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CHEMICAL STATUS EVALUATION OF THE ROMANIAN BLACK SEA MARINE ENVIRONMENT BASED ON BENTHIC ORGANISMS' CONTAMINATION

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ABSTRACT
The use of living organisms to provide information on the quality of aquatic environments is now a widely accepted methodology for assessing contaminant bioavailability. Molluscs have developed tolerance mechanisms towards environmental stressors and can accumulate a large range of contaminants. The assessment of marine environment quality was based on heavy metals (HM), persistent organic pollutants (organochlorine pesticides – OCPs and polychlorinated biphenyls - PCBs) and polynuclear aromatic hydrocarbons (PAHs) analysis in *Mytilus galloprovincialis, Rapana venosa, Anadara kagoshimensis* species sampled during 2016 - 2020 along Romanian Black Sea coast. Toxic metals (cadmium, lead) had bioaccumulation levels below threshold values in most molluscs samples investigated. Cadmium registered few values (15%) surpassing maximum admissible levels, in all three species. Data evaluation demonstrated the maintenance of a high level of concentrations of persistent organic pollutants in the mollusc tissue, but also of the exceedances of the values that characterize the good ecological status of these compounds. Polynuclear aromatic hydrocarbons showed a declining trend and no exceeding of the maximum allowed limit for benzo[a]pyrene was recorded in the last years. In consequence, the overview assessment based on "OneOutAllOut" (OOAO) approach, considering all groups of substances, indicates a bad chemical status for this period.
Keywords: *Black Sea, chemical status, benthic organisms, contaminants, pollution*

AIMS AND BACKGROUND

The marine environment is a vital resource for life on Earth covering 71 % of its surface and including 90 % of the biosphere, being a great source of biodiversity but also socio-economic welfare (EC, 2006). Utilizing their abiotic and biotic components and generating complex processes (nutrient uptake, photosynthesis, respiration, excretion, decomposition, food web interactions, etc.), marine ecosystems fulfil functions like primary production, carbon sequestration, resilience (Barbier, 2017; Hattam *et al.*, 2014; Chakraborty *et al.*, 2020) and provide multiple benefits as nutrition and maintaining food production and health, natural cleaning of water and sediments, flood and erosion protection, climate regulation, removal of unpleasant smells and visual nuisances, relaxation, cultural and spiritual fulfilment (Fig.1). Therefore, functioning ecosystems are critical for maintaining the healthy status of the sea. Unfortunately, despite our reliance on biodiversity and marine ecosystem services, population expansion, economic growth, and inappropriate management of human activities are leading to increasing anthropogenic pressures on coastal areas (Wilson *et al*., 2013) and accordingly, to a declining supply of ecosystem services worldwide (Costanza *et al.*, 2014) (Galparsoro *et al*., 2014).

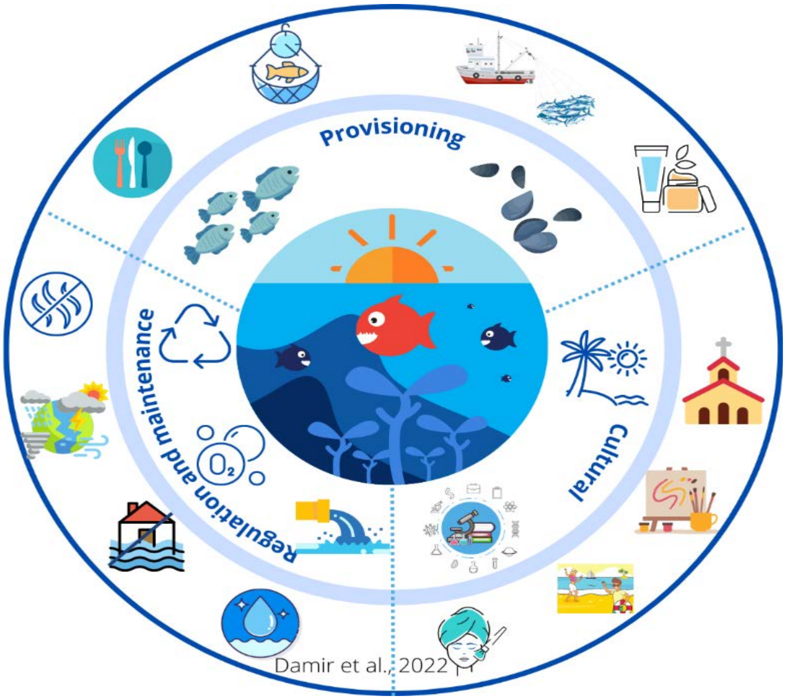

Fig.1. Marine ecosystems functions and benefits (source: NIMRD)

Anthropogenic pressures, uses, and human activities in or affecting the marine environment are grouped as biological, physical, substances, litter and energy (Directive 2017/845). From the latter theme, *the input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides) – diffuse sources, point sources, atmospheric deposition, and acute events* is one of the pressures with relevance for descriptors 8 and 9 (MSFD, 2008) and coming from the extraction of oil and gas, agriculture, transport, urban and industrial uses, waste treatment and disposal (Fig.2).

Benthic organisms play an important role in food chains, including as food for humans, and some play a critical role in the breakdown of organic matter (CSC, 2008). The benthic zone provides an area for spawning, foraging, and refuge for various fish species, and benthic habitats function in nutrient cycling and removal of contaminants from the water, such as with the removal by filter feeders (scallops, mussels, and so on) of pollutants, organic matter, and sediments (CSC, 2008). However, molluscs are vulnerable to anthropogenic pressures and have developed tolerance mechanisms towards environmental stressors and can accumulate a large range of contaminants. Consequently, the use of *Mytilus* as a sentinel organism is well established and further standardized in mussel monitoring documents, for example, from UNESCO (1992), National Oceanic and Atmospheric Administration (Lanksbury et al. 2010, Lanksbury & West 2012), International Credential Evaluation Service (ICES) (Davies & Vethaak 2012), OSPAR (2012), and European Commission (EC 2014) (for review and references see Beyer et al. 2017 and Pollutants section) (Baden et al., 2021). In this respect, the use of living organisms to provide information on the quality of aquatic environments is nowadays a widely accepted methodology for assessing contaminants' bioavailability.

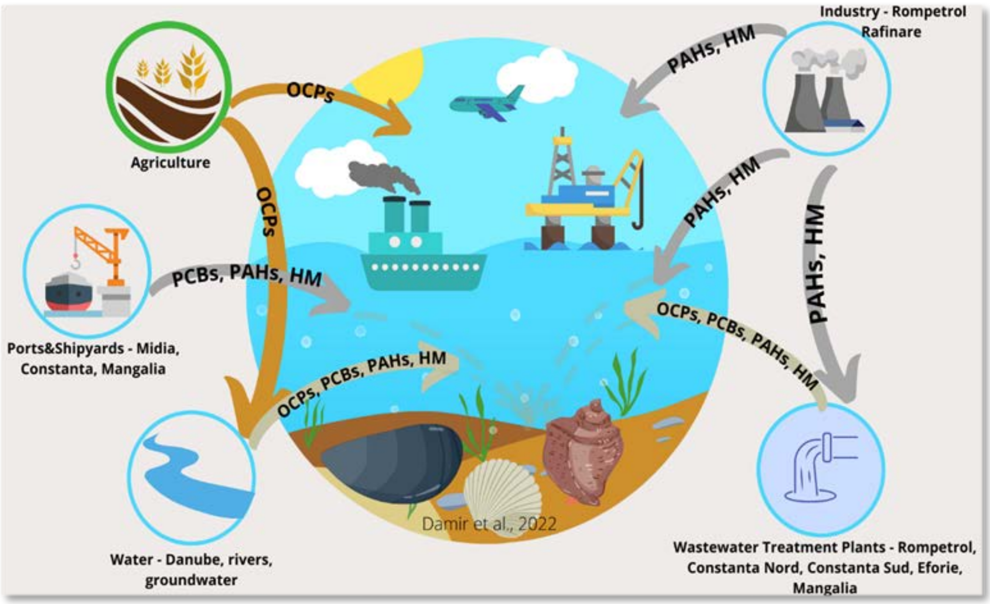

Fig.2. Main sources and pathways of contaminants introduced in the Romanian Black Sea (source: NIMRD)

