NET4mPLASTIC PROJECT

WP4 – Act. 2 Lab's analysis on plastic and microplastic wastes on coastal and marine environments

D 4.2.4 – Annex 1

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

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1 Introduction

This document is the annex of the Deliverable D4.2.4 MP contamination and correlation between the presence of PCBs/Dioxins/PAHs and MPs in bivalves. This document contains part of the results of the activity 4.2: Laboratory analysis on plastic and microplastic wastes on coastal and marine environments. The results of the chemical analysis of mussels and the number of MPs found in mussels are presented here.

The data presented here refer to the pilot sites selected by the NET4mPLASTIC project (for further information regarding the pilot site, please see the Deliverable 4.1.1):

1. CROATIA - Rijeka area;
2. CROATIA - Split area.

2 TIPH – Results of analysis of mussels from Rijeka and Split area

The TIPH-team performed some sampling campaigns for biota marine litter and MP data collection, in different sites close to Rijeka, 1 time in 2021 and 2 times in 2022. on 4 different locations. All the samples were collected on natural banks, since in Rijeka area there are no mussel farms. These mussels are not intended for human consumption and are collected near the populated sites. The RERA – IOF team collected samples of biota 2 times in 2021 and 2 times in 2022 in 2 different locations. The samples were collected on natural banks and farms in the same locations.

Chemical analysis of samples of mussels from Rijeka and Split area

Concentrations of PAHs, PCBs, heavy metals and dioxins and number of microplastic particles were analysed in the samples of mussels from Rijeka and Split area. Description of locations and dates of sampling are part of the Deliverable 4.1.1 and 4.1.2. Both laboratory of IZSAM and TIPH performed chemical analysis of mussels on PAHs, PCBs and heavy metals. Similar results were obtained by both laboratories. Determination of dioxins and dioxin-like PCBs in all the samples was performed by IZSAM only.

Methods used for the determination of the concentration of PAHs, PCBs and metals (except for the Mercury) in the laboratory of TIPH are standardised methods. For the determination of Mercury an adapted method according to the producer manual was used. The methods are listed in the Table 1. For the determination of number of microplastics in mussels a standard method was used.

Table 1. Methods for the determination of the concentration of PAHs, PCBs and heavy metals used by the laboratory of TIPH.

Indicators	Methods
Metals: Pb, As, Cd	HRN EN 14084:2005 (EN 14084:2003), HRN EN 13804:2013 (EN 13804:2013), Determination of lead, cadmium, zink, copper and iron by atomic absorption spectrometry (AAS) after microwave digestion (EN 14084:2003). This European standard specifies a method for the determination of Pb, Cd, Zn, Cu and Fe in foodstuffs by atomic absorption spectrometry (AAS) after microwave digestion. This method describes determination in various types of foods, with the exception of oils, fats and other extremely fatty products. The method was modified
Metal: Hg	Determination of mercury by Mercury analyser AMA 254, Adapted method according to the producer manual: AMA 254 Advanced Mercury analyser Operation manual, 2002
Determination of selected polycyclic aromatic hydrocarbons (PAH)	Modified HRN EN ISO 17993:2008, Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction (ISO 17993:2002; EN ISO 17993:2003). ISO 17993:2002 specifies a method using high performance liquid chromatography (HPLC) with fluorescence detection for the determination of 15 selected polycyclic aromatic hydrocarbons (PAH) [naphthalene, acenaphthene, phenanthrene, fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, fluorene, anthracene, pyrene, chrysene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene] in drinking and ground.
Gas chromatographic determination of selected polychlorinated byphenyls (PCBs:PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180, PCB 105, PCB 118, PCB 156, PCB 170)	Modified HRN EN ISO 6468:2002, Determination of certain organochlorine insecticides polychlorinated biphenyles and chlorobenzenes -- Gas chromatographic method after liquid-liquid extraction (ISO 6468:1996; EN ISO 6468:1996). Describes a method for determining certain organochlorine insecticides, polychlorinated biphenyls (PCBs) and chlorobenzenes (except the mono- and dichlorobenzenes) in drinking water, ground water, surface waters and waste waters. The method is applicable to samples containing up to 0,05 g/l of suspended solids. Modified method.

Determination of the concentration of PAHs in the samples of mussels from Rijeka and Split area

Determination of the concentration of PAHs was performed in all the samples from Rijeka and Split area. 2g of dry soft tissue of mussels was used for the analysis. The samples were prepared and analysed according to the method HRN EN ISO 17993:2008. The obtained values of PAHs from the samples of mussels from all the locations previously described were low or not detectable (values were lower than LOQ) (Table 2 and Table 3). Similar results were obtained by the laboratory of IZSAM (See the tables in the attachment to this document.). For quality assessment of mussels intended for consumption EU Commission Regulation (EC) No 1881/2006 of 19 December 2006 is used in Croatia.

