

## **“Impact of Algae That Produce Bio - Toxin on Shellfish" *Mytilus galloprovincialis* (Lamarck, 1819) Cultivated in Lake Butrinti”**

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### **Abstract**

This title, this piece of paper highlights the problematic issues arising out of the practice of Mussels cultivation. (*Mytilus galloprovincialis* (Lamarck, 1819), they are related to their plankton and the adverse effects on the consumption quality (Fig. 2 a, b, c, d, e, f). There are examined the factors thought to have contributed to the outbreak of several types waves of life-producing algae toxin, some of which also feed the above mentioned shellfish. The paper provides data on the quantity of Bio - Toxin which results from analyses identified among these mussel samples from the Laboratory of the Institute of Food Safety and Veterinary Medicine at the Ministry of Agriculture, at the early-season-market for 2012 and following, for this food product. The study provides some thoughts on the causes of identification on the rate of Bio - Toxin among these limpets in this season.

**KEYWORDS:** Shellfish (mussel), algae, Bio - Toxin, salinity, water.

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### **Introduction**

In our country, in the southern part of Albania, in the Butrinti Lake is cultivated as food element from human beings the shellfish known with the Latin term (*Mytilus galloprovincialis* (Lamarck, 1819).

Half salty waters of this lake are result of the connection with the sea waters and flows of sweet waters from the Bistrica spring.

AS the result of deviation last periods of this spring, salinity of the lake waters has changed its parameters, and this has favored the massive reproduction of algae which are the main source of Bio-Toxin production in this basin.

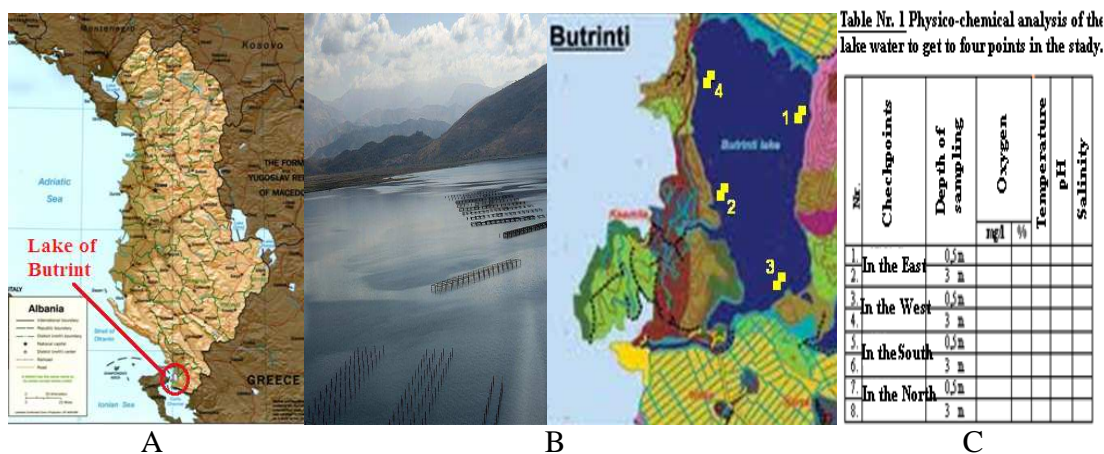
The use of such algae as food element from the shellfish known with the Latin term (*Mytilus galloprovincialis* (Lamarck, 1819) has contaminated its flesh with such Bio-Toxins increasing the pollution level allowed to be consumed from human beings

Based on this fact we initiate such research study in order to open a way to suggestions how to eliminate such phenomena and preservation of trading issues of such shellfish flesh.

### **Materials and methods:**

To fulfill this study, it is taken into consideration the mussel being cultivated in Lake Butrinti

(Fig.2<sub>a, b, c, d, e, f</sub>) Assigned and take samples for analysis of toxic contents in mussel flesh and for physical-chemical analysis of water from 4 sample points with reference to the articles of the following table (Fig.1<sub>A, B, and C</sub>):



**Fig. Nr. 1 (A, B, C)** – Natural and mapped view of Lake Butrinti with its 4 points of physical-chemical analysis of water.

During data processing, some of them were not involved in this work and it is just those dealing with physic-chemical analysis of water. These were used only for the realization of the results obtained in terms of the presence of Bio - Toxin in the flesh of this shellfish.