Information related to the assessment of hazardous substances in biota is rather limited in the Black Sea region, thus in the recent years' activities carried out in the framework of regional or international projects, like FP7 "Policy-oriented marine Environmental Research for the Southern European Seas" (PERSEUS) (Oros *et al.,* 2016), DG ENV "MSFD Guiding Improvements in the Black Sea Integrated Monitoring System" (MISIS) (Coatu *et al.,* 2016) or CBC "Assessing the vulnerability of the Black Sea marine ecosystem to human pressures" (ANEMONE) (Coatu *et al.,* 2020; Oros et al., 2021) aimed to fill knowledge gaps and to help focus future research efforts toward key domains, like the presence of hazardous substances in biota, impact of human pressures upon to the contamination status and trends, and biological effects monitoring.

For the Romanian marine waters, data on dangerous substances bioaccumulation in molluscs, fish, macroalgae, etc., are collected in the framework of national monitoring and various national research projects (Oros & Gomoiu, 2012; Coatu *et al.*, 2013; Coatu *et al.*, 2015; Abaza *et al*., 2016; Coatu *et al.,* 2018; Oros, 2019; Marin *et al.*, 2021), some of the information being also used for the national assessment of the Descriptors 8 and 9 during MSFD reporting cycles: Initial assessment (2012) (Boicenco *et al.,* 2012), and updates of the MSFD Articles 8, 9 and 10 (2018) (Boicenco *et al.,* 2018).

The scope of the study is to assess biota concentrations in order to evaluate the marine environment status as essential information for the integrated management of land, water and living resources that promotes conservation and sustainable Black Sea use in an equitable way (EcAP - Ecosystem approach).

EXPERIMENTAL

The assessment of marine environment quality was based on heavy metals (HM), persistent organic pollutants (organochlorine pesticides – OCPs and polychlorinated biphenyls - PCBs) and polynuclear aromatic hydrocarbons (PAHs)analysis in *Mytilus galloprovincialis, Rapana venosa* and *Anadara kagoshimensis* species sampled in 2016 - 2020 along Romanian Black Sea coast covering variable salinity, coastal and marine reporting units (MSFD) with bottom depths within 20-56 m (Fig.3).

The whole soft tissue of molluscs was 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).

For **trace metals** analysis, 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 a volume of 100 ml with deionized water. The analytical determination of the copper, cadmium, lead, nickel, and chromium was carried out by graphite furnace atomic absorption spectrometry method (GF-AAS), using a Solaar M6 DUAL Zeeman, Thermo Electron 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 an average value reported (IAEA-MEL, 1999).

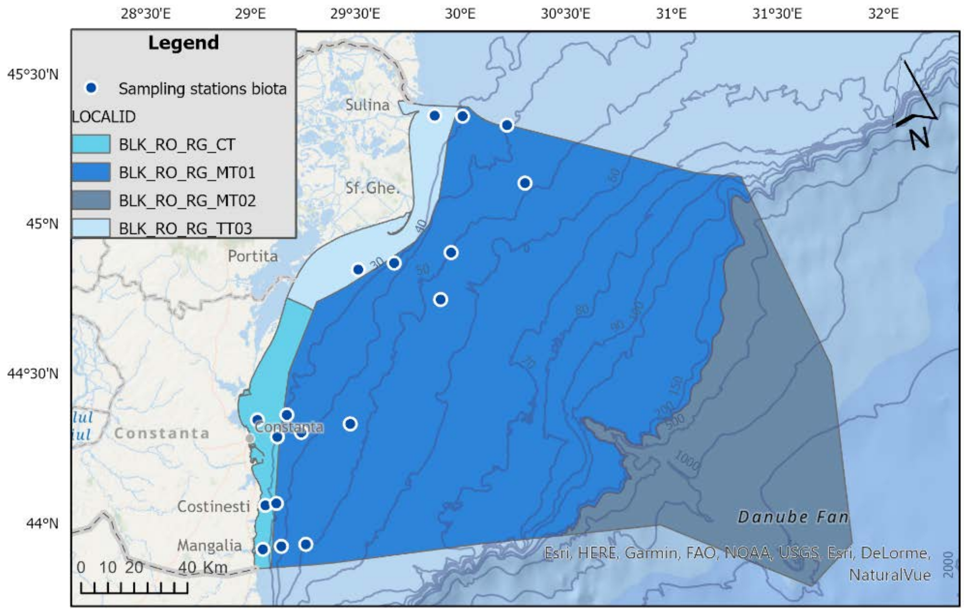

Fig.3. Map of network sampling stations – biota, Romanian Black Sea, 2016-2020

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 a microwave extraction system Start E Milestone for 30 min at 1200C. 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 the 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 aromatic hydrocarbons (PAHs) were combined for analysis. The fraction containing PAHs was evaporated using the Kuderna-Denish concentrator 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).

The status of the Black Sea ecosystem concerning MSFD is assessed by evaluating the 75% percentile of the data in the marine region units in a given period against threshold values that define good environmental status (MAC-EQS) following European legislation (EU Directive 2013/39, EC Regulation 1881/2006) or Environmental Assessment Criteria (EAC) values developed by OSPAR for assessing the ecological status (OSPAR, 2008). As a result, a “Good” or “Bad” status for each substance is obtained and the overall result is given by the worst-case using the “one out, all out” (OOAO) principle (Boicenco et al., 2018).

Data visualization and statistical analysis were accomplished with dedicated software STATISTICA 14.0 and ArcGIS Pro 2.9.3.

RESULTS AND DISCUSSION

For several decades, bivalve molluscs and other invertebrates have been widely included in long-term environmental monitoring programs as they accumulate different hazardous substances to a greater extent than other constituents of coastal ecosystems, mainly due to their filtering behavior. Since they are sessile or sedentary organisms, bivalves can provide greater spatial integration than sediment and provide information to identify sources of bioavailable contaminants (González-Fierro & Ponce-Vélez, 2018).

Metallic elements are found in all living organisms and play several roles. They may be structural elements, stabilizers of biological structures, components of control mechanisms (e.g., in nerves and muscles), and in particular, activators or components of redox systems (Nordberg *et al.*, 2007). Thus, some metals are essential elements, such as iron, copper, zinc, manganese, s.a., and their deficiency results in biological functions impairment (Hogstrand & Haux, 2001). But even essential metals, when present in excess, could become toxic. Non-essential metals, such as Pb, Cd and Hg are usually potent toxins even at lower concentrations and their bioaccumulation in tissues leads to intoxication, decreased fertility, cellular and tissue damage, cell death and organ malfunction (Oliveira Ribeiro et al., 2002). Such effects may lead to the modification of an entire population or species assembly in an ecosystem and may be of great significance to human life so they should be considered in the total evaluation of environmental pollution by metals and their compounds (González-Fierro & Ponce-Vélez, 2018).

The levels of metals in molluscs and other invertebrates are often considerably higher than in other constituents of the marine environment due to their habitat and feeding habits (Sun *et al.*, 2011). Compared to sediments, molluscs exhibit greater spatial sensitivity and therefore are the most reliable tool for identifying sources of available metal contamination (Hamed & Emara, 2006). Under certain environmental conditions, metals might accumulate up to toxic concentrations and cause ecological damage (Bai et al., 2011).

