Assessment of the Contaminants in Biota from the Western Black Sea Basin in respect with MSFD Requirements in the frame of the MISIS Project *(Valentina Coatu, Andra Oros, Daniela Ţigănuş, Galina Shtereva†, Levent Bat)*	"Cercetări Marine" Issue no. 46 Pages 82-97	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

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ABSTRACT

Within the Joint MISIS cruise biota samples were taken from Romanian, Bulgarian and Turkish transects in order to evaluate the presence of hazardous substances in respect to Marine Strategy Framework Directive criteria and indicators under Descriptor 8 and Descriptor 9.

The results for polycyclic aromatic hydrocarbons allow the classification of the biota samples as minimally and moderately contaminated. Except p, p' DDT and its metabolites organochlorine pesticides concentrations are below detection limits. The polychlorinated biphenyls levels may not pose a significant risk for the ecosystem or human health. Heavy metals assessed with respect to their human health risk were below the maximum admissible limits.

Keywords: *Black Sea, hazardous substances, biota, MSFD*

AIMS

The aim of the paper is to evaluate the status of the hazardous substances in biota from the western part of Black Sea (Romanian, Bulgarian and Turkish waters) in the light of Descriptor 8 and 9 of the MSFD, taking into account relevant existing environmental targets.

BACKGROUND

Within the scope of the Marine Strategy Framework Directive, Member States of one marine region and neighbouring countries which share the same marine waters, have to cooperate in order to protect more effectively the marine environment. A joint cruise of the western countries of the Black Sea was organized in the frame of MISIS project in July 2013 in order to improve availability and quality of data to provide for integrated assessments of the Black Sea state of environment.

Presence of hazardous substances in biota represent relevant criteria and indicators for assessing the status of the of the Black Sea environment under Descriptor 8 ("Concentrations of contaminants are at levels not giving rise to pollution effects") and 9 ("Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards").

MATERIALS AND METHODS

Thirteen samples of molluscs were collected - *Mytilus galloprovincialis, Rapana venosa* and *Scapharca inequivalvis* (4 from the Romanian transect, 6 from the Bulgarian transect, and 3 from the Turkish transect) (Table 1). The dredge was launched in order to collect biota samples for pollutants. One sample consisted of 10 – 15 individuals from the same species, shell length measured, whole soft tissue being separated on board in clean conditions, wrapped, frozen and subsequently analysed in the NIMRD laboratory for heavy metals and POPs.

Table 1. Biota sampling for pollutants

Station	**Start**		**End**		**Dredge length (M)**
	Latitude (° ')	**Longitude** (° ')	**Latitude** (° ')	**Longitude** (° ')	
M01-M02	44°10.125'N	028°48.973'E	44°10.121'N	028°49.349'E	500
M04-M05	44°10.103'N	029°40.399'E	44°10.099'N	029°40.756'E	490
M04-M05	44°10.096'N	029°41.903'E	44°10.101'N	029°42.918'E	1350
M12-M11	43°09.999'N	028°10.026'E	43°09.999'N	028°10.026'E	440
M12-M11	43°10.005'N	028°10.922'E	43°10.013'N	028°11.892'E	1300
M12-M11	43°10.058'N	028°16.684'E	43°10.074'N	028°17.349'E	895
M11-M10	43°15.329'N	028°18.425'E	43°15.470'N	028°18.771'E	535
M10-M09	43°08.790'N	028°27.147'E	43°09.066'N	028°27.110'E	512

M18	41°50.763'N	028°01.808'E	41°50.558'N	028°02.046'E	500
M18	41°49.810'N	028°02.540'E	41°50.234'N	028°02.191'E	920
M17	41°52.495'N	028°07.455'E	41°52.999'N	028°07.689'E	980

Metal analyses were performed according to IAEA-MEL[1]. The biota samples were freeze-dried, digested with nitric acid in Teflon vessels on hot plate,. The accuracy and precision of the analytical methodology was verified with the standard reference material SRM 2976 which was provided by the National Institute of Standards and Technology-USA (NIST).

For PCBs/OCPs and PAHs analysis the frozen mussel tissues were freeze-dried and homogenized. About 2 g of the dried tissue were used for analysis. Internal standard 2,4,5 TCB respectively 9,10 dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. Extraction of OCPs, PCBs and PAHs from biota samples was done with hexane/methanol, in Soxhlet apparatus. Further processing of the samples, followed the steps: concentration of the extracts to rotoevaporator, treatment of samples with concentrated sulphuric acid/0.7 M KOH to remove the lipids, clean-up on florisil, respectively, alumina/silica column and concentration of the samples using Kuderna- Denish concentrator and nitrogen flow. Analytical determination of the compounds was made by gas-chromatographic method with a Perkin Elmer gas chromatograph CLARUS 500 equipped with electron capture detector for OCPs and PCBs and mass spectrometer for PAHs[2].

