THE PROGRAM FOR MARITIME AFFAIRS AND FISHERIES 2014-2020
Project selection procedure BG14MFOP001-6.004 "Increasing knowledge of the state of the marine environment"
EUROPEAN UNION
EUROPEAN MARITIME AFFAIRS AND FISHERIES FUND
THE PROGRAM FOR MARITIME AFFAIRS AND FISHERIES 2014-2020
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INTERNATIONAL SEMINAR OF ECOLOGY- 2022
Actual problems of Ecology
September 29th - 30th, 2022
Online event

Evaluation of abundance of microplastics in Bulgarian coastal waters

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INTRODUCTION

Plastic pollution in sea waters is ubiquitous, but quantitative estimates on the floating microplastics in Black Sea are still limited. Plastics may adsorb persistent environmental contaminants, thus representing a potential risk for marine organisms.

The aim of the study was evaluation of the presence and characteristics of microplastic particles (MPs) in waters from the Black Sea coast of Bulgaria.

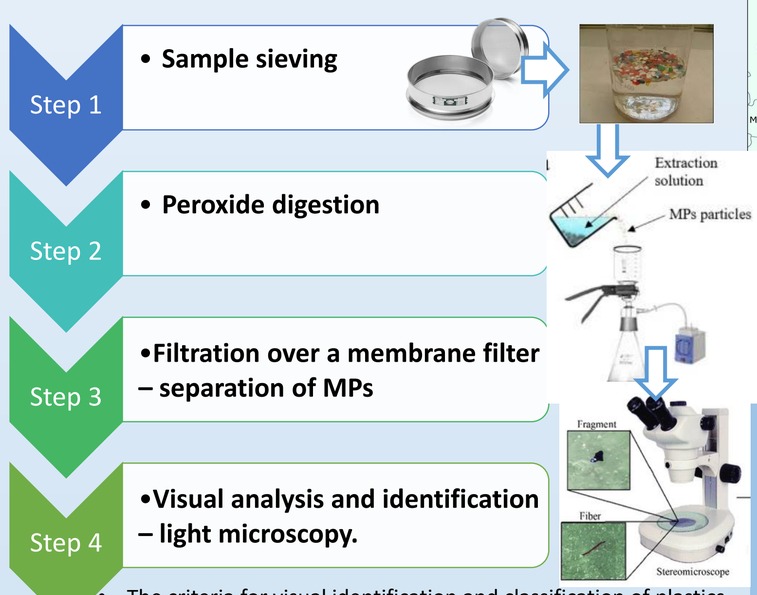
MATERIALS

Samples of coastal waters were collected from March 2021 to April 2022 from different station on Black Sea coast, including protected, aquaculture and industrial areas. In order to determine the number of plastic particles in water, 23 samples were collected from surface waters at depth of ca. 1 m close to the Bulgarian shore.

METHODS

Water samples were treated with H2O2, plastic particles were isolated by density separation and filtered over a membrane filter. Identification analysis of micro particles (< 5 mm) were performed visually by microscopy.

Statistical analysis - SPSS 16 software.



- The criteria for visual identification and classification of plastics were based on Hidalgo-Ruz et al. (2012) and Kovač Viršek et al. (2016).
- Characterization by morphological types, size classes (Ferret diam.) and color according Covernton et al. (2016).

Black sea map and sampling area

RESULTS

Composition of microplastics particles according to their:

size classes → **form type** → **color classes**

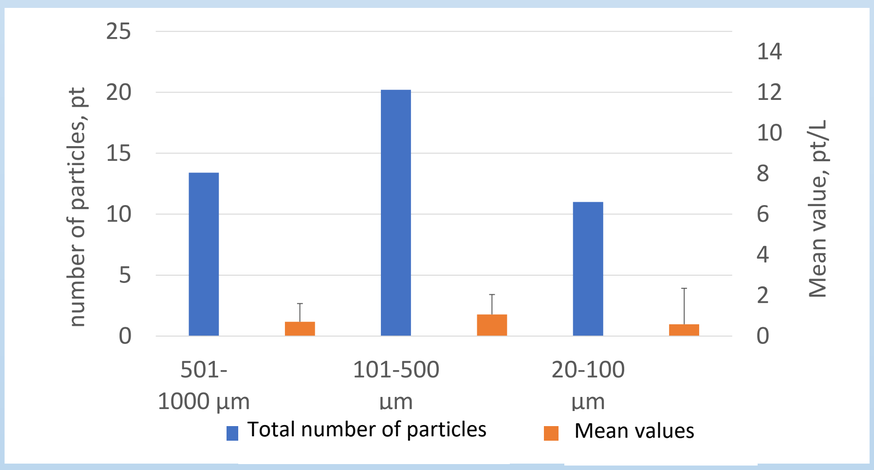


Figure 1. Size classes of MP particles (fragments and other*) in coastal waters

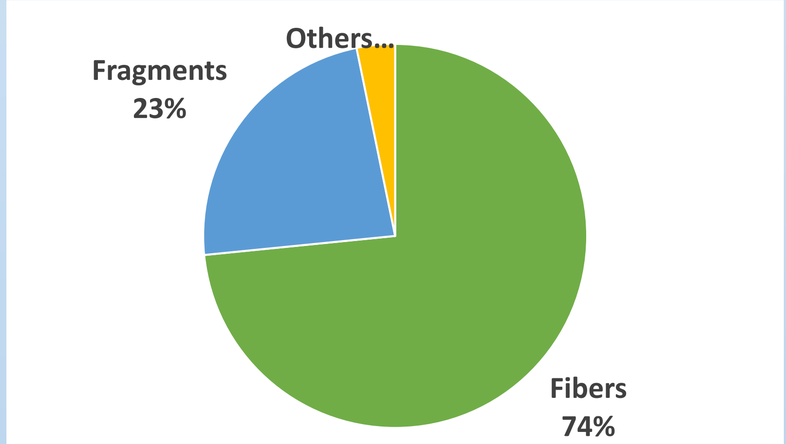


Figure 2. Distribution of MP particles by form type

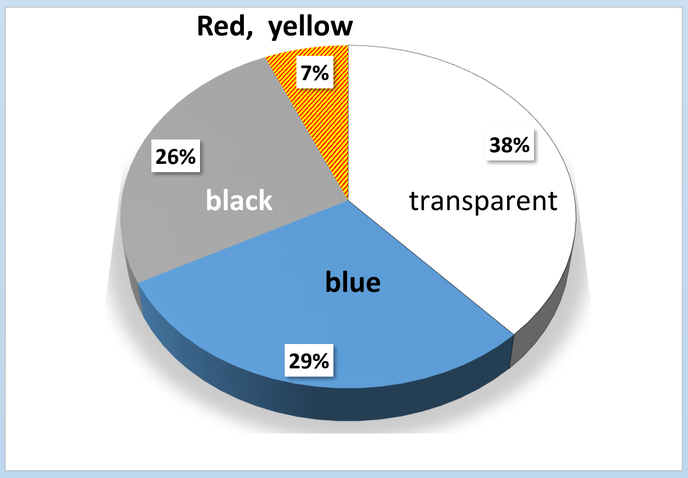


Figure 3. Composition of microplastics polymers according to their color classes

Results:

- Results indicated widespread presence of microplastics in coastal waters.
- Mean MPs concentration was calculated 7.2 ± 4.8 pt/L.
- The most dominant forms were fibers followed by fragments.
- The most abundant size class of fragments was 101-500 µm Ferret diameter.

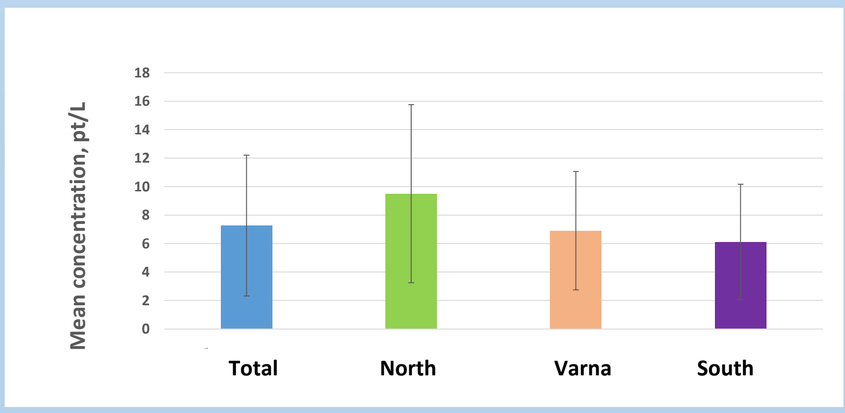


Figure 4 Distribution of microplastics pollution on the Bulgarian coast

Results:

- The highest concentrations of microplastics were observed in sea waters from industrial region (16.3 pt/L) and from protected area (12.7 pt/L).
- Comparison of the protected, aquaculture and industrial area showed that there is no significant difference in the abundance of plastic particles – 7.9, 7.8 and 6.3 pt/L resp.

Conclusions:

- The confirmation of the possible sources of pollution - further analyses, e.g. FTIR spectrometry
  - ➢ lack of spectroscopic validation of microplastic identity – drawback of the present study
  - ➢ could have resulted in an overestimation of the reported concentrations
- Fibrous particles may have a greater tendency for entanglement within complex feeding structures
  - ➢ accurately quantifying the concentrations of any types of MPs
  - ➢ bridging the gap between laboratory and field studies of ecological risk.