Table 2. Concentration of PAHs in the samples of mussels from Rijeka area (samples analised by the laboratory of TIPH).

Sample	Lopar	Lopar	Bakar	Bakar	Stinica	Smokvica
Date of sampling	24.09.2021.	02.02.2022.	08.02.2022.	04.05.2022.	03.02.2022.	03.05.2022.
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
PAHs						
Naphthalene	5,56	1,26	3,92	4,51	0,91	3,08
Acenaphthene	2,77	1,43	2,11	2,17	0,37	1,81
Fluorene	3,53	2,07	1,02	3,59	0,67	2,95
Phenanthrene	23,4	16,7	12,4	24,6	4,83	22,3
Anthracene	<0,1	<0,1	<0,1	0,15	<0,1	0,18
Fluoranthene	5,85	2,07	0,66	7,44	1,33	6,04
Pyrene	6,62	4,36	3,35	7,04	0,54	4,85
Benzo(a)anthracene	3,58	2,05	0,41	0,75	0,38	1,55
Chrysene	1,17	<0,04	<0,04	<0,04	<0,04	1,22
Benzo(b)fluoranthene	4,46	3,37	0,60	0,79	<0,1	3,63
Benzo(k)fluoranthene	<0,1	0,18	<0,1	<0,1	<0,1	<0,1
Benzo(a)pyrene	<0,1	0,19	0,26	0,25	<0,1	0,26
Dibenzo(a,h)anthracene	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Benzo(g,h,i) perylene	0,35	<0,1	0,12	<0,1	<0,1	<0,1
Indeno(1,2,3cd)pyrene	0,13	<0,1	<0,1	0,34	<0,1	<0,1
Acenaphthylene	<2	<2	<2	<2	<2	<2
TOTAL	57,42	33,68	24,85	51,63	9,03	47,87

Table 3. Concentration of PAHs in the samples of mussels from Split area (samples analised by the laboratory of TIPH).

Sample	Site A – mussel farm	Site A – natural bank	Site B – mussel farm	Site B – natural bank
Date of sampling	19.1.2022.	19.1.2022.	19.1.2022.	19.1.2022.
	µg/kg	µg/kg	µg/kg	µg/kg
PAHs				
Naphthalene	3,17	3,66	4,30	2,68
Acenaphthene	1,75	1,44	2,29	1,63
Fluorene	3,01	2,18	4,27	2,25
Phenanthrene	22,8	21,6	35,4	18,5
Anthracene	<0,1	<0,1	<0,1	<0,1
Fluoranthene	2,40	2,40	4,45	3,85
Pyrene	2,73	9,15	7,84	3,40
Benzo(a)anthracene	1,27	0,74	1,04	1,46
Chrysene	<0,04	<0,04	<0,04	1,34
Benzo(b)fluoranthene	0,73	<0,1	<0,1	<0,1
Benzo(k)fluoranthene	<0,1	<0,1	<0,1	<0,1
Benzo(a)pyrene	0,11	0,15	0,24	0,59
Dibenzo(a,h)anthracene	<0,1	<0,1	<0,1	<0,1
Benzo(g,h,i) perylene	<0,1	<0,1	<0,1	0,30
Indeno(1,2,3cd)pyrene	<0,1	<0,1	<0,1	<0,1
Acenaphthylene	<2	<2	<2	<2
TOTAL	37,97	41,32	59,83	36,00

Determination of the concentration of PCBs and dioxins in the samples of mussels

Determination of the concentration of PCBs, dioxins dioxin-like PCBs was performed in all the samples of mussels from Rijeka and Split area. 2g of dry soft tissue of mussels was used for the analysis of PCBs. The samples were prepared and analyzed according to the modified method HRN EN ISO 6468:2002. The obtained values of PCBs from the samples of mussels from all the locations previously described were low or not detectable (values were lower than LOQ) (Table 4 and Table 5). Similar results were obtained by the laboratory of IZSAM (See the tables in the attachment to this document.). Dioxins and dioxin – like PCBs' concentrations were also very low (See the tables in the attachment to this document.). For quality

assessment of mussels intended for consumption EU Commission Regulation (EC) No 1881/2006 of 19 December 2006 is used in Croatia. Dioxins and dioxin-like PCBs are not included in the legislative.

Table 4. Concentration of PCBs in the samples of mussels from Rijeka area (samples analised by the laboratory of TIPH).

