Hazardous Substances Assessment in the Black Sea Biota *(Andra Oros, Valentina Coatu, Leyla Gamze Tolun, Hakan Atabay, Yuriy Denga, Nicoleta Damir, Diana Danilov, Ertuğrul Aslan, Maryna Litvinova, Yurii Oleinik, Volodymyr Kolosov)*	"Cercetări Marine" Issue no. 51 Pages 27 - 48	2021
DOI:10.55268/CM.2021.51.27		

HAZARDOUS SUBSTANCES ASSESSMENT IN THE BLACK SEA BIOTA

Andra Oros[1], Valentina Coatu[1], Leyla Gamze Tolun[2], Hakan Atabay[2], Yuriy Denga[3], Nicoleta Damir[1], Diana Danilov[1], Ertuğrul Aslan[2], Maryna Litvinova[3], Yurii Oleinik[3], Volodymyr Kolosov[3]

[1]National Institute for Marine Research and Development "Grigore Antipa", 300 Mamaia Blvd., 900581, Constanta, Romania
[2]TUBITAK Marmara Research Center (MRC), Turkey
[3]Ukrainian Scientific Center of Ecology of the Sea, UkrSCES, Ukraine
E-mail: aoros@alpha.rmri.ro

ABSTRACT

The assessment of contaminants in biota is 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, activities carried out in the framework of CBC Project "Assessing the vulnerability of the Black Sea marine ecosystem to human pressures" (ANEMONE) contributed at filling knowledge gaps identified for the region. Thus, new data on chemical contamination (heavy metals, polycyclic aromatic hydrocarbons, organochlorinated pesticides, polychlorinated biphenyls) of marine organisms (mussels, veined rapa whelk, pelagic and demersal fish), collected in 2019 during specific pilot studies in the selected study areas (open sea, and coastal – in front of river mouths, hot-spots) from Ukraine, Romania, Bulgaria and Turkey were obtained. The HELCOM integrated hazardous substances assessment tool (CHASE) developed by NIVA Denmark was tested in the Black Sea with contaminants in biota data set and the overall scores evinced sub-regional differences in the status results, with worse status predominating in the north-western part of the Black Sea (rivers influenced coastal areas and hotspots) and better status in the open sea area and 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, almost half (46%) of biota samples being „unaffected by hazardous substances" state (good and high status), whereas the remaining 54% of biota samples are „affected by hazardous substances" state (bad, poor and moderate).

Key-Words: Black Sea, biota, heavy metals, organic pollutants, CHASE.

AIMS AND BACKGROUND

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 the 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. Background levels are key in establishing attribution of contaminants where multiple sources or contaminant contributors exist. A background level is the concentration of a hazardous substance that provides a reference point that can be used to evaluate whether a release from the site has occurred.

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 environment is under massive anthropogenic pressure caused by pollution with chemical substances and marine litter, overfishing, deterioration of the sea floor (by construction activities, extraction of minerals, bottom trawling), and introduction of noise, (by ships, construction, renewable energy, tourism) (Fliedner *et al.*, 2018), the European Union has adopted the Marine Strategy Framework Directive (MSFD) (2008/56/EC) that aims to the conservation and protection of the EU marine waters.

Descriptor 8 refers to contaminants in water, sediment, or biota which are assessed against threshold values (i.e., values set in accordance with Water Framework Directive (WFD, 2000/60/EC) and its daughter directives. 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, based on the risk assessments, Member States can choose to not consider contaminants and/or to 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).

Seafood monitoring related to human health is different from biota monitoring 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 assessment of compliance with reference values. In contrast, the monitoring of contamination level in seafood for human consumption relies on the edible fraction of a wide variety of commercially relevant species for which the precise origin is often unknown (Swartenbroux *et al*., 2010).

The MSFD, however, requires that the Good Environmental Status (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 conducted by the food safety authorities, which often are different from the environmental institutions implementing the MSFD and its associated monitoring.

Considering that information related to the assessment of hazardous substances in biota is rather limited in the Black Sea region, activities carried out in the framework of CBC Project “Assessing the vulnerability of the Black Sea marine ecosystem to human pressures” (ANEMONE) 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 the Black Sea region. The ultimate objective is to build more harmony, based on the agreed common indicators, and to obtain new information, thus focusing future research efforts toward key domains for the Black Sea region, like the presence of hazardous substances in biota, and impact of human pressures upon to the contamination status and trends.

Pilot investigations were mainly 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 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 the Black Sea region, common relevant species from the most consumed species of demersal and pelagic fish and other seafood (mollusks – *Mytilus galloprovincialis* and *Rapana venosa*) were selected for investigations.

EXPERIMENTAL

Samples of marine organisms (mollusks and fish) were collected in 2019 during specific pilot studies in the selected study areas: open sea (ANEMONE Joint Cruise: ANE-RO-1, ANE-BG-3, ANE-BG-5, ANE-BG-7, ANE-TR-1, ANE-TR-5, ANE-TR-7), and coastal areas (in front of river mouths, hot spots) from Ukraine, Romania, Bulgaria and Turkey (Fig. 1; Table 1).

