memory loss (amnesia) and can become severe in certain cases (Wright *et al.,* 1989).

Consequently, DA assays in shellfish have become systematic since 2003 in Morocco, in the context of the phytoplankton monitoring network, as soon as the threshold for Pseudo-nitzschia spp. rises above 10^5 cells/ litre (themean threshold for Europe) (Amzil, 2002).

Domoic acid is extracted from bivalve tissue with a mixture of methanol and water. The extract is filtered through a membrane filter and measured using HPLC equipment with isocratic elution and UV detection. The amount of domoic acid is calculated by the external standard method.

The main purpose of the present study is the assessment of the monitoring concerning amnesic toxins in Pseudo-nitzschia spp. blooms in Dakhla coast in terms of: domoïc acid accumulation in shellfish.

II. METHODS AND MATERIAL

Shellfish Samples

Tow shellfish species were sampled at different sites of dakhla coasts (fig.1): *Mytilus galloprovincialis and Crassostrea gigas.*

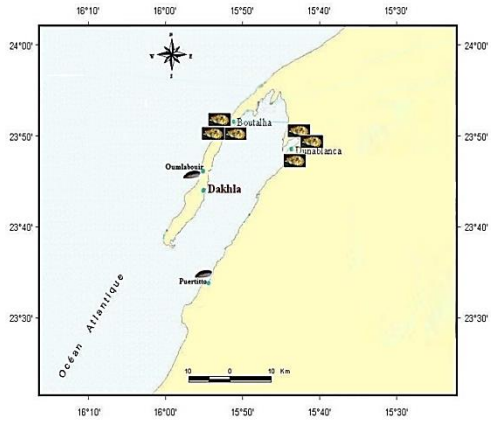

Figure 1 : Sampling locations of Dakhla coasts.

Shellfish Extracts

One hundred grams of total shellfish meat were ground in an Ultra-turrax. The assay amount (4 g) was precisely weighed in a graded centrifuge tube to which 16 ml MeOH/H O (1:1) were added. The sample was homogenized followed by centrifugation at 4,800 g for 10 min. The supernatant was filtered on 0.2 µm, and 20 µl were analysed by HPLC/UV.

High performance Liquid chromatography with ultraviolet detection (HPLC/UV).

High performance liquid chromatography with ultraviolet detection (HPLC-UV) was the first chemical analytical method for domoic acid and is still the most commonly used for monitoring shellfish. Domoic acid detection is facilitated by its strong absorbance at 242 nm (Quilliam *et al.*, 1995).

The DA assay by HPLC/UV was performed according to the method of (EU-Harmonised Standard Operating Procedure, version 1, 2008) using a C_{18} reverse phase (Vydac : 5 µm, 250 mm x 4,6 mm) at 40° C. The elution solvent for single pump systems was a 10% acetonetrile; 0,1% TFA and dilute to 1L with water with, a flow rate of 1 ml/min. DA detection was performed at a wavelength of 242 nm. The DA in the sample was quantified in duplicate using a certified standard DA provided by NRC, Halifax, Canada (SOP, 2008).

III. RESULT AND DISCUSSION

Results analysis during the study period showed that at 4 sampling sites, Mussel samples were the most contaminated with DA compared to Oyster samples. The maximum concentrations found (7.6 mgDA/Kg meat) were not harmful for consumers as they were largely below the legal threshold (20 mg DA /Kg of meat) (fig.3).

Fig. 2 summarises the results of analyses of the shellfish samples in 4 the study sites. Domoic acid was detected in different bivalve species from the 4 sites on the Dakhla coast. With a percent of toxicity that augments in Boutalha to Oum labouir. The maximum % of txicity found (75% in Oum labouir)

However, at the study areas, it was noted that the percentage of toxicity was much more important in the area and Puertitto Oum Labouir in areas of Boutalha and Duna Blanca.

Knowing that the DA is responsible for the ASP (Amnesic shellfish poisoning) syndrome is synthesized directly by Pseudo-Nitzshia which is a phytoplanktonic micro-alga, it is assumed that this high rate of toxicity is due to a phytoplankton bloom of this micro-alga, which is stimulated by different environmental factors. According to studies carried out on the phytoplacton, the periods of annual blooms are recorded around the middle of the year between spring and early summer. (Andrew et al, 2012). This may explain the rate of toxicity between April and June 2015.

