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Food safety status of mussels from Bulgarian coast in regard of marine biotoxins

Zlatina Peteva[1,2*], Bernd Krock[3], Stanislava Georgieva[1], Mona Stancheva[1] and Thomas Max[3]

[1] *Medical University Varna, 9002 Varna, Bulgaria*
[2] *Dobrudzha Technological College, 9302 Dobrich, Bulgaria*
[3] *Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Chemical Ecology, am Handelshafen 12, 27570 Bremerhaven, Germany*
[*]*Corresponding author:* zlatina_peteva@mail.bg

Abstract

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Seafood, such as mussels is rich of essential nutrients, but it could be also a source of contaminants that may cause adverse health effects. This article aimed to identify safety status of mussels harvested from Bulgarian coast.

Wild and cultivated mussels (N = 17) were collected from their breeding sites in the period throughout 2021, along the Bulgarian coast of the Black Sea. Among all six regulated marine biotoxins (domoic acid, dinophysistoxin-1 (DTX1), dinophysistoxin-2 (DTX2), azaspiracid-1 (AZA1), pectenotoxin-2 (PTX2) and yessotoxin (YTX)) were determined via high performance liquid chromatography-tandem mass spectrometry. Safety of investigated samples was assessed by calculating the human exposure to detected toxins and comparing it with the legislated acute reference doses. For calculations a body weight (66 kg) and mean portion size of 400 g, recommended by EFSA were used.

The presence of only PTX2, yessotoxin and some of their analogues (PTX2-seco acid (PTX2sa) and hydroxy-YTX) was confirmed in all samples. Highest calculated exposure was to YTX – 0,00013 microgram/kg bw, which is much lower than the accepted acute reference dose of 25 microgram/kg bw. Calculated exposures to pectenotoxins were more than thousand times lower than the acute reference dose of 0,8 microgram/kg bw. Estimated results indicate that no human health risk could be expected based on the analyzed mussels.

Although, no human health risk was identified, a running monitoring of marine biotoxins is important as mass proliferations of harmful microalgal are hard to predict. Thus, high levels of yessotoxins and pectenotoxins might be a certain danger to consumers. The insidiousness lies in the mechanism of action of these toxins, as it is still unknown and does not allow us to have reliable information on the symptoms and problems that it develops in humans.

This work presents that in mussel samples from Bulgarian coast from 2021, low levels of marine biotoxins were detected. No human health risk might be expected if mussels were consumed.

Keywords: yessotoxins; pectenotoxins; Black Sea; acute exposure

Introduction

Living organisms from marine environments provide economic, social, and cultural value as sources of food and recreation. The term “seafood” usually refers to edible marine organisms. The consumption of seafood is much more often discussed in terms of the global demand for additional food (Duarte et al., 2009; Béné et al., 2015).

A large number of studies have been dedicated to the benefits and risks for human health, resulting from the consumption of seafood (Hellberg et al., 2012; Frewer et al., 2015). In the past 30 years, the seafood consumption in Europe was variable – it decreased by 17% from 1985 to 1995, then increased by 11% from 1996 to 2006 and decreased again by 2 % from 2007 to 2017 (Our World in data, 2017). The latter decrease is discussable from the perspective of the sometimes-contradictory media releases about potential health benefits and risks from seafood consumption on one side, and the scientific investigations about consumers' perceptions about the seafood on the other side (Zander et al., 2008; Jacobs et al., 2015).

Despite of their excellent nutritional characteristics, mussels are considered by the consumers as a 'risky' seafood (Wessells et al., 1995; Brooks, 2021) due to their lifestyle as filter-feeders and the potential exposure to environmental pollutants. These concerns might be reasonable as different toxicants were registered and even in levels that could pose a health risk (Krstulović & Šolić, 1994). On the other hand, many studies show that contaminant levels in mussels are compliant with the legislations (Esposito et al., 2020; Parolini et al., 2020; Novakov et al., 2021).

Marine biotoxins represent a specific group of toxicants. They are natural toxic metabolites produced by certain marine microalgae. Marine biotoxins accumulate in marine organisms and migrate along the food chain (Otero et al., 2010). In addition, the risk of contamination is higher than marine biotoxins do not change the vitality and the taste of mussels. Human poisonings still take place due to the rapid increase in toxin levels, and/or disrespect of harvest bans (Vale, 2020; Costa et al., 2021).