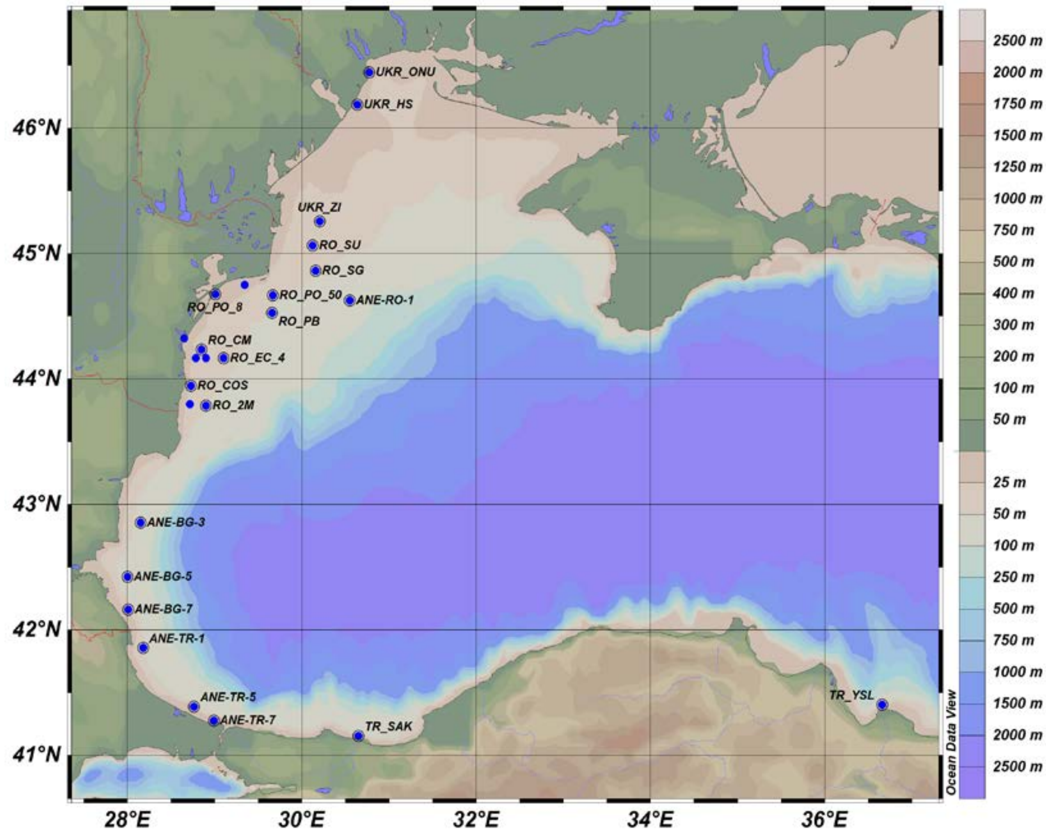

Fig. 1. Sampling stations (UA, RO, BG, TR) for biota (mollusks, fish) contamination studies, 2019

Table 1. Biota samples (mollusks, fish) from Black Sea region investigated for the presence of hazardous substances, 2019

Region	**Station code**	**Species** *(alphabetical order)*	**Date**	**Longitude**	**Latitude**
TR	TR_SAK	*Alosa fallax*	9/10/2019	30.6502	41.1541
RO	RO_PO_8	*Engraulis encrasicolus*	6/22/2019	29.0067	44.6767
RO	RO_PB	*Engraulis encrasicolus*	8/13/2019	28.6490	44.3231
TR	TR_SAK	*Merlangius merlangus euxinus*	9/10/2019	30.6502	41.1541
TR	TR_YSL	*Merlangius merlangus euxinus*	9/12/2019	36.6579	41.4034
TR	ANE-TR-1	*Merlangius merlangus euxinus*	10/5/2019	28.1781	41.8573
RO	RO_COS	*Merlangius merlangus euxinus*	5/19/2019	28.7267	43.9450
TR	TR_SAK	*Mullus barbatus ponticus*	9/10/2019	30.6502	41.1541
TR	TR_YSL	*Mullus barbatus ponticus*	9/12/2019	36.6579	41.4034
RO	RO_EC_4	*Mullus barbatus ponticus*	5/19/2019	29.1025	44.1667
RO	RO_SU	*Mytilus galloprovincialis*	5/11/2019	30.1252	45.0642
RO	RO_SG	*Mytilus galloprovincialis*	5/12/2019	30.1580	44.8603
RO	RO_PO_50	*Mytilus galloprovincialis*	5/13/2019	29.6682	44.6669
RO	RO_PB	*Mytilus galloprovincialis*	5/14/2019	29.6596	44.5270
RO	RO_PO_50	*Mytilus galloprovincialis*	8/1/2019	29.6682	44.6669
RO	RO_CM	*Mytilus galloprovincialis*	8/1/2019	28.8472	44.2347
RO	RO_EC_2	*Mytilus galloprovincialis*	8/1/2019	28.7833	44.1667
RO	RO_EC_3	*Mytilus galloprovincialis*	8/1/2019	28.9000	44.1667
RO	RO_COS	*Mytilus galloprovincialis*	8/1/2019	28.7267	43.9450
RO	RO_MAN	*Mytilus galloprovincialis*	8/1/2019	28.7156	43.7986
RO	ANE-RO-1	*Mytilus galloprovincialis*	10/1/2019	30.5490	44.6253
BG	ANE-BG-3	*Mytilus galloprovincialis*	10/3/2019	28.1496	42.8525
BG	ANE-BG-5	*Mytilus galloprovincialis*	10/4/2019	28.0001	42.4222
BG	ANE-BG-7	*Mytilus galloprovincialis*	10/4/2019	28.0072	42.1601
UKR	UKR_ONU	*Mytilus galloprovincialis*	9/30/2019	30.7746	46.4435
UKR	UKR_ONU	*Mytilus galloprovincialis*	11/16/2019	30.7746	46.4435
TR	TR_SAK	*Mytilus galloprovincialis*	9/10/2019	30.6502	41.1541
TR	ANE-TR-5	*Mytilus galloprovincialis*	10/6/2019	28.7636	41.3864
TR	ANE-TR-7	*Mytilus galloprovincialis*	10/6/2019	28.9883	41.2735
RO	RO_EC_4	*Neogobius melanostomus*	5/19/2019	29.1025	44.1667
UKR	UKR_ZI	*Neogobius melanostomus*	6/28/2019	30.2050	45.2575
UKR	UKR_ONU	*Neogobius melanostomus*	9/29/2019	30.7746	46.4435
RO	RO_2M	*Psetta maxima maeotica*	5/18/2019	28.9000	43.7871
TR	TR_SAK	*Psetta maxima maeotica*	9/10/2019	30.6502	41.1541
TR	TR_YSL	*Psetta maxima maeotica*	9/12/2019	36.6579	41.4034
RO	RO_EC_2	*Rapana venosa*	8/1/2019	28.7833	44.1667
UA	UKR_ZI	*Rapana venosa*	6/28/2019	30.2050	45.2575
UA	UKR_ZI	*Rapana venosa*	6/28/2019	30.2050	45.2575
UA	UKR_HS	*Rapana venosa*	9/14/2019	30.6347	46.1846
UA	UKR_ONU	*Rapana venosa*	11/16/2019	30.7746	46.4435
TR	TR_SAK	*Rapana Venosa*	9/10/2019	30.6502	41.1541
TR	TR_YSL	*Rapana Venosa*	9/12/2019	36.6579	41.4034
TR	TR_YSL	*Solea solea*	9/12/2019	36.6579	41.4034
TR	TR_SAK	*Sprattus sprattus*	9/10/2019	30.6502	41.1541
RO	RO_PO_50	*Sprattus sprattus*	6/21/2019	29.6682	44.6669
RO	RO_PO	*Squalus acanthias*	5/21/2019	29.3453	44.7492
TR	TR_SAK	*Trachurus mediterraneus ponticus*	9/10/2019	30.6502	41.1541
TR	TR_YSL	*Trachurus mediterraneus ponticus*	9/12/2019	36.6579	41.4034
RO	RO_PB	*Trachurus mediterraneus ponticus*	6/13/2019	28.6490	44.3231

