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ANEMONE

Black Sea CROSS BORDER COOPERATION

Hazardous substances assessment in Black Sea biota

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CROSS BORDER COOPERATION

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Acronyms

ANEMONE	Assessing the vulnerability of the Black Sea marine ecosystem to human pressures
BAC	Background Assessment Concentrations
BDL	Below detection limit
BG	Bulgaria
BS	Black Sea Integrated Monitoring and Assessment Programme
BSC	Commission on the Protection of the Black Sea Against Pollution
BSIMAP	Black Sea Integrated Monitoring and Assessment Programme
CHASE	Contaminants Status Assessment Tool
CIS	Cooled Injection System
CR	Contamination ratio
CS	Contamination score
CW1	Ukrainean water bodies - Area of the island Zmeinuy
CW2	Ukrainean water bodies - Tuzlovsky estuaries
CW3	Ukrainean water bodies - Budag estuary
CW6	Ukrainean water bodies - Odessa Bay
CW7	Ukrainean water bodies - From Odessa Bay to Tendra Bay
CW9	Ukrainean water bodies - Kinburn Spit
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DG ENV	Directorate-General for the Environment
EAC	Environmental Assessment Concentrations
EC	European Commission
ECD	Electron capture detector
EEA	European Environment Agency
EOM	Extractable organic matter
EPA	Environment Protection Authority
EQS	Environmental Quality Standard
EU	European Union
EUR	European
FAO	Food and Agriculture Organization
FP7	Framework Progamme 7
GC	Gas chromatography
GES	Good Environmental Status
GF-AAS	Graphite Furnace Atomic Absorption Spectrometry
HCB	Hexachlorobenzene
HELCOM	The Baltic Marine Environment Protection Commission
HM	Heavy metals
IAEA-MEL	International Atomic Energy Agency - Marine Environment Laboratory
IAEA-MESL	International Atomic Energy Agency - Marine Environmental Studies Laboratory
ICES	The International Council for the Exploration of the Sea
ICP-MS	Inductively coupled plasma mass spectrometry
IOC	Intergovernmental Oceanographic Commission of UNESCO
JRC	Joint Research Center
MAC	Maximum Admisible Limits
MISIS	MSFD Guiding Improvements in the Black Sea Integrated Monitoring System
MoEU	Ministry of Environment and Urbanization
MoFAL	Ministry of Food and Agriculture
MPC	Maximum permissible concentrations
MSFD	Marine Strategy Framework Directive
NIMRD	National Institute for Marine Research and Development "Grigore Antipa", Romania
OCP	Organochlorine pesticides
OO-AO	"One Out, All Out" principle from Water Framework Directive
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic (the 'OSPAR Convention')
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyls
PERSEUS	Policy-oriented Marine Environmental Research in the Southern European Seas (FP7 project)
PFC	Perfluorocarbons
PLE	Accelerated pressure extraction
POP	Persistent Organic Pollutants
RMS	Root mean square
TGK	Turkish Food Codex

TM	Trace metals
TR	Turkey
TUBITAK	Scientific and Technological Research Council of Turkey (Turkish: Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, TÜBİTAK)
TW5	Ukrainean water bodies - Danube region
UkrSCES	Ukrainian scientific center of Ecology of Sea
UA	Ukraine
UNEP	United Nations Environment Programme
WBS	Western Black Sea
WFD	Water Framework Directive
WP	Work package
WWTP	Waste Water Treatment Plant

Introduction

Hazardous substances are widespread in the marine environment. Many can be found at low concentrations in the Earth's crust and occur naturally in seawater. Synthetic hazardous substances such as PCBs, DDT, polybrominated diphenyl ethers (PBDEs) and perfluorocarbons (PFCs) are not found naturally in the environment. The main sources are generally waste/disposal, the burning of fossil fuels and industrial activities, including mining and production. Human activities have caused a general mobilization of these hazardous substances in the marine environment. The pathway of contamination is not always obvious, but it is primarily through riverine discharge and atmospheric deposition. Hence, although hot spots tend to be directly linked to particular human activities, the substances are also found in organisms that are collected far from point sources. The effects that some hazardous substances have on the environment and their potential risk to human health because of their toxic, bioaccumulative and persistent characteristics have led to considerable efforts (at the political, management and scientific levels) to address them. Specific policies and conventions aim to minimise the direct and indirect effects of these contaminants, generally by reducing emissions and discharges to the marine environment (EEA, 2019).

Anthropogenic contaminants reach the marine environment mostly directly from land-based sources, but there are cases in which they are emitted or re-mobilized in the marine environment itself. A recent review paper focused on the European environment compiled a list of contaminants potentially released into the sea from sea-based sources and provided an overview of their consideration under existing EU regulatory frameworks (Tornero and Hanke, 2016). The EU Marine Strategy Framework Directive Descriptor 8, together with the Water Framework Directive and the Regional Sea Conventions, provides the provisions against pollution of marine waters by chemical substances. The resulting list of contaminants identified from the literature as potentially released into the marine environment from sea-based anthropogenic activities it includes 276 substances (19 metals/metalloids, 10 organometallic compounds, 24 inorganic compounds, 204 organic compounds, and 19 radionuclides) and major sea-based sources. The offshore oil and gas operations contribute to this list with the highest number of substances, followed by shipping, dredging/dumping of dredged material, mariculture, historical dumping sites, shipwrecks and seabed mining activities (Tornero and Hanke, 2016).

Moreover, although most substances have been linked to only one sea-based activity, there are cases in which they are associated with more than one source, thus increasing their potential risks. While many substances are likely to be introduced into the marine environment from various sources, this does not implicitly mean that all of them have to be regarded as being very hazardous or discharged at levels of concern. The degree of concern must be evaluated in terms of a combination of factors, mainly the temporal and spatial scales over which the compound can be found together with their toxicity and adverse effects on marine organisms. With the increased use of the sea and its resources, a regularly updated inventory of the types and quantities of chemicals released is essential to understand the relative influence of each human activity and how they accumulate and interact to impact the marine ecosystems (Tornero and Hanke, 2016).

Marine ecosystems and organisms are influenced by many internal and external factors, including ecological processes and their interactions, fisheries, a changing climate, habitat modification, eutrophication and inputs of toxic chemicals. Exposure to contaminants has the potential to affect cellular and physiological processes in marine organisms, as well as fundamental processes in marine ecosystems.The health of individuals or integrity of ecological processes will depend on many environmental factors, not only the presence of contaminants (Hylland et al., 2017). Moreover, the consequences of contaminant exposure for the health of individual marine organisms will depend on the species, whether it is being exposed as adult, larvae, or embryo, and the life history of that species. Marine ecosystems are by nature dynamic and, particularly in temperate and polar regions of the globe, there is a pronounced annual seasonality in both abiotic and biological processes that modulate both partitioning of contaminants and effects caused by exposure to contaminants (Vijayan et al., 2006). Although it is close to impossible to single out how they influence marine organisms in any particular moment, it is important for regulatory reasons to be able to assess the extent to which contaminants actually cause effects and, whenever possible, to pinpoint the responsible contaminant(s)/sources. To this end it is crucial to be able to separate contaminant-related effects from changes caused by other environmental influences (Laane et al., 2012).

Measuring contaminants in biota is basic to ecotoxicology, both for understanding the movement of contaminants within organisms and through food chains, and for understanding and quantifying injuries to organisms and their communities. Measuring tissue concentrations is basic to studies on the kinetics of contaminants, which entails characterizing the rates of uptake and elimination in organisms, as well as redistribution (organs, lipid, and plasma) within them. In monitoring programs, tissue concentrations tell us about the geographical distribution of contaminants and how they change through time. Measuring contaminants in tissue can also be important for defining the background, or the uncontaminated condition, as well as identification of hot spots and gradients from point sources. Although analyses of sediments also provide information on the distribution of contaminants, analyses of tissues provide information that is more meaningful to ecotoxicologists. In some instances, chemical analyses of tissues gave the first hint of the global dispersion of chemicals (Beyer and Meador, 2011).

Acknowledging that marine environments are under massive pressure caused by anthropogenic exploitation and pollution, including pollution with chemical substances and marine litter, extensive fishing activities, deterioration of the sea floor, e.g., by construction measures, extraction of minerals, and fishing with ground nets, and introduction of noise, e.g., by ships, construction, renewable energy, and tourism (Fliedner et al., 2018), the European Union has adopted the Marine Strategy Framework Directive (MSFD) (2008/56/EC) that aims at the conservation and protection of the EU marine waters. Descriptors D8 and D9 both deal with contaminants. D8 refers to contaminants in marine water, sediment, or biota which are assessed against threshold values (i.e., values set in accordance with Water Framework Directive WFD (EC 2000) and its daughter directives or, if not applicable or no value is set under the WFD, values set by Member States through regional or subregional cooperation). Descriptor 9 focuses on contaminants in fish and other seafood for human consumption. The number of contaminants assessed under D9 is lower compared to D8 and comprises mainly those for which regulatory levels for foodstuffs are set under Regulation (EC) No 1881/2006 and its amendments. However, on the basis of risk assessments, Member States can choose to not consider contaminants and/or include additional contaminants, for which threshold values must then be established by the Member States through regional or subregional cooperation.

There is a link between Descriptors 8 and 9: because many contaminants are transferred along the food web those of concern to marine fish will likely also be of concern to humans (Fleming et al. 2006). On the other hand, concentrations exceeding the regulatory levels for food will probably also affect the ecosystem because food regulatory levels are usually higher than thresholds for assessing environmental pollution (Swartenbroux et al. 2010). Monitoring seafood related to human health is different from monitoring biota for environmental purposes. For the latter, a high degree of standardization and geographical traceability of the samples are crucial to the derivation of temporal trends and the assessment of compliance with reference values. In contrast, monitoring of seafood contamination for human consumption relies on the edible fraction of a wide variety of commercially relevant species for which the precise origin is not relevant and often unknown (Swartenbroux et al. 2010). The MSFD, however, requires that the GES has to be achieved or maintained for a specified region or subregion. The species monitored in the context of D9 shall be relevant to the marine region or subregion concerned, implying that the geographical origin of the samples should be known (Fliedner et al., 2018). In most countries, the monitoring of contaminants in seafood is executed by the responsible authorities in charge, which often are different from the environmental institutions implementing the MSFD and its associated monitoring. Thus, cooperation between authorities and environmental institutions in charge of health monitoring is strongly encouraged. Exchanging information on data, approaches and methodologies between environmental monitoring institutions and human health risk related monitoring institutions is very important.

Descriptor 9 from the Marine Strategy Framework Directive (MSFD) 2008/56/EC is referring to European regulations that provide for the protection of human health from harm potentially deriving from the consumption of contaminated seafood. Good Environmental Status (GES) would be achieved if all contaminants are at levels below the levels established for human consumption. Therefore, distinction should be made between contaminants for which regulatory levels have been set and other contaminants of relevance in fish and other seafood. Monitoring of Descriptor 9 consider measuring contaminants in fish and other seafood for which regulatory limits have been set, whereas monitoring for other contaminants should focus on trend analysis. The significance of an increase for specific contaminants under Descriptor 8 should be regarded as an important element for inclusion in monitoring under Descriptor 9.

1 Objectives

According to a recent European Environment Agency (EEA) report on Indicator Assessment: Hazardous Substances in marine organism (EEA, 2019), even there is a large number of potentially hazardous substances entering the seas, data with sufficient geographical and temporal coverage are available for only a few of them, which is insufficient to warrant a pan-European assessment of hazardous substances in marine organisms. Therefore, this EEA indicator is based on the assessment of eight substances: the metals cadmium, lead and mercury; the pesticides DDT and lindane; two other types of synthetic substance, HCB and PCBs; and the polycyclic aromatic hydrocarbon BaP, measured in organisms from the regional seas as follows: Baltic Sea — Atlantic herring (*Clupea harengus*); North-East Atlantic Ocean — blue mussel (*Mytilus app*), Atlantic cod (*Gadus morhua*), flounder (*Platichtys flesus*); Mediterranean Sea — Mediterranean mussel (*Mytilus galloprovinicialis*); Black Sea — Mediterranean mussel (*Mytilus galloprovinicialis*). Because of insufficient data coverage and/or lack of data, a comprehensive assessment of hazardous substances for the Mediterranean Sea and Black Sea regions could not be conducted in the last EEA assessment (EEA, 2019).

The assessment of contaminants in biota is the most important, not only for biomonitoring of the marine pollution, but also in case of biota used for human consumption there are further implications with respect to public health reasons. Since data on this topic are rather limited in the Black Sea region, **study was focused on the assessment of hazardous substances in biota, in addition to water and sediments, thus contributing with new relevant information for the region.** Some of the Black Sea countries have implemented programs in relation to contaminants monitoring in water, and/or sediments and/or biota, under the auspices of the Bucharest Convention and BSIMAP, however the scope and scale of this activity varies.

Activities conducted aimed to provide a broad survey of new data on chemical contamination of aquatic organisms and potential risks, thus filling knowledge gaps and provide new information for Black Sea region. The ultimate objective is to build more harmony between the various existing research initiatives, based on the agreed common indicators, and to obtain new data and information, focusing future research efforts toward key domains for the Black Sea region, like the presence of hazardous substances in biota for human consumption, impact of human pressures upon to the contamination status and trends, and biological effects monitoring.

Pilot studies investigations were especially focused on the following contaminants for which regulatory levels have been laid down: heavy metals (lead, cadmium, and mercury), polycyclic aromatic hydrocarbons, polychlorinated biphenyls, *dioxins* (including dioxin-like PCBs). Additionally, further contaminants of relevance were considered (e.g., organochlorinated pesticides). The selection of the species to be used for pilot studies considered the following criteria: species more prone to biomagnify / bio-accumulate specific classes of contaminants; species representative of the different trophic levels or habitats; species representative for entire region; species representing consumer habits. Moreover, in order to make pilot monitoring results more comparable within Black Sea region, a limited number of common target species from the most consumed species of demersal and pelagic fish and other seafood (mollusks – *Mytilus galloprovincialis* and *Rapana venosa*) was selected for investigations.

2 Overview of existing information and knowledge

2.1 Ukraine

The state of biological samples was assessed according to the content of the following pollutants: toxic metals (TMs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs). In the process of assessment, data from **2012-2017** were considered and compared.

In Ukraine, the national methodology to assess the ecological state is by calculation of a **pollution factor (Kz)**, developed by UkrSCES.

Kz reflects the concentration of all pollutants of the same type in a certain period in a given area. This factor represents the sum of the ratios of the concentration of each pollutant to its maximum permissible concentration, in accordance with EU Directive 2013/39/EU (EQS) for biota, to the number of measurements performed in a given period of time.

Formula for calculating the pollution factor Kz:

$$CR = \frac{C_{mon}}{C_{Treshold}}$$

$$\mathrm{Kz} = \frac{1}{n}\sum_{i=0}^{n} CR_i$$

Where: CR is contamination ratio; C_{mon} is measured concentration; $C_{threshold}$ is maximum permissible concentration;

General assessment of biological samples' ecological state in the studied area was made coming out of the worst grades of groups of pollutants.

The ecological condition of biota samples is estimated by means of Kz, as follows:

for TM:		**for Organic pollutants:**
Very good when Kz is less than 0,5;		Very good when Kz is less than 0,2;
Good when Kz is from 0,5 to 1,0;		Good when Kz is from 0,2 to 1,0;
Satisfactory when Kz is from 1,0 to 1,25;		Satisfactory when Kz is from 1,0 to 5,0;
Bad when Kz is from 1,25 to 2,5;		Bad when Kz is from 5,0 to 25;
Very bad when Kz is more than 2,5		Very bad when Kz is more than 25

Table 2.1 shows the values for pollution factor (Kz) for each pollutant's concentration (TMs, OCPs, PAHs) in biological samples from different water bodies and areas of the Ukrainian Black Sea, according to the monitoring data from 2012 to 2017. Figure 2.1 shows a map of monitoring sea waters in Ukraine and the position of water bodies.

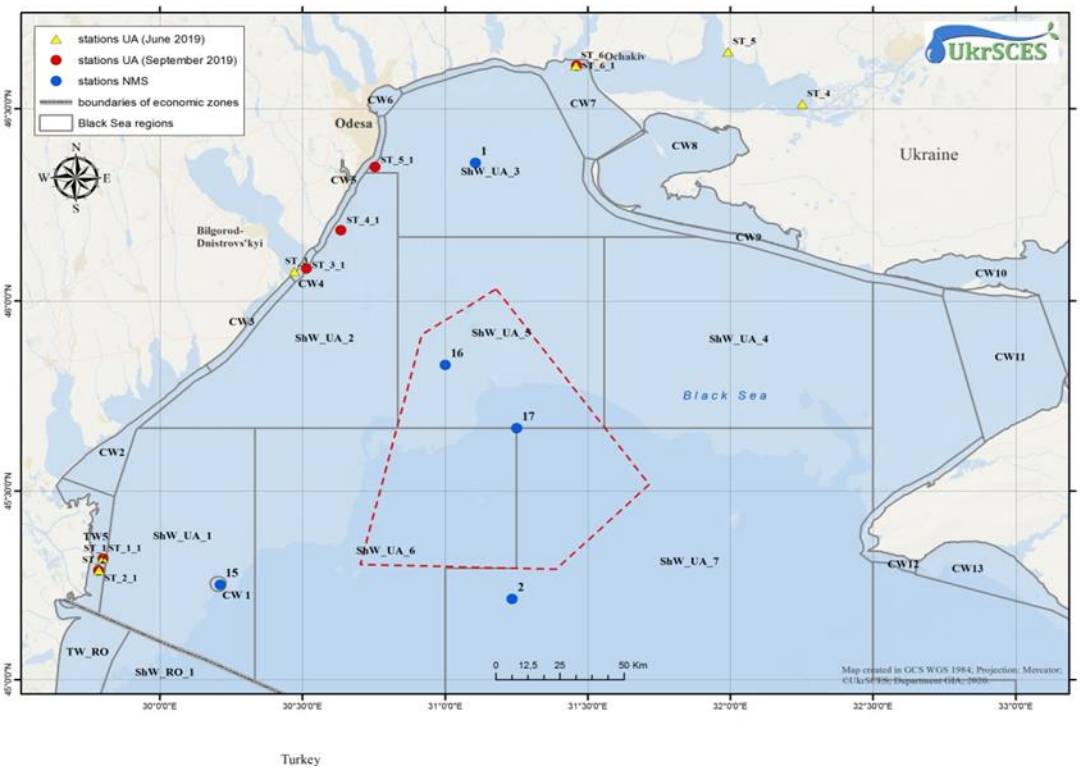


Figure 2.1 - Map of monitoring of sea waters of Ukraine

Name of the water bodies: CW1 - Area of the island Zmeinuy; CW2 – Tuzlovsky estuaries; CW3 – Budag estuary; CW6 – Odessa Bay; CW7 – From Odessa Bay to Tendra Bay; CW9 - Kinburn Spit; TW5 - Danube region.

As can be seen from Table 2.1, the average levels of contamination of bivalve molluscs from Ukrainian waters investigated during 2012 - 2017 are as follows:

- in the water body CW1 are in a very bad state, Kz TM (mercury) - very bad, Kz OCPs - very bad (Kz heptachlor corresponds to a very bad level, Kz hexachlorobenzene corresponds to a satisfactory level).
- in the water body CW3 are in bad condition, Kz TM (mercury) - bad, Kz PAHs - satisfactory (Kz benzo(a)pyrene corresponds to a bad level).
- in the water body CW6 are in a satisfactory condition, Kz PAHs - satisfactory (Kz benzo(a)pyrene corresponds to a satisfactory level). TM were not studied.
- in the water body CW7 are in bad condition, Kz TM (mercury) - bad, Kz PAHs - satisfactory, (Kz fluoranthene corresponds to a satisfactory level, Kz benzo (a) pyrene corresponds to a bad level).
- in the water body CW9 are in a very bad state, Kz OCPs - very bad (Kz heptachlor corresponds to a very bad level.
- in the Dniester region they are in a satisfactory condition, Kz OCPs - satisfactory (Kz hexachlorobenzene corresponds to a satisfactory level).
- in the mixing area are in a very bad state, Kz OP - very bad, (Kz heptachlor corresponds to a very bad (critical) level), Kz PAHs - satisfactory, (Kz benzo(a)pyrene and fluoranthene correspond to a satisfactory level).

Average levels of Rapana contamination investigated between 2012 and 2017 are as follows:

- in the water body CW1 are in a very bad state, Kz TM (mercury) - very bad, Kz OCPs - very bad (Kz heptachlor corresponds to a very bad level), Kz PAHs - satisfactory, (Kz benzo(a)pyrene and fluoranthene correspond to a satisfactory level).
- in the water body CW2 are in bad condition, Kz TM (mercury) - satisfactory, Kz PAHs - bad, (Kz benzo(a)pyrene corresponds to a bad level).
- in the water body CW3 are in bad condition, Kz TM (mercury) - bad, Kz PAH - good, but Kz benzo(a)pyrene corresponds to a satisfactory level.
- in the water body CW7 are in bad condition, Kz OCPs - bad (Kz heptachlor corresponds to a very bad level), Kz PAHs - good, but Kz fluoranthene corresponds to a satisfactory level.
- in the water body CW9 are in bad condition, Kz TM (mercury) - bad, Kz PAHs - satisfactory, (Kz benzo(a)pyrene corresponds to a satisfactory level).
- in the water body TW5 are in a very bad state, Kz TM (mercury) - very bad, Kz OCPs - bad (Kz heptachlor corresponds to a very bad level).
- in the area of mixing are in a very bad state, Kz TM (mercury) - satisfactory, Kz OCPs - very bad (critical) (Kz heptachlor corresponds to a very bad level).

The average levels of contamination of fish caught in the CW1 water body in the period from 2012 to 2017 (Table 2.1), are in critical condition, Kz TM (mercury) - very bad, Kz OCPs - very bad (Kz heptachlor corresponds to very bad level), Kz PAHs - good, but Kz benzo(a)pyrene corresponds to a satisfactory level.

Table 2.1 - Kz TM, OCPs, PAHs in biological samples from Ukrainian waters, 2012 – 2017

Region	Year	Kz Hg	Kz OCPs	Kz HCB	Kz Heptachlor	Kz PAHs	Kz Fluoranthene	Kz Benzo(a)pyrene
Bivalve molluscs (*Mytilus galloprovincialis*)								
CW1	2012	13.0	25.4	0.01	50.8			
	2013	0.17	2093	0.04	4185			
	2014	2.18	0.00	0.00	0.00			
	2015	0.28	1030	0.02	2060			
	2016	0.63	10.1	0.72	19.4	0.255	0.15	0.36
	2017	0.38	335	4.88	666	0.72	0.83	0.61
CW1 (Average)		2.76	582	0.95	1163	0.485	0.49	0.48
CW3	2017	1.55	0.14	0.28	0.00	3.56	0.60	6.52
CW6	2017		0.18	0.37	0.00	2.285	0.75	3.82
CW7	2017	1.60	0.25	0.49	0.00	3.2	1.14	5.26
CW9	2017	0.80	464	0.59	927	0.31	0.48	0.14
Dniester region	2016	0.60	2.45	4.89	0.00	0.355	0.13	0.58
	2017	0.20	0.35	0.71	0.00	0.515	0.58	0.45
The average for the Dniester region for the entire period		0.40	1.40	2.80	0.00	0.435	0.35	0.52
Mixing area	2016	0.80	54.8	0.57	110	0.28	0.14	0.42
	2017	1.18	509	1.40	1016	4.345	3.54	5.15
The average mixing area for the entire period		0.99	282	0.98	563	2.315	1.84	2.79
Sea snail (*Rapana venosa*)								
CW1	2012	27.5	27.6	0.02	55.2			
	2013	0.25	1189	0.10	2377			
	2014	1.13	22.8	0.00	45.7			
	2015	1.26	1.29	0.09	2.49			
	2016	2.29	19.5	0.47	38.4	0.325	0.23	0.42
	2017	0.54	176	0.07	351	6.46	9.73	3.19
CW1 (Average)		5.50	239	0.12	478	3.395	4.98	1.81
CW2	2017	1.10	0.47	0.94	0.00	15.52	0.64	30.4
CW3	2016	1.20	0.02	0.03	0.00	0.7	0.28	1.12
	2017	1.70	0.65	1.29	0.00	0.88	0.73	1.03
CW3 (Average)		1.45	0.33	0.66	0.00	0.79	0.51	1.07
CW7	2016	0.90	36.0	0.30	71.6	0.735	1.47	0.00
CW9	2017	1.55	0.20	0.41	0.00	1.605	0.57	2.64
TW5	2016	2.65	23.2	0.02	46.3	0.25	0.15	0.35
Mixing area	2016	1.25	117	0.50	233	0.14	0.10	0.18
Fish (*Gobiidae*)								
CW1	2012	20.5	153	0.01	305			
	2013	0.87	753	0.04	1507			
	2014	0.93	608	0.01	1215			
	2015	0.00	0.00	0.00	0.00			
	2016	2.49	17.3	0.27	34.3	0.94	0.42	1.46
	2017	1.73	1575	1.61	3149	1.01	0.73	1.29
CW1 (Average)		4.42	518	0.32	1035	0.98	0.58	1.38

2.2 Romania

In 2018, in the framework of the **Report on the ecological status of the Black Sea marine ecosystem in accordance with the requirements of Article 17 of the Marine Strategy Framework Directive (MSFD 2008/56/EC)** (Boicenco et al., 2018), for Descriptor 9 the assessment was made on the basis of criterion D9C1 "The level of contaminants in edible tissues (muscle, liver, or other soft parts, as appropriate) of seafood (including fish, crustaceans, mollusks, seaweed and other marine plants), caught or harvested from the natural environment (excluding fish from mariculture) does not exceed the maximum admissible levels".

Monitoring data available for the period **2012 - 2017** on the concentration of contaminants in mollusk species of commercial interest (*Rapana venosa* and *Mytilus galloprovincialis*), collected from the Romanian marine waters have been centralized, processed, statistically analyzed and evaluated against the proposed target values for defining good ecological status.

The definition of good environmental status (GES) for criterion D9C1 was based on the maximum permissible levels required by the legislation in force (Regulation EC nr. 1881/2006 modified by: Regulation (EC) nr. 1126/2007; Regulation (EC) nr. 565/2008; Regulation (EC) nr. 629/2008; Regulation (EC) nr.1259/2011; Regulation (EC) nr. 105/2010; Regulation (EC) nr. 165/2010; Regulation (EC) nr. 1259/2011, Order 147/2004).

Analysis of data available in the period 2012 to 2017 (N=44) shows that persistent organic pollutants (organochlorinated pesticides, polychlorinated biphenyls) have a good ecological status in the marine area (Table 2.2 and Table 2.3), percentile '75th being below the maximum allowable values for human consumption laid down by national and European legislation. The evaluation shows a trend of stability in concentrations of persistent organic pollutants in marine mollusks of commercial interest during 2012-2017, compared to the previous period (2006-2011).

Assessment of the polycyclic aromatic hydrocarbons indicator in mollusks of commercial interest (*Mytilus galloprovincialis* and *Rapana venosa*), following the processing of data for the period 2012-2017 (N=32), reflects in all cases a good ecological status (Table 2.4). Benzo(a)pyrene had low values in the marine area, below the maximum limit allowed by the (EC) nr.1881/2006 (10.0 µg/kg wet tissue).

Table 2.2 - Assessment of ecological status for organochlorinated pesticides in species of marine mollusks of commercial interest based on D9 in the marine area, 2012-2017

Assessment area	Compound	Percentile '75th (mg/kg wet tissue)	Threshold value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Marine waters	HCB	0.0019	0.0200	2.27	Good	Good
	Lindane	0.0043	0.1000	2.27	Good	
	Heptachlor	0.0025	0.0200	2.27	Good	
	Aldrin	0.0023	0.0200	2.27	Good	
	Dieldrin	0.0118	0.0200	11.36	Good	
	Endrin	0.0029	0.0050	20.45	Good	
	DDT (sum of p,p'DDE, p,p'DDD, p,p'DDT)	0.0177	0.1000	2.27	Good	

Table 2.3 - Assessment of ecological status for polychlorinated biphenyls in species of marine mollusks of commercial interest based on D9 in the marine area, 2012-2017

Assessment area	Compound	Percentile '75th (mg/kg wet tissue)	Threshold value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Marine waters	Sum of 6 PCBs (PCB28, PCB52, PCB101, PCB153, PCB138, PCB180)	19.14	75	5.71	Good	Good

Table 2.4 - Assessment of ecological status for polycyclic aromatic hydrocarbons in species of marine mollusks of commercial interest based on D9 in the marine area, 2012-2017

Assessment area	Compound	Percentile '75th (mg/kg wet tissue)	Threshold Value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Marine waters	Benzo(a)pyrene	1.6212	10.0	0	Good	Good

Analysis of data available in the period 2012 to 2017 (N=44) shows that toxic metals (cadmium, lead) have in marine mollusks of commercial interest a good ecological status (Table 2.5), the value of 75th percentile being below the maximum allowable values for human consumption laid down by European legislation. In the case of lead there was no exceedance of the maximum allowable value of European legislation in the mollusks analyzed between 2012 and 2017, while in the case of cadmium there were exceedances of the threshold value in 3% of the samples investigated.

The evaluation of the data obtained between 2012 and 2017, compared to the initial evaluation (2006-2011), shows a tendency to decrease and stability of lead concentrations in marine mollusks of commercial interest, while in the case of cadmium the multiannual variability is much more pronounced, overall, with growth trends during the evaluated period.

Table 2.5 - Assessment of the ecological status for heavy metals (cadmium, lead) in marine mollusk species of commercial interest based on D9 in the marine area, 2012-2017

Assessment area	Compound	Percentile '75th (mg/kg wet tissue)	Threshold Value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Marine waters	Cadmium	0.62	1.00	3	Good	Good
	Lead	0.12	1.50	0	Good	

The integration of the results obtained for individual compounds within each group of contaminants, based on the one-out-all principle, has revealed a good ecological status for all groups of contaminants.

By integrating the results obtained for each group of contaminants, based on the one-out principle, a good ecological status has been achieved (Table 2.6).

The evaluation shows in most cases a trend of stability of contaminant concentrations in marine mollusks of commercial interest compared to the previous period (2006-2011).

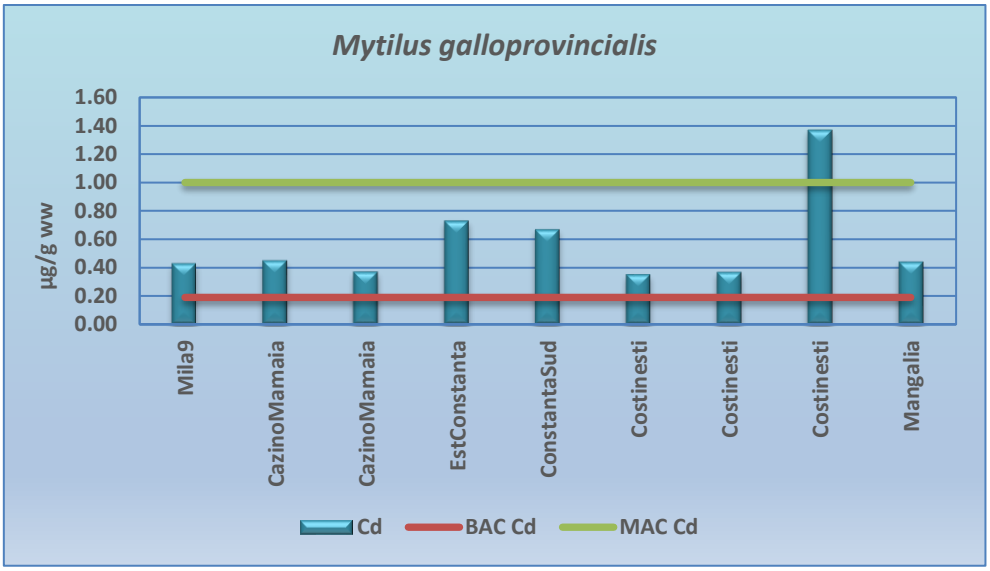
Table 2.6 - Environmental status assessment for the marine area for D9C1, 2012-2017

Assessment area	Compound	Ecological status for each group of compounds	Ecological status for the assessment area
Marine waters	Organochlorinated pesticides	Good	Good
	Polychlorinated biphenyls	Good	
	Polycyclic aromatic hydrocarbons	Good	
	Heavy metals	Good	

In the framework of the project **FP7 Policy-oriented Marine Environmental Research in the Southern European Seas (PERSEUS)**, mapping of contaminants levels in **marine mollusks, demersal and small pelagic fish** was carried out. Mollusks (38 samples) and **fish** (34 samples) were collected from Romanian Black Sea waters (Danube mouths and Constanta area) during 2012 - 2014. Pollution was assessed based on measurements of contaminants levels in biota (whole soft tissue for mollusks; muscle for fish): heavy metals (Cu, Cd, Pb, Ni, Cr) and organic pollutants (PAHs, OCPs, PCBs) (Oros A. et al., 2016).

Levels of **Cd** and **Pb** in mussels (*Mytilus galloprovincialis*), sampled from different locations along Romanian coast, were compared against Background Assessment Concentrations (BAC/OSPAR) and Maximum Admissible Levels from European legislation (EC nr. 1881/2006). Whereas Cd BAC were slightly exceeded in all samples, only one value higher than MAC was noticed, all the other samples presenting Cd concentrations below MAC. As for Pb, this element presented low values of accumulation in mussels, all samples being much below MAC. Background levels for Pb were exceeded in only 2 samples, from the locations situated in the vicinity of Constanta Port (Figure 2.2).

Fish samples belonging to 9 species frequently encountered in Romanian marine waters were investigated for heavy metals levels in their muscle tissues during 2012-2014. Determined concentrations presented high variability, interspecific differences between pelagic and demersal species being noticed. For instance, many elements presented higher concentrations in whiting, anchovy, and sprat.



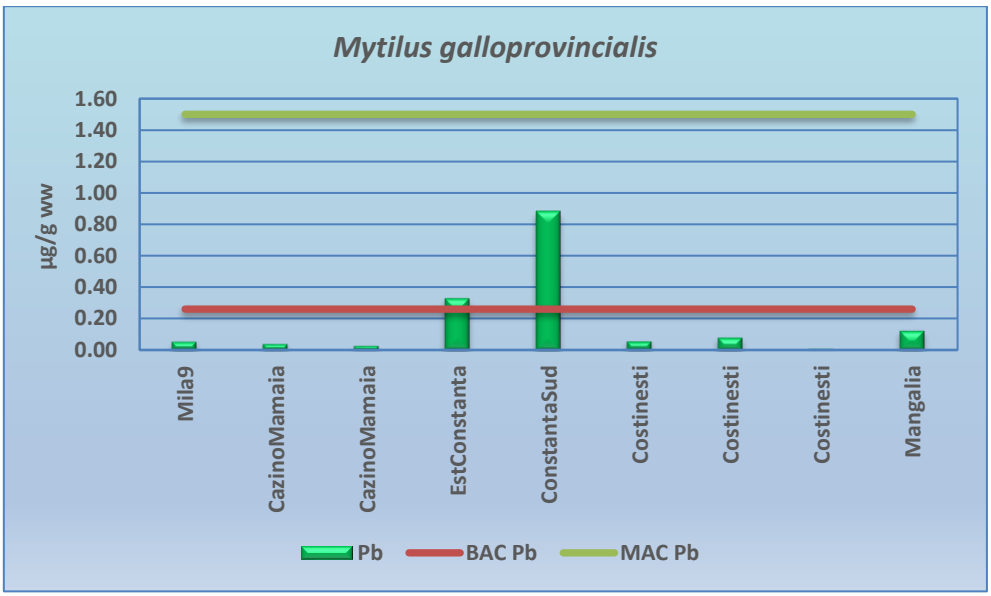


Figure 2.2 - Levels of Cd and Pb in mussels along Romanian Black Sea coast (2012-2014) against background assessment concentrations (BAC /OSPAR) and maximum admissible concentrations (MAC /EC nr. 1881/2006)

Concentrations of **Cd** and **Pb** in fish muscle were compared with Maximum Admissible Limits (MAC) from European legislation (EC no. 1881/2006). For Cd, the regulation proposes different thresholds, depending of the fish species: 0.30 µg/g ww Cd for species like anchovy, and 0.10 µg/g ww Cd for other species, like bluefish, sole or horse mackerel. All fish samples concentration were below 0.30 µg/g ww Cd, whereas the limit of 0.1 µg/g ww Cd was surpassed by 2 samples: whiting (sample no. 3) and horse mackerel (sample no. 34), the other samples exceeding the limit being anchovy and sprat, for which the other threshold is applied. Pb MAC (0.30 µg/g ww) was exceeded in 5 fish samples: whiting (sample no. 3), bluefish (sample no. 18), and sprat (samples no. 21, 26, 27) (Figure 2.3).

The **polycyclic aromatic hydrocarbons** (PAH) concentrations determined in mollusks -*Mytilus galloprovincialis,* and nine fish species (pelagic and demersal species) from different locations along Black Sea coast in 2012- 2014 have been determined.

In 88% of samples, total content of PAHs varied from 100.0 to 440.01 ng/g ww, indicating a moderately contamination level of marine organisms. The most important contributors to PAH burdens in biota were phenanthrene (60%) and naphthalene (23%), a low molecular weight PAHs with 2-3 aromatic rings, which are consistent with a composition profile following a petroleum exposure.

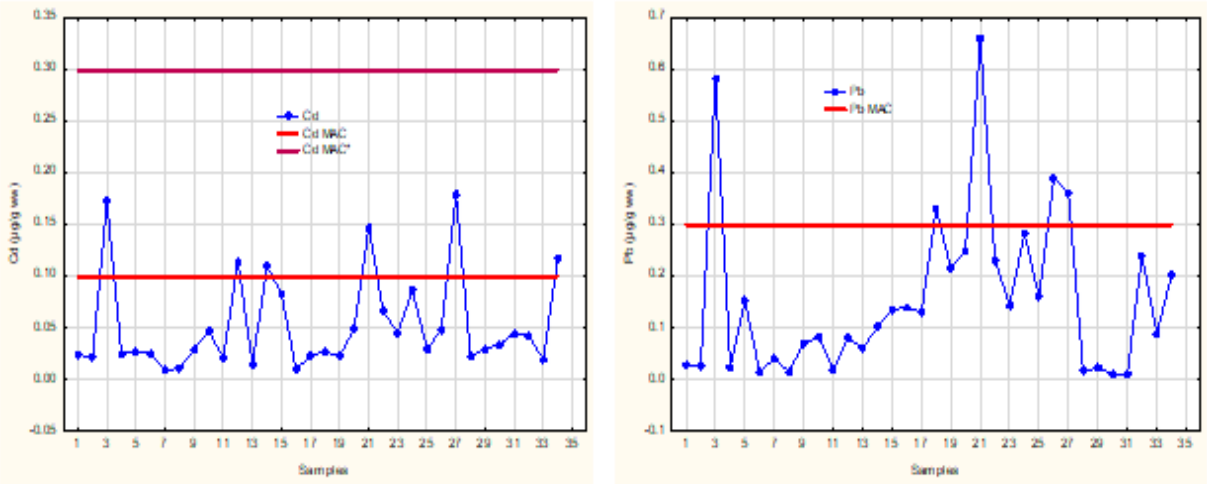


Figure 2.3 - Levels of Cd and Pb in fish samples from the Romanian Black Sea coast (2012-2014) against maximum admissible concentrations (MAC /EC no. 1881/2006)

Individual compounds showed no significant difference (p>0.05) between the means, except benzo[a]anthracene and benzo[a]pyrene. These compounds have been observed to be more readily accumulated by the mussels than fish.