RESULTS AND DISCUTIONS

In order to allow comparison with assessment criteria, it is necessary to choose the bases on which all concentrations must be expressed in the scope of each descriptor: dry weights for organochlorines and PAHs in soft body tissues in the scope of Descriptor 8 and wet weights for metals, organochlorines and PAHs in soft body tissues in the scope of Descriptor 9.

PAHs levels and composition

Total concentrations of the priority PAHs (sum of the 16 EPA priority pollutants) in the molluscs varied from 308.7 to 3756.9 µg/kg dw (Table 2), respectively 61.7 to 641.5 µg/kg ww (Table 3). This results are comparable to that obtained by Karacık[3] in the mussels of Istanbul Strait (215-3005 µg/kg dw, respectively 43.0 - 601.0 µg/kg ww).

According to Varanasi[4], the seafood can be classified into four categories, depending on the total content of PAHs: not contaminated (ΣPAHs < 50 µg/kg dw), minimally contaminated (ΣPAHs from 50 to 495 µg/kg), moderately contaminated (ΣPAHs from 500 to 5000 µg/kg dw) and highly contaminated (Σ PAHs > 5000 µg/kg dw). The results (Table 2) allow the classification of the biota samples as minimally (31%) and moderately contaminated (69 %).

The following individual compounds are dominant in the three molluscs species: phenanthrene, naphthalene, fluorene, benzo[a]pyrene, benzo[a]anthracene and dibenzo(a,h)anthracene. Petroleum PAHs (LMW) have been observed to be more readily accumulated by organisms than PAHs generated by combustion of organic matter (HMW)[5]. The results are in agreement with previous findings which show that the molluscs are enriched in low molecular weight PAHs relative to higher molecular weight PAHs (Table 2). Scatterplot of phenanthren against the total $\sum_{16}$PAHs (µg/kg dw) in bivalve shows a good correlation (Fig. 1).

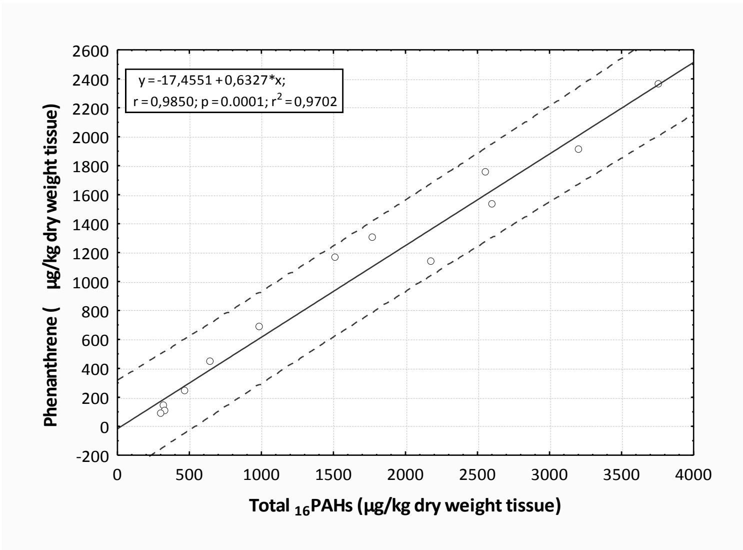

Figure 1. Scatterplot of phenanthrene against Total $\sum_{16}$PAHs (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013

According to Webster[6], PAH concentrations >250 µg/kg ww tissue are likely to be indicative of a more severe (e.g. emergency) or long term chronic exposure. Mussel tissue PAH concentrations in the range 150–250 µg/kg ww may indicate an acute exposure or low-level chronic exposure to PAH contaminants. PAH concentrations between 50 and 150 µg/kg ww can be considered as background for pre-spawning mussels and concentrations < 50 µg/kg ww is considered as typical background/reference values for post-spawning mussels[7]).

