

Microplastics and bisphenol A in mussels along Italian and Croatian coast of the Adriatic Sea

Bogdanović T.^{1*}, Di Renzo L.², Petričević S.¹, Mascilongo G.², Brkljača M.³, Cito F.⁴, Calistri P.⁴, Notarstefano V.⁵, Gioacchini G.⁵, Giorgini E.⁵, Giansante C.², Berti M.², Sokolić D.⁶, Ferri N.², Listeš E.¹, Di Giacinto F.²

¹Croatian Veterinary Institute, Poljička cesta 6, 21 000 Split, Croatia; ²Water Biology Unit, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM), Campo Boario, 64100 Teramo, Italy; ³ Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10 000 Zagreb, Croatia; ⁴ National Reference Centre for Veterinary Epidemiology, Programming, Sciences, Information and Risk Analysis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM), Campo Boario, 64100 Teramo, Italy; ⁵ Department of Life and Environmental Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy; ⁶ Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, 31000 Osijek, Croatia

Introduction

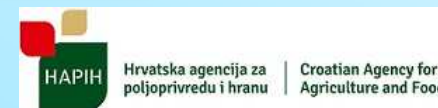
The Mediterranean basin is one of the most impacted area by marine litter and within it the Adriatic Sea even more. Recent studies detected floating and sinking microplastics (MPs) in the aquatic environment, as well as in biota (Di Renzo *et al*, 2021). From ancient times, Adriatic coasts are particularly devoted to the mussel farming. *Mytilus galloprovincialis* is one of the most commercial interest species, it is widely consumed by humans. Mussels could be considered as particularly good trusted candidates for the assessment of human exposure to MPs, as they are filter-feeders directly exposed to MPs present in the environment (Mercogliano *et al*, 2021). Nowadays, a broad range of complementary analytical methodologies has been applied regarding the detection and identification of MPs and their additives such as Bisphenol A (BPA), without any harmonized monitoring protocols by making difficult the comparison of disposable data on their occurrence in marine biota and the risk assessment for human health (Barboza *et al*, 2020).

Overall Objective

The study is focused on the assessment of the overall MP contamination status and BPA concentration in mussels (*Mytilus galloprovincialis*), simultaneously collected from aquaculture farms and natural beds located along the Italian and Croatian coasts of the Adriatic Sea, and their implications for human health.



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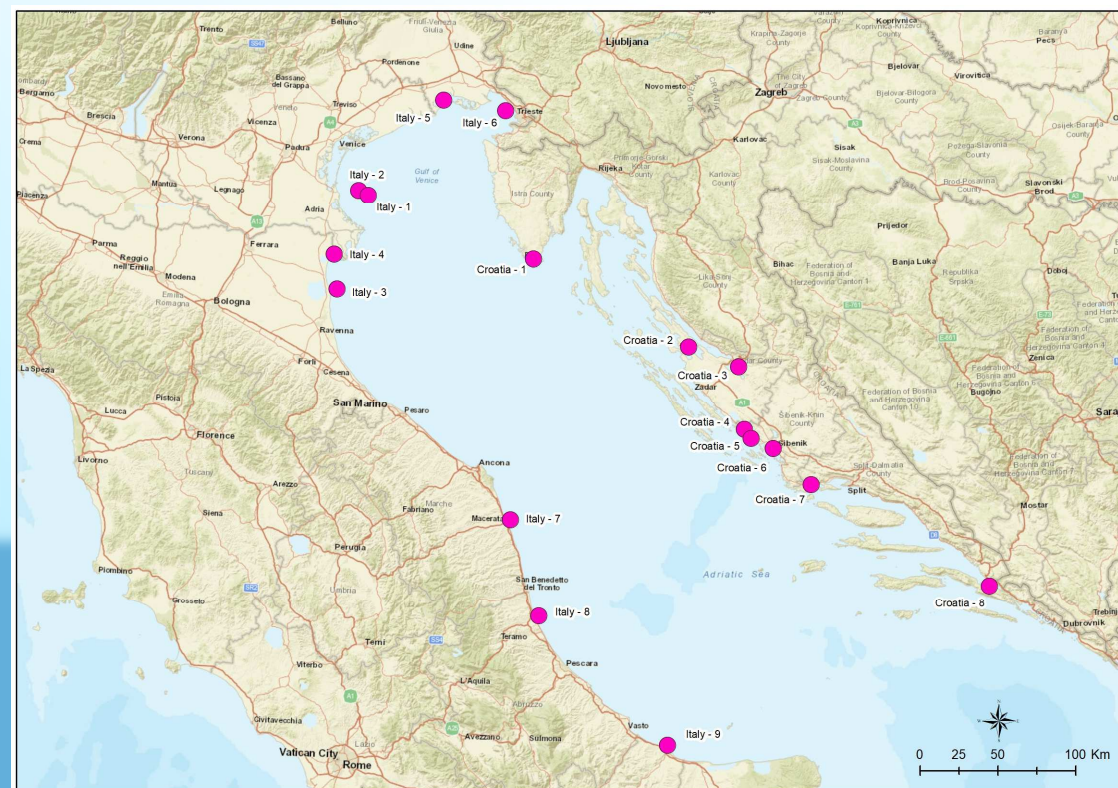
Study Area, Sampling, Quality assurance