The Commission Regulation (EC) No. 208/2005 sets maximum concentrations of 10.0 and 2.0 ng/g ww for **benzo[*a*]pyrene** in bivalve mollusks, respectively in muscle meat of fish. The concentrations of benzo[*a*]pyrene in marine organisms (percentile 75th of all data series) were situated below quality standards (Figure 2.4).

Bioaccumulation of **organochlorine pesticides** (OCP) and **polychlorinated biphenyls** (PCB) was investigated in four species of mollusks (three bivalves and one sea snail) and nine fish species (pelagic and demersal species) from different locations along Black Sea coast during 2012-2014. Concentrations of OCPs and PCBs in fish and mollusks were compared with Environmental Assessment Concentrations (EAC/OSPAR) and Environmental Quality Standards from European legislation (EC no. 36/2013) in respect to Descriptor 8 and with maximum levels from European legislation (EC no. 1259/2011) and national legislation in force (Order 147/2004), in respect to Descriptor 9.

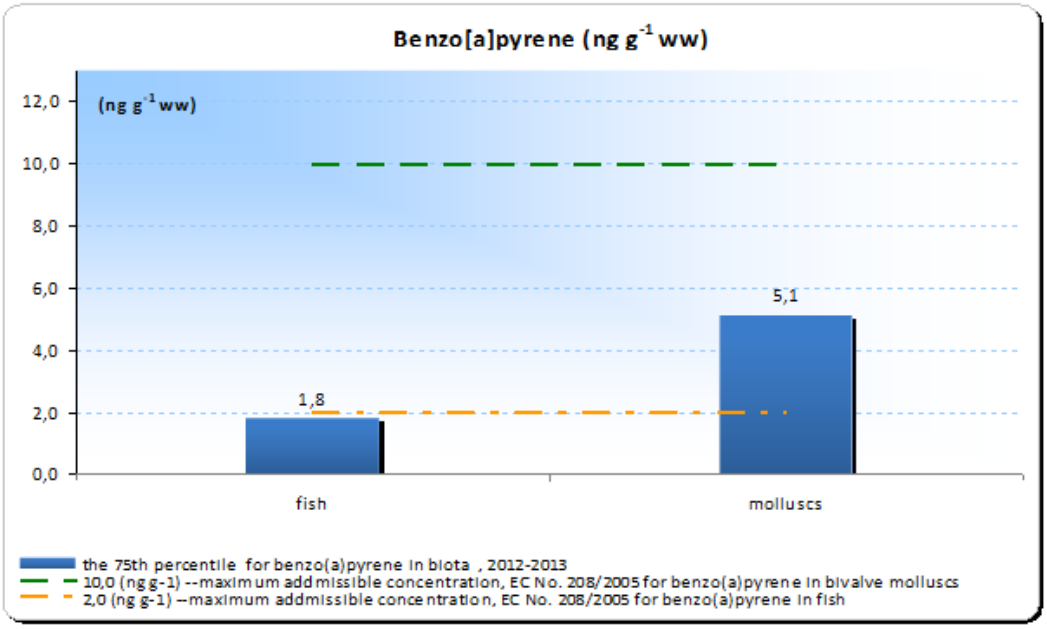


Figure 2.4 - Levels of benzo[a]pyrene (ng/g ww) in marine organisms along Romanian Black Sea coast (2012-2013) against maximum admissible concentrations (MAC /EC no. 208/2005)

In the scope of Descriptor 9, in fish, there were no exceeding for OCPs and only 4 % overruns of PCBs of the maximum acceptable concentrations in food for the protection of public health (Figure 2.5). In mollusks of commercial interest (*Rapana venosa* and *Mytilus galloprovincialis*), there were frequent (20 – 30 %) exceeding for OCPs and often (75%) overruns of PCBs of the maximum acceptable concentrations in food for the protection of public health, in 2012.

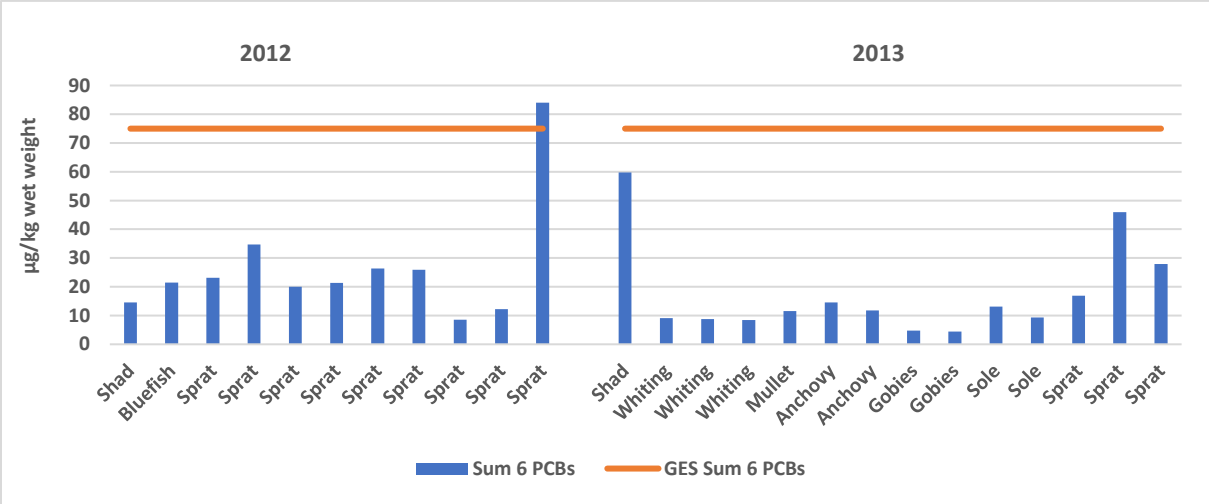


Figure 2.5 - Concentrations of PCBs in fish, 2012 -2013 (Constanta area and Danube mouths), in relation to the maximum acceptable concentrations in food for the protection of public health

During the Joint Cruise organized in 2013 in the framework of the project **DG ENV "MSFD Guiding Improvements in the Black Sea Integrated Monitoring System" (MISIS)**, 13 samples of mollusks – *Mytilus galloprovincialis, Rapana venosa* and *Scapharca inequivalvis* were collected (4 from the Romanian transect, 6 from the Bulgarian transect, and 3 from the Turkish transect) (Coatu et al., 2014; 2016).

The assessment according to Descriptor 9 was done considering European regulations that establish maximum admissible levels of contaminants in fish and other seafood for human consumption: EC no. 1881/2006 - only applicable to a few substances relevant for this indicator: benzo[a]pyrene, cadmium, lead, and mercury and EC no. 1259/2011 amending Regulation (EC) no. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs, and non-dioxin-like PCBs in foodstuffs.

Benzo[a]pyrene can be used as a marker for the occurrence and effects of carcinogenic PAHs in food. Only one sample of *Mytilus galloprovincialis* returned a benzo[a]pyrene concentration (14.2 ng/g wet weight) greater than maximum admissible limit of 10 µg/kg wet weight. EU legislation sets a maximum concentration of 75 ng/g wet weight for sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180. None of the mollusk's samples investigated in 2013, in the frame of MISIS project, exceeded the regulated level for **PCB**s (Figure 2.6).

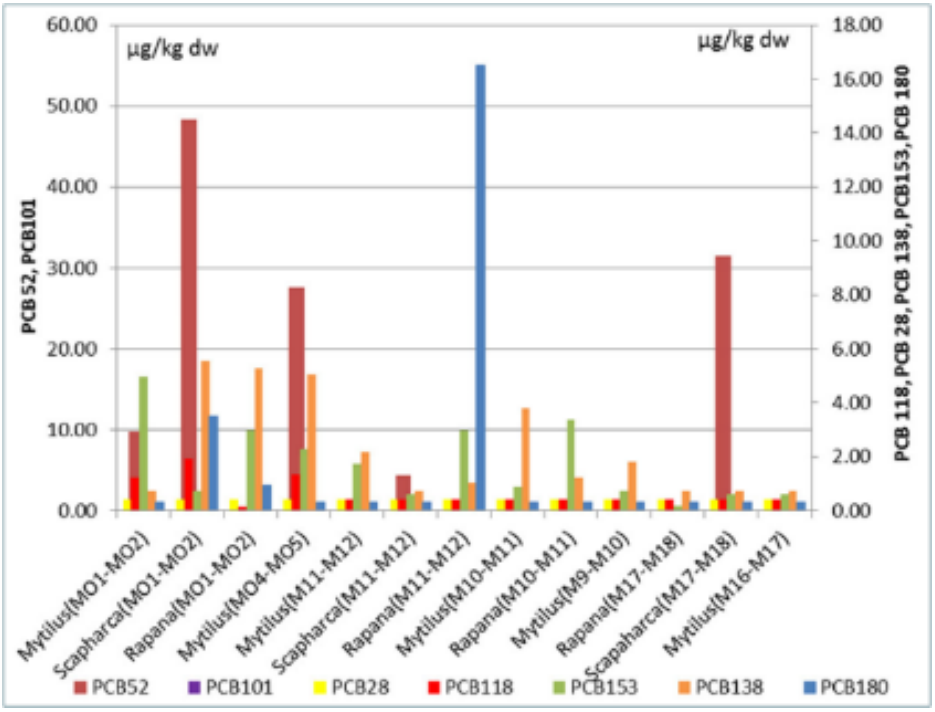
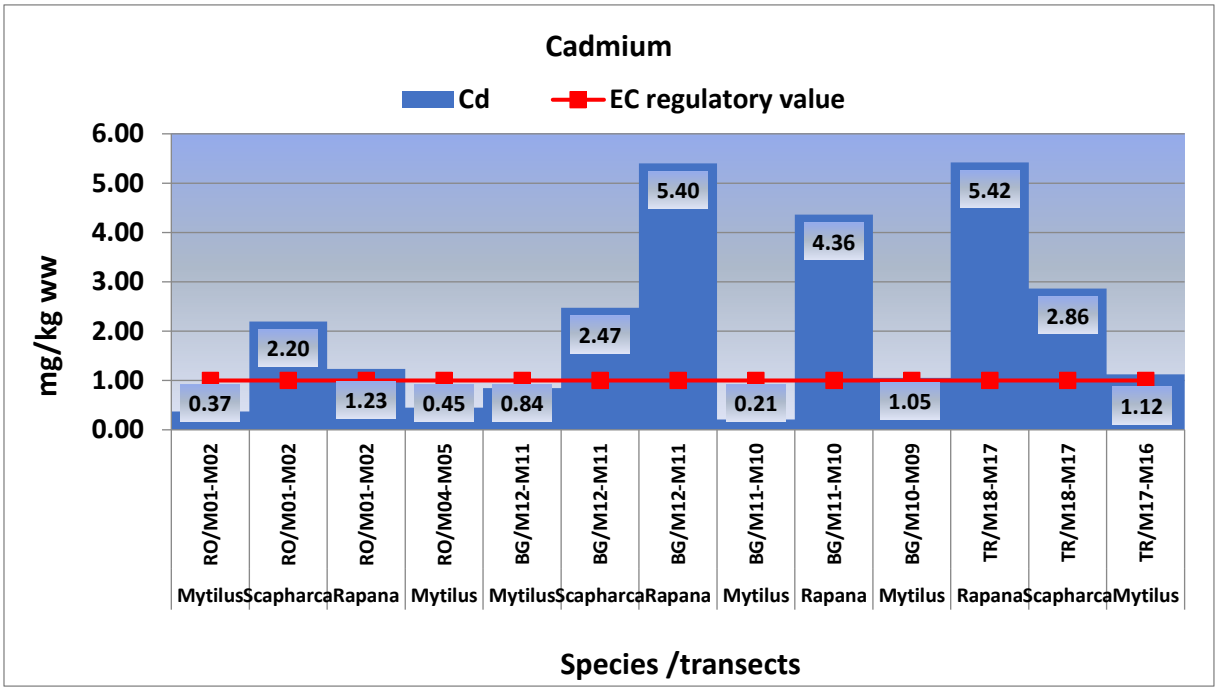


Figure 2.6 - Individual PCBs levels (µg/kg dry weight tissue) in mollusks from the Romanian, Bulgarian and Turkish waters, 2013

In comparison with EC regulatory value for **cadmium** in bivalve mollusks (1 µg/g ww), all *Mytilus* samples were below the limit, whereas *Scapharca* and *Rapana* from all transects presented higher bioaccumulation levels. We should mention that *Rapana* was analyzed as whole soft tissue, i.e., including viscera, where metals have the tendency to accumulate. In case of **lead,** all three species of mollusks were much below regulatory value (1.5 µg/g ww) (Figure 2.7).



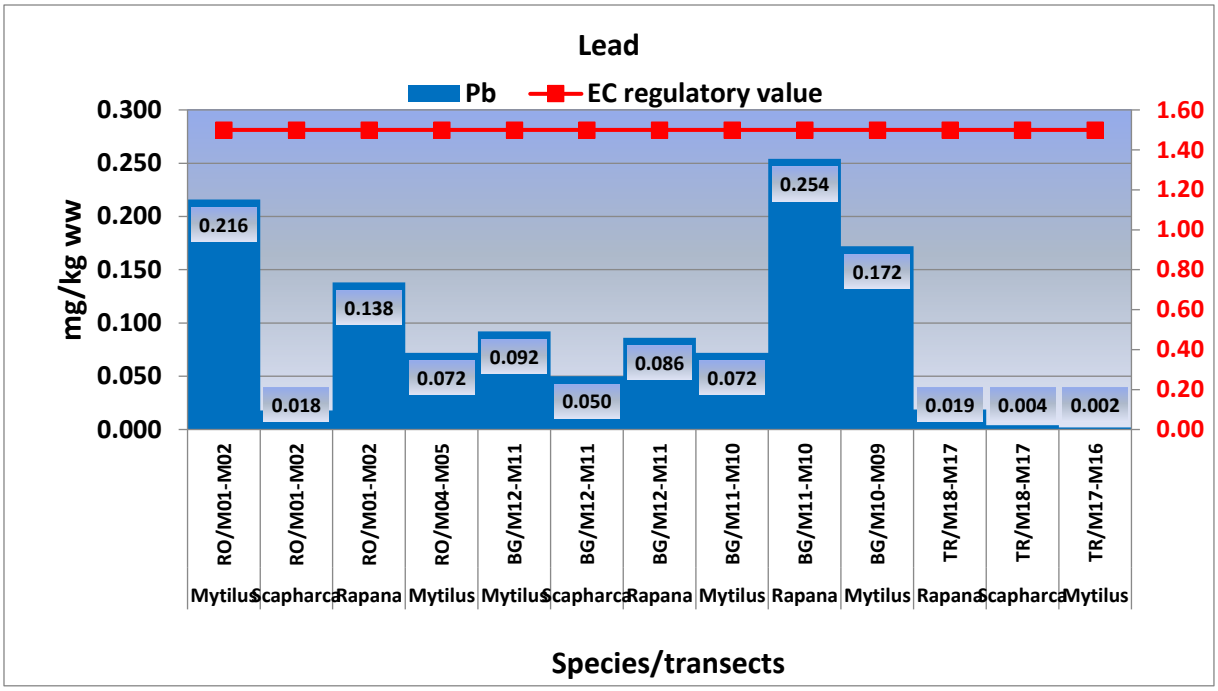


Figure 2.7 - Cadmium and lead concentrations in mollusks from the Romanian, Bulgarian and Turkish waters, 2013, in comparison with EC regulatory values

2.3 Turkey

Although MSFD is not legally binding yet, infrastructure has been developed for its implementation. TUBITAK was participated to the MarinTurk project (MarinTurk Report, 2016) aiming to develop the national infrastructure for MSFD implementation. In the mentioned project, initial assessment, good environmental status, targets and status monitoring on the related descriptors (including descriptor 9) were assessed and related gaps on the MSFD implementation were identified by the experts for the Black Sea and Mediterranean.

MoEU (Ministry of Environment and Urbanization) in cooperation with research organizations and MoFAL (Ministry of Food and Agriculture) General Directorate of Food Control is responsible for funding and undertaking the monitoring of contaminants in edible marine and aquaculture products according to the Turkish Food Codex (harmonized with EU Directives). In the framework of the National Monitoring project, contaminants levels (Pb, Cd, Hg, Benzo-a Pyrene, Dioxine like PCB's, Organochlorinated pesticides, DDT's) in selected target species were monitored once per year, in 2014-2016 and 2019-2020 periods. *Mullus barbatus* samples were collected by trawling from İgneada, Şile, Sinop, Bafra and Ordu, representing 5 marine assessment units, in 2014 and 2015.

Since there is very little data for separate years and marine assessment units, statistical analysis could be made for all stations using the national monitoring results for 2014, 2015, 2016 and 2019 (Table 2.7).

Table 2.7 - Descriptive statistics of selected contaminant concentration in biota in Turkish Black Sea waters during 2014-2019 (Tr-BS)

(2014 – 2015 - 2016 - 2019) BS stations of Tr	N	Mean	Std. Error of Mean	Median	Std. Dev.	Min.	Max.	Percentiles		
								25	50	75
Mullus barbatus										
Benzo(a) pyrene	81	0.31	0.08	0.04	0.73	0.00	2.63	0.03	0.04	0.11
PCBs total*	84	3.58	0.35	2.34	3.23	0.02	13.16	1.41	2.34	4.59
Cd	82	0.02	0.02	0.002	0.16	0.00	1.49	0.00	0.00	0.004
Pb	82	0.07	0.01	0.04	0.07	0.02	0.43	0.03	0.04	0.06
Mytilus galloprovincialis										
Benzo(a) pyrene	8	0.50	0.23		0.65	0.00	1.32	0.01	0.05	1.28
PCBs total	8	0.42	0.07		0.20	0.11	0.63	0.24	0.41	0.62
Cd	8	0.60	0.06		0.16	0.41	0.91	0.44	0.61	0.66
Pb	8	0.13	0.02		0.04	0.08	0.20	0.10	0.12	0.17

In 2016, the synthetic and non-synthetic pollutant contents of the *Mullus barbatus* and *Merlangius merlangus* samples collected from İğneada, Şile, Ereğli, Sinop, Samsun, Ordu-Fatsa and Perşembe, were evaluated using the threshold values given in the Turkish Food Codex (TGK no. 28157/2011). Main results of the National Monitoring Project are summarized below (National Marine Monitoring Program Reports, MoEU and TUBITAK-MRC, 2017; MoEU and TUBITAK-MRC, 2020):

- Total PCB (PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180) concentrations in edible tissues of both fish species were detected below the maximum limit values permitted by the Turkish food codex (TGK).
- PAH compounds such as benzo(a)pyrene and fluoranthene are specified as priority substances in the EU directive (2013/39/EU). Benzo(a)pyrene and fluorine content was found below the EQS biota values given in the directive 2013/39/EU and TGK.
- Generally, metal contents of above-mentioned biota samples, collected between 2014-2016, remained below the limit values given in the Turkish Food Codex in terms of Cd, Pb and Hg. The only exception is Ordu-Fatsa station where Pb contents of *Mullus barbatus* was determined slightly above the limit value specified in the TGK (Figure 2.8).
- Although the mercury values of fish samples in all regions are below the TGK threshold values, it is seen that they are above the limit value of 0.02 mg/kg ww for ecosystem health (2013/39/EU).

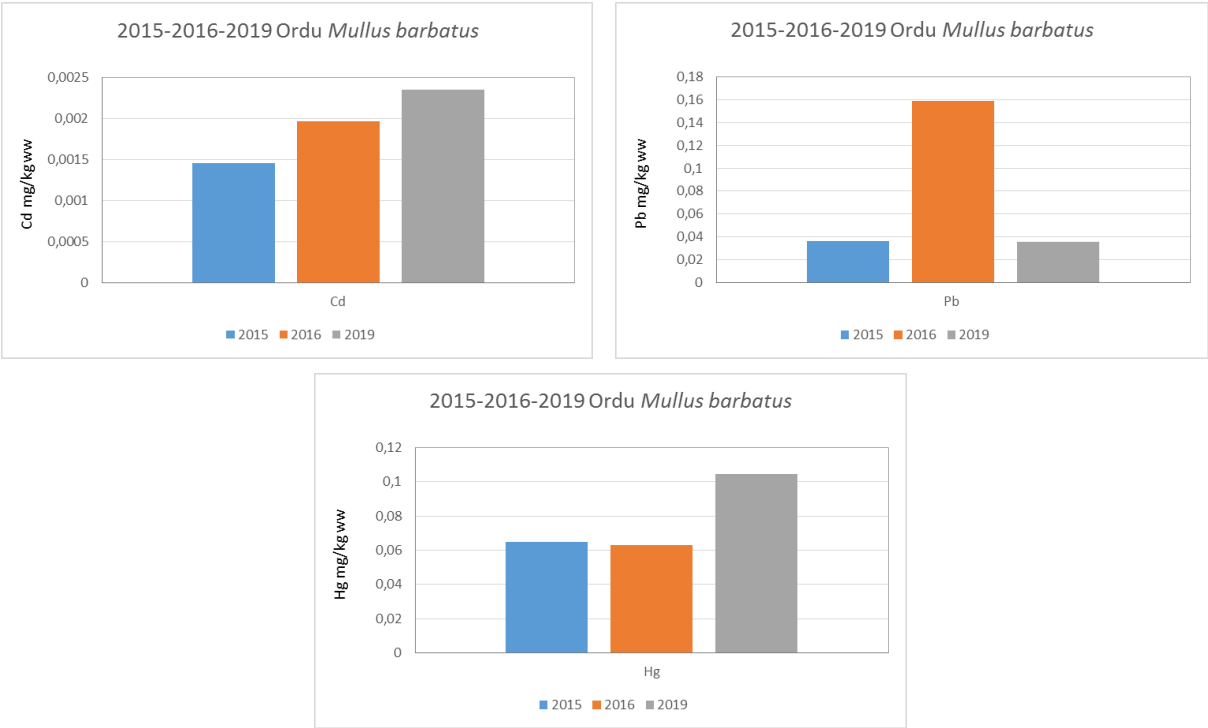


Figure 2.8 - Cd, Pb and Hg trends in demersal fish in Ordu station during 2015 – 2019 (MoEU and TUBITAK-MRC, 2017 and MoEU and TUBITAK-MRC, 2020)

National monitoring data available for the period 2014-2017 on the concentration of contaminants in fish species of commercial interest *Mullus barbatus*, collected from the Turkish Black Sea coastal waters have been centralized, processed, statistically analyzed, and evaluated against the proposed target values for defining good ecological status. Concentration of the contaminants in the mollusk species (*Mytilus galloprovincialis)* collected from Şile station were analyzed similarly for the same years.

The definition of good environmental status (GES) for criterion D9C1 was based on the maximum permissible levels required by the legislation in force (Commission Regulation no. 1881/2006, similar to TGK).

Analysis of data available in the above-mentioned period (N=84 and 8, for fish and mollusks, respectively) shows that persistent organic pollutants (PCBs and Benzo(a) pyrene) and heavy metals (Cd and Pb) indicates a good environmental status for the whole coastal area (assuming as one assessment unit) and Şile station (Table 2.8 and Table 2.9), percentile '75th being below the maximum allowable values for human consumption laid down by national and European legislation (similar to the Turkish Food Codex-TGK).

Table 2.8 - Environmental status assessment for D9C1 (all coastal water)

Mullus barbatus	BS Tr coastal stations (2014 -2015 / 2016-2019				
Compound	Percentile '75th (mg/kg wet tissue)	Threshold Value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Benzo(a)pyrene	0.11	2.00	0	Good	Good
PCBs total*	4.59	75.00	0	Good	
Cd	0.004	0.050	0	Good	
Pb	0.06	0.30	0	Good	

Table 2.9 - Environmental status assessment for D9C1 (Şile station)

Mytilus galloprovincialis	Şile station (2014 - 2015 / 2016-2019)				
Compound	Percentile '75th (mg/kg wet tissue)	Threshold Value (mg/kg wet tissue)	Threshold value exceedings (%)	Ecological status for individual compounds	Ecological status for the evaluation area
Benzo(a)pyrene	1.28	5.00	0	Good	Good
PCBs total*	0.62	75.00	0	Good	
Cd	0.66	1.00	0	Good	
Pb	0.17	1.50	0	Good	

Turkey (TUBITAK and Sinop University) participated to the DG ENV MISIS project coordinated by Romania (NIMRD), in which contamination of biota was analyzed with common methods and jointly assessed with BS countries. Results of the project was summarized in the chapter 2.2.

3 Activity performed - study areas, sampling campaigns and analytical methods

3.1 Ukraine

Biota samples (mollusks and fish) were investigated in June, September and November 2019 in the framework of contaminants pilot studies. The coordinates of biota sampling sites in Ukrainian waters are given in Table 3.1.

Table 3.1 - Biota samples for contaminants pilot studies, Ukrainian waters

Station	Species	Date (dd.mm.yyyy)	Longitude [degrees east]	Latitude [degrees north]
Zmeiniy Island	*Rapana venosa*	28.06.2019	30.2049	45.2575
Zmeiniy Island	*Rapana venosa*	28.06.2019	30.2049	45.2575
Zmeiniy Island	Round Bull Goby	28.06.2019	30.2049	45.2575
St 4 - Place of discharge from WWTP city and port Chornomorsk	*Rapana venosa*	14.09.2019	30.6347	46.1846
ONU_Biostation	Round Bull Goby	29.09.2019	30.7746	46.4435
ONU_Biostation	Mussel	30.09.2019	30.7746	46.4435
ONU_Biostation	Mussel	16.11.2019	30.7746	46.4435
ONU_Biostation	*Rapana venosa*	16.11.2019	30.7746	46.4435

Analytical methods

To determine contaminants in samples of bivalve molluscs and rapana for analysis, whole soft tissue samples were taken, whereas in fish samples, dorsal muscle tissue was investigated.

Trace metals

The samples were decomposed in sealed Teflon bombs (containers) with a mixture of concentrated hydrochloric and nitric acids (ratio 1/3) for a day, then hydrogen peroxide was added and kept for another day. After the end of the exposure, they were boiled for 3 hours in a water bath. The resulting solution was brought to 50 ml and analyzed on the AAS with ETI.

Calibration was performed with working standards for each element, starting from stock solutions of 1000 µg/L (Sigma-Aldrich). The work domains are as follows: Cd 0-10 µg/L; other metals 0-40 µg/L. At least 3 instrumental readings have been performed for each sample, with average value reported.

Organic pollutants

The samples were ground with calcined Na_2SO_4. The extraction was then carried out on an accelerated pressure extraction unit (PLE) with a mixture of hexane/dichloromethane/methanol (60%/20%/20%). Preliminary internal standards were added to the sample - anthracene D10 and PCB29.

The resulting extract was evaporated to 1 ml and purified from fat, and fractionation was carried out on a silica gel column. After fractionation, the obtained fractions were concentrated in a turbo evaporator under nitrogen flow.

Persistent organic pollutants were analyzed by gas chromatography. GC-ECD was used for OCPs and PCBs, and GC-MS was used for PAHs.

The calibration of instruments for the determination of the investigated pollutants was carried out using standard solutions for OCPs, PCBs, and PAHs.

3.2 Romania

During 10-15.05.2019, Research Vessel "Steaua de Mare 1" carried out a scientific expedition in the Northern area of the Romanian Black Sea coast, between 20 and 60 m bottom depth. Survey area, under the influence of the Danube mouths, was represented by 4 profiles: Sulina, Sf. Gheorghe, Portița and Periboina profiles. In the framework of this expedition dedicated to the analysis of the river Danube impact on the Black Sea, beside water and sediments, also samples of mollusks were taken from the 4 transects (between 40-50 m depth), as part of the pilot study to assess contaminants in marine organisms.

Mollusks - dredges were made to sample mussels (Figure 3.1). The species concerned was *Mytilus galloprovincialis*. A dredge of 100 x 30 cm was launched, the cord holding it had to be left with a length of 3 times the water. One person remained in command to record the coordinates, the depth, the speed of the vessel and the time, and one remained at the stern for launch.

When the dredge hits the bottom, the cable is checked. When the cable is stretched, the start time of the dredging is noted, and 5 minutes are needed as waiting time. Vessel speed during dredging must not exceed 1.2-1.5 Mn.

In each dredge, small individuals of the species *Mytilus galloprovincialis* were found in enough quantities for analysis.

Figure 3.1 - Mollusks sampling

During 6-11.08.2019, Research Vessel "Steaua de Mare 1" carried out a scientific expedition along the Romanian Black Sea coast, between 5 and 100 m bottom depth, in the framework of national monitoring. Mollusks (*Mytilus galloprovincialis and Rapana venosa*) were sampled by dredging from 5 transects (depths between 20-50 m), one in northern area of the littoral, the other four in southern area (Cazino Mamaia, Est Constanta, Costinesti and Mangalia).

Fish samples for contaminants analyses (8 species, pelagic and demersal) were collected in May, June and August 2019 in the framework of scientific expeditions for fishery resources assessment, carried out along the Romanian coast.

Also, in the framework of the ANEMONE Joint Cruise (30.09-7.10.2019), mussel samples were collected from ANE-RO-1 station and from 3 station from Bulgarian waters, that were also processed by NIMRD for the assessment of biota contamination.

Overall, for the pilot study regarding biota contamination in the Romanian waters, 21 samples (mollusks and fish) from various areas (river mouths, hot-spots, open sea) were investigated for the current levels of bioaccumulation of dangerous substances (Table 3.2, Figure 3.2).

Table 3.2 - Biota samples for contaminants studies, Romanian waters

Station	Species	Date (dd.mm.yyyy)	Longitude [degrees_east]	Latitude [degrees_north]	Depth (m)
RO_SU	*Mytillus galloprovincialis*	11.05.2019	30.1252	45.0642	40
RO_SG	*Mytillus galloprovincialis*	12.05.2019	30.1580	44.8603	50
RO_PO_50	*Mytillus galloprovincialis*	13.05.2019	29.6682	44.6669	50
RO_PB	*Mytillus galloprovincialis*	14.05.2019	29.6596	44.5270	50
RO_PO_50	*Mytillus galloprovincialis*	01.08.2019	29.6682	44.6669	50
RO_CM	*Mytillus galloprovincialis*	01.08.2019	28.8472	44.2347	30
RO_EC_2	*Rapana venosa*	01.08.2019	28.7833	44.1667	28
RO_EC_2	*Mytillus galloprovincialis*	01.08.2019	28.7833	44.1667	28
RO_EC_3	*Mytillus galloprovincialis*	01.08.2019	28.9000	44.1667	36
RO_COS	*Mytillus galloprovincialis*	01.08.2019	28.7267	43.9450	30
RO_MAN	*Mytillus galloprovincialis*	01.08.2019	28.7156	43.7986	39
RO_PO_8	*Engraulis encrasicolus*	22.06.2019	29.0067	44.6767	8
RO_PO_50	*Sprattus sprattus*	21.06.2019	29.6682	44.6669	54
RO_Z	*Squalus acanthias*	21.05.2019	29.3453	44.7492	14
RO_HP	*Trachurus mediterraneus ponticus*	13.06.2019	28.6490	44.3231	10
RO_HP	*Engraulis encrasicolus*	13.08.2019	28.6490	44.3231	10
RO_EC_4	*Neogobius melanostomus*	19.05.2019	29.1025	44.1667	43
RO_EC_4	*Mullus barbatus ponticus*	19.05.2019	29.1025	44.1667	43
RO_COS	*Merlangius merlangus euxinus*	19.05.2019	28.7267	43.9450	53
RO_2M	*Psetta maeotica*	18.05.2019	28.9000	43.7871	58
ANE-RO-1	*Mytillus galloprovincialis*	01.10.2019	30.5490	44.6253	78

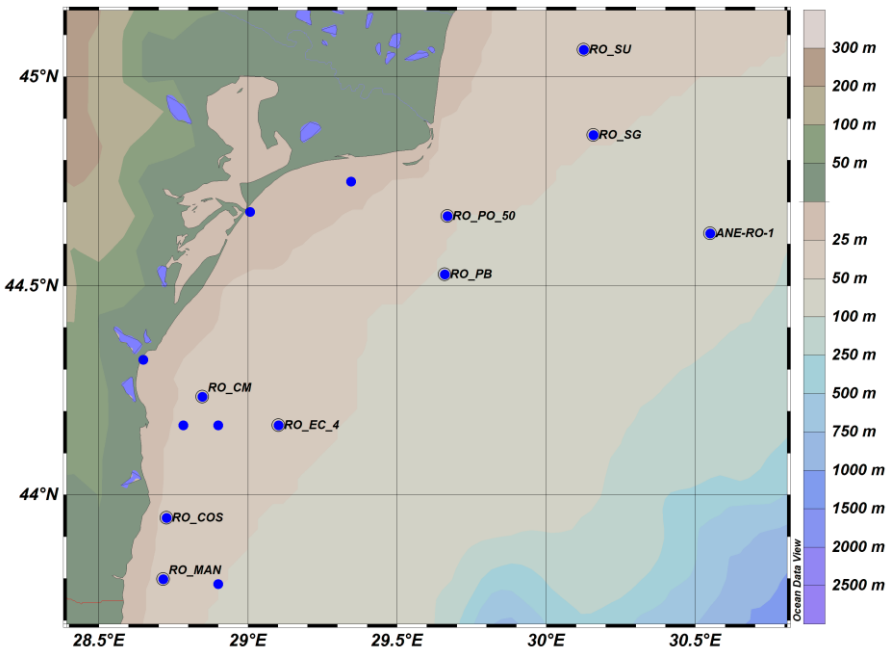


Figure 3.2 - Sampling stations for biota contamination study, Romanian waters, 2019

Analytical methods

Biota samples (whole soft tissue of mollusks, dorsal muscle of fish) were freeze-dried and further processed for heavy metals and organic pollutants. One composite sample represents tissues dissected from at least 5 - 10 individuals from each location (UNEP, 1990; 1993).

Trace metals

The biological samples were homogenized, weighed, and digested with concentrated nitric acid, in sealed Teflon vessels, on the electric plate at 120°C. At the end of mineralization, the samples were brought to volume 100 ml with deionized water. The analytical determination of the copper, cadmium, lead, nickel, and chromium was carried out by atomic absorption spectrometry method (GF-AAS), using a Solaar M6 DUAL Zeeman, Thermo Electron – Unicam model. Calibration was performed with working standards for each element, starting from stock solutions of 1000 µg/L. The work domains are as follows: 0-50 µg/L, Cd 0-10 µg/L, Pb 0-25 µg/L, Ni 0-50 µg/L, Cr 0-50 µg/L. At least 3 instrumental readings have been performed for each sample, with average value reported (IAEA-MEL, 1999).

Organic pollutants

For organic pollutants analysis (organochlorinated pesticides - OCPs, polychlorinated biphenyls – PCBs and polyaromatic hydrocarbons -PAHs), the freeze-dried tissues were homogenized and about 2 g of dried tissue was extracted for each class of compounds.

The extraction of OCPs and PCBs from biota samples was done with 30 ml acetone/hexane (1:1, v:v), in microwave extraction system Start E Milestone for 30 min at 120^0C. Internal standard 2,4,5-trichlorobenzene was added to the samples for quantifying the overall recovery of the analytical procedures. Further processing of the samples was done by clean-up on florisil column and concentration using the Kuderna-Denish concentrator and nitrogen flow. The analytical determination of the OCPs and PCBs was made by the gas-chromatographic method with a Perkin Elmer gas chromatograph CLARUS 500, equipped with electron capture detector (IAEA-MEL, 1995).

For PAHs analysis samples were extracted at Soxhlet for 8 h with 250 ml of methanol. Internal standard 9,10 dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. The extracts were then saponified by adding 20 ml of 0.7 M KOH and 30 ml of water and refluxing for 2 h. The resulting mixture was transferred into a separating funnel and extracted 3 times with hexane – once with 90 ml, twice with 50 ml. The extracts were concentrated by rotary evaporation down to 15 ml, and then further concentrated to about 5 ml under a gentle flow of clean nitrogen. Finally, the extract was cleaned up and fractionated by passing through a silica/alumina column. Elution was performed using 20 ml of hexane to yield the first fraction (containing the aliphatic hydrocarbons), then 30 ml of hexane:methylene chloride (90:10) and followed by 20 ml of hexane:methylene chloride (50:50). These two eluents containing the aromatic hydrocarbons (PAHs) were combined for analysis. The fraction containing PAHs was evaporated using the Kuderna-Denish concentrator and under a weak flow of nitrogen to 1 ml and it was subjected to quantitative analysis on GC/MS Perkin Elmer Clarus 600 (IAEA-MEL, 1995).

3.3 Turkey

Trawling operations were carried out at the marine areas close to the river mouths of Sakarya and Yeşilırmak rivers, in September 2019, in order to sample seafood, including fish. After obtaining necessary permissions from the Ministry of Agriculture and Forestry, a leased fishing boat was used for the trawl operations (Table 3.3, Figure 3.3, Figure 3.4).

The biota samples collected from the net were classified by species such as *Mullus barbatus, Alosa fallax, Merlangius merlangus euxinus, Trachurus mediterraneus, Sprattus sprattus, Psetta maxima, Solea solea, Rapana Venosa* and *Mytilus galloprovincialis* (Table 3.4). Each batch of specimen was counted, weighed and measured for their size (Figure 3.3). Whole soft tissues of the mollusks and dorsal muscle of the fish samples were separated onboard in clean conditions. Samples were kept in

freeze (at -20^{0}C) then subsequently analyzed in the TUBITAK MRC laboratory for heavy metals, Poly-Aromatic Hydrocarbons (PAHs) and Persistent Organic Pollutants (POPs) (

Table 3.5).

