NET4mPLASTIC PROJECT

WP4 - Activity 4.4 Microplastic and risks for Human Health

D.4.4.4

Guidelines and indicators for proper consumption of mussel and shellfish to prevent toxicity and human health risks

June, 2022 – Final Version

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1 Introduction

This WP applies the guidelines of the EU's chemicals strategy for sustainability towards a toxic-free environment, with a focus on microplastics present in mussels and shellfish. The Net4MPlastic is part of the EU's zero pollution ambition, which is a key commitment of the European Green Deal.

EFSA The European agency for communication on risks associated with the food supply chain in May 2022, published an opinion on the identification and prioritisation of plasticiser substances in food contact materials (FCMs) for reassessment and a protocol for assessing exposure.

EFSA carried out preparatory work to re-evaluate the health risks from plasticisers such as phthalates, structurally similar substances, and substances used to replace phthalates in FCMs. s. Protocols are being developed concerning the assess human health risks of micro-and nanoplastics in food. EFSA has highlighted that there is no legislation for microplastics and nanoplastics as contaminants in food. Methods are available for identification and quantification of microplastics in food, including seafood and the occurrence data are limited.

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Another theme addressed in the indications of EFSA concerns additives and contaminants adsorbed in the plastic. Both additives and contaminants can be of organic as well of inorganic nature. Based on a conservative estimate the presence of microplastics in seafood would have a small effect on the overall exposure to additives or contaminants.

The project fits into this knowledge gap reported by EFSA, and through severe demonstrative approaches proposes a sampling, analysis, relaying and data processing and certification protocol aimed at providing a useful guideline for the characterization of shellfish food products. These characterization and treatment methodologies can be adopted for reducing risks and guaranteeing plastic free conditions and for the protection of human health.

When discussing impact of microplastics on human health three aspects should be focused: the impact of well-known chemicals that can be adsorbed on the plastic particles such as PAHs, PCBs and heavy metals,

additives that are added to plastic material to create the desired characteristic of plastic materials and the impact of microplastic particles on human health.

Activity 4.4 Microplastic and risks for Human Health capitalizza i risultati delle activities carried out in the WP 3 and 4 of Interreg NET4mPLASTIC project concerning:

1) The Review of the literature discussing the toxicity mechanisms of microplastic and accompanying chemicals is given in the Deliverables D.3.4.1
2) Guidelines and indicators editing for proper consumption of mussel and shellfish to prevent toxicity and human health risk and D.3.4.2
3) Report of data collection about co-presence of contaminants (PAHs, PCB, dioxins and heavy metals) and microplastic in bivalves. Microplastic particles and their impact on human health are described in the Deliverable D.4.4.1
4) Determination of the fate of MPs within cells and in the D.4.4.2 Determination of the effects of contaminants at single cell levels in more details. Evaluation of clearance gut rate in bivalves for risk assessment associated with their consumption is discussed in the Deliverable D.4.4.3.

These previous Deliverables have deepened the microplastic impacts with an analysis of the literature, an analysis of the types, abundance and size of microplastics present in the various marine environments and in fish products, and the sampling and investigation methods defined with experimental and demonstrative activities. The changes induced on the cells increased in vitro by exposure to microplastics of 1 and 2 µm at different interaction times were analysed and the related risk factors were defined.

This Deliverables takes into consideration the possible presence of inorganic (heavy metals) and organic (POP, OCP, IPA and PCB) in mussels and shellfish from different locations in all coastal and maritime areas of the Adriatic, for the transferability of the results obtained at the scale of the Italy-Croatia CBC entire programme area and finally in the conclusions it capitalizes the results of all the Deliverables mentioned above to provide the "Guidelines and indicators for proper consumption of mussel and shellfish to prevent toxicity and human health risks".

Conclusions described in this document are based on all of the Deliverables mentioned before.

2 Possible presence of inorganic (heavy metals) and organic (POP, OCP, IPA and PCB) in mussels and shellfish from different locations in all coastal and maritime areas of the Italy-Croatia CBC Programme

In this Deliverable chemicals that can be adsorbed on the plastic particles (PAHs, PCB, dioxins and heavy metals) are discussed in more details. For number of years these parameters have been monitored in different locations on the Adriatic coast and in the Adriatic Sea. In this deliverable, the available data on the concentration of contaminants in the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 were summarized in a comprehensive overview.

Data was collected from available databases, published sources and retrieved from Croatian public institutions (Institute of Oceanography and Fisheries in Split, Croatian Waters, Ministry of Environmental Protection and Energetics). Extracted information included data on pollution with heavy metals, polycyclic aromatic hydrocarbons, organochlorine pesticides, and polychlorinated biphenyls in the Adriatic Sea emphasizing the eastern Adriatic with comparisons of relevant studies. It provides harmonized and useful datasets for further analyses of contaminations in the marine environment. The guidelines for the assessment of obtained values for the mentioned parameters exist but they are limited to only several parameters. They are established by the Codex Alimentarius Commission (CODEX), created in 1963 by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). In Europe, the EU Commission Regulation 1881/2006/EC sets the maximum limits for Hg, Cd, and Pb in bivalve molluscs. There is no European standard for other contaminants in shellfish so it is necessary to create guidelines and food standards for other contaminants as soon as possible.

Review of the literature discussing the toxicity mechanisms of microplastic and accompanying chemicals is given in the Deliverables D.3.4.1 Guidelines and indicators editing for proper consumption of mussel and shellfish to prevent toxicity and human health risk and D.3.4.2 Report of data collection about co-presence of contaminants (PAHs, PCB, dioxins and heavy metals) and microplastic in bivalves. Microplastic particles and their impact on human health are described in the Deliverable D.4.4.1 Determination of the fate of MPs within cells and in the D.4.4.2 Determination of the effects of contaminants at single cell levels in more details. Evaluation of clearance gut rate in bivalves for risk assessment associated with their consumption is discussed in the Deliverable D.4.4.3. Conclusions described in this document are based on all of the Deliverables mentioned before.

2.1 Definitions and abbreviations

- BIOACCUMULATION - accumulation and storage of substances from the environment in organisms
- BIOACCUMULATION FACTOR (BAF) - is the ratio of the concentration of substance in an aquatic organism (g/kg) and the concentration of that substance in water (surrounding medium) (g/kg) (is a result of both bio-concentration and bio-magnification. It takes into account of both the exposure from respiratory surface and the dietary exposure.)
- BIOCONCENTRATION – intake and retention of a water-borne substance by an aquatic organism
- BIOCONCENTRATION FACTOR (BCF) - the concentration of certain chemicals in living tissue versus the concentration of that chemical in the water surrounding the tissue - BCF = concentration of organism chemicals (mg/kg) / concentration of chemicals in the environment (water) (mg/L) (is the result of a balance between the rate of chemical uptake from the water via the respiratory surface of the test organism (i.e, gills and/or skin) and the elimination of the chemical from the organism. It may also be expressed as the ratio of the uptake rate constant (k1) to the depuration rate constant (k2)
- BIOINDICATORS - organisms or communities of organisms which respond to pollutants with changes in vital functions and accumulation of pollutants
- BIOMAGNIFICATION – increase of the concentration of harmful substances bio-accumulated in parts of the food chain, proportionally with the level of the food chain
- BIOMAGNIFICATION FACTOR (BMF) - ratio of the chemical concentrations in the organism (CB) and the diet of the organism (CD), i.e., BMF = CB (mg/kg)/CD (mg/kg), where the chemicals are usually expressed in units of mass of chemical per kg of the organism (in wet weight or in a lipid basis) and mass chemical per kg of food (in wet weight or in a lipid basis)
- BIOMARKERS - changes at the molecular, cellular or physiological level in an organism exposed to a toxic substance
- BIOMONITORING - the use of organisms in monitoring the degree of environmental pollution
- MED-POL - Mediterranean Pollution Assessment and Control Program under the Mediterranean Action Plan
- PCA – principal component analysis
- XENOBIOTIC - a substance that occurs in the environment, but did not originate from it, and is not an integral part of a particular biological system. It is commonly used for synthesized chemical compounds. It could also be referred as foreign substance to the organism, which stimulates some biochemical-physiological reaction once introduced into the body.

2.2 Literature search and data selection

The search for the available data on heavy metals and organic compounds in Mediterranean mussels *Mytilus galloprovincialis* within the Adriatic Sea comprehended extensive literature review of databases ISI Web of Knowledge (https://www.webofscience.com/) (5 papers), Scopus (https://www.scopus.com/) (9 papers including (Bajc and Kirbiš, 2019, Bajt et al., 2019, Bille et al., 2015, Jović and Stanković, 2014, Kanduč et al., 2018, Kljaković-Gašpić et al., 2010, Maulvault et al., 2015, Spada et al., 2013, Tavoloni et al., 2021), and Google Scholar (https://scholar.google.com/) database for any additional literature not found in previous databases. Keywords included in the search used to identify research papers containing the data on heavy metals in Mediterranean mussels in the Adriatic Sea were "heavy metals", "organic compounds", "mussels", "Adriatic" and "*Mytilus galloprovincialis*". The search included: (heavy AND metal*) AND (organic* AND compound*) AND (mussel* OR *mytilus* AND *galloprovincialis*) AND (adriatic*). Also, the database of indicators of the state of the marine environment, mariculture and fisheries (http://baltazar.izor.hr/azopub/bindex) from the Ministry of Environmental Protection and Energetics was consulted as were the Environmental Reports of the Republic of Croatia for data regarding xenobitotics in mussels. Data in the database were provided by the Institute of Oceanography and Fisheries, Split. Additionally, the data was obtained from "Hrvatske vode" legal entity for water management established by the Water Act (Official Gazette no. 107/95 & 150/2005) by the Republic of Croatia in charge of yearly monitoring of coastal and transitional waters.

3 Review of existing available data on heavy metals and organic compounds in mediterranean mussel (*Mytilus galloprovincialis* lamarck, 1819) in the adriatic sea

With constant human activity, several thousand chemical substances are reaching the ocean through the continental waters and/or atmosphere. The impact of a variety of substances in the environment is complex and can directly or indirectly affect different populations and ecosystems (Azizi et al., 2018). As an enclosed basin the Adriatic Sea is under constant pressure from land-based sources of contamination and from maritime activities such as fishery, aquaculture, gas and oil exploitation, tourism and, maritime transportation (Bajt et al., 2019, Combi et al., 2016). Due to the variety of pressures EU environmental legislation concerned with marine pollution includes EU Water Framework Directive (WFD, Directive 2000/06/EC), Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC), Nitrates Directive (Directive 91/676/EEC) and Urban Waste Water Treatment Directive (Directive 91/271/EEC). In order to assess the chemical pollution (metals, PAHs, PCBs, and organochlorine pesticides) in Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) in the Adriatic waters in this deliverable, we bring the review of existing available data.

4 Mussels as bio indicators of pollution in the coastal environment

In order to evaluate the status of coastal ecosystem chemical contamination in the 1970ies scientist suggested the use of organisms, specifically mussels from the genus *Mytilus*, as bioindicator species. Previously marine environmental monitoring was focused on abiotic parameters but with a rise of awareness that pollutants (something that causes biological effect) and contaminants (excess concentrations of materials from discharges to the environment) could affect mankind and the environment, monitoring was refocused on the detection of anthropogenic materials in order to obtain information on distribution, transport mechanisms, and fates of these materials (Azizi et al., 2018, Dame, 1996). The sampling of natural waters was inadequate because it failed to identify contaminants that were not in the routine analysis, did not relate directly to the impact that measured chemicals have on the biota and ecosystem and it failed to identify sporadic contamination of the ecosystem (Kramer and Foekema, 2001). In order to obtain more information, continuous and biological monitoring was developed. A number of bivalves characteristics have led to their use as “monitors”, “sentinels” or “indicators” of environmental stress such as dominance in coastal and estuarine systems with wide geographical distribution (*Figure 1*), sedentary lifestyle (integration of contamination in a specific area), tolerance to a wide range of environmental condition, suspension-feeding (pumping large volumes of water and concentrating many chemicals by several orders of magnitude over the concentrations in seawater), concentration of contaminants providing an assessment of biological availability, low level of activity for enzyme systems capable of metabolizing organic contaminants, stable populations of commercially important species, easiness of transplantation and maintenance, commercially important as seafood (their chemical contaminations is of interest to public health) and have few behavioral and physiological answers to stress that are quickly and easily measured. With multitudinous advantages, it is not surprising that bivalves are successfully used as short-term and long-term monitors of environmental stress in coastal and estuarine waters (Dame, 1996).

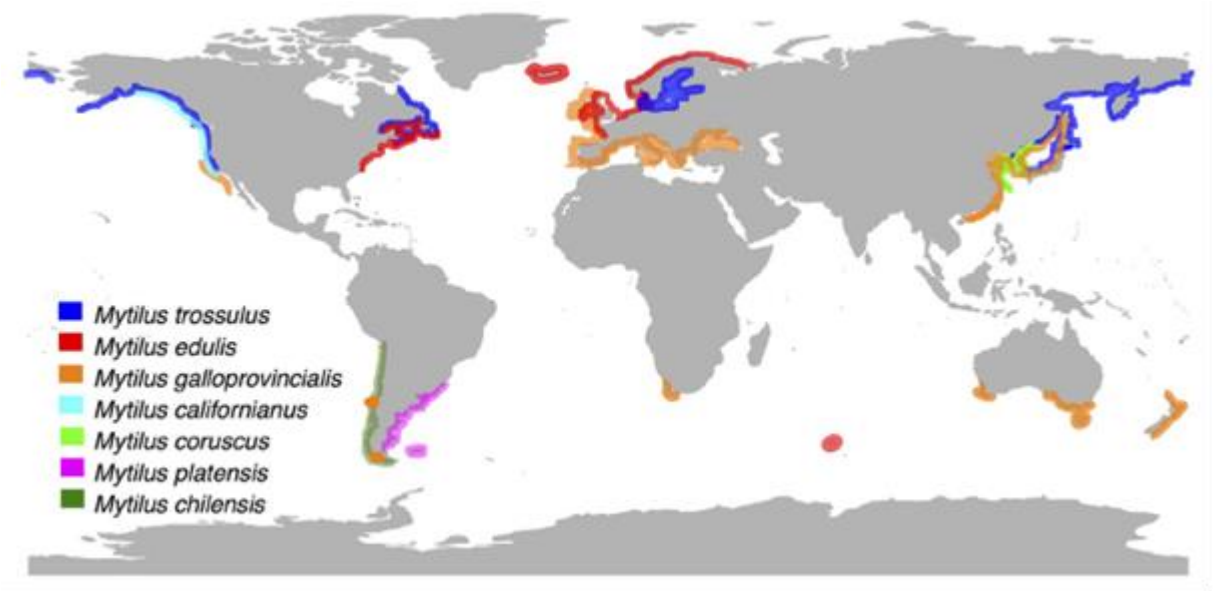

Figure 1: Geographic distribution of marine mussels of the genus Mytilus (Gaitán-Espitia et al., 2016).

Direct assessment of chemical pollution is difficult due to low concentrations in the seawater often below detection limits requiring chemical pre-concentration, clean sample collection, handling, and frequent sampling due to temporal and spatial variability. Alternatively, chemical concentrations in bivalve tissues (soft and hard) are often used as indicators for ambient water chemistry. Due to the way of bivalves feed they can concentrate a lot of chemical species by order of magnitude over seawater concentrations. Also, the level of pollutants in specific tissues indicates bioavailability (Pitts and Wallace, 1994). For the above reasons, bivalves have been used as monitors of chemical contamination in coastal waters, particularly in areas receiving waste discharges from point sources like municipal waste treatment plants or non-point sources such as storm water runoff.

There is strong experimental evidence supporting the usage of bivalves as biomonitors. A large number of laboratory experiments and field transplant experiments have demonstrated the accumulation of pollutants in bivalves (Fisher and Teyssie, 1986, Roesijadi et al., 1984). The degree to which inorganic and organic contaminants are accumulated by bivalves depends on abiotic physiochemical properties and biotic factors (pumping activity, growth, biochemical composition, reproductive condition, and metabolism). All the above factors influence the rates of the dynamic processes concerned with uptake, deposition, and depuration that together determine the degree of bioaccumulation (Gosling and Gosling, 1992).

Today the "Mussel Watch Monitoring Program" is developed successfully in many countries including the U.K., France, Canada, Australia, Japan, Taiwan, India, South Africa, etc. The initial program analysed trace metals, chlorinated hydrocarbons, petroleum hydrocarbons, and radionuclides and later included DDT and its metabolites, chlorinated pesticides other than DDT, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, the major elements aluminium, iron, manganese, and trace elements. Except for the major elements, all above are possible contaminants in the sense that their concentration in the environment has been altered by human activities (Dame, 1996). The mussel watch-type programs have not only quantified the degree of contamination but have identified unexpected contamination hot spots (Widdows and Donkin, 1992). As sentinel organism bivalves have proved to be useful tools in identifying variations in chemical contamination between sites and contributed to an understanding of trends in coastal contamination (Dame, 1996).

The Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 (*Figure 2*) is used as a sentinel organism in several biomonitoring programs under the patronage of UNEP in the Mediterranean Sea and OSPAR at the North and Baltic Sea (Kanduč et al., 2018). Based on demands of the WFD and MSFD in 2008 a first comprehensive study on the contamination burden of the Adriatic coastal waters was done using active mussel biomonitoring with *Mytilus galloprovincialis* for metals, organochlorine pesticides, polychlorinated biphenyls (PCB), and polycyclic aromatic hydrocarbons (PAH) (Bajt et al., 2019).

Figure 2: Three perspectives of mediterranean mussel Mytilus galloprovincialis Lamarck, 1819 1) shell appearance, 2) side view of both shells with the visible interior and 3) internal appearance of both shells (Paiva, 2014).

4.1 Bioaccumulation of contaminants in marine invertebrates

Bivalve molluscs are exposed to large volumes of water during respiration and feeding, and metals and organic contaminants will thus inevitably enter the bivalves through the gills and other exchange surfaces (*Figure 3*).

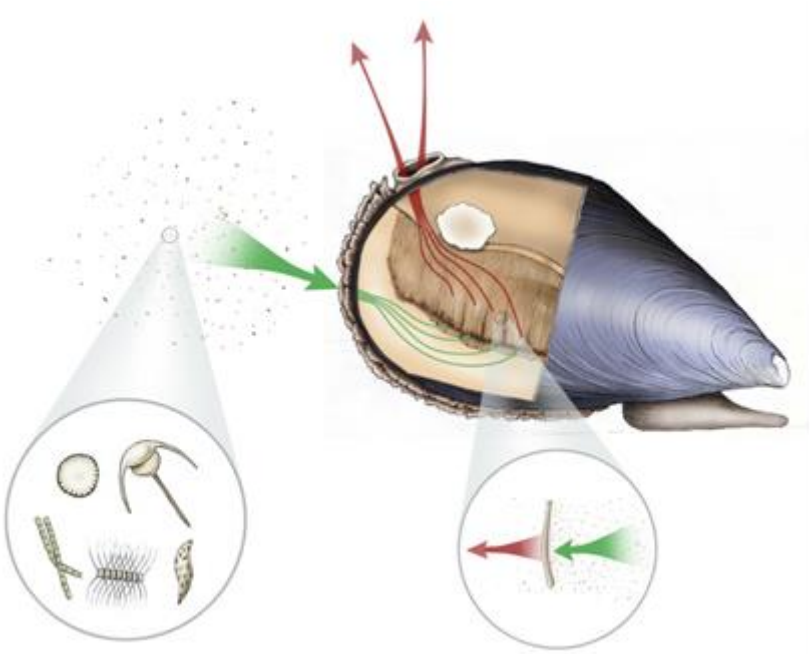

Figure 3: Suspension feeding in mussels by filtrating seawater (https://www.kimberly-andrews.com/filter-feeding-in-a-mussel.html).

However, the uptake mechanisms of metals in bivalves have surprisingly remained not well understood (Wang, 2009). There are several different routes across the apical membrane of epithelial cells how metals can be taken up (Tessier and Turner, 1995). Since the 1980s, the free-ion activity model (FIAM) has been the guiding principle behind the understanding of metal bioavailability from solutions (Morel and Hering, 1993). Rates of uptake by facilitated transport can be modelled by the availability of the free metal ions (*Figure 4*). Other routes may involve the transport of whole metal complexes across the apical membrane (without a role for the free metal ion). In addition, the involvement of the Ca channel in the transport of metals has also been reported in several species of bivalves (Vercauteren and Blust, 1999, Wang and Dei, 1999, Wang and Fisher, 1999).

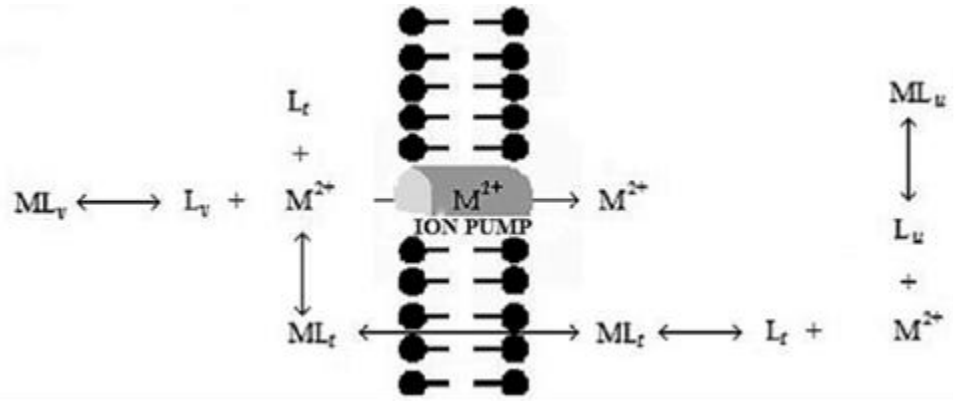

Figure 4: The uptake of a divalent trace metal into a cell across cell membrane in invertebrates; Lt - transport ligand, Lu - internal ligand, Lv - outer ligand, M- metal; modified from - (Rainbow, 1997).

After entering the body of bivalves, trace metals can perform a certain essential function in metabolism. However, if the metal is non-essential or the essential metal in excess it must be detoxified temporarily or permanently. Detoxification involves the binding of metals in such a way that they can no longer bind at any active site and are transported to organs such as the hepatopancreas, the digestive gland or, the excretory organ. Trace metals can be excreted from the body in detoxified form or stored permanently, which results in an increased concentration of metals in the body, that is in bioaccumulation. It should be emphasized that the high accumulated concentration of detoxified metal does not indicate a risk of toxicity. The risk of toxicity arises from a much lower concentration of the metabolically available metal that has not yet been detoxified (Rainbow, 1997).

The bioaccumulation of trace elements in mussel tissue depends not only on the concentration of the same elements in the marine environment but also on a number of biological and environmental factors. Biological factors, which are a consequence of the physiology of the organism itself, include size, sex, sexual maturity, reproductive phases, and cycles of seasonal growth. Environmental factors include temperature, salinity, pH, organic carbon, food availability, nutrient availability, dissolved oxygen, sediment granulation, and hydrological characteristics of the marine aquatic system. As they live in shallow coastal waters, in tidal zones, mussels are exposed to numerous stressful changes in the environment, such as natural variations in temperature, oxygen, and total organic carbon, but also anthropogenic factors, including water and sediment pollution. The concentration of metals in mussel tissue may also depend on geological characteristics, thus depicting geographical differences between individual areas (Perošević-Bajčeta, 2020).

Chemical contaminants accumulation including heavy metals and persistent organic pollutants (POPs) in bivalves for human consumption presents a permanent risk to humans as final consumers at the top of the food chain (Bogdanović et al., 2014). Today, the seafood standards for bivalves are limited to only several metals and established by the Codex Alimentarius Commission (CODEX), created in 1963 by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). The primary objectives are the protection of the health of consumers, the assurance of fair practices in the food trade, and the coordination of the work on food standards. Different countries, however, have different regulations on their safety standards. In Europe, the EU Commission Regulation 1881/2006/EC sets the maximum limits for Hg, Cd, and Pb in bivalve molluscs at 05, 1, and 1.5 mg/kg wet weight respectively. There is no European standard for other contaminants in shellfish (Wang, 2009).

Lipophilic organic compounds are taken up into the tissues of bivalves and concentrated to levels much above those of the seawater around. Due to practical difficulties in measuring low concentrations of contaminants in marine environments, chemical analysis of bivalves tissues has been extensively used as an indicator of contamination (Livingstone, 1991). The majority of urbanized coastal areas of the world are polluted with polycyclic aromatic hydrocarbons (PAHs) which accumulate in sediments and biota that are unable to efficiently eliminate them (Meador et al., 1995). As widely distributed environmental contaminants PAHs have detrimental biological effects, toxicity, mutagenicity, and carcinogenicity. Due to their ubiquity, recalcitrance, bioaccumulation potential, and carcinogenic activity, the PAHs have generated great environmental concern (Haritash and Kaushik, 2009). Organism physiology and bioavailability are the two important variables that have a major effect on chemical contaminant body burden where only the bioavailable fraction can enter the organism. Different from metals and ionizable organics, the bioavailability of PAHs is affected by sole a few environmental variables such as sediment surface area and organic carbon. Contaminant body burden is also determined by physiological factors, including lipid levels and the rates of uptake and elimination (metabolism, diffusion, and excretion). It is assumed that the process of uptake of hydrophobic compounds is passive (vis-a-vis active transport) and controlled by diffusion pressure (fugacity) because of the differential between the environmental matrix and tissue concentration. Controlling factors of the concentration of the free PAH (nonsorbed) are important for the uptake of PAHs from water. Variables like salinity and hydrogen ion concentration may also have an effect on the bioavailable fraction; however, these factors generally have a rather minor effect over the range found in marine and estuarine environments. For PAH accumulation, ingestion of prey, detritus, and sediment is also important; however, the factors that determine the bioavailable fraction taken up from ingested materials are less well known (Meador et al., 1995).

Depuration is thought to be mainly a passive process, including re-equilibration of the xenobiotic between the biotic and external compartments. Still, studies on the elimination of naphthalene from *M. edulis* have pointed out active excretion from the gills and kidneys (Widdows et al., 1983). Exponential depuration curves are normally beholding which can often be resolved into an initial faster phase and a longer-term slower phase of elimination of xenobiotic. For a number of distinct compounds, the pattern of elimination looks to be noticeably affected by the length of exposure to the xenobiotic. Short-term exposure results in rapid and almost complete or complete elimination, whereas longer-term exposure is followed by slower and often incomplete elimination. Therefore, the half-life of depuration following exposure increases with increasing time of exposure. The differences generally appear independent of tissue pre-depuration xenobiotic concentration, and the relationship is particularly evident for petroleum hydrocarbons. The mechanisms in the basis of such phenomena are unknown but have been interpreted in terms of the xenobiotic entering a more stable cellular or molecular section, with a lower rate of xenobiotic turnover. Chemical structure and hydrophobicity are factors affecting the differential elimination of different xenobiotics. Thus, higher molecular weight, less water-soluble compounds are generally eliminated at a lower rate, this being particularly evident for increasingly chlorinated PCB isomers. As in the uptake of xenobiotics, patterns of depuration are also affected by season and temperature, and species physiology (Livingstone, 1991).

5 Mussel biomonitoring in the Adriatic Sea

In order to assess the chemical pollution (metals, PAHs, PCBs, and organochlorine pesticides) in Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) in the Adriatic waters in this deliverable, we bring the review of existing available data.

The first comprehensive assessment of chemical contamination (metals, PAHs, PCBs, and organochlorine pesticides) in the Adriatic coastal waters by active mussel biomonitoring with *Mytilus galloprovincialis* was conducted in 2008., reported in 2019 in Bajt et. al., providing useful harmonized dataset to support a coordinated definition of baselines, targets and thresholds and further management of chemical contamination in the Adriatic Sea.

Figure 5: Sampling sites along the coastline in a comprehensive assessment of chemical contamination in the Adriatic Sea (Bajt et al., 2019).

The following chemicals were selected and measured from the priority list of WFD: metals (Hg, V, Ni, Cu, Zn, Cd, Pb, Cr); organochlorinated pesticides (α-and β- endosulfan, α-, β- and γ-HCH (hexachlorocyclohexane), p,p'DDT, p,p'DDE and p,p'DDD, aldrin, endrin, dieldrin and isodrin, hexachlorobenzene (HCB), hexachlorobutadiene (HCBD)); PCBs (congeners PCB28, PCB31, PCB52, PCB101, PCB105, PCB118, PCB138, PCB153, PCB156, PCB180); and 16 EPA PAHs. Sampling sites and samples are presented in *Figure 5* and *Table 1*.

Table 1: Sampling sites description and analysed Mytilus galloprovincialis samples (No. of specimens – used for CI calculation; CI – condition index) (Bajt et al., 2019).

Station name	Code	Region	Depth (m)	Longitude	Latitude	No. of specimens	Dry weight (%)	CI
Vlora	VL AL	SA	31	194,105	404,482	22	19.8	0.09
Kepi Rhodon	KR AL	SA	29	194,087	415,770	24	23.0	0.11
Bar	BA ME	SA	32	190,630	420,925	25	20.5	0.10
Kotor	KO ME	SA	15	187,617	424,368	24	23.9	0.09
Baošići	BO ME	SA	22	186,360	424,435	24	21.3	0.09
Dubrovnik	DU HR	SA	40	180,063	426,416	20	22.3	0.12
Neum	NE BA	CA	26	175,454	429,284	24	23.3	0.07
Neretva	NE HR	CA	15	174,830	430,380	22	19.3	0.10
Cetina	CE HR	CA	50	166,833	434,333	20	18.6	0.11
Split	SP HR	CA	31	163,873	435,100	20	22.4	0.14
Šibenik	SI HR	CA	22	157,707	437,372	22	20.1	0.11
Zadar	ZA HR	CA	26	152,483	440,891	22	20.3	0.11
Novigrad	NO HR	CA	29	155,380	441,995	23	19.5	0.09
Krk	KR HR	NA	29	144,565	450,999	20	19.7	0.10
Bakar	BA HR	NA	30	145,558	452,708	22	21.6	0.10
Rijeka	RI HR	NA	36	144,687	453,144	22	20.4	0.09
Pula	PU HR	NA	29	137,949	448,945	18	22.2	0.13
Lim	LI HR	NA	30	136,169	451,237	18	22	0.11
Piran	PI SI	NA	13	135,784	454,941	17	21.9	0.12
Strunjan	ST SI	NA	17	135,903	455,305	18	23.2	0.13
Koper	KO SI	NA	13	137,236	455,550	17	22.0	0.12
Trieste	TS IT	NA	14	135,635	456,862	15	24.3	0.15
Po Nord	PON IT	NA	26	125,579	450,905	15	27.7	0.17
Po	PO IT	NA	23	124,990	450,423	15	19.1	0.14
Port Garibaldi	PG IT	NA	13	123,389	446,969	15	26.0	0.20
Ravenna	RA IT	NA	12	123,441	445,096	15	24.8	0.20
Rimini	RI IT	NA	11	126,730	440,703	15	25.6	0.19
Fano	FA IT	NA	12	128,346	439,819	14	24.8	0.16
Falconara	FAL IT	CA	15	133,806	437,026	15	23.9	0.18
Ancona port	AN IT	CA	15	135,008	436,315	15	24.3	0.13
San Benedetto	SB IT	CA	13	139,467	429,193	17	24.3	0.15
Pescara	PE IT	CA	12	142,336	424,890	25	23.1	0.10
Vasto	VA IT	CA	28	146,844	422,376	19	22.0	0.11
Tremiti	TR IT	CA	35	155,032	421,229	25	21.9	0.09
Manfredonia	MA IT	SA	12	160,247	416,071	25	21.1	0.10

Bari	BA IT	SA	21	168,573	411,471	17	21.4	0.12
Brindisi	BR IT	SA	31	179,896	406,645	25	20.0	0.09
Taranto	TA IT	SA	13	171,382	404,866	17	21.5	0.11

Additional data was collected from the Environmental Report of the Republic of Croatia for the period from 2009 to 2012 (HAOP, 2022) and 2013 to 2016 (MINGOR, 2022). The study was conducted by the Institute of Oceanography and Fisheries in Split and the sampling stations are shown in *Figure 6*. In the period from 2009 to 2011, the concentration of hazardous substances in marine organisms was monitored in bivalves *M. galloprovincialis*. During interpretation of the results it should be taken into account that the stations on which the monitoring of hazardous substances was done are not spatially distributed throughout the Adriatic coast and that due to insufficient samples on some of them, monitoring of indicators of hazardous substances was not continuous. In addition, in 2012 analyses were not conducted due to a lack of funding.

As there were no defined environmental quality standards for pollutants in marine organisms, sampling and assessments in the mentioned period were made in accordance with international recommendations organization MED-POL (UNEP). All available data from national environmental reports were collected during the implementation of the Croatian National Project "Adriatic" until 2012, during 2013 they were collected within the scientific experimental monitoring of the project “Protection against water pollution in the coastal area - Adriatic Sea Monitoring Program Phase II (2014). The research was not conducted in the period from 2014 to 2016 so the data are only available for the year 2013. New data for 2016 to 2020 and later were not reported but through the oral communication (Ivana Ujević – Institute of Oceanography and Fisheries) it was found out that research was not done for heavy metals except in 2020 but due to data ownership issues the data was unattainable.

Transitional waters:

OC03 - in front of the Ploče
OC04 - in front of Omis
OC09 - Šibenik port

Coastal waters:

OC01 - in front of Dubrovnik
OC02 - Mali Ston Bay
OC05 - Brač Channel
OC06 - Kastela Bay
OC08 - in front of Primosten
OC10 - in front of Šibenik
OC11 - in front of Gaženica
OC12 - in front of Zadar
OC13 - in front of Petrčane
OC14 - in front of Crikvenica
OC15 - Bakar Bay
OC16 - in front of Rijeka
OC17- Kvarner
OC18 - in front of Rovinj

Sea waters:

OC19-5NM in front of Rovinj
OC20- in front of Umag

Figure 6: Position of measuring stations during the period from 2009 to 2012 (HAOP, 2022).

