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Evaluation of selected persistent organic pollutants in bivalves from the Bulgarian Black Sea Coast

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OVERVIEW

Bivalves are bioindicators of pollutants in marine environment due to their wide spread distribution and capability of bioaccumulation. Mussels accumulate persistent organic pollutants and concentrate them to levels well above those in the seawater

The concentrations of 13 PAHs, 15 PCBs and organochlorine pesticides (such as DDTs and HCHs) were determined in mussel soft tissues.

Simultaneous extraction of POPs in Accelerated solvent extractor (ASE) and detection by gas chromatography system with mass spectrometry (GC-MS).

The results confirm that the persistent organic pollutants still present in detectable levels in mussels from the Black Sea.

INTRODUCTION

In last decades the levels of persistent organic pollutants (POPs) in the environment are steadily declining, but residues of polychlorinated biphenyls (PCBs), organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) continue to wide spread in marine ecosystems and to bioaccumulate in animal tissues. Bivalves are often used as bioindicators of pollutants in marine environment due to their higher capability of bioaccumulation.

The AIM of the study was to assess the present contamination status of POPs using black mussel (*Mytilus galloprovincialis*).

MATERIALS

Wild and cultivated mussels were collected from different sites of the Bulgarian Black Sea coast in the period 2021 – 2022.

METHODS

- Soft tissue of mussels – 4 g for extraction.
- Accelerated solvent extractor (ASE) - hexane/acetone (3/1; v/v), lipid determination.
- Extract clean up - glass column packed with neutral and acid silica – elutents n-hexane and n-hexan/dichloromethane (4:1).
- **GC-MS determination** – GC FOCUS (Thermo Electron Corporation, USA) equipped POLARIS Q Ion Trap mass spectrometer.

Quality control

- Pure reference standard solution was used for instrument calibration, quantification of compounds and recovery determination.
- Procedural blanks were analyzed between each 5 samples.
- Internal standards PCB 30 and PCB 204 and Certified reference material (CRM): BB 350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European commission.

Statistical analysis - SPSS 16 software.

Black sea map and sampling area

RESULTS

Polycyclic aromatic hydrocarbon (PAHs)

- Organic compounds containing two or more condensed aromatic rings. **PAHs are genotoxic and cancerogenic.**
- Benzo[a]pyrene is a marker for availability of PAHs in foods and environment.
- The phenanthrene and fluorene were the most abundant compounds in all samples investigated.
- Benzo(a)pyrene was detected in 25% of analyzed samples, the concentrations do not exceed the limit set in EC Regulation.

DDTs (p,p-DDE + p,p-DDD + p,p-DDT)

- The metabolite p,p'-DDE was the abundant organochlorine contaminant in mussel samples (from 0.73 to 1.66 ng/g ww).
- DDT was present mainly in the form of its metabolites p,p'- DDE and p,p'- DDD, suggesting contamination in the past.

Plychlorinated biphenyls (PCBs)

•PCB 153 and 138 were the most abundant congener in mussel samples.
•The most of dioxin-like PCB congeners were found below the analytical detection limits in all mussel samples.

