NET4mPLASTIC PROJECT

WP4 – Act. N 4.2 Lab's analysis on plastic and microplastic wastes on coastal and marine environments

D 4.2.4 – Annex 2

MP contamination and correlation between the presence of PCBs/Dioxins/PAHs and MPs in bivalves

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24/06/2022	1.1	Pizzurro Federica, Nadia Barile, Giampiero Scortichini, Recchi Sara, Nerone Eliana, Diletti Gianfranco Riccardo Nardella, Rosaria Aloia, Romolo Salini	Barile Nadia	Barile Nadia	Final	Approved

INDEX

1 Introduction 1

2 Materials and methods 2

2.1 Qualitative and quantitative analysis of microplastics in biota 2

2.1.1 Delivery and storage of the sample 2

2.1.2 Oxidizing digestion 2

2.1.3 Filtration 4

2.1.4 Reading with the stereomicroscope 5

2.2 Chemical contaminants analysis 9

2.2.1 Delivery and storage of the sample 9

2.2.2 Qualitative and quantitative analysis of dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) in biota 12

2.2.3 Qualitative and quantitative analysis of lead, cadmium and mercury in biota 12

2.2.4 Qualitative and quantitative analysis of Polycyclic Aromatic Hydrocarbons (PAH) in biota .. 14

2.2.5 Statistical analysis 15

3 Results 16

3.1 Results of the qualitative-quantitative monitoring of microplastics in biota in the Goro Sacca area 16

3.2 Results of the qualitative-quantitative monitoring of microplastics in biota in the Pescara area . 22

3.3 Results of chemical contaminants' monitoring in biota in Goro Sacca area 27

3.4 Results of chemical contaminants' monitoring in biota in the Pescara area 34

3.5 Results of chemical contaminants' monitoring in biota in Croatia area 39

4 Discussion and Conclusion 53

4.1 Qualitative-quantitative monitoring of microplastics in biota 53

4.2 Chemical contaminants' monitoring in biota 60

5 References 61

1 Introduction

This document reports on the analyses carried out on biota samples collected by IZSAM relative to qualitative and quantitative research of microplastics and for the detection of chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and heavy metals (lead, cadmium and mercury).

2 Materials and methods

2.1 Qualitative and quantitative analysis of microplastics in biota

The following protocol used in the NET4mPLASTIC project for the "biota" matrix consists of 4 phases:

1. Delivery and storage of the sample;
2. Oxidizing digestion;
3. Filtration;
4. Reading with the stereomicroscope.

2.1.1 Delivery and storage of the sample

The taken samples, once they arrive at the IZSAM laboratory in refrigerated thermal containers (4 ± 2 °C), are either analyzed within 24 hours after collection or are stored in the freezer at -20 ± 5 °C until the test is carried out.

2.1.2 Oxidizing digestion

For each sampling, the number of bivalve organisms to be analyzed was equal to 50.
For each organism to be analyzed, the length of the valves and the weight of the soft tissue were measured. These data were then recorded in specific work sheets.

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ESAME QUALI -QUANTITATIVO DELLE MICROPLASTICHE

NRG			
Data		Operatore	

Colore	Bianco		Tipo	frammenti	?lm	Classe dimensionale	< 15 µm	15-50 µm	100-500 µm
	trasparente	rosso		pellet	schiuma		50-100 µm	> 500 µm	
	blu	verde		filamenti	granuli				
	giallo	nero		non categorizzato					

ID campione	n. particelle MP	Colore	Tipo	Dimensione (µm)	Classe dimensionale
1	1				
1	2				
1	3				
1	4				
1	5				
1	6				
1	7				
1	8				
1	9				
1	10				
2	1				
2	2				
2	3				
2	4				
2	5				
2	6				
2	7				
2	8				
2	9				
2	10				
3	1				
3	2				
3	3				
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4	1				
4	2				
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4	4				
4	5				
4	6				
4	7				
4	8				
4	9				
4	10				
5	1				
5	2				
5	3				
5	4				
5	5				
5	6				

Peso tessuto molle (g)	
n. particelle MP totali/individuo	
n. particelle MP/g	#DIV/0!

Peso tessuto molle (g)	
n. particelle MP totali/individuo	
n. particelle MP/g	#DIV/0!

Peso tessuto molle (g)	
n. particelle MP totali/individuo	
n. particelle MP/g	#DIV/0!

Peso tessuto molle (g)	
n. particelle MP totali/individuo	
n. particelle MP/g	#DIV/0!

Peso tessuto molle (g)	
n. particelle MP totali/individuo	
n. particelle MP/g	#DIV/0!

Subsequently, we proceeded as follows:

- Dissect each organism with the aid of a scalpel and remove the soft tissue including intravalvular fluid;
- Deposit the soft tissue and the intravalvular liquid inside the glass flasks, taking care to name each flask with a progressive number and cover the opening with aluminum foil;
- Add to each flask a quantity of 20 ml of hydrogen peroxide (H_2O_2) at 30% for each gram of soft tissue of the organism dissected and previously weighed;
- Proceed with the incubation of the flasks inside a specific thermostat, adjusting the temperature to 55-60 °C and for a duration of 4-7 days, depending on the digestive state of the soft tissues.
  If after 4 days of digestion the organic matter is not completely degraded, add 1-2 ml of H_2O_2 at 30% and leave the sample in incubation for another 2 days, until it is completely digested.

2.1.3 Filtration

At the end of the digestion phase, we proceeded with the filtration of the digested solution as follows:

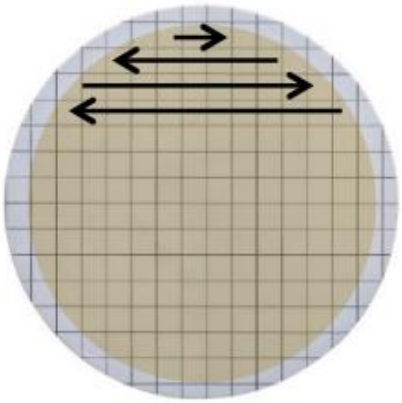
- Set up the filtration equipment with the appropriate accessories;
- Take a glass fiber filter with the help of tweezers and place it in the center of the base of the filter support;
- Fix the glass with suitable pliers to the filter support;
- After shaking the previously digested solution, pour it into the glass for filtration;
- Turn on the vacuum pump and proceed with sample filtration;
- Then rinse the flask containing the digested solution with filtered distilled water (100-150 ml) in order to collect all the material left inside the flask and add it to the solution to be filtered;
- After filtration, transfer the filter with the aid of tweezers inside appropriately labeled glass Petri dishes;
- Cover the Petri dishes with the appropriate lid and leave the filters to dry at room temperature for at least 24 hours before reading with the stereomicroscope.

In order to exclude any possible contamination with airborne microfibers in the work environment, a blank sample in three replicates was prepared simultaneously with the analysis of the samples. These consist of filtered distilled water treated with 30% H_2O_2, incubated together with the other samples. In the presence of environmental contamination, it was necessary to make a correction of the results obtained by subtracting the average of the microplastic particles found in the white control samples from the results obtained by reading the filters of the samples under examination.

2.1.4 Reading with the stereomicroscope

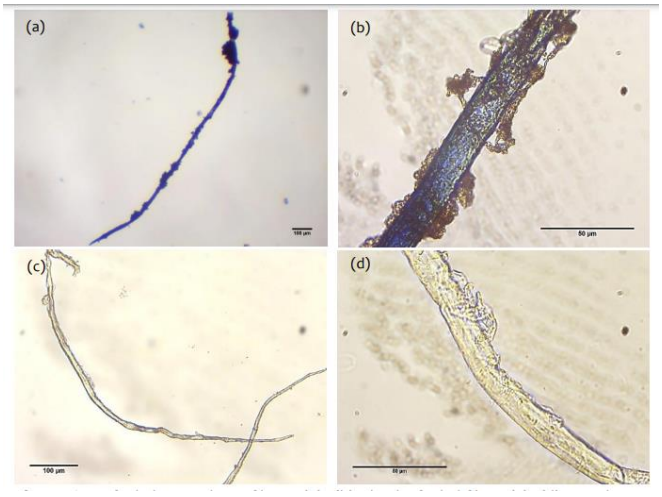
The final phase of the examination involved the use of a stereomicroscope equipped with a digital camera and special software that allowed to photograph and measure the microplastic elements observed.

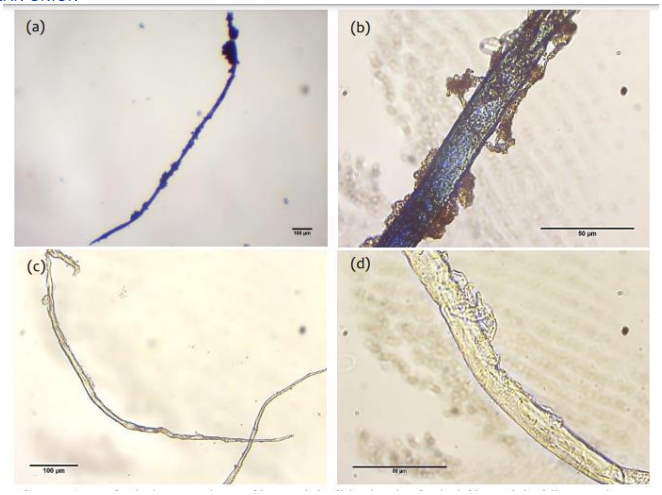
The visual observation of the filters was performed by reading from left to right, and continuing by moving down one row each time and taking care to classify each particle of a plastic nature found on the basis of shape, size class and color.