5.1 Metals

Concentrations of Hg, V, Ni, Cu, Zn, Cd, Pb, and Cr were measured in the tissues of *M. galloprovincialis* with levels of accumulated metals reported in *Table 2*. Mercury (Hg) concentrations ranged from 0,05 mg/kg (from Ravenna to Ancona port) to 0,13 mg/kg (Trieste) where the maximum concentrations were recorded. High concentrations were observed in the eastern Adriatic Sea (Vlora, Baošići, Neum, Cetina, Split, Novigrad, and Pula) and in Taranto (Ionian Sea) in a range from 0,11 mg/kg to 0,12 mg/kg while the sites with low concentrations from 0,05 mg/kg to 0,06 mg/kg were located along the western coastline (from Port Garibaldi to Pescara).

In comparison to other sites, the very high concentrations of vanadium (V) were recorded in station Termiti (10,2 mg/kg) which was according to Bajt et.al. peculiar because the site is near the island without an industrial area, due to the oil and gas platforms are northward so the currents might bring traces of oil loaded by vanadium southward. Sites Vlora, Lim, and Bari were characterized by high values from 5,4 mg/kg to 5,9 mg/kg.

Nickel (Ni) concentration varied from 0,6 mg/kg in Pula to 2,3 mg/kg in Vora and 2.9 mg/kg in Kepi Rhodon. High Ni concentrations were measured at Bar, Neum, Neretva, Novigrad, and Vasto while moderate concentrations were found at the remaining sites. Higher Ni levels in *Mytilus galloprovincialis* individuals were reported along the Albanian (2,19 mg/kg to 6,18 mg/kg), Montenegro (1,35 mg/kg to 7,50 mg/kg) and Croatian coast (0,8 mg/kg to 5,0 mg/kg) (Jović and Stanković, 2014).

Copper (Cu) concentrations were highest on the eastern Adriatic side with high value of 10.1 mg/kg found at Neretva river estuary. All stations had moderate values (*Table 2*) except Dubrovnik with a high 4.6 mg/kg value.

Zinc (Zn) concentrations varied from 94 mg/kg in Kepi Rhodon, Port Garibaldi) to a very high value of 174 mg/kg at Vasto. Southwestern Adriatic Sea had very high concentrations down to Brindisi. Semi enclosed gulfs and bays like Koper, Lim, Bakar, Krk, Cetina, Neum, Baošići and Taranto showed moderate concentrations from 131 mg/kg to 144 mg/kg. Human activity like industrial and urban wastes, harbor activities, nautical marinas, and road traffic do affect the above sampling sites.

The highest detected cadmium (Cd) concentration was at Novigrad Sea (1.03 mg/kg). Along the Italian coastline high concentrations of Cd were at fano, Vasto, Tremiti, Manfredonia and Brindisi from 0,78 mg/kg to 0,90 mg/kg. All other sampling stations were with moderate concentrations from 0,54 mg/kg to 0,78 mg/kg.

Chromium (Cr) concentrations were high in the south-eastern Adriatic Sea 0,8 mg/kg in Kotor to 2.4 mg/kg in Kepi Rhodon. In northern and south-western coasts, the results of concentrations were mostly below 1 mg/kg. Sites with very high Cr concentration were Kepi Rhodon with 2.4 mg/kg and Neum with 1.8 mg/kg while in southern Adriatic the concentrations were high in Vlora, Bar, Dubrovnik, Neretva and Cetina. On the northern Adriatic port of Koper had high concentrations of 1,3 mg/kg. Low concentrations were detected in Rimini and Taranto while other locations were in moderate concentration category.

Lead (Pb) concentratrions were from 0,5 mg/kg in Rimini to 1,8 mg/kg in Tremiti. Values that were high were found at Kotor, Baošići, Dubrovnik, Split, Novigrad, Rijeka, and Pula at the eastern Adriatic Sea. Other locations were with moderate concentration from 0,7 mg/kg to 1.2 mg/kg.

To compare the total content of metals at the different sampling sites the Metal Pollution Index (MPI) was used, obtained with the equation MPI = $(Cf1 \times Cf2 \ldots Cfn)^{L}/^{n}$, where Cfi--concentration for the metal i in the sample (Usero et al., 1996). MPI values of Bajt et al. study was from 1.08 mg/kg in Lim bay to 2.23 mg/kg at Vlora. From Vlora to Cetina the values were the highest. It is noticeable that the MPI index is higher in areas under anthropogenic influence like in urbanized, industrial areas, areas with port activities, touristic areas and river mouths paired with limited exchange of water masses enabling the accumulation of several compounds. The study indicated that there are few sites that are hotspots due to high metal concentrations. The hotspots comprehended Vlora (Hg, Ni, Cd, Cr, V), Kepi Rhodon (Ni, Zn, Cr), Kotor (Pb, Cr), Neretva (Cu), Dubrovnik (Cu, Pb), Neum (Cr, Ni), Novigrad (Cd, Hg, Ni, Pb), Vasto (Zn, Ni, Cd), Tremiti (V, Cd, Pb) and the most contaminated with mercury (0,13 mg/kg) Trieste.

Table 2: Concentration of analysed metals in Mytilus galloprovincialis samples in Adriatic Sea (mg/kg, d.w.) (Bajt et al., 2019).

Station name	Code	Region	Hg	V	Ni	Cu	Zn	Cd	Pb	Cr	MPI
Vlora	VL AL	SA	0.12	5.5	2.3	3.7	116	0.70	0.9	1.5	2.23
Kepi Rhodon	KR AL	SA	0.10	3.8	2.9	3.7	94	0.65	0.7	2.4	2.13
Bar	BA ME	SA	0.10	2.7	1.7	3.4	116	0.76	1.1	1.2	1.92
Kotor	KO ME	SA	0.10	1.8	1.0	2.9	119	0.57	1.5	0.8	1.60
Baošići	BO ME	SA	0.12	2.4	1.2	4.1	131	0.67	1.3	1.1	1.91
Dubrovnik	DU HR	SA	0.09	4.1	1.5	4.6	123	0.52	1.5	1.4	2.07

Neum	NE BA	CA	0.11	3.2	1.8	3.8	135	0.68	1.1	1.8	2.14
Neretva	NE HR	CA	0.09	3.4	1.6	10.1	110	0.65	1.2	1.2	2.18
Cetina	CE HR	CA	0.11	2.9	1.2	4.2	137	0.75	1.2	1.3	2.00
Split	SP HR	CA	0.12	2.2	0.8	3.9	118	0.54	1.4	0.6	1.61
Šibenik	SI HR	CA	0.08	2.3	0.8	3.4	124	0.62	1.0	0.6	1.48
Zadar	ZA HR	CA	0.10	2.3	0.8	3.7	129	0.64	1.1.	0.7	1.60
Novigrad	NO HR	CA	0.12	2.1	1.6	4.0	123	1.03	1.3	0.9	1.98
Krk	KR HR	NA	0.09	1.4	0.9	3.5	144	0.74	1.1	0.5	1.48
Bakar	BA HR	NA	0.09	1.5	0.9	3.7	133	0.63	1.2	0.5	1.48
Rijeka	RI HR	NA	0.09	1.2	0.9	3.8	130	0.69	1.4	0.6	1.52
Pula	PU HR	NA	0.11	3.3	0.6	2.9	116	0.45	1.3	0.4	1.43
Lim	LI HR	NA	0.09	5.9	1.1	2.9	136	0.66	1.1	1.1	1.08
Piran	PI SI	NA	0.09	4.2	1.2	3.1	128	0.61	1.0	0.8	1.75
Strunjan	ST SI	NA	0.09	4.6	1.0	3.2	118	0.59	1.1	0.4	1.59
Koper	KO SI	NA	0.08	3.5	1.3	3.5	139	0.61	1.1	1.3	1.92
Trieste	TS IT	NA	0.13	3.5	1.0	3.1	119	0.54	1.1	0.5	1.63
Po Nord	PON IT	NA	0.08	4.1	0.9	3.0	106	0.53	0.9	0.4	1.43
Po	PO IT	NA	0.09	3.4	1.0	3.2	127	0.64	0.9	0.5	1.56
Port Garibaldi	PG IT	NA	0.06	2.5	0.9	3.1	94	0.48	0.9	0.5	1.31
Ravenna	RA IT	NA	0.05	2.4	1.0	3.1	100	0.50	0.8	0.7	1.34
Rimini	RI IT	NA	0.05	2.3	0.9	3.1	96	0.59	0.5	0.3	1.13
Fano	FA IT	NA	0.05	2.9	0.8	2.8	101	0.66	0.6	0.4	1.23
Falconara	FAL IT	CA	0.06	4.0	1.0	2.7	107	0.84	0.7	0.4	1.42
Ancona port	AN IT	CA	0.05	2.8	1.1	3.1	121	0.72	0.5	0.6	1.37
San Benedetto	SB IT	CA	0.06	2.9	1.3	2.8	95	0.64	0.7	0.7	1.44
Pescara	PE IT	CA	0.06	4.3	1.2	2.8	127	0.65	1.1	0.6	1.62
Vasto	VA IT	CA	0.08	4.5	1.8	2.8	174	0.83	0.9	0.7	1.89
Tremiti	TR IT	CA	0.09	10.2	1.0	3.0	145	0.86	1.8	0.5	2.05

Manfredonia	MA IT	SA	0.09	3.2	1.5	3.2	152	0.78	0.8	0.6	1.73
Bari	BA IT	SA	0.08	5.4	1.2	3.1	153	0.67	0.8	0.6	1.73
Brindisi	BR IT	SA	0.10	4.6	1.2	3.3	161	0.89	1.0	0.5	1.84
Taranto	TA IT	SA	0.12	1.3	0.8	3.6	135	0.47	0.6	0.3	1.23

The data collected from the Environmental reports of the Republic of Croatia (HAOP, 2022) (*Figure 7*) showed elevated values of mass fractions of heavy metals in bivalve molluscs in areas with a strong anthropogenic impact, i.e. in the area of major ports, marinas, industrial areas etc. The highest average values of chromium mass fractions and cadmium were recorded at the station near Dubrovnik, and lead and copper at the station near Pula. In regards to the previous reporting period, average values of zinc mass fractions were increased at five stations (OT04, OT10, OT17, OT18 and OT24). The highest average values of mass shares of mercury in this reporting period, as in the period from 2005 to 2008 were measured in samples from station OT11 (Kaštela), near the former plant for PVC production.

Mass fractions of heavy metals (Cd, Cu, Pb, Zn and Hg) in the soft tissue of bivalves (*Mytilus galloprovincialis*) in national report from 2012 to 2016 were collected at 8 stations in Croatian coastal waters in 2013. For these metals, due to the different intensity of anthropogenic pressures, a heterogeneous spatial distribution of mass fractions was observed. Elevated values of the content of tested heavy metals in the soft tissue of shellfish were found in larger ports, marinas and near industrial plants, where the anthropogenic impact is more pronounced (ports Ploče and Rijeka, Šibenik Bay - Martinska Bay and Bakar Bay). The highest content of cadmium (Cd) was recorded in the Lim Channel, and copper (Cu) in the marina Rovinj, while lead (Pb) showed the highest concentration in the Vranje Bay (station OT10). The highest content of zinc (Zn) was recorded in shellfish from the area of Rijeka (station OT 22), and mercury (Hg) in the area of Bakar (station OT21). Comparing the obtained values of the share of ecotoxic metals with the results of long-term measurements (2000-2011), it can be concluded that the measured values in the 2013 samples are in a same range as previous surveys. The measured values of Pb and Cd in shellfish samples at all stations are lower than the permitted concentrations by national policy. Policy on maximum permitted quantities of certain contaminants in food (OG 146/12). The maximum permitted amount of cadmium is 1.0 mg/kg of wet weight of bivalve mollusc tissue, while the maximum permitted amount of lead is 1.5 mg/kg of wet weight. Among the elements whose content has been analysed, three of them (Cd, Pb and Hg) are listed as priority substances in accordance with the Priority Substances Directive 2008/105/EC. The measured values of the content of these three elements in shellfish from all investigated stations are below the maximum permitted levels in seafood, that is below the levels that lead to harmful effects on the environment.

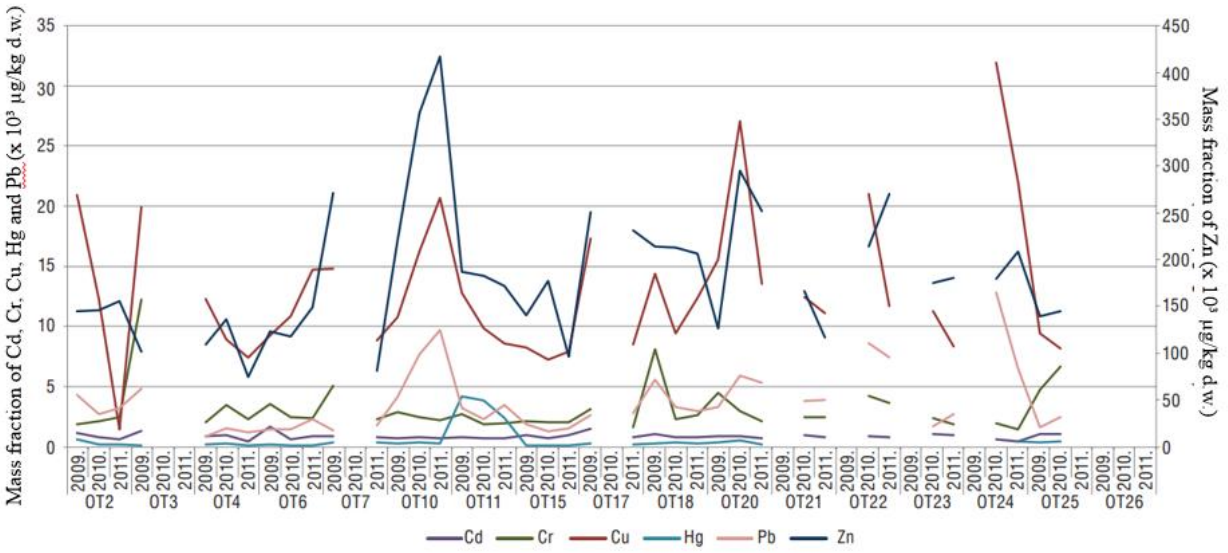

Figure 7: Mass fraction of Cd, Cr, Cu, Hg, Pb and Zn in the Eastern Adriatic from the years 2009 to 2011 on selected sampling stations (HAOP, 2022).

Continuous monitoring of ecotoxic metals in shellfish from Croatian coastal areas indicated a relatively low level of anthropogenic pressures at most of the investigated stations.

Table 3 presents mean and median concentrations of Pb, Cd, V, Ni, Cr, and As measured levels during 2008–2021 in *M. galloprovincialis* in the Adriatic Sea.

Hg was almost always below LOQ (LOQ = 0.025 mg/kg), while Cd, Pb, V, Ni, Cr, and As were quantified in most of the bivalves. Cd and Pb mean levels reported in mussels were similar in all Adriatic regions (Bajt et al., 2019, Bille et al., 2015, Kanduč et al., 2018, Maulvault et al., 2015). Further, Ni and Cr concentrations were lower than those reported by Maulvault et al. (2015) and Kanduč et. al. (2010-2013) in wild populations. In the Central Adriatic Sea vanadium levels were lower with respect to the concentrations reported by Bajt et al. (2019). Hg, Cd, and Pb (Table 1) were below the EU maximum limits (Reg 1881/2006, 2006) in all the samples analysed (MRL: 0.5, 1.0 and 1.5 mg/kg for Hg, Cd, and Pb, respectively). Hg EQS is 25 times lower (0.020 mg/kg) with respect to the maximum limit in bivalves, but Hg was measured at negligible levels in all the samples analysed, in agreement with what was previously reported in bivalves from the Italian Central Adriatic coast (Bajt et al., 2019). Travoloni et. al. 2021 had lower levels of Hg than previously reported for the same species harvested in areas closer to the Po Delta (Bille et al., 2015) and also had lower Cu concentrations than other regions of the Adriatic Sea. Ni concentrations were found similar in reported areas for Albania, Slovenia, Croatia, and Italy Lower values were recorded for the Venice lagoon and the western Adriatic coast of Italy (*Table 3*). The Cd concentrations in the mussels from the Boka Kotorska Bay were found to be similar to those previously

reported for the eastern, south-western and western Adriatic coast (Kljaković-Gašpić et al., 2002). In the case of the Pb concentrations in mussels, lower values were reported for the Albanian coast, the eastern Italian coast and the Trieste Gulf, while higher values were reported for the Mali Stone Bay and the Goro Bay, compared to Travoloni at al. 2021 (*Table 3*). Comparable Pb values were obtained for the eastern Croatian and south-eastern Italian coast (Kljaković-Gašpić et al., 2002, Oreščanin et al., 2006, Spada et al., 2013). Generally, the trace metal concentrations found in mussels were within the ranges of trace metal concentrations determined in low to moderately polluted areas.

Table 3: Review of metal concentrations (mg/kg w.w.) in mussels Mytilus galloprovincialis from the Adriatic Sea – – mean ± SD (min - max).