Concentrations of heavy metals in the three molluscs species from the Romanian marine waters investigated during 2016-2020 (*Anadara, Mytilus, Rapana*) varied within wide ranges, as follows: Cu 0.930-12.867 µg/g ww; Cd 0.143-2.915 µg/g ww; Pb 0.010-0.373 µg/g ww; Ni 0.020-5.744 µg/g ww; Cr 0.088-4.384 µg/g ww. Concerning maximum admissible concentration (MAC) from European regulation (EC No 1881/2006), concentrations of lead (Pb) were much below MAC value (1.500 µg/g ww) in all molluscs samples, whereas surpasses of cadmium (Cd) MAC (1.000 µg/g ww) were noticed in few cases (15%) from all 3 species (Fig. 4).

Copper (Cu) concentrations evinced some interspecific differences, as *Rapana* bioaccumulated higher concentrations (1.490-12.867 µg/g ww) (Fig. 5), compared with bivalve molluscs, values similar to variation ranges reported for *Rapana* from the Turkish coast (1.603-19.75 µg/g ww) (Bat, 2014). Nevertheless, high Cu content in marine gastropods and bivalve species is normal since Cu is an essential element and is present in hemocyanin, the blood pigment of these invertebrates, vital for respiration and oxygen transport (Lino et al., 2016).

Mussels' species are widely considered as one of the most suitable sentinels and biological indicators of pollution since they possess a multitude of useful characteristics for this purpose, being sessile filter feeders that accumulate contaminants in their tissues and having stable local populations in many places. (Azizi *et al.*, 2018).

In *Mytilus galloprovincialis* samples that were investigated during 2016-2020, the bioaccumulation values of heavy metals varied within the following limits: Cu 1.134-5.110 µg/g ww; Cd 0.143-2.018 µg/g ww; Pb

0.02-0.181 µg/g ww; Ni 0.232-5.744 µg/g ww; Cr 0.088-4.384 µg/g ww. Values were generally comparable with data reported in the literature for mussels from the Turkish Black Sea coast: 0.98-1.34 µg/g ww Ni; 1.14-2.46 µg/g ww Cu; 0.68-1.06 µg/g ww Pb; 0.04-1.15 µg/g ww Cd (Topcuoglu, 2004, cited by Bat, 2014).

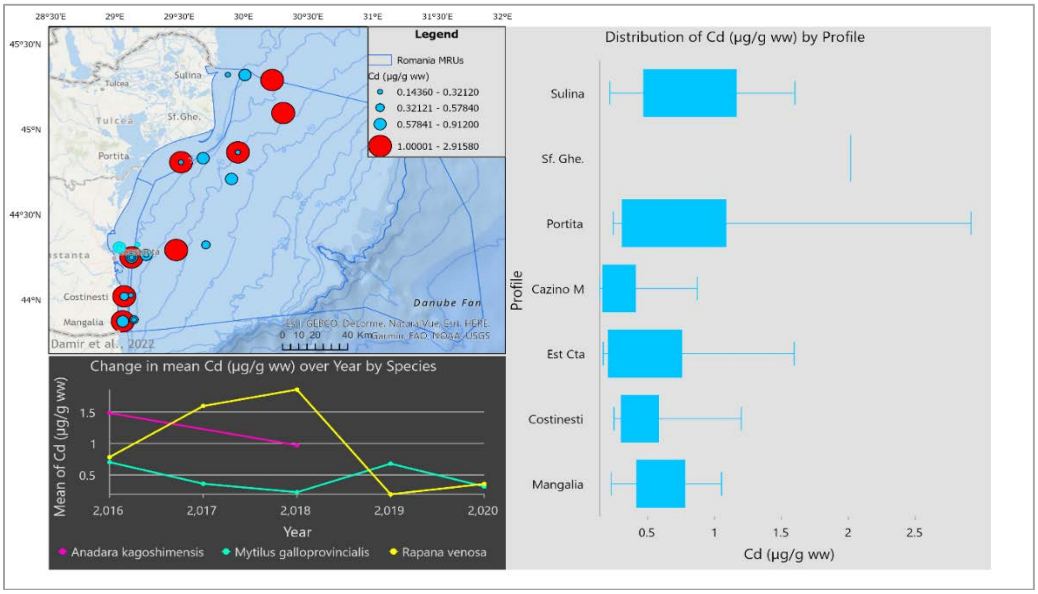

Fig. 4. Bioaccumulation of cadmium (Cd) in three molluscs species from the Romanian marine waters investigated during 2016-2020

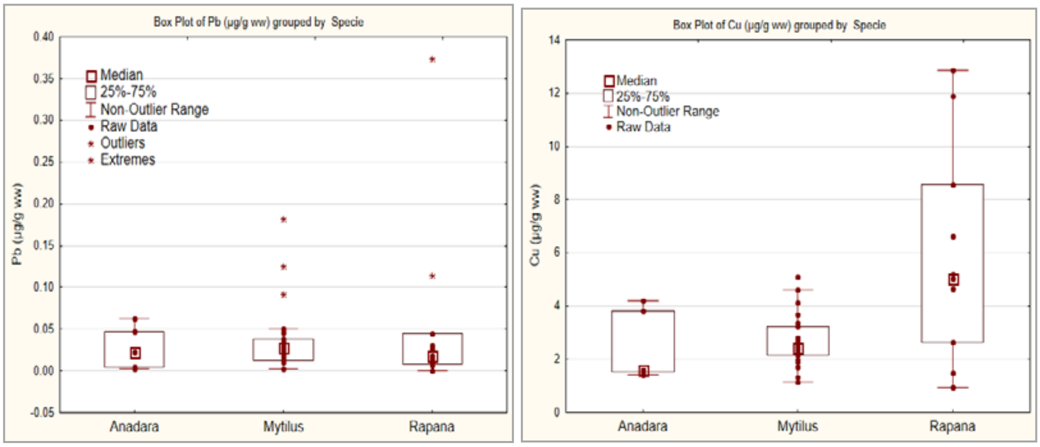

Fig. 5. Bioaccumulation of lead (Pb) and copper (Cu) in three molluscs species from the Romanian waters investigated during 2016-2020

Exceedances of the MAC value for Cd (1 µg/g ww) were observed in about 9% of the mussels' samples, all of which were collected in 2019.

Analysis of the spatial distribution evinced that the highest bioaccumulation values of Cd were measured in the mussels from the northern area of the coast (Sulina – Portita). Pb concentrations were well below the MAC value (1.5 µg/g ww) in all samples of mussels investigated and had a slightly decreasing tendency over the investigated period. Unlike Cd, the distribution of Pb did not show significant differences depending on the sampling location, north or south of the coastline (Fig. 6-7).