In order to identify most of the microplastics encountered, the rules of Hidalgo-Ruz et al. (2014) were followed:

- microplastics have no visible cellular or organic structure;



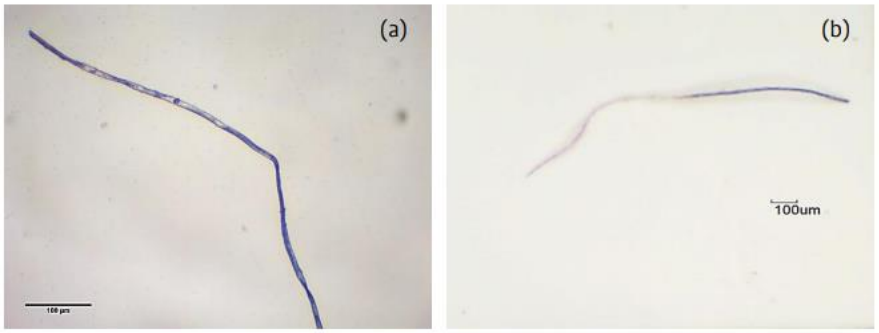


(a) and (b) microplastic filaments with no cell structure; (c) and (d) filaments of an organic nature

- the fibers must have an equal thickness along their entire length;

Microplastic filament

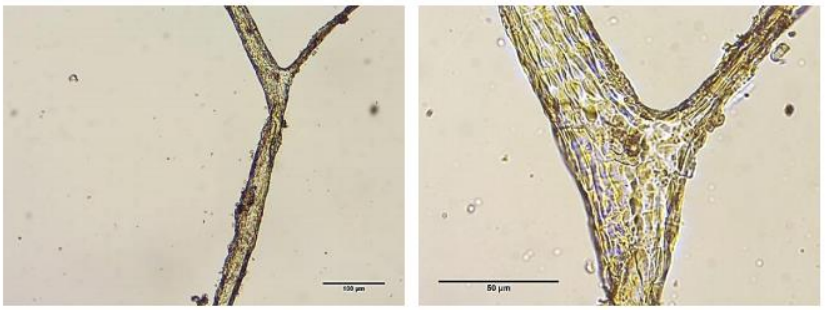
- microplastic particles generally show a homogeneous color, although some may have patterns or stripes.



Exceptions to the rules: (a) Partially bleached blue filament; (b) Red, white and blue filament.

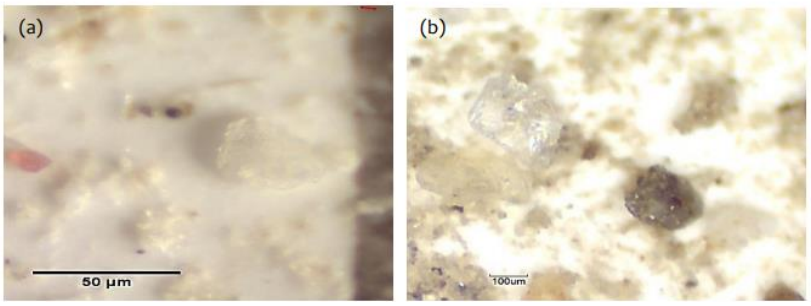
In addition to following the rules of Hidalgo-Ruz et al. (2012), it is important that the operator is able to recognize any other types of material on the filter:

- ***algae:*** sometimes they can be difficult to distinguish from plastic as even when stressed, these biological pieces may not break. It is important to look for the presence of cellular structures in the whole examined piece;



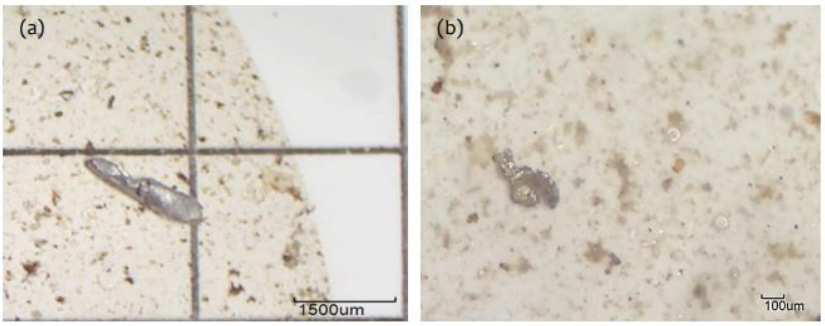
A piece of algae. Notice the cellular structure seen throughout the piece.

- ***Salt and Sand Crystals:*** These can sometimes appear similar to plastic, however when stressed they break.



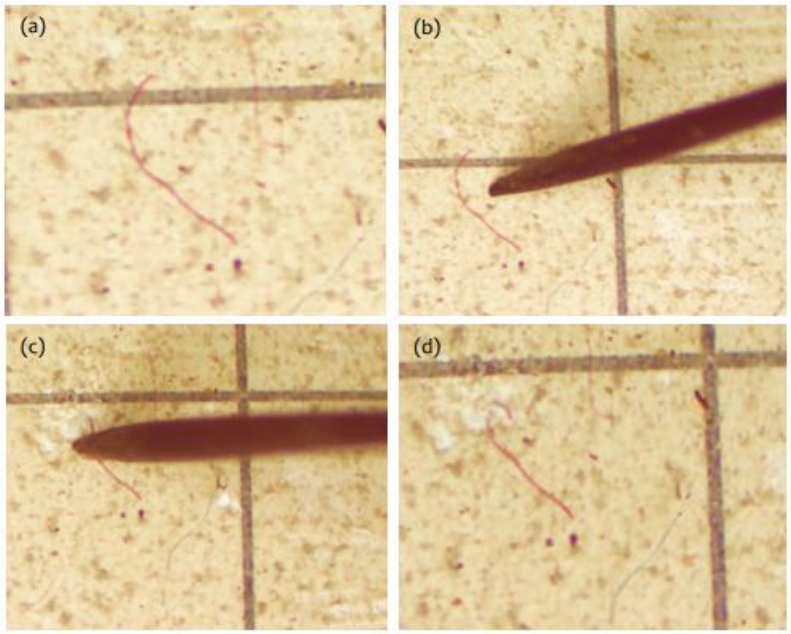
(a) Salt crystal; (b) Grain of sand.

- ***Aluminum foil:*** can flake off the lids. It is possible to identify these pieces due to their intense reflectivity and luster. They also do not show the characteristic features of the most plastic pieces such as flexibility.



Examples of aluminum pieces: (a) Large piece; (b) Smaller piece in detail.

In cases where the operator was unable to distinguish between plastic particles and other types of organic debris, the "Hot Needle" test was used: the test involves the use of a needle, which is first heated through a flame and then brought closer to the particle to be tested; in the case of particles of a plastic nature, it can be observed that these melt or curl. Biological debris and other non-plastic materials will not.



Hot Needle test on a red filament: (a) Pre-test; (b) Approaching the hot needle; (c) Contact between hot needle and filament; (d) Final result: the filament reacted and curled due to the heat.

Whenever the presence of a microparticles of a plastic nature was observed, the following procedure was carried out:

- Take and save a photograph;
- Observe the shape (fragment, sphere, pellet, filament, foam, film, unclassified shape) and the color (white, black, red, blue, transparent, green and other colors);
- Measure (measure the longest diagonal) of the detected particle;
- Make a note of the above in the appropriate worksheet.

The result is expressed individually for each organism subjected to analysis as

- "*Number of microplastic particles per gram of soft tissue of the bivalve mollusk (n. MP particles/g)*" and
- "*Number of microplastic particles per bivalve organism (n. MP particles/organism)*".

The result is calculated by dividing the total number of microplastic particles observed for each filter analyzed, by the total grams of soft tissue of the organism subjected to digestion and filtration.

2.2 Chemical contaminants analysis

2.2.1 Delivery and storage of the sample

The taken samples were stored in the freezer at -20 ± 5 °C until the test is carried out. All the samples were homogenized by a knife mill Grindomix GM-200 (Retsch, Dusseldorf, Germany) before analysis

2.2.2 Qualitative and quantitative analysis of dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) in biota

2.2.2.1 Chemicals

Solvents such as n-hexane, dichloromethane, acetone, toluene and isooctane were organic residue analysis quality (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). Other reagents included anhydrous sodium sulphate, concentrated sulphuric acid and sodium chloride, all at reagent grade (Honeywell Burdick & Jackson, Seezle, Germany).

Prepacked multilayer silica, alumina, and carbon columns were obtained from Fluid Management Systems (Massachusetts, USA).

All standard solutions were supplied by Wellington Laboratories (Guelph, Ontario, Canada). Calibration solutions DF-CVS (CS1 through CS4), 13C12-labeled internal standard DF-LCS-C200, and recovery standard DF-IS-J were used for PCDD/Fs analysis. Calibration solutions WP-CVS (CS1 through CS7), 13C12-labeled internal standard WP-LCS, and recovery standard P48-RS-STK were prepared for DL-PCB analysis. Calibration solutions P48-M-CVS (CS1 through CS5), 13C12-labeled internal standard P48-M-ES, and recovery standard P48-RS-STK were selected for NDL-PCB analysis.

2.2.2.2 Analytical Methodology

The 17 PCDD/Fs, the 12 DL-PCBs and the 6 indicator NDL-PCBs (Table 1) were determined through accredited methods in accordance with ISO EN 17025, based on EPA Method 1613 for PCDD/Fs (US EPA 1994) and EPA Method 1668 for PCBs (US EPA 2010). Both methods are based on isotopic dilution and high resolution mass spectrometry (HRMS) detection. In order to adapt the analytical procedures to the matrix under examination, variations have been made in the extraction and purification phases of the sample. Aliquot weights of approximately 10,00 ± 2,00 g were taken from each sample, mixed with anhydrous sodium sulphate in a ratio of 1:3 (w/w) and fortified with a mixture of the internal standards containing: 17 PCDD/Fs 13C12-labeled

(0.2-0.4 ng); 12 DL-PCBs 13C12-labeled (1.0 ng); 6 NDL-PCBs 13C12-labeled (2.0 ng). The samples were dried for at least 8 hours in an oven at a temperature of 40±5 °C, before extraction.

An ASE 350 Thermo Scientific Dionex (Sunnyvale, California, USA) accelerated solvent extraction system using a mixture of n-hexane and acetone 80:20 (v/v) was used to extract the samples (three extraction cycles at a temperature of 125 ◦C and a pressure of 1500 psi). The extract was filtered through anhydrous sodium sulphate and collected in a volumetric flask. Solvent evaporation was carried out on a rotary evaporator with a water bath at 40 ± 5 °C.

The extract was dissolved in hexane and subjected to liquid-liquid partitioning process with sulphuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride to exclude the lipid component. Then, it was purified on an automated Power-Prep™ system (Fluid Management System (FMS) Massachusetts, USA) using disposable columns (multilayer silica, alumina and activated carbon). The fraction containing ortho-PCBs and NDL-PCBs was eluted from alumina column while dioxins and non-ortho PCBs were collected from carbon colums.

The two eluates were concentrated by evaporation in nitrogen stream and dissolved in the corresponding recovery standards solutions (13C12-labeled PCDD/Fs and PCBs different from the previous ones).

