



Fund “Nauka” Project № 16012 Resume

“Determination of marine biotoxins in shellfish and plankton from the Black Sea”

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In recent years, there has been an increase in the consumption of seafood, as it contains valuable substances such as vitamins and polyunsaturated fatty acids. Therefore, it is important to assess seafood safety as food.

Marine biotoxins are produced by certain types of toxic plankton and can be found in mussels that use plankton for food. Consumption of seafood contaminated with toxins can cause acute intoxication, leading to paralytic, amnesic and diarrheic poisoning.

In the EU, there are health standards for the tolerable content of marine biotoxins in bivalve mollusks. Acute poisoning occurs when mussels containing toxins above these levels are consumed. If a person is exposed to low concentrations of marine biotoxins, but for a long period of time, then chronic exposure is observed. For example, people who collect mussels and often consume them are at such risk.

The main goal of the project is:

Determination of the concentration of marine biotoxins (phycotoxins) – PSP (paralytic toxins), ASP (amnesic toxins), DSP (diarrheic toxins) in mussel samples and health risk assessment for mussel consumers.

Main tasks:

- Study of clinical cases of shellfish poisoning – diagnosis T61.2.
- Determination of marine biotoxins in samples of Black Sea mussels and plankton from the North Black Sea coast
- **Health risk assessment of mussel consumption**
- Dissemination of project results

The study of medical records of patients with diagnosis “shellfish poisoning” systematizes data from clinical cases, which is beneficial for public health, as the results contribute to:

- Clarify the frequency of this type of poisoning in order to be assessed the significance of this type of intoxication.
- Raising the awareness of the public and clinical specialists about the symptoms and possible side effects of the consumption of contaminated mussels;

The health risk assessment is made by comparing the determined concentrations of marine biotoxins with the norms in the European legislation, as well as by calculating the acute and chronic exposure when consuming mussels containing toxins.

Samples of wild and cultivated mussels as well as plankton from the Northern Black Sea coast were analyzed in the study. In June 2017 and June 2018, two scientific expeditions were conducted with the sailing yacht “Hygia” of MU-Varna for sampling plankton and wild mussels from the region of Varna Bay.

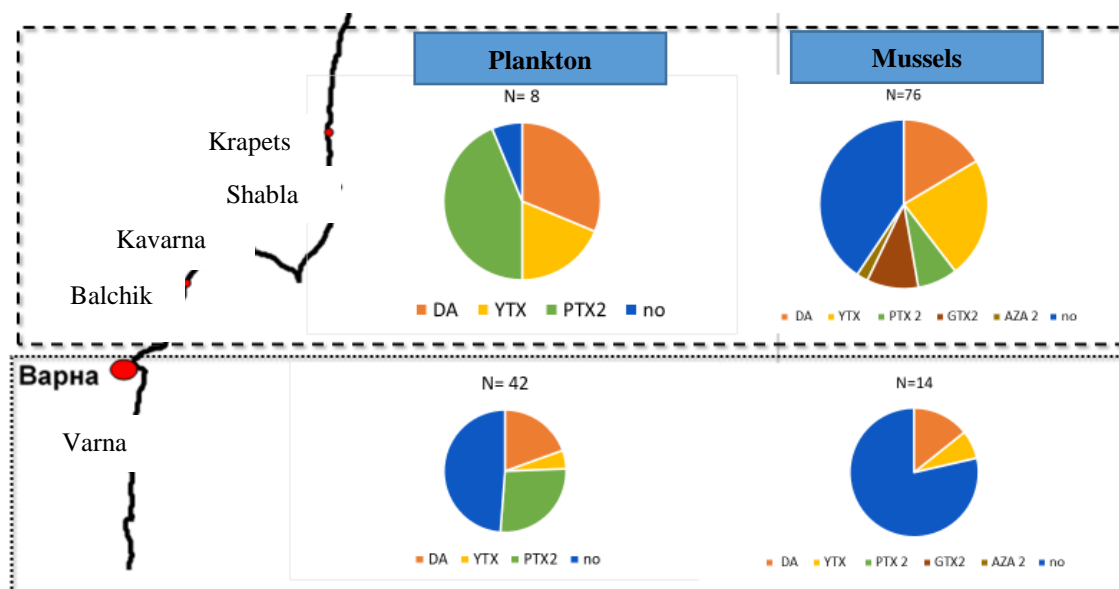
Main results:

The results show the presence of toxins that can cause paralytic (PSP) and amnesic poisoning (domoic acid). Marine biotoxins identified are goniatotoxin-2, domoic acid, yessotoxin, pectenotoxin-2 and azaspiracid-2. Yessotoxin, pectenotoxin-2 and azaspiracid-2 are registered for the first time on the Northern Bulgarian Black Sea coast.

Domoic acid is detected in plankton and mussel samples of from spring 2017, as well as in the samples from winter-spring 2018. In the other seasons, the toxin was not detected in the analyzed samples.

The comparison of the results by years shows that e.g. paralytic toxins are found in some of the samples from 2017, but not in the analyzed samples from 2018 and 2019. Pectenotoxin - 2 is registered in samples of mussels and plankton from all studied seasons.