While viewing the information, the temperature was considered the average among all water depths for this life form (0.5 to 3 m). The hydro-chemical analysis of Lake Butrinti are conducted by the Laboratory itself (The Laboratory of Collection and Depuration of Mussels Butrint - Sarandë) based on the feeler method by means of the apparatus “MULTI 340 i” composed of three drills. One is used to measure O<sub>2</sub>, the other one for pH and one for Salinity in %. The oxygen in water is measured by digital ox meter YSI-85 supplied with a cable of 15 m in length. As the results taken from our analyze are the temperature and salinity, the drill level of salinity is 0, 01 mol/KCL

And together with the drill of pH the temperature is felt as well. To measure the value of Ph, it is conducted by using Pocked-Sized pH meter “Checker I” model HI 98103. [2] The bacterial analysis and the Bio - Toxin evidence at the flesh of this mussel above the norm are carried out and evidenced by the Laboratory of the Institute of Food Safety and Veterinary Medicine at the Ministry of Agriculture and Consumer Protection in Tirana, following the method *Yassumoto 1984* while testing mice. Following the analysis at the species which produce this Bio - Toxin is done with the help of the method *EN 15204 having its measure unit cells/liter*.

**The general part:**

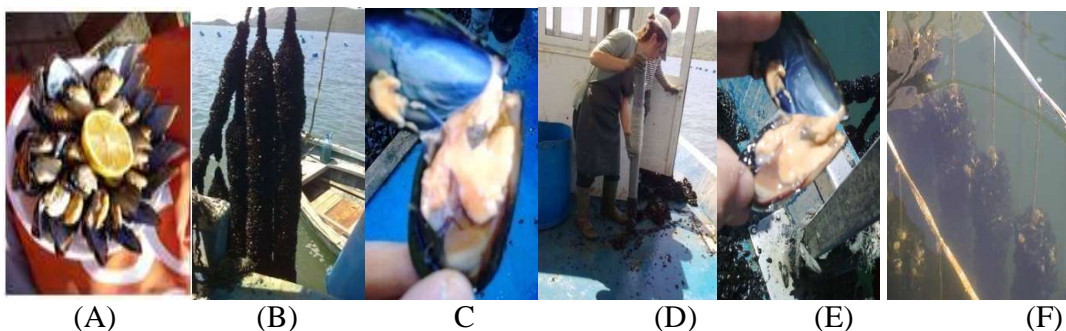
To have a good cultivation of this species, it is clear that some factors are of great importance such as: temperature, air, the force of water wave, quality and quantity of zooplankton and phytoplankton as well as water circulation (Van Erkom

Schurink&Griffiths 1993). Worth mentioning is the level of water salinity. [1] At its optimal conditions, the mussel (*M. galloprovincialis*) usually reproduces not only in November-December but also in February-March. The effect of water waves proves the fact that the great majority of small mussels is said to be found precisely among the stratification from 30-100 cm under the water surface. It provides not only the fact that the wave effect influences the transport of a great amount of larvae but it also brings to light the fact that this is the richest stratification with phytoplankton which is so necessary for their growth.

At its biogenic phase, the normal cultivation of mussels requires

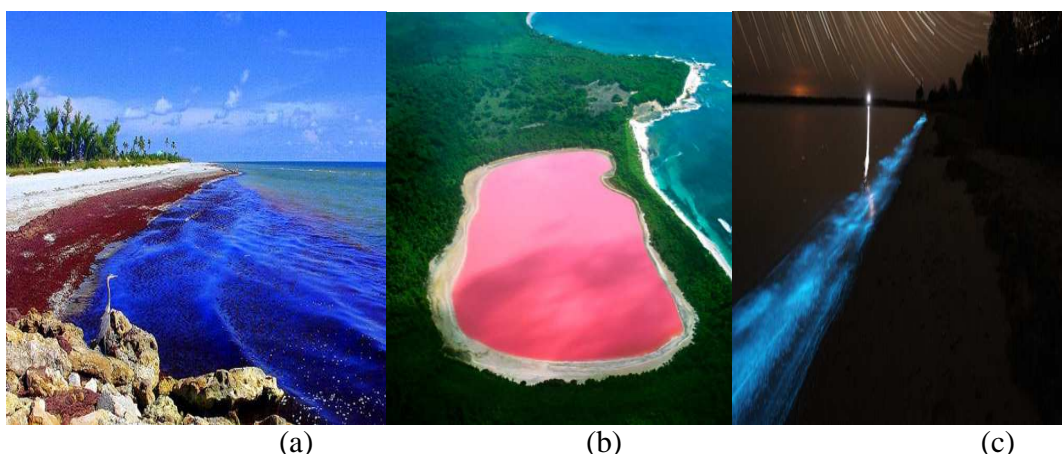
- A great percentage of oxygen (5 – 6 cm<sup>3</sup> per liter)
- A great percentage of zooplankton and phytoplankton
- Water temperature 13-25<sup>0</sup>C
- A percentage of salinity 15 – 30/000 (we think 12 – 15/000) [7]