PCB	LOQ	Lopar	Lopar	Bakar	Bakar	Stinica	Smokvica
Date of sampling		24.09.2021.	02.02.2022.	08.02.2022.	04.05.2022.	03.02.2022.	03.05.2022.
PCB 28 2,4,4´-triklorobifenil	1,0	< LOQ	< LOQ	< LOQ	2,2	< LOQ	< LOQ
PCB 33 2´,3,4-triklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 53 2,2´,5,6´-tetraklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 52 2,2´,5,5´-tetraklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 101 2,2´,4,5,5´-pentaklorobifenil	1,0	2,6	< LOQ	3,4	4	< LOQ	< LOQ
PCB 81 3,4,4´,5-tetraklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 77 3,3´,4,4´-tetraklorobifenil	1,0	1,0	1,6	1,9	2,2	4,2	1,1
PCB 110 2,3,3´,4´,6-pentaklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 123 2´,3,4,4´,5-pentaklorobifenil	1,0	< LOQ	< LOQ	2,7	< LOQ	< LOQ	< LOQ
PCB 149 2,2´,3,4´,5´,6-heksaklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 118 2,3´,4,4´,5-pentaklorobifenil	1,0	1,1	1,5	1,1	7,4	< LOQ	< LOQ
PCB 114 2,3,4,4´,5-pentaklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 153 2,2´,4,4´,5,5´-heksaklorobifenil	1,0	6,3	4,6	13,3	15,3	1,7	3,6
PCB 105 2,3,3´,4,4´-pentaklorobifenil	1,0	8,0	5,6	3,7	4,3	2,9	4,7
PCB 138 2,2´,3,4,4´,5´-heksaklorobifenil	1,0	2,9	4,2	9,5	10,9	1,4	2,4

PCB 126 3,3´,4,4´,5´-pentaklorobifenil	1,0	1,4	2,0	1,5	2,1	< LOQ	1,0
PCB 167 2,3´,4,4´,5,5´-heksaklorobifenil	1,0	2,5	3,6	1,8	1,9	< LOQ	1,5
PCB 156 2,3,3´,4,4´,5-heksaklorobifenil	1,0	< LOQ	< LOQ	1,1	1,8	< LOQ	< LOQ
PCB 157 2,3,3´,4,4´,5´-heksaklorobifenil	1,0	< LOQ	1,3	< LOQ	< LOQ	< LOQ	< LOQ
PCB 180 2,2´,3,4,4´,5,5´-heptaklorobifenil	1,0	< LOQ	< LOQ	1,8	2,1	< LOQ	< LOQ
PCB 169 3,3´,4,4´,5,5´-heksaklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 170 2,2´,3,3´,4,4´,5-heptaklorobifenil	1,0	2,1	6,3	10,7	8,5	4,6	5,3
PCB 189 2,3,3´,4,4´,5,5´-heptaklorobifenil	1,0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PCB 194 2,2´,3,3´,4,4´,5,5´-oktaklorobifenil	1,0	1,9	1,8	< LOQ	1,1	< LOQ	1,5

Table 5. Concentration of PCBs in the samples of mussels from Split area, collected in January 2022. (samples analised by the laboratory of TIPH).

PCB	Site A – mussel farm	Site A – natural bank	Site B – mussel farm	Site B – natural bank
PCB 28 2,4,4´-triklorobifenil	2,0	< LOQ	< LOQ	1,3
PCB 33 2´,3,4-triklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 53 2,2´,5,6´-tetraklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 52 2,2´,5,5´-tetraklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 101 2,2´,4,5,5´-pentaklorobifenil	< LOQ	< LOQ	< LOQ	1,1
PCB 81 3,4,4´,5-tetraklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ

PCB 77 3,3´,4,4´-tetraklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 110 2,3,3´,4´,6-pentaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 123 2´,3,4,4´,5-pentaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 149 2,2´,3,4´,5´,6-heksaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 118 2,3´,4,4´,5-pentaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 114 2,3,4,4´,5-pentaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 153 2,2´,4,4´,5,5´-heksaklorobifenil	1,3	1,9	< LOQ	1,9
PCB 105 2,3,3´,4,4´-pentaklorobifenil	9,3	5,0	1,3	7,6
PCB 138 2,2´,3,4,4´,5´-heksaklorobifenil	< LOQ	1,2	< LOQ	< LOQ
PCB 126 3,3´,4,4´,5´-pentaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 167 2,3´,4,4´,5,5´-heksaklorobifenil	< LOQ	1,5	< LOQ	< LOQ
PCB 156 2,3,3´,4,4´,5-heksaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 157 2,3,3´,4,4´,5´-heksaklorobifenil	4,9	1,0	< LOQ	< LOQ
PCB 180 2,2´,3,4,4´,5,5´-heptaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 169 3,3´,4,4´,5,5´-heksaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 170 2,2´,3,3´,4,4´,5-heptaklorobifenil	< LOQ	< LOQ	2,8	< LOQ
PCB 189 2,3,3´,4,4´,5,5´-heptaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ
PCB 194 2,2´,3,3´,4,4´,5,5´-oktaklorobifenil	< LOQ	< LOQ	< LOQ	< LOQ