Figure 3.3 - Trawling operation (Sakarya river and Yeșilırmak river mouths marine area) and biota sampling/classification

Table 3.3 - Sakarya and Yeșilırmak River mouth coastal area biota sampling stations, coordinates and depths

Station	Date (dd.mm.yyyy)	Depth station	Speed (knots)	Time (min)	Start		End	
					Latitude [degrees_north]	Longitude [degrees_east]	Latitude [degrees_north]	Longitude [degrees_east]
Sakarya River	10.09.2019	38.0	2.9	30.0	41.154083 N	30.650217 E	41.154150 N	30.650100 E
Yeșilırmak River	12.09.2019	38.0	3.1	30.0	41.156233 N	36.556817 E	41.156217 N	36.556750 E

Figure 3.4 - Sakarya and Yeşilırmak Rivers trawl transect location for biota sampling

Table 3.4 - Biota samples for contaminants studies, Turkish waters

Station	Species	Date (dd.mm.yyyy)	Longitude [degrees_east]	Latitude [degrees_north]	Depth (m)
Sakarya River	*Mullus barbatus*	10.09.2019	30.6502	41.1541	38
	Alosa fallax	10.09.2019	30.6502	41.1541	38
	Mytilus galloprovincialis	10.09.2019	30.6502	41.1541	38
	Merlangius merlangus euxinus	10.09.2019	30.6502	41.1541	38
	Trachurus mediterraneus	10.09.2019	30.6502	41.1541	38
	Sprattus sprattus	10.09.2019	30.6502	41.1541	38
	Psetta maxima	10.09.2019	30.6502	41.1541	38
	Rapana Venosa	10.09.2019	30.6502	41.1541	38
Yeşilırmak River	*Mullus barbatus*	12.09.2019	36.5568	41.1562	38
	Trachurus mediterraneus	12.09.2019	36.5568	41.1562	38
	Merlangius merlangus euxinus	12.09.2019	36.5568	41.1562	38
	Psetta maxima	12.09.2019	36.5568	41.1562	38
	Solea solea	12.09.2019	36.5568	41.1562	38
	Rapana Venosa	12.09.2019	36.5568	41.1562	38

Table 3.5 - Sampling Methodology

Matrix	Parameter	Sampling Method	Storage Method	Reference
Pollutants biota	Metals (Cu, Cd, Cr, Ni, As, Pb, Mn, Co, Zn, Fe, Hg) PAHs, OCPs and PCBs	Mussel: Whole soft body (pooled sample consisting of at least 20 individuals) Fish: Muscle tissue (fillet) Composite samples are prepared in a manner that all biota samples will be as 3 replicates in the same length group.	Glass jar, samples were kept in freeze (at -20 C°)	EU, 2010. Guidance on Chemical Monitoring of Sediment and Biota under the WFD. CIS for WFD, Guidance Document No. 25. UNEP, 1999. UNEP/FAO/IOC/IAEA: Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. Ref. Method No. 12

Analytical methods

Biota samples (fish and mollusks) were transferred to pre-cleaned glass jars and freeze-dried. The analysis method for each of the parameters are summarized in Table 3.6.

Table 3.6 - Analysis methods

Matrix	Parameter	Method	Device	Reference	Unit
BIOTA	Cu, Cd, Pb, Ni, Cr, As, Mn, Co, Zn, Fe	Digested with nitric acid and hydrofluoric acid in microwave digestion system	ICP-MS	EPA Method 3052. EPA 6020 A 2007 – 02	µg/g
	Hg	Direct Mercury Analyzer	DMA 80 Mercury Analyzer	EPA Method 7473	µg/g
	OCPs and PCBs	Soxhlet Extraction (Hexane-dichloromethane) Removal of lipids by concentrated Sulphuric Acid Clean up technique: Florisil column (two fractions)	GC-MS-MS	UNEP/IOC/IAEA,1996 EPA8082 A EPA 3545 A	ng/g
	PAH	Soxhlet Extraction (Methanol) Saponification (n-Hexane) Clean up technique: Silica column (two fractions)	GC-MS-MS	UNEP/IOC/IAEA No:20; 1992 EPA 3630C Silica Gel Cleanup	ng/g
	EOM	Hexane-Dichloromethane Extraction	Gravimetric	EPA 3540 C	mg/lipids

Trace metals

The biological samples were homogenized, weighed, and digested with concentrated nitric acid and hydrochloric acid mixture (3/1) in the microwave digestion system at 180°C under higher pressure for 35 minutes. At the end of digestion, the filtered samples were brought to volume 50ml with deionized water. The analytical determination of the trace elements (such as copper, cadmium, lead, nickel, and chromium) was carried out by İnductively Coupled Plasma Mas Spectroscopy (ICP-MS) (Perkin Elmer- Neixon300x model (Figure 3.5). Calibration was performed with working standards for each element, starting from stock solutions of 100 mg/L (High Purity Standards). Calibration curves were prepared at 9 points, between 0.5-150 µg/L concentration level for all elements. At least 3 instrumental readings have been performed for each sample, with average value reported.

Recovery of the results varied between 91 and 110%. TUBITAK MRC - Marine laboratory participates in intermediate calibration tests (IAEA-MESL) twice a year in order to obtain better quality control / assurance regarding analysis results and successful results are obtained in these tests.

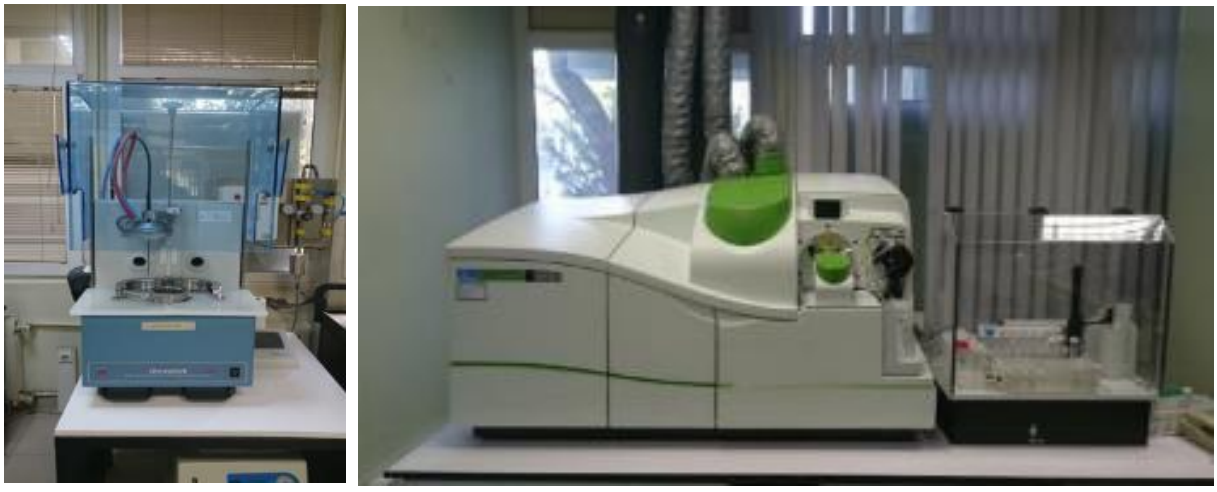


Figure 3.5 - ICP-MS instrument and microwave combustion system

Mercury contents of the pre-dried biota samples were determined via DMA80 Mercury analyzer, based on controlled heating in oxygenated environment (Figure 3.6).

Figure 3.6 - DMA 80 Mercury Analyzer

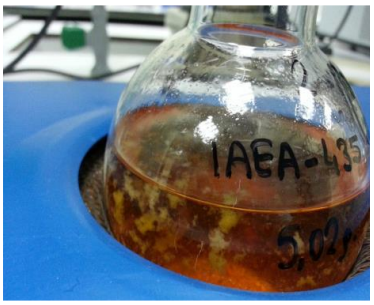
Organic pollutants

Samples were Soxhlet extracted for 8 hours using 250 ml of mixture of hexane and dichloromethane (50:50) for the organochlorinated compounds. PCB29 and PCB198 were used as internal standards for organochlorine compounds. The extract was concentrated by rotary evaporation down to 10-15 ml. The extract was dried with anhydrous sodium sulphate. The extractable organic matter (EOM) was determined by evaporating a measured small volume of this extract. Then the lipids removed with concentrated sulfuric acid. Extract was concentrated to about 1-2 ml under a gentle flow of clean nitrogen. Finally, the extract was purified and fractionated by passing it through a florisil column.

Soxhlet extraction was also used in PAH analysis. About 5 g sample Soxhlet extracted for 8 hours using 200 ml methanol. Chrysene D12, Acenapthene-D10, Napthhalene-D8, Perylene-D12 and Phenathrene-D10 were used as internal standards for PAHs. Then, 20 ml of 0.7 M KOH and 30 ml of distilled water were added and boiled for 2 more hours (Saponification process). Then the extract took into a separating funnel, 90 ml hexane is added on it and the upper phase is taken into a clean balloon. Extract is rinsed with 40 ml hexane 2 more times and the upper phase (hexane phase) is taken and transferred to the clean flask. The extract concentrated by rotary evaporation down to 10-15 ml. The extract dried with anhydrous sodium sulphate. Extract was concentrated to about 1-2 ml under a gentle flow of clean nitrogen. Finally, the extract was purified and fractionated by passing it through a silica column (Figure 3.7).

Appropriate blanks were analyzed with each set of biota samples. The organochlorinated compounds and polycyclic aromatic hydrocarbons in the extracts were measured using GC- MS (Figure 3.7).

Soxhlete Extraction

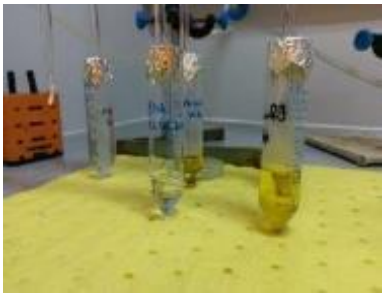


Saponification (with KOH)

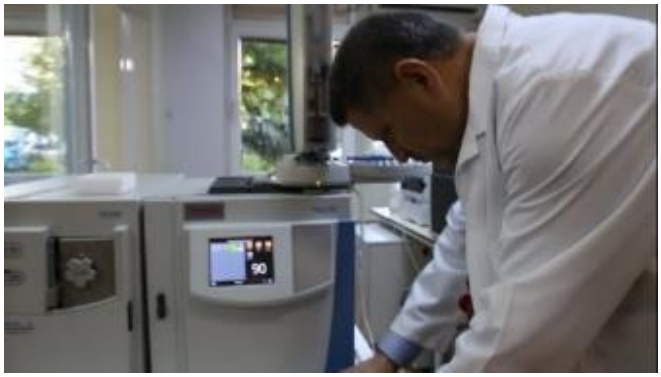
Extraction and separation process with Hexan

Evaporation

Clean Up (with silica column)



N_2 gas



GC/MS/MS

Figure 3.7 - PAH analysis in biota

4 Results and discussions

4.1 Ukraine

Biota samples investigated in 2019 within the framework of the biota contaminants pilot studies were evaluated using the Kz and CHASE methodology.

The maximum allowable concentrations of pollutants were taken from Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 (descriptor 8, ecological status) and from Commission Regulation (EC) No 1881/2006 of 19 December 2006 and Commission Regulation (EC) 1259/2011 of 2 December 2011 and national legislation (MAC UA) (Klyachko & Belenky, 1988) (descriptor 9, human consumption).

4.1.1 Trace metals

Table 4.1 presents the results of laboratory studies of the concentrations of toxic metals in selected biological samples.

In mussels and Rapana, the content of toxic metals exceeded the MAC concentrations:

- Cd (EC No 1881/2006) in a sample of Rapana caught in the area of discharge from the treatment facilities of the city and the port of Chornomorsk.
- As (MAC UA) in samples of Rapana and mussels caught in the area of the ONU biological station, located near the coastline of the urban zone of Odessa; in a sample of Rapana caught in the area of the wastewater treatment plant discharge of the city and the port of Chornomorsk; in one of the samples of Rapana caught in the area of the Zmeiniy Island, which is under the influence of the Danube River.
- Hg (Directive 2013/39/EU) in all samples of biological objects, except for one sample of mussels caught in the area of the ONU biological station.

In fish, the content of toxic metals exceeded the MAC:

- Pb (EC No 1881/2006) in a sample of Round goby caught in the area of the ONU biological station located near the coastline of the urban area of Odessa.
- Hg (Directive 2013/39/) in all samples.

Metals for which MAC were not established (Ni, Cr, Mn, Co, Fe) were present in low concentrations.

4.1.2 Organic pollutants

Table 4.2 presents the results of laboratory studies of OCPs concentrations in selected biological samples.

In mussels, Rapana and fish, an excess of the thresholds concentrations (Directive 2013/39/EU) was recorded only for heptaclor in one sample of mussel and a sample of round goby caught in the area of the ONU biological station located near the coastline of the urban zone of Odessa, and in a sample of a round goby caught in the area of the Zmeiniy Island, which is under the influence of the Danube River.

Among the OCPs for which no MAC have been established, it should be noted that lindane concentrations were higher in mussel and Rapana samples than in fish, in all areas.

The p,p'DDE concentrations were at a high level in the sample of round goby caught in the area of the ONU biological station, the p,p'DDT concentrations were at a high level in the mussel sample in the

area of the ONU biological station and were extremely high in the sample of Rapana caught in the area of the discharge from the wastewater treatment plant the city and port of Chornomorsk.

Table 4.3 presents the results of laboratory studies of PCBs concentrations in selected biological samples.

In mussels, Rapana and fish, no excess of the limit concentrations of PCBs was recorded (Directive 2013/39/EU and EC No 1881/2006 and EC 1259/2011.

Since, as polychlorinated biphenyls are incorporated into biological food chains, there is a progressive loss of low chlorinated components due to their selective biotransformation, the most dangerous highly chlorinated PCBs accumulate in human and animal organisms (Klyuev & Brodsky, 2000). Table 4.3 shows that the concentration of highly chlorinated biphenyls is lower than the concentration of low chlorinated biphenyls in all biological samples. It can be assumed that contaminants containing weakly chlorinated transformed PCBs are constantly supplied to the regions where samples were taken, and the accumulation of highly chlorinated biphenyls due to their fresh supply and accumulation is not observed.

Table 4.4 presents the results of laboratory studies of PAHs concentrations in selected biological samples.

An excess of the maximum available concentrations of PAHs was recorded in mussels and Rapana (according to Directive 2013/39/EU): benzo [a] pyrene in a sample of Rapana caught in the area of discharge from the wastewater treatment plant the city and port of Chornomorsk, in one sample of mussels caught in the area of the ONU biological station.

In biological samples, the accumulation of concentrations of carcinogenic PAHs is not observed.

There is an increased contamination of the sample of mussels and Rapana caught in the area of the ONU biological station with those PAHs for which maximum available concentrations have not been established, and this is evident from the total concentration of PAH. In the area of the ONU biological station, located near the coastline of the urban zone of Odessa, PAHs can come from the discharge of rainwater from city streets and from gas emissions from automobile transport.

In the sample of the Round goby caught in the area of the Zmeiniy Island, there is also showed a high concentration of total PAH.

Table 4.1 - Concentrations of TM in mg/kg ww in selected samples of biological objects

Station	MAC for mussel	Zmeiniy Island	Zmeiniy Island	St 4 - Place of discharge from WWTP city and port Chornomorsk	ONU Biostation	ONU Biostation	ONU Biostation	MAC for fish	ONU Biostation	Zmeiniy Island
Species		Rapana venosa	Rapana venosa	Rapana venosa	Mussel	Mussel	Rapana venosa		Round Goby	Round Goby
Date		28.06.2019	28.06.2019	14.09.2019	30.09.2019	16.11.2019	16.11.2019		29.09.2019	28.06.2019
Cu	30.0	9.52	12.7	19.1	1.21	0.608	10.6	10.0	0.94	0.41
Cd	1.00	0.511	0.808	1.80	0.026	0.092	3.38	0.10	0.053	0.019
Pb	1.50	0.13	0.46	0.48	0.29	0.293	0.396	0.30	0.66	0.06
Ni		0.22	0.34	0.27	0.36	0.32	0.29		0.16	0.08
Cr		0.14	0.24	0.28	0.25	0.14	0.29		0.30	0.09
As	2.00	0.33	9.68	5.68	5.39	5.06	69.9	5.00	1.43	1.33
Hg	0.02	0.026	0.047	0.048	0.050	0.006	0.050	0.020	0.042	0.027
Mn		7.48	0.91	1.95		1.10	1.05		26.5	1.07
Co		0.04	0.13	0.24	0.35	0.50	0.18		0.06	<0.04
Zn	200	20.4	37.4	32.4	15.3	15.1	13.8	40.0	16.5	7.53
Fe		30.3	35.8	67.8	36.0	12.4	55.2		16.9	18.9

Table 4.2 - Concentrations of OCPs in µg/kg ww, in selected biological samples

Station	MAC for mussel	Zmeiniy Island	Zmeiniy Island	St 4 - Place of discharge from WWTP city and port Chornomorsk	ONU Biostation	ONU Biostation	ONU Biostation	MAC for fish	ONU Biostation	Zmeiniy Island
Species		Rapana venosa	Rapana venosa	Rapana venosa	Mussel	Mussel	Rapana venosa		Round Goby	Round Goby
Date		28.06.2019	28.06.2019	14.09.2019	30.09.2019	16.11.2019	16.11.2019		29.09.2019	28.06.2019
HCB	**10**	0.79	<0.05	<0.05	<0.05	<0.05	<0.05	**10**	2.03	6.17
α-HCH		2.78	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
ß-HCH		2.42	<0.05	<0.05	<0.05	0.56	8.38		<0.05	<0.05
Lindane		27.7	5.79	5.82	0.10	8.48	5.76		<0.05	1.29
Heptachlor	**0.0067**	<0.05	<0.05	<0.05	<0.05	**9.37***	<0.05	**0.0067**	**8.92***	**87.4***
Aldrin		2.67	<0.05	<0.05	<0.05	<0.05	4.93		<0.05	<0.05
Dieldrin		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		5.02	3.88
p,p'DDE		7.47	<0.05	<0.05	<0.05	<0.05	<0.05		22.8	<0.05
p,p'DDD		<0.05	<0.05	<0.05	10.9	7.17	0.63		0.34	<0.05
p,p'DDT		<0.05	<0.05	250	<0.05	24.4	14.0		10.9	0.34

*** Concentrations exceeding MAC are highlighted in bold**

Table 4.3 - Concentrations of PCBs in selected samples of biological objects

Station	MAC for mussel	Zmeiniy Island	Zmeiniy Island	St 4 – discharge of WWTP city and port Chornomorsk	ONU Biostation	ONU Biostation	ONU Biostation	MAC for fish	ONU Biostation	Zmeiniy Island
Species		Rapana venosa	Rapana venosa	Rapana venosa	Mussel	Mussel	Rapana venosa		Round Goby	Round Goby
Date		28.06.2019	28.06.2019	14.09.2019	30.09.2019	16.11.2019	16.11.2019		29.09.2019	28.06.2019
Sum PCB (28,52,101,138, 153,180)	**75**	1,17	1,71	**97,9***	2,73	19,4	22,9	**75**	21,4	21,3
PCB 8		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 18		0.23	<0.05	17.6	0.41	2.11	1.02		4.23	2.50
PCB 31		149	124	<0.05	0.66	0.34	0.23		2.70	100
PCB28		<0.05	<0.05	<0.05	<0.05	2.74	0.2		<0.05	<0.05
PCB52		1.17	<0.05	21.1	0.67	3.11	3.21		5.75	6.91
PCB 49		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 44		<0.05	<0.05	7.36	0.26	0.59	0.13		1.60	2.27
PCB 66		<0.05	1.57	44.1	1.95	4.89	6.28		9.08	11.9
PCB 77		<0.05	3.24	158	6.1	11.4	35.3		<0.05	39.0
PCB101		<0.05	0.35	47.0	0.26	7.17	8.43		6.74	4.10
PCB 110		8.58	1.31	<0.05	1.61	2.61	5.38		8.74	13.5
PCB 149		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		21.2	<0.05
PCB118		1.29	<0.05	36.6	<0.05	<0.05	0.45		1.15	4.33
PCB153		<0.05	1.36	7.82	1.80	6.35	11.1		8.87	10.3
PCB 105		<0.05	0.06	6.76	0.15	1.76	4.47		3.41	7.00
PCB 187		<0.05	0.55	<0.05	0.60	3.66	8.48		5.76	10.4
PCB 126		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 128		0.06	<0.05	<0.05	0.28	0.19	0.77		0.34	0.82
PCB 196		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 206		<0.05	0.25	<0.05	<0.05	1.59	1.71		1.48	1.31
PCB138		<0.05	<0.05	22.0	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 183		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 174		<0.05	<0.05	<0.05	0.09	0.39	1.30		0.19	1.25
PCB 177		<0.05	<0.05	<0.05	0.38	0.69	0.82		0.38	1.22
PCB180		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 170		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		0.21	<0.05
PCB 199		<0.05	<0.05	<0.05	0.09	<0.05	1.49		<0.05	0.99
PCB 194		<0.05	<0.05	5.57	<0.05	<0.05	<0.05		<0.05	<0.05
PCB 209		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
PCB total		160.33	132.69	374.32	15.31	49.59	90.77		81.83	217.81

*** Concentrations exceeding MAC are highlighted in bold**

Table 4.4 - Concentrations of PAHs in µg/kg ww, in selected samples of biological objects

Station	MAC for mussel	Zmeiniy Island	Zmeiniy Island	St 4 - Place of discharge from WWTP city and port Chornomorsk	ONU Biostation	ONU Biostation	ONU Biostation	MAC for mussel	ONU Biostation	Zmeiniy Island
Species		Rapana venosa	Rapana venosa	Rapana venosa	Mussel	Mussel	Rapana venosa		Round Goby	Round Goby
Date		**28.06.2019**	**28.06.2019**	**14.09.2019**	**30.09.2019**	**16.11.2019**	**16.11.2019**		**29.09.2019**	**28.06.2019**
Naphthalene		3.30	13.2	39.0	76.3	13.3	21.5		21.8	13.1
Acenaphthylene		2.32	5.29	<0.05	<0.05	7.49	378		18.7	<0.05
Acenaphthene		4.49	2.05	<0.05	<0.05	5.8	566		188	3.36
Fluorene		33.6	10.8	15.2	8.09	175	8550		12.7	10.3
Phenanthrene		71.6	17.4	114	118	276	8171		226	65.8
Anthracene		2.28	0.92	3.37	4.31	46.6	428		1.15	1.74
Fluoranthene	**30**	3.43	1.94	10.6	29.7	3.90	26.1	**30**	21.5	6.68
Pyrene		1.99	0.68	5.32	20.0	3.25	22.7		4.11	4.72
Benzo[a]anthracene*		1.69	0.93	<0.05	9.69	1.65	30.9		1.92	2.37
Crysene*		<0.05	<0.05	<0.05	<0.05	2.62	64.2		4.07	4.28
Benzo[b]fluoranthene*		<0.05	1.70	8.17	<0.05	2.02	25.9		<0.05	3.73
Benzo[k]fluoranthene*		1.73	5.89	28.2	22.7	0.73	10.4		16.5	3.93
Benzo[a]pyrene*	**5**	2.56	<0.05	**17.0***	**10.2***	0.80	<0.05	**5**	1.11	0.58
Benzo (g,h,i)perylene		<0.05	4.18	11.3	33.3	0.87	2.21		7.93	3.24
Dibenzo(a,h)anthracene*		<0.05	1.48	<0.05	11.1	<0.05	1.89		1.90	6.64
Indeno(1,2,3-c,d)pyrene*		<0.05	10.2	<0.05	38.9	0.74	7.14		<0.05	5.06
PAH total		128.93	76.66	254.16	382.29	540.77	18305.94		527.39	135.53

*** Concentrations exceeding MAC are highlighted in bold**

Assessment of the ecological state of biological samples using Kz and CHASE is presented in Table 4.5.

Table 4.5 - Assessment of the ecological state of biological samples using Kz and CHASE

Assessment by Kz	Kz TM	Kz OCP`s	Kz PCB`s	Kz PAH`s	Assessment by CHASE
Mussels - *Mytilus galloprovincialis*					
ONU_Biostation 30.09.2019	0.92	0	0.04	1.52	2.59
ONU_Biostation 16.11.2019	0.54	699.26	0.26	0.15	422.8
Sea snail - *Rapana venosa*					
Zmeiniy Island 28.06.2019	0.95	0.02	0.02	0.18	1.84
St 4 - Place of discharge from WWTP city and port Chornomorsk 14.09.2019	1.36	0	1.31	2.07	4.1
ONU_Biostation 16.11.2019	6.92	0	0.31	0.44	12.87
Fish - Round Bull Goby					
Zmeiniy Island 28.06.2019	0.37	6522.7	0.28	0.17	3934.19
ONU_Biostation 29.09.2019	0.94	665.77	0.28	0.47	403.54

As can be seen from Table 4.5, mussels sampled in the ONU_Biostation area are characterized by increased organic pollution. In September 2019, the pollution level of PAH`s corresponds to a satisfactory level, Kz PAH`s = 1.52, pollution with benzo[a]pyrene prevails, the general assessment of the state is satisfactory.

In November, the pollution of OCP`s corresponds to a very bad level, Kz OCP`s = 699, Heptachlor pollution prevails, the general assessment of the state is very bad. The assessment of the state using the CHASE formula is identical to the assessment of Kz.

Regarding *Rapana venosa* sampled in the Zmeiniy Island area - overall state using Kz is good, whereas the overall assessment using CHASE is satisfactory.

Rapana venosa sampled in Station 4 - Place of discharge from WWTP city and port Chornomorsk, are characterized by increased metal contamination, PCB`s and PAH`s. The contamination level of TM corresponds to a bad ecological state, Kz TM=1.36, Hg, Cd and As contamination prevails, the level of PCB`s and PAH`s contamination corresponds to a satisfactory ecological state. Kz PCB`s=1.31, Kz PAH`s=2.07, benzo[a]pyrene contamination predominates (Table 4.5). The overall state is bad, and according to the CHASE - satisfactory.

Rapana venosa sampled in the ONU_Biostation area are characterized by increased metal contamination. The level of TM contamination corresponds to a very bad level, Kz TM=6.92, Hg and Cd contamination prevails. The overall assessment of the state is very bad, the assessment using the CHASE is identical to the assessment of Kz.

Fish (round bull goby) sampled in the Zmeiniy Island and ONU_Biostation areas are characterized by increased pollution with OCP`s, Kz OCP`s=6522 for Zmeiniy Island and 665 for ONU_Biostation, which corresponds to a very bad level, Heptachlor pollution prevails (Table 4.5). The overall assessment of the state is very bad. The assessment of the state using the CHASE formula is identical to the assessment of Kz.

Conclusions

- The results of the studies carried out in 2019 showed a high level of pollution of biological samples with toxic metals, in particular arsenic, cadmium and mercury.
- The maximum concentration of arsenic in Rapana (69,9 mg/kg), exceeding the MAC by almost 30 times, was found in November 2019 in the area of the ONU biological station, increased concentrations (more than 2-4 MAC) were also detected in Rapana samples from the area of wastewater discharge from the city and port Chornomorsk and in mussel samples from the ONU biological station.
- One case of exceeding the MAC by 1,8 times in the concentration of cadmium in Rapana in September 2019 in the area of wastewater discharge from the city and port Chornomorsk was recorded.
- Mercury concentrations exceeded MAC by 2-2,5 times in all biological objects (Mussel, Rapana, fish).
- Among organic pollutants, MACs were established only for hexachlorobenzene, heptachlor, the sum of 6 individual PCBs (28, 52, 101, 138, 153, 180), fluoranthene and benzo(a)pyrene.
- No cases of exceeding the MAC for the concentration of hexachlorobenzene in biological objects were recorded.
- The maximum concentration of heptachlor (87,4 µg/kg) was found in a sample of a Round goby caught in the area of Zmeinyi Island. High concentrations (9-10 µg/kg) were also recorded in the sample of Rapana and Round goby from the area of the ONU biological station.
- Exceeding the MAC for the amount of 6 PCBs was detected only in one Rapana sample in the place of discharge from WWTP city and port Chornomorsk.
- No cases of exceeding the MAC for the concentration of fluoranthene in biological objects were detected.
- Concentrations of benzo(a)pyrene exceeded the MAC in only one Rapana sample in the place of discharge from WWTP city and port Chornomorsk and one mussel sample from the area of ONU biological station.

4.2 Romania

4.2.1 Trace metals (TM)

Measured TM concentrations in mollusks and fish samples are presented in Table 4.6 and Table 4.7.

Table 4.6 - TM concentrations in µg/g ww, in Mytilus galloprovincialis, 2019

	Valid N	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std.Dev
Cu	11	2.824	2.486	1.134	5.110	1.970	4.122	1.258
Cd	11	0.643	0.311	0.143	2.018	0.202	1.086	0.647
Pb	11	0.020	0.019	0.002	0.077	0.002	0.031	0.022
Ni	11	1.303	0.930	0.232	5.744	0.574	1.230	1.518
Cr	11	2.015	1.570	0.322	4.384	0.860	3.324	1.415

Table 4.7 - TM concentrations in µg/g ww, in fish samples, 2019

	Valid N	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std.Dev
Cu	9	1.309	1.275	0.565	2.052	1.097	1.545	0.434
Cd	9	0.017	0.016	0.007	0.032	0.008	0.021	0.009
Pb	9	0.041	0.002	0.002	0.182	0.002	0.026	0.072
Ni	9	0.736	0.332	0.092	3.221	0.212	0.575	0.992
Cr	9	0.169	0.155	0.062	0.310	0.120	0.215	0.077

Rapana venosa was sampled only from 1 location, and the measured concentrations were as follows: Cu 4.640 µg/g ww; Cd 0.193 µg/g ww; Pb 0.007 µg/g ww; Ni 0.744 µg/g ww; Cr 2.376 µg/g ww.

TM bioaccumulation levels in mussels presented a wide spatial variability, with a pronounced tendency of higher concentrations being measured in area under the Danube influence (Northern sector) for most of the elements, especially Cd, Cu and Pb. Maximum value of Ni was measured in front of Sf. Gheorghe branch discharge, whereas maximum for Cr was noticed in Southern sector, in front of Constanta city and harbor (EC_2 station). Mussels from higher depth (78 m) (ANE-RO-1 joint cruise station) were characterized by low levels of HM, except for Pb, that had the maximum value here. (Figure 4.1;Figure 4.2; Figure 4.3).

TM bioaccumulation levels in dorsal muscle of pelagic and demersal fish species investigated in 2019 highlighted some interspecific differences, depending on the position along trophic chain, physiological state, diet, age, environmental conditions.

Cu concentrations were rather homogeneous distributed among fish species, with slightly higher values being measured in *Engraulis encrasicolus* and *Psetta maeotica*, and the minimum value in *Merlangius merlangus*. Cd presented maximum values in *Trachurus mediterraneus ponticus* and in two demersal species, *Neogobius melanostomus* and *Psetta maeotica*. Pb registered low bioaccumulation levels in the majority of fish samples, except for *Trachurus mediterraneus ponticus* and *Psetta maeotica*. Turbot presented also higher values of Ni, in comparison with other species, followed by *Squalus acanthias*. Cr maximum value was measured in *Sprattus sprattus*, and minimum in *Engraulis encrasicolus* (Figure 4.4).

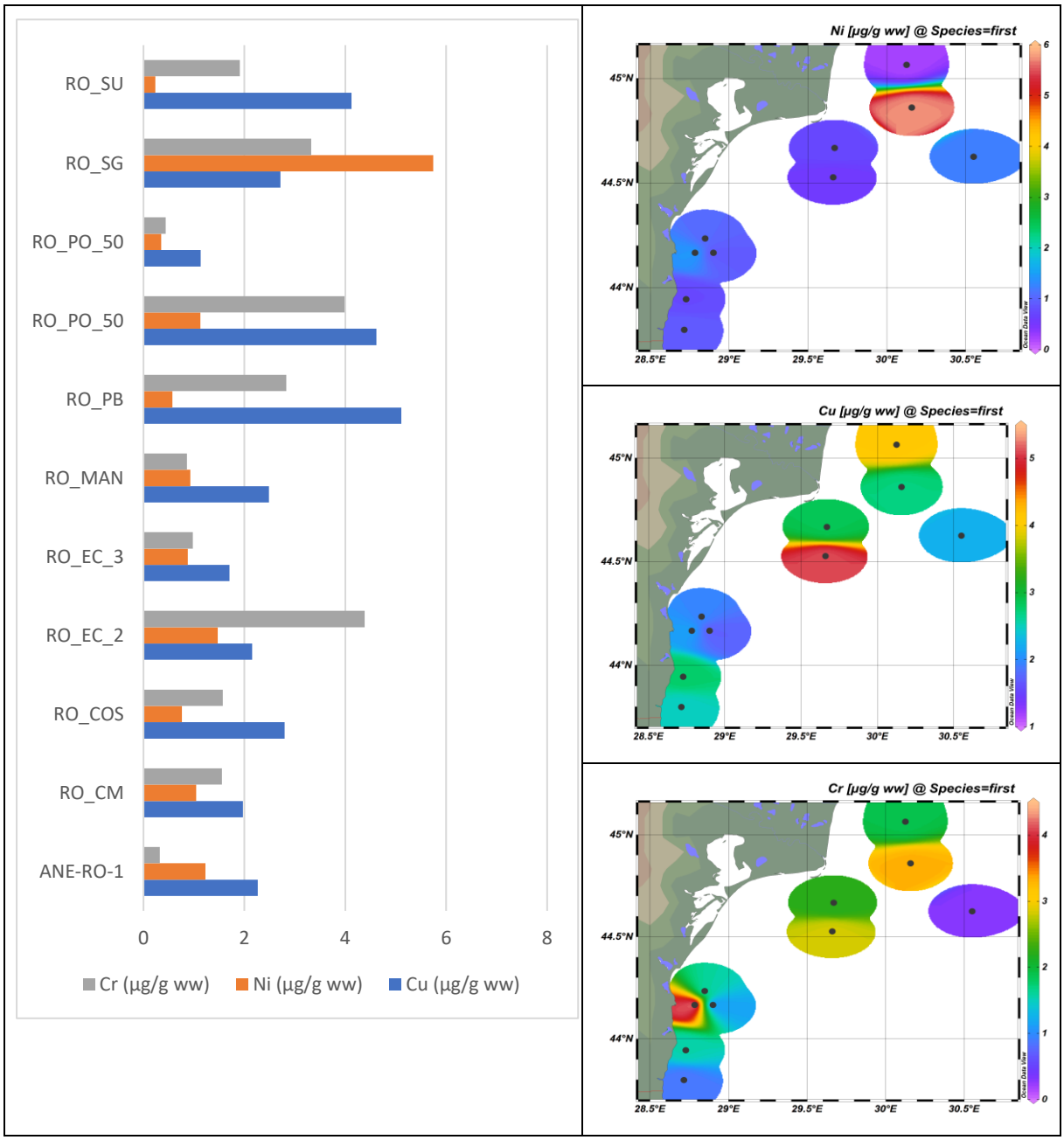


Figure 4.1 - Cu, Ni and Cr concentrations in Mytilus galloprovincialis from Romanian waters, 2019

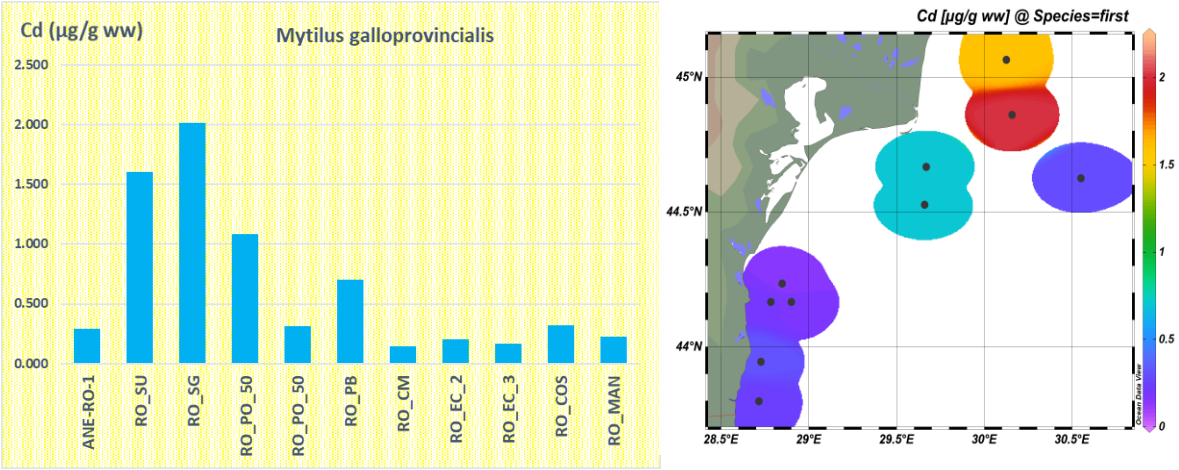


Figure 4.2 - Cd concentrations in Mytilus galloprovincialis from Romanian waters, 2019

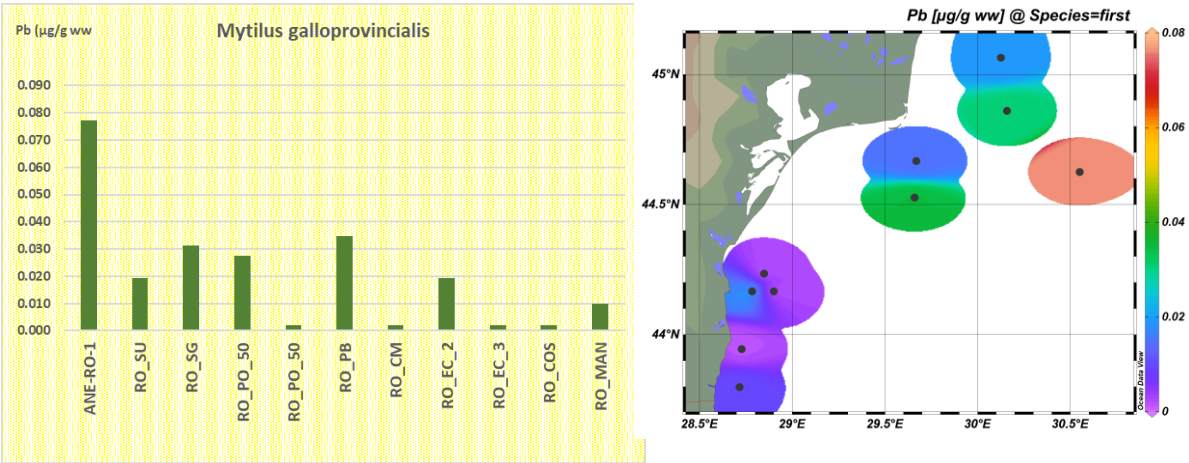


Figure 4.3 - Pb concentrations in Mytilus galloprovincialis from Romanian waters, 2019

Regarding compliance with maximum admissible concentrations stipulated by EC regulation 1881/2006, and further amendments, Pb bioaccumulation levels were much below MACs (1.5 µg/g ww mussels; 0.3 µg/g ww fish) in all investigated biota samples. In the case of Cd, surpassing of regulated levels (1 µg/g w.w. mussels; 0.1 µg/g ww fish) was noticed in only 3 samples of mussels, all from the Northern sector of the littoral, whereas all fish samples were below MAC (Figure 4.5).

Overall, measured HM concentrations in fish were lower in comparison with mussels, varying in much narrower ranges, especially in the case of Cd, Cu, and Cr (Figure 4.6).