N.	Reference	Sampling area	Time frame	Cd	Pb	Hg	Ni	Cr	V	As
1	(Maulvault et al., 2015)	NW Adriatic Sea (Italy) Po river delta	2013	0.144	0.173	0.014	1.38	0.873	1.57± 0	
2	(Bille et al., 2015)	NW Adriati Sea (Italy) Veneto region	2007 - 2012	0.13±0 .06 (0.02-0.58)	0.22±0 .1 (0.03-0.08)	0.03±0 .02 (0.002-0.18)				
3	(Bajt et al., 2019)	Adriatic Sea	2008	0.128 (0.087-0.199)	0.200 (0.097-0.349)	0.017 (0.010-0.025)	0.233 (0.116 - 0.563)	0.153 (0.058 - 0.466)	0.657 (0.233 -1.98)	
4	(Spada et al., 2013)	S Adriatic (Italy) Apulia region	2009	(0.074-0.357)	(0.072-0.631)	(0.019-0.157)	(0.198 -2.94)	(0.186 -1.83)	(0.255 -1.07)	(1.23 - 14.8)
5	(Kanduč et al., 2018)	NE Adriatic Sea (Croatia) Mariculture	2010 - 2013	0.4 ±0.1	0.5 ±0.3		0.9 ±0.7	1.3 ±1.6		18.1 ±4.6
6	(Kanduč et al., 2018)	NE Adriatic Sea (Croatia) Port area	2010 - 2013	0.6 ±0.2	6.2 ±3.5		1.7 ±1.1	1.8 ±1.0		22.1 ±12.8

7	(Bajc and Kirbiš, 2019)	N Adriatic Sea (Slovenia)	2015	(0.072-0.26)						(1.66 - 4.97)
8	(Tavoloni et al., 2021)	C Adriatic Sea (Italy) Marche region	2008 - 2018	0.158 (0.023-0.849)	0.185 (0.059-0.616)	<0.025	0.478 (0.105 -1.14)	0.268 (0.065 - 0.632)	0.489 (0.063 -1.38)	3.5 (2.19 - 7.01)
9	(Jović and Stanković, 2014)	SE Adriatic, (Montenegro) BK Bay	2009	0.17-0.64	0.34-1.85	0.024-0.371	0.17-1.26	-	-	-

a Published result were converted in mg/kg wet weight taking as reference the moisture content reported from USDA for mussels and clams

b Mean (converted in mg/kg wet weight) was calculate from the raw data.

5.2 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) are hydrophobic organic compounds whose concentrations were determined from *Mytilus galloprovincialis* individuals (whole mussel) for 16 individual PAHs and total PAH including Naphthalene (NA); acenaphthene (ACE); acenaphthylene (ACY); fluorine (FL); phenanthrene (PHE); anthracene (AN); fluoranthene (FLU); pyrene (PY); chrysene (CHR); benzo[a]anthracene (BAN); benzo[b]fluoranthene (B(b)FLU); benzo[k]fluoranthene (B(k)FLU); benzo[a]pyrene (BPY); dibenzo[a,h]anthracene (DAN); benzo [g,h,i]perylene (BPE), and indeno[1,2,3-c,d]pyrene (IPY) (*Table 4*). Distribution concentrations were random for all sampling stations. The highest values were mostly found in areas affected by intense maritime traffic, mainly close to big ports and with areas with river inputs. Therefore, the sites with the highest total PAH concentrations were Vlora, Kotor, Baošići, Dubrovnik, Split, Bakar, Rijeka, Pula, Piran, Strunjan, Koper, Trieste, Ancona, Taranto, Neretva and the Po River where they exceeded 30 µm/kg, with the highest values >50 µg/kg (Split, Trieste, and Taranto). Lowest values < 10 µg/kg were found in Novigrad, Po Nord, and Ravenna. The south-western Adriatic Sea showed lower PAH concentrations mostly <20 µg/kg except in the before mentioned ports than the eastern Adriatic Sea.

Specific PAHs - BPE, BPY, B(k)FLU, DAN and IPY concentrations were below the detection limits at all locations, except at Taranto for B(k)FLU. ACY, AN, BAN, CHR and B(b)FLU were heterogeneously distributed at some stations, mostly those with higher total PAH content. At almost all sampling sites the following PAHs were detected: NA (1.6–12.5 µg/kg), ACE (1.5–10.7 µg/kg), FL (1.2–14.1 µg/kg), PHE (2.3–10.8 µg/kg), FLU (1.1–6.1 µg/kg), and PY (1.1–3.4 µg/kg).

According to Bajt et. al. at all sampling sites low molecular weight (LMW) PAHs were more abundant in comparison to the high molecular weight (HMW) PAHs (Figure 8). The proportion between LMW and HMW PAHs was almost equal only at Taranto. The LMW fraction of PAHs is mainly composed of congeners with two and three aromatic rings, while the HMW PAHs are those with four, five and six aromatic rings. PAHs with four rings like CHR, FLU could be either of petrogenic or pyrogenic origin. Petrogenic sources like crude oil and oil derivatives are usually connected with LMW PAHs while HMW PAHs are usually derived from combustion processes of organic matter like wood, coal, and oil (pyrogenic origin). Mixed PAH origin with a significant contribution of petrogenic origin (*Figure 8*).

Chemical characteristics and structure of PAHs define susceptibility to go through physical, chemical and biological transformations in the marine environment. The degradation of PAH isomers could be different, due to their dependence on thermodynamic stability. Many processes can take place after the release of organic pollutants into the natural environment. In coastal waters the spreading of pollutants by seawater currents can be very efficacious. Organic compounds can be transformed during their transport (photooxidation, microbial degradation, evaporation, dissolution, adsorption on particulate matter, and sedimentation) (Haritash and Kaushik, 2009). These processes can also happen after deposition in marine bottom sediments, leading to a different accumulation connected to the molecular weight. It is well known that LMW hydrocarbons are more soluble, volatile, and adequate substrates for microbial degradation (MacRae and Hall, 1998). PAHs with high molecular weights are more likely to end up in sediment after the adsorption on suspended matter. The composition of accumulated compounds does not reflect their origin completely because of different behaviour of hydrocarbons in the marine environment. In this way, their availability in the water column to the biota can be reduced. For gaining more in-depth insight into possible PAH origins, their isomer pairs with similar molecular weight have also been widely used like AN/PHE, FLU/PY; AN/PHE+AN; FLU/FLU+PY. They are based on the thermodynamic stability of isomers in the natural environment.

Figure 9 presents the cross-plot of diagnostic ratios for determining the origin of PAHs in the Adriatic Sea. There is no single PAH origin since the whole Adriatic Sea is under the influence of many pollution sources like industry, maritime traffic, cities and agriculture. Sampling sites were mostly in the range of a pyrogenic origin. However, Kepi Rhodon, Vlora, and Dubrovnik showed a highly expressed pyrogenic origin. A significant group of sites was characterised by relatively low AN/AN+PHE ratio, approaching 0.1, indicating less significant but still prevailing pyrogenic origin. Some stations exhibited mixed origin, among them Kotor, Bar, Neum, Neretva, Trieste, and Falconara. In Ancona were evident mixed origins, with petroleum combustion as prevailing pyrogenic origin. Only Fano appeared as having a prevailing petrogenic origin.

Table 4: Concentration of PAHs in Mytilus galloprivincialis samples in the Adriatic Sea (µm/kg, d.w.) (Bajt et al., 2019).

Code	PAH																
	NA	**ACE**	**ACY**	**FL**	**AN**	**PHE**	**CHR**	**BAN**	**FLU**	**PY**	**BPY**	**B(b)FLUU**	**B(k)FLUU**	**DAN**	**BPE**	**IPY**	**Tot. PAH**
VL AL	1.8	4.2	<5	4.2	5.5	8.8	1.9	1.7	6	2.7	<0.5	1.5	<0.5	<2.5	<2.5	<2.5	**38.3**
KR AL	1.6	3	<5	4.2	4.5	6.5	<0.5	<0.5	2.6	1.2	<0.5	1.1	<0.5	<2.5	<2.5	<2.5	**24.7**
BA ME	2	4	<5	5.3	<0.5	6	1.5	<0.5	2.5	1.8	<0.5	1.1	<0.5	<2.5	<2.5	<2.5	**24.2**
KO ME	4.2	4.9	<5	11.8	<0.5	5.9	2.1	1.2	3.1	2.7	<0.5	1.6	<0.5	<2.5	<2.5	<2.5	**37.5**
BO ME	3.9	4.4	<5	4.3	1.4	6.7	2	1.4	3.4	2.8	<0.5	2.1	<0.5	<2.5	<2.5	<2.5	**32.4**
DU HR	3.4	4.2	<5	7.3	3.9	8.4	2.3	1.1	5	3	<0.5	2.3	<0.5	<2.5	<2.5	<2.5	**40.9**
NE BA	4.7	5	<5	7.7	<0.5	6.1	<0.5	<0.5	2	1.3	<0.5	1.2	<0.5	<2.5	<2.5	<2.5	**28.0**
NE HR	9.7	2.8	11.8	9.1	<0.5	6.9	<0.5	<0.5	2.7	2.4	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**45.4**
CE HR	2.2	4.4	<5	4.9	1.1	6.8	1.3	1.1	2.8	2.3	<0.5	2	<0.5	<2.5	<2.5	<2.5	**28.9**
SP HR	12.5	7.5	12.3	14.1	1.2	7.9	<0.5	<0.5	3.1	1.8	<0.5	1.4	<0.5	<2.5	<2.5	<2.5	**61.8**
SI HR	3	4.2	<5	2.7	<0.5	4.2	<0.5	<0.5	2	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**16.1**

ZA HR	4	5.1	<5	5.4	1.2	8	<0.5	<0.5	2.7	1.6	<0.5	1.5	<0.5	<2.5	<2.5	<2.5	**29.5**
NO HR	<0.5	<0.5	<5	1.2	<0.5	2.3	<0.5	<0.5	1.3	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**5.3**
KR HR	4.4	4.9	<5	2.3	1	5	<0.5	<0.5	2.4	1.3	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**21.3**
BA HR	5.3	6.6	<5	4.6	1.3	8.3	2.9	<0.5	3.6	1.7	<0.5	1.3	<0.5	<2.5	<2.5	<2.5	**35.6**
RI HR	4.8	4.5	<5	2.6	1.3	6.1	2.6	<0.5	3.5	2.1	<0.5	1.6	<0.5	<2.5	<2.5	<2.5	**29.1**
PU HR	6.2	6.3	<5	4.2	1.4	7.5	2.2	<0.5	3.9	2.4	<0.5	1.5	<0.5	<2.5	<2.5	<2.5	**35.6**
LI HR	2.8	5.4	<5	2.8	1.2	5.6	1.1	<0.5	2.4	1.3	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**22.6**
PI SI	1.7	6	<5	3.8	1.3	8.5	1.9	1.3	4.3	2	<0.5	1.5	<0.5	<2.5	<2.5	<2.5	**32.3**
ST SI	5.9	6.5	<5	5.6	1.2	6.2	1.6	<0.5	3.4	1.8	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**32.2**
KO SI	3.5	6.6	<5	3.6	1.1	7.5	2.2	<0.5	5.3	3.2	<0.5	1.8	<0.5	<2.5	<2.5	<2.5	**34.8**
TS IT	6.9	10.7	<5	6.7	<0.5	10.8	3.3	1.2	6.1	3.4	<0.5	2.8	<0.5	<2.5	<2.5	<2.5	**51.9**
PON IT	<0.5	1.5	<5	1.5	<0.5	2.9	<0.5	<0.5	1.4	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**7.3**
PO IT	6.5	6	<5	4.8	1.4	9.9	<0.5	<0.5	4.1	2.6	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**35.3**
PG IT	4.8	5.2	<5	2.9	<0.5	4.7	<0.5	<0.5	1.2	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**18.8**
RA IT	<0.5	2	<5	2.2	<0.5	3.2	<0.5	<0.5	1.1	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**8.5**
RI IT	4.9	5.7	<5	4.4	1.1	5.9	<0.5	<0.5	1.2	1.1	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**24.3**
FA IT	8.2	7.1	<5	6.5	<0.5	6.3	<0.5	<0.5	1.3	2	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**31.4**
FAL IT	6.7	6.9	<5	5.7	<0.5	6.3	<0.5	<0.5	1.3	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**26.9**
AN IT	3.5	6.9	<5	5.2	<0.5	7.4	1.7	<0.5	3.1	3.4	<0.5	1.6	<0.5	<2.5	<2.5	<2.5	**32.8**
SB IT	8.6	10.1	<5	7	1.4	6.3	<0.5	<0.5	2.4	1.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**37.3**
PE IT	2.9	6.4	<5	3.3	<0.5	4.6	<0.5	<0.5	1.4	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**18.6**

VA IT	4.8	3.6	<5	1.9	<0.5	3.3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**13.6**
TR IT	3.5	2.6	<5	1.7	<0.5	2.9	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**10.7**
MA IT	3.5	2.9	<5	3.3	1	4.3	<0.5	<0.5	1.4	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**16.4**
BA IT	4	2.2	<5	2	<0.5	2.9	<0.5	<0.5	1.1	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**12.2**
BR IT	8	1.8	<5	2.2	<0.5	2.7	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<2.5	<2.5	<2.5	**14.7**
TA IT	10.3	3.5	<5	3.1	1.2	6.5	6.6	1.4	5.8	3.2	<0.5	6.7	2.9	<2.5	<2.5	<2.5	**51.2**

Naphthalene (NA); acenaphthene (ACE); acenaphthylene (ACY); fluorine (FL); phenanthrene (PHE); anthracene (AN); fluoranthene (FLU); pyrene (PY); chrysene (CHR); benzo[a]anthracene (BAN); benzo[b]fluoranthene (B(b)FLU); benzo[k]fluoranthene (B(k)FLU); benzo[a]pyrene (BPY); dibenzo[a,h]anthracene (DAN); benzo [g,h,i]perylene (BPE), and indeno[1,2,3-c,d]pyrene (IPY).

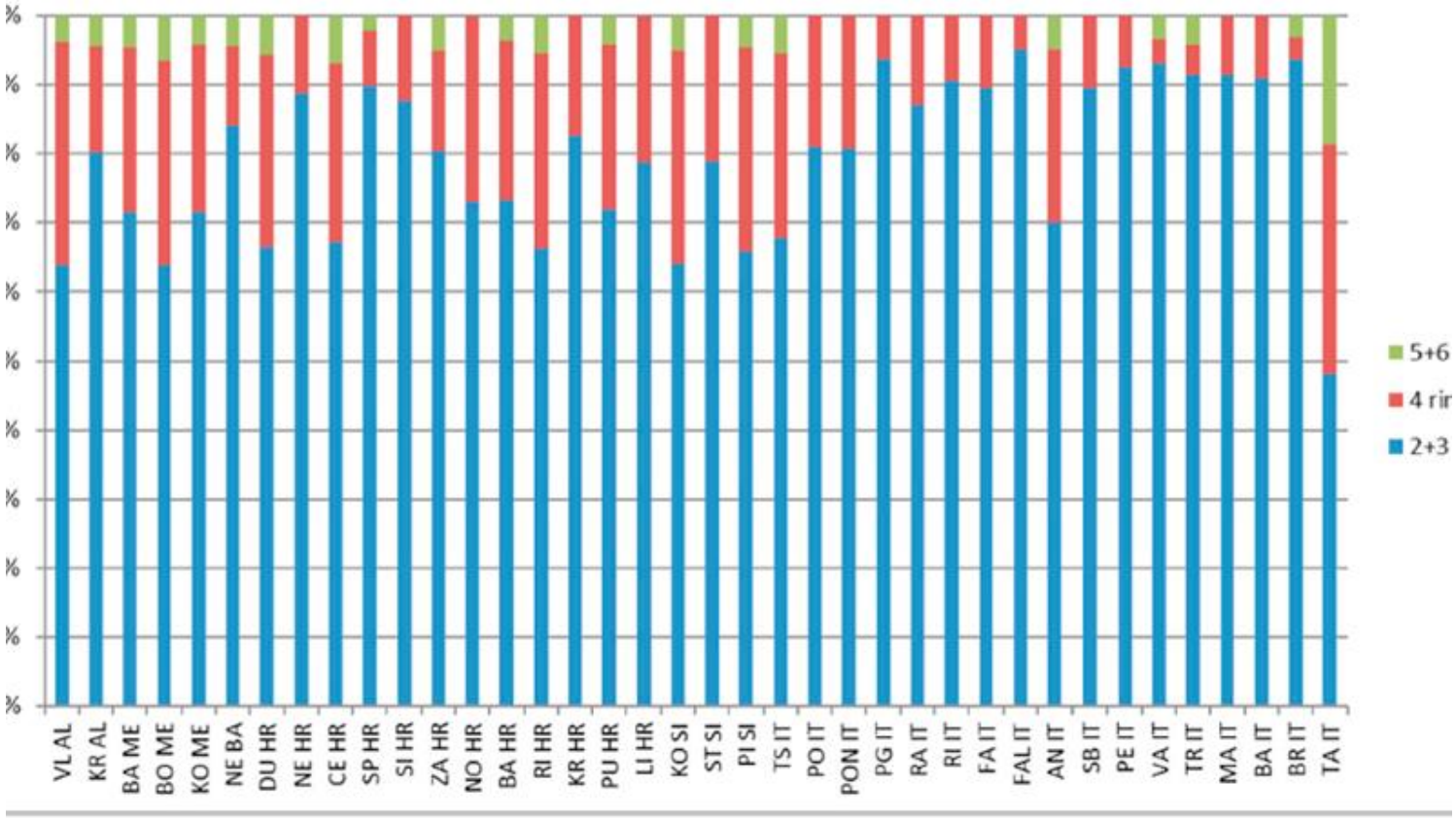

Figure 8: PAH distribution of the number of aromatic rings.