Determination of the concentration of metals in the samples of mussels

Determination of the concentration of heavy metals (lead, cadmium, mercury and arsenic) was performed in all the samples from Rijeka area. 0.5 g of dry soft tissue of mussels was used for the analysis. The samples were prepared and analysed according to the modified methods HRN EN 14084:2005 (EN 14084:2003) and HRN EN 13804:2013 (EN 13804:2013). The obtained values of metals from samples of mussels from all the locations previously described were low (Table 6). The concentrations of Cd on every location and the concentration of Pb in samples of mussels from Bakar were low but out of maximum allowed limit according to EU Commission Regulation (EC) No 1881/2006. These samples are collected in populated areas, near small ports used by local people and are not intended for consumption. Similar results were obtained by the laboratory of IZSAM (See the tables in the attachment to this document).

Table 6. Concentration of heavy metals in the samples of mussels from Rijeka area (analysis preformed by laboratory of TIPH).

Location		Pb (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	As (mg/kg)
Lopar	1	0,81	1,9	0,11	14
Lopar	2	0,80	1,8	0,15	18
Bakar	3	/	2,4	0,46	19
Bakar	4	3,1	2,0	0,55	19
Stinica	5	0,66	1,9	0,35	20
Smokvica	6	0,67	1,6	0,13	15

Determination of microplastics in the mussel samples

Number of microplastics was determined in the samples of mussels collected in Rijeka and Split area. The mussels were collected on the locations described in the Deliverable 4.1.1. Determination of number of microplastics in the samples of mussels was preformed according to the methodology described in the Deliverable D4.1.1. Ten mussels of each sample were used for the analysis. Shell length and weight of soft tissue were measured. 30 % hydrogen peroxide was used for the oxidation of the soft tissue of mussel samples. Solution was then filtered through glass fiber filters. For the analysis of microplastics on the filters stereo zoom microscope was used. The average results are given in the tables below (Table 7 and Table 8). For evaluation of the quality of mussels intended for consumption according to this parameter, there are no specific guidelines.

Table 7. Average number of microplastics in the samples of mussels from Rijeka area (samples analised by the laboratory of TIPH).

Locations	Lopar (24.09.2021.)	Lopar (02.02.2022.)	Stinica (03.02.2022.)	Smokvica (03.05.2022.)
Average maximum shell lenght (cm)	5,3	5,12	5,31	7,5
Average soft tissue (g)	3,33	3,67	3,85	4,85
Average number of microplastics per organism	1,0	0,2	0,4	0,8
Average n° of microplastics per g of soft tissue	3,0	0,54	1,04	1,8

Table 8. Average number of microplastics in the samples of mussels from Split area (samples analised by the laboratory of TIPH).

Dates	06.05.21.	19.01.22.	06.05.21.	19.01.22.	06.05.21.	19.01.22.	06.05.21.	19.01.22.
Locations	Site A - farm	Site A - farm	Site A - natural bank	Site A - natural bank	Site B - farm	Site B - farm	Site B - natural bank	Site B - natural bank
Average maximum shell lenght (cm)	7,4	7,3	6,48	4,9	7,18	6,08	5,9	6,7
Average soft tissue (g)	6,13	5,95	3,62	3,44	4,97	4,13	5,79	4,31
Average number of microplastics per organism	0,4	0,4	0,2	0,2	0,3	0,3	0,7	0,9
Average n° of microplastics per g of soft tissue	0,65	0,67	0,55	0,58	0,603	0,73	1,12	2,09

The dominant form of microplastics is filament (100% of all the microplastic items collected in the samples of mussels), and the dominant colour is blue (74% of all collected microplastic items collected in the samples of mussels).

3 Conclusion

Concentrations of PAHs, PCBs, Dioxins and Dioxin-like PCBs were low. They were not detected in every sample of mussels collected. Heavy metals were detected in every sample but the concentrations were low. The concentrations of Cd and Pb in Bakar were low but out of maximum allowed limit according to EU Commission Regulation (EC) No 1881/2006. Microplastic particles were found in all the collected samples from Rijeka and Split area. The average number of MPs in the samples were low.