Table 2. Concentrations of individual (µg/kg dry weight tissue), total PAHs ($\sum_{16}$PAHs) (µg/kg dry weight tissue) and LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings), in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station -M*	Nap**	Acl	Ac	Fl	Phe	An	Fa	Py	B[a]a	Chry	B[b]fl	B[k]fl	B[a]pyr	B(g,h,i)p	D(a,h)a	Ip	$\sum_{16}$PAHs	LMW / HMW
Mytilus BG(11-10)	48.6	6.4	8.3	34.8	85.9	26.4	11.8	9.7	7.7	5.9	2.4	8.7	16.8	8.1	8.5	18.8	**308.7**	2.14
Mytilus RO(01-02)	13.9	6.7	8.7	30	144.4	19	5.1	8.2	15.1	4.2	4.8	5.4	36.7	6.2	13.6	2.8	**325.0**	2.18
Rapana RO(01-02)	24.9	5.4	6	31.1	102.6	24.2	8.2	13.8	11.4	7.4	1.1	7.1	33	14.2	22	15.7	**328.0**	1.45
Rapana TR(18-17)	14.8	5.2	7,3	13.5	241.4	15.7	7.6	8.7	14.9	10	7.2	14.1	32.9	21.7	40.5	19.9	**475.4**	1.68
Mytilus BG(12-11)	62.2	5.7	6.1	36.3	446.4	23.1	6.8	12.5	5	3.6	4.4	6.4	7.2	8.9	6.6	8.5	**649.6**	8.29
Mytilus BG(10-09)	107.4	5.1	11.5	62.9	687.9	37.3	4.6	9.6	8.9	3.1	5.4	8.3	11.3	5.7	16.5	3.6	**989,1**	11.86
Mytilus TR(17)	50.2	5	10.5	54,1	1165.1	42.3	14.3	11.4	15.3	11.2	4.9	13.2	71.0	15.6	24.1	10.2	**1518.7**	6.93
Scapharca RO(01-02)	90.8	8.3	11.3	160.4	1297.9	28.4	19.7	21.7	16.2	5.6	16.7	17	35.2	8.8	27.3	12.6	**1778.1**	8.83
Rapana BG(11-10)	707.6	18.3	21.6	222.1	1137.5	15.1	10	10.6	9.2	3.4	2,3	4.9	4.8	7.4	5.1	4.9	**2184.5**	33.99
Scapharca BG(12-11)	497.4	12.6	10.3	167.5	1751.4	39.3	18.3	18.7	5.3	3.3	4.6	7.9	9.5	4.5	4.8	5.9	**2561.2**	29.99
Mytilus RO(04-05)	633.9	23.8	27.6	279.4	1532.0		9.1	14.5	5	5.2	6.3	13.3	17.8	6.3	15.6	12.6	**2602.4**	23.63
Rapana BG(12-11)	896.5	18.4	13.7	257.6	1905.4	26.9	17.5	13.4	7.1	4.4	2.2	7.1	6.0	7.1	20.1	4.2	**3207.8**	34.96
Scapharca TR(18-17)	788.0	11	15.7	119.4	2363.9	8.8	18.2	17.9	92.6	9.3	9.1	21.5	222.9	16.6	21.6	20.3	**3756.9**	7.35
EAC***(µg/kg)	340				1700	290	110	100	80				**600**	110				

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 - M 17).

** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, 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

*** **EAC -** Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species

Table 3. Concentrations of individual (µg/kg wet weight) and total PAHs ($\sum_{16}$PAHs) (µg/kg wet weight) in biota with relevance for human consumption from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station -M*	Nap**	Acl	Ac	Fl	Phe	An	**Fa**	Py	B[a]a	Chry	B[b]fl	B[k]fl	B[a]pyr	B(g,h,i)p	D(a,h)a	Ip	$\sum_{16}$PAHs
Mytilus BG(11-10)	9.7	1.3	1.7	7.0	17.2	5.3	2.4	1.9	1.5	1.2	0.5	1.7	3.4	1.6	1.7	3.8	**61.7**
Mytilus RO(1-2)	2.8	1.3	1.7	6.0	28.9	3.8	1.0	1.6	3.0	0.8	1.0	1.1	7.3	1.2	2.7	0.6	**65.0**
Rapana RO(1-2)	5.0	1.1	1.2	6.2	20.5	4.8	1.6	2.8	2.3	1.5	0.2	1.4	6.6	2.8	4.4	3.1	**65.6**
Rapana TR(18-17)	3.0	1.0	1.5	2.7	48.3	3.1	1.5	1.7	3.0	2.0	1.4	2.8	6.6	4.3	8.1	4.0	**95.1**
Mytilus BG(12-11)	12.4	1.1	1.2	7.3	89.3	4.6	1.4	2.5	1.0	0.7	0.9	1.3	1.4	1.8	1.3	1.7	**129.9**
Mytilus BG(10-9)	21.5	1.0	2.3	12.6	137,6	7.5	0.9	1.9	1.8	0.6	1.1	1.7	2.3	1.1	3.3	0.7	**197.8**
Mytilus TR(17-16))	10.0	1.0	2.1	10.8	233.0	8.5	2.9	2.3	3.1	2.2	1.0	2.6	14.2	3.1	4.8	2.0	**303.7**
Rapana BG(11-10)	141.5	3.7	4.3	44.4	227.5	3.0	2.0	2.1	1.8	0.7	0.5	1.0	1.0	1.5	1.0	1.0	**436.9**
Mytilus RO(4-5)	126.8	4.8	5.5	55.9	306.4	0.0	1.8	2.9	1.0	1.0	1.3	2.7	3.6	1.3	3.1	2.5	**520.5**
Rapana BG(12-11)	179.3	3.7	2.7	51.5	381.1	5.4	3.5	2.7	1.4	0.9	0.4	1.4	1.2	1.4	4.0	0.8	**641.6**
EC regulatory level***	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 - M 17).
** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, 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
*** maximum admissible levels for human consumption according to Commission Regulation (EC) no. 1881/2006

Of the ten samples analysed, four showed $\sum_{16}$PAHs greater than 250 µg/kg ww and six samples contained between 50 and 150 µg/kg ww PAH. There was no significant difference ($p>0.05$) between the means of the total PAH concentrations (µg/kg ww) determined in the two species sampled of *Mytilus galloprovincialis* (213 ± 175.9) and *Rapana venosa* (309.8 ± 278.0).