In Ukraine biota samples were collected from Zmeiniy Island area (UKR_ZI), a hot-spot area - place of discharge from Chornomorsk WWTP (UKR_HS) and Odessa National Mechnikov University Hydrobiological station /ONU Biostation (UKR_ONU). In Romania biota samples were collected from the Northern area of the coast, under the influence of Danube – Sulina (RO_SU), Sf. Gheorghe (RO_SG), Portita (RO_PO) and Periboina (RO_PB), and also from the Southern part of the coast – Cazino Mamaia (RO_CM), East Constanta (RO_EC), Costinesti (RO_COS), Mangalia (RO_MAN), 2 Mai (RO_2M). In Turkey, biota sampling was carried out at the marine areas close to the river mouths of Sakarya (TR_SAK) and Yeşilırmak (TR_YSL) rivers (Fig. 1; Table 1).

Overall, 49 biota samples from the Black Sea region were investigated for hazardous substances presence (23 samples of pelagic and demersal fish samples, 19 samples of mussels and 7 samples of rapa whelk) (Table 1).

In order to get more comparable results within the Black Sea region, partners agreed on a common set of contaminants (heavy metals, polycyclic aromatic hydrocarbons - PAHs, organochlorinated pesticides - OCPs, polychlorinated biphenyls - PCBs) and selected relevant species (mussels, rapa whelk, pelagic and demersal fish) to be investigated (Table 2).

Table 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	β-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	

To determine contaminants in samples of bivalve mollusks and rapa whelk for analysis, whole soft tissue samples were taken, whereas in fish samples, dorsal muscle tissue was investigated (UNEP, 1990; 1993). Contaminants were analyzed in UkrSCES, NIMRD and TUBITAK laboratories, using the analytical methods summarized in Table 3. Quality assurance and quality control (QA/QC) procedures involved certified reference materials (CRMs).

Table 3. Analytical methods

Contaminant	Analytical method	Pre-treatment method	References
Trace metals	Atomic Absorption Spectrometry (AAS); Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	Acid digestion	IAEA-MEL, 1999; EPA Method 3052; EPA Method 6020 A 2007;
Organic pollutants	Gas Chromatography (GC): Gas Chromatography with Electron Capture Detector (GC-ECD) - for OCPs and PCBs Gas Chromatography – Mass Spectrometry (GC-MS) - for PAHs	Extraction with a mixture of organic solvents, purification, fractionation, and concentration	IAEA-MEL, 1995; UNEP/IOC/IAEA,1996; EPA Method 8082 A; EPA Method 3545 A;

RESULTS AND DISCUSSION

For the assessment of contamination status, the maximum allowable concentrations (MAC) of pollutants were taken from Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, with amendments, Directive 2013/39/EU as regards priority substances in the field of water policy, and national legislation (Klyachko & Belenky, 1988; Order 147/2004; Turkish Food Codex).

Ukraine

The results of pilot studies carried out in Ukraine showed a high level of pollution with toxic metals, in particular arsenic, cadmium and mercury. The maximum concentration of arsenic in rapa whelk, exceeding MAC by almost 30 times, was found in November 2019 in samples collected from ONU Biostation (UKR_ONU).