Indicator PCBs (I-PCBs) IUPAC № 28, 52, 101, 138, 153, 180

Dioxin-like PCBs IUPAC № 77, 105, 118, 126, 156, 169

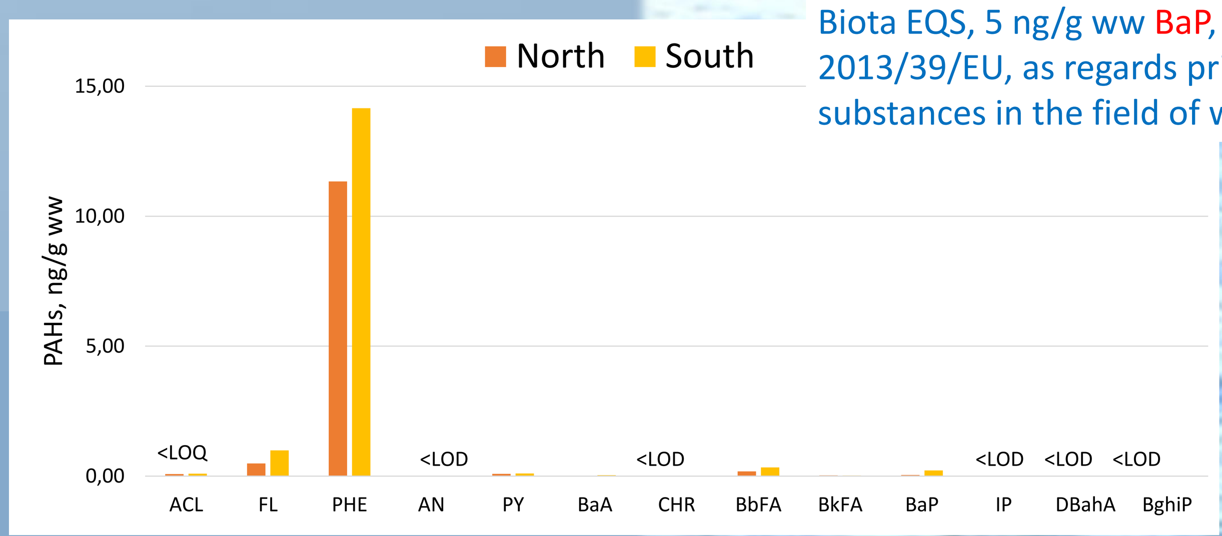


Figure 1 Individual PAHs levels (mean values) in mussels (*Mytilus galloprovincialis*) from North and South part of the Bulgarian Black Sea Coast

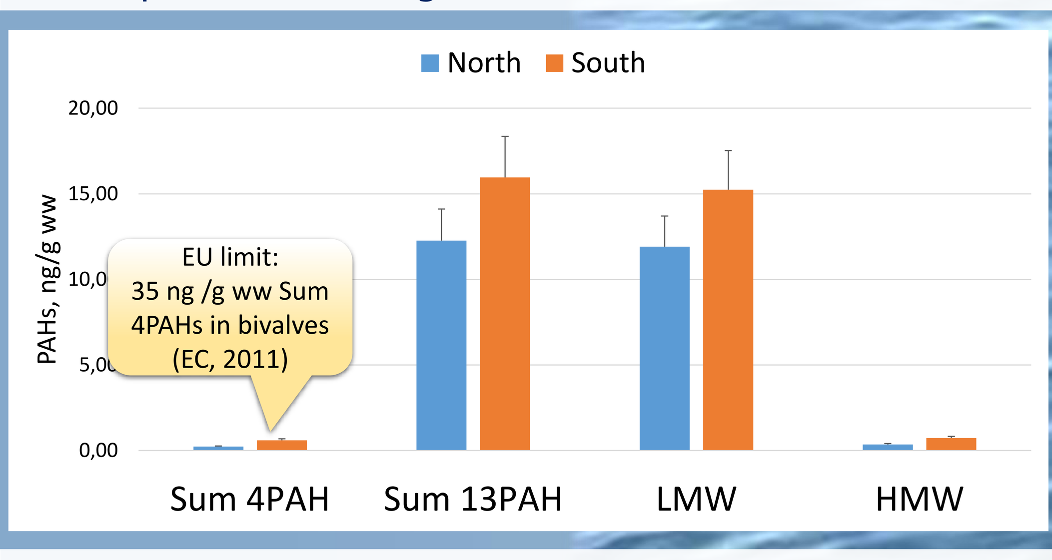
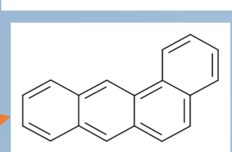
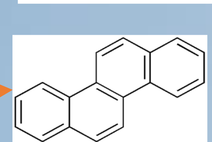


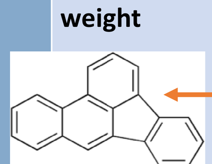
Figure 2. PAHs level, ng/g ww in mussel from different sampling regions.