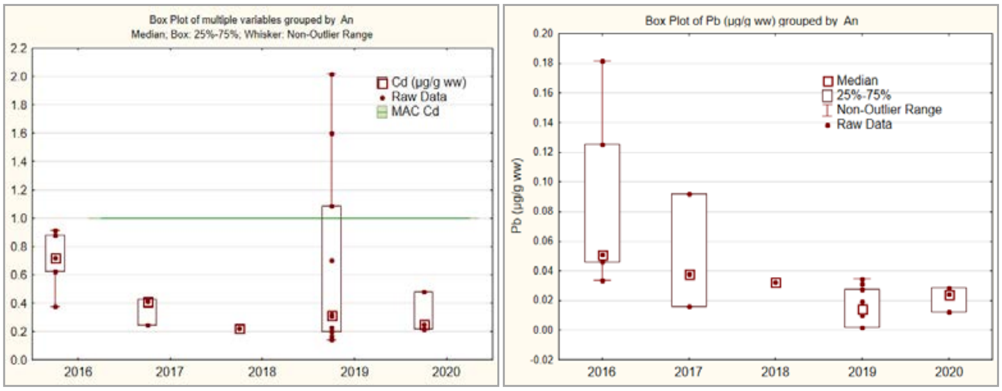

Fig. 6. Evolution of cadmium (Cd) and lead (Pb) concentrations in *Mytilus galloprovincialis* from the Romanian waters during 2016-2020

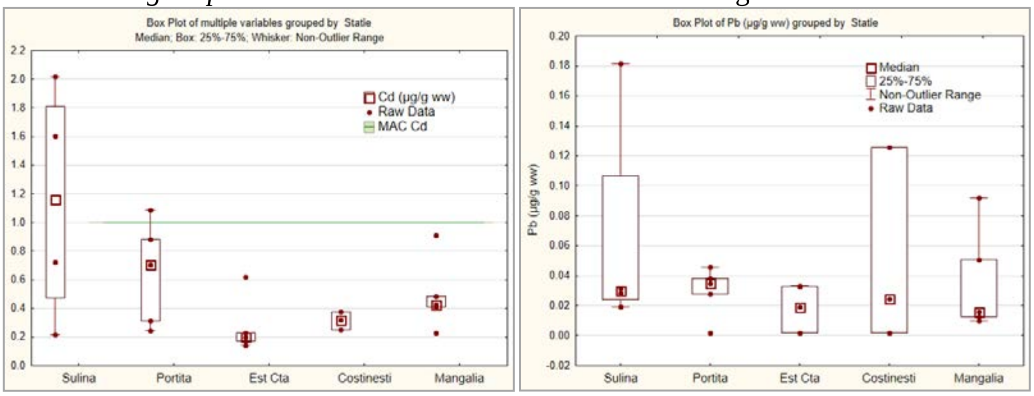

Fig. 7. Spatial distribution of cadmium (Cd) and lead (Pb) concentrations in *Mytilus galloprovincialis* from the Romanian waters during 2016-2020

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have caused deep concern for both developed and developing countries due to their toxicity, persistence, bioaccumulation, high long-range atmospheric transport and potentially adverse health effects on marine organisms and humans (Kilunga *et al.*, 2017). Despite their ban or restricted use, PCBs and OCPs are still found in various environmental matrices due to their low degradation (Syakti *et al.*, 2013).

POPs originate mainly from anthropogenic sources such as waste burning, fossil fuel combustion, industrialization, commercial exploitation of the marine environment, and agrochemicals (Bouwman *et al.*, 2012; Sun *et al.*, 2013; Bouwman, 2003).

Bivalve and gastropod molluscs are among the most useful organisms for environmental biomonitoring because of their spatial and temporal abundance and ease of sampling (Phillips and Rainbow 1994). In addition to the above criteria, these organisms are relatively site-specific, being either attached to the substrate or very restricted in their motility (Bresler *et al.*, 2003).

Organochlorine pesticides varied in wide ranges in molluscs species. High values were observed in *Mytilus* mainly for endrin, heptachlor, p,p'DDD and p,p' DDT. The same compounds recorded big concentrations in the other species, too (Fig. 8-10).

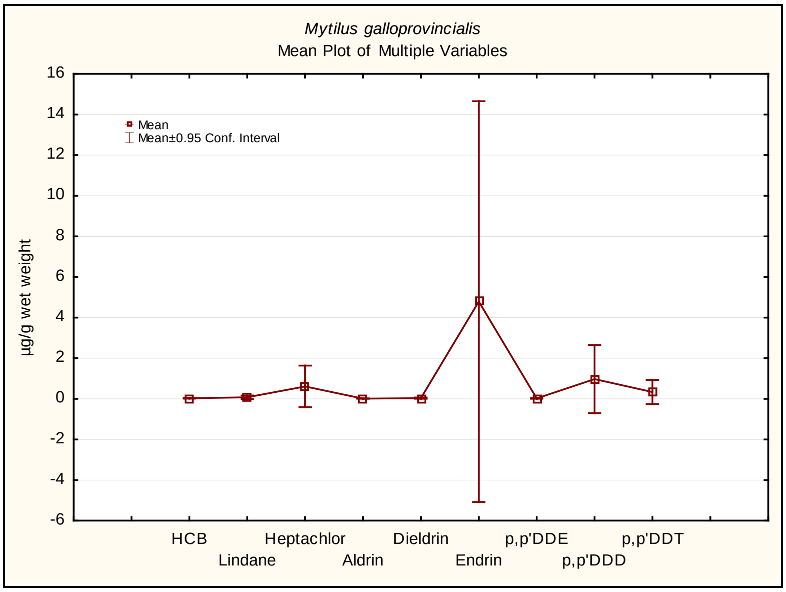

Fig. 8. Organochlorine pesticides concentrations in *Mytilus galloprovincialis* from Romanian waters, 2016 – 2020

Concerning the maximum admissible concentrations stipulated by European legislation (EC regulation 39/2013, which refers only to HCB and heptachlor in relation to a good ecological state), HCB surpassed the regulated levels in 33% of the samples in this period. Concentrations over EQS were more frequent lately, with significant increases in 2019-2020 in *Mytilus* and 2018-2020 in *Rapana* (Fig.11).

As regards heptachlor, EQS is lower than the detection limit, so exceedances cannot be correctly evaluated. Significant concentrations over the detection limit were noticed in the last year (2020) in the southern area (Mangalia, Costinesti) (Fig.12).

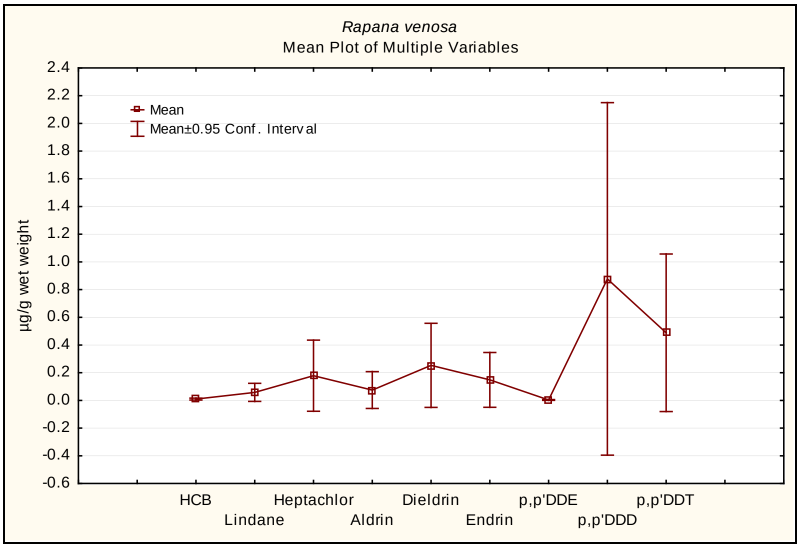

Fig. 9. Organochlorine pesticides concentrations in *Rapana venosa* from Romanian waters, 2016 - 2020