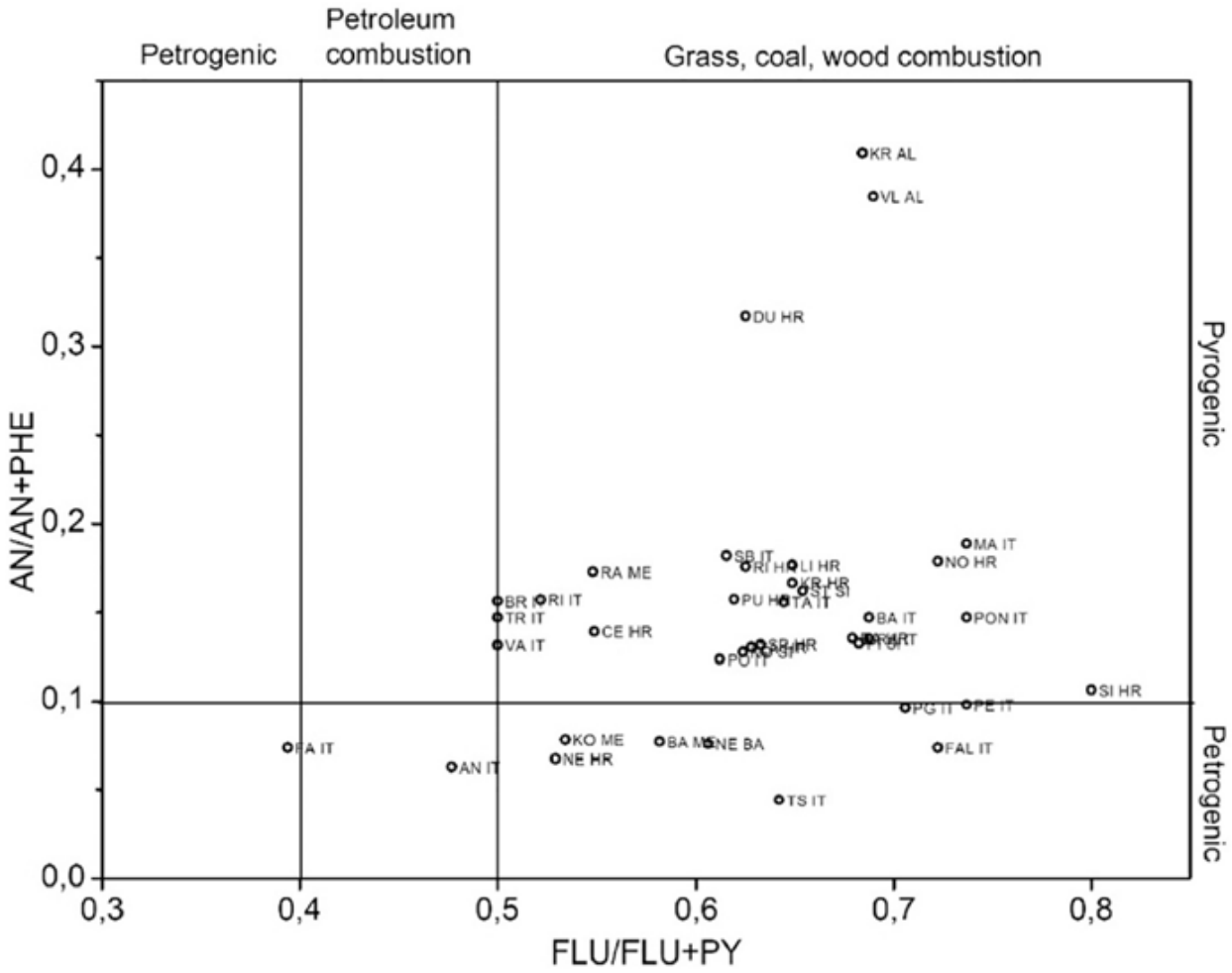

Figure 9: PAH origin determination in the Adriatic Sea (Bajt et al., 2019).

Monitoring data of PAHs received from the "Hrvatske vode" are presented in *Table 5* and *Table 6* for the years 2016 – 2020. Elevated concentrations of fluoranthene and benzo(a)pyrene were recorded in mussels at the measuring station in the Raša water body. Environmental quality standards for biota according to Croatian Official Gazette 78/15 are 5 µg/kg w.w. for benzo(a)pyrene and 30 µg/kg w.w. for fluoranthene which are measured in bivalvia.

Table 5: Concentration of PAHs in Mytilus galloprivincialis samples from the Croatian part of the Adriatic Sea in 2016, 2018 and 2020 in transitional waters (µm/kg, w.w.) (Izvor: Hrvatske vode, 2022).

YEAR	2016			2018			2020		
ESTUARY (TRANSITIONAL WATERS)	Average mussel dry matter (%)	(PAH) (benzo (a) pyrene) (µg/kg)	(PAH) (fluoranthene) (µg/kg)	Average mussel dry matter (%)	(PAH) (benzo (a) pyrene) (µg/kg)	(PAH) (fluoranthene) (µg/kg)	Average mussel dry matter (%)	(PAH) (benzo (a) pyrene) (µg/kg)	(PAH) (fluoranthene) (µg/kg)
Ombla	10.8	1,1	3,6	19.9	0.1	4.4	17.6	0.5	3.6
Ombla	15.1	0,5	2,3	15.3	0.2	11.0	15.2	0.4	2.4
Neretva	8.4	-	-	19.8	0.4	4.7	20.1	0.2	2.1
Neretva	11.1	0,6	2,4	17.2	0.1	3.0	15.9	0.1	1.0
Neretva	15.3	1,8	2,0	15.8	1.0	9.2	14.8	3.0	8.3
Neretva	10.0	1,0	1,9	15.5	0.3	9.5	14.4	0.3	1.3
Cetina	11.8	2,3	8,1	16.0	0.4	3.8	14.9	0.2	1.8
Cetina	12.0	1,8	2,5	16.3	2.7	8.7	15.8	0.4	1.1
Jadro	15.6	2,9	3,5	-	-	-	-	-	-
Jadro	14.2	1,1	2,3	15.3	0.5	3.6	14.2	2.8	3.8
Krka	13.2	1,0	1,7	19.5	2.3	13.7	18.1	0.4	1.4
Krka	13.2	4,2	2,7	20.0	0.1	2.2	19.2	0.4	1.5
Zrmanja	5.1	0,1	0,7	8.7	0.6	2.2	15.3	0.4	1.5

Zrmanja	4.4	0,1	0,7	13.4	0.9	4.8	15.6	0.1	0.7
Rječina	13.9	1,2	3,1	11.9	0.1	3.1	-	-	-
Raša	18.7	0,2	2,1	13.4	18.9	30.0	14.5	0.1	0.7
Mirna	10.1	1,2	1,2	11.1	0.1	2.2	-	-	-
Dragonja	15.0	1,1	2,3	14.4	0.3	2.9	19.9	0.1	0.7

Table 6: Concentration of PAHs in Mytilus galloprivincialis samples from the Croatian part of the Adriatic Sea in 2017 and 2019 in coastal waters (µm/kg, w.w.) (Izvor: Hrvatske vode, 2022).

YEAR	**2017**			**2019**		
DESCRIPTION OF THE WATER BODY (COASTAL WATERS)	**Average mussel dry matter (%)**	**(PAH) (benzo (a) pyrene) (µg/kg w.w.)**	**(PAH) (fluoranthene) (µg/kg w.w.)**	**Average mussel dry matter (%)**	**(PAH) (benzo (a) pyrene) (µg/kg w.w.)**	**(PAH) (fluoranthene) (µg/kg w.w.)**
Župski bay-Cavtat	14.3	0.1	2.3	85.1	3.0	9.0
Mj, Hv, Ko, Vi channel	12.6	0.5	3.5	87.2	4.3	10.9
Mali Ston bay	13.6	0.1	1.6	86.3	1.2	3.6
Malo more and Neretva channel	12.2	0.3	1.8	84.3	0.2	1.6
Brač and Split channel	13.6	0.6	3.7	86.2	0.8	2.8
Port Split	12.3	0.4	5.4	84.5	1.3	7.5
Trogir, Kastela and Marina bay	17.8	0.6	5.7	83.5	0.8	6.2
Kaštelanski bay	16.9	0.7	3.6	81.9	0.3	3.1
Kornati and Šibenik area	16.3	1.2	5.7	89.6	2.9	7.5
Pašman and Zadar bay	12.8	1.6	11.4	88.1	2.2	4.6
From Kvarnerić to Pag channel	14.4	0.8	3.0	82.0	0.2	3.3
South part of Istrian peninsula to Long Island	18.2	0.2	3.0	86.4	0.4	3.0
South part of Velebit channel	15.6	0.7	5.6	90.4	1.9	4.3
Inlet of Pag settlement	14.6	0.9	12.2	87.3	1.1	7.7
Dio Kvarnerića i dio Veleb channel	12.6	0.5	4.4	86.9	1.2	5.5

Pag channel	17.2	0.5	7.6	86.6	1.4	5.4
Vinodol channel	16	0.3	3.6	86.6	0.1	2.2
Bakar bay	19.3	0.2	5.2	89.5	0.9	6.2
Port Rijeka	14.3	0.3	3.9	90.8	0.9	5.0
Rijeka bay	10.2	0.3	1.7	89.3	0.5	3.1
Kvarner	16.1	0.3	5.6	84.7	0.2	3.6
Inner part of Raša	14.2	0.2	2.4	85.6	0.3	3.3
Port Pula	13.1	0.9	6.4	88.7	1.6	5.6
West coast of Istrian peninsula	13.7	0.8	5.5	84.6	0.9	5.8
West coast of Istrian peninsula	-	-	-	89.8	2.9	5.7
Lim channel	15.1	0.1	2.2	88.2	0.1	1.4

5.3 Chlorinated pesticides

Chlorinated pesticides including α- and β- endosulfan, α-, β- and γ-HCH, aldrin, dieldrin, endrin, isodrin, hexachlorobenzene, p,p'-DDT, p,p'-DDE, and p,p'-DDD (13 in total) were determined in caged mussel samples, collected along the Adriatic coast (*Table 7*). Concentrations of α-endosulfan (<limit of quantification (LOQ) - 2.8 µg/kg in Dubrovnik) and β-endosulfan (< LOQ - 4.9 µg/kg in San Benedetto) were very low at all sites, with concentrations below LOD (limits of detection) at 17 stations. The highest total endosulfan concentrations were found in general along the Italian Adriatic coast, at stations like San Benedetto (5.4 µg/kg), Po (5.3 µg/kg), Pescara (5.1 µg/kg), Trieste (4.7 µg/kg), and Bakar (5.1 µg/kg) in the Croatian coast. The lowest values from 1.2 µg/kg to 2.4 µg/kg were found at Manfredonia, Piran, Pula, and Neretva. Levels of α-, β- and γ-HCH, as well as hexachlorobenzene, were below LOQ (0.5 µg/kg) at all sampling sites.

Data for the cyclodiene pesticides were high concentrations of endrin ranging from 3.0 µg/kg in Šibenik to 32.1 µg/kg in Kotor, moderate concentrations of aldrin (< LOQ - 11.1 µg/kg in Bakar) and dieldrin (< LOQ - 8.4 µg/kg in Porto Garibaldi), and low concentrations of isodrin (< LOQ - 4.0 µg/kg in Zadar). The most abundant pesticide in the Adriatic was endrin (*Figure 10*). Relatively high endrin concentrations sites were Po (28.1 µg/kg), Split (24.0 µg/kg), Neretva (19.9 µg/kg), Manfredonia (19.5 µg/kg), and Pula (19.0 µg/kg). All the other stations had average values, ranging from 9.6 µg/kg (Taranto) to 17.7 µg/kg (Fano).

Table 7: Concentration of pesticides in Mytilus galloprovincialis species in Adriatic Sea (µg/kg/d.w.) (Bajt et al., 2019).

Code	Aldrin	α-endosulfan	α-HCH	β-endosulfan	β-HCH	p,p'-DDD	p,p'-DDE	p,p'-DDT	Dieldrin	Endrin	γ-HCH	Hexachlorobenzene	Isodrin	Hexachlorobutadiène
VLAL	0.5	0.5	0.5	0.5	0.5	2.2	4.3	2.7	0.5	15	0.5	0.5	0.5	5
KRAL	0.5	0.5	0.5	0.5	0.5	6.9	4.1	4	0.5	5.6	0.5	0.5	0.5	5
BAME	1.1	0.5	0.5	0.5	0.5	1.4	2.3	1.1	0.5	11.6	0.5	0.5	0.5	5
KOME	0.5	0.5	0.5	0.5	0.5	5.3	12.2	3.5	1.5	32.1	0.5	0.5	1.2	5
BOME	0.5	0.5	0.5	0.5	0.5	1.6	3.4	1.9	4.4	17	0.5	0.5	0.5	5
DUHR	0.5	2.8	0.5	0.5	0.5	0.5	2.3	2.9	0.5	15.6	0.5	0.5	3.5	5
NEBA	0.5	0.5	0.5	0.5	0.5	0.5	1.8	0.5	1.9	3.2	0.5	0.5	0.5	5
NEHR	1.7	1.9	0.5	0.5	0.5	0.5	2.3	5.4	0.5	19.9	0.5	0.5	1.2	5
CEHR	1.3	0.5	0.5	0.5	0.5	0.5	1.5	1.3	0.5	10.8	0.5	0.5	0.5	5
SPHR	1.1	1.2	0.5	2.4	0.5	2.8	1	0.5	1.9	24	0.5	0.5	0.5	5
SIHR	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.8	0.5	3	0.5	0.5	0.5	5
ZAHR	2.6	0.5	0.5	3.3	0.5	1.6	1.2	2	2.6	14.7	0.5	0.5	4	5
NOHR	0.5	0.5	0.5	0.5	0.5	0.5	1.1	1.7	2.2	4.7	0.5	0.5	0.5	5

KR HR	1.1	0.5	0.5	0.5	0.5	0.5	3.3	0.5	2.3	10.1	0.5	0.5	0.5	5
BA HR	11.1	2.7	0.5	2.4	0.5	0.5	1.1	1.3	1.8	10.7	0.5	0.5	1.8	5
RI HR	0.5	0.5	0.5	3.1	0.5	0.5	1.6	1.2	0.5	16.3	0.5	0.5	2.5	5
PU HR	1.5	0.5	0.5	1.7	0.5	0.5	4.8	1.3	0.5	19	0.5	0.5	0.5	5
LI HR	0.5	0.5	0.5	2.4	0.5	0.5	3.6	0.5	1.4	12.4	0.5	0.5	0.5	5
PI SI	1.6	1.4	0.5	0.5	0.5	0.5	0.5	0.5	2.6	13.3	0.5	0.5	0.5	5
ST SI	0.5	1.3	0.5	1.6	0.5	0.5	0.5	0.5	0.5	14.9	0.5	0.5	0.5	5
KO SI	1.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.4	12.1	0.5	0.5	0.5	5
TS IT	4	2.5	0.5	2.2	0.5	0.5	0.5	0.5	2.6	15.8	0.5	0.5	2.2	5
PO N IT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4.2	0.5	0.5	0.5	5
PO IT	3	1.8	0.5	3.5	0.5	0.5	0.5	0.5	6.7	28.1	0.5	0.5	0.5	5
PG IT	2	2.2	0.5	1.9	0.5	0.5	0.5	0.5	8.4	12.7	0.5	0.5	2	5
RA IT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.3	4.7	0.5	0.5	0.5	5
RI IT	1.1	1.4	0.5	1.1	0.5	0.5	0.5	0.5	2.5	11.9	0.5	0.5	3.2	5
FA IT	2.8	0.5	0.5	3.5	0.5	0.5	0.5	0.5	4.1	17.7	0.5	0.5	0.5	5
FAL IT	2.2	1.6	0.5	3.3	0.5	0.5	0.5	0.5	7	12.1	0.5	0.5	1.5	5

AN IT	0.5	1.6	0.5	2.5	0.5	0.5	0.5	0.5	4	16.3	0.5	0.5	2	5
SB IT	1.2	0.5	0.5	4.9	0.5	1.6	5	0.5	1.1	7.9	0.5	0.5	3.6	5
PE IT	0.5	2.3	0.5	2.8	0.5	0.5	3.3	0.5	2.1	15.8	0.5	0.5	1.5	5
VA IT	0.5	0.5	0.5	0.5	0.5	0.5	1.7	0.5	0.5	5.7	0.5	0.5	0.5	5
TR IT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	6	0.5	0.5	0.5	5
MA IT	0.5	0.5	0.5	1.2	0.5	0.5	1.9	0.5	0.5	19.5	0.5	0.5	2.6	5
BA IT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	5.8	0.5	0.5	0.5	5
BR IT	0.5	0.5	0.5	0.5	0.5	0.5	1.1	0.5	0.5	6.9	0.5	0.5	0.5	5
TA IT	2.5	0.5	0.5	0.5	0.5	0.5	4	0.5	1.8	9.6	0.5	0.5	0.5	5

Drins sum revealed the highest concentrations at Kotor 34.8 µg/kg, Baošići 19.6 µg/kg, and Dubrovnik 19.6 µg/kg. A similar concentration of all drins was measured in the central Adriatic Sea, at Neretva (22.8 µg/kg), Split (27.0 µg/kg), and Zadar (23.9 µg/kg). The highest value in the northern Adriatic was measured at Po (37.8 µg/kg), followed by the same extent at Bakar (25.4 µg/kg), Trieste (24.6 µg/kg), Port Garibaldi (25.1 µg/kg), and Fano (24.6 µg/kg). Along the Italian coast, similar concentrations were observed at Falconara, Ancona, Pescara, and Manfredonia from 22.1 µg/kg to 22.8 µg/kg.