Organochlorine Pesticides and Polychlorinated Biphenyls

Total concentrations of the organochlorine pesticides in the tissues of the three collected species varied from 4.87 to 52.91 µg/kg dw (Table 4). Total concentrations of the polychlorinated biphenyls in the tissues of the three collected species varied from 2.86 to 61.08 µg/kg dw (Table 5). *Mytilus galloprovincialis* and *Scapharca ineqiuvalvis* accumulated the higher quantity of OCPs and PCBs, probably because of their filter-feeding system.

Table 4. Concentrations of individual and total OCPs (∑OCPs) (µg/kg dry weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station -M*	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	Total OCPs
Rapana RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	10.14	6.11	7.00	25.44
Rapana BG(10-11)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	10.01	0.20	3.10	15.51
Rapana BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	<0.20	<0.20	2.60	5.20
Rapana TR(17-18)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	2.17	0.20	<0.30	4.87
Mytilus RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	7.26	43.15	<0.30	52.91
Mytilus RO(04-05)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	17.84	7.79	7.60	35.43
Mytilus BG(09-10)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	9.57	<0.20	4.66	16.63
Mytilus BG(10-11)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	<0.20	15.82	9.76	27.98
Mytilus BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	22.28	0.20	5.55	30.23
Mytilus TR(16 -17)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	7.75	4.64	<0.30	14.89
Scapharca RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	13.77	<0.20	14.21	30.38
Scapharca BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	14.72	<0.20	<0.30	17.42
Scapharca TR(17-18)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	12.52	<0.20	<0.30	15.22

*13 samples of molluscs - *Mytilus, Rapana* and *Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 - M 17)

Table 5. Concentrations of individual and total PCBs (∑PCBs) (µg/kg dry weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station-M*	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	Total PCBs
Rapana RO(01-02)	<0.40	<0.30	<0.60	0.15	2.98	5.28	0.95	10.66
Rapana BG(10-11)	<0.40	<0.30	<0.60	<0.40	3.38	1.21	<0.30	6.59
Rapana BG(11-12)	<0.40	<0.30	<0.60	<0.40	2.98	1.01	16.49	22.18
Rapana TR(17-18)	<0.40	<0.30	<0.60	<0.40	0.16	<0.70	<0.30	2.86
Mytilus RO(01-02)	<0.40	9.74	<0.60	1.23	4.97	<0.70	<0.30	17.95
Mytilus RO(04-05)	<0.40	27.56	<0.60	1.34	2.28	5.03	<0.30	37.51
Mytilus BG(09-10)	<0.40	<0.30	<0.60	<0.40	0.73	1.82	<0.30	4.55
Mytilus BG(10-11)	<0.40	<0.30	<0.60	<0.40	0.89	3.80	<0.30	6.69
Mytilus BG(11-12)	<0.40	<0.30	<0.60	<0.40	1.74	2.16	<0.30	5.91
Mytilus TR(16 -17)	<0.40	<0.30	<0.60	<0.40	<0.60	<0.70	<0.30	3.30
Scapharca RO(01-02)	<0.40	48.40	<0.60	1.93	0.72	5.53	3.49	61.08
Scapharca BG(11-12)	<0.40	4.38	<0.60	<0.40	<0.60	<0.70	<0.30	7.38
Scapharca TR(17-18)	<0.40	31.52	<0.60	<0.40	<0.60	<0.70	<0.30	34.52
EAC**(µg/kg)	1.7	2.7	3	0.6	40	7.9	24	

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 – M 17).
** **EAC-** Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species

The major OCPs compounds are p, p' DDE, p, p' DDD and p, p' DDT. The other investigated pesticides were under detection limit (Fig. 2). The concentration detected for p, p' DDT and its metabolites are comparable with other results obtained for Black Sea mussels and previous data obtained in the Romanian Black Sea waters[8,9,10,11]. Except PCB 28 and PCB 101 that were under detection limit in all samples, the other PCBs were detected in concentrations between detection limits and 48.40 µg/kg dw. Higher levels were measured for PCB 138, PCB 153 and PCB 180 and especially PCB 52 in some samples (Fig. 3).

