Comparative methods for the determination of PSP toxins in bivalve mollusks of Albania

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SUMMARY

The present contribution provides extensive data regarding a four year period of monitoring along the Adriatic seashore of Albania, for the presence of organic and inorganic pollutants. The monitoring has been conducted in eight selected monitoring areas, where sampling of water and bivalve mollusks was performed.

As a result of the monitoring, PSP biotoxins were detected for the first time in clams. In five out of eight monitoring areas, PSP levels surpassing the maximal permissible levels of 80ug/100g were detected in clams.

In two areas considerable levels of Cr and Hg were also detected, indicating most probably the presence of the pollutants from the nearby industrial sites.

The detection and the quantitative assessment of the PSP biotoxins were performed by the official AOAC mouse i/p test. However the positive cases were further confirmed by the rapid immunochromatography test.

Keywords: PSP toxins, bivalve mollusks, ecosystem

INTRODUCTION

The aqueous ecosystems in general and the coastal ones in particular, can be considered as the most utilized and critical areas in all countries. In fact, they bear the consequences of lots of human activities originating from the earth resources such as the urban depositions, irrigative channels, industrial remains etc. All these cause a degradation of the underground waters, of the lake, bog, river and sea waters.

It’s becoming more and clearer that the evaluation of dangerous impacts in the environment and also on the species and their habitats required understanding and evaluating the stress on the whole hierarchy of biological organization beginning since the molecular levels, the cellular ones till to the organisms and their populations. (Viarengo et al, 2000).

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In the past, the environmental damages have mainly identified and evaluated in a retrospective way and mainly as a reaction towards the events with accidental character such as (Seveso and Bhopal accidents, or naphtha inflow from Amoco Cadiz and Exxon Valdez) (Amiard et al, 1986).

Their evaluation has been made principally on the viewpoints of their impacts on the human health and it has been measured with the losses caused in human populations or in species of the affected ecosystems. However, the long-term and the chronic exposure towards the environmental stress where there are included also other anthropogenic factors, very rarely can cause fats and catastrophic changes. The impact will be gradual, insusceptible and frequently not distinguishable from the natural processes and effects of the environmental changes. Among the main problems in this direction we can mention the industry role as the main discharger of the pollutants in environment and the fact that the environmental pollutants and their negative impact do not know the state limits. The presence of biotoxin PSP in bivalve mollusks makes up a powerful bio-indicator of the aqueous ecosystems disorders (Harding et al, 2004). The presence of this biotoxin is related to a powerful and abnormal increase of the toxic phytoplankton in the presence of organic substances in the form of pollutants in ecosystem. For this reason, its determination is important for the evaluation of the aqueous ecosystems’ condition but also for the protection of the consumers from one of the toxins known mostly till now. In its essences, this makes up the scope of this study.

MATERIAL AND METHODS

**Analysis of mussels samples for the presence of sea biotoxin PSP, saxitoxin and derivates**

The samples of bivalve mollusks *Mytilis galloprovincialis* will come under the qualitative and quantitative analysis for the determination of the sea biotoxins PSP (saxitoxin and derivates). The biotoxins PSP (saxitoxin and derivates) are toxins which are produced from several kinds of toxic phytoplankton, they accumulate pre-emptively in the bivalve molluscs as a result of the continuous filtration of the nutritive substances from these organisms and concentrate mainly in the lysosomes’ membranes harming their functions seriously (Viarengo, 1999).

Among others, these sea toxins are powerful neuroparalytic toxic substances and create reversible connection with sodium ports at the level of neurons’ membrane.

Therefore, they occlude the depolarization of membranes and occlude the rise of action mechanism. So, it is occluded the rise and distribution of neural impulses Cajaraville et al, 2000).

Saxitoxin is the most poisonous non-protein substance known till now. It is 1000 times more powerful than the sarin neural gas. The deadly dose 50% in mouse is 8ug, while 1 mg would be sufficient to cause the immediate death in a
human with average body weight 70kg (Koukouzika et al, 2005).

Intraperitoneal injection method in mice
This is still the official method of biotoxins PSP determination in albino mice Balb (Dailianis et al, 2003). The mice must weigh 19-21 g. This will make up the basic method which was used in this study’s frame.

It was created a group of 5 mice that came under the intra-peritoneal injection of the derived extract, while another group composed of the same number of individuals serves as control.