Increased concentrations were also detected in rapa whelk samples from the area under the influence of wastewater discharge from the city and port Chornomorsk (UKR_HS) and in mussel samples from the UKR_ONU station. One case of exceeding MAC value for cadmium by 1.8 times was recorded in rapa whelk in September 2019, in the area of wastewater discharge from the city and port Chornomorsk (UKR_HS).

Mercury concentrations exceeded MAC by 2-2.5 times in all biota samples (mussel, rapa whelk, fish) (Table 4).

No cases of exceeding MAC for the concentration of hexachlorobenzene in biota samples were recorded. The maximum concentration of heptachlor was found in a fish sample (goby) caught in the area of Zmeinyi Island (UKR_ZI).

High concentrations of heptachlor were also recorded in the sample of rapa whelk and goby from the UKR_ONU station (Table 5).

Sum of 6 PCBs exceeded the MAC only in one rapa whelk sample from station UKR_HS, affected by Chornomorsk WWTP discharge.

No cases of exceeding MAC for the concentration of fluoranthene in biota samples were detected. Concentrations of benzo(a)pyrene exceeded the MAC in only one rapa whelk sample in station UKR_HS and one mussel sample from UKR_ONU biological station (Table 6).

Romania

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

Fig. 2. - Pb and Cd concentrations in *Mytilus galloprovincialis* from Romanian waters, 2019

Table 4. Concentrations of heavy metals (mg/kg ww) in biota samples from Ukrainian pilot studies, 2019

Station	MAC for mussel	Zmeiniy Island (UKR_ZI)	Zmeiniy Island (UKR_ZI)	Place of discharge from WWTP city and port Chornomorsk (UKR_HS)	ONU Biostation (UKR_ONU)	ONU Biostation (UKR_ONU)	ONU Biostation (UKR_ONU)	MAC for fish	ONU Biostation (UKR_ONU)	Zmeiniy Island (UKR_ZI)
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 5. Concentrations of OCPs (µg/kg ww) in biota samples from Ukrainian pilot studies, 2019

Station	MAC for mussel	Zmeiniy Island (UKR_ZI)	Zmeiniy Island (UKR_ZI)	Place of discharge from WWTP city and port Chornomorsk (UKR_HS)	ONU Biostation (UKR_ONU)	ONU Biostation (UKR_ONU)	ONU Biostation (UKR_ONU)	MAC for fish	ONU Biostation (UKR_ONU)	Zmeiniy Island (UKR_ZI)
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
β-HCH		2,42	<0,05	<0,05	<0,05	0,56	8,38		<0,05	<0,05
Lindan		27,7	5,79	5,82	0,10	8,48	5,76		<0,05	1,29
Heptaclor	**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

Table 6. PAHs (µg/kg ww) in biota samples from Ukrainian pilot studies, 2019

Station	**MAC for mussel**	**Zmeiniy Island (UKR_ZI)**	**Zmeiniy Island (UKR_ZI)**	**Place of discharge from WWTP city and port Chornomorsk (UKR_HS)**	**ONU Biostation (UKR_ONU)**	**ONU Biostation (UKR_ONU)**	**ONU Biostation (UKR_ONU)**	**ONU Biostation (UKR_ONU)**	**Zmeiniy Island (UKR_ZI)**
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	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	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

Trace metals bioaccumulation levels in dorsal muscle of pelagic and demersal fish species investigated in 2019 highlighted some interspecific differences, depending of 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 concentrations in most fish samples, with the exception of *Trachurus mediterraneus ponticus* and *Psetta maeotica* (Fig. 3*)*. 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*

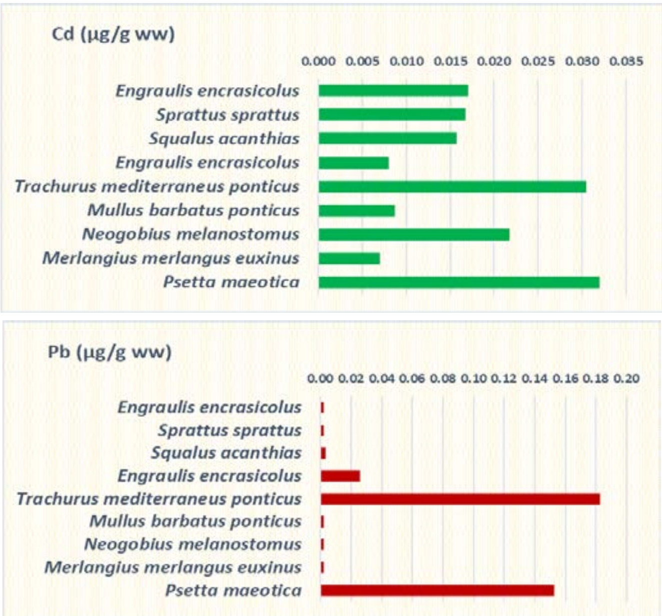

Fig. 3. Cd and Pb concentrations in dorsal muscle of pelagic and demersal fish species from the Romanian waters, 2019

Regarding compliance with maximum admissible concentrations stipulated by EC regulation 1881/2006, and further amendments, Pb concentrations 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 ww 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.

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.

In *Mytilus galloprovincialis,* OCPs concentrations varied in a large range, from detection limit to 335.2 ng/g ww. 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. 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 (Fig. 4).