The organochlorine pesticides and polychlorinated biphenyls concentrations determined in the tissues of the two species with relevance for human consumption (*Mytilus galloprovincialis* and *Rapana venosa)* are presented in Table 6 and Table 7, where ∑OCPs (µg/kg ww tissue) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p' DDD and p,p' DDT) and ∑PCBs (µg/kg wet weight tissue) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

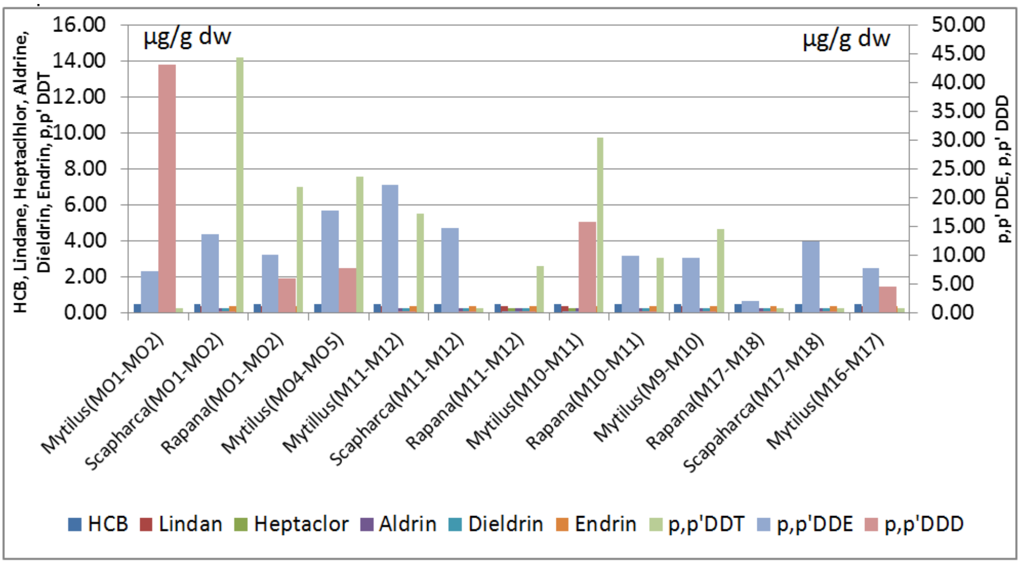

Fig. 2. Individual OCPs levels (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013

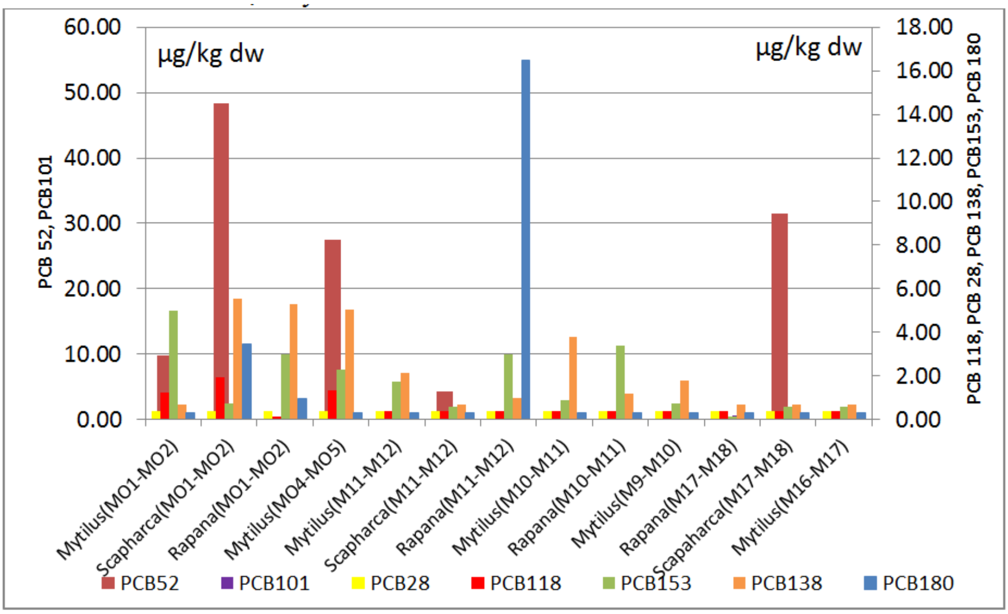

Fig. 3. Individual PCBs levels (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013

The organochlorine pesticides and polychlorinated biphenyls concentrations determined in the tissues of the two species with relevance for human consumption (*Mytilus galloprovincialis* and *Rapana venosa)* are presented in Table 6 and Table 7, where ∑OCPs (µg/kg ww tissue) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p' DDD and p,p' DDT) and ∑PCBs (µg/kg wet weight tissue) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

Table 6. Concentrations of individual and total OCPs (∑OCPs) (µg/kg wet weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station -M*	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	Total OCPs
Rapana RO(01-02)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	2.535	1.528	1.750	6.360
Rapana BG(10-11)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	2.503	<0.050	0.775	3.878
Rapana BG(11-12)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	<0.050	<0.050	0.650	1.300
Rapana TR(17-18)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	0.543	0.050	<0.075	1.218
Mytilus RO(01-02)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.090	6.479	<0.045	7.944
Mytilus RO(04-05)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	2.679	1.170	1.141	5.320
Mytilus BG(09-10)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.437	<0.030	0.700	2.497
Mytilus BG(10-11)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	0.030	2.375	1.465	4.201
Mytilus BG(11-12)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	3.345	0.030	0.833	4.539
Mytilus TR(16 -17)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.164	0.697	<0.045	2.236