The aim of this study is to identify safety status of mussels harvested from Bulgarian coast in regard of marine biotoxins.

Materials and Methods

Sampling area and sample collection

Wild and cultivated mussels (N = 17) were collected along the Bulgarian coast of the Black Sea. The sampling sites were divided into two categories – mussel farms (N = 3) and sites of natural growth (N=2) in both North and South coast. The sampling includes two periods winter-spring 2021, and summer 2021.

Mussels were harvested by the mussel farmers using a collecting machine. It surrounded the pole and scraped the mussels off, depositing them on the vessel being used. Recreational harvesters collected the mussels by hand.

After sampling, the mussels were stored in cool boxes and delivered immediately in the laboratory. The biometrical data was recorded. Thereafter, the mussel meat was removed from the shell. The hepatopancreas (digestive gland) was dissected with a scalpel. Digestive glands were homogenized and frozen until further analysis.

Toxin analysis

Mussel homogenates (4.16±0.13 g) were subjected to liquid-liquid extraction with methanol extraction by high-speed blending (POLYMIX®PT 1200E, KINEMATIKA AG, Germany). The procedure is in detail described by Peteva et al. (2018a). An aliquot (~ 1000 μL) of each extract was analyzed by LC-MS/MS according Krock et al. (2008) for the presence of okadaic acid (OA), dinophysistoxins-1 and -2 (DTX1,2), pectenotoxins (PTX2, PTX2-sa, epi-PTX-sa), yessotoxins (YTX, OH-YTX), azaspiracid-1 (AZA1), spirolides (SPX1) and gymnodimine A (GYMA) on a LC-MS/MS system. In addition to the above-mentioned method, transitions for the detection of goniodomin A (GDA) were included (Krock et al., 2018), the lc-MS/MS system, consisted of a liquid chromatograph (model 1100 LC, Agilent, Waldbronn, Germany), coupled to a triple quadrupole mass spectrometer (API 4000 QTrap, Sciex, Darmstadt, Germany), equipped with a Turbo Spray interface.

The quality control was performed by regular analysis of procedural blanks and certified reference material (National Research Council, Canada). Limits of detection (LOD) for lipophilic toxins and DA were determined based on 3:1 signal-to-noise ratio.

Calculations

For the purpose seasonal changes to be described and analysed, the toxin load of each sample was calculated as follows:

PTXs = PTXs2 + PTX-sa + Epi-PTX-sa

YTXs = YTX + OH-YTX,

where PTXs is the load of the sample with toxins from the PTX group,

YTXs is the load of the sample with toxins from the YTX group.

Statistical analysis

SPSS 16 was used for statistical processing of the results.

Descriptive statistical analysis was applied using tabulated graphical method, mean values and the standard deviation (in absolute value), within each group.

Statistical hypothesis test (t-test) was applied to establish the existence of a statistically significant difference between the mean values of toxin loads, depending on the sampling season and between the toxin variants in the two different sampling regions. Results were reported by p-values. The groups, for which we proved statistically significant difference $p \leq 0.05$ are indicated with*.

Safety assessment

Determined toxin levels were recalculated for whole shellfish meat by applying a conversion factor 5 proposed by EFSA (EFSA 2008a; 2008b). The calculated levels were compared with the legislative limits.

Safety of investigated samples was also assessed by comparison of calculated human exposure to detected toxins with the legislated acute reference doses. For calculations an average body weight (66 kg) and a mean portion size of 400 g recommended by EFSA were used.

Results and Discussion

The Black Sea is considered the largest semi-closed marine ecosystem and anaerobic water body in the world (area 434 400 km^2, maximum depth 2212 m, water volume 547 000 km^3) (Murray et al., 1989). It has very low salinity (~ 17 psu), as it receives more than 30 000 km^3 of riverine input every year, and suffers serious pollution from agriculture, urbanization and industry (Ludwig et al., 2009). The Bulgarian coastline extends for 432 km from Cape Sivriburun in the north (marking the state border with Romania), to the Rezovska River in the south (marking the state border with Turkey).

In the period February-May and June-July 2021, in total 17 mussel samples were collected from the Bulgarian shore (Table 1).

With the aim to identify marine biotoxins in mussel samples, only the hepatopancreas was processed. The digestive gland has been shown to accumulate these substances due to its adhesive nature (MacKenzie et al., 2002; O'Driscoll et al., 2014; Mafra Jr et al., 2015).