- No 17 sampling points were established along Croatian and Italian Adriatic coasts;
- altogether 30 individuals of commercial size (average condition index $29,4 \pm 5,88$) have been sampled per sampling point, 15 individuals for MPs identification method using Raman Microspectroscopy (RMS) and 15 individuals for polyethylene terephthalate (PET), polycarbonate (PC), para phthalic acid (PTA) and bisphenol A (BPA) determination by UPLC-MS/MS.
- QA/QC applied: the external environment and the prevention of contamination have been assessed through operator, the work environment, the cleaning procedures and preparations of solutions and blanks; spiked sample, duplicate sample.

Methodology

Mussel analysed by UPLC-MS/MS

1. Depolymerization via hydrolysis within 30 min in 1-pentanol solution with 1 g KOH heating at 135 °C and extraction in MS/MS grade water
2. SPE extraction (STRATA-X 6 cc/200 mg)
3. Sample reconstitution in a 2 mL of water/MeOH (8.5:1.5 v/v),
4. Freely available concentrations of PTA and BPA were extracted after the same procedure as above except heating
5. Chromatographic separations were performed on Poroshell 120 EC-C18 column (100×2.1mm internal diameter, 2.7 µm particle size) and Polaris C18-Ether column (100x3.0 mm internal diameter, 3.0 µm particle size) from Agilent (Santa Clara, CA) for BPA and PTA respectively in LC gradient elution programs for BPA (H₂O (water with 0.1 % v/v ammonium and methanol) and PTA (water with 0.1 % v/v formic acid and acetonitrile with 0.1 % v/v formic acid)
6. Internal standard method at concentrations ranged from 0.1 to 750 ng/mL for BPA and PTA with the concentrations of 2 IS fixed at 200.0 ng/ml