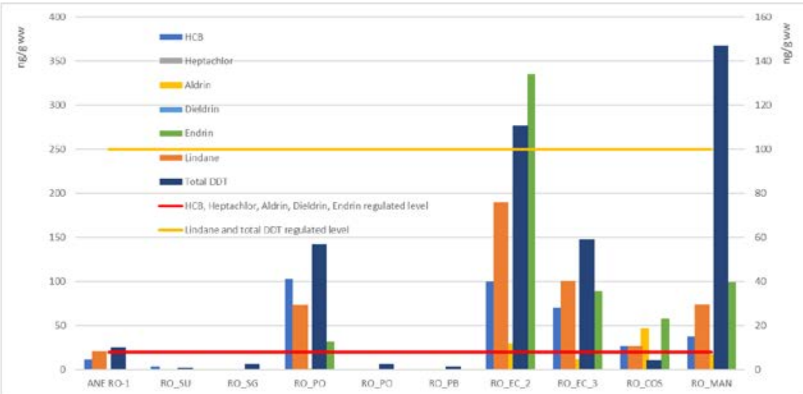

Fig. 4. Organochlorine pesticides concentrations in *Mytilus galloprovincialis* from Romanian waters, 2019 in relation to maximum admissible levels (national legislation Ord. 147/2004)

The sum of 6 PCBs regulated by European legislation (EC regulation 1259/2011) surpassed the maximum admissible level in 80% of the analyzed mussels' samples (Fig. 5).

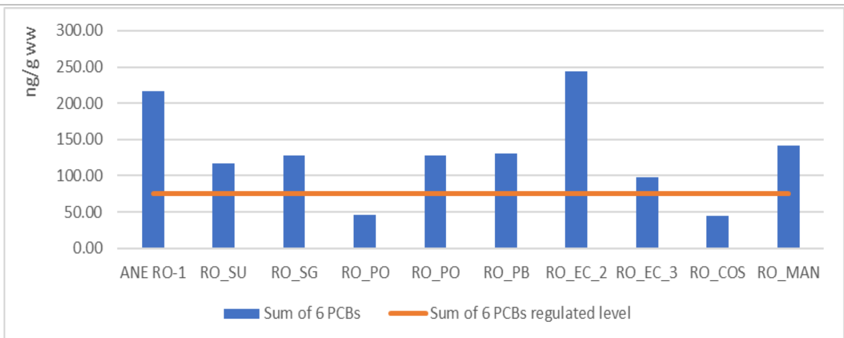

Fig. 5. Polychlorinated biphenyls concentrations in *Mytilus galloprovincialis* from Romanian waters, 2019 in relation to maximum admissible levels (EC regulation 1259/2011)

The concentration of total PAHs in mussels varied from 0.32 to 81.8 ng/g ww. Most of the PAHs analyzed were below detection limit. Higher concentrations of acenaphthylene, acenaphthene, anthracene, and naphthalene were measured in the northern part under the influence of the Danube. Only the sample collected in front of Constanta city and harbor exceeded the maximum admissible level for benzo(a)pyrene stipulated by European legislation.

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. 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 (Fig. 6).

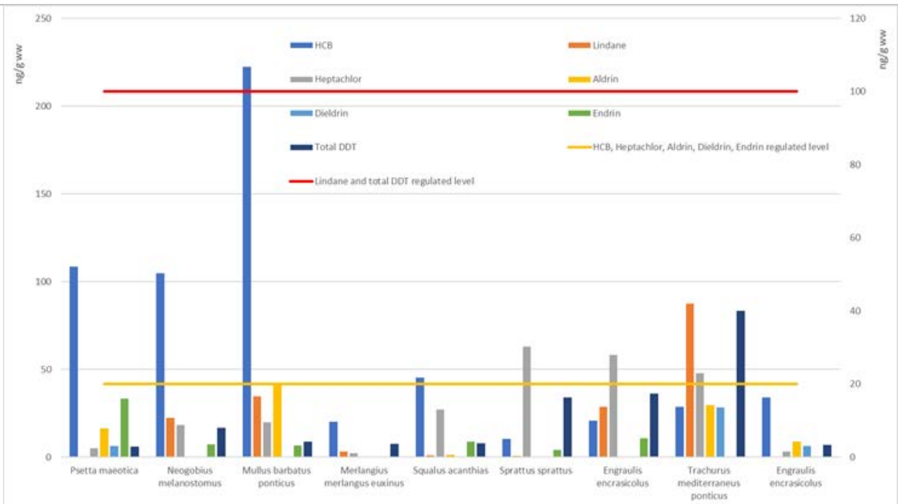

Fig. 6. Organochlorine pesticides concentrations in fish species from Romanian waters, 2019, in relation to maximum admissible levels (national legislation Ord. 147/2004)

The sum of 6 PCBs regulated by European legislation surpassed the maximum admissible level in 89% of the analyzed fish samples.

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

Turkey

The average amount of heavy metals measured in the samples of the fish and mollusk species investigated during Turkish pilot studies are given in Table 7. The values above or close to the maximum levels permitted for human consumption (Turkish Food Codex, EC 1881/2006) are highlighted in bold. Cadmium (Cd) content of the *Rapana venosa* samples (av. 2.728 µg/g ww) collected from Sakarya river impact area was the only one 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.

In general, higher levels of metals were detected in mollusks than in edible fish tissues and varied according to the species.