Range of DDT concentrations were from <LOQ to 5.4 µg/kg at Neretva, while its metabolites DDE were from <LOQ to 12.2 µg/kg at Kotor and DDD from <LOQ to 6.9 µg/kg at Kepi Rhodon (*Figure 11*). Before mentioned three compounds presented as DDTs had relatively higher concentrations, (1.5 µg/kg–21 µg/kg), were detected along the Albanian coast at Vlora, 9.2 µg/kg and Khepi Rhodon, 15.0 µg/kg, whereas the highest value was found at Kotor Bay with 21 µg/kg. At Baošići (6.9 µg/kg) and Dubrovnik (5.2 µg/kg) concentrations were a bit lower. In the central Adriatic Sea, the highest value was found at the Neretva River mouth in Croatia (7.7 µg/kg), followed by Zadar (4.8 µg/kg) and Split (3.8 µg/kg). At the northern Adriatic Sea values detected were from 2.4 kg to 6.1 µg/kg, the highest level was observed at Pula. In mussels from Krk, Bakar, Rijeka, and Lim, DDTs concentrations were from two to three times lower in comparison to the sites mentioned above. In the Slovenian and northern Italian coasts, no DDTs were detected. On the other hand, low levels (< LOQ - 3.3 µg/kg) were measured in the central Italian coast, with the highest level at Pescara (3.3 µg/kg), followed by two times lower values at Falconara and Ancona. In southern Italy (< LOQ - 4.0 µg/kg), the highest level was measured at Taranto (4.0 µg/kg), and the lowest at Manfredonia (1.9 µg/kg) and Brindisi (1.1 µg/kg).

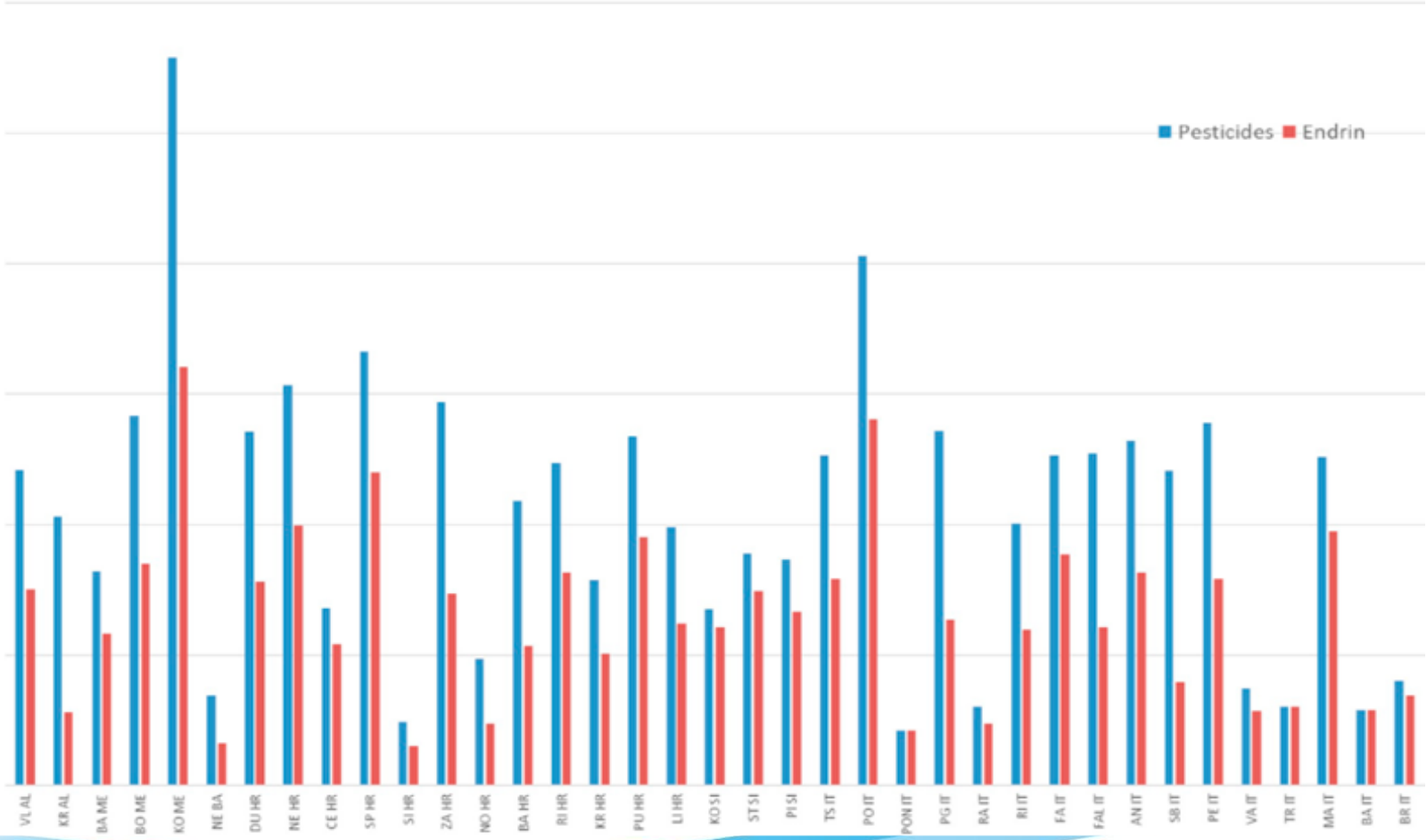

Figure 10: Total concentrations of pesticides (including endrin) in Mytilus galloprovincialis samples (µm/kg, d.w.), and concentration of endrin alone (Bajt et al., 2019).

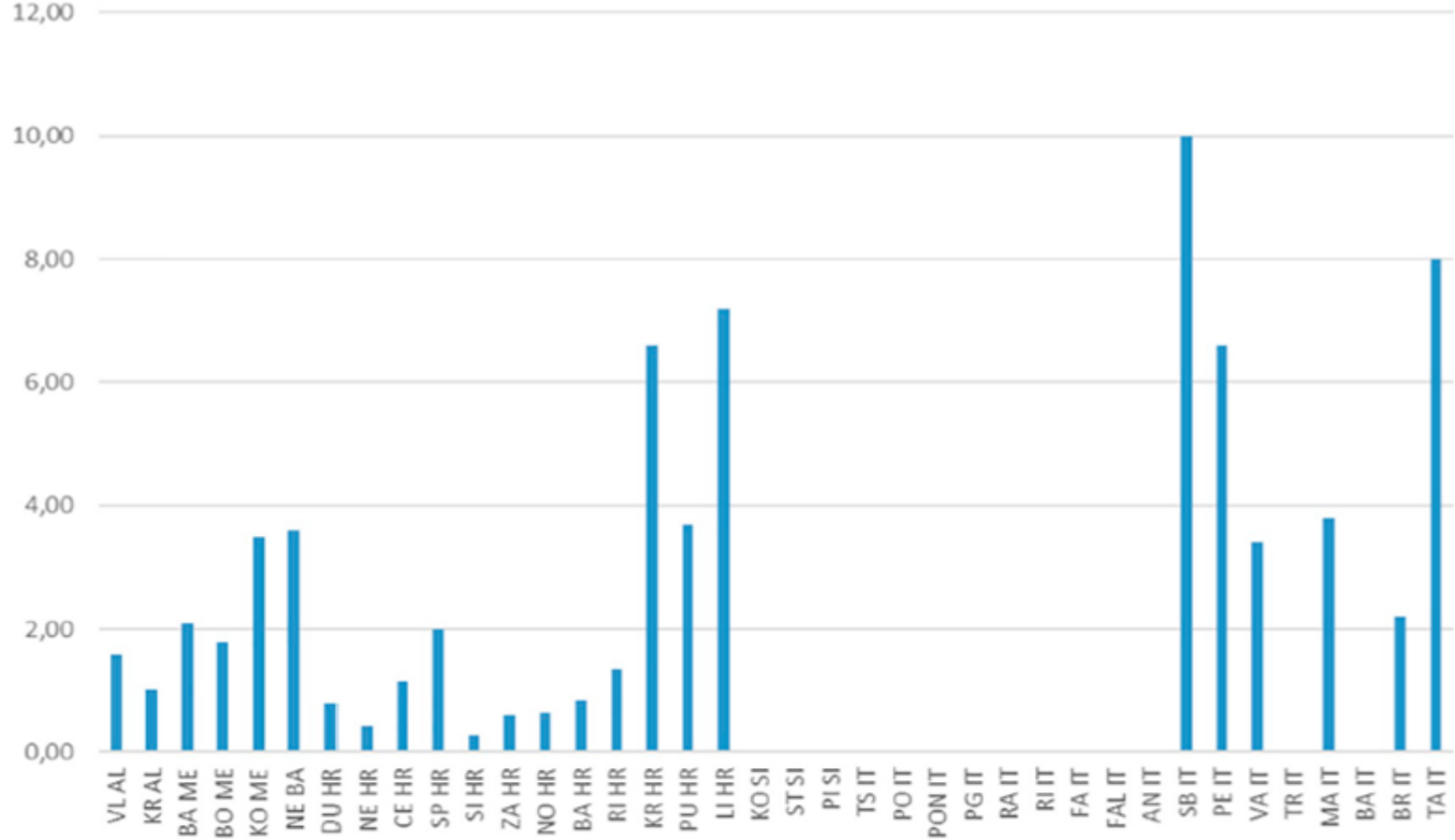

Figure 11: Ratio between DDE and DDT at sampling sites in the Adriatic Sea, sites without bars were below detection limit (µm/kg, d.w.), (Bajt et al., 2019).

Data collected from the Environmental reports of Republic of Croatia (HAOP, 2022) on pesticides dichloro-diphenyl-trichloroethane (DDT) are presented in *Figure 12* and PCBs in *Figure 13*. Previously mentioned compounds are in the environment in low levels, but are transmitted over long distances by water and by air, and as such are widespread. The results of monitoring are indicating that PCBs are present in significantly larger quantities compared to DDTs, which points on the predominant impact of industrial pollution over agricultural throughout the area.

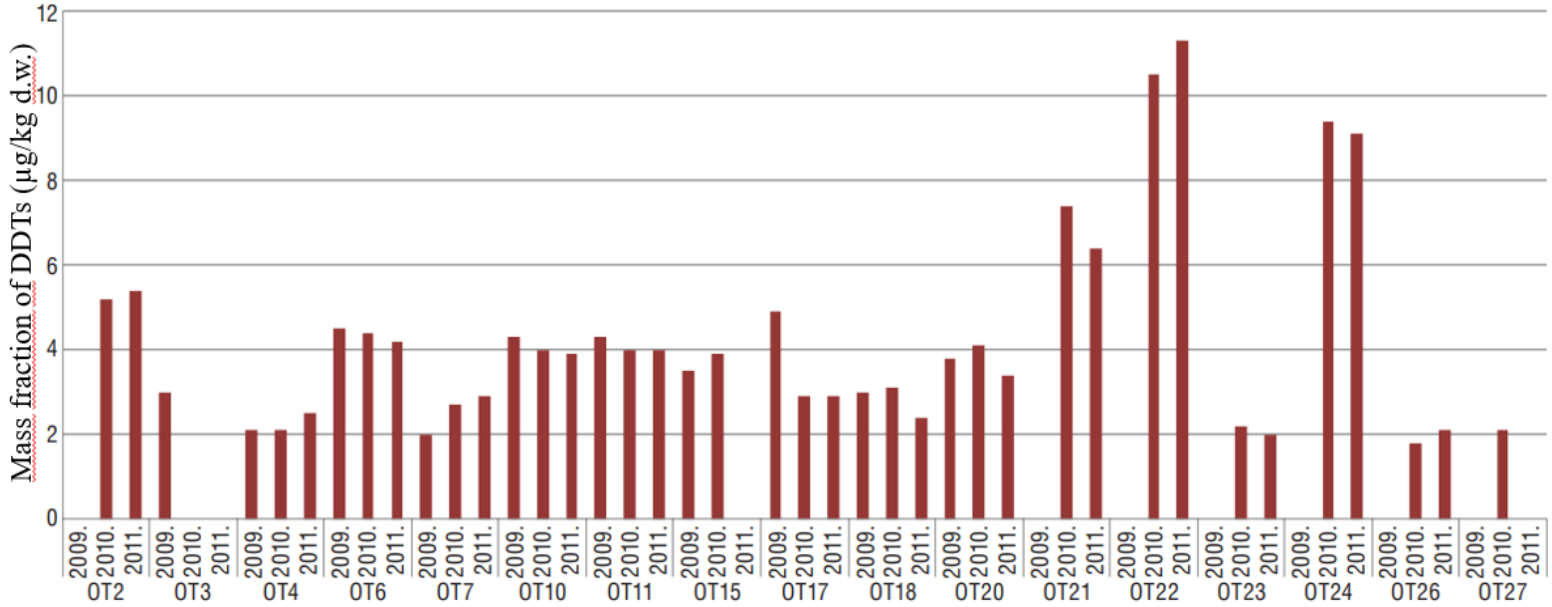

Figure 12: DDT mass fraction at sampling sites along the Croatian coast from 2009 to 2011 (HAOP, 2022).

5.4 Polychlorinated biphenyls

Data on polychlorinated biphenyls are presented in Table 8 according to Bajt et al. 2019. PCB accumulation in transplanted individuals of *M. galloprovincialis* including PCB 28; PCB 31; PCB 152; PCB 101; PCB 105; PCB 118; PCB 138; PCB 153; PCB 156; and PCB 180 (*Figure 14*) is presented as a sum of ten PCB congeners (Figure 13). The sum of the ten PCBs ranged from 3.7 µg/ kg in sampling station Po Nord to 114.4 µg/kg in Taranto. Detection of slightly higher accumulations in the south-eastern Adriatic Sea included Baošići (66.0 µg/kg) and Kotor (59.6 µg/kg) in Montenegro and Neretva (41.1 µg/kg) in Croatia. All analysed locations had PCB 153, ranging from 2.6 at Po Nord to 46.4 µg/kg at Taranto, while PCB 138 was<LOQ only at Neretva, with the maximum value always found at Taranto 18.9 µg/kg. PCB 52 and 180 were detected at eight locations out of thirty-nine. PCB 105 was found at seven sampling stations, whereas 156 was found only at three. The tri-chlorinated congeners PCB 31 and PCB 28 were not detected at any analysed station.

Table 8: Concentration of PCB-s in Mytilus galloprovincialis samples from the Adriatic Sea (µg/kg/d.w) (Bajt et al., 2019).