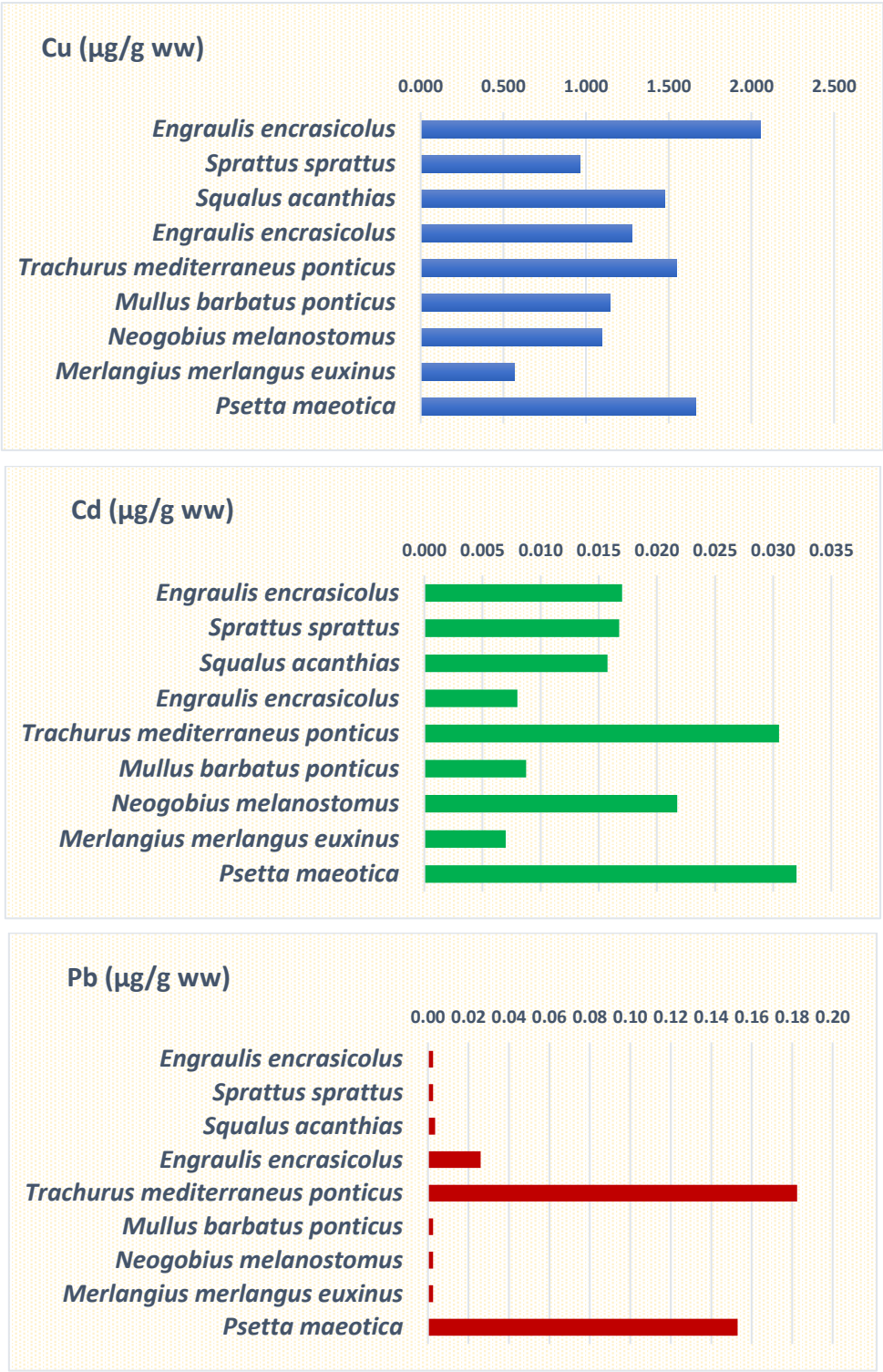


Figure 4.4 - TM concentrations in dorsal muscle of pelagic and demersal fish species from the Romanian waters, 2019

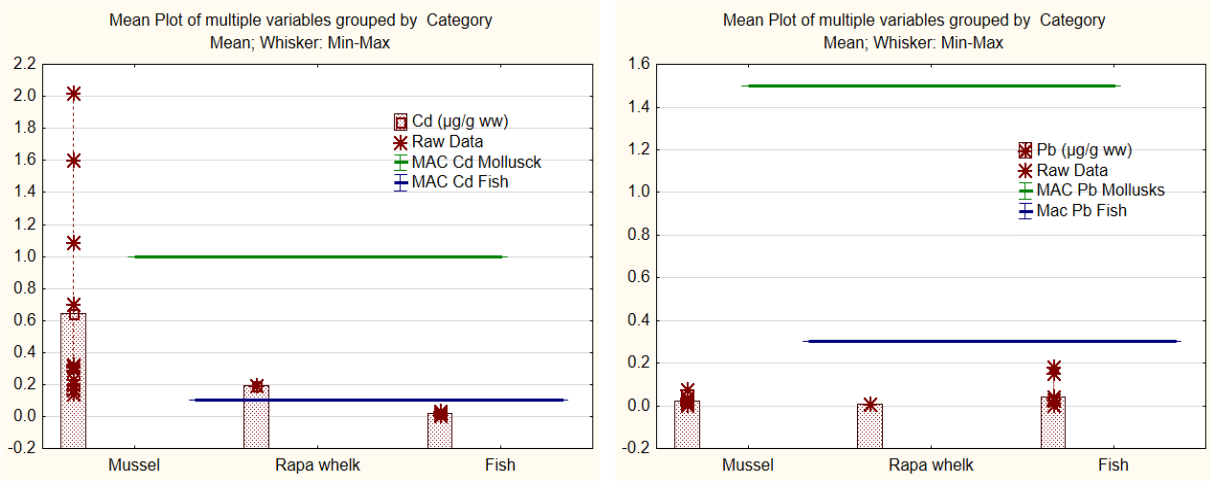


Figure 4.5 - Cd and Pb concentrations in biota (mollusks, fish) in comparison with maximum admissible levels (MAC, EC Regulation nr. 1881/2006)

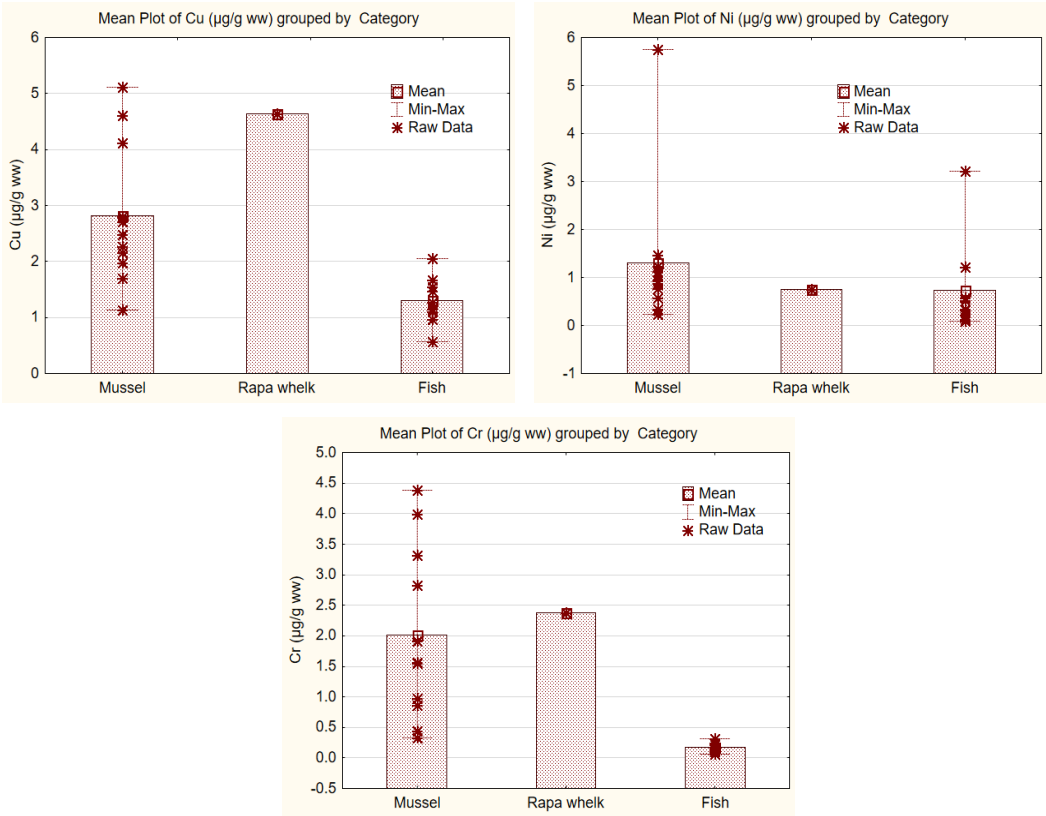


Figure 4.6 - Cu, Ni and Cr concentrations in biota (molluscs, fish) from Romanian waters, 2019

In comparison with previous data (2012-2017), it could be noticed for *Mytilus galloprovincialis* that median value in 2019 is closed to multiannual median value for most metals. Only Cr presented an increasing trend in 2019. Also, variation ranges in 2019 for Cu and Pb were much narrow, with less outlier values, whereas for Cd some values outside normal limit of variation were noticed (Figure 4.7). In *Rapana venosa* sample, Ni concentration in 2019 was similar to multiannual median, Cu, Cd and Pb were below overall median (2012-2017), and only Cr presented an increasing trend (Figure 4.8).

Available data for fish evinced for 2019 data a decreasing trend for Cd and Pb, or at least maintaining within the same variation ranges observed in the previous period (Figure 4.9).

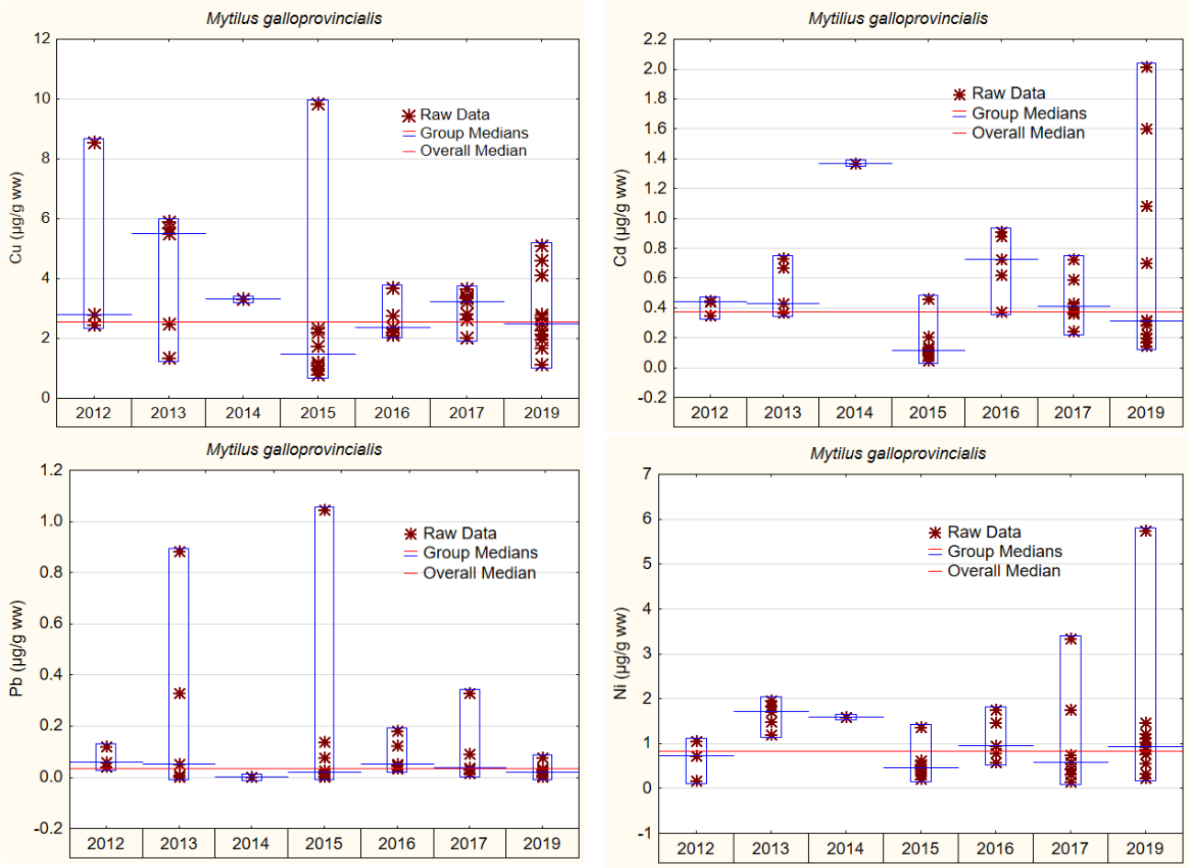


Figure 4.7 - TM trends in Mytilus galloprovincialis in Romanian waters during 2012 - 2019

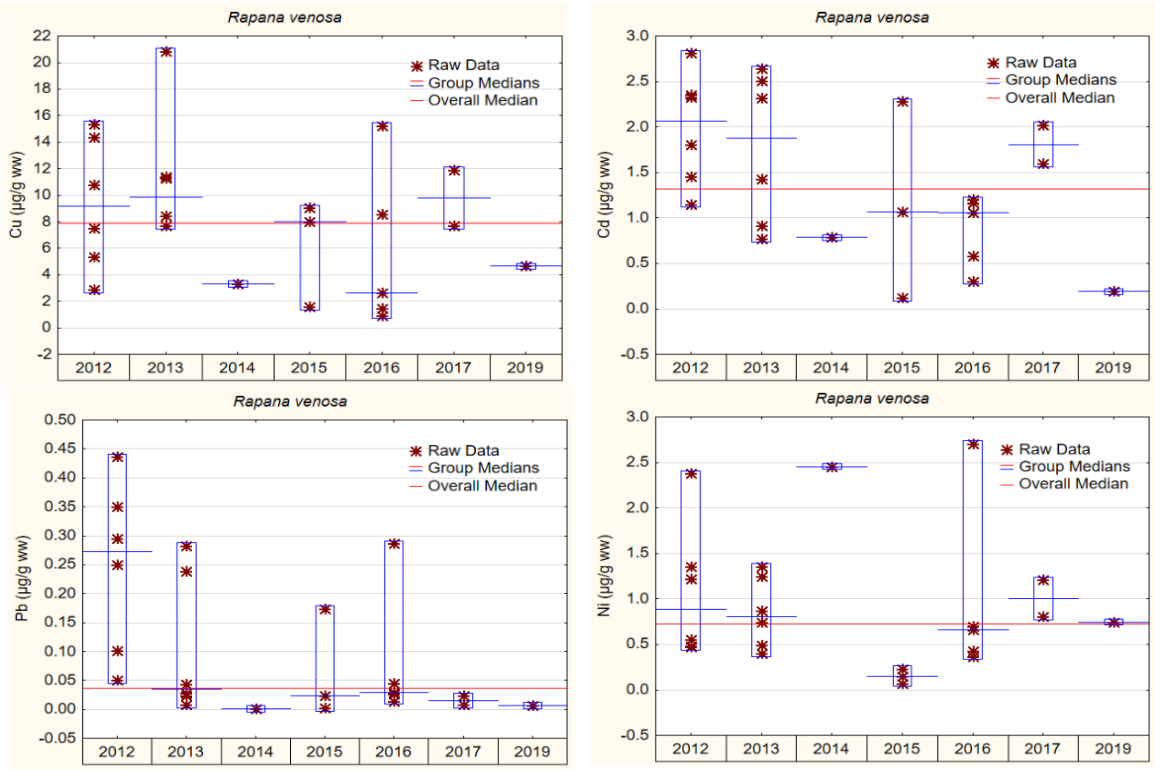


Figure 4.8 - TM trends in Rapana venosa in Romanian waters during 2012 - 2019

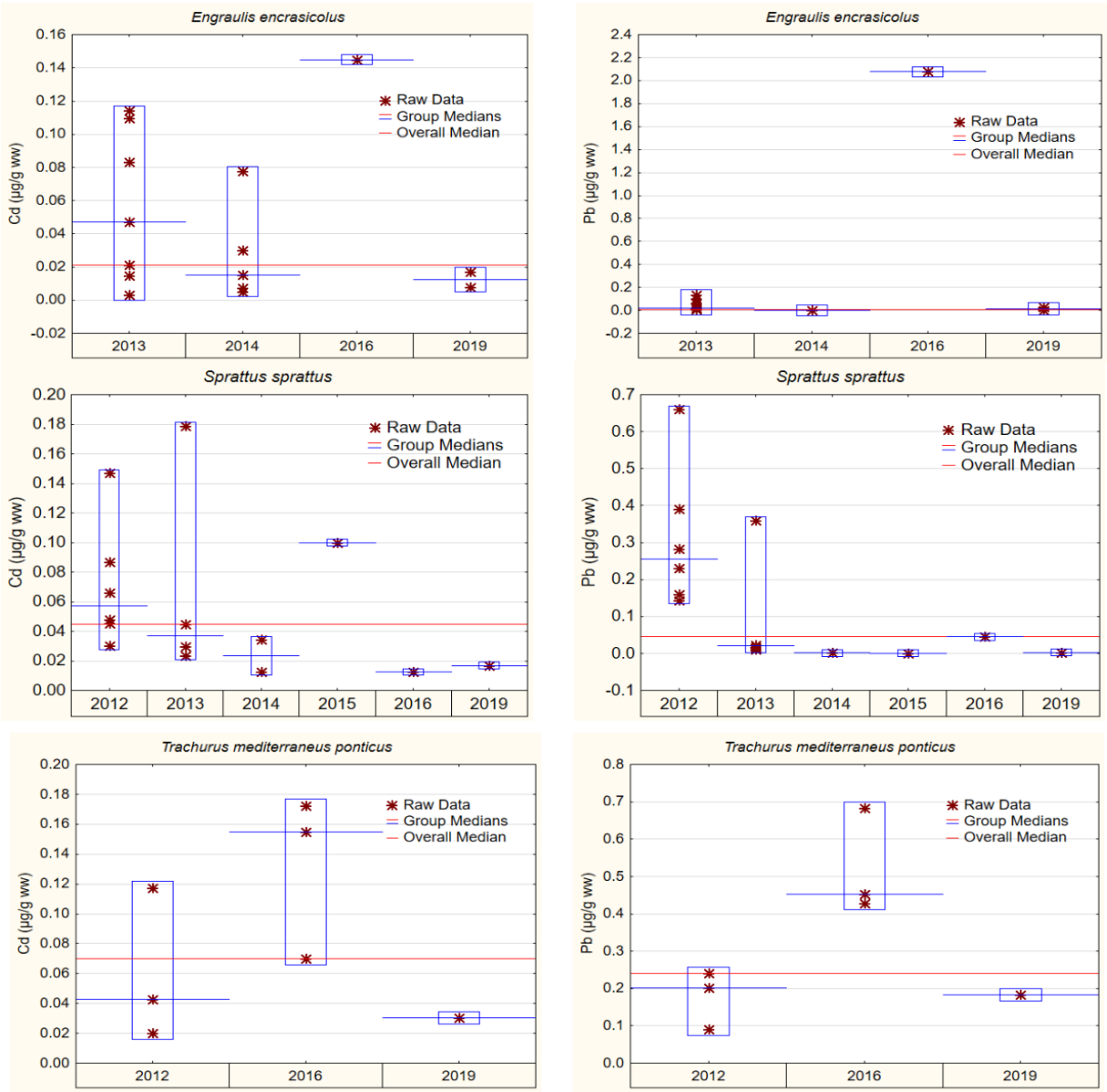


Figure 4.9 - TM trends in fish in Romanian waters during 2012 - 2019

4.2.2 Organic pollutants

Organic pollutants concentrations, respectively organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons measured (PAHs) in molluscs and fish sampled in 2019, are presented in Table 4.8 to Table 4.13.

Table 4.8 - Summary statistics of organochlorine pesticides concentrations in Mytilus galloprovincialis, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile ^{25}th	Percentile ^{75}th	Std. Dev
HCB	11	41.92	26.73	<0.08	116.08	<0.08	100.49	46.77
Lindane	11	28.25	10.86	<0.06	116.10	<0.06	40.40	37.43
Heptachlor	11	12.54	<0.05	<0.05	46.16	<0.05	21.12	17.39
Aldrin	11	11.00	1.73	0.21	47.25	0.27	17.19	15.34
Dieldrin	11	16.24	2.75	<0.05	83.45	0.24	11.94	28.96
Endrin	11	64.87	32.36	<0.06	335.20	<0.06	98.49	99.02
p,p'DDE	11	26.29	1.48	<0.03	101.30	0.49	55.34	35.52
p,p'DDD	11	26.93	7.05	0.33	101.30	1.13	55.34	35.06
p,p'DDT	11	1.21	0.19	<0.03	7.46	<0.03	1.41	2.27

Table 4.9 - Summary statistics of polychlorinated biphenyls concentrations in Mytilus galloprovincialis, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile ^{25}th	Percentile ^{75}th	Std. Dev
PCB28	11	31.36	19.22	<0.06	86.35	<0.06	73.60	35.81
PCB52	11	16.73	4.01	<0.05	73.77	<0.05	35.90	25.86
PCB101	11	30.74	14.00	<0.09	141.92	2.80	35.63	43.20
PCB118	11	50.80	54.23	4.43	89.75	25.81	78.40	29.62
PCB153	11	27.39	23.83	<0.09	68.79	<0.09	59.84	29.53
PCB138	11	34.64	29.01	<0.11	132.18	<0.11	58.32	42.85
PCB180	11	11.23	5.87	<0.05	54.72	3.57	8.15	16.38

Table 4.10 - Summary statistics of polyaromatic hydrocarbons concentrations in Mytilus galloprovincialis, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile ^{25}th	Percentile ^{75}th	Std. Dev
Naphthalene	11	1.30	<0.02	<0.02	7.35	<0.02	2.29	2.27
Acenaphthylene	11	2.43	<0.02	<0.02	26.55	<0.02	<0.02	8.00
Acenaphthene	11	2.58	<0.02	<0.02	28.22	<0.02	<0.02	8.50
Fluorene	11	0.03	<0.02	<0.02	0.17	<0.02	0.03	0.05
Phenanthrene	11	0.66	0.17	<0.02	4.36	<0.02	0.21	1.35
Anthracene	11	4.74	<0.02	<0.02	24.39	<0.02	7.19	8.46
Fluoranthene	11	0.03	<0.02	<0.02	0.17	<0.02	<0.02	0.05
Pyrene	11	0.04	<0.02	<0.02	0.18	<0.02	0.04	0.05
Benzo[a]pyrene	11	0.75	<0.02	<0.02	7.33	<0.02	<0.02	2.19
PAHs total	11	18.59	0.86	0.32	81.81	0.36	27.50	30.51

Table 4.11 - Summary statistics of organochlorine pesticides concentrations in fish, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std. Dev
HCB	9	66.06	33.97	10.35	222.27	20.86	104.73	68.74
Lindane	9	19.72	3.25	<0.10	87.47	0.49	28.50	28.85
Heptachlor	9	46.89	27.12	2.34	197.39	5.16	58.22	61.12
Aldrin	9	10.90	1.38	<0.08	41.53	<0.08	16.46	15.35
Dieldrin	9	4.60	0.08	<0.08	28.26	<0.08	6.31	9.28
Endrin	9	7.89	6.60	<0.10	33.42	0.28	8.67	10.34
p,p'DDE	9	10.87	3.97	3.04	40.58	3.31	15.37	12.40
p,p'DDD	9	10.87	3.97	3.04	40.58	3.31	15.37	12.40
p,p'DDT	9	1.37	0.81	<0.05	5.50	0.72	1.30	1.67

Table 4.12 - Summary statistics of polychlorinated biphenyls concentrations in fish, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std. Dev
PCB28	9	24.35	17.43	<0.10	51.56	11.86	36.06	17.45
PCB52	9	29.88	7.38	<0.08	163.64	0.46	17.15	54.02
PCB101	9	60.07	18.34	6.14	238.97	10.47	23.52	90.82
PCB118	9	79.32	82.78	<0.10	193.89	22.75	88.80	66.08
PCB153	9	35.63	0.34	<0.15	99.88	<0.15	85.39	45.15
PCB138	9	47.34	49.97	<0.18	112.64	17.21	62.96	37.15
PCB180	9	3.86	1.89	<0.08	17.16	1.01	3.33	5.33

Table 4.13 - Summary statistics of polycyclic aromatic hydrocarbons concentrations in fish, 2019 (all values are in ng/g ww)

	Valid N	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std. Dev
Naphthalene	9	2.95	3.11	<0.03	7.17	<0.03	4.46	2.51
Acenaphthylene	9	0.16	<0.03	<0.03	1.14	<0.03	<0.03	0.37
Acenaphthene	9	0.22	<0.03	<0.03	1.55	<0.03	<0.03	0.51
Fluorene	9	0.08	<0.03	<0.03	0.37	<0.03	<0.03	0.12
Phenanthrene	9	0.66	0.04	<0.03	2.99	<0.03	0.19	1.19
Anthracene	9	8.27	<0.03	<0.03	55.40	<0.03	3.20	18.16
Fluoranthene	9	0.08	<0.03	<0.03	0.40	<0.03	<0.03	0.13
Pyrene	9	0.09	<0.03	<0.03	0.44	<0.03	<0.03	0.14
Indeno(1,2,3-c,d)pyrene	3.32	0.03	<0.03	29.71	<0.03	<0.03	9.90	3.32
PAHs total	16.01	8.15	0.41	60.21	3.88	23.33	19.42	16.01

The levels of organic pollutants varied in similar ranges in molluscs and fish samples collected in 2019. In respect with maximum admissible concentrations stipulated by European (EC regulation 1881/2006, with further amendments, completed by EC regulation 1259/2011) and national legislation (Order 147/2004), organic pollutants surpassed the regulated levels both in molluscs and fish.

In *Mytilus galloprovincialis* OCPs concentrations varied in a large range, from detection limit to 335.2 ng/g wet weight. The highest values were recorded for endrin, HCB, Lindane, p,p' DDE and p,p' DDD in the samples collected in the southern part, from Constanta to Mangalia, strongly influenced by anthropogenic activities: endrin – 335.2 ng/g ww in EC_2 station, 89.33 ng/g ww in EC_3 station, 99.38 ng/g ww in Mangalia station; HCB – 100.49 ng/g ww in EC-2 station and 70.15 ng/g ww in EC-3 station; Lindane – 75.80 ng/g ww in EC_2 station and 40.40 ng/g ww in EC_3 station; 73.53 ng/ g ww p,p' DDE and p,p' DDD in Mangalia station and 55.34 ng/ g ww p,p' DDE and p,p' DDD in EC_2 station. OCPs exceeded the regulated levels in 20% of the samples for Aldrin, dieldrin and total DDT, in 40% of the samples for endrin and in 50% of the samples for HCB (Figure 4.10).

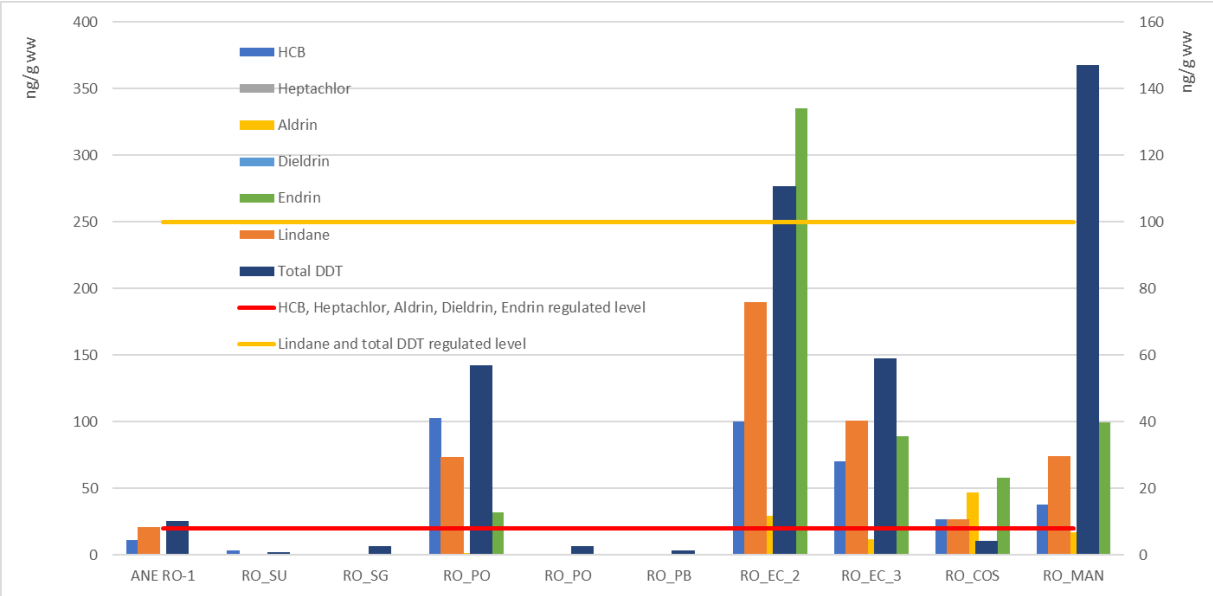


Figure 4.10 - Organochlorine pesticides concentrations in* Mytilus galloprovincialis *from Romanian waters, 2019 in relation to maximum admissible levels (national legislation Ord. 147/2004)

PCBs had also a large variation, from detection limit to 141.92 ng/g wet weight. High concentration of different compounds was observed in almost all samples (Figure 4.11). In the northern part under the influence of the Danube, the highest values were recorded for PCB 118 (83.97 ng/g ww at Portita station, 78.4 ng/g ww in Periboina station and 73.36 ng/g ww in Sf. Gheorghe station) and PCB 28 (81.40 ng/g ww at Sulina station, 86.35 ng/g ww in Periboina station and 73.6 ng/g ww in Sf. Gheorghe station). In the southern part, the highest values were recorded in Mangalia station for PCB 101 (141.92 ng/g ww) and PCB 153 (68.79 ng/g ww), in EC_2 station for PCB 138 (132.18 ng/g ww) and PCB 52 (73.77 ng/g ww) and in EC_3 station for PCB 138 (81.98 ng/g ww).

The sum of 6 PCBs regulated by European legislation (EC regulation 1259/2011) surpassed the maximum admissible level in 80% of the analysed samples (Figure 4.12).

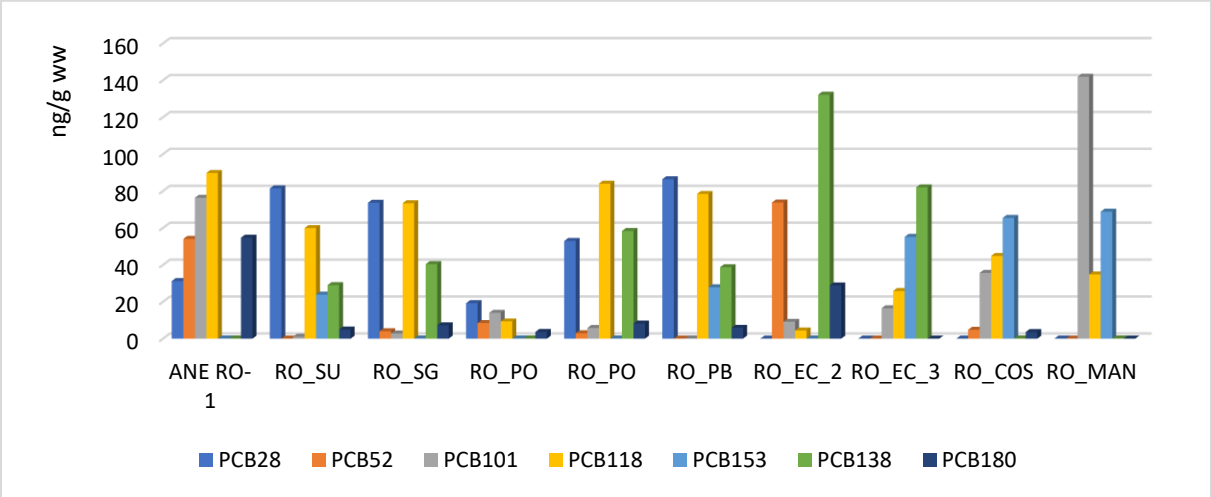


Figure 4.11. Polychlorinated biphenyls concentrations in Mytilus galloprovincialis from Romanian waters, 2019

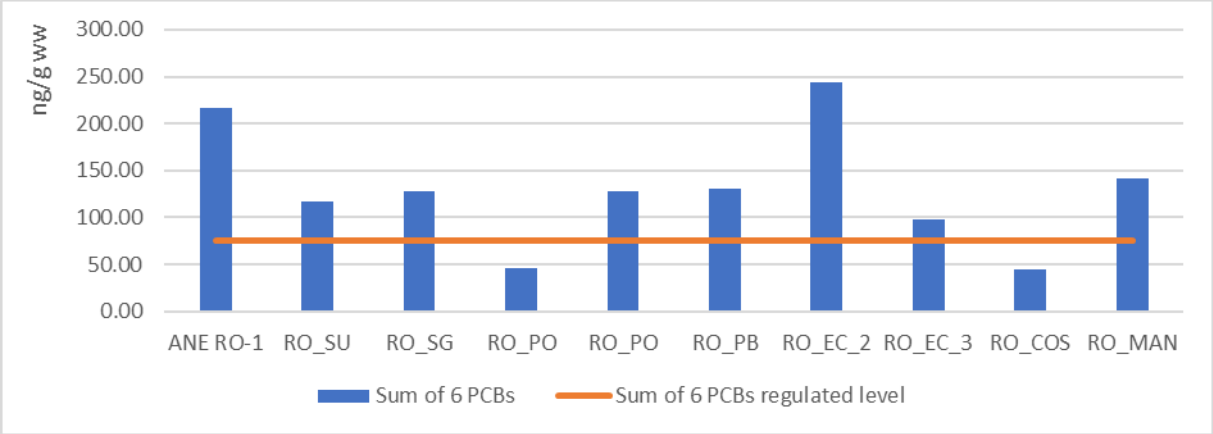


Figure 4.12 - Polychlorinated biphenyls concentrations in* Mytilus galloprovincialis *from Romanian waters, 2019 in relation to maximum admissible levels (EC regulation 1259/2011)

The concentration of total PAHs varied from 0.32 to 81.8 ng/g wet weight. Most of the PAHs analyzed were below detection limit. Higher concentrations were measured in the northern part under the influence of the Danube. Maximum values were recorded in samples collected from Portita (Acenaphthylene - 26.55 ng/g ww, Acenaphthene - 28.22 ng/g ww, Anthracene -17.51 ng/g ww, Naphthalene - 7.35 ng/g ww) and Sf. Gheorghe (Anthracene - 24.39 ng/g ww) stations, under the influence of Sf. Gheorghe branch and EC_2 station (Benzo(a)pyrene - 7.33 ng/g ww) in front of Constanta city and harbor (Figure 4.13). Only the sample collected in front of Constanta city and harbor (EC_2 station) exceeded the maximum admissible level stipulated by European legislation (EC regulation 1881/2006 with further amendment).

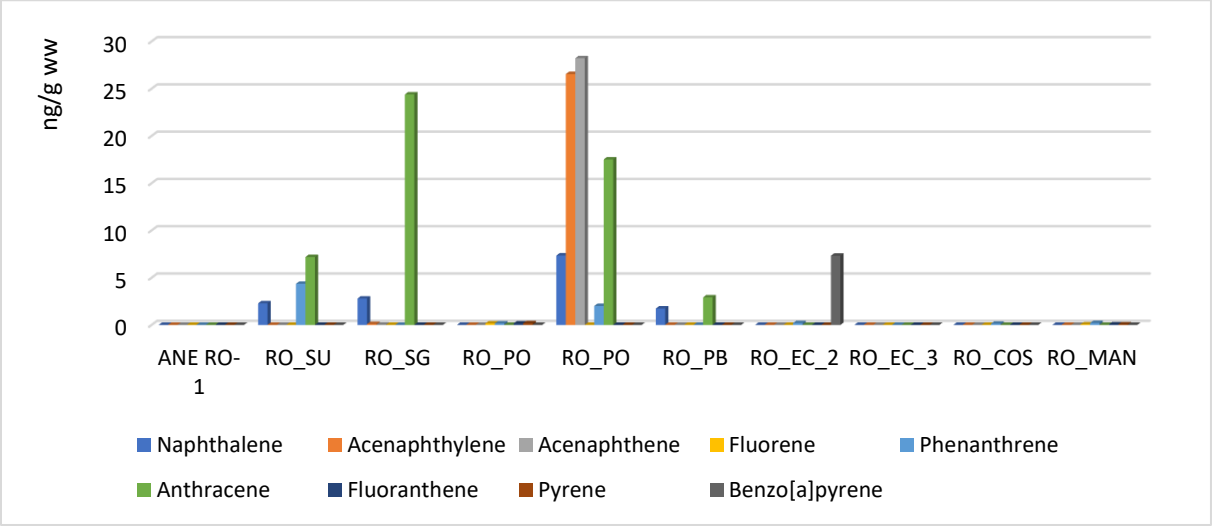


Figure 4.13 - Polycyclic aromatic hydrocarbons concentrations in Mytilus galloprovincialis from Romanian waters, 2019

Rapana venosa was sampled only from 1 location. The detected PAHs were fluorene, phenanthrene and fluoranthene in concentration of 0.28 ng/g ww and pyrene in concentration of 0.3 ng/g ww. OCPs ranged from 0.65 ng/g ww (p,p' DDT) to 28.29 ng/ g ww (HCB) and PCBs from 0.1 ng/g ww (PCB 118) to 54.93 ng/g ww (PCB 153). Only HCB exceeded the maximum admissible level.

Organic pollutants levels in the dorsal muscle of pelagic and demersal fish species investigated in 2019 varies by species, age, lipid contents, habitat, their position in the trophic chain.

Higher concentrations of OCPs were observed in benthal species (*Psetta maeotica, Neogobius melanostomus* and *Mullus barbatus ponticus*) especially for HCB (222.26 ng/g ww in red mullet, 108.35 ng/g ww in goby and 104.72 ng/g ww in turbot). OCPs exceeded the regulated levels in 10% of the

samples for dieldrin and endrin, in 20% of the samples for aldrin, in 40% of the samples for heptachlor and in 80% of the samples for HCB (Figure 4.14).