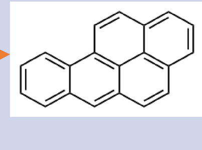
Classification	PAHs	Abbreviation	Source of pollution	IARC group
Low molecular weight	Acenaphthylene	ACL	**Petrogenic PAHs** as a result of spillage of diesel oil and fuel oil	-
	Fluorene	FL		3
	Phenanthrene	PHE		3
	Anthracene	AN		3
High molecular weight	Pyrene	PY	**Pyrolitic PAHs** as products of the incomplete combustion of organic matter	3
	Benzo[a]anthracene	BaA		2B
	Chrysene	CHR		2B
	Benzo[b]fluoranthene	BbFA		2B
	Benzo[k]fluoranthene	BkFA		2B
	Benzo[a]pyrene	BaP		1
	Dibenzo[a,h]anthracene	DBahA		2A
	Indeno[1,2,3-cd]pyrene	IP		2B
	Benzo[g,h,i]perylene	BghiP		3

Priority PAHs (PAH 4), EFSA 2008









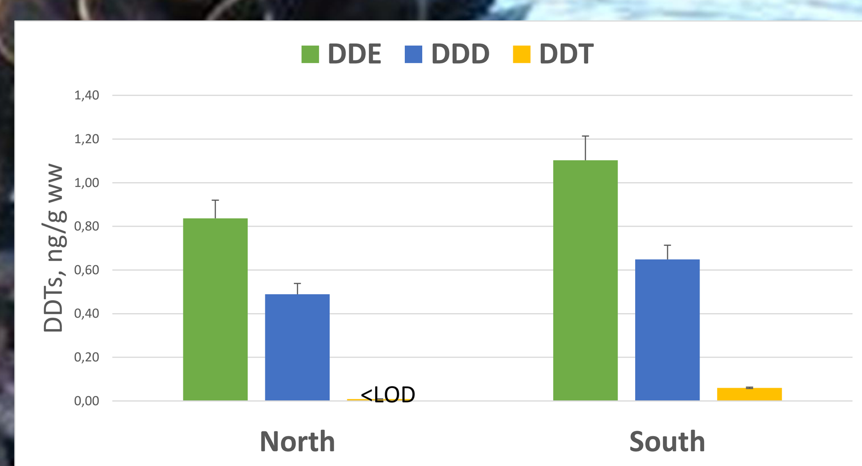


Figure 3. DDT and its metabolites, ng/g ww in mussel from different sampling regions

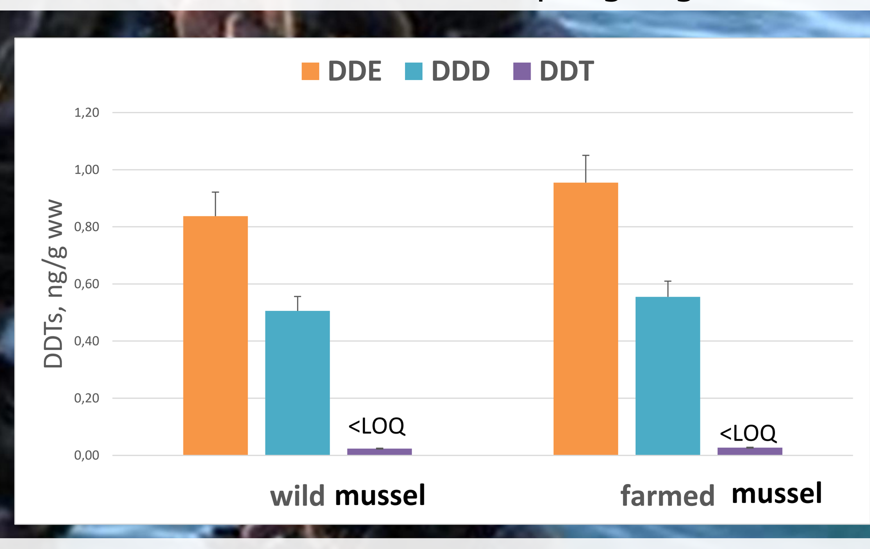


Figure 4. Distribution pattern of DDE, DDD and DDT in wild and farmed mussel.