Code	PCB 101	PCB 105.	PCB 118.	PCB 138.	PCB 153	PCB 156	PCB 180	PCB 28	PCB 31	PCB 52	Pentachlorobenzene
VL AL	1.3	0.5	1	4.8	12.4	0.5	0.5	0.5	0.5	0.5	5
KR AL	0.5	0.5	0.5	3.1	8.2	0.5	0.5	0.5	0.5	0.5	5
BA ME	0.5	0.5	1.5	4	9	0.5	0.5	0.5	0.5	0.5	5
KO ME	3.6	2	15.3	12.7	24.1	0.5	1.9	0.5	0.5	0.5	5
BO ME	7.2	1.2	5.5	17.5	31.3	1.1	2.2	0.5	0.5	0.5	5
DU HR	2.4	2.9	4	3.6	17.5	0.5	2.1	0.5	0.5	0.5	5
NE BA	1.8	0.5	1.6	5.4	13	0.5	0.5	0.5	0.5	0.5	5
NE HR	0.5	1.2	15.1	0.5	10.2	13.5	1.1	0.5	0.5	0.5	5
CE HR	0.5	4.8	2.1	2.4	8.9	0.5	0.5	0.5	0.5	1	5
SP HR	2.6	1.1	2.8	4.7	13.2	0.5	1.7	0.5	0.5	1.7	5
SI HR	4.3	1.4	7.6	1.1	4.6	0.5	0.5	0.5	0.5	1.2	5
ZA HR	0.5	0.5	1.1	2.4	7.7	0.5	1	0.5	0.5	0.5	5

NO HR	0.5	0.5	1.7	1.1	5.2	0.5	0.5	0.5	0.5	0.5	5
KR HR	0.5	0.5	1.2	3.7	8.4	0.5	0.5	0.5	0.5	0.5	5
BA HR	1.9	0.5	2.8	8.8	17.8	0.5	1.6	0.5	0.5	0.5	5
RI HR	1.6	1	3.1	9.9	20.9	0.5	1.2	0.5	0.5	0.5	5
PU HR	1.7	0.5	1.9	6.4	14	0.5	0.5	0.5	0.5	0.5	5
LI HR	0.5	0.5	0.5	3.5	8.6	0.5	0.5	0.5	0.5	0.5	5
PI SI	1.1	0.5	1.2	4.2	10.3	0.5	0.5	0.5	0.5	0.5	5
ST SI	1.5	0.5	1.5	4	9.3	0.5	0.5	0.5	0.5	0.5	5
KO SI	2.8	0.5	3.2	7.8	17.7	0.5	0.5	0.5	0.5	1.1	5
TS IT	2.7	0.5	2.3	5.7	12.6	0.5	0.5	0.5	0.5	0.5	5
PON IT	0.5	0.5	0.5	1.1	2.6	0.5	0.5	0.5	0.5	0.5	5
PO IT	2.4	0.5	1.7	5.1	11.2	0.5	0.5	0.5	0.5	0.5	5
PG IT	1.7	0.5	1.3	3.8	6.8	0.5	0.5	0.5	0.5	0.5	5
RA IT	1.2	0.5	0.5	2.8	5.5	0.5	0.5	0.5	0.5	0.5	5
RI IT	2.7	0.5	2.1	5.7	11.2	0.5	0.5	0.5	0.5	1	5
FA IT	2.8	0.5	2.3	5.9	12.3	0.5	0.5	0.5	0.5	0.5	5
FAL IT	2.2	0.5	1.7	4.4	9.5	0.5	0.5	0.5	0.5	0.5	5
AN IT	2.5	0.5	1.3	4.2	6	0.5	0.5	0.5	0.5	1.1	5
SB IT	2.3	0.5	2.8	7.3	13.7	0.5	0.5	0.5	0.5	1.1	5
PE IT	2.4	0.5	0.5	3.3	7	0.5	0.5	0.5	0.5	0.5	5
VA IT	0.5	0.5	1	1.7	5	0.5	0.5	0.5	0.5	0.5	5
TR IT	0.5	0.5	0.5	1.7	5.3	0.5	0.5	0.5	0.5	0.5	5
MA IT	1.3	0.5	1.9	2.6	10.1	0.5	0.5	0.5	0.5	0.5	5
BA IT	1.5	0.5	1.3	2.3	5.8	0.5	0.5	0.5	0.5	0.5	5
BR IT	0.5	0.5	0.5	2.4	7.2	0.5	0.5	0.5	0.5	0.5	5
TA IT	17.7	3	16	18.9	46.4	1.2	4.2	0.5	0.5	7	5

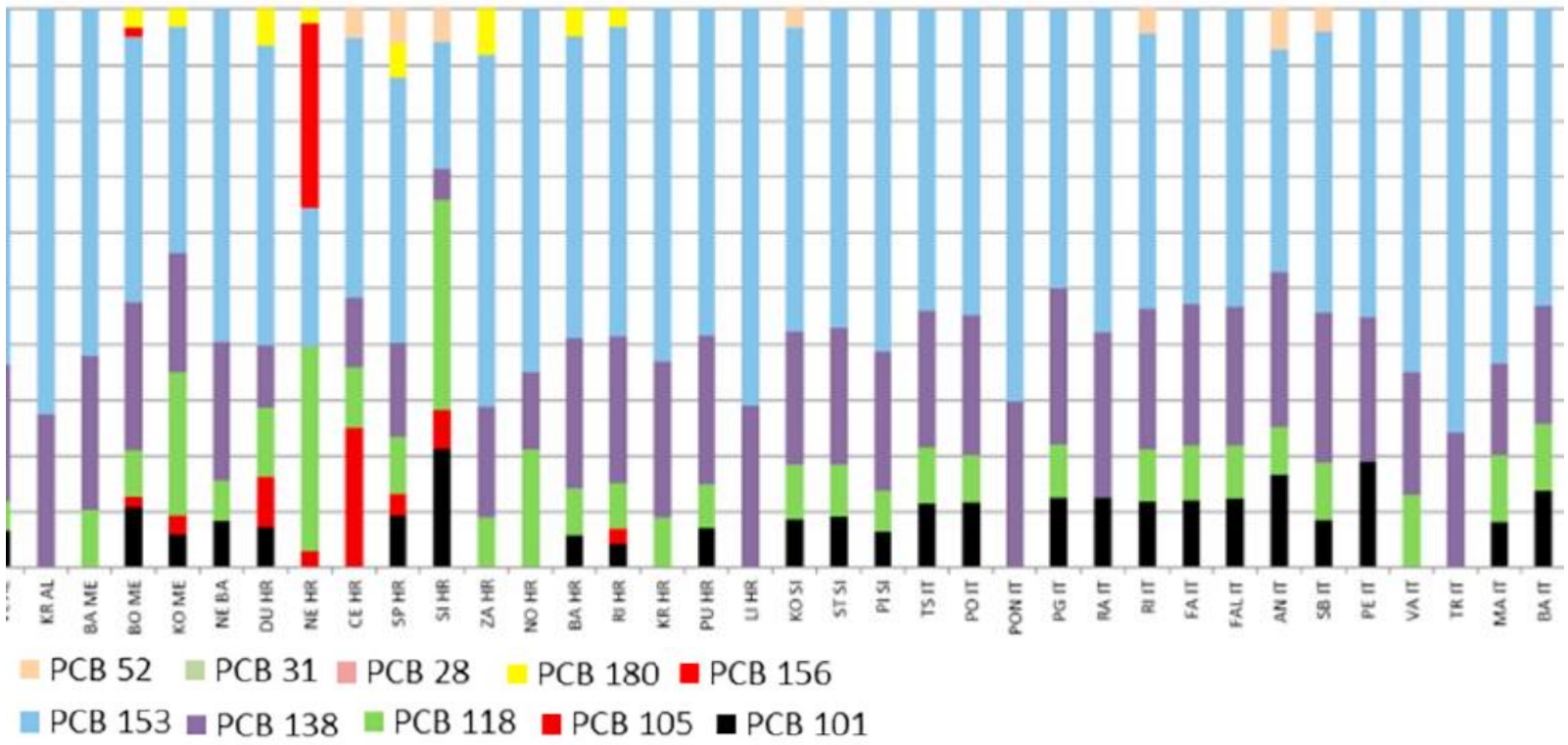

Figure 13: Proportions of different PCB congeners in Mytilus galloprovincialis samples (Bajt et al., 2019).

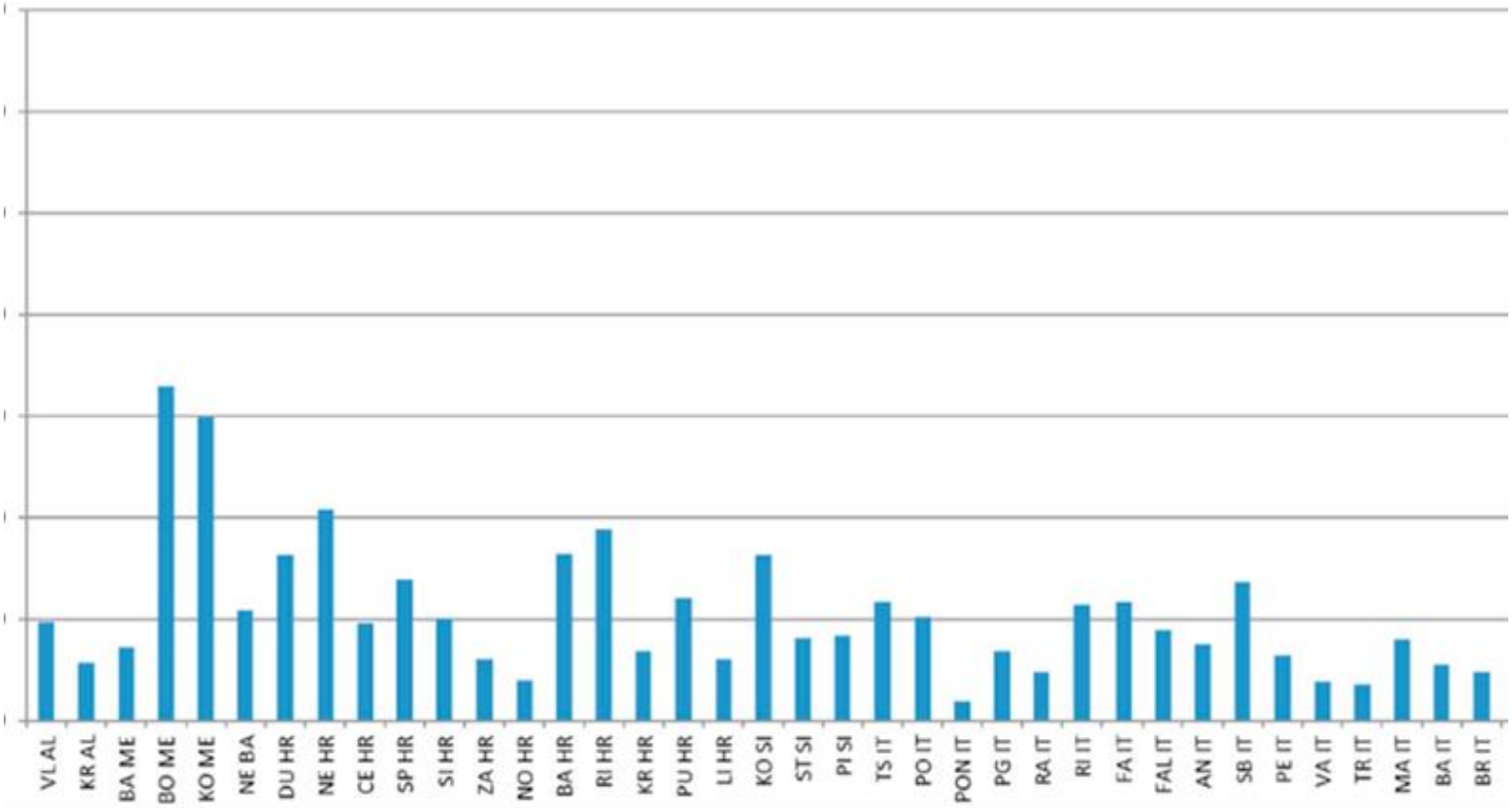

Figure 14: Concentrations of total PCB in Mytilus galloprovincialis samples (µg/kg, d.w.) (Bajt et al., 2019).

Based on the spatial distribution of the mass fractions of hazardous substances in the eastern Adriatic coast (*Figure 15*), the largest mass fractions are primarily for PCBs, and significantly less for chlorinated pesticides in the area Dubrovnik (OT02, OT03), Split (Kaštela Bay): OT10, OT11), Bakar Bay (OT21), Rijeka (OT22) and Pula (OT24). This is the result of many years of discharging industrial and municipal wastewater in coastal areas, respectively by law prohibited production and use of chlorinated pesticides.

The values of mass fractions of lindane are generally below or slightly above the detection limit of the test method (<0.05 µg/kg d.w.), and values of mass fractions of DDT compounds, as and PCB contamination levels do not show significant changes. By analysing the collected data, considering their time distribution, an increase in mass fractions was generally found mainly for all ecotoxic metals, especially in areas under the immediate anthropogenic impact, while a mass fractions decrease was found in polychlorinated biphenyls, lindanes, and DDTs on the majority of sampling stations.

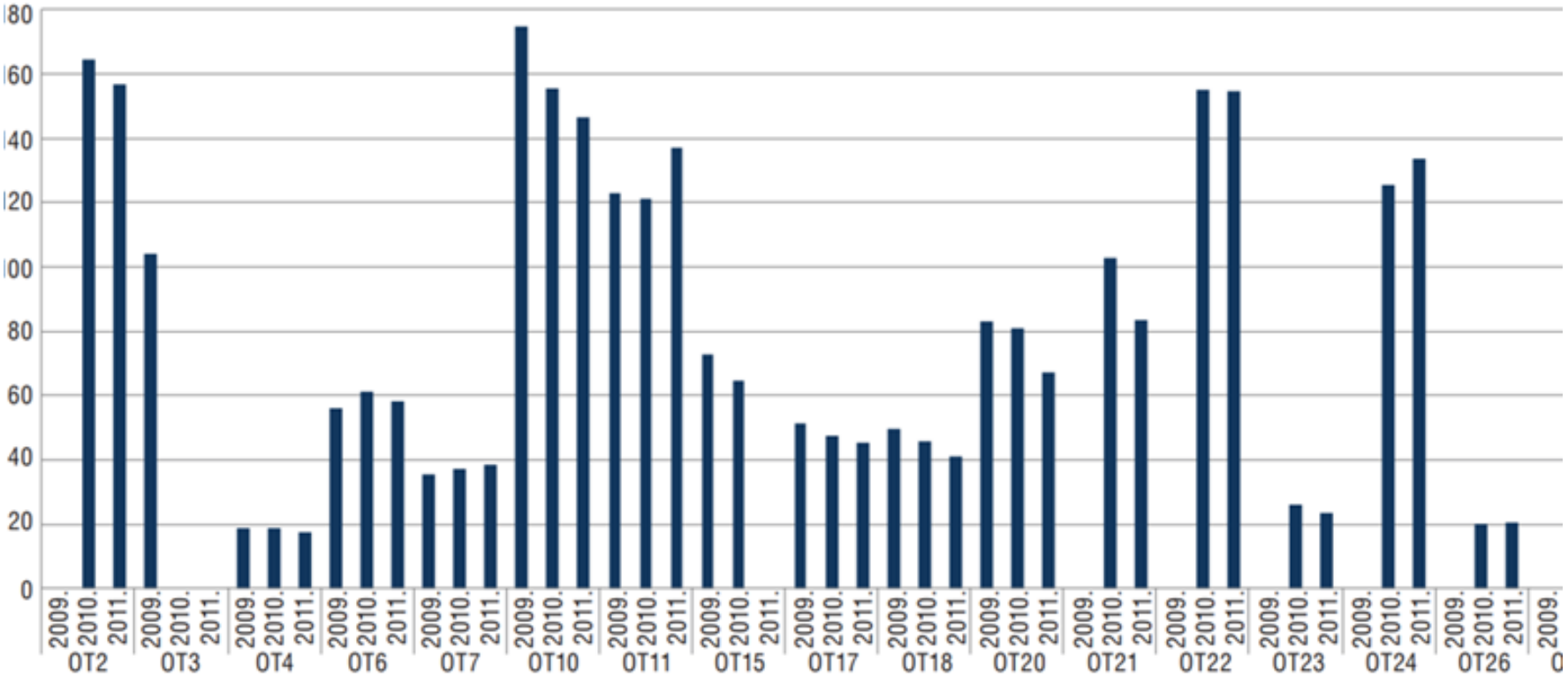

Figure 15: Concentrations of total PCB in Mytilus galloprovincialis samples in the Croatian Adriatic Sea (µg/kg, d.w.)(HAOP, 2022).

5.5 Statistical analysis

Statistical analysis of the variability between the studied sampling sites from 2008. in Bajt at. al. 2019 was done using PCA for metals, total PAH, total PCB, and total organochlorinated pesticides. Standardized concentrations were considered in PCA. PCA analysis factor scores are presented in Figure 16. The first PC accounted for 27% and the second for 22%, together explaining 49% of the total variance. Metals showed high positive factor scores for PC1 and/or PC2 but organic pollutants showed high negative loading of PC1. Based on these two components, it is difficult to divide the sampling sites into separate groups. PCA shows that the majority of sites on the Croatian and Slovenian coasts and some in the Italian coast of the northern Adriatic Sea, with low PC1 and PC2 loadings, did not have any significant accumulation of the studied compounds. Although, some of the sites formed a separate group in the lower part of the PC2 (Ancona, Falconara, Po Nord, Port Garibaldi, Fano, Rimini, Ravenna). They are geographically situated along the western central Adriatic coast. South-western Adriatic Sea including Bari, Brindisi, Vasto, Tremiti, and Novigrad in Croatia showed higher accumulation of particular metals, like Zn, Cd, and V. A higher accumulation of particular metals is characteristically for the south-eastern Adriatic Sea, including Cu and Hg in Neretva, Dubrovnik, Baošići, and Cr and Ni in Kepi Rhodon, Vlora, and Neum. A high accumulation of organic pollutants (PAH and/or chlorinated compounds) was detected in Split, Kotor, Pula, Trieste, and Taranto, which are sites affected by intense maritime traffic and/or industrial activities.