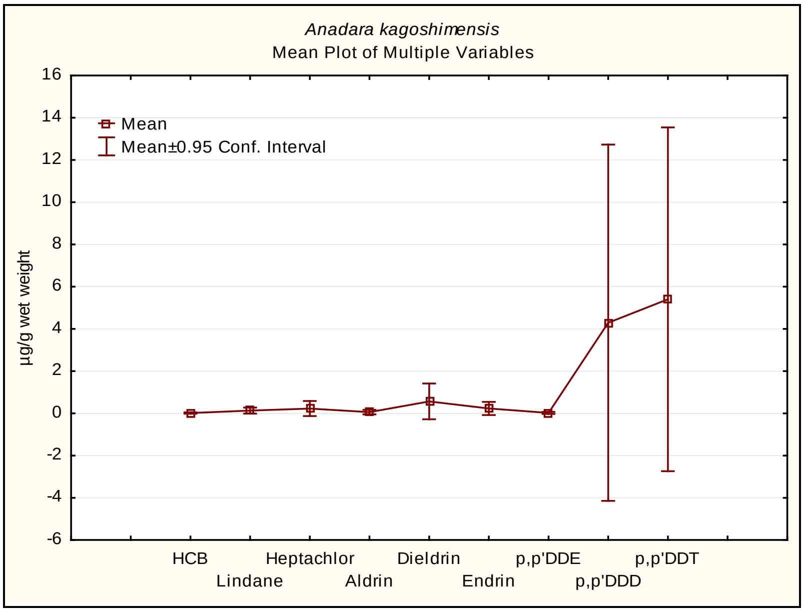

Fig. 10. Organochlorine pesticides concentrations in *Anadara kagoshimensis* from Romanian waters, 2016 - 2020

However, an ascendant trend was noticed in the last years for total organochlorine pesticides in all investigated species, with the highest concentration in the southern area (from Cazino Mamaia to Mangalia) (Fig.13).

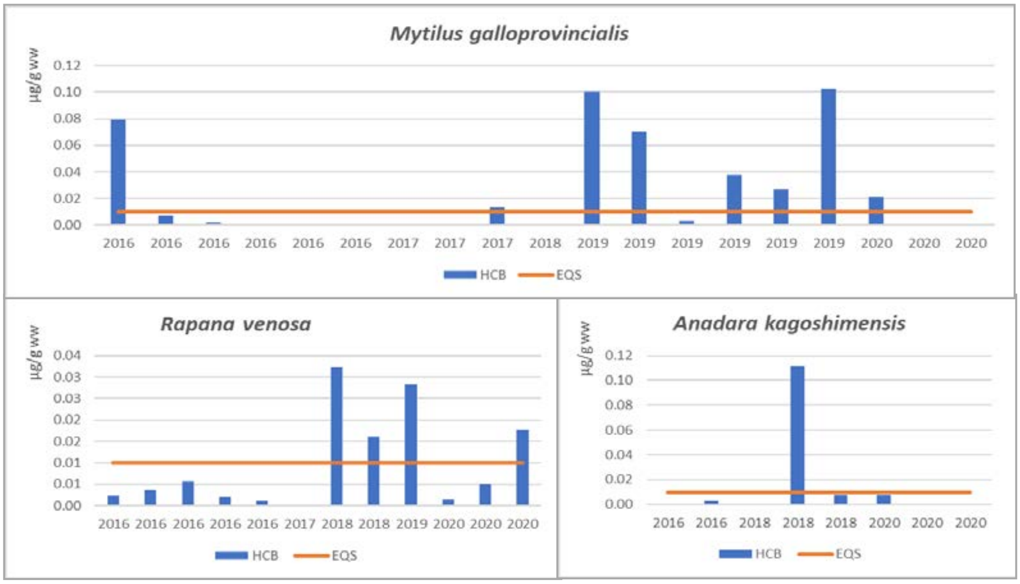

Fig. 11. HCB values in molluscs against the ecological quality standard, 2016-2020

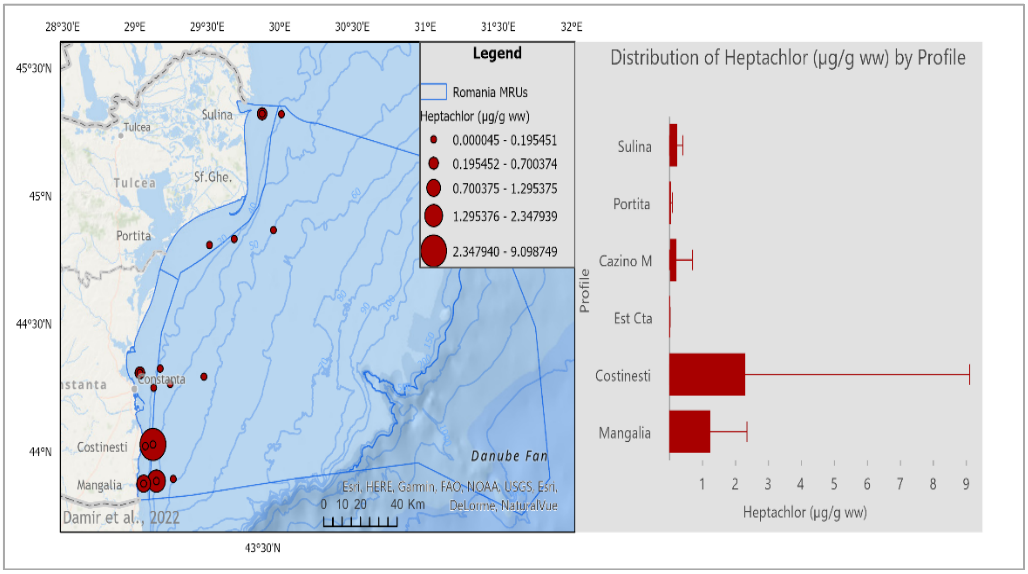

Fig.12. Spatial distribution of Heptachlor, 2016-2020

Even if concentrations were lower compared with OCPs, values higher than good ecological status thresholds were recorded for PCBs in 36 to 69 % of the samples (Table 1).

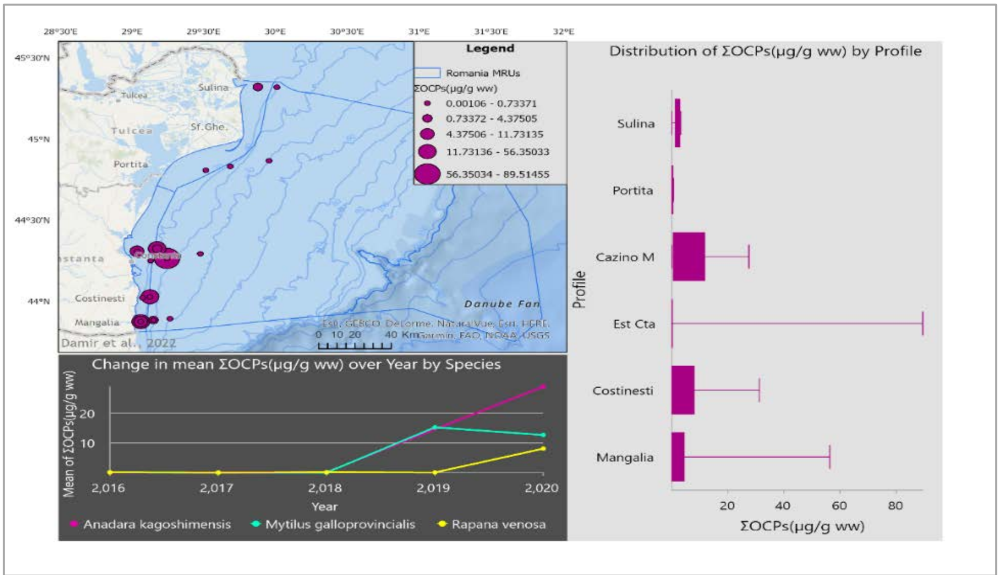

Fig.13. Spatial distribution and trend of OCPs by species, 2016-2020

Table 1. Exceeding the proposed threshold values that define the good ecological status for polychlorinated biphenyls in molluscs 2016 - 2020

PCB	Threshold values (µg/g)	Exceeding the threshold values (%)
PCB 28	0.0032	36
PCB 52	0.0054	69
PCB101	0.0060	46
PCB118	0.0012	36
PCB153	0.0800	53
PCB138	0.0158	38
PCB180	0.0240	49

PCB 52 was the dominant compound in all species. Higher values were also observed for PCB 101,118,153,138 in *Mytilus* and PCB 153,138, 180 in *Anadara* and *Rapana* (Fig.14,15,16).