Table 7. Heavy metal concentrations in biota from the areas of river impact

Sakarya River (µg/g ww) (mean) (TR_SAK)									
Species	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**Hg**
Mullus barbatus	1.473	< 0.00001	0.056	0.153	0.425	0.270	0.076	6.736	0.037
Alosa fallax	1,152	< 0.00001	0.007	0.076	0.950	0.040	0.050	5.700	0.019
Merlangius merlangus euxinus	0.841	< 0.00001	0.022	0.128	0.320	0.143	0.039	4.130	0.008
Trachurus mediterraneus	0.755	< 0.00001	0.015	0.078	0.591	0.131	0.036	8.753	0.010
Sprattus sprattus	1.114	0.008	0.033	0.204	0.503	0.205	0.045	11.34	-
Psetta maxima	1.261	< 0.00001	0.010	0.095	0.160	0.079	0.027	8.287	0.014
Mytilus galloprovincialis	2.879	0.459	0.436	1.182	1.214	1.714	**0.28**	43.98	-
Rapana Venosa	7.077	**2.728**	0.081	0.324	16.73	0.365	0.124	28.56	0.020
Yesilirmak River (µg/g ww) (mean) (TR_YSL)									
Species	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**Hg**
Mullus barbatus	1.679	< 0.00001	0.025	0.067	0.369	0.086	0.034	5.778	0.112
Trachurus mediterraneus	0.934	< 0.00001	0.009	0.053	0.766	0.056	0.033	8.553	0.048
Merlangius merlangus euxinus	0.806	< 0.00001	0.009	0.057	0.269	0.055	0.032	3.756	-
Psetta maxima	1.839	< 0.00001	0.004	0.059	0.215	0.058	0.026	5.866	0.039
Solea solea	0.886	< 0.00001	0.011	0.066	0.427	0.077	0.037	10.73	-
Rapana venosa	5.494	0.103	0.012	0.152	15.16	0.274	0.051	13.85	0.033

Higher Pb, Hg and Ni contents were detected in the *Mullus barbatus* (Mb) then the other fish species (Fig. 7). *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 in terms of Cd contents. Hg, Ni and Pb contents 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 Yeșilırmak impact area were found higher than those obtained from Sakarya impact area.

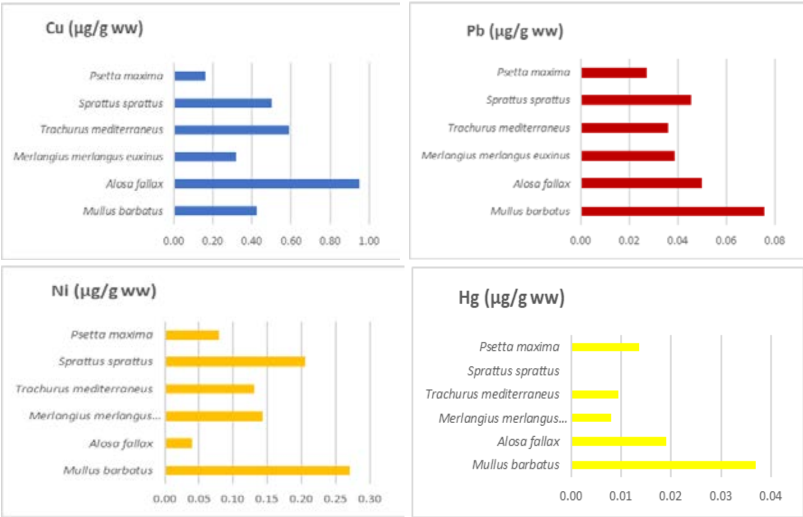

Fig. 7. Heavy metals concentrations in muscle tissue of fish species from the Sakarya River, 2019

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.

As a marker compound of carcinogenic PAHs, the concentrations of benzo[a]pyrene in marine organisms were detected below the acceptable limits of the Turkish Food Codex (5.0 µg/kg ww for bivalve mollusks, 2.0 µg/kg ww for fish meat) (Fig. 8).

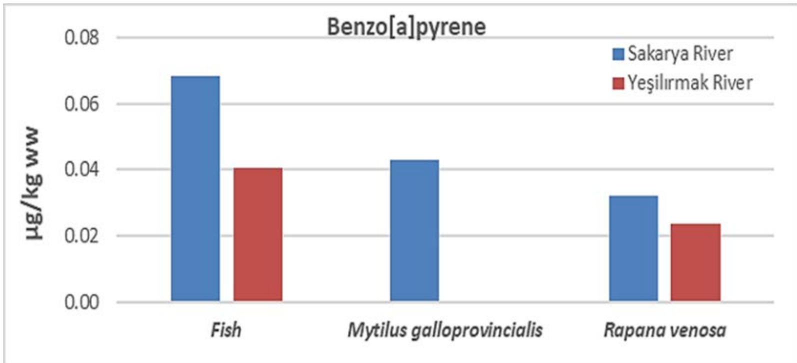

Fig. 8. Average benzo[a]pyrene concentrations in biota samples affected by Sakarya and Yeşilırmak Rivers

Similarly, none of the mollusk and fish samples were found to have any level of PCBs that could pose a risk to human health (Fig. 9). 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 in both Sakarya and Yesilırmak river sea impact areas (Fig. 10). Other pesticides investigated were either low levels or below the detection limit.