Recent research showed the presence of lipophilic toxins in mussels harvested from the same region (Peteva et al., 2018b; Peteva et al., 2020; Dzhembekova et al., 2022). Also, some other toxins like AZA, GDA, GYM and SPX1 are more often detected in European waters (Zurhelle et al., 2018; Kremp et al., 2019; Bacchiocchi et al., 2020; Tillmann et al., 2020; Lamas et al., 2021; McGirr et al., 2021). As these compounds are usually registered in very low concentrations, it is important that appropriate LODs (Table 2) are achieved. Nevertheless, in the investigated samples, only PTX2, PTX2-sa, epi-PTX2-sa, YTX and OH-YTX in small concentration ranges were registered (Table 2). Among the detected toxins, YTX reached the highest level (0,122 μg/g in sample ME1) and epi-PTX2-sa the lowest level (0.0012 μg/g in sample ME46).

The significant effect of seasons on metabolic processes of mussels is often attributed to a number of biological and environmental inter-related factors. Based on apparent in-

Table 2. LODs and levels of detected toxins in investigated samples

Studied toxins	LOD, μg/g	Number of positive samples	Concentration range in positive samples, μg/g
DA	0.0012	0	nd
OA	0.0062	0	nd
DTX2	0.0088	0	nd
DTX1	0.0144	0	nd
PTX2	0.0009	2	0.0015-0.0016
PTX2-sa[†]		17	0.0014-0.0295
Epi-PTX2-sa[†]		16	0.0012-0.0140
GDA	0.0073	0	nd
YTX	0.0240	7	0.0280-0.1222
OH-YTX[†]		8	0.0261-0.0750
AZA1	0.0002	0	nd
GYMA	0.0004	0	nd
SPX1	0.0006	0	nd

[†]no reference material is available for PTX2-sa. Epi-PTX2-sa and OH-YTX and the LOD of PTX2 and YTX, respectively, were used for quantification of these toxins; nd- not detected

Table 1. Number and biometric characteristics of mussel samples

Season	Characteristics	North coast		South coast	Total
		Cultivated mussels	Wild mussels	Cultivated mussels	
Winter-spring 2021	Number of samples	5	1	3	9
	Biometrical data	Mean length (cm)		Mean weight (g)	
		5,9 ±0,4		13,9±1,7	
Summer 2021	Number of samples	4	2	2	8
	Biometrical data	Mean length (cm)		Mean weight (g)	
		5,8 ±0,3		15,2±2,5	
In total		9	3	5	17

crease in heavy metals (Azizi et al., 2018; 2021) and polycyclic aromatic hydrocarbons (Grigoriou et al., 2021) levels during winter, it is even proposed that mussels can be used as bioindicator for pollution and monitoring. In regard to the marine biotoxins it was suggested that the dynamic of phytoplankton responsible for the toxin production was mainly driven by temperature and salinity (Dhanji-Rapkova et al., 2018; Draredja et al., 2019).

In the present study, we investigated the seasonal changes in PTX and YTX levels (Figure 1). It was found that for both toxin groups – PTXs and YTXs the winter-spring toxin loads are significantly higher than the summer toxin loads.

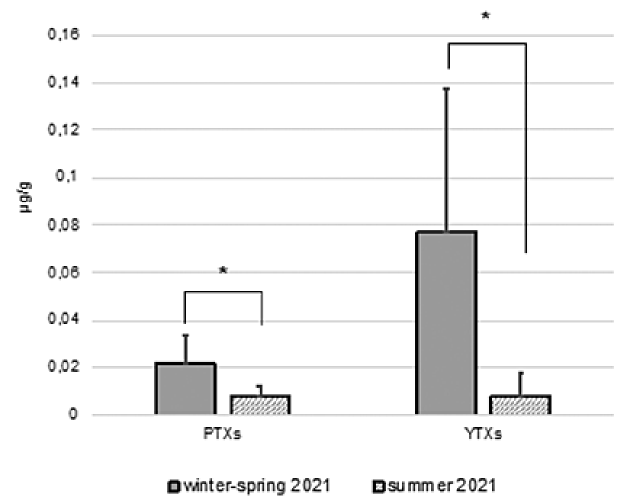

Fig. 1. Mean toxin loads of samples by sampling season

Pectenotoxins were previously detected in mussel samples from Bulgarian coast, as well as in plankton samples (Peteva et al., 2018a; 2018b; Peteva et al., 2020; Dzhembekova et al., 2022) in lower levels. This suggests the persistent availability of PTX-producing microalgae. In the cited studies, a similar seasonal pattern was established – the levels of PTXs increase in the cold months. In another study conducted, in the winter seasons of 2019 and 2020 PTX2, is reported in 0.92% (Mudadu et al., 2021), whereas the toxin content was more than 2 times higher than in the present study.