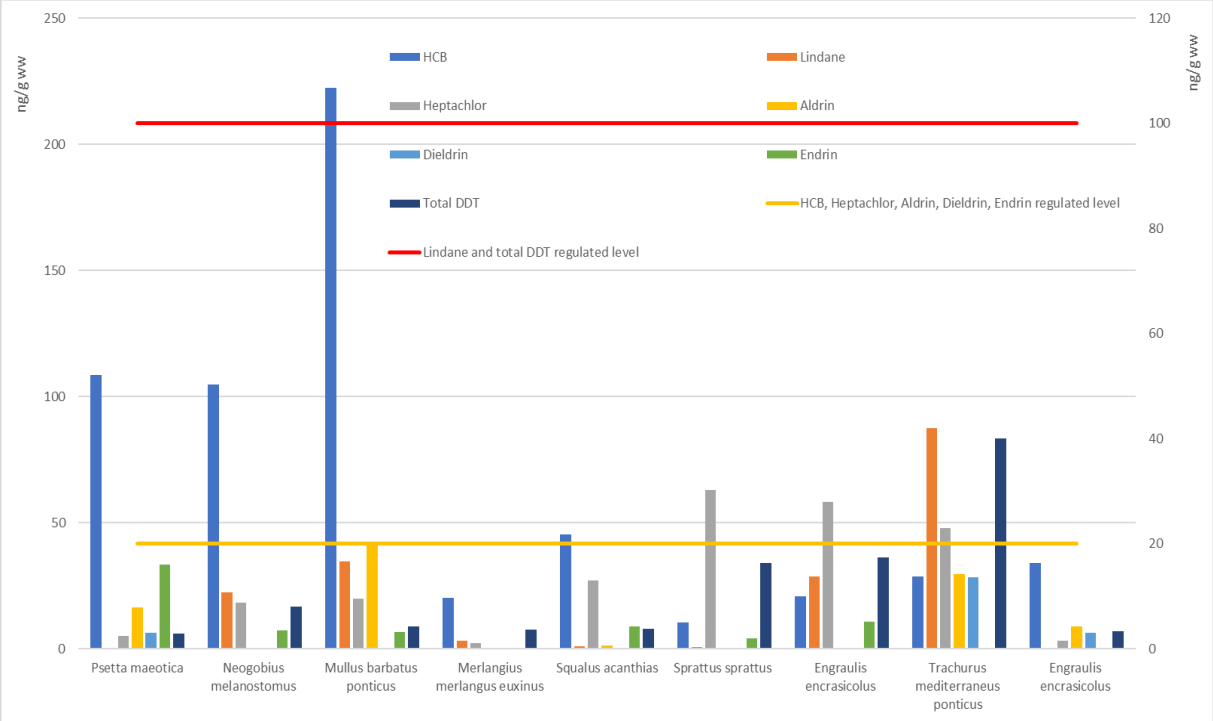


Figure 4.14 - Organochlorine pesticides concentrations in fish species from Romanian waters, 2019, in relation to maximum admissible levels (national legislation Ord. 147/2004)

PCBs distribution is relatively similar among the pelagic and demersal species (Figure 4.15). The highest concentrations were recorded for PCB 101 (238.97 ng/g ww in turbot, 198.97 ng/g ww in achovy), PCB 118 (193.58 ng/g ww in shark, 156.87 ng/g ww in whiting) and PCB 52 (163.63 ng/g ww in anchovy).

The sum of 6 PCBs regulated by European legislation (EC regulation 1259/2011) surpassed the maximum admissible level in 89% of the analysed samples (Figure 4.16).

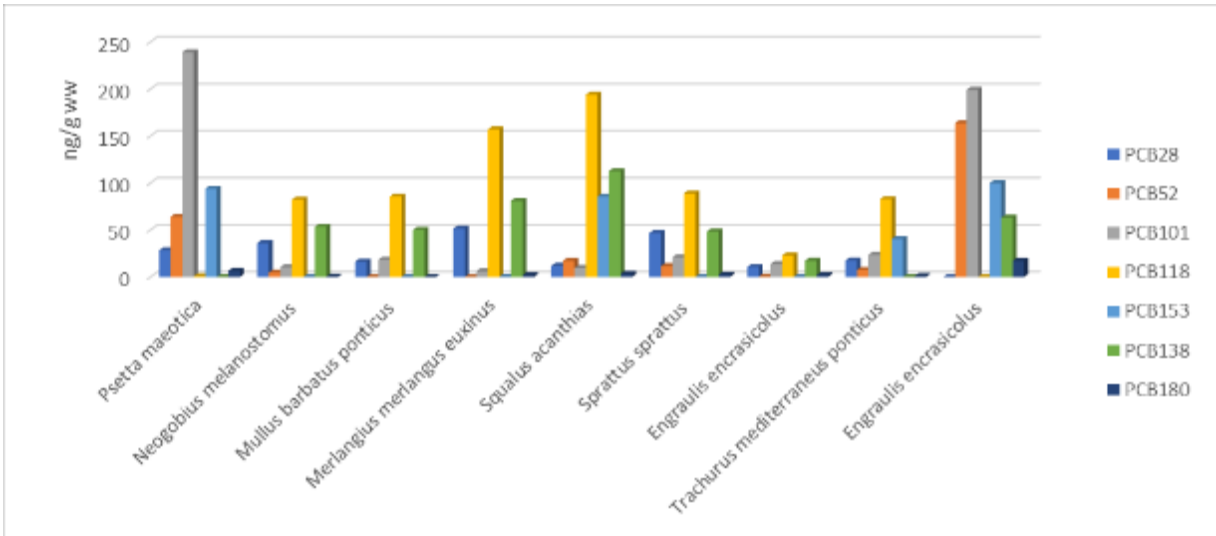


Figure 4.15 - Polychlorinated biphenyls concentrations in fish species from Romanian waters, 2019

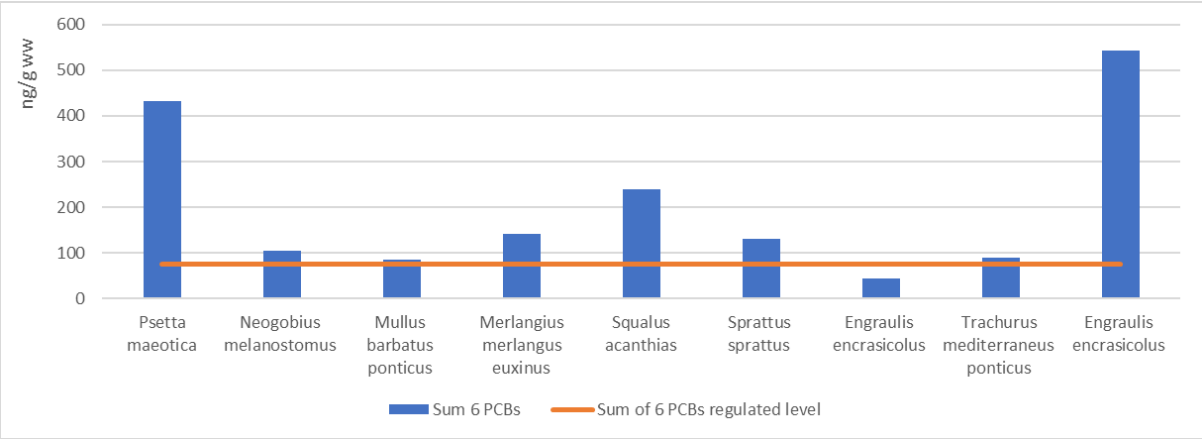


Figure 4.16 - Polychlorinated biphenyls concentrations in fish species from Romanian waters, 2019 in relation to maximum admissible levels (EC regulation 1259/2011)

Low levels of polycyclic aromatic hydrocarbons (from detection limit to 55.4 ng/g ww) were detected in fish species collected from Romanian waters in 2019 (Figure 4.17). The highest values were recorded for anthracene in goby (55.4 ng/g ww) and anchovy (12.84 ng/g ww) and indeno(1,2,3-c, d)pyrene in horse mackerel (29.71 ng/g ww). No exceeding of the maximum admissible level stipulated by European legislation was recorded.

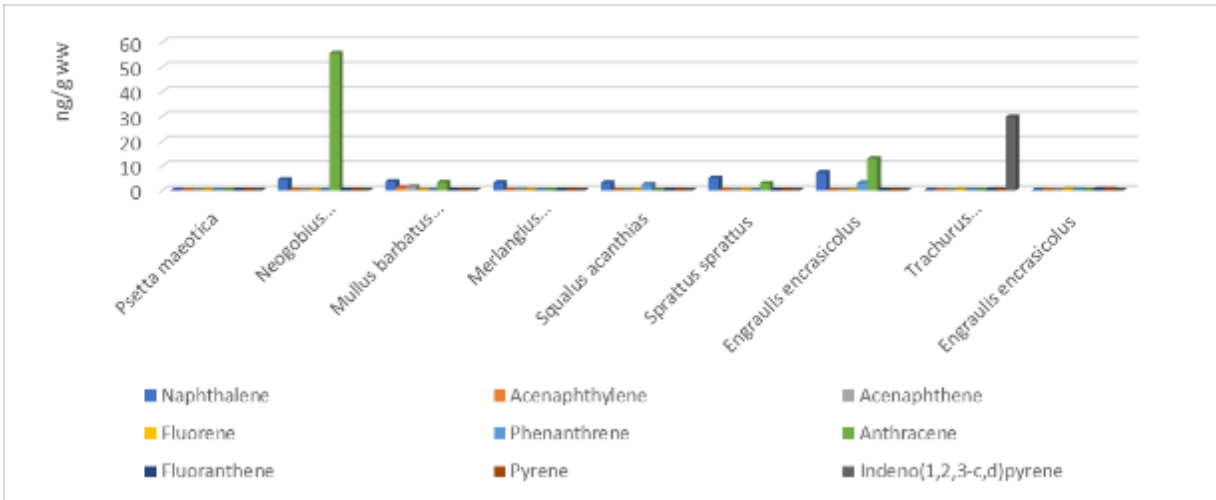


Figure 4.17 - Polycyclic aromatic hydrocarbons concentrations in fish species from Romanian waters, 2019

In comparison with previous data (2012-2017), it was observed that except for endrin, lindane and HCB which show a slight growth tendency in *Mytilus galloprovincialis*, as well as total OCPs, the rest of the median values of organochlorine pesticides are within the same limits of variability (Figure 4.18). Similarly, the concentrations measured in *Rapana venosa* in 2019, both for the individual compounds and for the total OCPs, are within the limits of variability observed in the previous period. PCBs show an increase tendency both in Mytilus galloprovincialis (Figure 4.19) and *Rapana venosa* for most of individual compounds and total PCBs. In the case of PAHs, there is a decreasing trend for benzo[a]pyrene, but especially for total PAHs in *Mytilus galloprovincialis* (Figure 4.20). In *Rapana venosa*, the values measured in 2019 for both total PAHs and benzo[a]pyrene are within the same limits of variability as in the previous period.

Given the small number of samples, it is difficult to assess a trend by species for fish. Therefore, the evolution of organic pollutants was evaluated based on the results obtained for all the analyzed species. Although many individual compounds show an increasing trend in 2019, total OCPs concentration has a decreasing trend (Figure 4.21). In the case of PCBs except PCB 52 and PCB 153, individual compounds have an increasing trend in 2019, and same total PCBs (Figure 4.22). Compared to 2016, both benzo[a]pyrene and total PAHs had lower median concentrations (Figure 4.23).

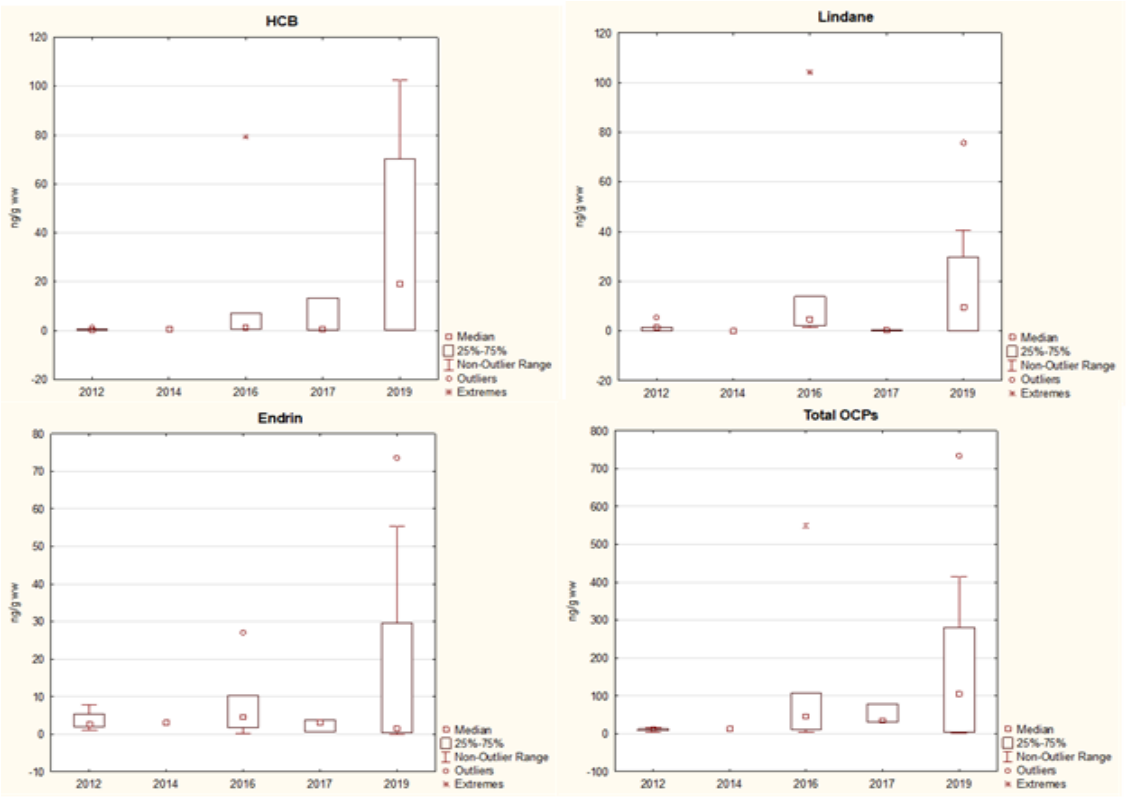


Figure 4.18 - Organochlorine pesticides trends in Mytilus galloprovincialis in Romanian waters during 2013 – 2019

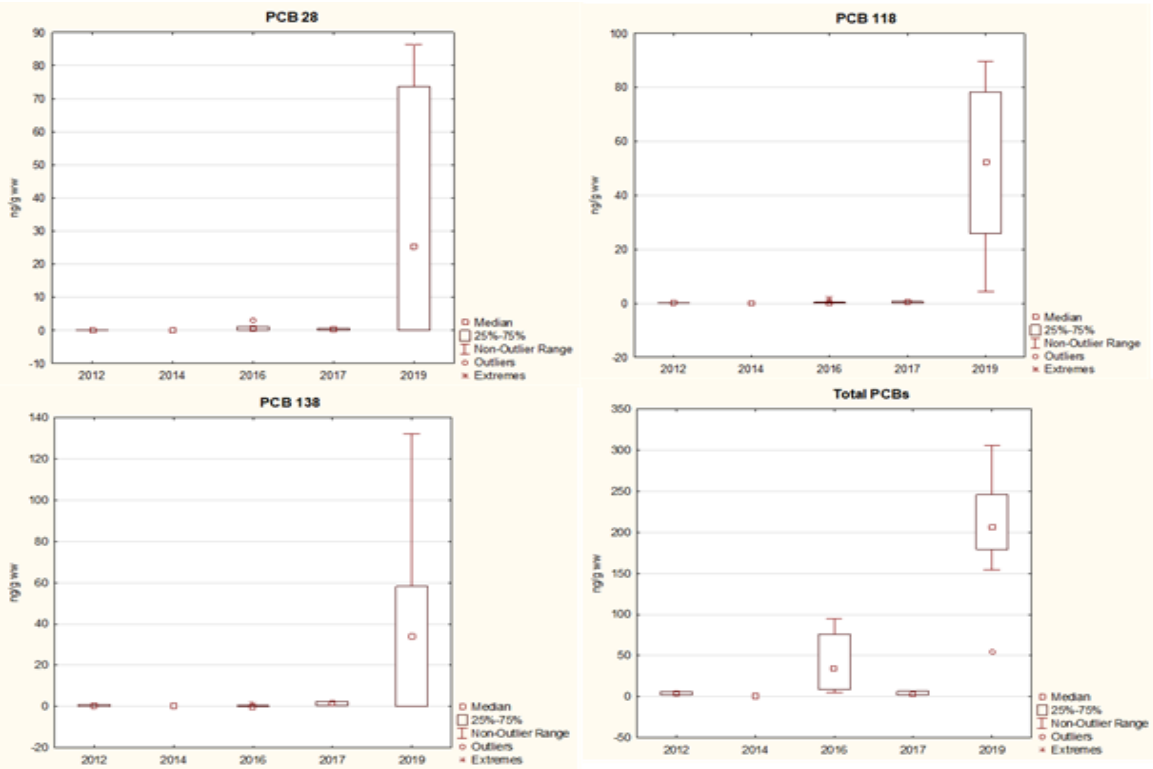


Figure 4.19 - Polychlorinated biphenyls trends in Mytilus galloprovincialis *in Romanian waters during 2013 - 2019*

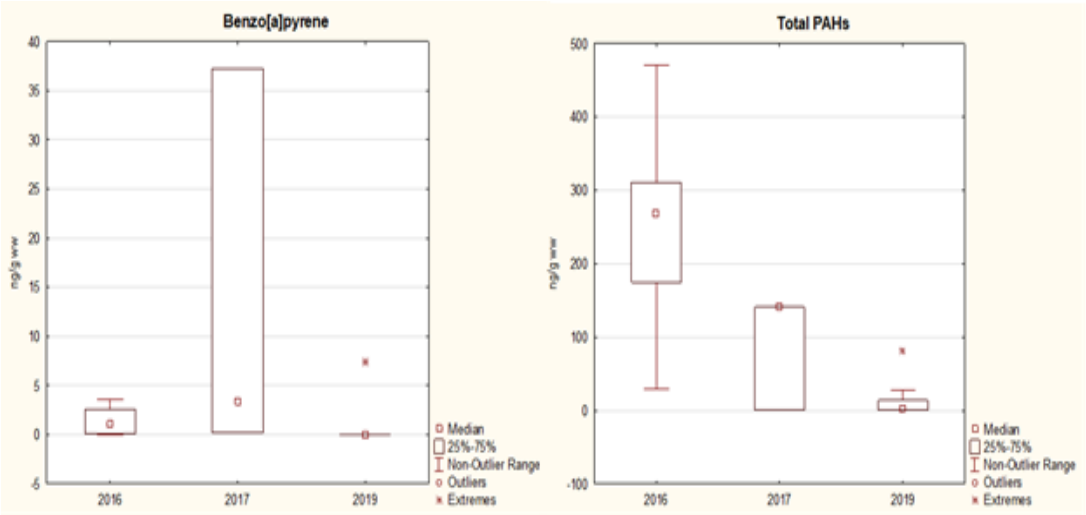


Figure 4.20 - Polycyclic aromatic hydrocarbons trends in Mytilus galloprovincialis in Romanian waters during 2013 - 2019

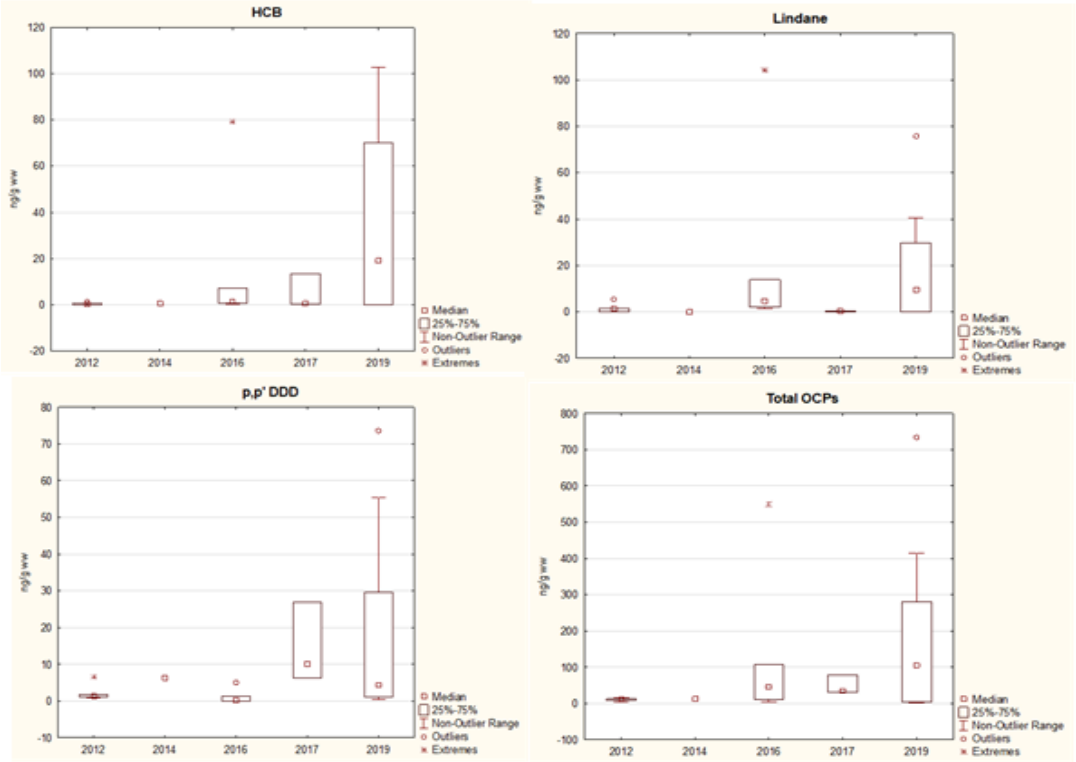


Figure 4.21 - Organochlorine pesticides trends in fish in Romanian waters during 2013 - 2019

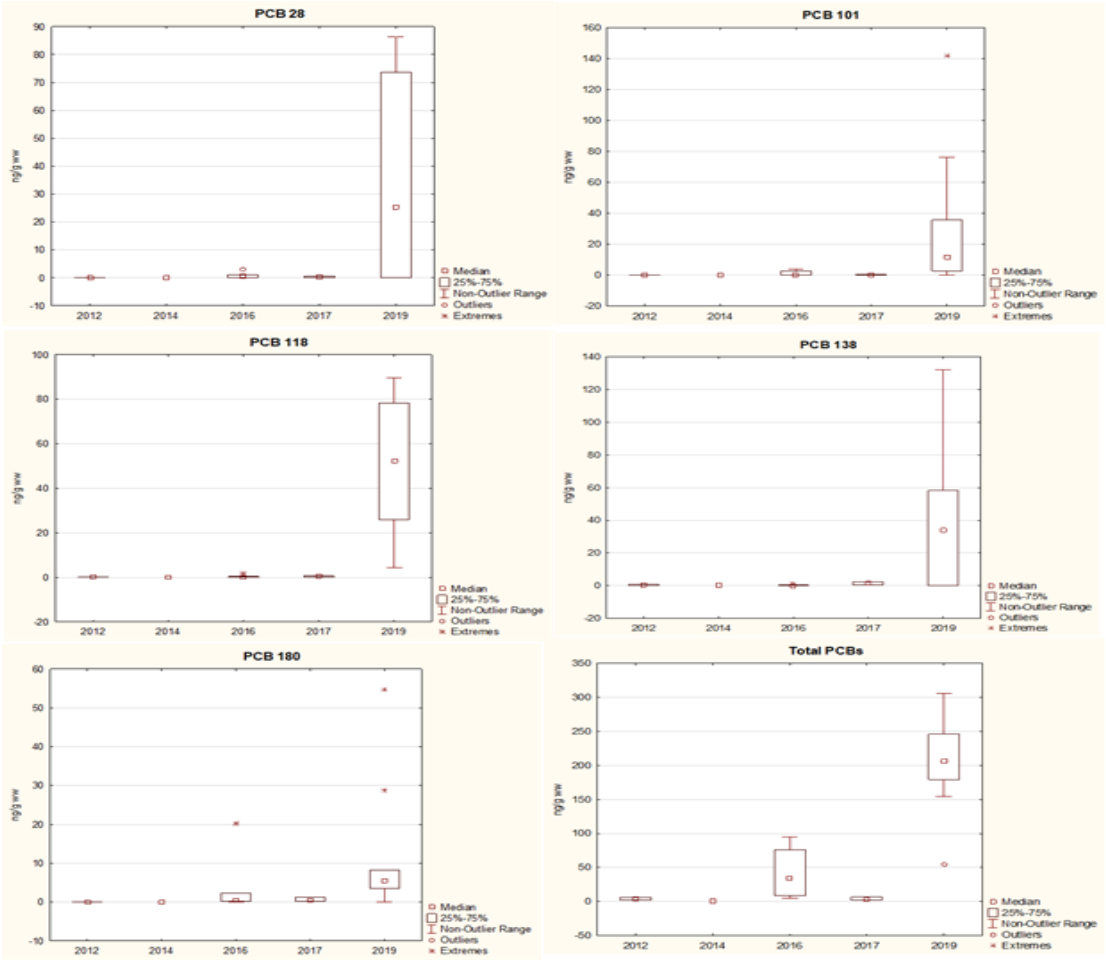


Figure 4.22 - Polychlorinated biphenyls trends in fish in Romanian waters during 2013 - 2019

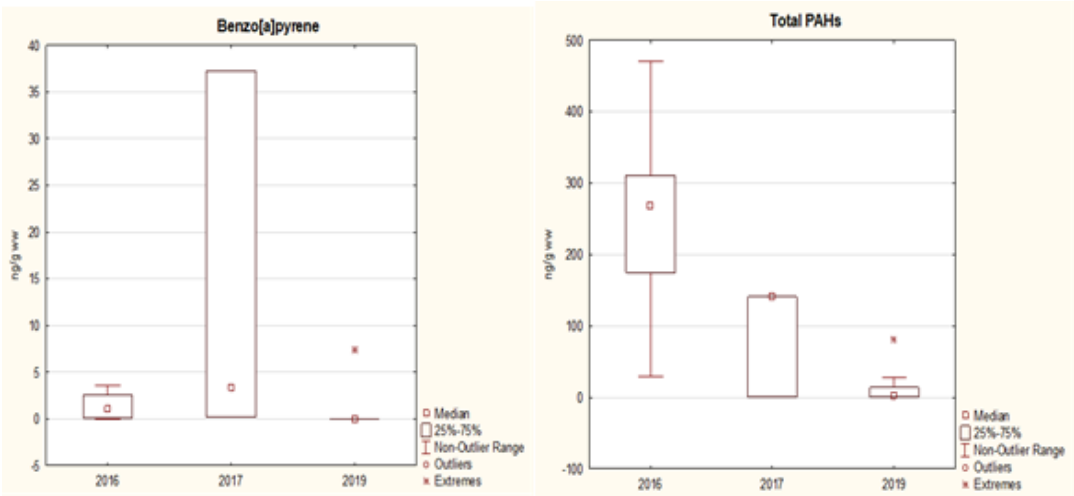


Figure 4.23 - Polycyclic aromatic hydrocarbons trends in fish in Romanian waters during 2013 - 2019

Conclusions

- Organic pollutants varied in similar ranges in mollusks and fish samples collected in 2019. The highest levels of organic pollutants were observed in samples collected from the northern

part under the influence of the Danube or in front of Constanta city and harbor and in benthal fish species.

- Organic pollutants surpassed the regulated levels both in mollusks and fish. The highest percent of samples that exceeded maximum admissible concentration was recorded for PCBs.
- Except PCBs which present an increase tendency in 2019, the other organic pollutants are within the same limits of variability or show a decreasing tendency compared with 2012 - 2017 period, both in mollusks and fish.

4.3 Turkey

Seven commercial fish and two mollusk species were assessed for their heavy metal and organic pollutant contamination.

4.3.1 Trace elements

The average amount of heavy metals measured in the samples of the fish and mollusk species are given in the Table 4.14. The maximum levels permitted for human consumption for Cd, Pb and Hg are also included in the same table. The values above or close to the threshold values were highlighted in bold. Other metals that do not have any maximum level for human consumption in the EU regulation are considered for information purposes.

Table 4.14 - Heavy metal concentrations in biota from the areas of river impact

Sakarya River (µg/g ww) (mean)									
Species	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**Hg**
Mullus barbatus (1,5,8)	1.473	< 0.00001	0.056	0.153	0.425	0.270	0.076	6.736	0.037
Alosa fallax (1,5,9)	1,152	< 0.00001	0.007	0.076	0.950	0.040	0.050	5.700	0.019
Merlangius merlangus euxinus (1,5,9)	0.841	< 0.00001	0.022	0.128	0.320	0.143	0.039	4.130	0.008
Trachurus mediterraneus (2,5,9)	0.755	< 0.00001	0.015	0.078	0.591	0.131	0.036	8.753	0.010
Sprattus sprattus (1,5,9)	1.114	0.008	0.033	0.204	0.503	0.205	0.045	11.34	-
Psetta maxima (1,5,9)	1.261	< 0.00001	0.010	0.095	0.160	0.079	0.027	8.287	0.014
Mytilus galloprovincialis (3,6,9)	2.879	0.459	0.436	1.182	1.214	1.714	**0.28**	43.98	-
Rapana Venosa (3,6,9)	7.077	**2.728**	0.081	0.324	16.73	0.365	0.124	28.56	0.020
Yesilirmak River (µg/g ww) (mean)									
Species	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**Hg**
Mullus barbatus (1,5,8)	1.679	< 0.00001	0.025	0.067	0.369	0.086	0.034	5.778	0.112
Trachurus mediterraneus (2,5,9)	0.934	< 0.00001	0.009	0.053	0.766	0.056	0.033	8.553	0.048
Merlangius merlangus euxinus (1,5,9)	0.806	< 0.00001	0.009	0.057	0.269	0.055	0.032	3.756	-
Psetta maxima (1,5,9)	1.839	< 0.00001	0.004	0.059	0.215	0.058	0.026	5.866	0.039
Solea solea (2,5,9)	0.886	< 0.00001	0.011	0.066	0.427	0.077	0.037	10.73	-
Rapana venosa (3,6,9)	5.494	0.103	0.012	0.152	15.169	0.274	0.051	13.85	0.033
Turkish Food Codex (EC 1881/2006)		(1) 0.05 (2) 0.1 (3) 1.00					(5) 0.3 (6) 1.5		(8) 1 (9) 0.5

In general, higher levels of metals were detected in mollusks than in edible fish tissues and varied according to the species (Table 4.14, Figure 4.25 and Figure 4.26).

It is also clearly shown in the Figure 4.24. that higher metal contents in Rapana venosa were detected in the samples collected from Sakarya than Yesilırmak river impact area.

Cadmium (Cd) content of the *Rapana venosa* samples (av. 2.728 µg/g ww) collected from Sakarya river impact area (Figure 4.24 and Table 4.14) was detected above the threshold value, 0.5 µg/g ww. Similarly, Lead (Pb) content of the Mytilus galloprovincialis samples (av. 0.28 µg/g ww) collected from the same area was also detected close to the threshold value, 0.3 µg/g ww.

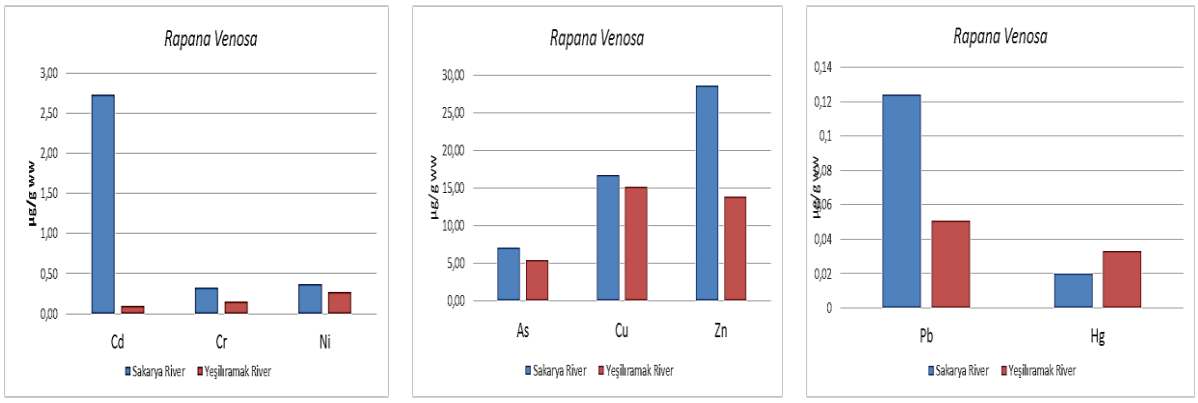


Figure 4.24 - Metal concentrations in Rapana Venosa from Sakarya and Yeşilırmak Rivers, 2019

Higher Pb, Hg and Ni contents were detected in the Mullus barbatus (Mb) then the other fish species. Sprattus sprattus (Ss) is the only fish species containing Cd concentration above the detection limit (0.00001 µg/g ww). There is no any difference in the other fish species for their Cd contents. Hg, Ni and Pb contens of the fish species Merlangius merlangus euxinus (Mm), Trachurus mediterraneus (Tm) and Psetta maxima (Pm) are decreasing in the following order: Mm>Tm>Pm. Cu, Cr and Zn contents of these fish species decrease as follows: Tm>Mm>Pm; Mm>Pm>Tm and Tm>Pm>Mm respectively. Hg levels of the fishes from Yeşilırmak impact area were found higher than those obtained from Sakarya impact area. The content of other metals such as Ni, Cr, Zn and Pb were higher in the above fish species collected from Yeşilırmak River's impact area (Figure 4.25 and Figure 4.26).

Figure 4.25 - HM concentrations in muscle tissue of demersal fish species from the Sakarya River, 2019

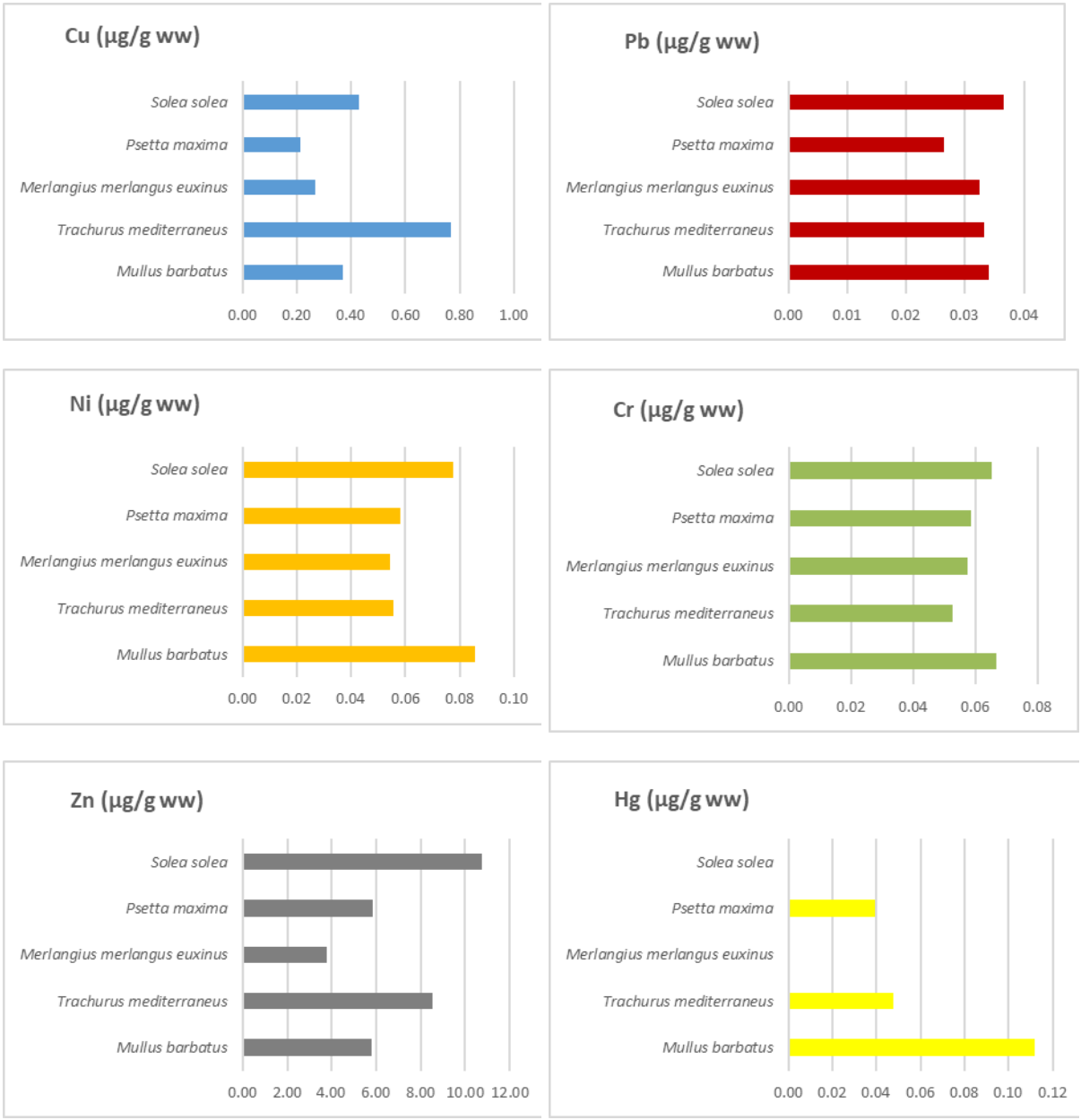
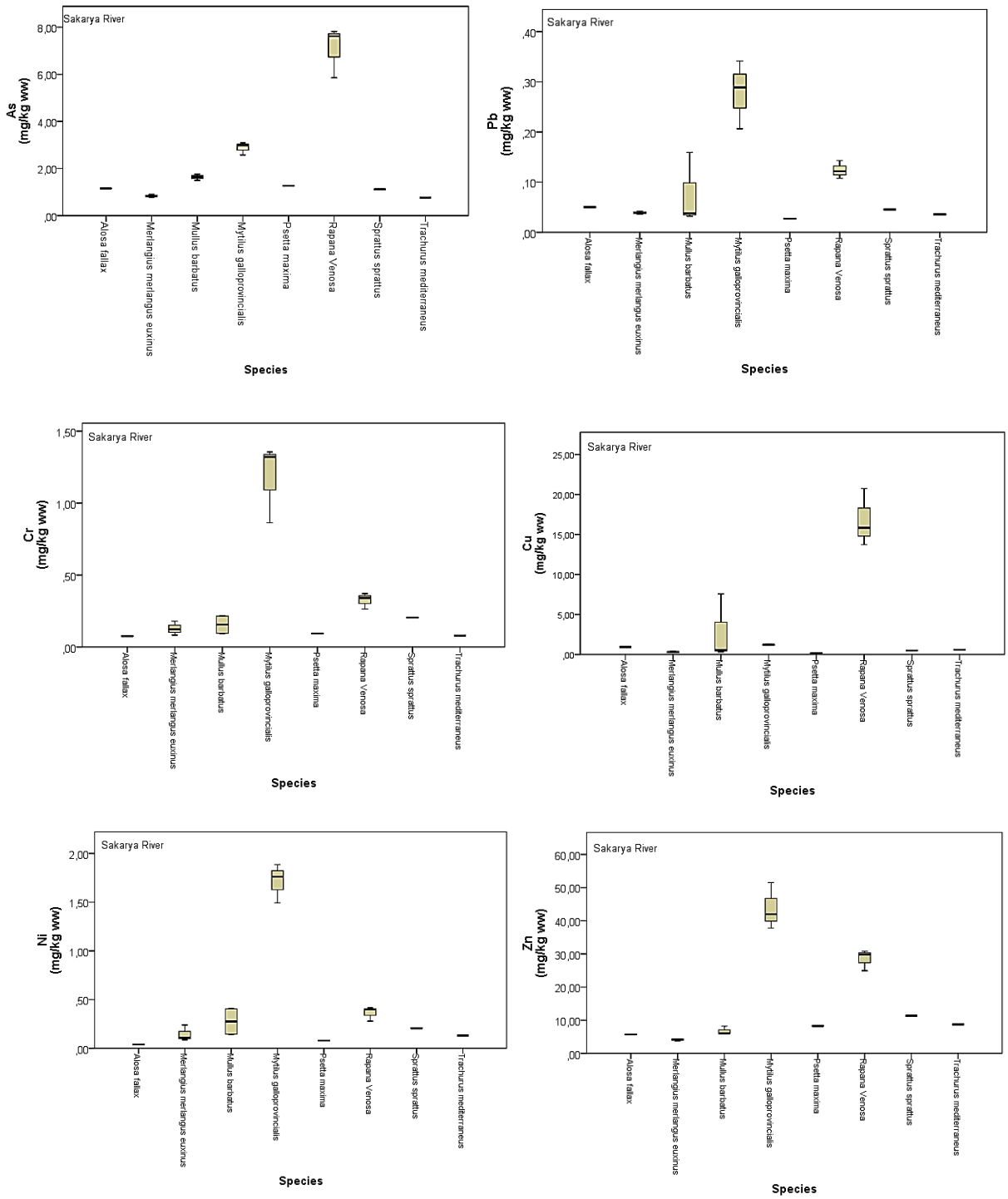
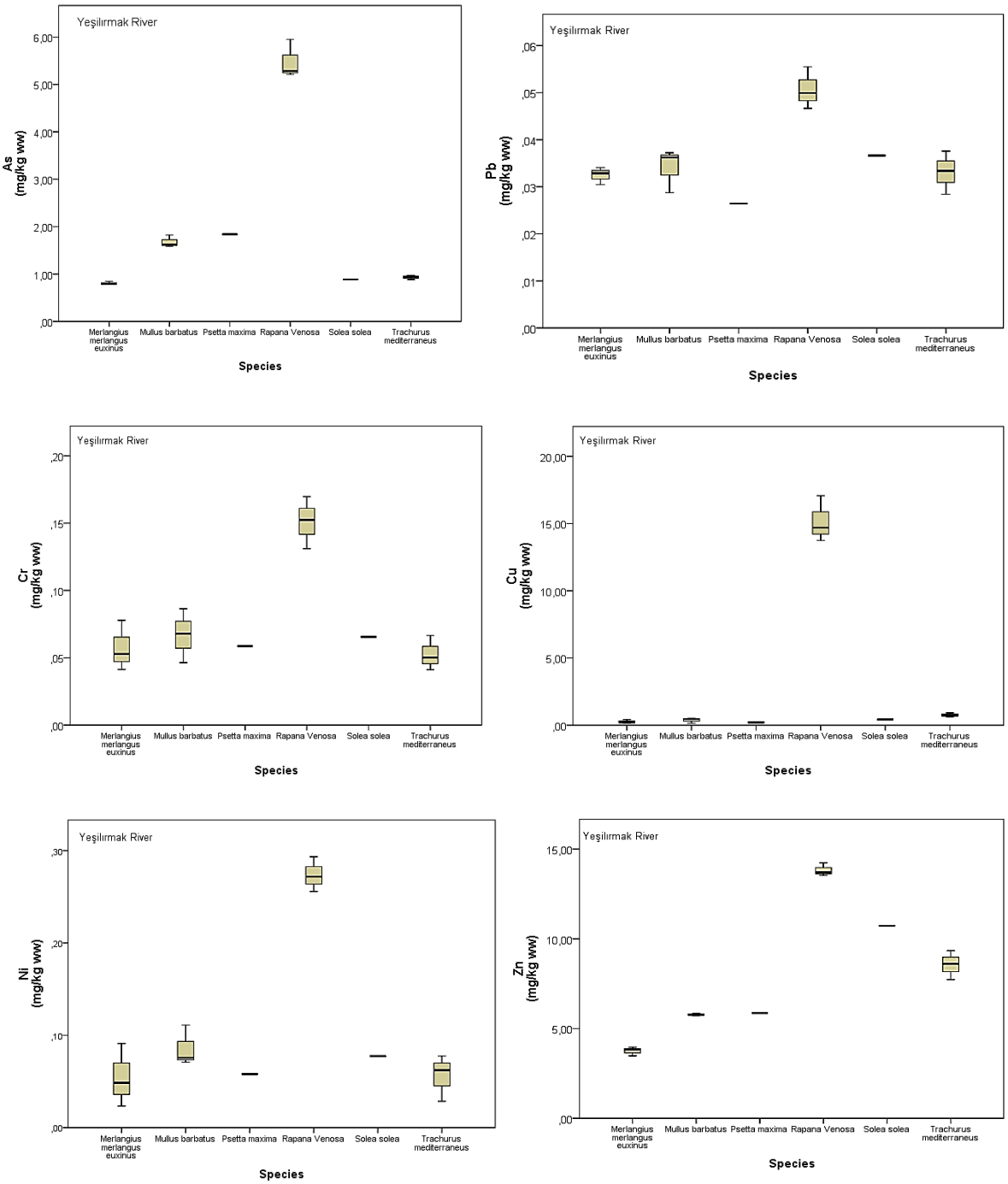


Figure 4.26 - HM concentrations in muscle tissue of demersal fish species from the Yesilırmak River, 2019


Sakarya River
As (mg/kg ww)
Pb (mg/kg ww)
Cr (mg/kg ww)
Cu (mg/kg ww)
Ni (mg/kg ww)
Zn (mg/kg ww)
Alosa fallax
Merlangius merlangus euxinus
Mullus barbatus
Mytilus galloprovincialis
Psetta maxima
Rapana Venosa
Sprattus sprattus
Trachurus mediterraneus
Species

a) Sakarya



b) Yesilırmak

Figure 4.27 - HM concentrations in different fish and mollusk species from Sakarya and Yeşilırmak River impacted marine areas, 2019

Concentrations and standard deviations of different fish, mussel and Rapana sp. were compared in the Figure 4.27. Highest mean values of Cd (1.415 µg/g ww) and Cu (15.97 µg/g ww) were found in Rapana sp., while highest values for Pb (0.087 µg/g ww), Cr (1.18 µg/g ww) and Ni (1.17 µg/g ww) were found in mussel (Figure 4.28 and Figure 4.29). Highest standard deviations were calculated for Cd and Cu contents of Rapana samples as 1.454 and 2.667 µg/g ww respectively.