The instrumental analysis was performed using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS), using GC Trace Series 2000 coupled to a MAT 95 XL (Thermo Fisher Scientific, USA) and a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA). The chromatographic separation of the 17 PCDD/Fs was carried out on a DB-5 MS capillary column 60m x 0.25mm x 0.10µm (J&W Scientific, California, USA). The chromatographic separation of DL-PCBs and NDL-PCBs was carried out on HT8-PCB capillary column 60m x 0.25mm x 0.25µm (SGE Analytical science, Melbourne, Australia). The acquisition of the masses was carried out in Single Ion Monitoring (SIM) mode at a resolution of 10,000, selecting the masses indicated by the method. As regards 17 PCDD/Fs and 12 DL-PCBs, TEQ concentration were determined by multiplying the analytical result of each congener by the corresponding WHO TEF (Van den Berg et al. 2006), while for NDL-PCBs, the result was reported as the sum of the 6 indicator congeners. All values have been reported as upper bound concentrations, that is all values below the limit of quantification (LOQ) are supposed to be equal to the respective LOQ (European Commission 2006). A laboratory blank was analyzed for each batch of samples. Recovery of labeled congeners ranged from 60% to 90%, and the analytical uncertainty was in the order of ± 20% for WHO-TEQs and the sum of six NDL-PCBs. Method performance was in agreement with the requirements for method of analysis used in official control of the levels of PCDD/Fs and PCBs in foodstuff (European Commission 2017) and has been successfully verified in many proficiency tests over 15 years.

PCDD/Fs	DL-PCBs	NDL-PCBs
Polychlorinated dibenzo-p-dioxins (PCDDs)	*non-ortho-PCBs*	
2,3,7,8-TCDD	PCB-77	PCB-28
1,2,3,7,8-PeCDD	PCB-81	PCB-52
1,2,3,4,7,8-HxCDD	PCB-126	PCB-101
1,2,3,6,7,8-HxCDD	PCB-169	PCB-138
1,2,3,7,8,9-HxCDD		PCB-153
1,2,3,4,6,7,8-HpCDD		PCB-180
OCDD		
Polychlorinated dibenzofurans (PCDFs)	*mono-ortho-PCBs*	
2,3,7,8-TCDF	PCB-105	
1,2,3,7,8-PeCDF	PCB-114	
2,3,4,7,8-PeCDF	PCB-118	
1,2,3,4,7,8-HxCDF	PCB-123	
1,2,3,6,7,8-HxCDF	PCB-156	
2,3,4,6,7,8-HxCDF	PCB-157	
1,2,3,7,8,9-HxCDF	PCB-167	
1,2,3,4,6,7,8-HpCDF	PCB-189	
1,2,3,4,7,8,9-HpCDF		
OCDF		

Table1. Analytes determined in mussel samples

2.2.3 Qualitative and quantitative analysis of lead, cadmium and mercury in biota

2.2.3.1 Chemicals

High-purity deionised water (resistivity 18.2 MΩ.cm, ELGA LabWater, High Wycombe, UK), concentrated nitric acid HNO3 (≥ 67%, trace-metal grade, CHEM-LAB NV, Zedelgem, Belgium) and hydrogen peroxide H2O2 (30%, Merck, Darmstadt, Germany) were used for sample preparation.

Mix elements stock solution of lead (Pb) and cadmium (Cd) at 100 mg/L in 2% HNO3 and traces HF was provided by Panreac Química SLU (Castellar del Vallès, Barcelona, Spain) and single element stock solution of Mercury (Hg) in 12% HNO3, Rhenium (Re) and Indium (In) in 2% HNO3 at 1000 mg/l were purchased from Merk KGaA (Darmstadt, Germany).

Argon gas of 99.9995% purity was supplied by Sapio (Monza, Italy).

The reference materials DOLT 5 (dogfish liver) and DORM 4 (fish protein) were provided by National Research Council Canada (Ottawa, Canada).

2.2.3.2 Analytical Methodology

Prior to analysis all the apparatus intended to come into direct contact with the sample and glassware were treated with 1÷2% HNO3 and then rinsed with high-purity water.

Every sample was analyzed to determine levels of lead, cadmium and mercury.

The samples of mussels were homogenized and mineralized according to the standard methods UNI EN 13804:2013 and UNI EN 13805:2014, respectively.

About 1 g of homogenized sample was weighed into PTFE vessels and dissolved in 5ml HNO3≥67% and 1mL H2O2 at 30%. In each vessel was added 100µL of gold and lutetium solution at 50mg/L as suggested in AOAC Method 2015.01-2015. Mineralization was performed in a Ethos Up microwave digestion system (Milestone Srl, Sorisole, Bergamo, Italy) according to the program shown in Table 2.

Step	Time (min)	MW (Watt)	T(°C)	Vent
1	20	1800	210	3
2	15	1800	210	3
3	60	-	cooling	-

Table 2. Microwave oven program

After cooling, the resulting clear solutions were quantitatively transferred into decontaminated

volumetric flasks and diluted exactly to a volume of 15mL with high-purity water.
Blank and quality control samples were prepared with the same procedure.
Analysis of Pb, Cd, Hg in the digested solutions were carried out by the inductively coupled plasma mass spectrometer Q-ICP-MS Nexion2000 (Perkin Elmer, Waltham, Massachusetts, USA) according to standard method UNI EN 15763:2010.
Sample introduction was carried out using a peristaltic pump connected to a SeaSpray nebulizer (2ml/min) with a glass cyclonic spray chamber. The radio frequency (RF) power was set at 1550W, the plasma gas flow was 15 L Ar/min, whereas the nebulizer gas flow was daily optimized in the range (0.90 ÷ 1.20) L Ar/min.
The isotopes of Pb, Cd and Hg were selected according to a compromise between the maximum of natural abundance and the minimum of interferences. They corresponded to: 206Pb, 111Cd and 199Hg.
The sample solutions were analyzed in ICP-MS in standard mode.
The internal standards and the standard addition calibration method were used to quantify the element of interest minimizing the matrix effect.
A mixture of internal standards was chosen to cover the entire range of masses of the isotopes considered: 187Re for high-mass isotopes such as 206Pb, 199Hg and, 115In for medium-low masses as 111Cd.
The calibration curve was performed using the matrix matching technique in order to reproduce in the plasma the same conditions as the real samples: a matrix, with a composition similar to the sample being analyzed and with a low content of the investigated elements, was used to prepare solutions of calibration.
Six standard additions ranging from 1 to 50 µg/L were made and 187Re and 111In were used as internal standards at the relative concentrations of 4 and 2 µg/L in all solutions (standards, blanks and samples), in order to compensate any random fluctuations of the signals.
Diluted solutions were prepared with the same nitric acid concentration of calibration standards (1÷2% v/v).
To avoid Hg memory effects in the sample introduction system (tube of peristaltic pump, nebulizer, spray chamber) after each solution injected, a rinse with a solution at 2 mg/L AuCl3 and 2% HNO3 was performed for 45 s at least.
Instrumental general operating parameters are summarized in Table 3.
In each analytical session, alternatively, two certified reference materials were processed as quality control samples for all determined elements: Dogfish Liver DOLT 5 and Fish Protein DORM 4.

Parameters	Value
Spray chamber	Glass Cyclonic
Nebulizer	SeaSpray (2ml/min)
RF Power (W)	1550
Plasma Ar flow (L/min)	15
Nebulizer Ar flow (L/min)	0.90 ÷ 1.20 (optimized daily)
Mathieu parameters	RPq =0.25; RPa=0
Dwell time (ms)	50
Correction equation	(-0.014038x^{118}Sn) for ^{115}In (-0.121362x^{189}Os) for ^{187}Re

Table 3. Instrumental parameters ICP-MS

2.2.4 Qualitative and quantitative analysis of Polycyclic Aromatic Hydrocarbons (PAH) in biota

2.2.4.1 Chemicals

Solvents such as acetonitrile and toluene were organic residue analysis quality (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). QuEChERS Extraction Packets (cat. no. 5982-7650), including prepacked mixture of anhydrous magnesium sulfate (4,0 g), sodium chloride (1,0 g), sodium citrate (1,0 g) and sodium hydrogencitrate sesquihydrate (0,5 g), were obtained from Agilent Technologies (Santa Clara, USA). Supel™QuE Z-SEP+ cleanup dispersive phase was purchased by Supelco® (Merck KGaA, Darmstadt, Germany).
All standard solutions were supplied by NEOCHEMA GmbH (Bodenheim, Germany): PAH-MIX-4 (1 µg/mL of each analyte in toluene) and deuterium-labeled internal standard PAH-Dx-MIX-4 (1 µg/mL of each D12-labeled compound in toluene) were used for PAH analysis.

2.2.4.2 Analytical Methodology

The analytical methodology used for the determination of four polycyclic aromatic hydrocarbons PAHs chrisene (Chr), benzo[b]fluoranthene (BbF), benzo[a]anthracene (BaA) and benzo[a]pyrene (BaP) was based on isotope dilution, using deuterated labelled surrogates. The analytes were isolated applying the modified QuEChERS method and measured by high-resolution gas chromatography-tandem mass spectrometry (HRGC-MS/MS).Aliquot weights of approximately2.00 ± 0.10 g were transferred in a 50 mL Falcon tubes and fortified with 50 µL of

the internal standard mixture containing each D12-labeled compound at 1 µg/mL.
Ultra-pure water (2.0 mL) and acetonitrile (10.0 mL) were added to the samples and the mixture was shaken for 15 min using an Agytax axial mixing shaker (AgytaxCirta, Madrid, Spain). Then, a QuEChERS prepacked salts mixture was added in the Falcon tube and the mixture was shaken again with the automatic axial extractor.After centrifugation at 4500 rpm for 10 min at 4 °C using a centrifuge (Heraeus Megafuge, Thermo Fisher Scientific), an aliquot of 5.0 mL of supernatant extract was purified using a Supel™QuE Z-SEP+ dispersive phase in a 12 mL centrifuge tube. The mixture was shaken manually for 30 s and centrifuged again at 4500 rpm for 10 min.A volume of 4.0 mL of the supernatant extract was concentrated to dryness under a gentle stream of nitrogen, followed by re-dissolution in in 300 µL of toluene.
The instrumental analysis was performed by high resolution gas chromatography - tandem mass spectrometry (HRGC-HRMS), using Trace GC Ultra coupled to a TSQ Quantum XLS (Thermo Fisher Scientific, USA). The chromatographic separation of the four PAHs was carried out on a DB-5 MS capillary column 30m x 0.25mm x 0.25µm (Agilent Technologies, Inc.). Mass filtering in the in Selected Reaction Monitoring (SRM) mode was carried out using electron ionization (EI) at 70 eV. A laboratory blank and a Quality Control (QC), fortified at level of 1 µg/kg, were analyzed for each batch of samples. For the quantification of analytes, four calibration solutions were analyzed in the range between 1.33 and 6.66 µg/L for the native compounds and a fixed levels of 6.7 µg/L for D12-labeled compounds. Recovery of quality controls ranged from 80% to 110%, and the analytical uncertainty was in the order of ± 14% for each PAH. Method performance was in agreement with the requirements used in official control of PAHs in foodstuffs and reported in EU Commission Regulation 333/2007.