Studies in other countries report different inter-seasonal intensities of YTX events. YTXs generally appeared in British coastal waters in higher quantities during summer months (Dhanji-Rapkova et al., 2019), late spring in Norway (Aasen et al., 2005), or in the autumn/winter period in the Mediterranean (Bacchiocchi et al., 2015).

YTXs were also detected in mussel samples during previous periods in the same region – in spring 2017 and summer-fall 2017, in comparable concentrations and were not detected in the winter-spring samples from 2018 (Peteva et al., 2018a; 2018b; 2020). Thus, YTX variants were registered in plankton samples from spring 2019 (Dzhembekova et al., 2022). In the latter study is suggested that YTX variants show distinct relationships with environmental variables (temperatures, salinity, water transparency and fluorescence), which might present a specific toxin profile in the Black Sea.

The study area was divided into two main regions – North and South. This was done because of the different hydrographic conditions and levels of physicochemical parameters (Dimitrov et al., 2019; Pavlova et al., 2020). Both pectenotoxins (PTXs) and yessotoxins (YTXs) were detected in the two regions. The mean levels of PTXs were lower than YTXs (Figure 2). Mean levels of PTX2-sa and epi-PTX2sa in both regions were similar. YTX and OH-YTX levels were slightly higher in the samples from North, but no statistical significance ($p>0,05$) was established. Rubini et al. (2021) conducted a study on much more shellfish samples from Northwestern Adriatic Sea. The obtained results have similar ranges as hereby reported.

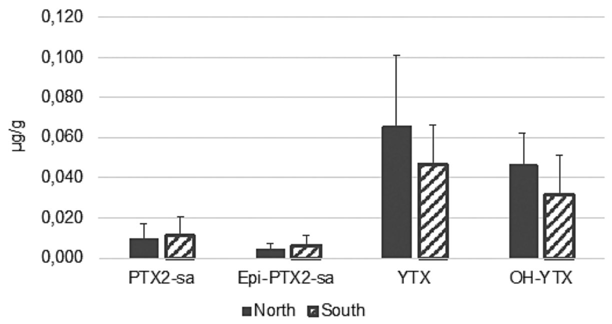

Fig. 2. Mean levels of PTXs and YTXs

Seafood, such as mussels is currently accepted as an essential food for humans (FAO, 2010). Mussel meat is known for its nutritional value due to the high quality of proteins, the presence of essential free amino acids and water-soluble and fat-soluble vitamins (Chi et al., 2012; Fuentes et al., 2009).

Risk-benefit approach is used of to assess the combined health impact of nutritional risks and benefits by consumption of the food. Important health concerns have recently appeared around shellfish. Poisoning can result from the ingestion of shellfish contaminated with phycotoxins. Various types of poisoning (diarrheic, paralytic, amnesic, neurologic and azaspiracid) may occur, each of which is caused by a group of toxins produced by a particular microalga (Garthwaite, 2000; Campàs et al., 2007).

The European Commission has set maximum legal limit for marine toxins in edible mussels (EC, 2004; 2013). In the present study, only the digestive glands of the mussels were investigated because this organ tends to accumulate the toxins (MacKenzie et al., 2002; O'Driscoll et al., 2014; Mafra et al., 2015). Normally, the whole mussel is consumed and therefore, the determined levels of marine biotoxins need to be expressed in terms of whole mussel meat. A factor of 5 is proposed to convert the value to whole shellfish meat (EFSA 2008a; 2008b).

We registered PTXs and YTXs in the digestive glands of the mussel samples. The mean toxin levels (MTL) determined in the digestive gland ($MTL_{PTX2\text{-}sa}$ = 0.010±0.008 µg/g, $MTL_{EPI\text{-}PTX2\text{-}sa}$ = 0.005±0.003 µg/g, MTL_{YTX} = 0.058±0.034 µg/g, $MTL_{OH\text{-}YTX}$ = 0.045±0.014 µg/g) were recalculated for whole mussel meat. A worst-case scenario is also developed by calculating the theoretical toxin levels in mussel samples, where the maximal concentrations are found. The obtained results are compared with the legislative limits (Figure 3). This analysis shows that no risk for human health might be expected if the studied mussels were consumed.