*10 samples of molluscs – *Mytilus and Rapana*: 3 from the Romanian transect (M 01-M 05), 5 from the Bulgarian transect (M12-M 09), and 2 from the Turkish transect (M 18 - M 17)

Table 7. Concentrations of individual and total PCBs (∑PCBs) (µg/kg wet weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013

Species/ Sampling station -M*	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	Total PCBs	Total six PCBs (except PCB118)
Rapana RO(01-02)	<0.100	<0.075	<0.150	0.038	0.745	1.320	0.238	2.628	2.59
Rapana BG(10-11)	<0.100	<0.075	<0.150	<0.100	0.845	0.303	<0.075	1.548	1.448
Rapana BG(11-12)	<0.100	<0.075	<0.150	<0.100	0.745	0.253	4.123	5.445	5.345
Rapana TR(17-18)	<0.100	<0.075	<0.150	<0.100	0.040	<0.175	<0.075	0.615	0.515
Mytilus RO(01-02)	<0.060	1.462	<0.090	0.308	0.746	<0.105	<0.045	2.509	2.201
Mytilus RO(04-05)	<0.060	4.138	<0.090	0.335	0.342	0.755	<0.045	5.431	5.096
Mytilus BG(09-10)	<0.060	<0.045	<0.090	<0.060	0.110	0.273	<0.045	0.623	0.563
Mytilus BG(10-11)	<0.060	<0.045	<0.090	<0.060	0.134	0.571	<0.045	0.944	0.884
Mytilus BG(11-12)	<0.060	<0.045	<0.090	<0.060	0.261	0.324	<0.045	0.826	0.766
Mytilus TR(16 -17)	<0.060	<0.045	<0.090	<0.060	<0.090	<0.105	<0.045	0.435	0.375
EC regulatory level**	-	-	-	-	-	-	-	-	75

*10 samples of molluscs – *Mytilus and Rapana*: 3 from the Romanian transect (M 01-M 05), 5 from the Bulgarian transect (M12-M 09), and 2 from the Turkish transect (M 18 – M 17)

** maximum admissible levels for human consumption according to Commission Regulation (EC) no. 1259/2011

Heavy metals

Heavy metals concentrations determined in the whole soft tissue of the molluscs species investigated in July 2013 registered the following averages and variation ranges (reported as wet weight tissue) (Table 8):

- *Mytilus galloprovincialis*: 2.04 (1.13 - 4.48) µg/g Cu; 0.68 (0.21 - 1.12) µg/g Cd; 0.104 (0.002 - 0.216) µg/g Pb; 2.48 (0.97 - 6.00) µg/g Ni; 0.34 (0.07 - 1.31) µg/g Cr;

- *Rapana venosa*: 7.32 (4.76-9.35) µg/g Cu; 4.10 (1.23 - 5.42) µg/g Cd; 0.124 (0.019 - 0.254) µg/g Pb; 0.64 (0.33 - 0.92) µg/g Ni; 0.30 (0.14 - 0.57) µg/g Cr;
- *Scapharca inequivalvis*: 2.03 (1.93 - 2.15) µg/g Cu; 2.51 (2.20 - 2.86) µg/g Cd; 0.024 (0.004 - 0.050) µg/g Pb; 2.47 (1.10 - 4.47) µg/g Ni; 0.29 (0.19 - 0.47) µg/g Cr.

With respect to interspecific differences, *Rapana* samples had a higher bioaccumulation capacity for copper and cadmium, whereas nickel levels were diminished, in comparison with the other molluscs (Fig. 4).

For mussels (*Mytilus edulis*) from North Sea and Baltic Sea the following threshold values, corresponding to normal background for metals, were proposed: Cu 2.0 µg/g ww; Cd 0.4-0.8 µg/g ww; Pb 0.4-1.0 µg/g ww; Ni 0.8-1.0 µg/g ww; Cr 0.4-0.6 µg/g ww[13]. In reference to these values, concentrations of heavy metals in mussels investigated in July 2013 were included in their variation range, with the exception of nickel.

The data obtained during MISIS cruise, July 2013, concerning heavy metals in molluscs are comparable with other data reported for the Black Sea or other marine regions, even showing diminished levels, as in case of lead[11, 14, 15, 16, 17, 18, 19].