2.2.5 Statistical analysis

The statistical analyses of data were performed using R sofware version 3.6.3 (R Core Team, 2020, R Foundation for Statistical Computing, Vienna, Austria).and Excel (2016, Microsoft, Silicon Valley, CA, USA). Normality of data set was tested with Shapiro-Wilk test.
Chi-square or Fisher tests were performed to verify the presence of statistically significant differences in the frequencies detected for the type, color and size class of the microplastics.
A Kruskal-Wallis test was applied to verify any differences between the sampling sites and sampling season with respect to the variable "weight of mussels (g)" and "MPs particles/g". When the Kruskal-Wallis test was significant, Dunn's post-hoc tests were carried out for comparisons between all possible pairs and Bonferroni's correction was applied to it.
The Spearman rank correlation test was performed to test any correlation between size of the mussels and the number of MPs. The analysis with $p < 0.05$ were considered statistically different.

3 Results

3.1 Results of the qualitative-quantitative monitoring of microplastics in biota in the Goro Sacca area

Below the results of analysis for each analyzed sample are reported in detail.

Autumn/Winter 2019	**ID sample 1641 (December 2019)**	**ID sample 1643 (December 2019)**
Number of mussels tested	50	50
Average maximum shell length (cm)	5,32	4,81
Average soft tissue (gr)	2,84	2,64
Number of mussels contaminated with MPs	43	36
MPs frequency of occurrence (%)	86	72
Number of microplastics detected for sampling	151	69
Prevaling type of MPs	Fiber (82%)	Fiber (55%)
Prevaling size range of MPs	> 500 µm	> 500 µm
MPs mean size (µm)	1892,7	1343,9
Minimum particle size (µm)	21,6	38
Prevaling color of MPs	Black (67%)	Black (62%)
Abundance of MPs *(mean value ± standard deviation)*		
a) Average n° microplastics/organism	3,02 ± 2,28	1,38 ± 1,24
b) Average n° microplastics/ g of soft tissue	1,11 ± 0,92	0,55 ± 0,56

Spring/Summer 2020	ID sample 612 (June 2020)	ID sample 617 (June 2020)
Number of mussels tested	50	50
Average maximum shell length (cm)	27	27
Average soft tissue (gr)	6,97	6,24
Number of mussels contaminated with MPs	7,46	7,90
MPs frequency of occurrence (%)	54	54
Number of microplastics detected for sampling	55	46
Prevaling type of MPs	Fiber (58%)	Fiber (59%)
Prevaling size range of MPs	100 - 500 µm	100 - 500 µm
MPs mean size (µm)	1162,02	496,15
Minimum particle size (µm)	22	25
Prevaling color of MPs	Black (54%)	Black (54%)
Abundance of MPs *(mean value ± standard deviation)*		
a) Average n° microplastics/organism	1,1 ± 1,46	0,92 ± 1,04
b) Average n° microplastics/g of soft tissue	0,17 ± 0,21	0,12 ± 0,13

Autumn/Winter 2020	ID sample 1348 (November 2020)	ID sample 1349 (November 2020)
Number of mussels tested	50	50
Average maximum shell length (cm)	32	18
Average soft tissue (gr)	4,67	4,48
Number of mussels contaminated with MPs	5,15	3,04
MPs frequency of occurrence (%)	64	36
Number of microplastics detected for sampling	60	31
Prevaling type of MPs	Fiber (81,7%)	Fiber (84%)
Prevaling size range of MPs	> 500 µm	> 500 µm
MPs mean size (µm)	1576,27	1005,42
Minimum particle size (µm)	30	38
Prevaling color of MPs	Black (68%)	Black (68%)
Abundance of MPs *(mean value ± standard deviation)*		
a) Average n° microplastics/organism	1,18 ± 1,27	0,62 ± 1,01
b) Average n° microplastics/ g of soft tissue	0,26 ± 0,29	0,21 ± 0,34

Spring/Summer 2021	ID sample 621 (May 2021)	ID sample 623 (May 2021)
Number of mussels tested	50	50
Average maximum shell length (cm)	5,80	5,98
Average soft tissue (gr)	6,00	5,98
Number of mussels contaminated with MPs	25	21
MPs frequency of occurrence (%)	50	42
Number of microplastics detected for sampling	53	28
Prevaling type of MPs	Fiber (87%)	Fiber (96%)
Prevaling size range of MPs	> 500 µm	> 500 µm
MPs mean size (µm)	852,9	1401,929
Minimum particle size (µm)	69	127,4
Prevaling color of MPs	Black (68%)	Blue (57%)
Abundance of MPs *(mean value ± standard deviation)*		
a) Average n° microplastics/organism	1,06 ± 1,31	0,58± 0,78
b) Average n° microplastics/ g of soft tissue	0,22 ± 0,29	0,11± 0,16

Autumn/Winter 2021	ID sample 1929 (December 2021)	ID sample 1931 (December 2021)
Number of mussels tested	50	50
Average maximum shell length (cm)	4,72	4,51
Average soft tissue (gr)	3,29	3,10
Number of mussels contaminated with MPs	24	25
MPs frequency of occurrence (%)	48	50
Number of microplastics detected for sampling	55	50
Prevaling type of MPs	Fiber (100%)	Fiber (92%)
Prevaling size range of MPs	> 500 µm (82%)	> 500 µm (82%)
MPs mean size (µm)	1933,6	1485,98
Minimum particle size (µm)	230	50
Prevaling color of MPs	Black (76%)	Black (80%)
Abundance of MPs *(mean value ± standard deviation)*		
a) Average n° microplastics/organism	1,10± 1,37	1,00± 1,35
b) Average n° microplastics/ g of soft tissue	0,37± 0,50	0,36± 0,48

Spring/Summer 2022	ID sample 379 (May 2022)
Number of mussels tested	50
Average maximum shell length (cm)	5,9
Average soft tissue (gr)	5,5
Number of mussels contaminated with MPs	5
MPs frequency of occurrence (%)	10
Number of microplastics detected for sampling	7
Prevaling type of MPs	Fiber (85,5%)
Prevaling size range of MPs	100-500µm (43%)
MPs mean size (µm)	632
Minimum particle size (µm)	74
Prevaling color of MPs	Blue (71%)
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	0,14± 0,45
b) Average n° microplastics/ g of soft tissue	0,03± 0,10

3.2 Results of the qualitative-quantitative monitoring of microplastics in biota in the Pescara area

Below the results of analysis for each analyzed sample are reported in detail.

Spring/Summer 2020	**ID sample 556 (June 2020)**
Number of mussels tested	556
Average maximum shell length (cm)	5,17
Average soft tissue (gr)	3,51
Number of mussels contaminated with MPs	23
MPs frequency of occurrence (%)	46
Number of microplastics detected for sampling	37
Prevaling type of MPs	Fiber (73%)
Prevaling size range of MPs	> 500 µm (54%)
MPs mean size (µm)	1959,39
Minimum particle size (µm)	25
Prevaling color of MPs	Black (78%)
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	0,74± 1,07
b) Average n° microplastics/ g of soft tissue	0,20± 0,26

Autumn/Winter 2020	ID sample 1502 (December 2020)
Number of mussels tested	50
Average maximum shell length (cm)	5,75
Average soft tissue (gr)	6,56
Number of mussels contaminated with MPs	25
MPs frequency of occurrence (%)	50
Number of microplastics detected for sampling	38
Prevaling type of MPs	Fiber (92%)
Prevaling size range of MPs	> 500 µm (74%)
MPs mean size (µm)	1417,368
Minimum particle size (µm)	65
Prevaling color of MPs	Black (60,5%)
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	0,70± 1,07
b) Average n° microplastics/ g of soft tissue	0,12± 0,16

Spring/Summer 2021	ID sample 698 (May 2021)
Number of mussels tested	50
Average maximum shell length (cm)	5,94
Average soft tissue (gr)	6,20
Number of mussels contaminated with MPs	32
MPs frequency of occurrence (%)	64
Number of microplastics detected for sampling	84
Prevaling type of MPs	Fiber (80%)
Prevaling size range of MPs	> 500 µm (55%)
MPs mean size (µm)	859,25
Minimum particle size (µm)	68
Prevaling color of MPs	Black (69%)
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	1,68± 1,71
b) Average n° microplastics/ g of soft tissue	0,33± 0,40

Autumn/Winter 2021	ID sample 1742 (October 2021)
Number of mussels tested	50
Average maximum shell length (cm)	5,22
Average soft tissue (gr)	3,63
Number of mussels contaminated with MPs	17
MPs frequency of occurrence (%)	34
Number of microplastics detected for sampling	30
Prevaling type of MPs	Fiber (80%)
Prevaling size range of MPs	> 500 µm (73%)
MPs mean size (µm)	1338,2667
Minimum particle size (µm)	34
Prevaling color of MPs	Black (60%)
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	0,60± 1,03
b) Average n° microplastics/ g of soft tissue	0,16± 0,27

Spring/Summer 2022	ID sample 411 (May 2022)
Number of mussels tested	50
Average maximum shell length (cm)	6,54
Average soft tissue (gr)	7,40
Number of mussels contaminated with MPs	15
MPs frequency of occurrence (%)	30
Number of microplastics detected for sampling	21
Prevaling type of MPs	Fibers (71%)
Prevaling size range of MPs	> 500 µm (67%)
MPs mean size (µm)	1067
Minimum particle size (µm)	54
Prevaling color of MPs	Blue (62%).
Abundance of MPs *(mean value ± standard deviation)*	
a) Average n° microplastics/organism	0,42±0,78
b) Average n° microplastics/ g of soft tissue	0,06±0,12

3.3 Results of chemical contaminants' monitoring in biota in Goro Sacca area

Below the results of analysis for each analyzed sample are reported in detail.
Furthermore the maximum levels of chemical contaminants allowed by current legislation are shown below for ease of reading.