At the early-season-market for 2012 for this food product ( May 2012), based on the analysis conducted at the Laboratory of the Institute of Food Safety and Veterinary Medicine at the Ministry of Agriculture and Consumer Protection in Tirana, it was observed that the product was contaminated with Bio - Toxin at a level beyond the norms of legal consumption.



Picture Nr.2 (A, B, C, D, E, F) – Working hours, cultivating mussels at Lake Butrinti

Bio - Toxin, which is presented by laboratory examinations, is mainly of the type DSP (Diarrheic Shellfish Poisoning). These toxins deposit in the animal tissues as a result of consuming a great amount of proteins of Dinoflagellate group, having been classified on the base of the International Code of Botanical Nomenclature ICBN, or the International Code of Zoological Nomenclature ICZN. Today, it is widely accepted to classify them on the base of the system ICBN [6]. A great variety of toxins is very strong, and if not seriously fatal, they can still cause neurological effects plus other negative ones (we can mention Bio - Toxin PSP- paralytic poison of mollusk, PSK-neurotic toxicity from mollusk, ASP- amnesic poison from shellfish etc). Almost the half of them is autotrophic and the rest is heterotrophic. Under suitable conditions, it is often observed a flow of their number, thus producing what it is called “*Tides red*”. These forms are reproduced in such number that the water turns to be seen as golden or red, thus producing the so-called “*Tides red*”. [14, 15] (Picture.3<sub>a, b, c</sub>) This effect is produced by Dinoflagellate of marine species which bloom along summer time. [16]



**Fig. Nr.3** (a,b,c) - Phenomenon “Tides red” at sea water (a,b – day view; c – night view)

Autotrophic forms have got the ability to emigrate through the stream making use of the depths of water which are rich in food, during the night and floating during the day to feed in an autotrophic way on the surface of the water ( as photosynthetic living forms).[12]

The massive blooms of these living forms usually prefer to have a temperature of water 5-8 °C.

When the temperature of water goes down to + 4° C and this Dinoflagellate will continue to live like cists sinking above the upper layer of sediment [11]. Some other types are able to produce their light through the process of bioluminescence (Fig.3). [16]

#### **The specific part:**

There are three types of these algae:

**(PSP)**

**PARALYTIC SHELLFISH POISONING**

caused by Sax - Toxin

**(ASP)**

**AMNESIC SHELLFISH POISONING**

caused by domoic acid

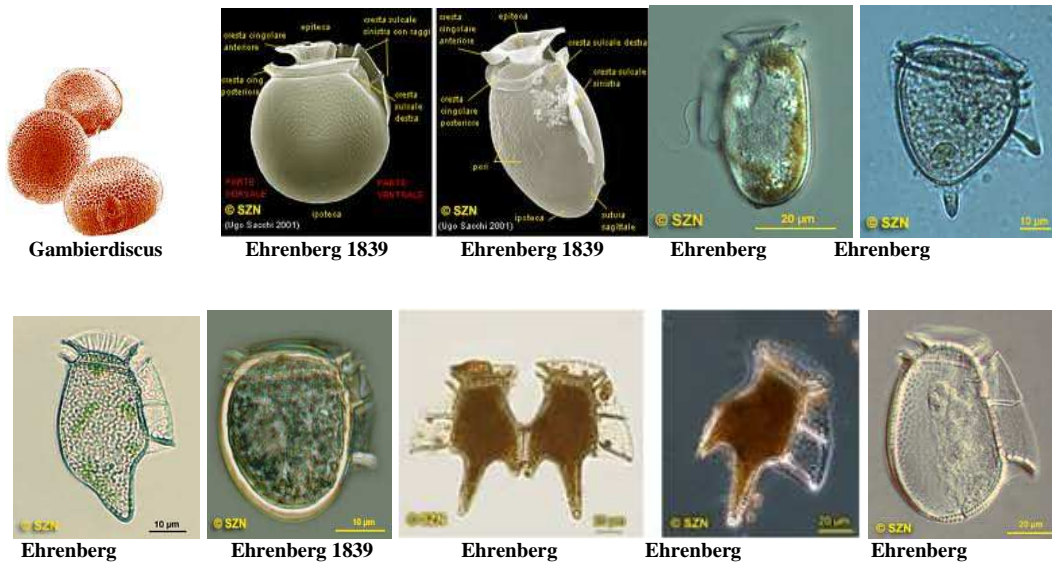
**(DSP)**

**DIARRHETIC SHELLFISH POISONING**

caused by kadaic acid

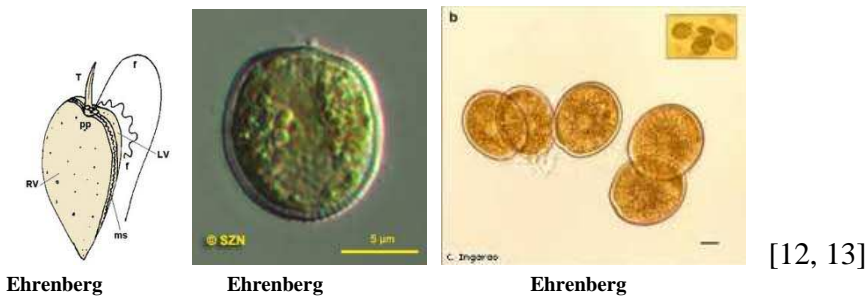
The toxins of type DSP, taken from the analysis conducted at our samples are mainly produced from Dinoflagellate of two main types: Dinophysis and Prorocentrum (Picture .nr.4 a, b).