Table 8. Concentration of heavy metals in three species of marine molluscs from the Western Black Sea, July 2013

Species	Station	UM	Cu (µg/g ww)	Cd (µg/g ww)	Pb (µg/g ww)	Ni (µg/g ww)	Cr (µg/g ww)
Mytilus galloprovincialis	RO/M01-M02	µg/g ww	2.20	0.37	0.216	1.95	0.14
Mytilus galloprovincialis	RO/M04-M05	µg/g ww	1.75	0.45	0.072	0.97	0.07
Mytilus galloprovincialis	BG/M12-M11	µg/g ww	4.48	0.84	0.092	1.00	0.17
Mytilus galloprovincialis	BG/M11-M10	µg/g ww	1.17	0.21	0.072	2.69	1.31
Mytilus galloprovincialis	BG/M10-M09	µg/g ww	1.51	1.05	0.172	6.00	0.15
Mytilus galloprovincialis	TR/M17-M16	µg/g ww	1.13	1.12	0.002	2.30	0.22
Rapana venosa	RO/M01-M02	µg/g ww	7.48	1.23	0.138	0.33	0.23
Rapana venosa	BG/M12-M11	µg/g ww	4.76	5.40	0.086	0.65	0.24
Rapana venosa	BG/M11-M10	µg/g ww	7.68	4.36	0.254	0.92	0.57
Rapana venosa	TR/M18-M17	µg/g ww	9.35	5.42	0.019	0.66	0.14
Scapharca inequivalvis	RO/M01-M02	µg/g ww	2.15	2.20	0.018	1.85	0.22
Scapharca inequivalvis	BG/M12-M11	µg/g ww	2.03	2.47	0.050	4.47	0.47
Scapharca inequivalvis	TR/M18-M17	µg/g ww	1.93	2.86	0.004	1.10	0.19
EC regulatory value*				1	1.5		

** maximum admissible levels for human consumption according to Commission Regulation (EC) no. 1881/2006

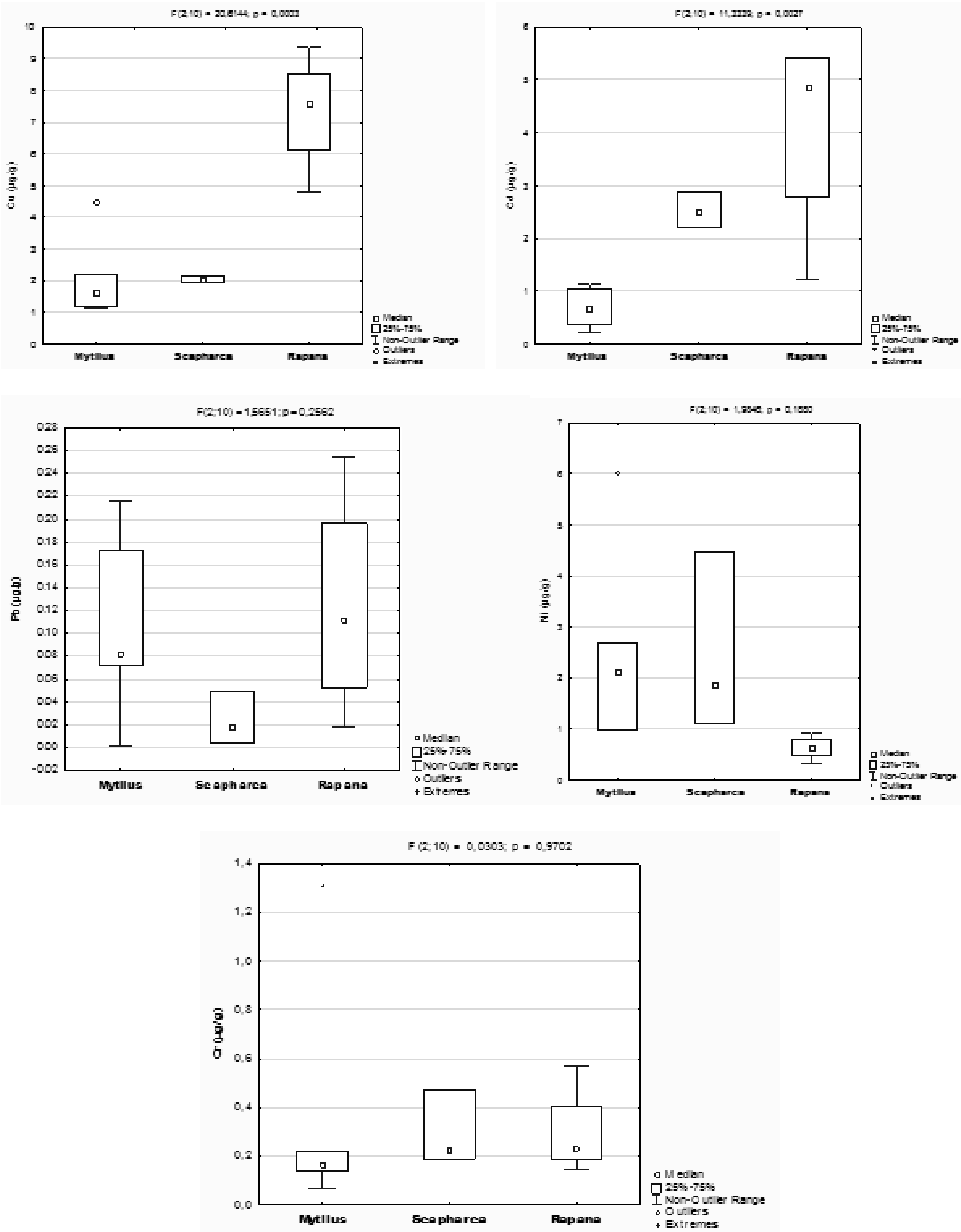

Fig. 4. Box plot of heavy metals (µg/g wet weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013

Comparison to Assessment Criteria

In the scope of **Descriptor 8** the results of the biota analyses for persistent organic pollutants were compared to Environmental Assessment Criteria (EACs) proposed by OSPAR as a means for assessing the significance of concentrations of hazardous substances in the marine environment. EACs (lower) are concentrations below which it is reasonable to expect that there will be an acceptable level of protection of marine species from chronic effects from specific hazardous substances (Table 2, Table 5).