Maximum levels in *Bivalve molluscs*	
Metals	
Lead	1,5mg/kg
Cadmium	1,0 mg/kg
Mercury	0,50 mg/kg
Dioxins and PCBs	
Sum of dioxins	3,5 pg-TEQ/g
Sum of dioxins and dioxin-like PCBs	6,5 pg-TEQ/g
Sum of non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180)	75 ng/g
Polycyclic aromatic hydrocarbons	
Summ of Benzo(a)pyrene, Benzo(a)anthracene, Benzo(a)fluoranthene and Chrysene	30 µg/Kg

Autumn/Winter 2019	ID sample 1640 (December 2019)	ID sample 1642 (December 2019)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	2	2,2
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,104 ± 0,017	0,058 ± 0,010
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,305 ± 0,050	0,178 ± 0,029
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	5,02 ± 0,66	2,78 ± 0,37
Metals		
Mercury (mg/kg)	0,04	Not quantifiable (< 0,025)
Lead (mg/kg)	0,58	0,25
Cadmium (mg/kg)	0,26	0,1

Spring/Summer 2020	ID sample 611 (June 2020)	ID sample 615 (June 2020)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	Not detectable	Not detectable
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,111 ± 0,024	0,071 ± 0,016
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,706 ± 0,120	0,573 ± 0,096
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	6,48 ± 0,92	6,50 ± 0,89
Metals		
Mercury (mg/kg)	Not quantifiable (<0,020)	Not quantifiable (< 0,020)
Lead (mg/kg)	0,12	0,12
Cadmium (mg/kg)	0,062	0,07

Autumn/Winter 2020	ID sample 1350 (November 2020)	ID sample 1351 (November 2020)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	Not detectable	Not detectable
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,044 ± 0,007	0,051 ± 0,008
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,315 ± 0,051	0,298 ± 0,048
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	4,35 ± 0,61	4,28 ± 0,57
Metals		
Mercury (mg/kg)	Not quantifiable (< 0,010)	Not quantifiable (< 0,033)
Lead (mg/kg)	0,098	0,096
Cadmium (mg/kg)	0,048	0,045

Spring/Summer 2021	ID sample 622 (May 2021)	ID sample 624 (May 2021)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	Not detectable	Not detectable
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,055 ± 0,009	0,077 ± 0,013
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,419 ± 0,067	0,403 ± 0,065
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	5,78 ± 0,76	5,09 ± 0,68
Metals		
Mercury (mg/kg)	Not quantifiable (<0.024)	Not quantifiable (<0.024)
Lead (mg/kg)	0,26	0,18
Cadmium (mg/kg)	0,12	0,093

Autumn/Winter 2021	ID sample 1930 (December 2021)	ID sample 1934 (December 2021)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	1,5	1,6
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,112 ± 0,019	0,136 ± 0,023
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,271 ± 0,044	0,290 ± 0,047
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	3,60 ± 0,52	3,48 ± 0,49
Metals		
Mercury (mg/kg)	0,013	0,11
Lead (mg/kg)	0,26	0,20
Cadmium (mg/kg)	0,081	0,058

Spring/Summer 2022	ID sample 380 (May 2022)	ID sample 382 (May 2022)
Polycyclic aromatic hydrocarbons		
IPA (µg/kg)	0,6	Not detectable
Dioxins and PCBs		
Dioxins (pg-TEQ/g)	0,083 ± 0,014	0,099 ± 0,017
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,457 ± 0,074	0,414 ± 0,067
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	5,55 ± 0,73	5,34 ± 0,71
Metals		
Mercury (mg/kg)	0,0090	0,0088
Lead (mg/kg)	0,15	0,17
Cadmium (mg/kg)	0,048	0,050

3.4 Results of chemical contaminants' monitoring in biota in the Pescara area

Below the results of analysis for each analyzed sample are reported in detail.

Spring/Summer 2020	**ID sample 555 (June 2020)**
Polycyclic aromatic hydrocarbons	
IPA (µg/kg)	Not detectable
Dioxins and PCBs	
Dioxins (pg-TEQ/g)	0,022 ± 0,004
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,205 ± 0,033
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,47 ± 0,21
Metals	
Mercury (mg/kg)	Not quantifiable (<0,022)
Lead (mg/kg)	0,13
Cadmium (mg/kg)	0,1

Autumn/Winter 2020	ID sample 1501 (December 2020)
Polycyclic aromatic hydrocarbons	
IPA (µg/kg)	Not detectable
Dioxins and PCBs	
Dioxins (pg-TEQ/g)	0,068 ± 0,011
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,220 ± 0,036
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,69 ± 0,22
Metals	
Mercury (mg/kg)	Not quantifiable (<0,033)
Lead (mg/kg)	0,14
Cadmium (mg/kg)	0,054

Spring/Summer 2021	ID sample 699 (May 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/kg)	Not detectable
Dioxins and PCBs	
Dioxins (pg-TEQ/g)	0,014 ± 0,002
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,176 ± 0,028
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,93 ± 0,28
Metals	
Mercury (mg/kg)	Not quantifiable (<0,016)
Lead (mg/kg)	0,22
Cadmium (mg/kg)	0,10

Autumn/Winter 2021	ID sample 1743 (October 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/kg)	Not detectable
Dioxins and PCBs	
Dioxins (pg-TEQ/g)	0,049 ± 0,008
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,187 ± 0,030
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,16 ± 0,16
Metals	
Mercury (mg/kg)	0,010
Lead (mg/kg)	0,14
Cadmium (mg/kg)	0,13

Spring/Summer 2022	ID sample 412 (May 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/kg)	Not detectable
Dioxins and PCBs	
Dioxins (pg-TEQ/g)	0,053 ± 0,009
Dioxins and dioxin-like PCBs (pg-TEQ/g)	0,262 ± 0,042
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,63 ± 0,23
Metals	
Mercury (mg/kg)	0,0096
Lead (mg/kg)	0,057
Cadmium (mg/kg)	0,057

3.5 Results of chemical contaminants' monitoring in biota in Croatia area

Our unit also performed analyzes for chemical contaminants on biota samples taken from Croatian sites.
Below is the table of the samples carried out and the results.

ID sample	Sampling date	Country - Macroarea	Habitat (natural bank /mussel farm)
979	27/05/2021	Croatia-site B	Mussel farm- site B
980	27/05/2021	Croatia-site B	Natural bank-site B
1027	07/07/2021	Croatia-island of Brac Maslinova	Mussel farm- site A
1028	07/07/2021	Croatia-site A	Natural bank-site A
76	19/01/2022	Croatia-site A	Natural bank-site A
77	19/01/2022	Croatia-site A	Mussel farm-site A
96	20/01/2022	Croatia-site B	Natural bank -site B
97	20/01/2022	Croatia-site B	Mussel farm-site B

ID sample	Sampling date	Country - Macroarea	Habitat (natural bank /mussel farm)
416	30/09/2021	Croatia - Lopar	Mussel farm
417	02/02/2022	Croatia - Lopar	Mussel farm
418	09/05/2022	Croatia - Bakar	Mussel farm
419	03/05/2022	Croatia - Smorvic	Mussel farm
479	03/02/2022	Croatia - Stinica	Mussel farm
480	08/02/2022	Croatia - Bakar	Mussel farm

Spring/Summer 2021	ID sample 979 (May 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not rivel.
Dioxins and PCBs	
Dioxins (pg/g)	0,029 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,176 ± 0,028
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,57 ± 0,09

Spring/Summer 2021	ID sample 980 (May 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not rivel.
Dioxins and PCBs	
Dioxins (pg/g)	0,028 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,140 ± 0,023
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,58 ± 0,09

Spring/Summer 2021	ID sample 1027 (July 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not rivel. (<0,31)
Dioxins and PCBs	
Dioxins (pg/g)	0,014 ± 0,002
Dioxins and dioxin-like PCBs (pg/g)	0,333 ± 0,054
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,93 ± 0,14

Spring/Summer 2021	ID sample 1028 (July 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not quantifiable (<0,51)
Dioxins and PCBs	
Dioxins (pg/g)	0,033 ± 0,006
Dioxins and dioxin-like PCBs (pg/g)	0,677 ± 0,109
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,85 ± 0,28

Autumn/Winter 2022	ID sample 76 (January 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	2,4
Dioxins and PCBs	
Dioxins (pg/g)	0,077 ± 0,013
Dioxins and dioxin-like PCBs (pg/g)	0,404 ± 0,065
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	1,40 ± 0,23
Metals	
Mercury (mg/Kg)	0,013
Lead (mg/Kg)	0,10
Cadmium (mg/Kg)	0,10

Autumn/Winter 2022	ID sample 77 (January 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	0,7
Dioxins and PCBs	
Dioxins (pg/g)	0,032 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,091 ± 0,015
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,33 ± 0,05
Metals	
Mercury (mg/Kg)	0,010
Lead (mg/Kg)	0,11
Cadmium (mg/Kg)	0,081

Autumn/Winter 2022	ID sample 96 (January 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	2,4
Dioxins and PCBs	
Dioxins (pg/g)	0,063 ± 0,011
Dioxins and dioxin-like PCBs (pg/g)	0,117 ± 0,019
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,28 ± 0,04
Metals	
Mercury (mg/Kg)	0,015
Lead (mg/Kg)	0,085
Cadmium (mg/Kg)	0,093

Autumn/Winter 2022	ID sample 97 (January 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	0,6
Dioxins and PCBs	
Dioxins (pg/g)	0,032 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,078 ± 0,013
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,37 ± 0,06
Metals	
Mercury (mg/Kg)	0,015
Lead (mg/Kg)	0,12
Cadmium (mg/Kg)	0,085

Autumn/Winter 2022	ID sample 416 (September 2021)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not rivel.
Dioxins and PCBs	
Dioxins (pg/g)	0,029 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,106 ± 0,017
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,49 ± 0,05
Metals	
Mercury (mg/Kg)	0,019
Lead (mg/Kg)	0,058
Cadmium (mg/Kg)	0,055

Autumn/Winter 2022	ID sample 417 (February 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	1,6
Dioxins and PCBs	
Dioxins (pg/g)	0,079 ± 0,013
Dioxins and dioxin-like PCBs (pg/g)	0,186 ± 0,030
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,84 ± 0,11
Metals	
Mercury (mg/Kg)	0,019
Lead (mg/Kg)	0,16
Cadmium (mg/Kg)	0,14