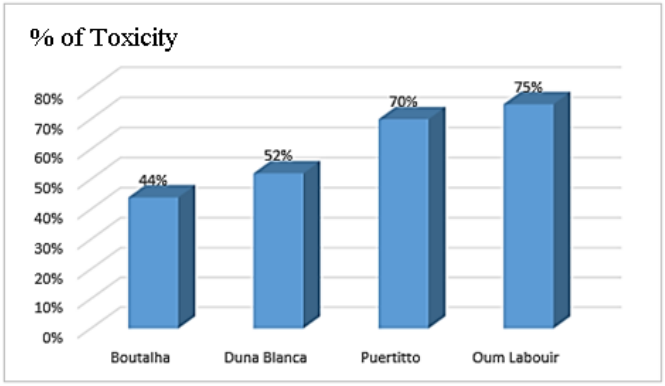

Figure 2 : Domoic acid concentration in shellfish at different sites of dakhla coasts.

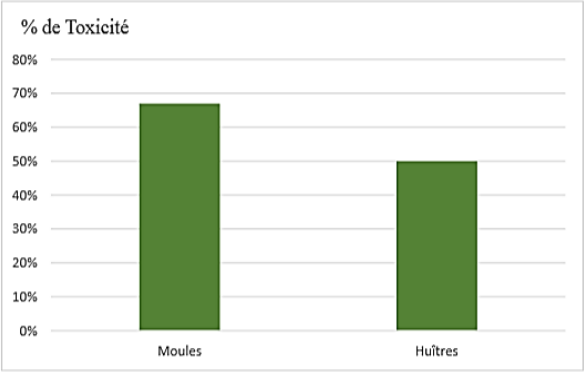

Figure 3 : Domoic acid concentration in shellfish extracts analysed by HPLC/UV.

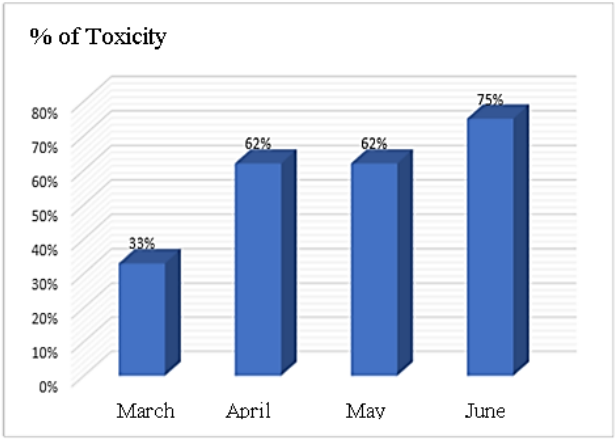

Figure 4 : Domoic acid concentration in shellfish extracts since March to June .

The occurrence of amnesic toxins in Moroco shellfish lends support to the idea that phycotoxins can be disseminated throughout the world via ballast waters and shellfish transfers. In fact, potentially toxic species of spp. have already been observed and implicated in shellfish toxicity events in neighbouring or nearby countries such as Spain and Portugal (Arévalo *et al.*,1998; Vale *et al.*, 1998).

IV. CONCLUSION

During the monitoring programme of harmful algal blooms established along the dakhla coast, the weekly determination of phycotoxins analysis in Mussels and Oyster is carried out from marsh 2015 to June 2015. Results analysis during the study period showed that at 4 sampling sites, Mussel samples were the most contaminated with DA compared to Oyster samples. However, at the study areas, it was noted that the percentage of toxicity was much more important in the area and Puertitto Oum Labouir in areas of Boutalha and Duna Blanca. the greatest percentage of toxicity of domoic acid (DA) was recorded in June, followed by the months of April and May, while the month of Mars was marked by a low DA toxicity. According to studies carried out on the phytoplacton, periods of annual blooms are recorded around the middle of the year between spring and early summer. This may explain the rate of toxicity between April and June 2015.

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