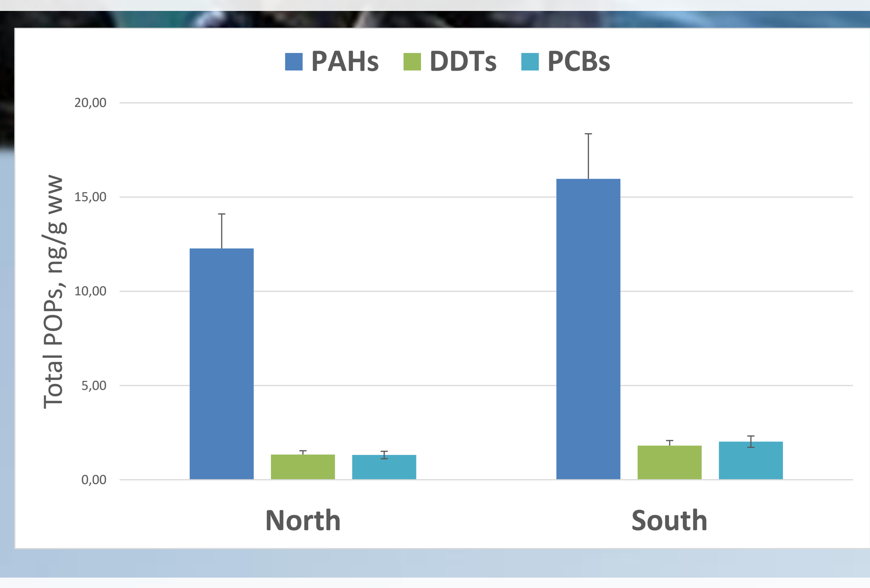


Figure 5. Total POPs pollution in mussel samples from Black Sea.

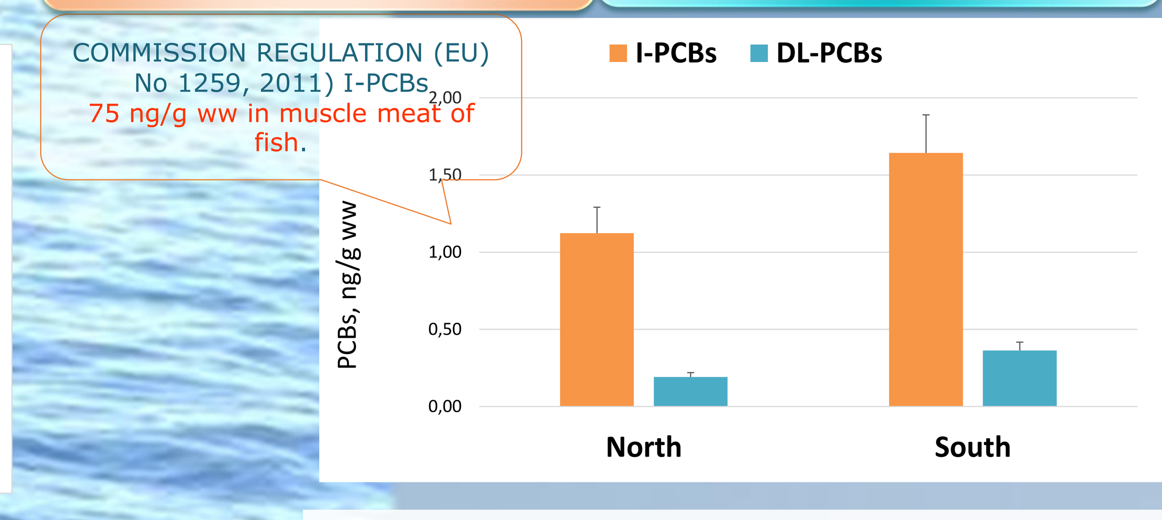


Figure 6. Levels of I-PCBs and DL-PCBs in mussel from different sampling regions.