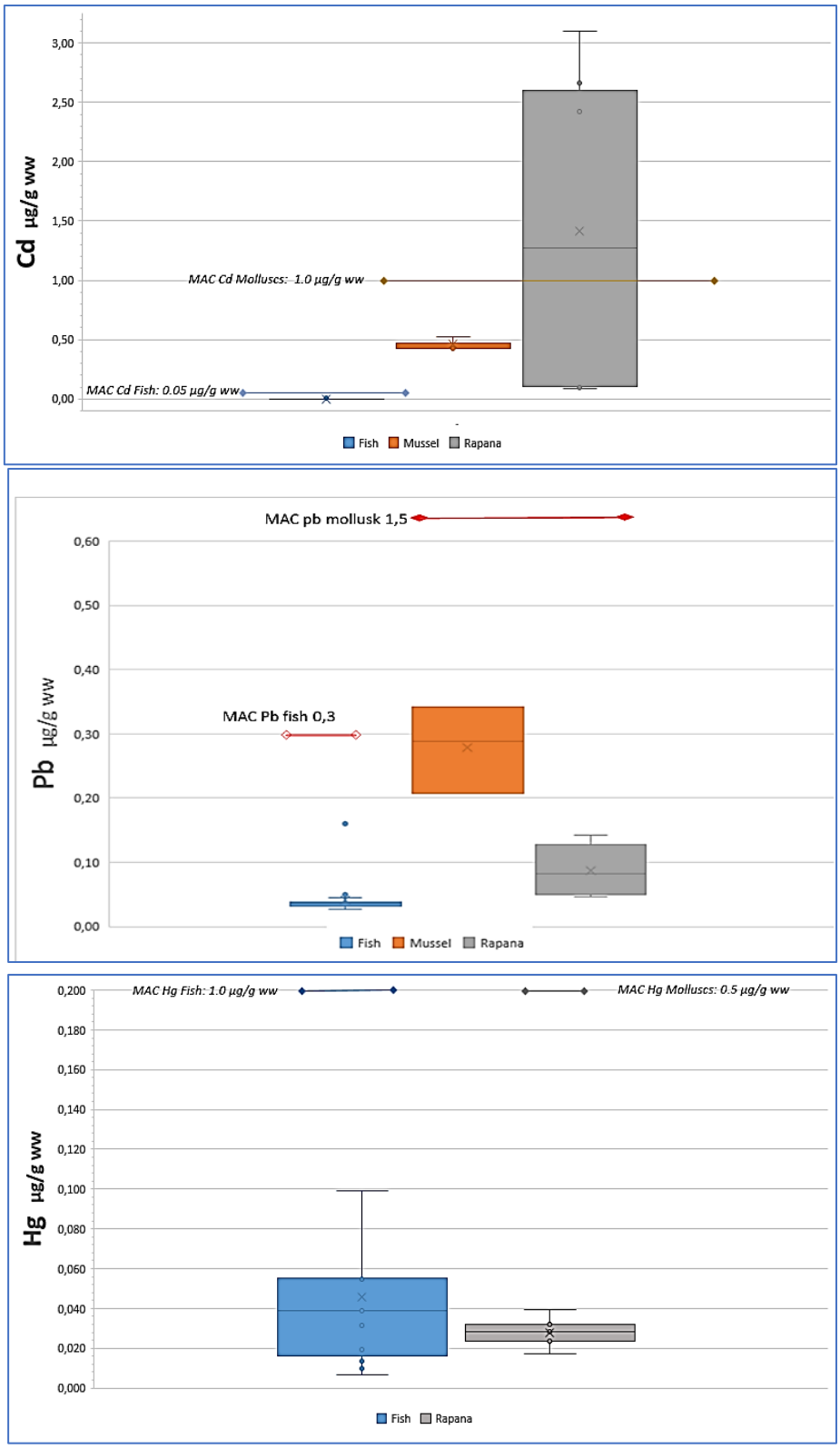


Figure 4.28 - Cd, Pb and Hg concentrations in total fish and mollusks from the river impacted marine areas, 2019.

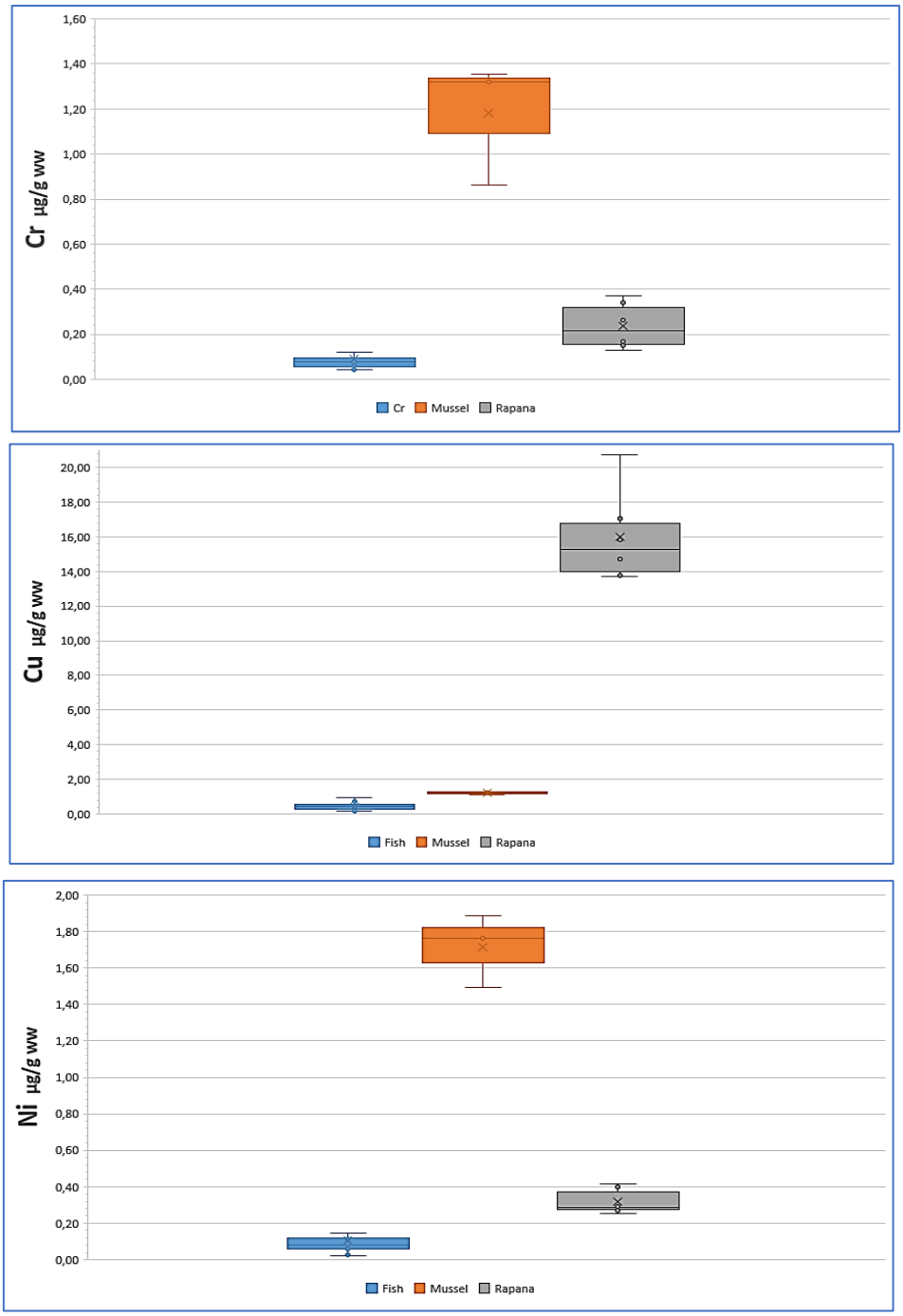


Figure 4.29 – Cr, Cu and Ni concentrations in total fish and mollusks from the river impacted marine areas, 2019

4.3.2 Organic Pollutants

Although a significant part of petroleum hydrocarbons entering the marine environment is removed by evaporation, part of it disperses in water, accumulated in sediment, and transferred to biota (Chouksey et al., 2004).

The presence of above the accepted level contaminants in fish and other seafoods, negatively affects in terms of both public health and other consumers being fed over seafood and also the sustainability of other marine resources.

The polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) concentrations determined in the tissues of mollusks and fish samples are presented in Table 4.16 and Table 4.17.

Total PAH in fish samples showed a distribution between 30.4-285.7 µg/kg ww in Sakarya River and 25.6-842.6 µg/kg ww in Yeşilırmak. Mytilus galloprovincialis samples were collected only from the trawl work in front of the Sakarya River. Total PAH of 45.9 µg/kg ww was measured in the Mytilus galloprovincialis sample. In Rapana Venosa samples, 22.84 µg/kg in Sakarya River and 31.66 µg/kg in Yeşilırmak River total PAH were determined.

The most important contributors to PAH components in biota were phenanthrene (43%) and naphthalene (20%), a low molecular weight PAHs with 2-3 aromatic rings, which are consistent with a composition profile following a petroleum exposure.

In Table 4.15, a comparison is made with the Turkish Food Codex limit values. Benzo(a)pyrene can be used as a marker for the occurrence and effect of carcinogenic PAH in food. The maximum limit for Benzo (a) pyrene is 5.0 µg/kg wet weight for bivalve mollusks, 2.0 µg/kg wet weight for fish meat in Turkish Food Codex. The concentrations of benzo[a]pyrene in marine organisms were situated below quality standards (Figure 4.30, Figure 4.31 and Table 4.15).

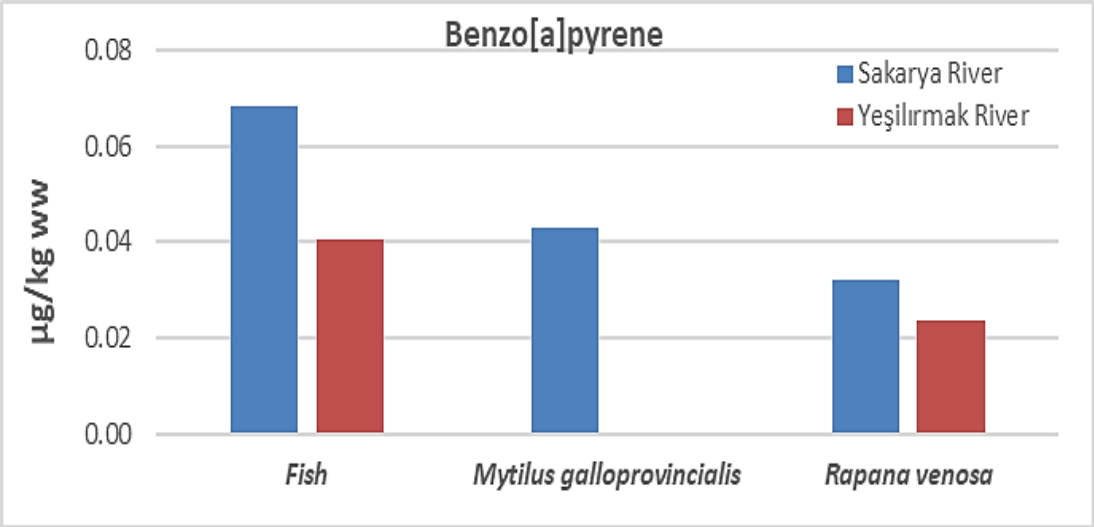


Figure 4.30 - Average Benzo[a]pyrene concentrations in biota samples affected by Sakarya and Yeşilırmak Rivers

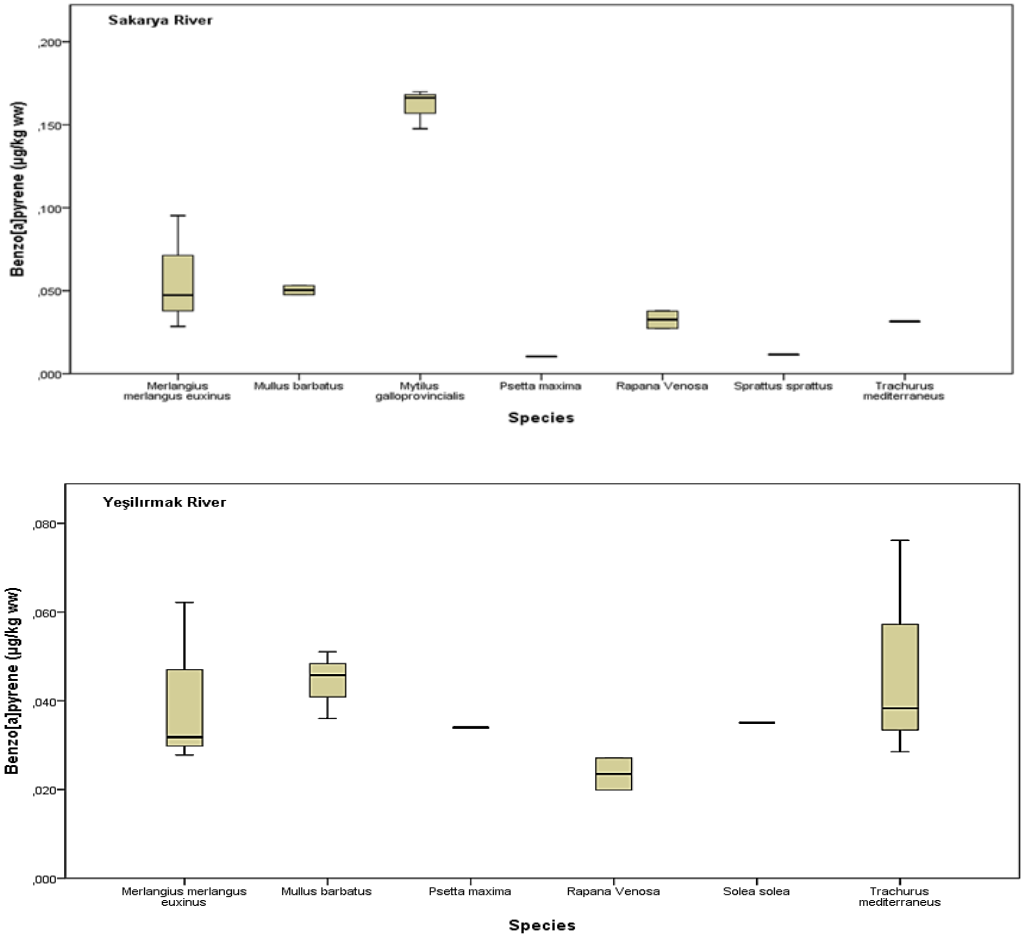


Figure 4.31 - Interspecific differences in Benzo[a]pyrene accumulation in 6 species of fish, Mytilus galloprovincialis and Rapana venosa from Sakarya River and Yeşilırmak River

Table 4.15 - Comparison of biota samples (Mytilus galloprovincialis, Rapana venosa and fish) affected by Yeşilırmak and Sakarya Rivers, with TGK limit value

Species	Benzo[a]pyrene ng/g wet weight	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6) ng/g wet weight
Sakarya River		
Mullus barbatus (1)	0.050	7.977
Merlangius merlangus euxinus (1)	0.161	0.618
Trachurus mediterraneus (1)	0.056	0.996
Sprattus sprattus (2)	0.031	2.864
Psetta maxima (1)	<0.043	10.23
Mytilus galloprovincialis (2)	<0.043	7.428
Rapana venosa (2)	0.032	1.753
Yesilirmak River		
Mullus barbatus (1)	0.044	3.406
Trachurus mediterraneus (1)	0.048	3.869
Merlangius merlangus euxinus (1)	0.041	0.710
Psetta maxima (1)	0.034	1.402
Solea solea (1)	0.035	2.530
Rapana venosa (2)	0.023	0.130
Turkish Food Codex	(1) 2 ng/g ww (2) 10 ng/g ww	75 ng/g ww (1)(2)

Table 4.16 - PAH concentrations (µg/kg ww) in fish and mollusks samples in Yeşilırmak and Sakarya Rivers

Sakarya River (ng/g wet weight)																	
Species	**Nap**	**Acl**	**Ac**	**Fl**	**Phe**	**An**	**Fa**	**Py**	**B[a]a**	**Chry+ Trip**	**B[b]fl**	**B[k]fl**	**B[a]pyr**	**B(g,h,i)p**	**D(a,h)a**	**I(1,2,3-c,d)p**	**Σ16PAHs**
Mullus barbatus	7.529	1.641	0.148	2.106	13.14	0.863	2.089	2.086	0.147	0.364	0.101	0.049	0.050	0.052	0.009	0.041	30.41
Merlangius merlangus euxinus	9.125	2.164	0.105	1.854	18.74	0.986	4.221	4.812	0.168	0.388	0.131	0.086	0.056	0.052	0.005	0.051	42.94
Trachurus mediterraneus	10.12	0.635	<BDL	1.585	15.39	0.374	3.418	3.763	0.111	0.24	0.080	0.033	0.031	0.026	0.005	0.024	35.84
Sprattus sprattus	45.22	22.91	1.023	24.07	120.8	11.09	28.22	30.45	0.434	1.018	<BDL	0.205	<BDL	0.171	<BDL	<BDL	285.7
Psetta maxima	9.136	1.372	<BDL	2.130	24.66	1.629	4.487	4.208	0.177	0.44	0.125	0.064	<BDL	0.048	0.008	0.039	48.52
Mytilus galloprovincialis	7.714	0.830	0.075	1.280	19.47	0.722	5.136	8.131	0.291	0.814	0.598	0.256	0.161	0.261	0.027	0.196	45.96
Rapana venosa	6.724	0.137	0.049	1.048	6.70	0.497	2.864	3.819	0.141	0.57	0.134	0.046	0.032	0.034	0.011	0.032	22.84
Yesilirmak River (ng/g wet weight)																	
Mullus barbatus	7.409	1.770	0.170	2.444	12.53	0.847	2.202	2.312	0.157	0.402	0.111	0.054	0.044	0.040	0.008	0.036	30.53
Trachurus mediterraneus	6.764	1.098	0.120	1.639	10.59	0.468	2.053	2.216	0.129	0.291	0.104	0.049	0.048	0.040	0.007	0.030	25.64
Merlangius merlangus euxinus	5.592	1.468	0.099	1.706	24.07	1.169	5.271	6.183	0.147	0.309	0.111	0.048	0.041	0.043	0.004	0.035	46.29
Psetta maxima	8.514	0.811	0.064	1.378	14.30	0.792	4.178	5.225	0.114	0.287	0.085	0.038	0.034	0.032	0.005	0.024	35.88
Solea solea	11.93	105.3	3.784	123.1	457.5	59.95	47.39	32.98	0.129	0.29	0.078	0.036	0.035	0.032	<BDL	0.025	842.6
Rapana venosa	5.659	0.262	0.048	0.935	10.96	0.680	4.968	7.621	0.097	0.25	0.082	0.028	0.023	0.025	0.005	0.022	31.66

Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene+Triphenylene: Chry+Trip, Benzo[b]fluoranthene: B[b]fl, Benzo[k]fluoranthene: B[k]fl, Benzo[a]pyrene: B[a]pyr, Benzo (g,h,i)perylene: B(g,h,i)p, Dibenzo(a,h)anthracene: D(a,h)a, Indeno(1,2,3-c,d)pyrene: I(1,2,3-c,d)p

Table 4.17 - OCPs and PCBs concentrations (µg / kg ww) in fish and molluscs samples in Yeşilırmak and Sakarya Rivers

Sakarya River (ng/g ww)															
Species	**α-HCH**	**β-HCH**	**Lindan**	**Heptaclor**	**Aldrin**	**p,p'DDT**	**p,p'DDE**	**p,p'DDD**	**PCB28**	**PCB52**	**PCB101**	**PCB118**	**PCB138**	**PCB153**	**PCB180**
Mullus barbatus	0.081	4.172	0.041	<BDL*	<BDL	2.526	17.737	4.426	0.140	0.458	0.430	1.403	2.975	3.046	0.928
Merlangius merlangus euxinus	0.007	0.365	0.013	<BDL	<BDL	0.306	2.445	1.380	0.056	0.112	0.185	0.213	0.286	0.297	0.061
Trachurus mediterraneus	0.048	2.201	0.019	<BDL	<BDL	1.248	7.632	3.554	0.098	0.181	0.353	0.539	0.960	1.010	0.263
Sprattus sprattus	0.070	6.188	0.046	<BDL	<BDL	4.631	25.222	20.335	0.432	0.954	1.484	2.098	3.270	3.321	0.766
Psetta maxima	0.039	2.121	0.017	<BDL	<BDL	2.802	17.696	10.770	0.255	0.511	1.052	1.479	2.536	2.558	0.700
Mytilus galloprovincialis	0.016	0.590	<BDL	<BDL	<BDL	0.328	1.353	1.088	0.030	0.043	0.072	0.114	0.221	0.227	0.025
Rapana venosa	0.015	0.529	0.009	<BDL	<BDL	0.222	2.290	0.918	0.029	0.065	0.160	0.203	0.706	0.729	0.065
Yesilirmak River (ng/g ww)															
Mullus barbatus	0.100	4.994	0.049	<BDL	<BDL	1.266	12.874	6.214	0.137	0.220	0.277	0.614	1.231	1.215	0.326
Trachurus mediterraneus	0.027	1.515	0.025	<BDL	<BDL	0.897	6.862	2.409	0.073	0.162	0.444	0.665	1.382	1.394	0.413
Merlangius merlangus euxinus	0.012	0.231	0.017	<BDL	<BDL	0.170	1.519	0.637	0.042	0.096	0.159	0.129	0.171	0.196	0.046
Psetta maxima	0.012	0.203	0.009	<BDL	<BDL	0.360	3.705	1.199	0.045	0.065	0.149	0.283	0.497	0.513	0.133
Solea solea	0.008	0.280	0.009	<BDL	<BDL	0.505	4.418	1.385	0.034	0.069	0.276	0.497	0.922	1.027	0.203
Rapana venosa	0.005	0.026	0.007	<BDL	<BDL	<BDL	0.142	0.064	0.012	0.012	0.015	0.012	0.041	0.043	0.007

* Below Detection Limit

Average PCB concentrations in Sakarya River are 7.08 µg/kg ww in fish, 0.73 µg/kg ww in *Mytilus galloprovincialis*, 1.96 µg/kg ww in *Rapana venosa* (Table 4.17, Figure 4.32 and Figure 4.33). In PCBs (ICES-6), this limit value is 75 µg/kg ww for biota in Turkish Food Codex. None of the mollusks and fish samples investigated in 2019 exceeded the Turkish Food Codex limit value for PCBs, so there is no risk for human health in respect with PCBs compounds (Table 4.15).

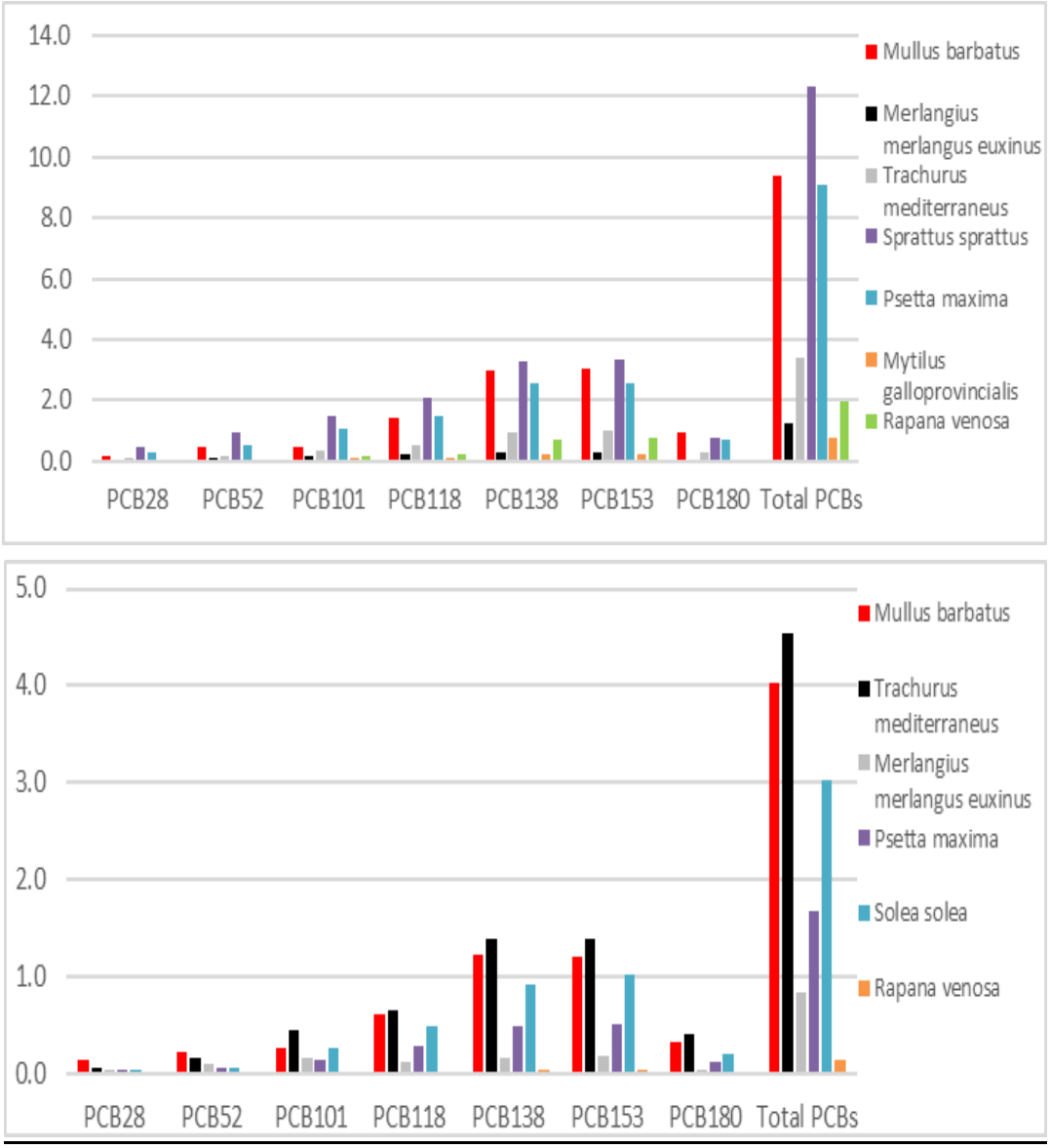
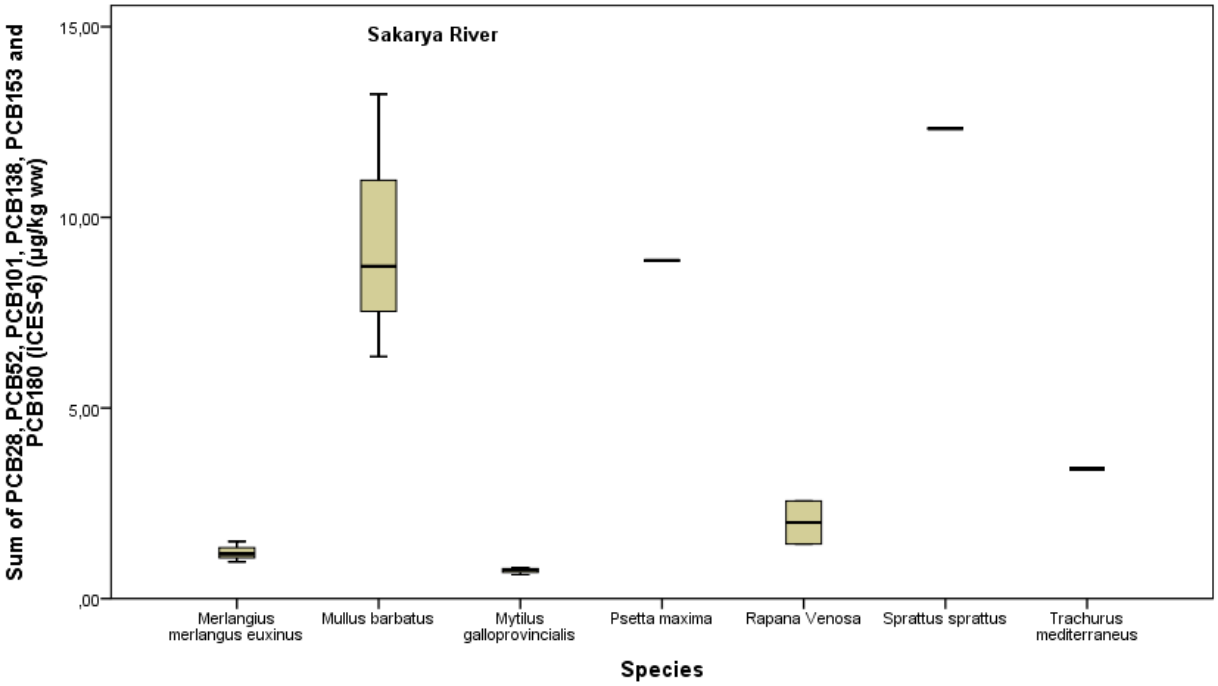


Figure 4.32 - PCB distributions of Sakarya (up) and Yeşilırmak (down) biota samples



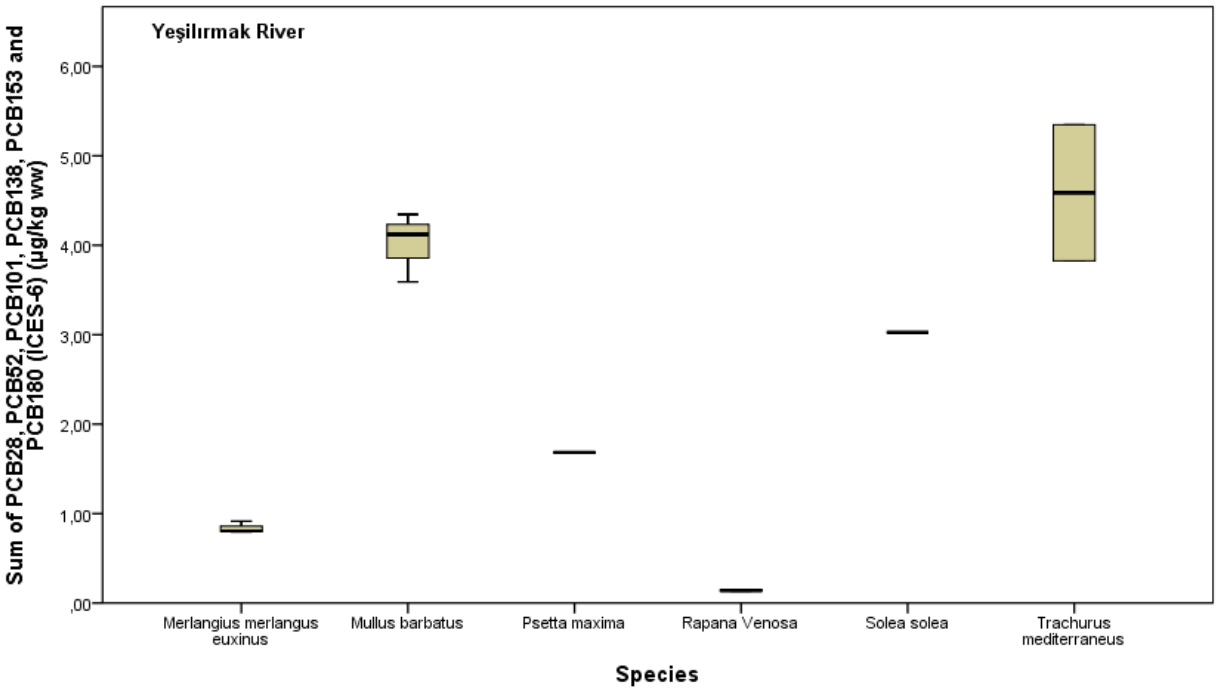


Figure 4.33 - Interspecific differences in Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6) accumulation in 6 species of fish, Mytilus galloprovincialis and Rapana venosa from Sakarya River and Yeşilırmak River

The major OCPs compounds are p, p' DDE, p, p' DDD and p, p' DDT. Among DDT and its derivatives, DDE is the most dominant (Figure 4.34). Other pesticides investigated were either low levels or below the detection limit.

There are not any limit value for organochlorine pesticides in the Turkish Food Codex, so it could not make a comparison.