Acknowledgments: This work was supported by the Maritime Affairs and Fisheries Program 2014-2020 co-financed by the European Union through the European Maritime Affairs and Fisheries Fund. Project "Investigation of priority chemical pollutants and biotoxins and assessment the state of the marine environment", No BG14MFOP001-6.004-0006, contract No МДР-ИП-01-13/25.01.2021.

International Seminar of Ecology – 2022, "ACTUAL PROBLEMS OF ECOLOGY"
9-30 September 2022

Recent knowledge about the occurrence of marine biotoxins on Bulgarian coast in 2021

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INTRODUCTION

Marine biotoxins are produced by certain phytoplankton species and use to accumulate in filter feeding marine organisms. Marine biotoxins' occurrence in all aquatic environments and latitudes is variable in time and space. Thus, it is an essentially natural phenomena, but its spread cannot be completely avoided or eliminated. A serious concern appears if these substances accumulate at high levels in seafood. If it is consumed severe illness might be developed.

The aim of this study is to assess the presence of marine biotoxins in plankton samples from 2021 and to compare the determined levels with previous period.

MATERIALS AND METHODS

Plankton samples (N=21) were collected in **2021** along the whole **Bulgarian coastline** (Black Sea). The presence of hydrophilic (***domoic acid*** (DA)) and lipophilic toxins (***okadaic acid, dinophysis toxin – 1, dinophysis toxin -2, azaspiracid-1, goniodomin A, pectenotoxin-2*** (PTX2) and ***yessotoxin***) was investigated via liquid chromatography – tandem mass spectrometry (**LC-MS/MS**).

Table. 1 LODs of studued toxins

Studied Toxins	Standard solution concentration, pg/µL	LOD, pg/µL
DA	100	4,93
YTX	500	100,00
PTX2	500	3,73
OA	500	25,71
DTX1	100	60,00
DTX2	1000	3,73
GonA	400	30,56
AZA1	100	0,92

RESULTS

✓ Presence of only **DA** in three samples

✓ **PTX2** in 2 samples

✓ Positive samples were sporadically distributed along the study period.

Table. 2 Levels of studued toxins in plakton samples

Retention time	312/266-7,17	786/607-12,67	822/223-11,57	822/223-11,87	836/237-12,57	842/824-12,62	876/213-12,14
Studied toxins / Sample	**DA** pg/µL	**GONA** pg/µL	**OA** pg/µL	**DTX2** pg/µL	**DTX1** pg/µL	**AZA1** pg/µL	**PTX2** pg/µL
ME6	**170,94**	nd	nd	nd	nd	nd	nd
ME8	nd	nd	nd	nd	nd	nd	nd
ME9	nd	nd	nd	nd	nd	nd	**9,23**
ME15	**138,48**	nd	nd	nd	nd	nd	nd
ME17	nd	nd	nd	nd	nd	nd	nd
ME38	nd	nd	nd	nd	nd	nd	nd
ME47	nd	nd	nd	nd	nd	nd	nd
ME48	nd	nd	nd	nd	nd	nd	nd
ME49	nd	nd	nd	nd	nd	nd	nd
ME57	nd	nd	nd	nd	nd	nd	nd
ME58	nd	nd	nd	nd	nd	nd	nd
ME59	**13,38**	nd	nd	nd	nd	nd	nd
ME66	nd	nd	nd	nd	nd	nd	nd
ME68	nd	nd	nd	nd	nd	nd	nd
ME74	nd	nd	nd	nd	nd	nd	nd
ME75	nd	nd	nd	nd	nd	nd	nd
ME76	nd	nd	nd	nd	nd	nd	**6,51**
ME83	nd	nd	nd	nd	nd	nd	nd
ME86	nd	nd	nd	nd	nd	nd	nd
ME89	nd	nd	nd	nd	nd	nd	nd

DISCUSSION AND CONCLUSIONS

- During 2018-2019 LC-MS/MS analysis confirmed the presence of DA, PTX2, SPX1 and GDA in plankton net samples collected from the same locations reported here.
- The matching toxins (DA and PTX2) are at comparable levels in both period of investigation, thus lower than in other European water where harmful algal blooms are registered.
- There results show the persistent appearance of some marine biotoxins in Bulgarian waters.
- Although concentration levels are low a constant monitoring is required in order toxic events by seafood consumption to be avoided.

Acknowledgements:
This work was supported by the Maritime Affairs and Fisheries Program 2014-2020 co-financed by the European Union through the European Maritime Affairs and Fisheries Fund. Project No BG14MFOP001-6.004-0006-C01, contract No МДР-ИП-01-13/25.01.2021 "Investigation of priority chemical pollutants and biotoxins and assessment the state of the marine environment".