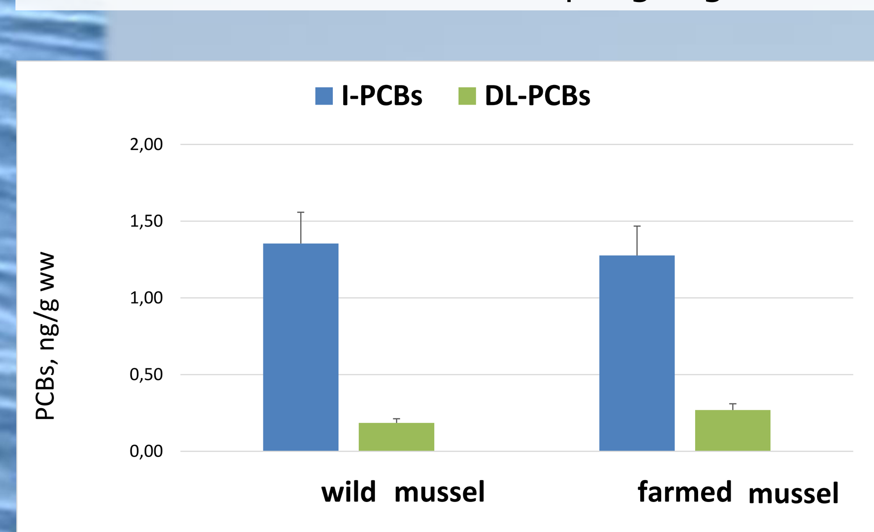


Figure 7. I-PCBs and DL-PCBs in wild and farmed mussel.

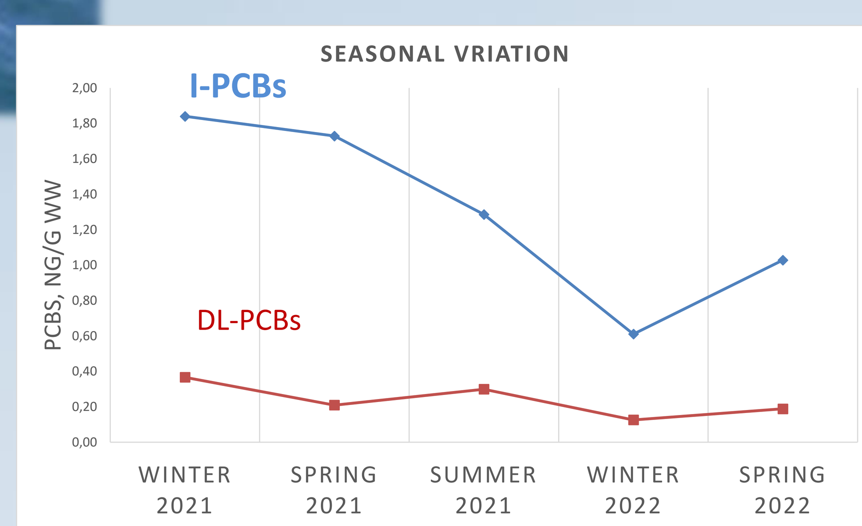


Figure 8. Seasonal variation in levels of I-PCBs and DL-PCBs in wild and farmed mussel.

Results:

- ✓ Relatively low PAH levels (Sum 14 PAHs) in mussels were observed in sampling points (Kavarna and Ravda) far from strongly urbanized and industrial areas.
- ✓ In contrast, the sum of PAH 4 in *Mytilus galloprovincialis* from Sozopol (close to Bourgas harbor area) was found higher (17.89 ng/g ww).