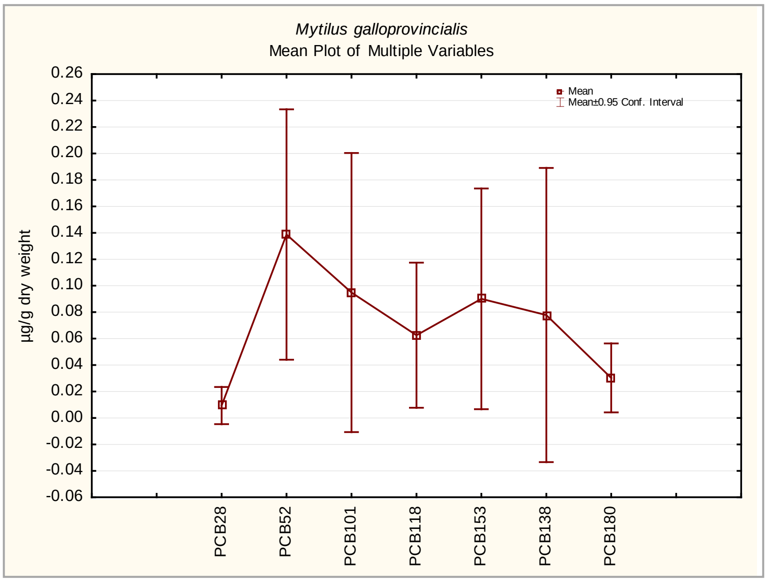

Fig. 14. Polychlorinated biphenyls concentrations in *Mytilus galloprovincialis* from Romanian waters, 2016 - 2020

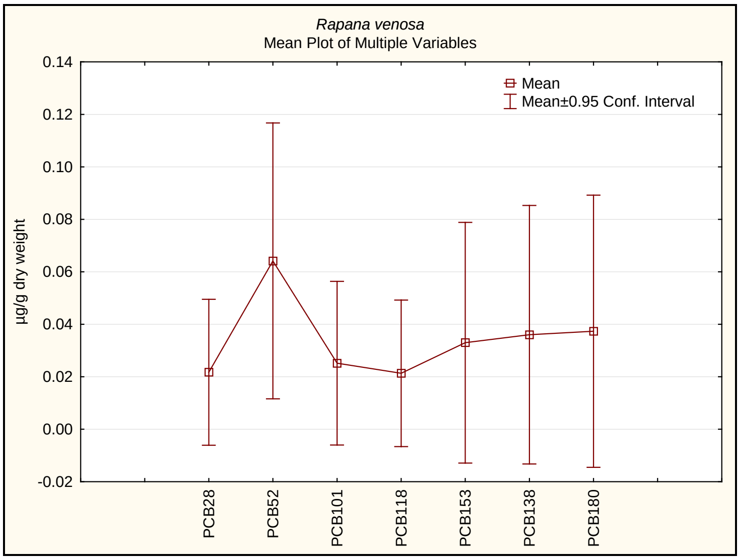

Fig. 15. Polychlorinated biphenyls concentrations in *Rapana venosa* from Romanian waters, 2016 - 2020

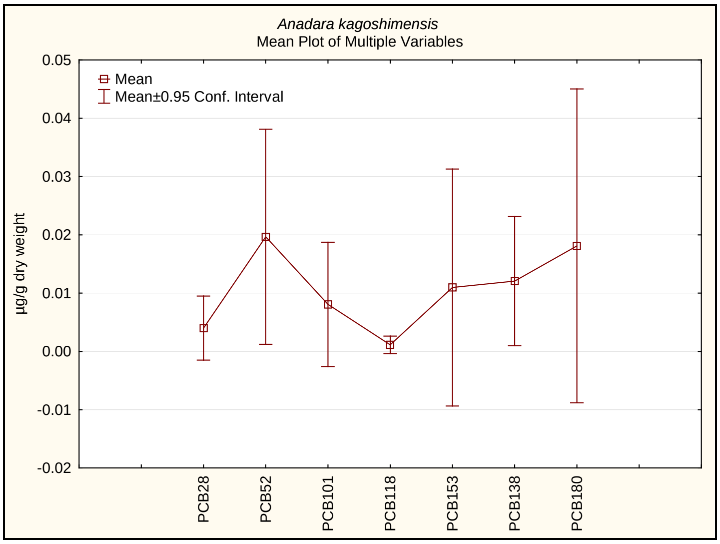

Fig. 16. Polychlorinated biphenyls concentrations in *Anadara kagoshimensis* from Romanian waters, 2016 – 2020

Total PCBs also showed an ascendant trend, the highest values being recorded in 2019. Their spatial distribution highlighted the more industrialized southern area (from Cazino Mamaia to Mangalia) as more polluted with PCBs compared to the northern part (Sulina – Portita) (Fig.17).

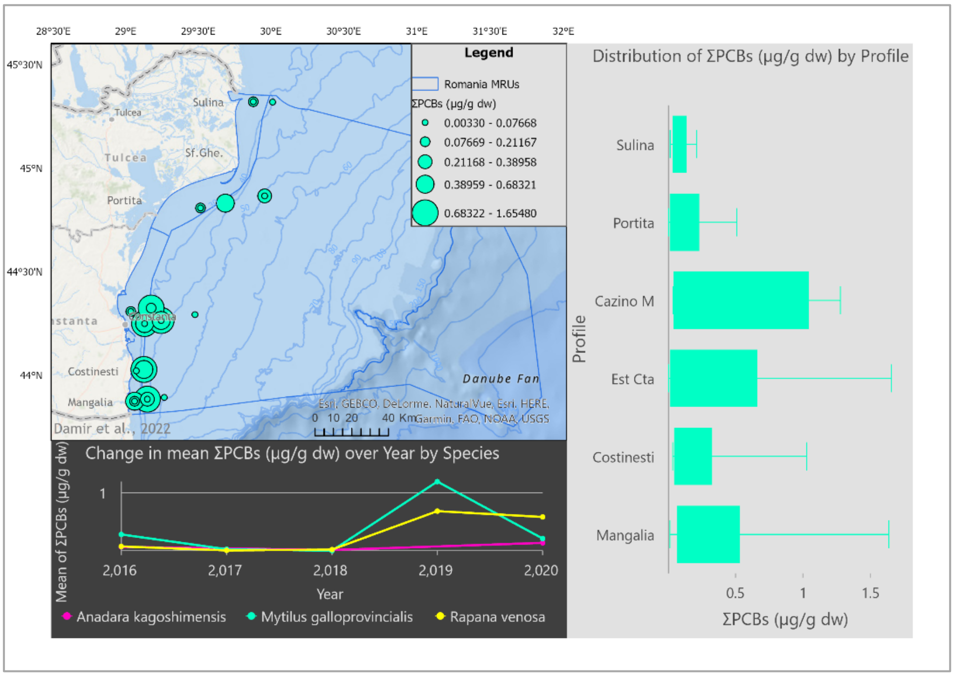

Fig.17. Spatial distribution and trend of PCBs by species, 2016-2020

Due to their properties and negative impact on the environment, PAHs are included in the list of priority pollutants (Kissao *et al.*, 2011). PAHs may come from anthropogenic or natural sources. Anthropic sources include the release into the environment by incomplete combustion of carbon-containing materials (coal, oil, wood), direct leakage of crude oil or refined petroleum products, by erosion of oil-contaminated materials in the aquatic environment (Kissao *et al.*, 2011). In addition to anthropogenic sources, their natural development is known. It is also known that perylene can be generated by diagenesis under anaerobic conditions or that naphthalene, phenanthrene and perylene can be produced naturally as a result of intense biological activity (Tautua *et al.*, 2013).