Most of the individual PAH concentrations do not exceed the EAC values in samples except naphthalene (38%,) phenanthrene (23%) and benzo[a]anthracene (8%). According to EACs proposed by OSPAR and the total content of PAHs the biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters in July 2013 can be classified as:

- minimally contaminated (31%) with ΣPAHs from 308.7 to 475.4 µg/kg dw found in *Mytilus* galloprovincialis and *Rapana thomasiana;*
- moderately contaminated (31 %) with ΣPAHs from 649.6 to 1778.1 µg/kg dw found in *Mytilus* and *Scapharca;*
- highly contaminated (38%) with ΣPAHs from 2184.5 to 3756.9 µg/kg dw found in *Mytilus, Rapana* and *Scapharca* with long-term biological effects (e.g. impaired growth, reproduction and survival) and acute biological effects (survival).

There are no EACs values available for OCPs in molluscs. As regard to PCBs, only PCB 52 and PCB 118 exceeded the EAC values in 30% and respectively 15% of samples.

The EU Marine Strategy Framework Directive – **Descriptor 9** requires that "*contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards*".

At this moment the EU legislation that establish maximum admissible levels of contaminants *in fish and other seafood for human consumption* are 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. The Commission Regulation (EC) no. 1881/2006 sets a maximum concentration of 10 µg/kg wet weight for benzo[*a*]pyrene in bivalve molluscs.

Only one sample of *Mytilus galloprovincialis* returned a benzo[*a*]pyrene concentration (14.2 µg/kg wet weight) greater than 10 µg/kg wet weight (Table 3).

EU legislation doesn't refer to OCPs. The Commission Regulation (EC) no. 1259/2011 sets a maximum concentration of 75 ng/g wet weight for sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 in muscle meat of fish and fishery products and products thereof. None of the samples exceeded the regulated level for PCBs (Table 6).

For the time being, there are no recommended Environmental Assessment Concentrations (EACs) for metals in fish and shellfish, with respect to descriptor 8. The alternative approach proposed by Webster[6], which have been adopted by OSPAR[20], is to assess the contaminant concentrations in marine biota with respect to their human health risk. The Commission Regulation (EC) no. 1881/2006 (and subsequent additions and amendments) sets maximum concentrations for contaminants in foodstuffs to protect public health, i.e. to ensure that contaminant concentrations are toxicologically acceptable. This regulation includes maximum levels for Pb, Hg and Cd in bivalve molluscs and fish muscle, and these are the values that can be selected for the assessment. It is recognized that this approach is not fully satisfactory in the context of an assessment addressing environmental risk, but their use an interim solution for addressing the need for criteria until a more appropriate approach and values can be define and agreed[20].

Thus, in comparison with EC regulatory value for cadmium in bivalve molluscs (1 µg/g ww), all *Mytilus* samples were below the limit, whereas *Scapharca* and *Rapana* from all transects presented higher bioaccumulation level. We should mention that *Rapana* was also analysed as whole soft tissue, i.e. including viscera, where metals have the tendency to accumulate. In case of lead, all three species of molluscs were much below regulatory value (1.5 µg/g ww) (Table 8).

CONCLUSIONS

The polycyclic aromatic hydrocarbon source for the three mollusc species (*Mytilus galloprovincialis, Rapana venosa and Scapharca inequivalvis)* may be mostly an acute petroleum exposure.

Benzo[*a*]pyrene and total content of PAHs not present a risk of adverse health effects for 40% of samples (*Mytilus galloprovincialis)*, the remainder of 60% indicate a significant health risks associated with the consumption of these bivalves.

In the absence of maximum admissible levels for OCPs in fish and other seafood for human consumption and EACs values proposed for OCPs it’s hard to assume the ecological status from this point of view.

None of the samples exceeded the regulated level for PCBs, so there is no risk for human health in respect with this class of compounds. The occasional PCB levels which exceed EACs may not pose a significant risk for the ecosystem.

Since for the moment there are no recommended Environmental Assessment Concentrations (EACs) for metals in fish and shellfish, the contaminant concentrations were assessed with respect to their human health risk. Thus, in comparison with EC regulatory values for cadmium and lead, *Mytilus galloprovincialis* samples were below the maximum admissible limits.

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