• **4-a. Type of Dinophysis**

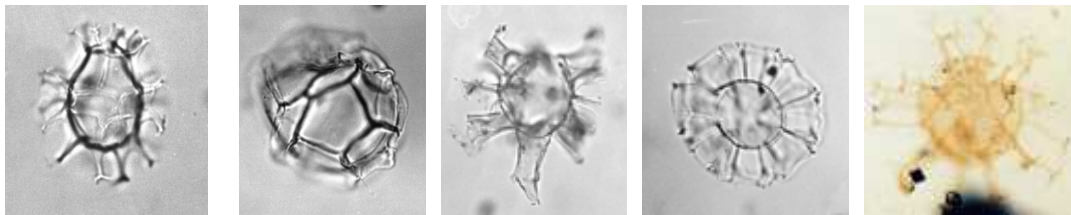


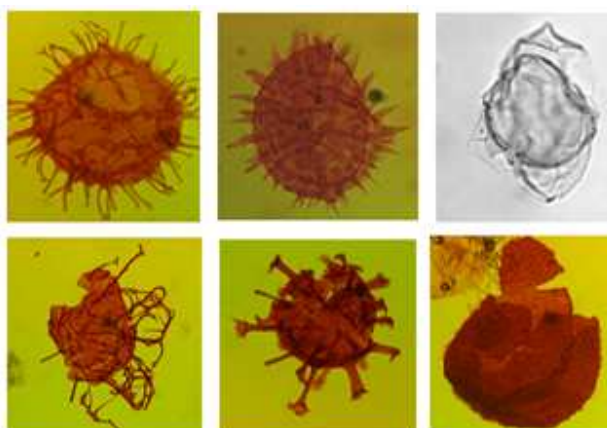
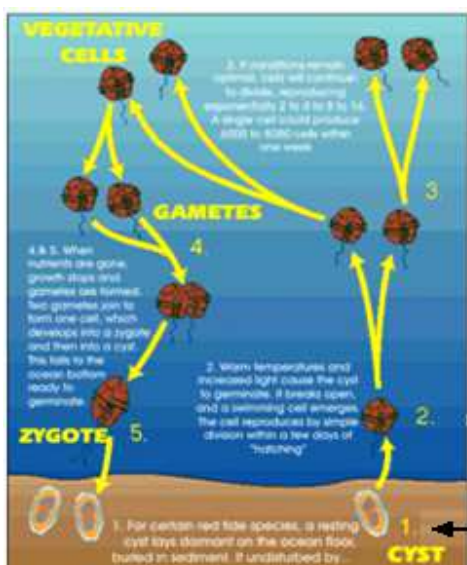
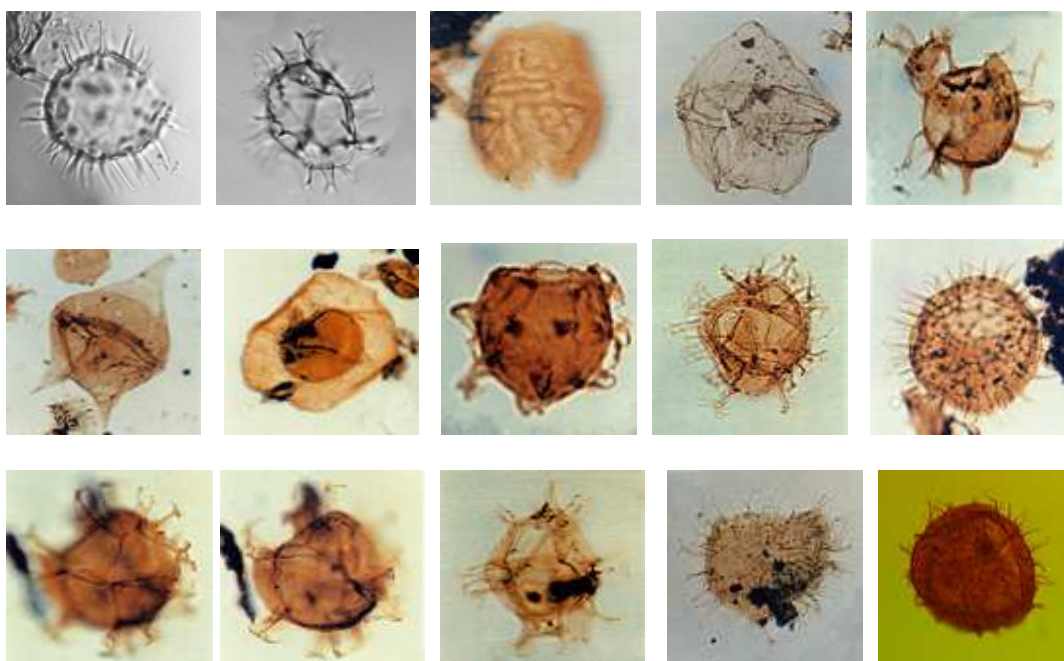
It is observed that a minor presence of some types of Dinophysis (*D. fortii*), simply 200 cells per liter water, has influenced the level of toxin at people. The period of toxin usually lasts from April to September. The effective doses of toxin are individual but the older people and younger ones are more exposed to danger. [3,4,13]

• **4-b. Type of Prorocentrum**



But the group Dinoflagelates polymorphism is demonstrated by a very large and a more productive development cycle, (Fig. follows):





The cycle of development of a cell of the group of these algae

Picture 4 (a, b) - Dinoflagellate of two main types: Dinophysis and Prorocentrum.

12 chemical components of DSP group are known lately and regarding their chemical characteristics, they are grouped under three main branches:

1. Ocadaic Acid (OA) and its associating toxin derivations (dinophysistoxins – DTX 1- 4)
2. Pectenotoxins (PTX) and
3. Yessotoxins (YTX)

Toxin being produced by them (DSP Diarrhetic Shellfish Poisoning) proves to be the cause of consumers poison since 1978 in Japan. [10].Afterwards, it affected the European zone like Spain, France and Ireland and in 1989 the first symptoms of these toxins were




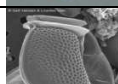
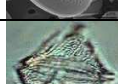
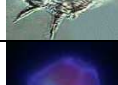