For safety assessment acute exposure to detected toxins also should be determined. The limits are set to protect human health from acute toxicity in the context of one meal of 400 g mussels for a 60 kg adult. Hereby the acute exposure is calculated using the highest registered levels.

Among the determined toxins only PTX2, YTX and OH-YTX are regulated in Europe (EFSA, 2009) and therefore, the acute exposure is estimated for them. Although, the toxicity of these toxins is debatable (Yoon et al., 1997; Miles et al., 2004; Tubaro et al., 2008; Ferreiro et al., 2017), they are still listed in the official legislative documents and should be monitored. Results show that even if the mussel containing the maximum levels of the toxins were consumed the calculated acute exposure is much lower than established acute reference doses (Figure 4). This might not affect the consumers' health.

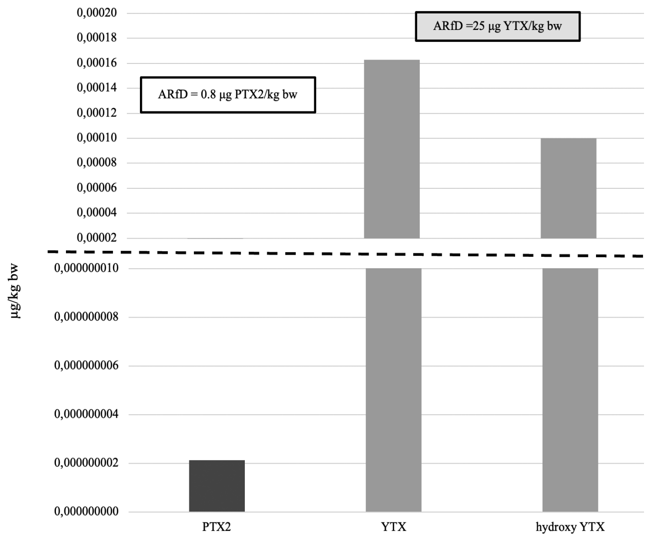

Fig. 4. Calculated acute exposure based on the maximal toxin concentration (ARfD- acute reference dose)

Conclusions

Different PTXs and YTXs were detected in mussel samples from Bulgarian coast. The determined concentrations vary significantly in time, but not between the regions of investigation. YTXs and PTXs concentrations in mussels are below the recommended value of the European Food Safety Authority; thus, the consumption of this seafood was not

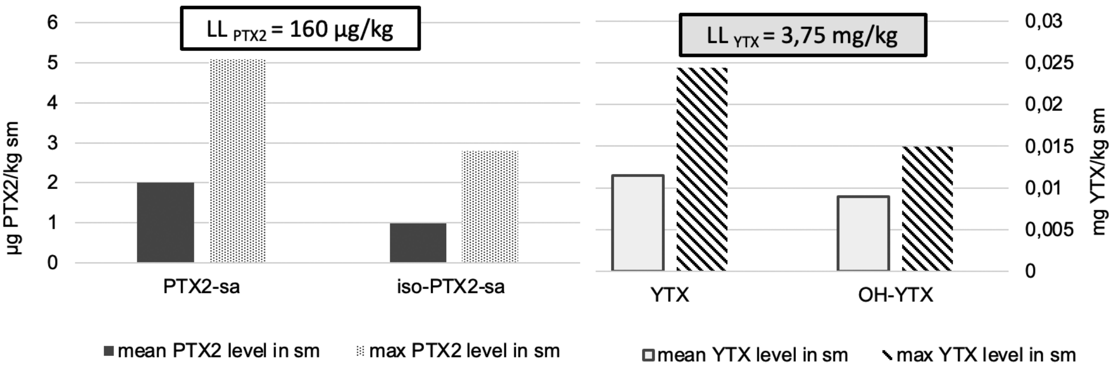

Fig. 3. Calculated mean and maximum levels of detected toxins for whole mussel meat (sm – whole mussel meat, LL – legal limit), * no mean levels for PTX2 are calculated because the positive samples are only two

considered to be a significant risk for human health. Based on these data and in relation to the ARfDs proposed by ESFA, it appears that Bulgarian residents are not currently at risk for acute poisoning.

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