Conclusions:

- The ratio LMW/HMW PAHs was higher than one, suggesting pollution predominantly of petrogenic origin.
- The Sum 4 PAH in mussels from Black Sea coast of Bulgaria was found below legislation limit.
- The levels of PCBs, DDTs and PAHs in mussels were found lower or comparable to levels measured in the similar species from other aquatic ecosystems.
- These results confirm that the persistent organic pollutants continue to be present in marine environment in the Black Sea.

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Levels of lipophilic marine biotoxins registered in Black Sea mussels from Bulgarian coast in 2021

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Overview:

- Marine biotoxins are found in some seafood worldwide
- At certain levels marine biotoxins can be extremely dangerous to human health
- Investigated is the presence of marine biotoxins in mussels from Bulgarian coast
- LC-MS/MS applied
- PTX-sa, 7-epi-PTX-sa, hydroxy-YTX, YTX (0,028-0,122 µg/g hp and PTX-2 were detected
- The levels of detected toxins do not exceed the regulatory levels
- Marine biotoxin distribution data shows that all four identified toxins occurred in both study areas
- Monitoring on their levels in most preferred shellfish is required to keep the consumers' health safe

Introduction: Marine biotoxins are produced by certain microalgae species. Based on their chemical structure they are devided into lipophilic and hydrophilic. Both could cause human illness and therefore represent a serious threat to public health. Shellfish such as mussels are the main dietary source of marine biotoxins. The digestive gland (hepatopancreas) is the organ where toxins accumulate and concentrate.

➢ **The aim of this study was to estimate the levels of multiple lipophilic marine biotoxins in mussel samples and to compare them with the regulatory levels.**

Methods: Harvested were most consumed mussels (N = 17) - from natural populations as well as from aquaculture regions. Sampling was performed in 2021. In order to develop the worst-case scenario, the hepatopancreas (hp) of mussels dissected und subjected to further analysis. The samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Table 1).

Table 1. Lods of analysed marine biotoxins

Analysed marine biotoxins	LOD, pg/µL
DA	4,93
YTX	100,00
PTX2	3,73
OA	25,71
DTX1	60,00
DTX2	3,73
GonA	30,56
AZA1	0,92

Results:

✓ in all samples were detected at least two of the determined biotoxins
✓ The most abundant toxin was PTX-sa (0,001- 0,030 µg/g hp), followed progressively by 7-epi-PTX-sa (0,001-0,14 µg/g hp), hydroxy-YTX (0,026-0,075 µg/g hp), YTX (0,028-0,122 µg/g hp)and PTX-2 (0,002 µg/g hp)
✓ the regulatory levels of both toxins from pectenotoxin group (160 µg/kg) and yessotoxin group (3,75 mg/kg) were not exceeded.
✓ The registered concentrations were much lower than the levels set in the EU legislation.
✓ The distribution of the content of marine biotoxins in samples of wild and cultivated mussels from two areas of catch - the Northern Black Sea (North) and the Southern Black Sea (South) was also investigated
✓ more comprehensively determination of the marine environment state
✓ This analysis is important because of the known differences in temperature, thermodynamic, hydrographic and salinity regimes of surface waters in the North and South regions.
✓ Analysis of marine biotoxin distribution data (Fig. 2) indicated that all four identified toxins occurred in both study areas.
✓ YTX OH-YTX levels were significantly higher than PTX2-sa and iso-PTX2-sa levels.
✓ When comparing the average concentrations of the phycotoxins, it was registered that the PTX2 derivatives had close levels in both regions.
✓ Average concentrations of YTX and OH-YTX were higher in the North region.