Analytic procedure
It is taken 5 g mollusk, which is marcered and homogenized with 20 ml of unionized and distillated water. The mixture must boil for 5 minutes in a baker. We add muriatic acid (HCL) 10% to the homogenized mass till that pH falls in the values 2-3.

The mixture is filtrated and then passed in a rotary-vapor where it is made its complete drying. Then, the precipitation is gathered with 1 ml Tween 60 and is injected to mice through the intra-peritoneal way. The result is considered positive if the mouse pegs out within 5 minutes since the injection moment. The mouse’s pegging out time is registered and then converted in m.u. (mouse unit) multiplying it with some table coefficients determined by Sommers (1956). Then, these are converted into ug/100 saxitoxin.

Method of immunochromatography with lateral afflux (ALERT)
This analytic method will be used as an alternative technique in the frame of this study for the determination of saxitoxin and derivates concentration. The analytic kites will be ordered by Biotech Ltd, Ontario Canada together with the standards of biotoxin PSP.

Analytic procedure
About 1 ml of mollusk acid aqueous extract “Mytilis galloprovincialis” was dropped in the sample’s pockmark on the test panel’s right hand. The test was left 20 minutes fro its realization in room temperature. Then we made the quantitative evaluation of the toxin concentration making the comparison of the colorimetric reaction to the three respective toxin levels as presented in the figure.

From the standard solution of saxitoxin di-hydrochloride (National Research Council STX004) were prepared the respective dilutions which were tested from in the test panels to valuate the threshold of the method’s sensitivity. The methods reached to discover the saxitoxin and derivates under the allowed limit determined by European Union just as presented in the photo.

RESULTS AND DISCUSSIONS
The sensitivity control of the method of immune-chromatography with lateral afflux, towards the progressive dilutions of saxitoxin di-hydrochloride resulted satisfactory. The indicators of the test positive results were presented since in the concentration 600 nM. This concentration is below the concentration 1075 nM or 80µg/100 g mollusk which represents the maximal limit of the allowed saxitoxin and its derivates in bivalve mollusks (fig 1). Consequently, the method is considered to complete the main requirements of the analytic monitoring in PSP biotoxins in bivalve mollusks.

![Figure 1. The reaction of smear test proof of the chromatography with lateral afflux towards the progressive concentrations of saxitoxin standard](image1)

![Figure 2. The classification of toxin from PSP in a positive case in bivalve mollusk of Narta lagoon](image2)

As it regards to the aqueous extract analysis, there were certified cases of a strong positivism of the saxitoxin and its derivates presence in the mollusks gathered in Narta lagoon, a fact that was not certified in the same type gathered in Butrinti Lake (fig.2).

The positivism concerning the saxitoxin and its derivates presence in the mollusks gathered in Narta lagoon regard to the third level/degree, that is the highest one. This certifies indirectly an emphasized breach of the trophic
equilibriums in this aqueous ecosystem which may come from the affluent presence of the poisonous alga.

Both methods used in this study’s frame resulted very effective in the discovery of toxins PSP in the mollusks prevailed by Narta zone, mainly teline.

The test in mice is considered positive if the mouse pegs out within 5 minutes since the intra-peritoneal injection of the acidified mollusk aqueous extract (Bolognesi et al, 1999).

Till now, a big positivism has been given from two teline samples taken from Narta lagoon in distance 50 m far from the coast in the period April/May 2008.

These samples resulted positive with the test of the immune-chromatography with lateral afflux too, a fact that show a good correspondence between the two proofs applied in this study’s frame.

It was not possible for us to study the poisonous alga, responsible for such high levels of sea biotoxin. Other researches are necessary for this purpose.

**CONCLUSIONS**

1. The evaluation method of the stability of lyssosomes’ membranes and the immune-chromatography method were applied successfully for the first time in site for the evaluation of biological stress level of the aqueous ecosystems.

2. In the selected sample of bivalve mollusks *Mytilus galloprovincialis* in the sampling centers – especially in Narta lagoon, were ascertained expressed damages in hemocytes level, a fact that expresses a high degree of the biological stress.

3. The indicators of the membrane damages of the hemocytes’ lysosomes of *Mytilus galloprovincialis*, expressed in retention time of neutral dipper, do correlate significantly with the monitored respective values of the pollutants with heavy metals Hg dhe Cr.

4. The concentration of sea biotoxins PSP (saxitoxin and derivates) in the type *Mytilus galloprovincialis* has a seasonal character and is evidently affected from the water temperature and wasted oxygen.

**REFERENCES**