As chemically stable and lipophilic compounds, they can easily cross lipid membranes and have the potential to bioaccumulate in aquatic organisms (Nwaichia *et al.*, 2016).

During the studied period (2016-2020), polycyclic aromatic hydrocarbons total content ($\sum_{16}$PAHs) varied between 0.00160 and 20.16548 (µg/g dry weight) in molluscs species. Among the species of molluscs analyzed, *Anadara kagoshimensis* accumulated the highest level of polyaromatic compounds (Fig.18).

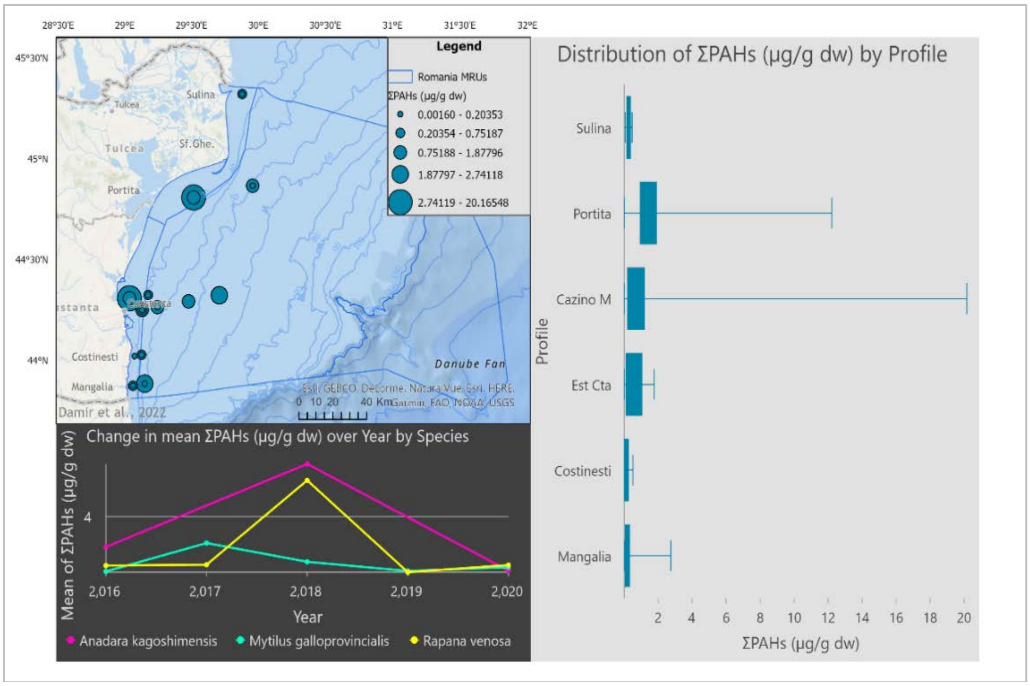

Fig. 18. Polycyclic aromatic hydrocarbons total content ($\sum_{16}$PAHs) spatial distribution by species, 2016-2020

The highest values were observed in *Mytilus galloprovincialis* mainly for fluorene. The same compound recorded big concentrations in *Rapana venosa and Anadara kagoshimensis* species (Fig 19, 20, 21).

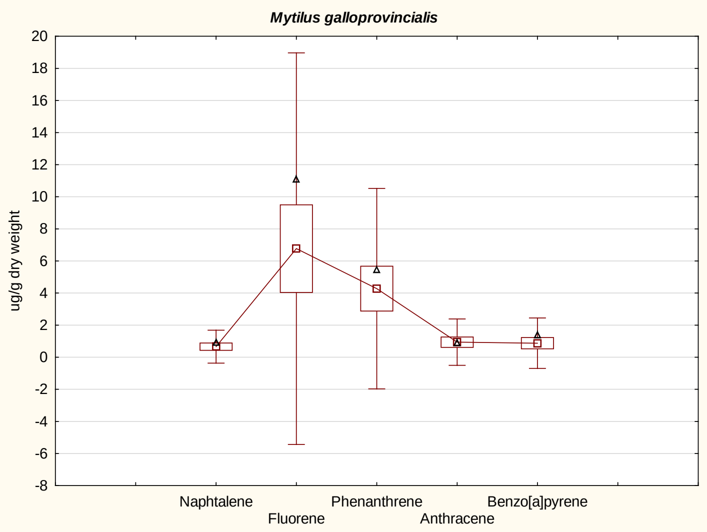

Fig. 19. Polyaromatic dominant compounds values in *Mytilus galloprovincialis* from Romanian waters, 2016 – 2020

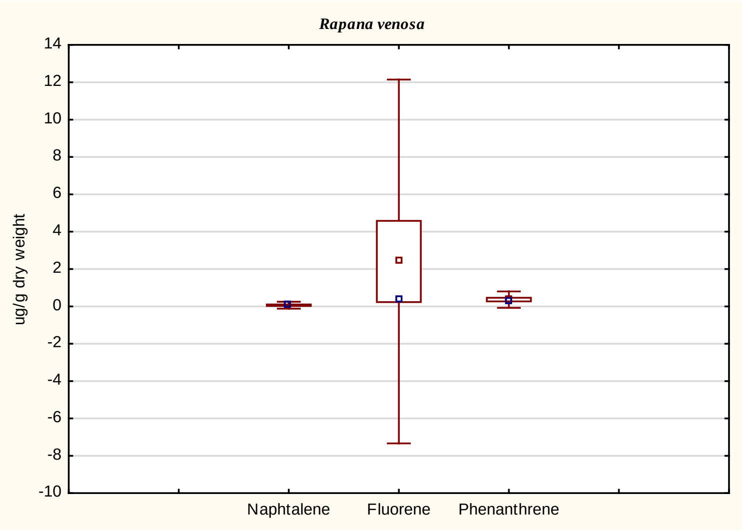

Fig. 20. Polyaromatic dominant compounds values in *Rapana venosa* from Romanian waters, 2016 - 2020

Benzo(a)pyrene is the only polyaromatic compound of the sixteen determined for which a maximum permissible limit (0.01 µg/g dry weight) is established under European Regulation 1881/2006/EC. The evaluation of polycyclic aromatic hydrocarbons indicator in molluscs species (*Mytilus* *galloprovincialis, Rapana venosa, Anadara kagoshimensis*), after processing data for the period 2016-2020 (N=43), reflects in benzo(a)pyrene case a good ecological status (Table 2).

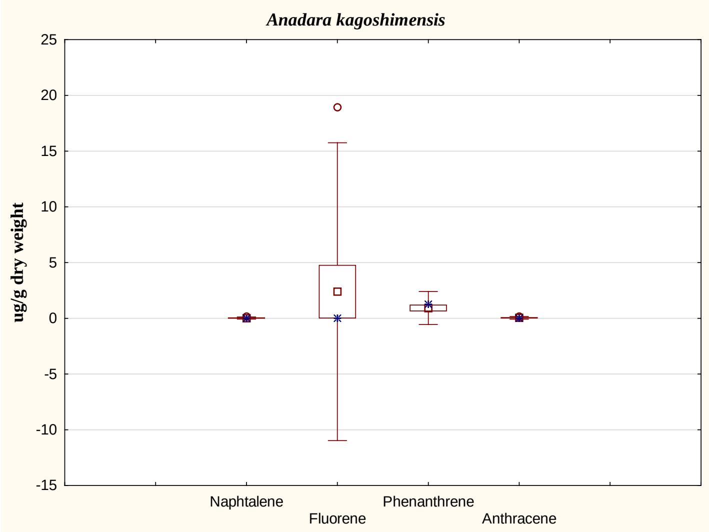

Fig. 21. Polyaromatic dominant compounds values in *Anadara kagoshimensis* from Romanian waters, 2016 - 2020

Table 2. Exceeding the proposed threshold values that define the good ecological status for polycyclic aromatic hydrocarbons in molluscs, 2016 – 2020

PAH	Threshold values (µg/g dry weight)	Exceeding the threshold values (%)
Benzo[a]pyrene	0.01	5.12

In molluscs species from Romanian waters, benzo(a)pyrene values ranged between 0.00003 and 0.59048 (µg/g dry weight). The highest values were recorded for *Mytilus galloprovincialis* (Fig. 22).