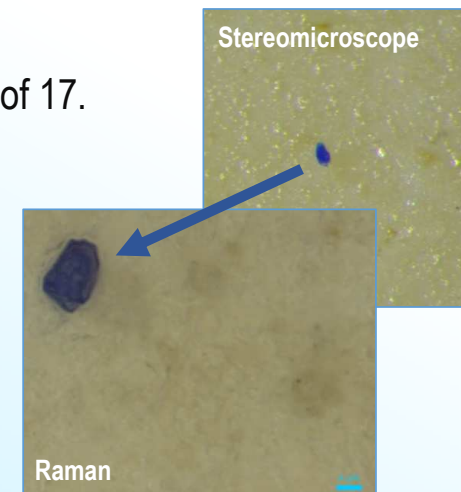
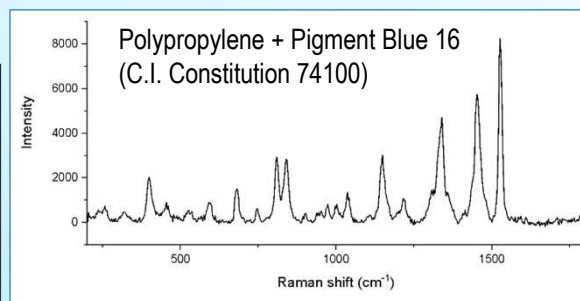
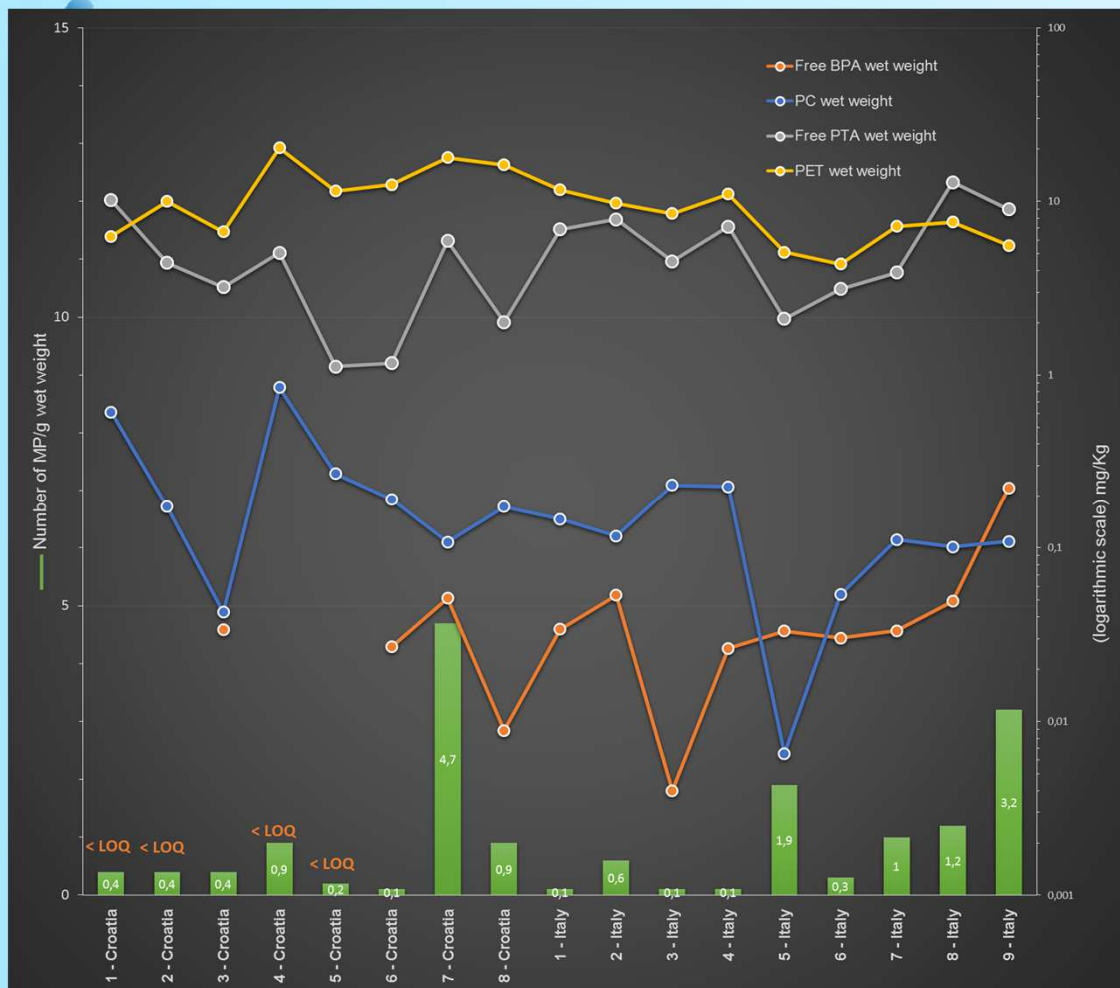


Mussel analysed by Stereomicroscope and RMS

1. mixing 100 gr of mussels of which 10 gr to be digested with KOH 10% for 24 hours at 60 °C stirred constantly at 300 rpm.
2. Density separation with KI 50% and filtration with vacuum system using nitrate cellulose filter at 1 µm. Then filtered particles were observed by Stereomicroscope and chemically characterized by Raman.