Autumn/Winter 2022	ID sample 418 (May 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	4,9
Dioxins and PCBs	
Dioxins (pg/g)	0,126 ± 0,021
Dioxins and dioxin-like PCBs (pg/g)	0,670 ± 0,109
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	6,88 ± 0,78
Metals	
Mercury (mg/Kg)	Not quant (<0,018)
Lead (mg/Kg)	0,22
Cadmium (mg/Kg)	0,032

Autumn/Winter 2022	ID sample 419 (May 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	Not rilev
Dioxins and PCBs	
Dioxins (pg/g)	0,067 ± 0,011
Dioxins and dioxin-like PCBs (pg/g)	0,384 ± 0,062
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	3,47 ± 0,40
Metals	
Mercury (mg/Kg)	0,046
Lead (mg/Kg)	0,12
Cadmium (mg/Kg)	0,11

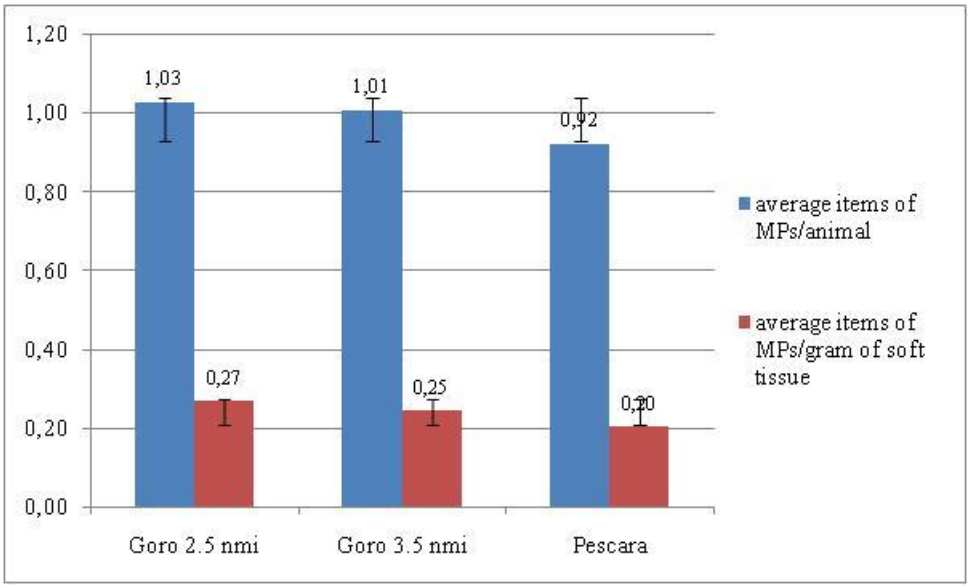
Autumn/Winter 2022	ID sample 479 (February 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	1,5
Dioxins and PCBs	
Dioxins (pg/g)	0,030 ± 0,005
Dioxins and dioxin-like PCBs (pg/g)	0,070 ± 0,012
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	0,25 ± 0,03
Metals	
Mercury (mg/Kg)	0,024
Lead (mg/Kg)	0,14
Cadmium (mg/Kg)	0,11

Autumn/Winter 2022	ID sample 480 (February 2022)
Polycyclic aromatic hydrocarbons	
IPA (µg/Kg)	36,2
Dioxins and PCBs	
Dioxins (pg/g)	0,490 ± 0,082
Dioxins and dioxin-like PCBs (pg/g)	1,199 ± 0,196
Non-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) (ng/g)	13,40 ± 1,72
Metals	
Mercury (mg/Kg)	0,26
Lead (mg/Kg)	3,3
Cadmium (mg/Kg)	1,0

4 Discussion and Conclusion

4.1 Qualitative-quantitative monitoring of microplastics in biota

Several authors reported the intake of MPs by mussels (Browne et al., 2008; Renzi et al., 2018; Santana et al., 2016; Vandermeersch et al., 2015). In this study, the organism of choice to evaluate risks associated to MPs in marine habitats was the mussel because they can absorb MPs due to their filter-feeding behavior (Wesch et al., 2016) and efficient water purification capacity (Xu et al., 2017). Therefore, the present study has had the following aims: (a) increase knowledge related to levels and principal features (type, size, colour) of MPs in mussels (Mytilus galloprovincialis) from mussel farming; (b) comparing MPs levels and different features in mussel farming from two different Italian sites (Goro Sacca and Pescara area).

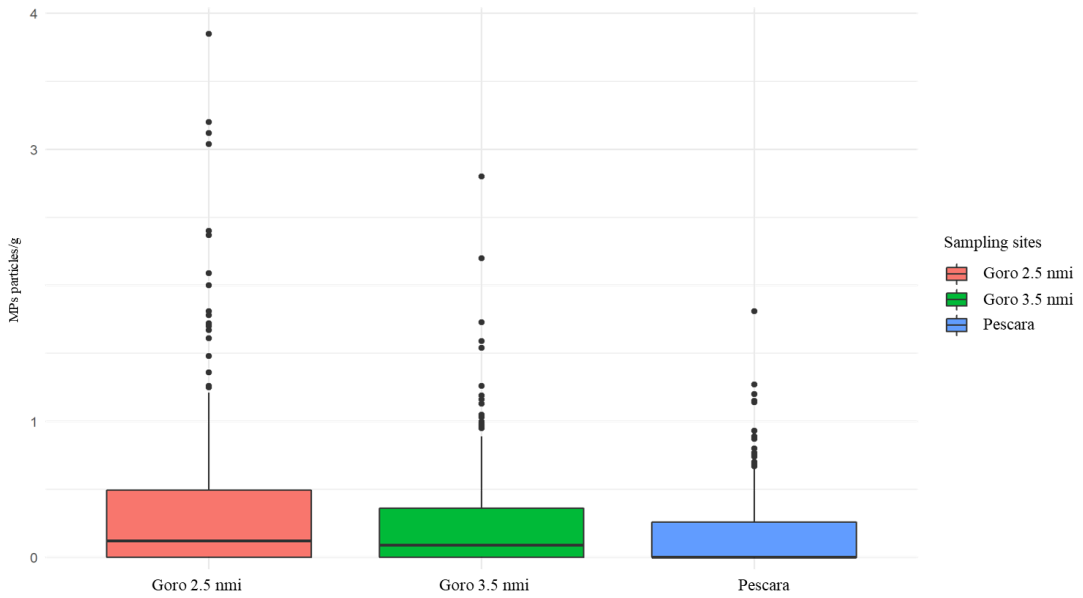


Graph. 1. Average values (with standard deviation) of microplastics recovered in mussels from the three sampling sites. Data are reported both as average items per animal and as average items per gram of soft tissue.

In Graph. 1, average (with standard deviation) items of MPs recovered per animals in each sampling sites are reported. The three mussels' sampling sites show average values of the microplastics found very similar.

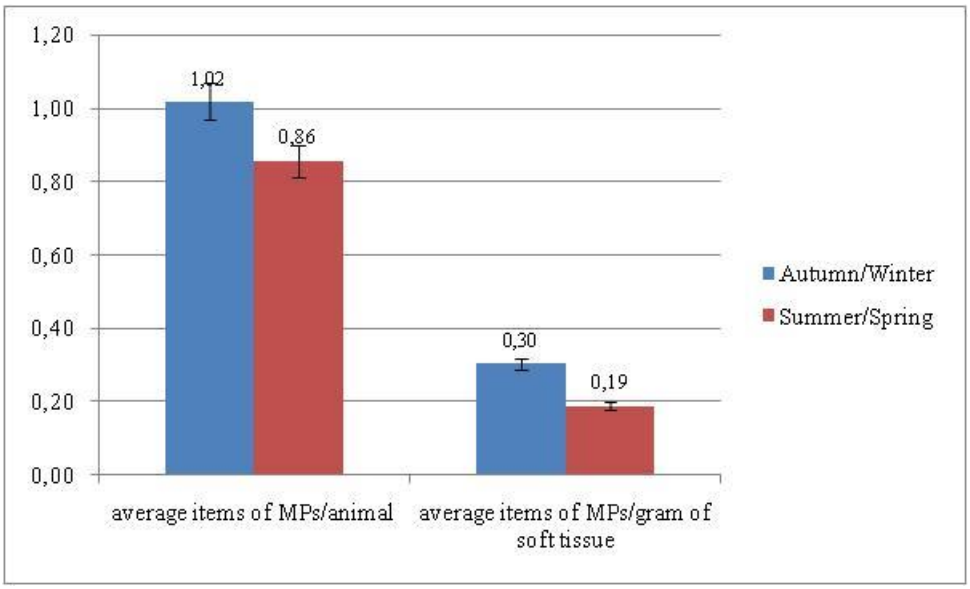
To standardize collected data, in Graph. 1 and are also shown average (with standard deviation) items of MPs recovered per gram of soft tissue in each sampling sites are reported. Significant differences (p. value 0,004) are recorded between Goro 2.5 nmi (0.27 average MPs particles/g) and Pescara (0.20 average MPs particles/g). Instead, no significant difference (Graph 2) is among couples Goro 2.5 nmi-Goro 3.5 nmi (p. value 0,467) and Goro 3.5 nmi-

Pescara (p. value 7,86E-02).