Human activities in the Romanian coastal area generate contaminants that, either from the point or diffuse sources, through different pathways, reach the Black Sea where they are deposited in sediments and affect benthic organisms. Our evaluations showed exceedances of the maximum allowable concentrations in the biota for most of the analyzed parameters, in different amounts, notably in the southern area, which is also the most industrialized. Thus, based on the "OOAO" principle, the chemical state of the marine environment in the study area is assessed as "Bad" (Fig.23).

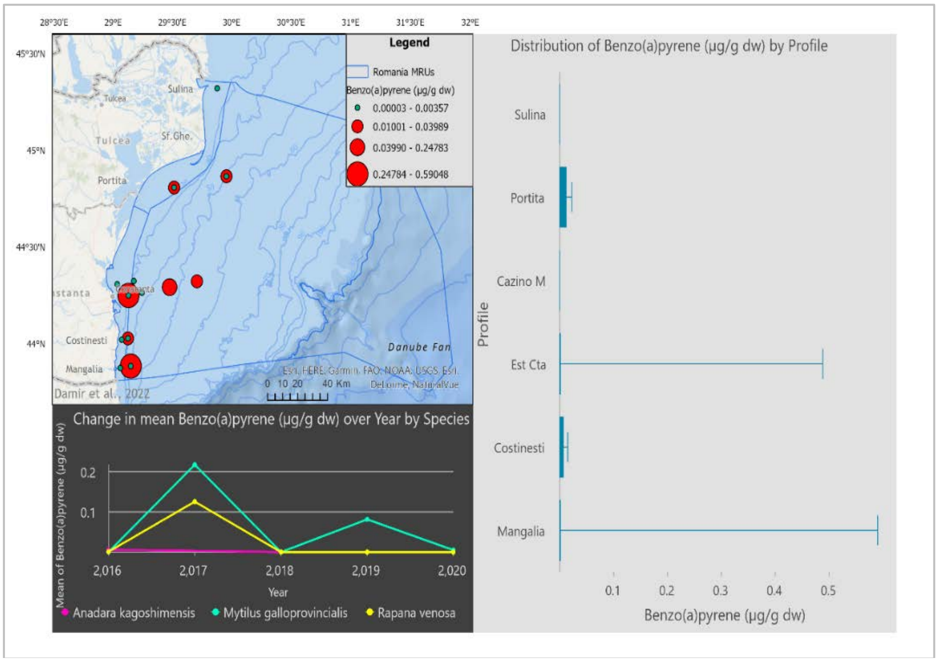

Fig. 22. Spatial distribution of Benzo(a)pyrene, 2016-2020

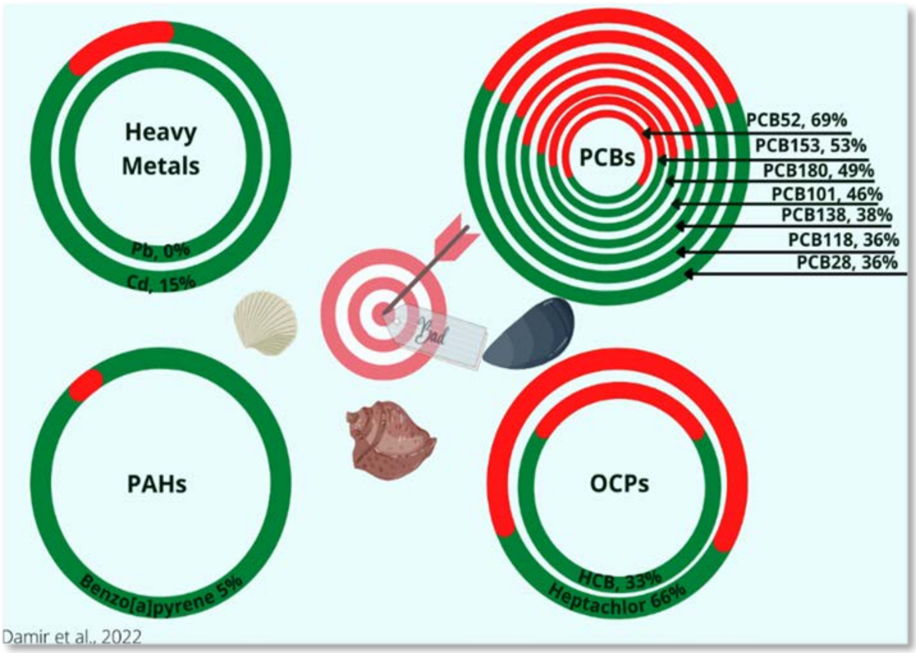

Fig.23. Chemical status of the Romanian Black Sea environment based on benthic organisms contamination, 2016-2020 (source: NIMRD)

CONCLUSIONS

The presence of contaminants in the Black Sea ecosystem, at values that exceed the thresholds, is an indicator of chemical pollution that requires quick and appropriate management actions. Their efficiency should be quantified by dedicated monitoring programs that target the pressures and measures taken to reduce them. These programs must be carried out regularly and using the latest techniques, equipment and trained personnel. Also, given that many pollutants (e.g., PCBs, Heptachlor, HMs) enter via the atmospheric pathway (Jurado et al., 2004; Lammel & Stemmler, 2012; Wania & Daly, 2002), urgent measures are required to reduce and monitor the air pollution, including the efficiency of waste disposal and hazardous waste management (ATSDR, 2007).

In relation to maximum admissible concentration (MAC) from European regulation (EC No 1881/2006), concentrations of Pb were much below MAC value (1.500 µg/g ww) in all molluscs samples, whereas surpassed Cd MAC (1.000 µg/g ww) were noticed in few cases (15%) from all 3 species. Thus, considering GES target of <25% samples exceeding MAC, for molluscs studied during 2016-2020, chemical status could be considered “moderate-good” concerning heavy metals.

Concerning the good ecological status thresholds, HCB surpassed this level in 33% of the samples and PCBs in 36 to 69 % of the samples. Even if the threshold of heptachlor is lower than the detection limit, overpasses of this value were recorded in 66% of the samples. In consequence, the status was evaluated as “bad” with respect to chlorinated compounds.

In relation to the maximum admissible limit (0.01 µg/g dry weight) established under European Regulation (1881/2006/EC), benzo(a)pyrene values exceeded the thresholds in 5% of the total molluscs species studied. Therefore, given the GES target of <25% of samples exceeding the maximum admissible limit, for molluscs species studied in the period 2016-2020, the chemical status could be considered “good” for polyaromatic compounds.

However, in consequence, the overview assessment based on OOAO approach, considering all groups of substances, indicates a “bad” chemical status in this period.

Accordingly, we agree that anthropogenic impacts on marine ecological functioning must be placed within the context of changes occurring in response to anthropogenic pressures and environmental variability. So, to successfully apply new biomonitoring tools to the assessment of human impacts on a large scale, the nature of the relationship between functioning and environmental conditions should be examined (Bremmer, 2005) by applying ecotoxicity experiments and modelling tools.

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