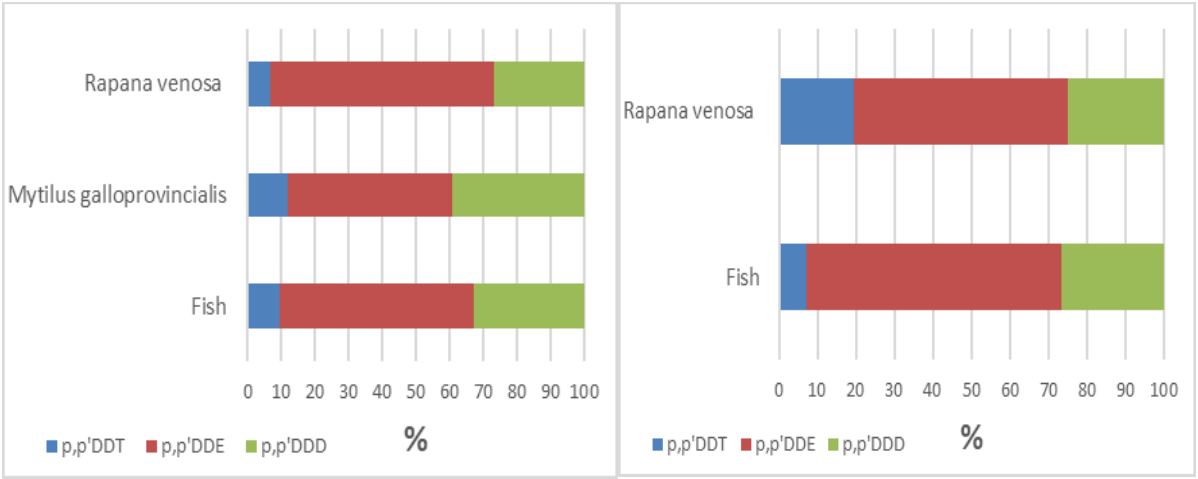


Figure 4.34 - Distribution of DDT and its derivatives in Sakarya (left) and Yeşilırmak (right) river

Conclusions

In general, higher levels of metals were detected in mollusks than in edible fish tissues and varied according to the species. Furthermore, higher levels were also detected in samples collected from the region under the influence of the Sakarya river compared to the Yeşilırnak samples. The amount of Cd in *Rapana venosa* and Pb in *Mytillus galloprovincialis* collected from Sakarya River mouth were found above the permissible limits for human consumption. As a marker compound of Polyaromatic Hydrocarbons, the concentrations of benzo[a]pyrene in marine organisms were detected below the acceptable limits of the National Food Codex. Similarly, none of the mollusc and fish samples were found to have any level of PCBs that could pose a risk to human health. Among DDT and its derivatives, DDE is the most dominant OCPs compounds in both Sakarya and Yesilırmak river sea impact areas.

5 Integrated assessment of biota contaminants data

Chemical Status Assessment Tool (CHASE)

The HELCOM Chemical Status Assessment Tool (CHASE) (Andersen et al., 2016) integrates data on hazardous substances in water, sediments and biota as well as bio-effect indicators and is based on a substance- or bio-effect-specific calculation of a '**contamination ratio' (CR)** being the ratio between an observed concentration and a threshold value. **Values <1.0** indicate areas potentially 'unaffected', while **values >1.0** indicate areas potentially 'affected'. These ratios are combined within matrices, i.e. for water, sediment and biota and for biological effects. The overall assessment used a 'one out, all out principle' with regard to each matrix. The CHASE tool can in combination with temporal trend assessments of individual substances be advantageous for use in remedial action plans and, in particular, for the science-based evaluation of the status and for determining which specific substances are responsible for a status as potentially affected.

Assessments of the environmental health of marine environments with regard to hazardous substances have traditionally been carried out on a substance-by-substance basis, focusing on thresholds for toxic effects, background concentrations and temporal trends (OSPAR 2010; EEA 2011). In Europe, following recent EU legislation, member states are required to carry out integrated assessments of 'chemical status' (Water Framework Directive) and 'contamination status' (Marine Strategy Framework Directive).

In the framework of an integrated thematic assessment of hazardous substances in the Baltic Sea (HELCOM 2010) it was developed a tool for integrated assessment of chemical status. The rationale for this new tool was twofold. Firstly, the tool should enable comparison between areas with differences in monitoring activities. Secondly, the new tool would fall in line with the HELCOM approach to develop and use indicator-based assessment tools for assessing eutrophication, hazardous substances and biodiversity. The prototype tool was named the HELCOM Chemical Status Assessment Tool (CHASE). In the implementation of the MSFD, EU member states are required to assess 'good environmental status' of marine waters. For this purpose, CHASE was further developed, where substances are combined under four themes: (1) contaminants in water, (2) contaminants in sediments, (3) contaminants in biota and (4) biological effects of contaminants. CHASE tool provides a unique approach to data-driven integrated assessments.

The benefit of using integrative tools is that they give a larger picture of the assessed elements by using numerous indicators and allowing inclusion of different substances, matrices, species and analytical methods to a single assessment (Andersen et al., 2016). There are four elements in the CHASE tool—water, sediment, biota and biological effects. The elements 'water' and 'sediment' include concentrations in the environment which reflect short term and long-term pollution, respectively. The elements 'biota' and 'biological effects' show the levels accumulated in organisms. All four elements combined provide a broad picture of the status of environmental contamination. The four groups are first assessed separately, and the final status is defined as the lowest status of the four elements. Thus, this status is based on the 'one out, all out principle' (OO-AO), which was considered appropriate as the four elements represent different aspects of the contamination status. Moreover, the approach adopted gives equal weight to all the elements because contamination in any of the four groups is seen as potentially equally harmful to the ecosystem.

The integrated assessment provides a final status for an assessment unit (i.e. a spatial unit), placing it in one of five classes: bad, poor, moderate, good and high. The classifications of bad, poor and moderate status indicate an environmental state which is '**affected**' (i.e. affected by hazardous substances). The classifications of good and high status indicate an environmental state '**unaffected**' (i.e. unaffected by hazardous substances). Thus, this classification system is essentially binomial (unaffected vs. affected) and is distinguished by a threshold value. The other classes are based on defined deviations from the unaffected/affected boundary.

In CHASE, each indicator is assessed against a specific threshold level and the results of the indicators are then combined to obtain the status for each element. For each of the indicators (n) at an assessment unit, the **contamination ratio (CR)** of the measured concentration (C_m) to a relevant assessment criterion for good environmental status ($C_{Threshold}$) is calculated. Integration of the CRs of the indicators within an element could be done in different ways: (1) the arithmetic mean of indicator CR values, (2) the root mean square (RMS) of CR values, (3) a contamination score (CS) and (4) the pollution level index. The contamination score it is considered the most appropriate for CHASE tool,

as this minimizes the problem of 'dilution' of high values when several substances from an area are analyzed (Andersen et al., 2016).

Generally, more reliable results are produced if data from both abiotic and biotic environment are incorporated and if indicator selection is more harmonized in the assessment areas. It was noticed that the number of elements in CHASE affected the assessment result. If an assessment unit had only few data from one matrix, it is more likely to end up with a positive status result. The CHASE assessment comprises two abiotic matrices (water and sediment), that represent contamination of habitats, and two biotic matrices (concentrations in biota and effects observed in biota), that provide a direct link to marine life (i.e. populations, communities, food web) (Andersen et al., 2016).

Although it is recommended that both aspects should be included in an assessment of contamination status, for the purpose of WP-3 ANEMONE, CHASE was applied for biota contaminants data in order to assess the status across stations/assessment units/various species (mollusks, fish)/various contaminants and to identify what hazardous substances poses the higher risk for not achieving good environmental status.

In order to make monitoring results more comparable within Black Sea region, partners agreed on a common set of contaminants (cca 70 individual hazardous substances) (heavy metals, polycyclic aromatic hydrocarbons, organochlorinated pesticides, polychlorinated biphenyls) and selected relevant species (mussels, Rapana, pelagic and demersal fish), collected from specific study areas (river influenced areas, coastal stations and open sea). Overall, 49 biota samples from Black Sea region (26 stations from Ukraine, Romania, Bulgaria, Turkey) were investigated for hazardous substances presence (23 samples of pelagic and demersal fish samples, belonging to 10 species, 19 samples of mussels and 7 samples of Rapana) (Figure 5.1, Table 5.2).

The CHASE assessment tool was tested in the Black Sea with contaminants in biota data set and the assessment results were produced, as overall scores related to assessment units (stations and regions), and matrix /species related scores. Generally, results could be influenced by the number of samples and type of species investigated in the assessment units, number of indicators, thresholds that were used. (Table 5.3, Table 5.4).

- There were evinced sub-regional differences in the status results, with worse status predominating in the north-western part of the Black Sea and better status in the southern part of the Black Sea. (*Figure 5.2*, Figure 5.3, Figure 5.4, Figure 5.5).

- Across the investigated biota samples, the CHASE test assessment showed a range of status results from bad to high, the majority of them (54 %) being in the „affected by hazardous substances"state (bad, poor and moderate), whereas the remaning 46% of biota samples are „unaffected by hazardous substances"state (good and high status). (Figure 5.6).

In order to enable back-tracking of the integrated result to the substance results, the CHASE tool shows the indicators behind the assessment results, and these can be used to identify sources of pollution or substances that potentially cause the greatest harm to environment (Andersen et al., 2016).

- For the ANEMONE biota contaminants data, the hazardous substances with the highest contamination ratio (CR>1) are ranked as follows: Sum of 6 PCBs (in 46% of samples), HCB (38%), heptaclor (33%), benzo(a)pyrene (12.5%), Hg (12.5%). Less frequent were aldrin, Cd and As (in 8% of samples), whereas endrin and Pb presented CR>1 in 4% of biota samples. (Figure 5.7).

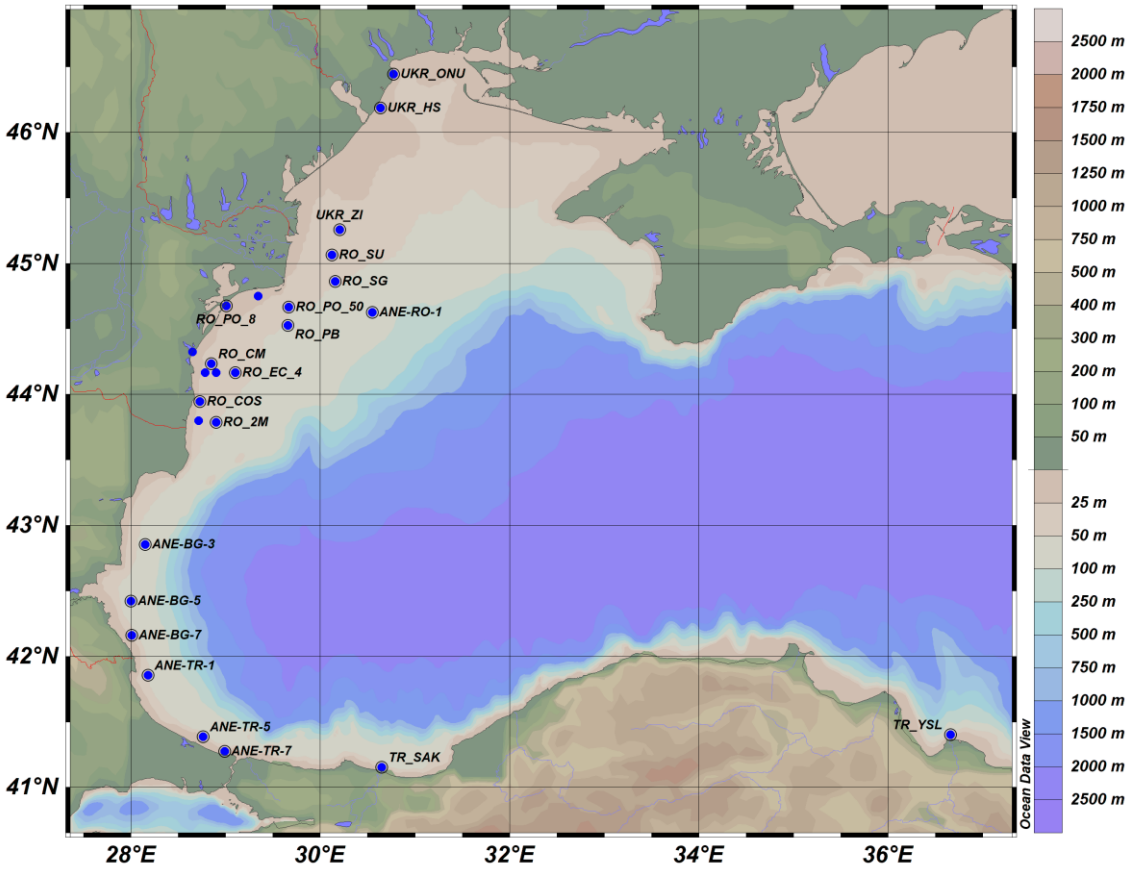


Figure 5.1 - Sampling stations (UA, RO, BG, TR) for biota (mollusks, fish) contamination studies, 2019

Table 5.1 - Biota samples (mollusks, fish) from Black Sea region investigated for the presence of hazardous substances, 2019

Region	Station code	Species *(alphabetical order)*	Date (dd.mm.yyyy)	Longitude [degree_ east]	Latitude [degree_ north]	Bot. Depth [m]
TR	TR_SAK	*Alosa fallax*	9/10/2019	30.6502	41.1541	38
RO	RO_PO_8	*Engraulis encrasicolus*	6/22/2019	29.0067	44.6767	8
RO	RO_HP	*Engraulis encrasicolus*	8/13/2019	28.6490	44.3231	10
TR	TR_SAK	*Merlangius merlangus euxinus*	9/10/2019	30.6502	41.1541	38
TR	TR_YSL	*Merlangius merlangus euxinus*	9/12/2019	36.6579	41.4034	38
TR	ANE-TR-1	*Merlangius merlangus euxinus*	10/5/2019	28.1781	41.8573	75
RO	RO_COS	*Merlangius merlangus euxinus*	5/19/2019	28.7267	43.9450	53
TR	TR_SAK	*Mullus barbatus ponticus*	9/10/2019	30.6502	41.1541	38
TR	TR_YSL	*Mullus barbatus ponticus*	9/12/2019	36.6579	41.4034	38
RO	RO_EC_4	*Mullus barbatus ponticus*	5/19/2019	29.1025	44.1667	43
RO	RO_SU	*Mytilus galloprovincialis*	5/11/2019	30.1252	45.0642	40
RO	RO_SG	*Mytilus galloprovincialis*	5/12/2019	30.1580	44.8603	50
RO	RO_PO_50	*Mytilus galloprovincialis*	5/13/2019	29.6682	44.6669	50
RO	RO_PB	*Mytilus galloprovincialis*	5/14/2019	29.6596	44.5270	50
RO	RO_PO_50	*Mytilus galloprovincialis*	8/1/2019	29.6682	44.6669	50
RO	RO_CM	*Mytilus galloprovincialis*	8/1/2019	28.8472	44.2347	30
RO	RO_EC_2	*Mytilus galloprovincialis*	8/1/2019	28.7833	44.1667	28
RO	RO_EC_3	*Mytilus galloprovincialis*	8/1/2019	28.9000	44.1667	36
RO	RO_COS	*Mytilus galloprovincialis*	8/1/2019	28.7267	43.9450	30
RO	RO_MAN	*Mytilus galloprovincialis*	8/1/2019	28.7156	43.7986	39
RO	ANE-RO-1	*Mytilus galloprovincialis*	10/1/2019	30.5490	44.6253	78
BG	ANE-BG-3	*Mytilus galloprovincialis*	10/3/2019	28.1496	42.8525	57
BG	ANE-BG-5	*Mytilus galloprovincialis*	10/4/2019	28.0001	42.4222	49
BG	ANE-BG-7	*Mytilus galloprovincialis*	10/4/2019	28.0072	42.1601	48
UA	UA_ONU	*Mytilus galloprovincialis*	9/30/2019	30.7746	46.4435	6
UA	UA_ONU	*Mytilus galloprovincialis*	11/16/2019	30.7746	46.4435	6
TR	TR_SAK	*Mytilus galloprovincialis*	9/10/2019	30.6502	41.1541	38
TR	ANE-TR-5	*Mytilus galloprovincialis*	10/6/2019	28.7636	41.3864	77
TR	ANE-TR-7	*Mytilus galloprovincialis*	10/6/2019	28.9883	41.2735	35
RO	RO_EC_4	*Neogobius melanostomus*	5/19/2019	29.1025	44.1667	43
UA	UA_ZI	*Neogobius melanostomus*	6/28/2019	30.2050	45.2575	9
UA	UA_ONU	*Neogobius melanostomus*	9/29/2019	30.7746	46.4435	6
RO	RO_2M	*Psetta maxima maeotica*	5/18/2019	28.9000	43.7871	58
TR	TR_SAK	*Psetta maxima maeotica*	9/10/2019	30.6502	41.1541	38
TR	TR_YSL	*Psetta maxima maeotica*	9/12/2019	36.6579	41.4034	38
RO	RO_EC_2	*Rapana venosa*	8/1/2019	28.7833	44.1667	28
UA	UA_ZI	*Rapana venosa*	6/28/2019	30.2050	45.2575	9
UA	UA_ZI	*Rapana venosa*	6/28/2019	30.2050	45.2575	9
UA	UA_HS	*Rapana venosa*	9/14/2019	30.6347	46.1846	10
UA	UA_ONU	*Rapana venosa*	11/16/2019	30.7746	46.4435	6
TR	TR_SAK	*Rapana Venosa*	9/10/2019	30.6502	41.1541	38
TR	TR_YSL	*Rapana Venosa*	9/12/2019	36.6579	41.4034	38
TR	TR_YSL	*Solea solea*	9/12/2019	36.6579	41.4034	38
TR	TR_SAK	*Sprattus sprattus*	9/10/2019	30.6502	41.1541	38
RO	RO_PO_50	*Sprattus sprattus*	6/21/2019	29.6682	44.6669	54
RO	RO_Z	*Squalus acanthias*	5/21/2019	29.3453	44.7492	14
TR	TR_SAK	*Trachurus mediterraneus ponticus*	9/10/2019	30.6502	41.1541	38
TR	TR_YSL	*Trachurus mediterraneus ponticus*	9/12/2019	36.6579	41.4034	38
RO	RO_HP	*Trachurus mediterraneus ponticus*	6/13/2019	28.6490	44.3231	10

Table 5.2 - List of hazardous substances measured in biota samples from Black Sea region, 2019

Heavy metals	Polycyclic aromatic hydrocarbons	Pesticides	Polychlorinated byphenyls	
Cu	Naphthalene	HCB	PCB 8	PCB 128
Cd	Acenaphthylene	α-HCH	PCB 18	PCB 196
Pb	Acenaphthene	ß-HCH	PCB 31	PCB 206
Ni	Fluorene	Lindan	PCB28	PCB138
Cr	Phenanthrene	Heptaclor	PCB52	PCB 183
As	Anthracene	Aldrin	PCB 49	PCB 174
Hg	Fluoranthene	Dieldrin	PCB 44	PCB 177
Mn	Pyrene	Endrin	PCB 66	PCB180
Co	Benzo[a]anthracene	p,p'DDE	PCB 77	PCB 170
Zn	Crysene	p,p'DDD	PCB101	PCB 199
Fe	Crysene+Triphenylene	p,p'DDT	PCB 110	PCB 194
	Benzo[b]fluoranthene	Atrazine	PCB 149	PCB 209
	Benzo[k]fluoranthene	Dursban	PCB118	
	Benzo[a]pyrene		PCB153	
	Benzo (g,h,i)perylene		PCB 105	
	Dibenzo(a,h)anthracene		PCB 187	
	Indeno(1,2,3-c,d)pyrene		PCB 126	

Table 5.3 - Status by station following application of CHASE on ANEMONE biota contaminants data

Region	Station code	Species/Matrix	CHASE Matrix Score	CHASE OVERALL Score
UA	UA_HS	Rapana	4	4
UA	UA_ONU	Fish	5	5
UA		Rapana	5	
UA		Mussel	5	
UA	UA_ZI	Fish	5	5
UA		Rapana	3	
RO	RO_2M	Fish	3	3
RO	RO_CM	Mussel	3	3
RO	RO_COS	Fish	2	3
RO		Mussel	3	
RO	RO_EC_2	Rapana	2	4
RO		Mussel	5	
RO	RO_EC_3	Mussel	3	3
RO	RO_EC_4	Fish	4	4
RO	RO_HP	Fish	3	3
RO	RO_MAN	Mussel	3	3
RO	RO_PB	Mussel	2	2
RO	RO_PO_50	Fish	3	3
RO		Mussel	3	
RO	RO_PO_8	Fish	3	3
RO	RO_SG	Mussel	3	3
RO	RO_SU	Mussel	3	3
RO	RO_Z	Fish	3	3
RO	ANE-RO-1	Mussel	3	3
BG	ANE-BG-3	Mussel	3	3
BG	ANE-BG-5	Mussel	3	3
BG	ANE-BG-7	Mussel	3	3
TR	ANE-TR-1	Fish	1	1
TR	ANE-TR-5	Mussel	1	1
TR	ANE-TR-7	Mussel	1	1
TR	TR_SAK	Fish	1	2
TR		Rapana	3	
TR		Mussel	1	
TR	TR_YSL	Fish	1	1
TR		Rapana	1	

*** Legend - CHASE scores**

1	2	3	4	5
High	Good	Moderate	Poor	Bad

Table 5.4 - Status by region following application of CHASE on ANEMONE biota contaminants data and number of investigated species and samples

Region	Species/Number of samples	Status (by matrix/specie)	Overall status
ANE-UA	Fish (2 samples, 1 species)	Bad	Bad
ANE-UA	*Rapana venosa* (4 samples)	Poor	
ANE-UA	*Mytilus galloprovincialis* (2 samples)	Bad	
ANE-RO	Fish (9 samples, 8 species)	Poor	Poor
ANE-RO	*Rapana venosa* (1 sample)	Good	
ANE-RO	*Mytilus galloprovincialis* (11 samples)	Moderate	
ANE-BG	Fish -	-	Moderate
ANE-BG	*Rapana venosa* -	-	
ANE-BG	*Mytilus galloprovincialis* (3 samples)	Moderate	
			Good
ANE-TR	Fish (12 samples, 7 species)	High	
ANE-TR	*Rapana venosa* (2 samples)	Good	
ANE-TR	*Mytilus galloprovincialis* (3 samples)	High	

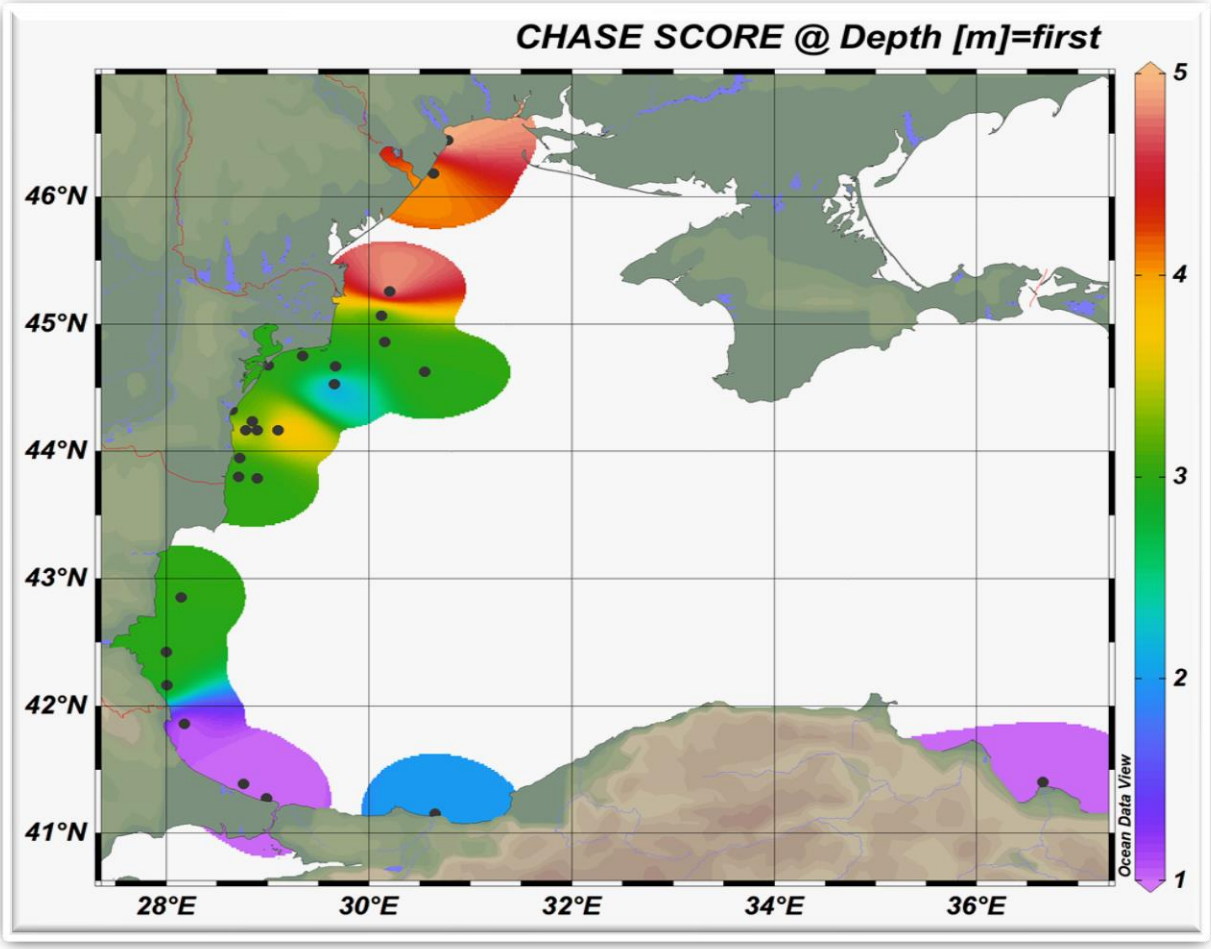


Figure 5.2. Overall status following application of CHASE on ANEMONE biota(mussels, Rapana and fish) contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

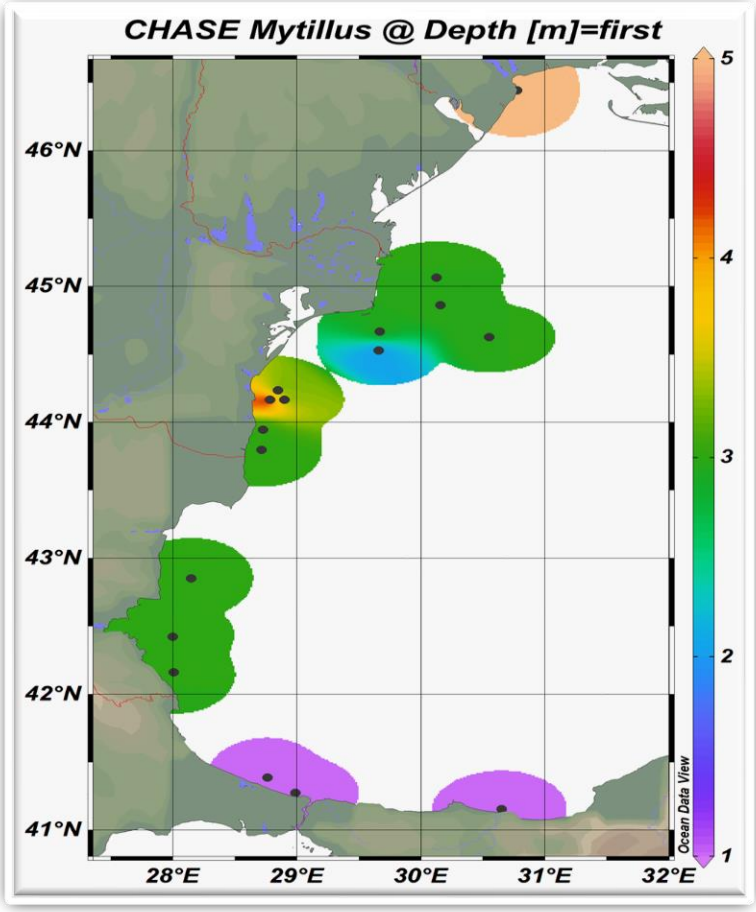


Figure 5.3 - Mytilus galloprovincialis status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

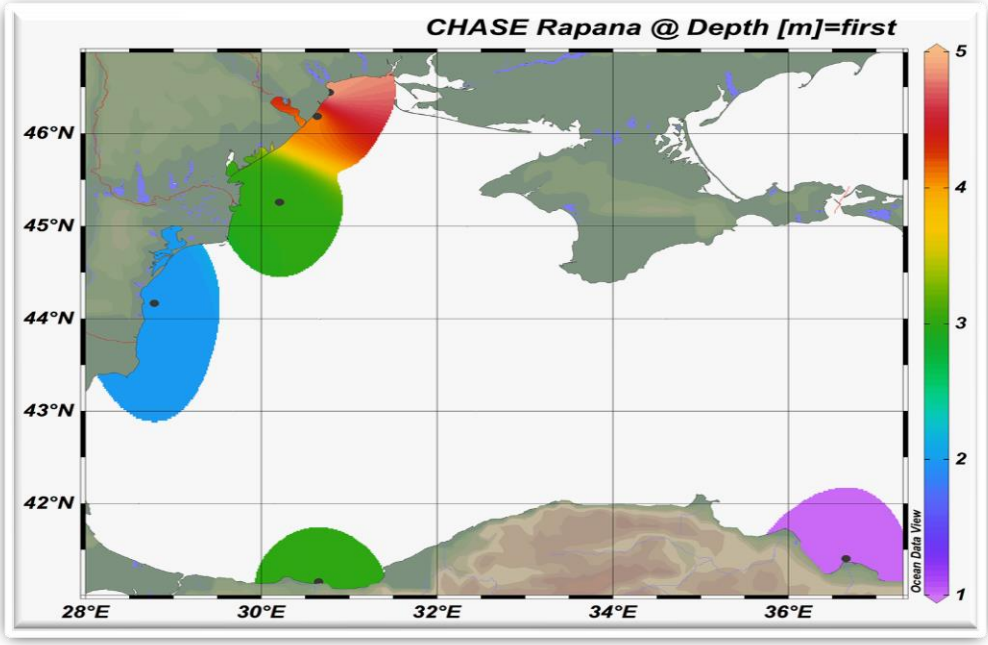


Figure 5.4 - Rapana venosa status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

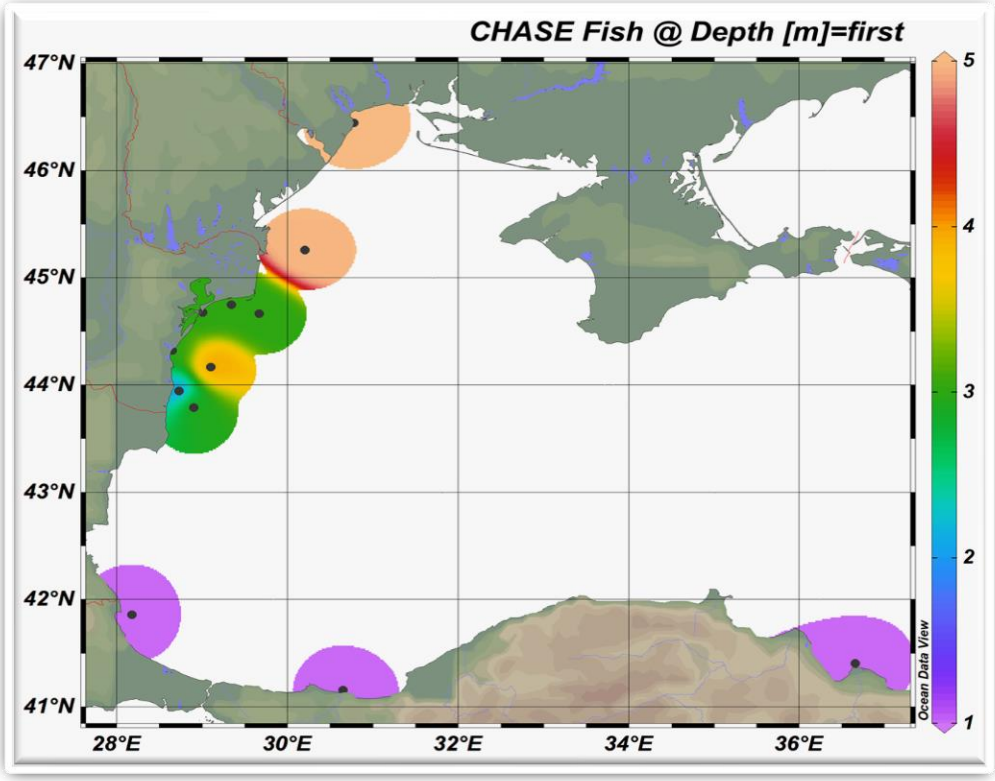


Figure 5.5 - Fish status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

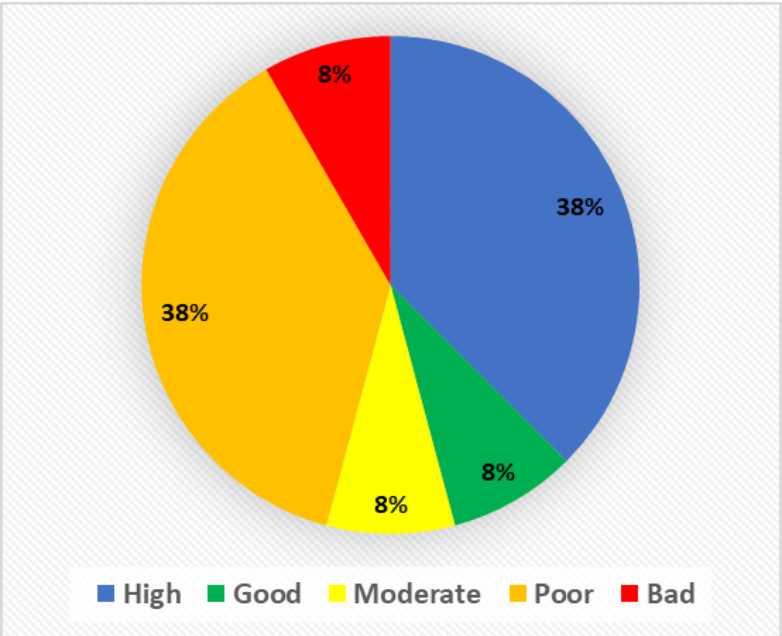


Figure 5.6 - CHASE status classification of biota samples based on hazardous substances bioaccumulation levels

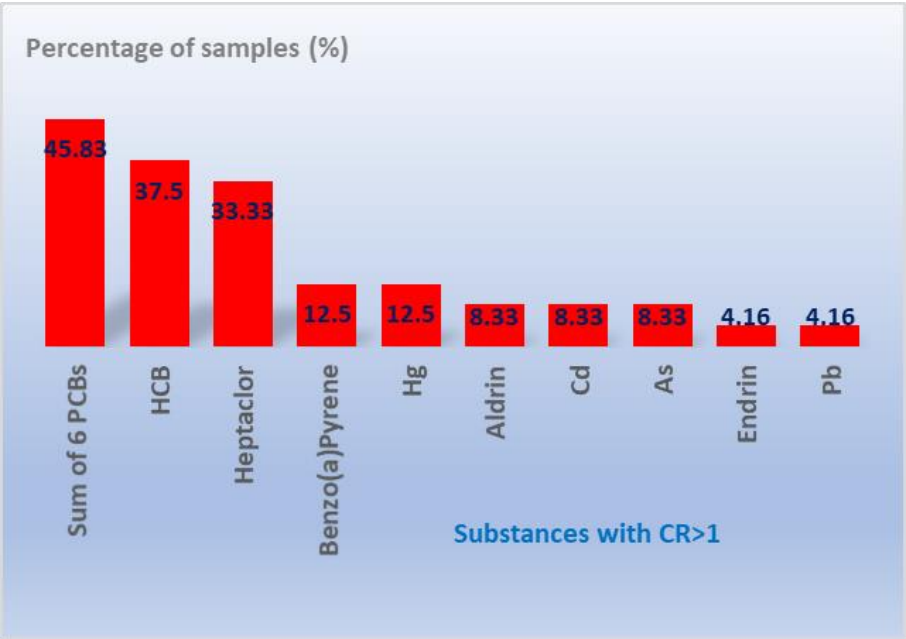


Figure 5.7. Frequency of occurrence of hazardous substances with the highest contamination ratios (CR > 1)

Spatial distribution of individuals substances concentrations (HM, OCPs, PAHs, PCBs) in marine mollusks (mussels and Rapana) showed a high variability among substances, species and geographical areas (Figure 5.8 to Figure 5.15), thus evincing the benefit of using integrative tools, like CHASE, as they give a larger picture of the assessed elements by using numerous indicators and allowing inclusion of different substances, matrices, species and analytical methods to a single assessment (Andersen et al., 2016).

Bioaccumulation of hazardous substances in dorsal muscle of demersal and pelagic fish that were investigated evinced various inter-specific differences, depending on element, species (demersal, pelagic), position along the trophic chain. For example, HMs (Cd, Pb), PAHs - B (a)P and OCPs (HCB, hepataclor) registered higher concentrations in two representants of demersal fish (*Neogobius sp.* and *Mullet sp.)* in comparison with other species, whereas PCBs (PCB153, PCB118) presented increased bioaccumulation level in a specie higher ranked in the food chain, namely *Squalus sp.* (Figure 5.16 to Figure 5.19).