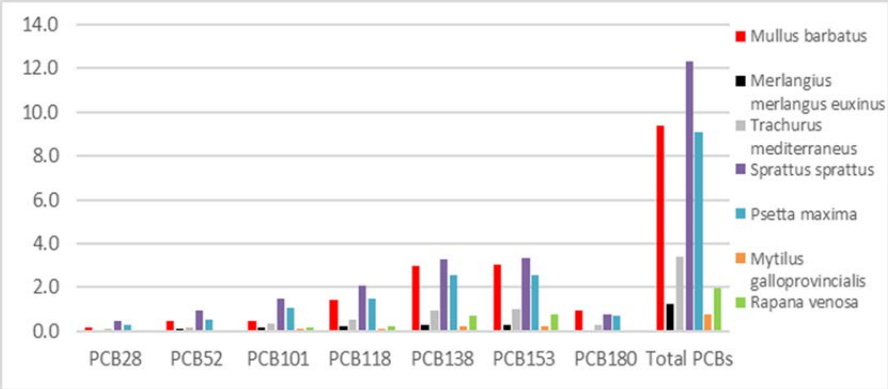

Fig. 9. PCB distributions of Sakarya biota samples

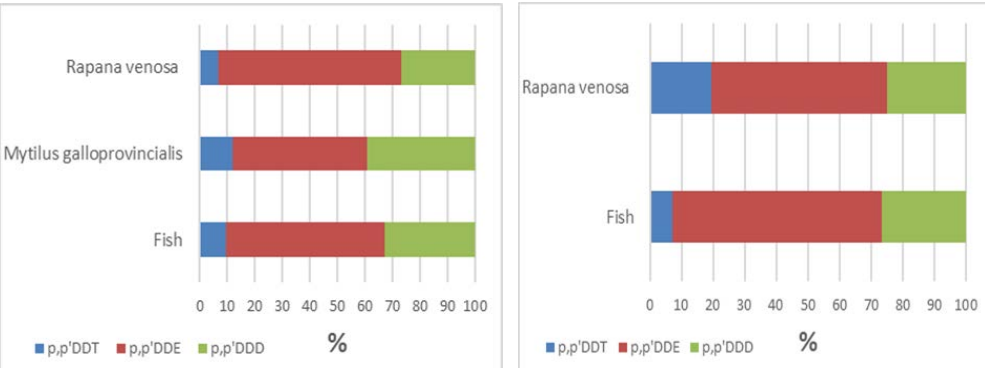

Fig. 10. Distribution of DDT and its derivatives in Sakarya (left) and Yeşilırmak (right) river

Integrated assessment of biota contaminants data

The HELCOM Chemical Status Assessment Tool (CHASE) (Andersen *et al*., 2016) integrates data on hazardous substances in water, sediments and/or biota, as well as bio-effect indicators, depending on data available for each category. It is based on the calculation of a 'contamination ratio' (CR), being the ratio between an observed concentration and a threshold value. When the observed value exceeds the threshold value, the resulting contamination ratio will be greater than 1.0, and if it is below the threshold value, the contamination ratio will be 1.0 or less.

These ratios are combined within matrices, i.e., for water, sediment and/or biota and for biological effects, meaning that an aggregated contamination score (CS) is calculated separately for each matrix category. If the aggregated contamination score (CS) is less than 1.0 within one matrix, the status for that individual matrix is determined to be good (matrix status). If above 1.0 that matrix is classified as not good. This is reflected as a ‘low’ or ‘high’ respective contamination status.

The low contamination status class is further subdivided into two categories, and the high contamination status class is subdivided into three categories, based on the value of the aggregated contamination score. The five categories (high, good, moderate, poor, bad) give a coarse estimate of how far the obtained result is from the ‘target’ and can help distinguish an area with a very high contamination score from an area with a score closer to 1.

The overall status assessment result is determined by the “One-out-all-out” approach, so that the matrix category with the worst status of the investigated categories (water, sediment and/or biota) determines the overall status for an individual assessment unit (Andersen *et al*., 2016). The CHASE tool can be used in remedial action plans, for the science-based evaluation of the status and for determining which specific substances are responsible for a status as potentially affected.

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 more reliable results, it is possible to use available data from only one matrix.

Thus, in ANEMONE project, the CHASE tool was preliminary tested in the Black Sea region using contaminants in biota data set, 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.

The CHASE 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 8; Table 9). 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 (Fig. 11 – 14). Across the investigated biota samples, the CHASE test assessment showed a range of status results from bad to high, most of them (54 %) being in the „affected by hazardous substances” state (bad, poor and moderate), whereas the remaining 46% of biota samples are „unaffected by hazardous substances” state (good and high status) (Fig. 15).

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).

Table 8. Status by station following application of CHASE on ANEMONE biota contaminants data

Region	Station code	Species/Matrix	CHASE Matrix Score	CHASE OVERALL Score
UA	UKR_HS	Rapana	4	4
UA	UKR_ONU	Fish	5	5
UA	UKR_ONU	Rapana	5	
UA	UKR_ONU	Mussel	5	
UA	UKR_ZI	Fish	5	5
UA	UKR_ZI	Rapana	3	
RO	RO_2M	Fish	3	3
RO	RO_CM	Mussel	3	3
RO	RO_COS	Fish	2	3
RO	RO_COS	Mussel	3	
RO	RO_EC_2	Rapana	2	4
RO	RO_EC_2	Mussel	5	
RO	RO_EC_3	Mussel	3	3
RO	RO_EC_4	Fish	4	4
RO	RO_PB	Fish	3	3
RO	RO_MAN	Mussel	3	3
RO	RO_PB	Mussel	2	2
RO	RO_PO_50	Fish	3	3
RO	RO_PO_50	Mussel	3	
RO	RO_PO_8	Fish	3	3
RO	RO_SG	Mussel	3	3
RO	RO_SU	Mussel	3	3
RO	RO_PO	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	TR_SAK	Rapana	3	
TR	TR_SAK	Mussel	1	
TR	TR_YSL	Fish	1	1
TR	TR_SAK	Rapana	1	

**** Legend - CHASE scores***

1	**2**	**3**	**4**	**5**
High	Good	Moderate	Poor	Bad

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%), heptachlor (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 (Fig. 16).