ascertained in Italy. The above mentioned substance exposes different toxic effects. For example, **Kadaic Acid and its derivations** cause diarrhea, vomit and the gripes. This kind of acid is thought to be a stimulant to tumors. The toxic reaction of *pectenotoxins* is observed at the liver while that of *yessotoxins* is made evident at harming the cardiac muscle.[5,10]. An in toxin of DSP is characterized by gripe disorders which quite often are sudden outbreaks as a result of consuming such mollusks thus being called “Diarrheic Shellfish Poisoning” [9, 11].The symptom frequencies of DSP are: diarrhea (92% of cases), nausea (80% of cases), vomit (79% of cases), abdominal pains (53% of cases) and deaths (10% of cases).The symptoms appear from 30 min the least to 12 hours the most. They appear within 4 hours at 70% of patients [9, 11]. Bio-toxicological tests have proved that a dose with a range of 40µg/100 gram to 80µg/100 gram of the adult tissue has been toxic at the mouse. The level of the ascertained Bio - Toxin at the mussels is toxic when the level of Dinophysis bacteria reaches a low density up to 200 cells per liter water, while the norm of consuming mussels as food is less than such a density. Toxicity is identified through positive or negative tests at mice following the method “**Yassumoto 1984**” Shellfish which feed on toxic Dinoflagellate keep their toxic effect for different periods depending on the type of shellfish. Some of them tend to send away toxin and thus they are thought to be toxic only during the period blooms. Others keep this effect for a long time, sometimes years on end [9, 11]. Toxic components are soluble in water and resistant to heat. If you cooked them to 5 min, it would lessen the toxicity of product to 30%. If it was to 20 min, it would increase the effect of denaturalization to 10%, therefore increasing it to 40% [11].

From analysis to verify the content of Bio - Toxin in mussel flesh conducted by The Laboratory of Collection and Depuration of Mussels Butrint- Sarandë, we are given the following data: (Tab.1, 2)

**Table Nr. 1** Analysis of the content of toxins in the mussel meat, according to the main groups of these algae.

Nr.	Test	Methods	Measuring Unit	Results
1.	Paralytic Shellfish Poison (PSP)	AOAC official method 959.08	µg ek.STX/Kg	Negative
2.	Diarrheic Shellfish Poison (DSP)	Yassumato 1984	Tests in mice	<b><i>Positive</i></b>
3.	Amnesic Shellfish Poison (ASP)	AOAC official method 959.08	Mg/Domoic acid/Kg	Negative

**Table Nr. 2** Sorts and quantity of some of these algae DSP group meeting in water of Lake Butrint.

Nr.	Test	Methods	Measuring Unit	Results	Panorama
1.	<i>Pseudo-nitzschia spp</i>	EN 15204	Cells/liter	740	
2.	<i>Alexandrium spp.</i>	EN 15204	Cells/liter	< 60	
3.	<i>Dinophysis sacculus</i>	EN 15204	Cells/liter	< 60	
4.	<i>Dinophysis fortii</i>	EN 15204	Cells/liter	< 60	
5.	<i>Gonyaulax spinifera</i>	EN 15204	Cells/liter	< 60	
6.	<i>Lingulodinium polyedrum</i>	EN 15204	Cells/liter	< 60	
7.	<i>Karenia spp.</i>	EN 15204	Cells/liter	< 60	
8.	<i>Gymnodinium catenatum</i>	EN 15204	Cells/liter	< 60	
9.	<i>Protoceratium reticulatum</i>	EN 15204	Cells/liter	< 60	

### Conclusions and suggestions:

It is already known that 1 ton mussels produce 0, 6 ton feces which influences the level of accumulating sediments thus causing ecological and reproduction problems.

1. It is an urgent need to continuously observe the decomposition process of this lake and the deviation of Bistrica to urgently control the level of salinity and the oxygen of the water. [8].
2. Dinoflagellate is among the most essential components of the marine phytoplankton regarding the unicellular beings with a size of 40-50  $\mu\text{m}$  in diameter. Thus, a high level of salinity which would be not so favorable on one hand becomes very effective on the other hand for new blooms of these unicellular being thus giving an advantage to contaminate the mussels with the Bio - Toxin of the type DSP. Consequently, it is felt a need to control the freshwater of Bistrica in Lake Butrinti.[11]



3. At all control points, what strikes you most is that during 2012 and following, the ranges of salinity are higher than those of 2011. They are noticeable differences. The high level of salinity at the water of the lake, which might result from the high and low tides or from the low flow of freshwaters in this basin, could mostly be an advantage to have a favorable environment to encourage the super flow of productive Dinoflagellate of Bio - Toxin. It has proved to us that the cultivation of this mollusk at the beginning of the session resulted to be contaminated with Bio - Toxin of the type DSP. [Table 1, 2]

4. Although the range of salinity while cultivating this mollusk can vary from 15/000 to 30/000, the maximum of this number is still about 12 – 15/000, which cannot help the cultivation of the marine Dinoflagellate.

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