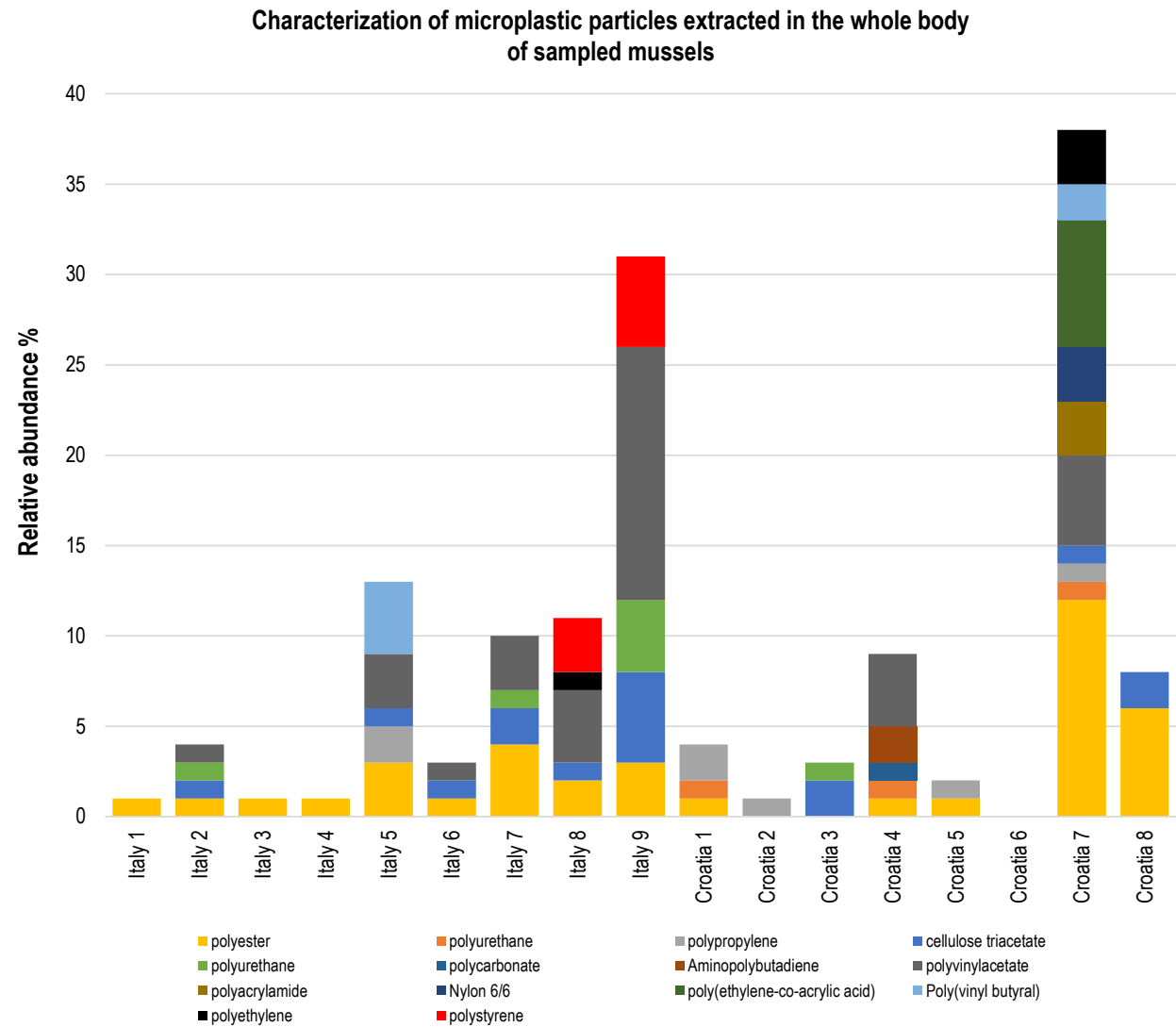
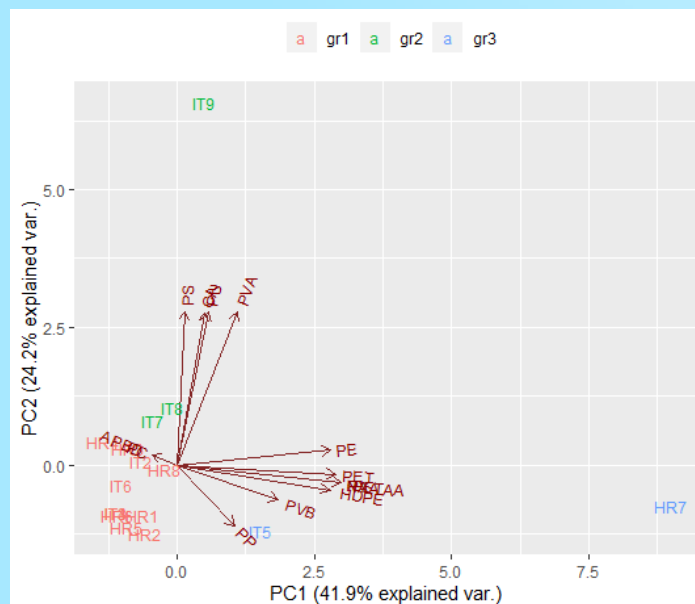
Results