Comparison of the toxin profiles of cultured and wild mussels shows the presence of all five identified toxins: domoic acid, yessotoxin, pectenotoxin-2, GTX2 and AZA-2 in both types of samples. DA and PTX2 levels are higher in wild mussels than in cultured mussels, while inverse dependence is observed for YTX.



Distribution of marine biotoxins on the Northern Bulgarian Black Sea coast

HEALTH RISK ASSESSMENT FROM MUSSEL CONSUMPTION

The safety of mussels in relation to marine biotoxins has been assessed by:

- comparison of the obtained results for toxin concentrations with limit values set in the European regulations: acute reference dose (RfD) and tolerable daily intake (TDI), respectively.
- calculation of acute and chronic exposure of the population to domoic acid and hazard quotient.
- determination of acute exposure of the population to YTX, pectenotoxins, azaspiracids and paralytic toxins.
- Exposure to domoic acid (amnesia toxin) is significantly higher in spring 2017 compared to spring 2018

These results are in accordance with the data from the studied medical documentation, which show the absence of clinical cases with the relevant diagnosis.

CONCLUSIONS:

- The levels of marine biotoxins in wild and farmed mussels are below the maximum permitted limits for the European Union.
- The calculated acute exposures are significantly lower than the reference values (ARfD) proposed by EFSA, therefore no health risk is expected from mussel consumption.
- Periodic monitoring of toxin levels in Black Sea mussels is necessary to prevent acute poisoning and chronic exposure.
- The control should cover more places for trade in mussels and control of biotoxins in wild mussels by modern methods.

CONTRIBUTIONS:

Contributions to the enrichment of scientific knowledge:

- A reference has been made for clinical cases of shellfish poisoning in clinical bases of MU-Varna;
- An analytical procedure for sample preparation and quantification of marine biotoxins in mussel and plankton samples has been developed;
- Liquid chromatographic methods for determination of marine biotoxins in mussels and plankton are modified and applied;
- Systematic data for the concentration of various marine toxins (domoic acid, yesotoxin, pectenotoxin-2 and azaspiracid-2, goniautoxin-2) in samples of wild and cultivated mussels from the Northern Bulgarian Black Sea coast were obtained;
- Data on the presence of toxins formed by plankton in the Black Sea - domoic acid, yesotoxin and pectenotoxin-2 were obtained.
- Yesotoxin, pectenotoxin-2 and azaspiracid-2 can be classified as emerging toxins, as they were first registered in plankton samples from the Northern Bulgarian Black Sea coast.
- The safety and health risk of cultivated and wild mussels has been assessed through several approaches.

Career development:

- The experimental results of the project are part of a successfully defended dissertation of Zlatina Veselinova Peteva as a member of the research team.

Publicity of the results:

- The scientific results are presented at a number of scientific forums in Bulgaria and abroad and scientific publications are prepared.

Scientific publications:

The results of the project are systematized, summarized and published in 6 scientific publications (4 in scientific journals and 2 in conference proceedings). Two of the scientific publications are in journals with impact factor.

- Peteva S., Georgieva St., Stancheva M., Makedonski L. (2017) Recreational angler exposure to domoic acid via consumption of contaminated shellfish from the Black sea, Bulgaria: a preliminary study, archives of the Balkan Medical Union, 52 (3), 291-297 **sjr=0.19**
- Peteva, Z., Georgieva, St., Krock, B., Stancheva, M. (2018). Selected contaminants in fish and mussels from Bulgarian Black sea. Innovations in science and education, Prague, Czech Republic, march 21-23. 1144 – 1149, doi: <http://dx.doi.org/10.12955/cbup.v6.1307>
- Peteva, Z; Stancheva, M; Georgieva, St; Gerasimova, A; Makedonski, L. (2018). Health risks associated with consumption of marine biotoxins-contaminated seafood from the North Bulgarian Black sea coast Proceedings from the Seventh Scientific Session of the Medical College of Varna, 2018, 7(3) [In Bulgarian]
- Zl. Peteva, B. Krock, M. Stancheva, St. Georgieva (2019), Evaluation of paralytic shellfish poisoning toxin profile of mussels from Bulgarian north Black Sea coast by HPLC-FL with postcolumn derivatisation, Bulgarian Chemical Communications, Volume 51, Special Issue D (pp. 233 – 240) **IF= 0.242**
- Peteva, Z; Stancheva, M; Krock, B; Georgieva, St; Gerasimova, A (2018). Empirical determination of conversion factor for depicting phycotoxin concentration in whole mussel *Mytilus galloprovincialis* meat. Food Science and Applied Biotechnology, 2019, 2(2), 166-174
- Stancheva, M; Peteva, Z; Krock, B (2019). Study on risk of exposure of seafood consumers in Bulgaria to hydrophilic marine toxins, Scripta Scientifica Medica, 51(1):24-31

Participation in scientific forums: the project team has participated in 21 scientific forums - prepared and presented are a total of 8 oral presentations and 14 posters.