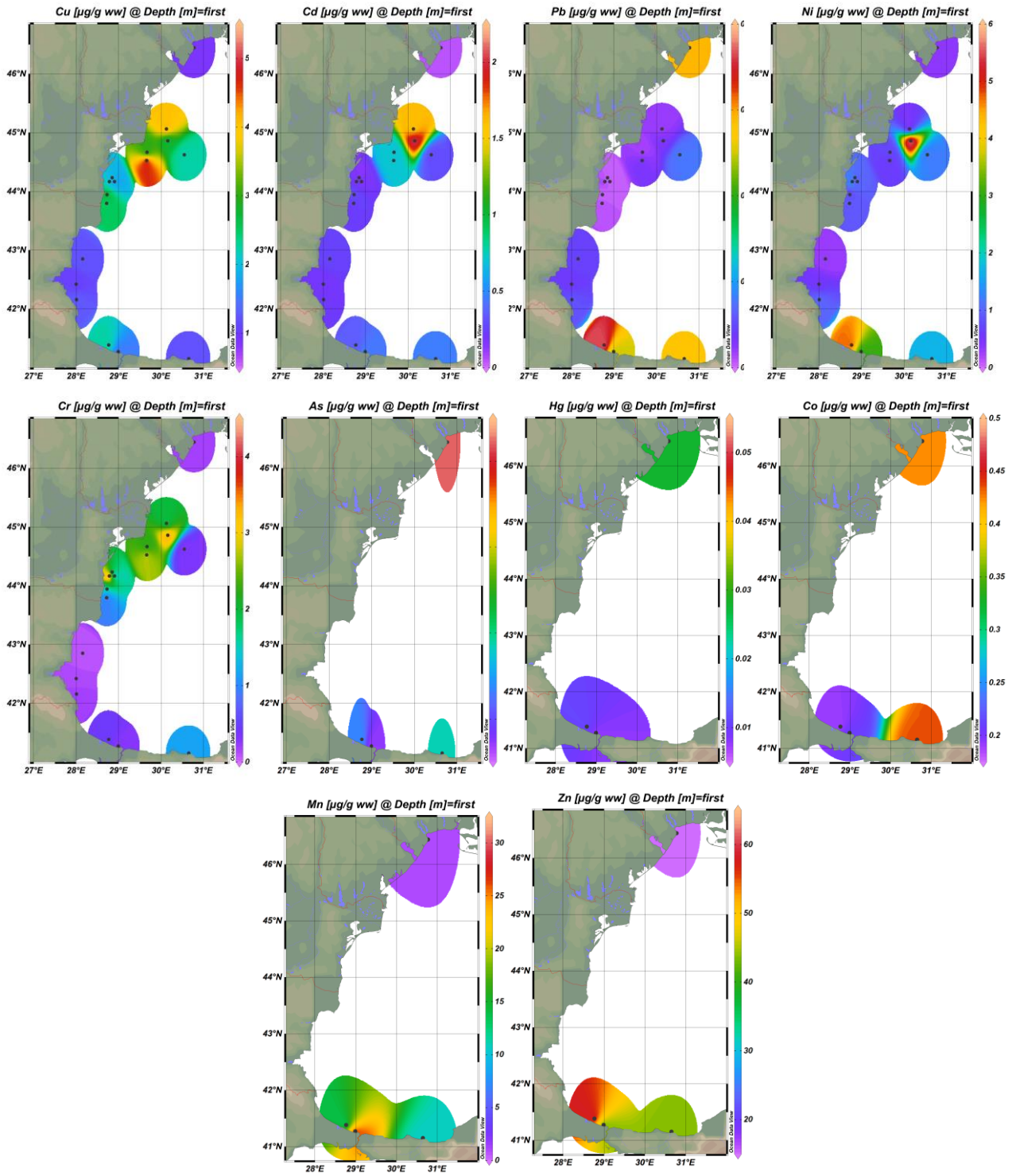


Figure 5.8 - Heavy metals bioaccumulation in Mytilus galloprovincialis from Black Sea region, 2019

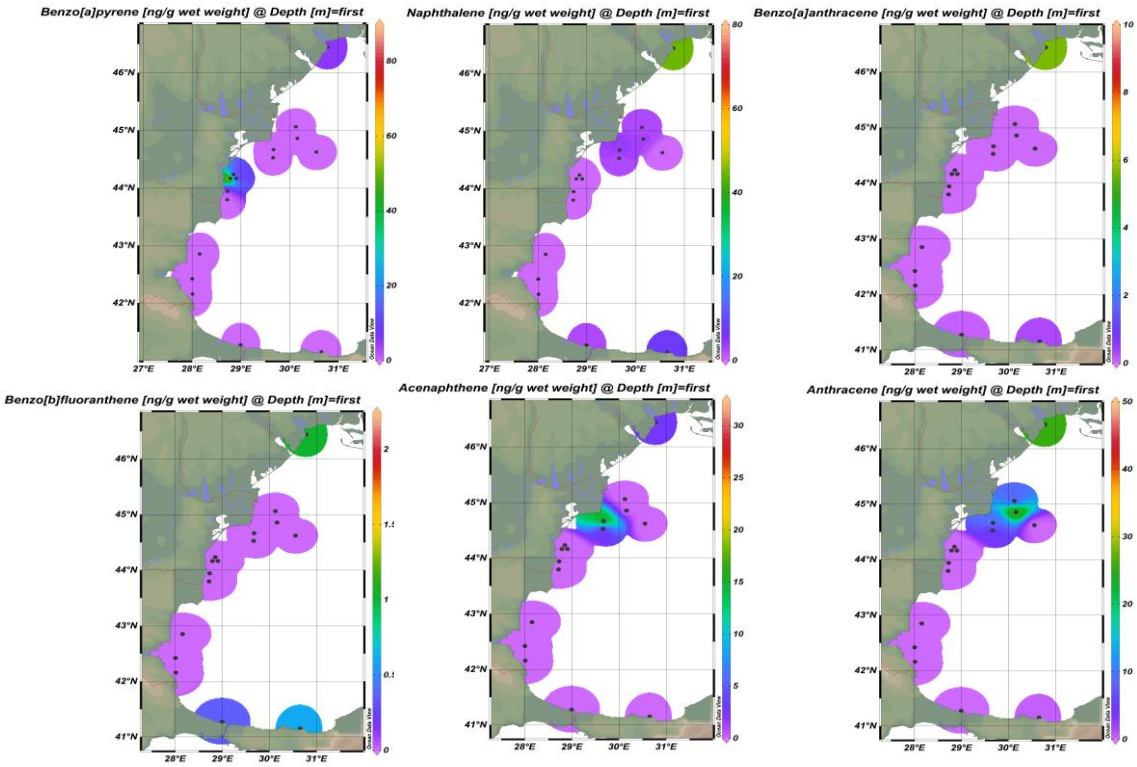


Figure 5.9 - PAHs bioaccumulation in Mytilus galloprovincialis from Black Sea region, 2019

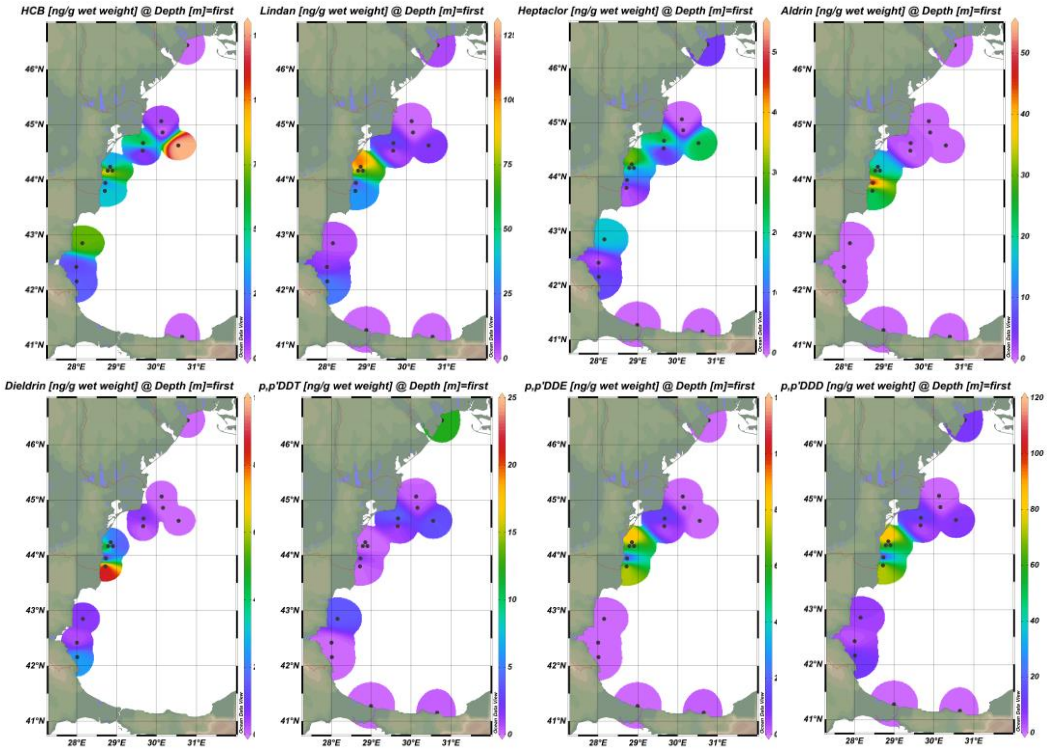


Figure 5.10 - OCPs bioaccumulation in Mytilus galloprovincialis from Black Sea region, 2019

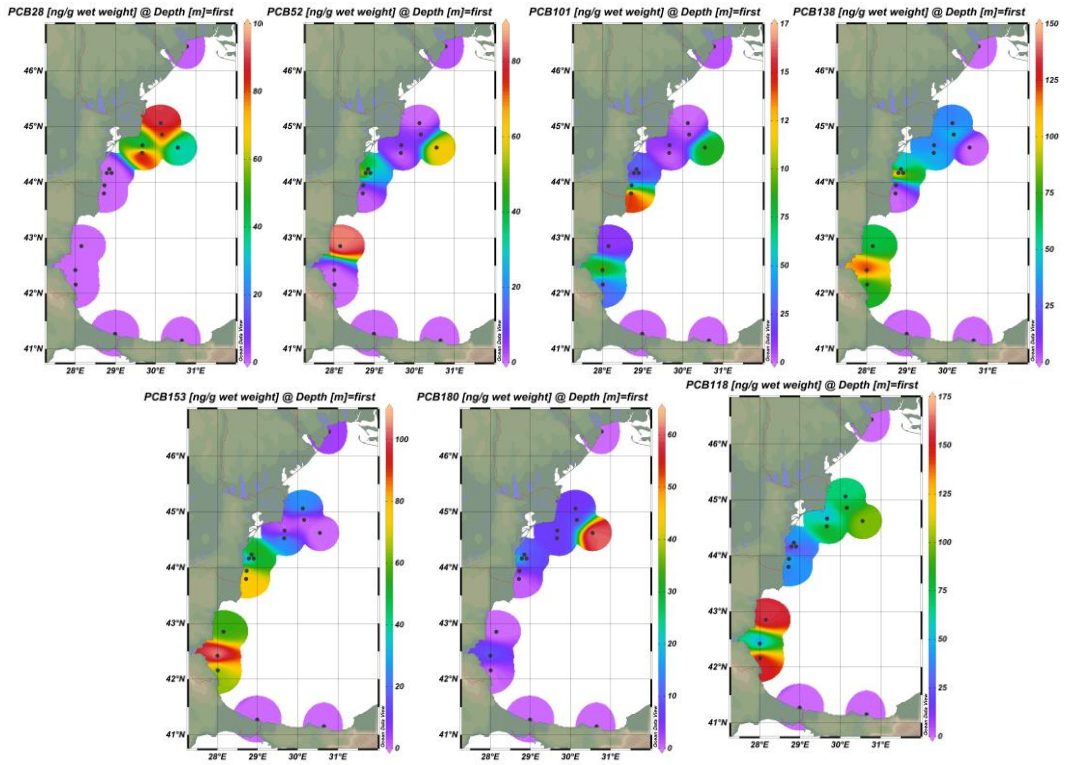


Figure 5.11 - PCBs bioaccumulation in Mytilus galloprovincialis from Black Sea region, 2019

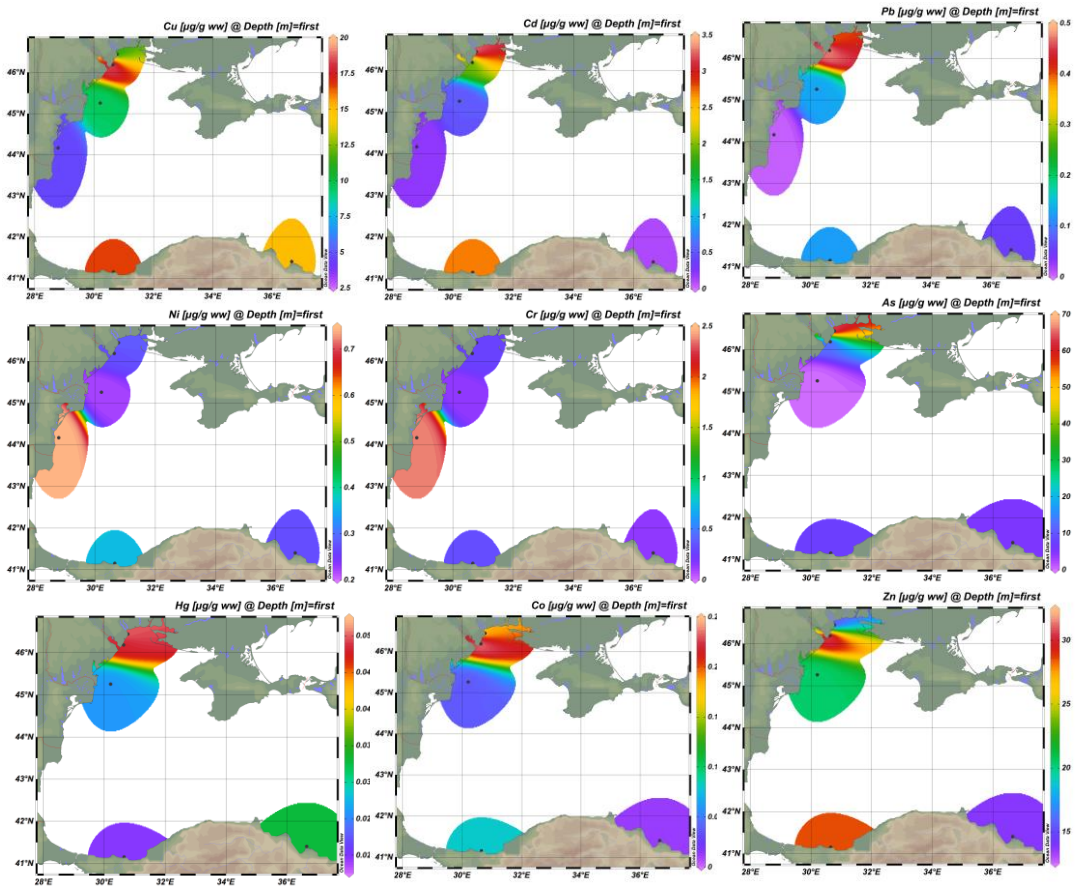


Figure 5.12 - Heavy metals bioaccumulation in Rapana venosa from Black Sea region, 2019

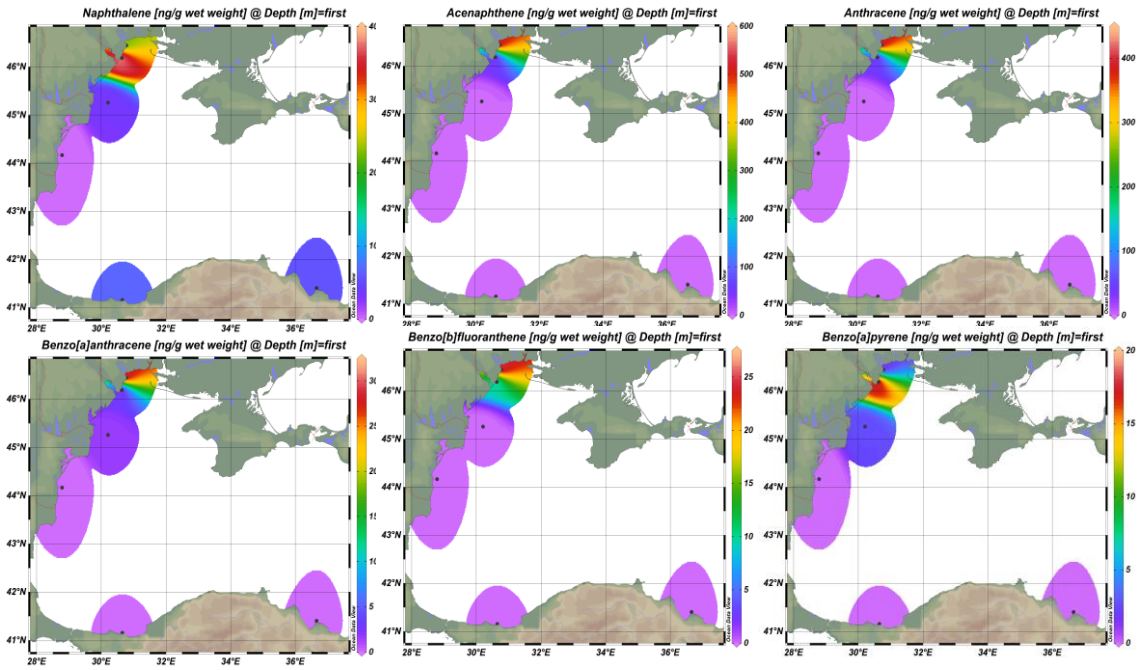


Figure 5.13 - PAHs bioaccumulation in Rapana venosa from Black Sea region, 2019

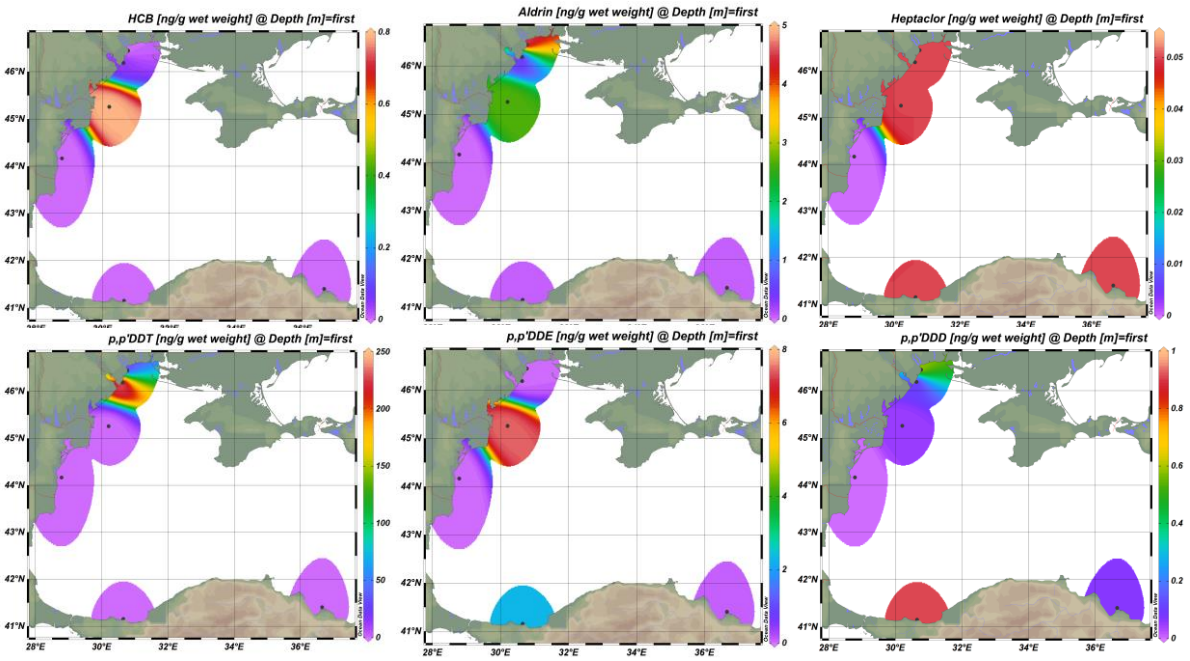


Figure 5.14 - OCPs bioaccumulation in Rapana venosa from Black Sea region, 2019

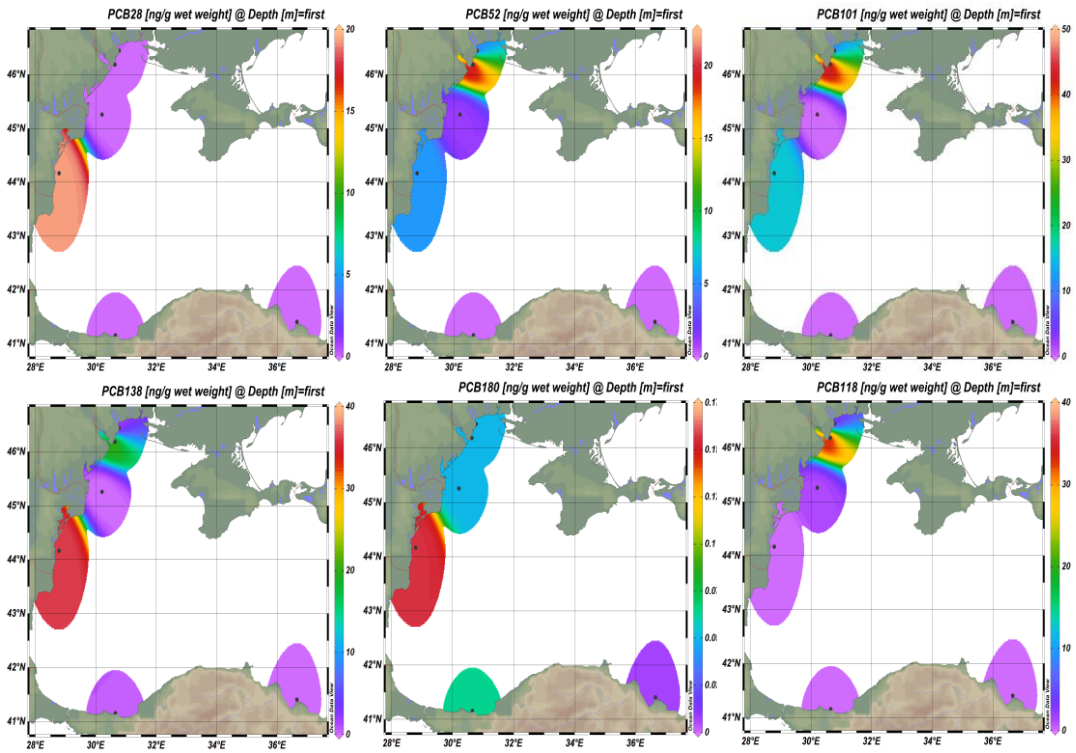


Figure 5.15 - PCBs bioaccumulation in Rapana venosa from Black Sea region, 2019

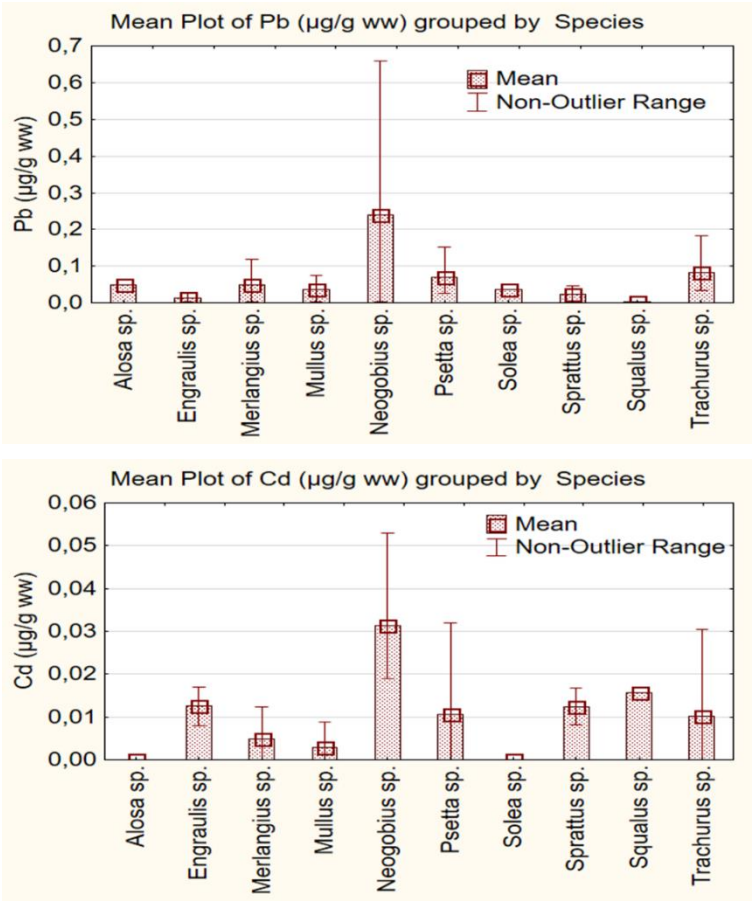


Figure 5.16 – Pb and Cd bioaccumulation in marine fish species from Black Sea region, 2019

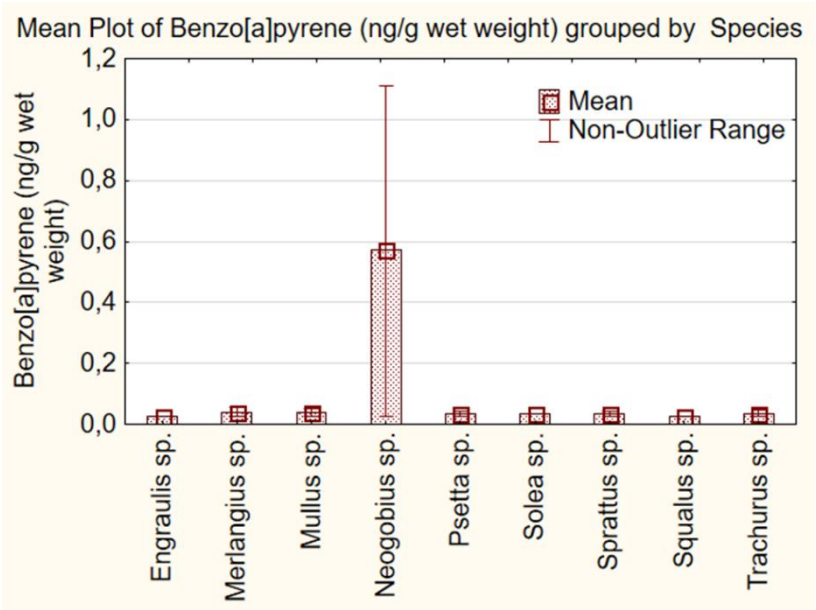


Figure 5.17 – Benzo(a)pyrene bioaccumulation in marine fish species from Black Sea region, 2019

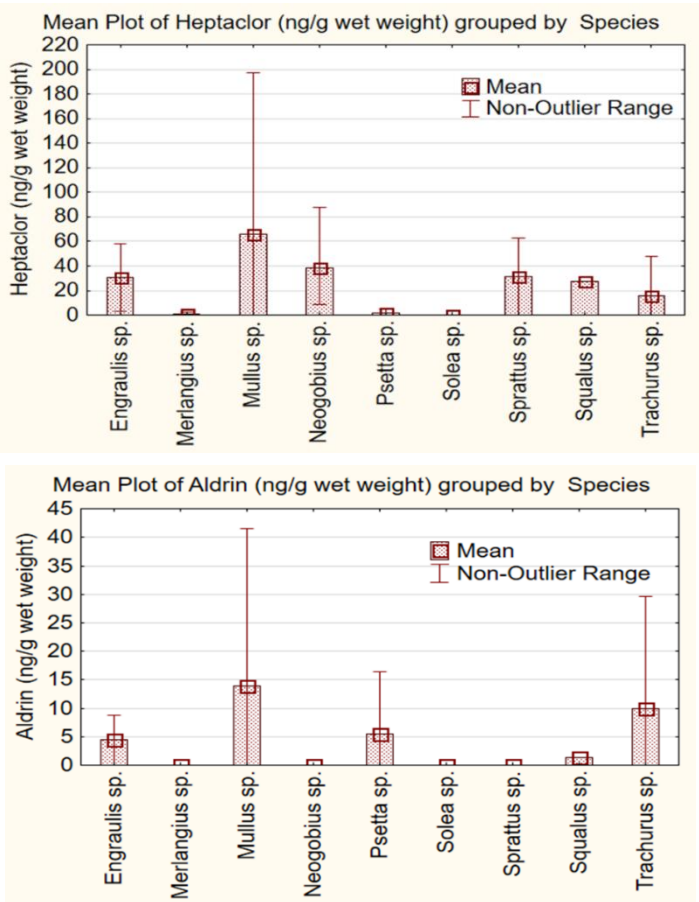


Figure 5.18 – OCPs bioaccumulation in marine fish species from Black Sea region, 2019

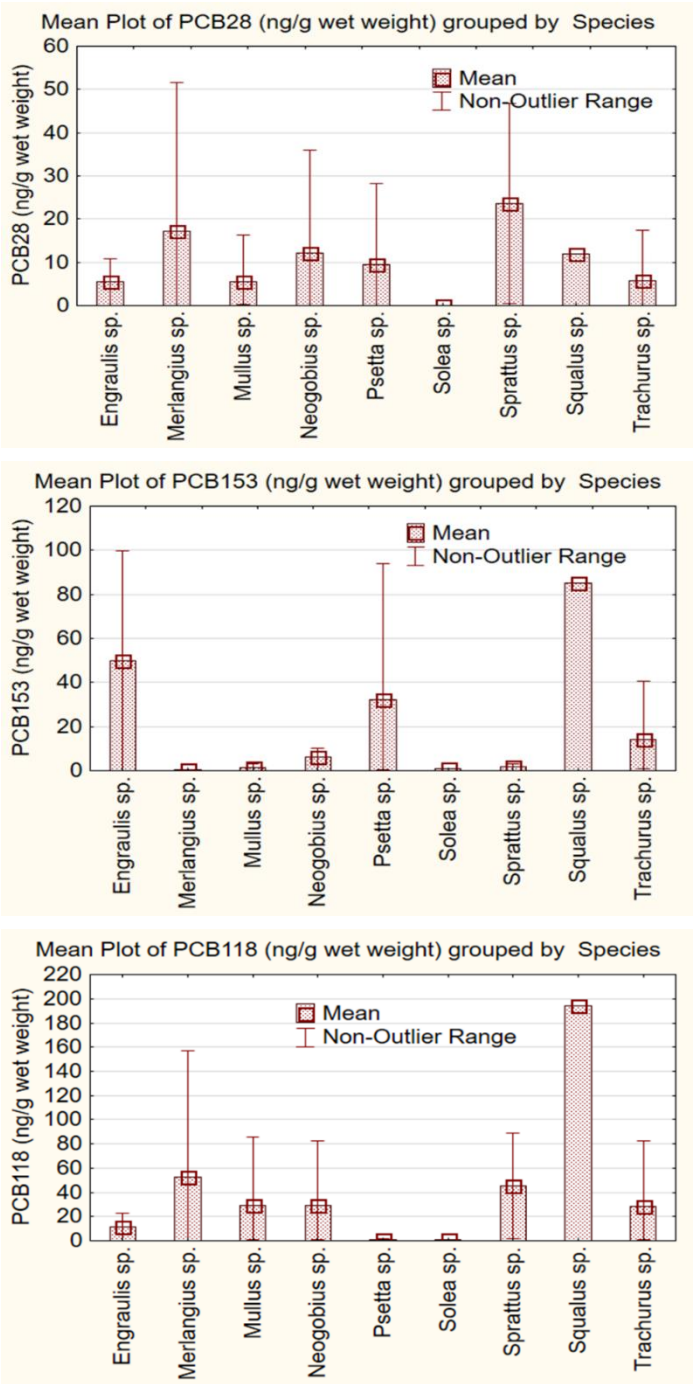


Figure 5.19 – PCBs bioaccumulation in marine fish species from Black Sea region, 2019

6 Conclusions and recommendations

- ✓ WP-3 contributed with new data on chemical contamination of aquatic organisms, collected during specific studies in the selected study areas, thus filling knowledge gaps identified for Black Sea region.
- ✓ The CHASE assessment tool was tested in the Black Sea with contaminants in biota data set and the assessment results were produced, as overall scores related to assessment units (stations and regions), and matrix /species related scores.
- ✓ There were evinced sub-regional differences in the status results, with worse status predominating in the north-western part of the Black Sea and better status in the southern part of the Black Sea.
- ✓ Across the investigated biota samples, the CHASE test assessment showed a range of status results from bad to high, the majority of them (54 %) being in the „affected by hazardous substances"state (bad, poor and moderate), whereas the remaning 46% of biota samples are „unaffected by hazardous substances"state (good and high status).
- ✓ CHASE assessment tool of contamination status enables 'back-tracking' of information in order to support monitoring and management actions. Thus, one can identify the indicators with the highest contamination ratios and, thus, the substances responsible for the contamination status in the assessment units.
- ✓ For the ANEMONE biota contaminants data, the hazardous substances that potentially cause the greatest harm to the environment, with the highest frequency of contamination ratio greater than one (CR>1), are ranked as follows: Sum of 6 PCBs, HCB, heptaclor, benzo(a)pyrene, Hg. Less frequent were aldrin, Cd, As, endrin and Pb.
- ✓ In order to increase the resolution of the assessment result and to allow comparisons of chemical status between subregions, there is a need for a higher level of harmonization regarding target levels, substances list, indicators and matrices. A main factor affecting the integrated assessment results is the quality of the threshold values, which need further improvement for many substances across European seas, not only for Black Sea region.
- ✓ Generally, the monitoring of contaminants in seafood is executed by the responsible authorities in charge (e.g., Food authorities, Sanitary – veterinary agencies, s.a), which often are different from the environmental institutions implementing the MSFD and its associated monitoring (D8, D9).
- ✓ Thus, cooperation between authorities and environmental institutions in charge of health monitoring is strongly encouraged. Exchanging information on data, approaches and methodologies between environmental monitoring institutions and human health risk related monitoring institutions is very important.
- ✓ An improved knowledge on how contaminants make their way through the marine environment and are taken up by different marine organisms would help scientists to assess the risks of eating contaminated seafood and raise awareness on this issue.

References

Andersen Jesper H., Ciarán Murray, Martin M. Larsen, Norman Green, Tore Høgåsen, Elin Dahlgren, Galina Garnaga-Budrė ,Kim Gustavson, Michael Haarich, Emilie M.F. Kallenbach, Jaakko Mannio, Jakob Strand, Samuli Korpinen, 2016. Development and testing of a prototype tool for integrated assessment of chemical status in marine environments. Environ Monit Assess, 188:115, DOI 10.1007/s10661-016-5121-x

Boicenco, L., Abaza, V., Anton, E., Bișinicu, E., Buga, L., Coatu, V., Damir, N., Diaconeasa, D., Dumitrache, C., Filimon, A., Galațchi, M., Golumbeanu, M., Harcotă, G., Lazăr, L., Marin, O., Mateescu, R., Maximov, V., Mihailov, E., Nenciu, M., Nicolaev, S., Niță, V., Oros, A., Pantea, E., Radu, G., Spînu, A., Stoica, E., Tabarcea, C., Timofte, F, Țiganov, G., Țoțoiu, A, Vlas, O., Vlăsceanu, E., Zaharia, T., 2018. Studiu privind elaborarea raportului privind starea ecologică a ecosistemului marin Marea Neagră conform cerințelor art. 17 ale Directivei Cadru Strategia pentru mediul marin (2008/56/EC), 331 pp.

Beyer, W. & Meador, James, 2011. Environmental Contaminants in Biota: Interpreting Tissue Concentrations. Publisher: Taylor and Francis CRC Press.

BSC, 2019. State of the Environment of the Black Sea (2009-2014/5). Edited by Anatoly Krutov. Publications of the Commission on the Protection of the Black Sea Against Pollution (BSC) 2019, Istanbul, Turkey, 811 pp.

Coatu Valentina, Oros Andra, Daniela Tiganus, Galina Shtereva, Levent Bat, 2014. Chapter VI. Contaminants In Biota. In MISIS Joint Cruise Scientific Report, 2014. State of Environment Report of the Western Black Sea based on Joint MISIS cruise (SoE-WBS), Moncheva S. and L. Boicenco [Eds], Ed. ExPonto, 401 pp. (ISBN 978-606-598-367-0).

Coatu Valentina, Oros Andra, Daniela Țigănuș, Galina Shtereva, Levent Bat, 2016. Assessment of the Contaminants in Biota from the Western Black Sea Basin in respect with MSFD Requirements in the frame of the MISIS Project. Cercetari Marine Nr. 46, 82-97. (ISSN 0250-3069).

Chouksey M. K., Kadam A.N., Zingde M.D. Petroleum hydrocarbon residues in the marine environment of Bassein–Mumbai. Marine Pollution Bulletin 49 (2004) 637–647.

EEA, 2019. Hazardous substances in marine organisms. CSI 049 , MAR 001 Published Oct 2019. https://www.eea.europa.eu/data-and-maps/indicators/hazardous-substances-in-marine-organisms-3/assessment.

Fliedner, A., Rüdel, H., Knopf, B. *et al.*, 2018. Assessment of seafood contamination under the marine strategy framework directive: contributions of the German environmental specimen bank. *Environ Sci Pollut Res* **25,** 26939–26956. https://doi.org/10.1007/s11356-018-2728-1.

Fleming LE, Broad K, Clement A, Dewailly E, Elmira S, Knap A, Pomponi SA, Smith S, Solo Gabriele H, Walsh P, 2006. Oceans and human health: emerging public health risks in the marine environment. Mar Pollut Bull 53(10–12):545–560.

HELCOM, 2010. Hazardous substances in the Baltic Sea—an integrated thematic assessment of hazardous substances in the Baltic Sea. Baltic Sea Environment Proceedings, 120B,1–116.

Hylland Ketil, Thierry Burgeot, Concepción Martínez-Gómez, Thomas Lang, Craig D. Robinson, Jörundur Svavarsson, John E. Thain, A. Dick Vethaak, Matthew J. Gubbins, 2017. How can we quantify impacts of contaminants in marine ecosystems? The ICON project, Marine Environmental Research, Volume 124, 2017, Pages 2-10, ISSN 0141-1136.

IAEA-MEL Marine Environmental Studies Laboratory: Training manual on the measurement of organochlorine and petroleum hydrocarbons in environmental samples (1995)

IAEA-MEL/Marine Environmental Studies Laboratory, 1999. Training manual on the measurement of heavy metals in environmental samples.

Klyachko Yu.A., Belenky S.M., 1988. Methods of analysis of food products, Moscow, Science.

Klyuev N.A., Brodsky E.S., 2000. Determination of polychlorinated biphenyls in the environment and biota. Polychlorinated biphenyls. Supertoxicants of the XXI century. Inf. Issue No. 5 VINITI, Moscow, p. 31-63.

Larsen J.C., Larsen P.B., 1998. Chemical carcinogens. In: Hester R.E., Harrison R.M., editors. Air Pollution and Health. The Royal Society of Chemistry; Cambridge, UK: 1998. pp. 33–56.

Laane, R.W.P.M., Slijkerman, D., Vethaak, A.D., Schobben, J.H.M., 2012.Assessment of the environmental status of the coastal and marine aquatic environment in Europe: A plea for adaptive management. Estuarine, Coastal and Shelf Science, 96, 31-38.

Ministry of Environment and Urbanization, TUBITAK-MRC (2017). "Integrated Marine Pollution Monitoring 2014-2016 Programmeme: 2016 The Black Sea Final Report, TÜBİTAK-MRC Press, Kocaeli.

Ministry of Environment and Urbanization, TUBITAK-MRC (2020). "Integrated Marine Pollution Monitoring 2017-2019 Programmeme: 2019 The Black Sea Final Report, TÜBİTAK-MRC Press, Kocaeli.

Oros Andra, Lazăr Luminita, Coatu Valentina, Țigănuș, Daniela, 2016. Recent Data From Pollution Monitoring And Assessment Of The Romanian Black Sea Ecosystem, Within Implementation Of The European Marine Strategy Framework Directive. 16th International Multidisciplinary Scientific GeoConference SGEM 2016, www.sgem.org, SGEM2016 Conference Proceedings, ISBN 978-619-7105-62-9 / ISSN 1314-2704, June 28 - July 6, 2016, Book3 Vol. 2, 821-828 pp.

Swartenbroux F, Albajedo B, Angelidis M, Aulne M, Bartkevics V, Besada V, Bignert A, Bitterhof A, Hallikainen A, Hoogenboom R, JorhemL, Jud M, Law R, Licht Cederberg D, McGovern E, Miniero R, Schneider R, Velikova V, Verstraete F, Vinas L, Vlad S, 2010. Marine strategy framework directive – task Group 9 Report Contaminants in fish and other seafood. EUR – Scientific and Technical Research series, Luxembourg, JRC/ICES, EUR 24339 EN, Pp 36. [http://publications.jrc.ec.europa.eu/repository/bitstream/JRC58103/tg9%20report%20final_vii.pdf]

Tornero V, Hanke G, 2016. Chemical contaminants entering the marine environment from sea-based sources: A review with a focus on European seas, Marine Pollution Bulletin, Volume 112, Issues 1–2, Pages 17-38, ISSN 0025-326X.

UNEP, 1990. Contaminant monitoring programmes using marine organisms: quality assurance and good laboratory practice. Reference Methods for Marine Pollution Studies, **57,** p1-23.

UNEP, 1993. Guidelines for monitoring chemical contaminants in the sea using marine organisms. *Reference Methods* for Marine Pollution Studies. **6,** p1-28.

Vijayan, M. M., Aluru, N., Maule, A. G., Jørgensen, E. H. 2006. Fasting augments PCB impact on liver metabolism in anadromous arctic char. Toxicological Sciences. 91, 431–439.

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