- The average number of MPs in Adriatic mussel is 1 item/g.
- Polyester and High Density Polyethylene were detected in 10 sampling sites, out of 17.
- Free PTA, PC and PET were revealed in all samples, free BPA in 76,5%.



RAMAN result	Croatia								Italy								
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9
Polyester																	
Polyurethane																	
Polyvinylacetate																	
Polypropylene																	
High density polyethylene																	
Polyethylene																	
Polystyrene																	
Aminopolybutadiene																	
Polycarbonate																	
Nylon 6/6																	
Poly(ethylene-co-acrylic acid)																	
Poly(vinyl butyral)																	
Polyacrylamide																	
Cellulose triacetate																	
Aluminium oxide																	





Risk assessment and Conclusion

	ITALY				CROATIA			
	EDI $\mu\text{g/kg}$ body weight/day		THQ		EDI $\mu\text{g/kg}$ body weight/day		THQ	
	Median	95th Percentile	Median	95th Percentile	Median	95th Percentile	Median	95th Percentile
BPA $\mu\text{g/kg}$	4.707	21.983	1.176	5.495	1.214	45.102	0.303	1.975
PTA mg/kg	0.977	1.599	1.954	3.199	0.668	1.521	1.337	3.042

$$EDI = (C_i \times B_f) \div BW_{\text{Barboza et al., 2020}}$$

$$THQ (BPA) = EDI / TDI (4 \mu\text{g/kg})$$

$$THQ (PTA) = EDI / RfD (0.5 \text{ mg/kg})$$

- This study provides evidences of microplastics contamination of mussels farmed along the Italian and Croatian coastline of the Adriatic Sea with Polyester (PE) and Polypropylene (PP) as the main polymer types.
- The median of bisphenols detected in Italian mussel resulted with considerably higher EDI values (3-fold higher) compared to Croatian results and the THQ values above 1.
- The THQ values of BPA based on the percentiles, representing a higher scenario of exposure, were above one suggesting that there is risk of exposure higher than the safety limits recommended by the EFSA.
- The obtained results also revealed a potential risk of exposure to microplastics and associated contaminants in mussels as a food source.
- PCA analysis showed the existence of the differences in the polymer abundance inside the mussel's tissue along the Adriatic coast despite the growth of mussels in the clear seawater and at distance from urban settlements. Probably, factors such as marine currents and sea depth affect the microplastics content in sessile organisms like mussels.
- Identification data should be combined with quantification data in order to estimate the mass of MP present in bivalves what is of great value for both environmental and human health risk assessment.