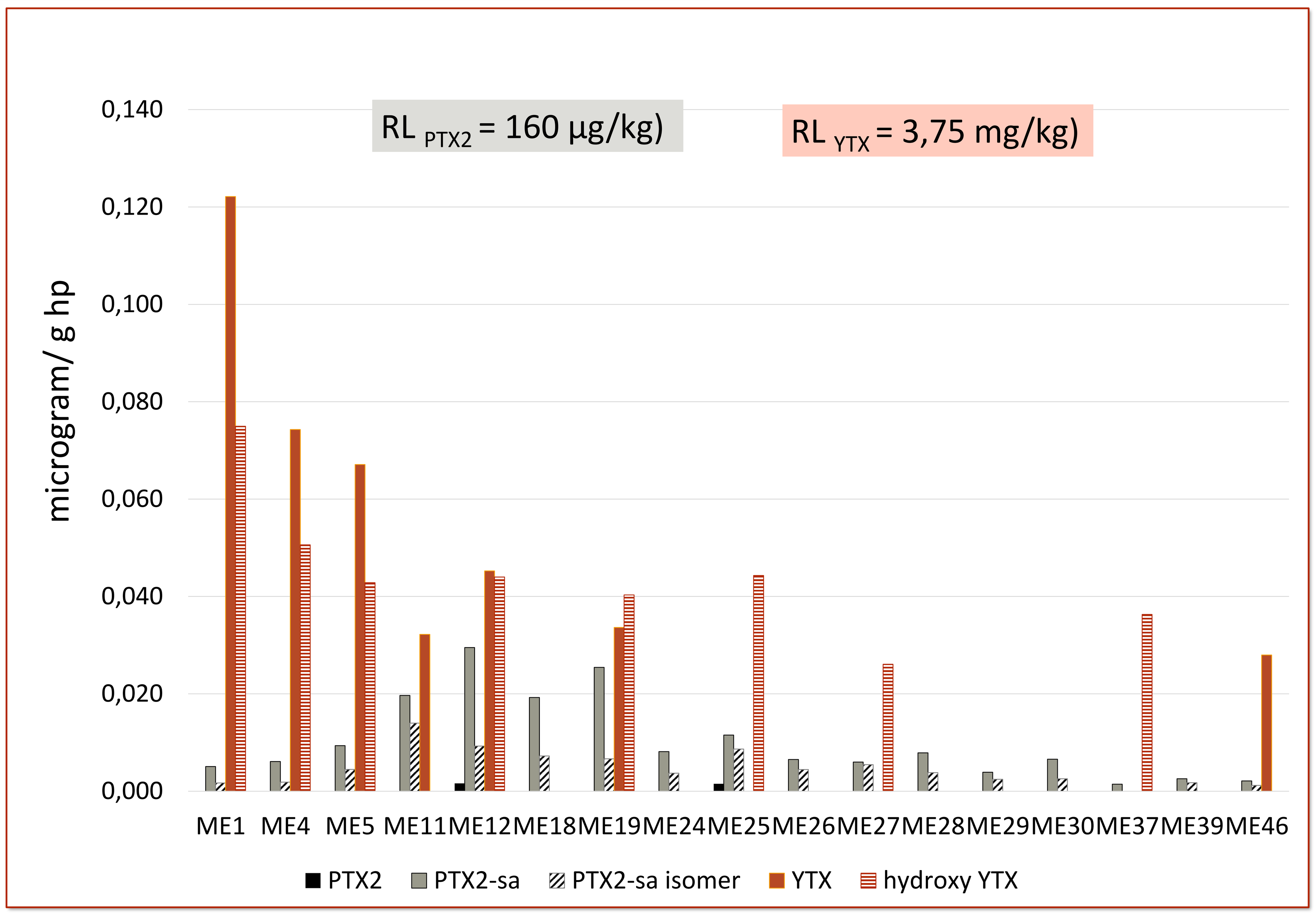

Figure 1. Marine biotoxins detected in mussel samples

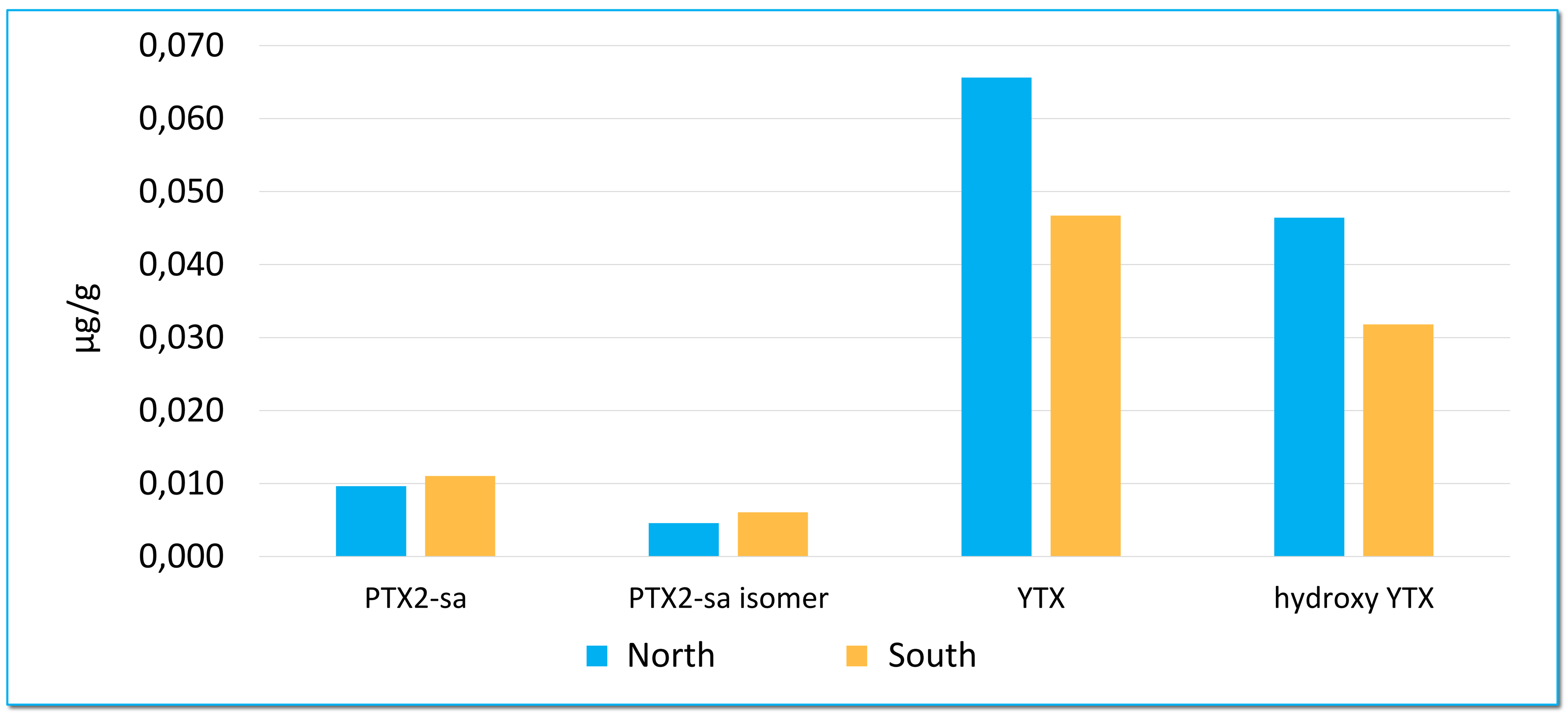

Figure 2. Comparison of the average levels of the detected marine biotoxins in the two sampling regions

Conclusion: Thus, as toxicokinetics, oral toxicity and relative potency of individual PTX and YTX-group is still being investigated, monitoring on their levels in most preferred shellfish is required to keep the consumers' health safe.

Acknowledgements

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