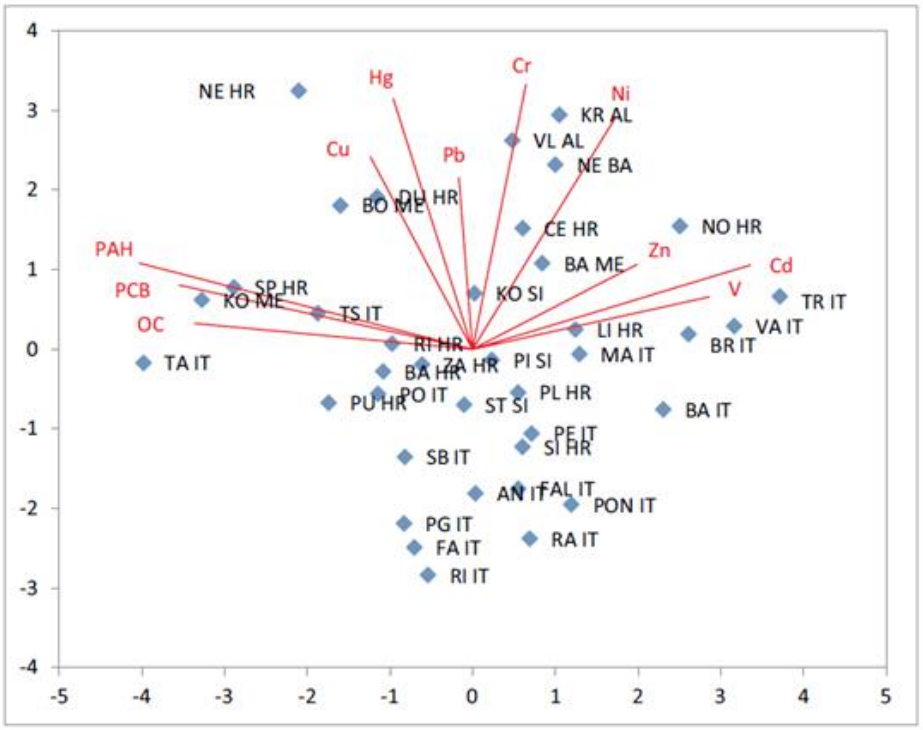

Figure 16: PCA analysis (Bajt et al., 2019).

6 Guidelines and indicators for proper consumption of mussel and shellfish to prevent toxicity and human health risks

The estimated public health risk related to a specific food / microplastic combination concerning the following factors:

1) **The dose / response relationships** to detected the exposure assessment. These data initially obtained from the literature ("DeFishGear project" - IPA Adriatic Cross-border Cooperation Program), were subsequently verified with tests of biological effects in relation to the concentration of microplastics used in experiments ("number of MPs particles per gram") and to time of exposure. For lowering the number of MPs in the mussels and shellfish it is possible to use methodology described in the Deliverable D.4.4.3. In the Deliverable D.4.4.3 evaluation of clearance gut rate in bivalves for risk assessment associated with their consumption is described. In WP4 - Act. N. 4.4 Microplastics and risks for human health It has been demonstrated by Prof. Barile that very short exposure times (10, 20 and 40 minutes) to spheres of microplastics do not give evident results because three exposure times are too short to allow mussels to accumulate these contaminants. While 24 hours to 3 days are significant. At the end of the 3-day exposure period, mussels were removed from the exposure tanks and thoroughly rinsed to avoid any transfer of microplastics. 20 mussels (0-time group) were removed and prepared for digestion and qualitative-quantitative analysis of the MPs, while other organisms were moved to another tank with clean seawater and subsequently undergone to purification process. The purification phase lasted a total of 7 days during which monitoring of mortality, renewal of water and feeding of the organisms were carried out on a daily basis. At the end of the 2-day purification period, 20 mussels (2-time group) were removed and prepared for digestion and qualitative-quantitative analysis of MPs, while other organisms continued purification for up to 7 days. At the end of the 7-day purification period, 20 mussels (7-time group) were removed and prepared for digestion and qualitative-quantitative analysis of MPs.7 days of purification (experimental purification) it was possible to highlight a statistically significant decrease (p value 2.5E-14) in the presence of the number of microplastic particles found per gram of soft tissue of the analysed mussels (Group T0: 2.17 MPs/g; Group T2: 0.49 MPs/g; Group T7: 0.27 MPs/g).
2) **Prevalence or exposure assessment** which depends on the consumption of mussel and shellfis frequency and serving size in the in the food combined with the concentration of microplastiche time of consumption.
3) **Composition of microplastics.** There are different types of plastics on the market, the most common are polystyrene (PS), polypropylene (PP), high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET) and polyvinyl chloride (PVC). Based on the types of plastics identified by UNITS in WP 4.4.3, three different shapes were selected to simulate the microplastic particles type observed in the real environment, such as in the study of Qu et al., 2018.

MPs Type	Type polimer	Size class	Purchased
Beads	*Polystyrene RED*	- 100 µm - 200 µm	ThermoScientific™
Fibers	*Polypropylene*	- < 1000 µm - 1000 -2000 µm - 2000 -3000 µm - > 3000 µm	LA MATASSINA
Fragments	*Polyethyleneterephthalate (PET)*	- 10 - 50 µm - 50 – 100 µm - 100 – 500 µm - 500 -1000 µm - > 1000 µm	Montello SPA

In the experiments from Defmar mussel farm located in Termoli were used microplastic purchased on the market and/or supplied by PET recycling companies that have flakes or pellets as the final products. Reducing the 'probability of occurrence' corresponds to the probability of observing an adverse effect specific to a particular hazard and associated with the consumption of a mussel containing plastic that housing times are required. These housing times can be assessed in relation to the amount of plastic actually present in the mussel farming environment. Compared to the experiment conducted in the tank, in the marine environment the plastics present are much lower so that it may be necessary the housing times much lower to guarantee plastic-free mussels. So the housing times must be estimated in relation to the actual pollution encountered. This depuration time allows disability-adjusted years of life (DALY) to approach zero. The life (DALY) is a common metric or indicator of time lived with a disability and time lost due to premature mortality associated with the adverse effect.

4) **Plastic particle size and "Determination of the fate of microplastics within cells".** Evidence suggests that MPs of size <20 µm can penetrate organs, and MPs <10 µm can penetrate cell membranes and cross the placental barrier in exposed cells or laboratory animals. Therefor more research is very much needed to understand its toxic mechanisms. Determination of the fate of MPs within cells and the effects of contaminants at single cell levels are described in the Deliverable D.4.4.1 Determination of the fate of MPs within cells and in the Deliverable D.4.4.2 Determination of the effects of contaminants at single cell levels. In the Deliverable 4.4.1 coordinated by Prof Neri of the University of Ferrara which is related to in vitro experimental researches performed to evaluate the uptake of microplastics (MPs) by human cell lines and their distribution within the cells.

The main aims of the Deliverable 4.4.1 were:

- Analysis of internalization of MPs by different human cell lines.
- Analysis of the influence of parameters, such as MPs concentration, size and exposure time, in the interaction between MPs and human cells.

In the Deliverable 4.4.1 was investigated the internalization of PS-MPs with different sizes and at different concentrations by three human cell lines (colorectal, lung and hepatocellular carcinoma) that could represent the human tissue mainly exposed to human plastic intake. The uptake of PS-MPs was evaluated at single-cell level highlighting the internalization of beads by cells in a dose- and time-dependent manner (especially for the 1 µm beads) but with differences among the three cell lines. Here, a major uptake of beads was identified for the hepatocellular carcinoma cells.

Further studies will be necessary to clarify and complete the set of in vitro experiments useful to determine the potential internalization of all the dimensions of PS-MPs by the three cell lines. This study has highlighted the extreme danger of 1 and 2 µm size microplastic materials so it is recommended in fish and aquaculture farms to perform analyses that exclude the presence of very small microplastics within fish products.

5) **Possible presence of inorganic (heavy metals) and organic (POP, OCP, IPA and PCB) in mussels and shellfish**

In this Deliverable the concentrations of PAHs, PCBs, dioxins, dioxin-like PCBs and heavy metals in mussel samples were studied in both the Croatian and the Italian NET4mPLASTIC project and the results show that these pollutants are very low or undetectable at the moment in the locations examined in this project.

As a reference, the EU Regulation 1259/2011 which sets the maximum levels of dioxins and PCBs in food - EUR-Lex and the EU Regulation 277/2012 which sets the maximum levels of dioxins and PCBs in the tariff have been used. The data in the project are reassuring as they also comply with the new 2015 of the differences between the tolerable intake levels established by the various scientific consultancy bodies.

The main reasons for the decrease in the former EU tolerable intake were the availability in EFSA of new epidemiological and experimental animal data on the toxicity of these substances and more refined modelling techniques to predict levels in the human body over time. The products marketed in fishmongers and supermarkets are certified with traceability so there are no risk factors as they are subject to rigorous controls and monitoring in certified laboratories that refer to the limits defined by the EU directives and by the EFSA tables. Risk factors can emerge from the consumption of fish, shellfish and crabs in restaurants purchased by local amateur fishermen. These parameters showed high values in bivalve molluscs in areas with a strong anthropogenic impact, i.e. in the area of large ports, marinas, industrial areas, etc. In these areas it is forbidden to collect shellfish, crabs and fish.

In a complete bibliographic search of the entire Adriatic Sea, they found several hotspots due to the high concentrations of metals including the stations of Valona (Hg, Ni, Cd, Cr, V); Kepi Rhodon (Ni, Zn, Cr; Kotor Pb, Cr); Neretva (Cu); Ragusa (Cu, Pb); Neum (Cr, Ni); Novigrad (Cd, Hg, Ni, Pb); Vasto (Zn, Ni, Cd); Tremiti (V, Cd, Pb); and Taranto, which stands out as the site most contaminated by Hg. The datasets collected for heavy metal pollution in *M. galloprovincialis* showed high values in areas with a strong anthropogenic impact, i.e. in the area of the main ports, marinas, industrial areas, etc. In general, high concentrations of all trace metals have been recorded in areas with a high contribution

of industrial, port and urban waste. Most of the values, when expressed on the basis of wet weight, were below the maximum permitted levels (MPL) prescribed by law for the trace metal content in fresh shellfish. In general, trace metal concentrations detected in mussels through monitoring were within the ranges of trace metal concentrations determined in low to moderately polluted areas. This guideline provides for an action to raise awareness among citizens and restaurants in the areas of the INTERREG Italy Croatia program in compliance with prohibitions in the practices of fishing in port areas or in areas polluted due to the presence of industrial waste. There is a need to raise awareness of the risks associated with the harvesting of molluscs in pollutant areas as they expose the population to risks. Often the indications of the prohibitions are not well defined so it is suggested to introduce applications that signal via mobile phone that the navigation area is forbidden for fishing as it is polluted.

7 Conclusion

A number of bivalves' characteristics have led to increased interest of science for these organisms. Among many of their characteristics, their sedentary lifestyle and suspension-feeding, as well as stable populations of commercially important species are the important reasons why these organisms are used as indicator organisms of environmental stress in coastal and estuarine waters (Dame, 1996). Also, they are commercially important as seafood and because of that their chemical and microbiological contamination has been of interest to public health for many decades. Monitoring of chemical parameters has been carried out for many years, and based on the obtained results, maximum acceptable concentration for some chemical indicators have been determined. According to existing legislation all the mussels that are intended for consumption are under some kind of control and there for safe for the consumption.

In the last decade scientists are sending a warning about the new threat that has come our way and that is the microplastics. Microplastic particles are present in every environment on the Planet. They are ubiquitous (Farady, 2019; Exposito et al., 2021). It is necessary to conduct a series of studies in order to demonstrate a cause-and-effect relationship between the impact on human health and the consumption of mussels containing microplastic particles and to produce new food standards.

Microplastics has become part of the human food web as one of the possible sources of microplastics for humans. Microplastic <150 µm in diameter have the potential to translocate into human tissues, trigger a localized immune response (EFSA, 2016; Wright and Kelly, 2017; Prata et al., 2020). To date neither safety threshold, nor tolerable daily intake, have been established and the risk to humans from dietary exposure must be still assessed (Exposito et al., 2022).

Microplastic poses a threat to human health in several ways. When ingested, microplastic can release toxic chemicals, that are either adsorbed on the particles from the environment or are added to it during production, into human body or any other organism. Also, little is known about the toxic effects of MPs in humans, although some evidence suggests that these particles are a threat to human health as well and that they can make a significant damage to human body. This toxic effect could depend on many different factors such as the type, size, shape, concentration, and charge of MPs. Evidence suggests that MPs of size <20 µm can penetrate organs, and MPs <10 µm can penetrate cell membranes and cross the placental barrier in exposed cells or laboratory animals (Ragusa et al., 2021). Therefor more research is very much needed to understand its toxic mechanisms. Determination of the fate of MPs within cells and the effects of contaminants at single cell levels are described in the Deliverable D.4.4.1 Determination of the fate of MPs within cells and in the Deliverable D.4.4.2 Determination of the effects of contaminants at single cell levels.

It is necessary to continuously monitor the presence of microplastic in the environment as well as the presence of MPs in human food, especially the one that comes from the sea, like mussels. Results of this Project show that microplastic is present in the Adriatic Sea in all the pilot sites investigated in this

research: in both Italian and Croatian sites, in surface sea water, on the beach sediment and in the mussels. Since there are many indications that MPs could impair health of humans and other organisms, this topic should become an urgent public health goal. It is absolutely necessary to determine the maximum acceptable concentration for all the chemicals described as well as the maximum acceptable number of MPs in food, especially mussels and other shellfish. It is important to create food standards that will take into consideration new scientific information on the subject of toxicity and the impact of MPs on human health.

Humans are exposed through food to chemical contaminants such as heavy metals and persistent organic pollutants (POPs) which tend to accumulate in many organisms including in bivalves which are intended for human consumption. This presents a permanent risk to humans as final consumers at the top of the food chain. To avoid the danger for the human health it is necessary to continuously monitor the concentrations of these chemicals on different locations in the Adriatic Sea as well as the concentration of these chemicals in the soft tissue of mussels intended for human consumption.

As the results of this project show - the concentrations of PAHs, PCBs, dioxins, dioxin-like PCBs and heavy metals in the samples of mussels from both Croatian and Italian side are very low or no detectable at the moment in the locations examined in this Project. Although, these parameters showed elevated values in bivalve molluscs in areas with a strong anthropogenic impact, i.e. in the area of major ports, marinas, industrial areas etc. in some previous researches. It is necessary to produce guidelines and food standards and further improve existing legislation to minimise the risk for human health.

The collected data based on harmonised active biomonitoring of mussels from Adriatic Sea were presented in this document considering the metals, organochlorine pesticides, PCBs, and PAHs. Data for eight relevant metals measured at sampling sites along the Adriatic Sea coastline were extracted including Hg, V, Ni, Cu, Zn, Cd, Cr and Pb. Bajt at al. (2019), in comprehensive research of whole Adriatic Sea, detected several hotspots due to high metal concentrations including stations Vlora (Hg, Ni, Cd, Cr, V); Kepi Rhodon (Ni, Zn, Cr; Kotor Pb, Cr); Neretva (Cu); Dubrovnik (Cu, Pb); Neum (Cr, Ni); Novigrad (Cd, Hg, Ni, Pb); Vasto (Zn, Ni, Cd); Tremiti (V, Cd, Pb); and Taranto, which stands out as the most contaminated site with Hg. Collected datasets for heavy metal pollution in *M. galloprovincialis* showed elevated values in areas with a strong anthropogenic impact, i.e. in the area of major ports, marinas, industrial areas etc. Generally, elevated concentrations of all trace metals were recorded in the areas with high input of industrial, harbor and urban wastes. Most of the values, when expressed on wet weight basis, were lower than the maximum permissible levels (MPL) prescribed by legislative provisions for trace metal content in fresh shellfish. Generally, the trace metal concentrations found in mussels through monitoring were within the ranges of trace metal concentrations determined in low to moderately polluted areas.

Taranto appears to be a hotspot even regarding PCB contamination, showing the highest bioaccumulation level (114.4 µg/kg), followed by Baošići and Kotor, with values slightly exceeding 60 µg/kg. The Neretva River and Šibenik showed significant differences in the distribution pattern of PCB

congeners, with a predominance of the 118 congener instead of 153, as at the other sites. Previously mentioned compounds are in the environment in low levels, but are transmitted over long distances by water and by air, and as such are widespread. PCBs are present in significantly larger quantities compared to DDTs, which points on the predominant impact of industrial pollution over agricultural throughout the researched area.

The “drins” compounds (aldrin, dieldrin, endrin, and isodrin) were the most abundant among the 13 pesticides, with values from 4.5 µg/kg at Šibenik to 37.8 µg/kg at the Po River. Endrin showed the highest values in almost all the sites investigated.

The highest PAH values were found in areas affected by intense maritime traffic near big ports. The riverine input of these compounds seems to be intense as well. The PAH composition revealed a mixed PAH origin with a significant contribution of petrogenic origin. Most of the values for PAHs were lower than the environmental quality standards for biota according to legislatives.

Considering the time distribution mass fractions decrease was found in polychlorinated biphenyls, lindanes and DDTs on the majority of locations while an increase of mass fractions was generally found mainly for all ecotoxic metals, especially in areas under immediate anthropogenic impact.

An important issue that should be considered when the pollution of seas is in question is the identification of all the sources of pollution including MPs and other contaminants on land that can easily end up in the seas. This issue should be considered immediately through implementation of the appropriate legislation.

Education through Projects, where a great number of Stakeholders is involved, is of great importance. It is the only way to encourage the change in people’s minds and to really start working on the implementation of sustainable use of plastic. Through Projects like this one it is possible to include different communities in search for the solution to this great crisis that humans have produced. Since people created this enormous problem, it is our obligation to try and make it smaller since at this point there is no evidence that we could solve it completely. People should be aware of the consequences of their action regarding the pollution of the environment with plastic items of all sizes. Adriatic Sea as well as other seas and oceans is endangered together with all the creatures that live there.

8 Literature

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