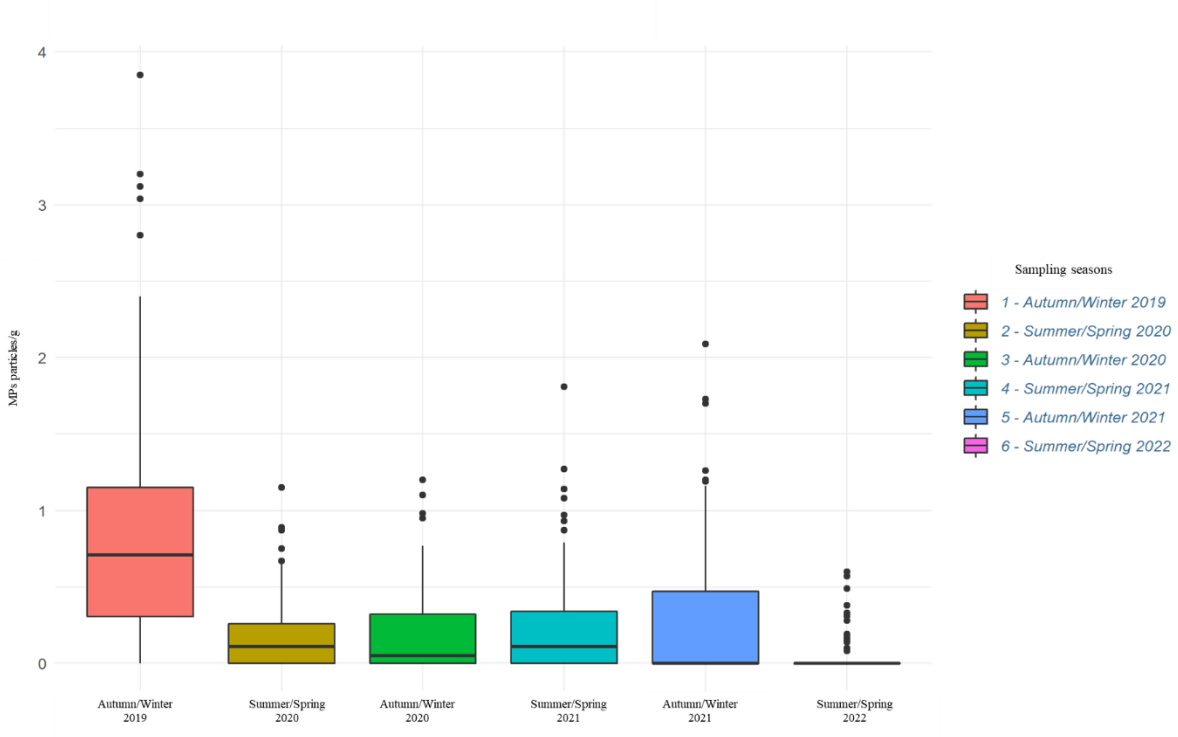
Graph. 2. Distribution of MPs particles/g of mussel soft tissue with respect to sampling sites.

In general, MPs particles found in mussels from Goro Sacca and Pescara are in line with other scientific studies (De Witte et al., 2014: 0.35/g of wet tissue (*M.edulis* farmed); Van Cauwenberghe and Janssen, 2014: 0.36/g of wet tissue (*M.edulis* farmed); Van Cauwenberghe et al., 2015: 0.2/g of wet tissue (*M.edulis* wild); Phuong et al., 2018: 0.23/g of wet tissue (*M.edulis*).



Graph. 3. Average values (with standard deviation) of microplastics recovered in mussels compared to the two sampling seasons (Autumn/Winter - Summer/Spring). Data are reported both as average items per animal and as average items per gram of soft tissue.

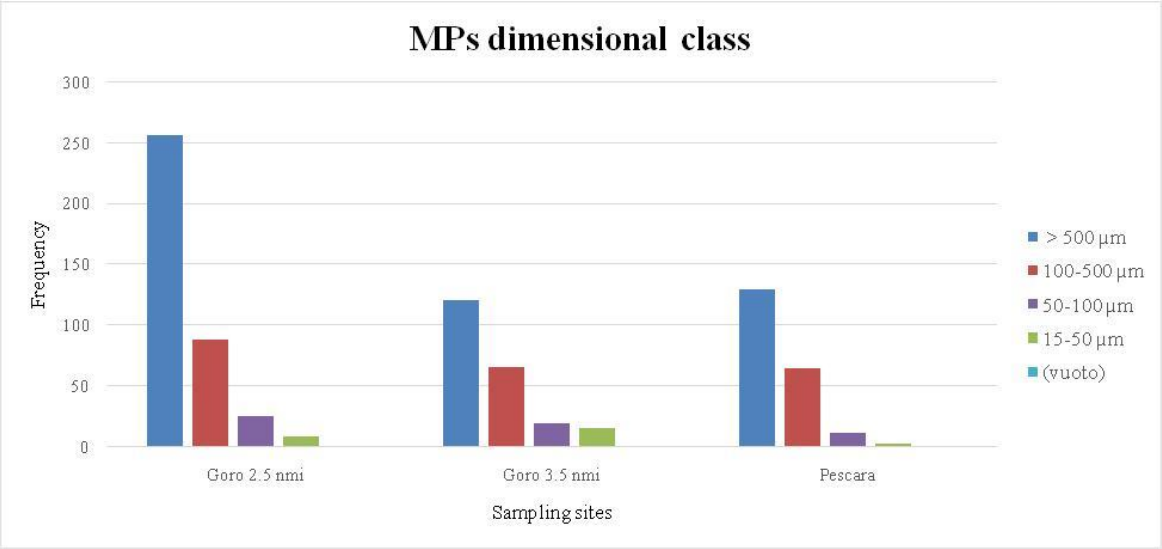
In Graph. 3, average (with standard deviation) items of MPs recovered per animals and per gram of soft tissue in the two seasons' samplings are reported.



Graph. 4. Distribution of MPs particles/g of mussel soft tissue with respect to sampling seasons.

There's not significant difference between the two season sampling periods (Autumn/Winter vs Summer/Spring) (Graph. 3-4). This could lead to suppose that the presence of MPs inside the bivalves is not strictly correlated with the meteorological conditions of the sampling site (bibliographic).

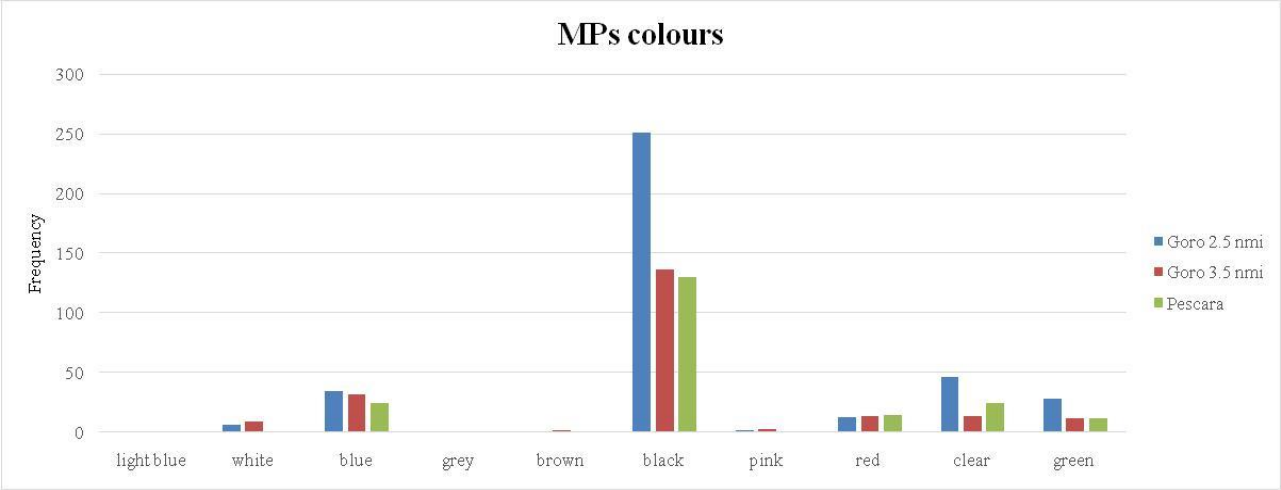
In all tested sites (Graph. 5) the prevailing size class is > 500 µm (Goro 2.5 mm 67.5%; Goro 3.5 mm 54%; Pescara 62%), followed by 100-500 µm (Goro 2.5 mm 23.5%; Goro 3.5 nmi 29.5%; Pescara 31%). The chi-square test performed on the collected data found that these differences are statistically significant (p. value 0.0012).



Graph. 5. Graphical representation of MPs' size class' detection frequencies for each sampling site.

Black is the colour prevailing in all sites (Graph.6) (Goro 2.5 mmi 93%; Goro 3.5 nmi95%; Pescara 94%), followed by blue, clear and green; while red, white, grey, brown, pink coloured microplastics were only occasionally recorded.

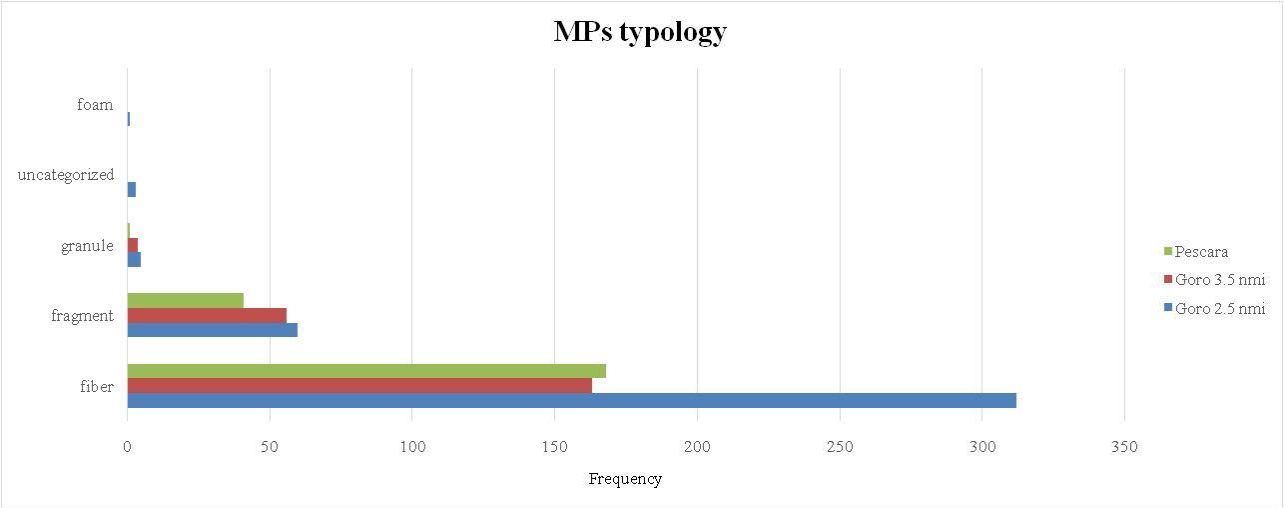
The chi-square test performed on the collected data found that these differences are statistically significant (p. value 0.014).



Graph. 6. Graphical representation of MPs' colours detection frequencies for each sampling site.