Table 9. Status by region following application of CHASE on ANEMONE biota contaminants data and number of investigated species

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	

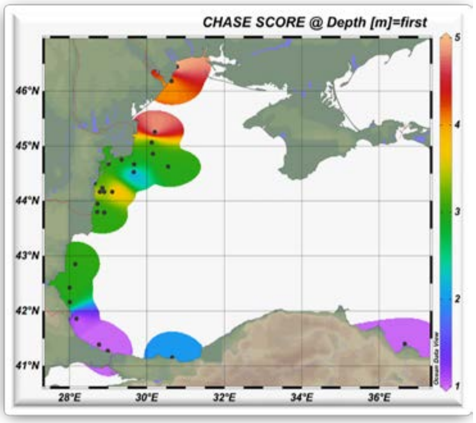

Fig. 11. Overall status following application of CHASE on ANEMONE biota (mussels, Rapana and fish) contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

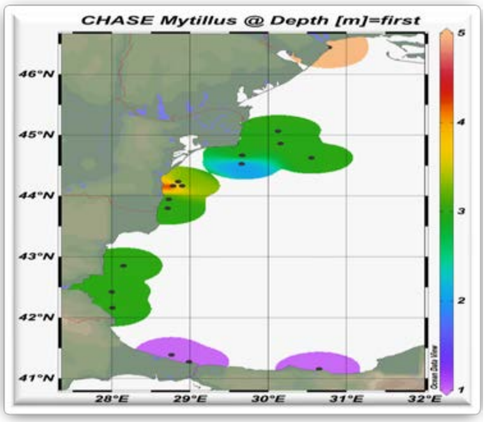

Fig. 12. *Mytilus galloprovincialis* status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

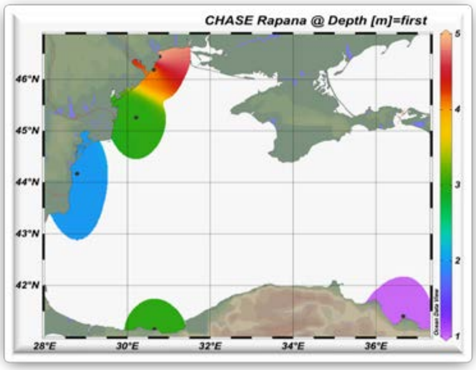

Fig. 13. *Rapana venosa* status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

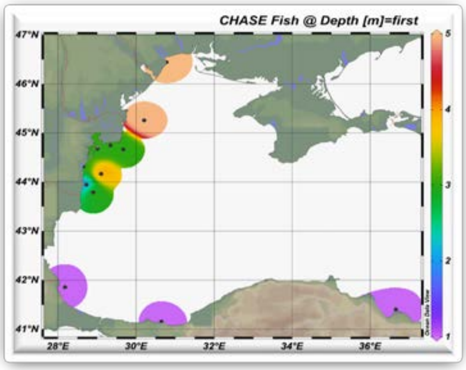

Fig. 14. Fish status following application of CHASE on ANEMONE contaminants data (1-High; 2-Good; 3-Moderate; 4-Poor; 5-Bad)

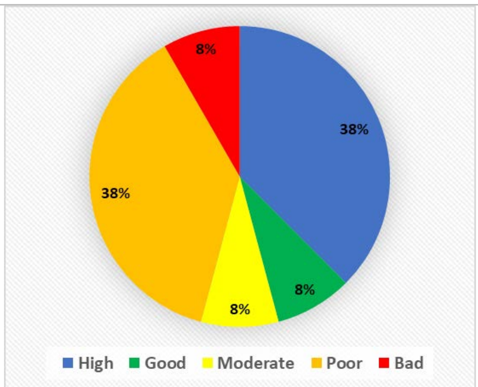

Fig. 15. CHASE status classification of biota samples based on hazardous substances bioaccumulation levels

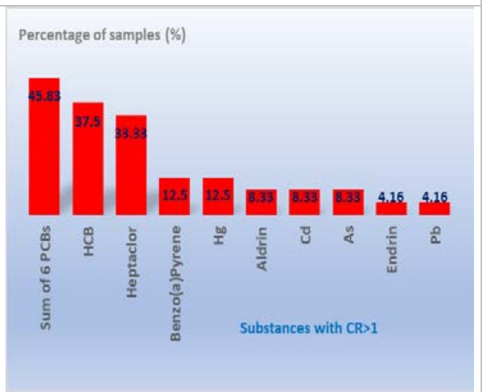

Fig. 16. Frequency of occurrence of hazardous substances with the highest contamination ratios (CR > 1)

CONCLUSIONS

This study 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.

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, most of them (54%) being in the „affected by hazardous substances” state (bad, poor and moderate), whereas the remaining 46% of biota samples are „unaffected by hazardous substances” state (good and high status).

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.

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.

Acknowledgment. This study has been supported by the ANEMONE project (funded by the EU under ENI CBC Black Sea basin Program 2014-2020, grant contract 83530). We would like to express our very great appreciation to Dr. Ciaran Joseph Murray, NIVA Denmark, for his valuable guidance and constructive suggestions for testing the Contaminants Status Assessment Tool (CHASE) in the Black Sea region. His willingness to give his time so generously has been very much appreciated.

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