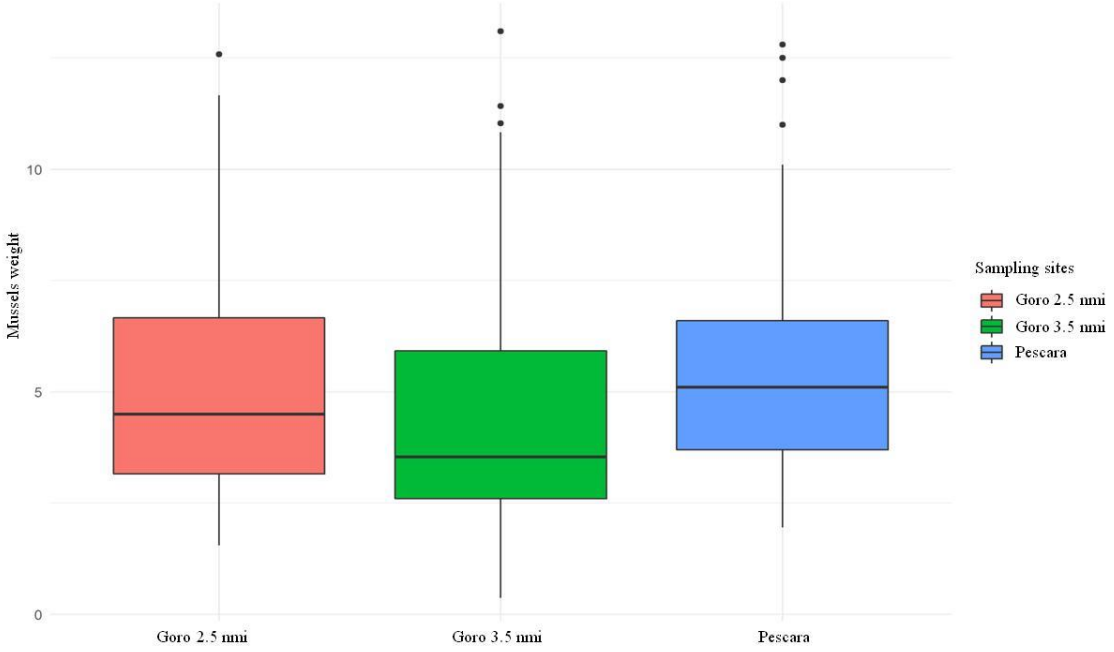
In all sampling sites the fibers (Graph. 7) (Goro 2.5 mm 77%; Goro 3.5 nmi 73%; Pescara 79%) were the type of microplastics most present, followed by fragments (Goro 2.5 mm 14%; Goro 3.5 nmi 25%; Pescara 19%).

Fibers are the only MPs type recorded in tested stocks and this could be probably due to plastics's geometry that allow them to better reach the considered species. Furthermore, fibers get trapped into gills and hepatopancreas and cannot be easily removed by animals accumulating into them (Renzi et al., 2018).

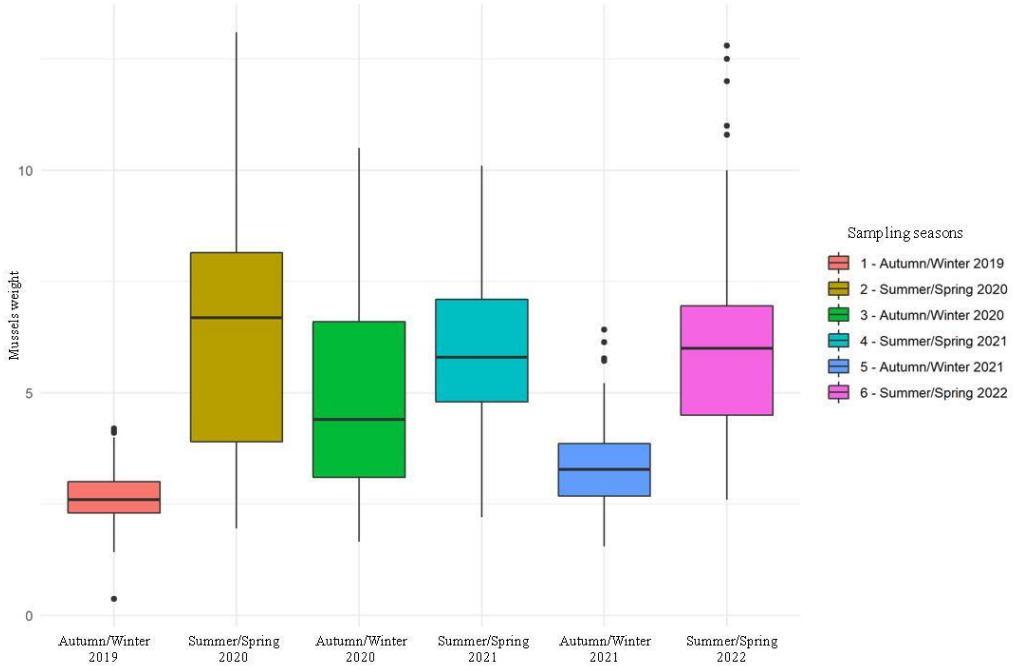


Graph. 7. Graphical representation of MPs' type detection frequencies for each sampling site.

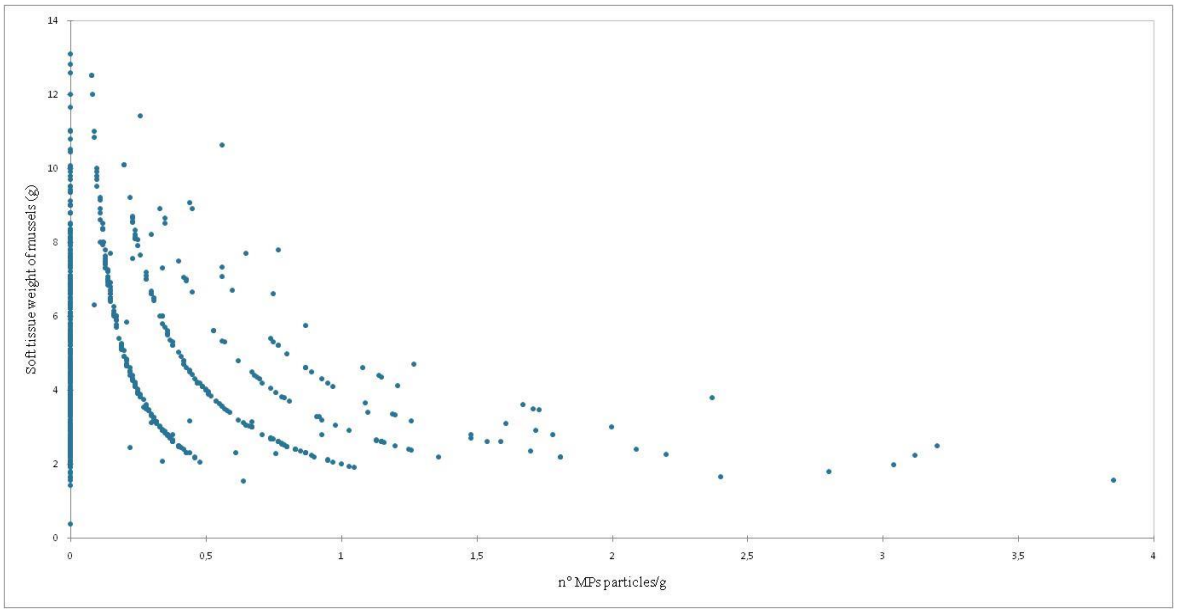
Regarding the variable "mussels' weight" (Graph. 8-9), statistical differences were detected between sampling sites, specifically between Goro 2.5 and 3.5 nmi (p.value 0.001) and between Pescara and Goro 3.5 nmi (p.value 1.16E-07). There was also a significant difference in sampling seasons (Autumn/Winter vs Summer/Spring) (p.value 3.33E-04), different mussel weight is clearly due to gametes' emission during autumn season.



Graph. 8. Weight distribution of sampled mussels with respect to sampling sites.



Graph. 9. Weight distribution of sampled mussels with respect to sampling seasons.



Graph. 10. Graphical representation of trend of n. particles MPs/g in sof tissue of mussels sampled and analysed.

There appears to be an inversely proportional correlation (Spearman's rho -0.257; p. Value

<0.0001) between the soft tissue weight of the analyzed mussels and the number of MPs found per gram of soft tissue (Graph. 10), i.e. when to increase the soft tissue weight of mussels, the number of MPs/g decreases. This result could lead to the hypothesis that mussels with a greater weight filter higher quantities of water and therefore are able to eliminate any microplastic particles accumulated in their tissues more quickly. This data is in contrast with our own purification experiments where no significant correlation was highlighted between the two variables (mussels' soft tissue and MPs/g), confirming the fact that the ability to accumulate microplastic particles inside the bivalve, as well as the ability to eliminate them, is not strictly correlated with the size of the organism itself, as also reported in other experiments (Birnstiel et al., 2019).

In conclusion our results suggest that MPs contamination is widespread among mussels in Italian Adriatic coast. Our work provides further data concerning microplastics' monitoring in marine-coastal waters using mussels; these bivalves are useful bioindicators of the 'health' of coastal ecosystems for their characteristics such as extensive filter feeding activity, broad geographical distribution, limited movement capacity and good tolerance to environmental pollution.

4.2 Chemical contaminants' monitoring in biota

In the present study, samples of *Mytilus galloprovincialis*, from the Adriatic Sea, were collected from the autumn/winter 2019 to spring/summer 2022 and analyzed for organic contaminants, such as PCDD/Fs, DL-PCBs, NDL-PCBs and PAH, and inorganic contaminants, including Mercury, Lead and Cadmium. All contamination levels were below the maximum limits reported in the Commission Regulation (EC) N. 1881/2006.

Considering chlorinated compounds, levels ranged from 0.014 to 0.14 pg-TEQ/g for dioxins, from 0.078 to 0.71 pg-TEQ/g for dioxins and dioxin-like PCBs and from 0.28 to 6.5 ng/g for sum of non dioxin-like PCBs.

Regarding toxic metals, levels were comprised between 0.085 and 0.58 mg/kg for lead, 0.045 and 0.26 for cadmium, while for mercury contamination levels were below the limit of quantification in a large part of samples and the maximum detected levels was 0.11 mg/kg.

For the sum of four PAHs, concentrations ranged from the quantification limit to 2.4 µg/kg. Among the four analysed PAHs, chrysene was the most detected followed by benzo[b]fluoranthene.

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