Bioaccumulation of Contaminants in the Main Links of the Pelagic Trophic Chain at the Romanian Black Sea Coast (Valentina Coatu, Andra Oros, Nicoleta Damir, Florin Timofte, Luminița Lazăr)	"Cercetări Marine" Issue no. 48 Pages 118-134	2018

BIOACCUMULATION OF CONTAMINANTS IN THE MAIN LINKS OF THE PELAGIC TROPHIC CHAIN AT THE ROMANIAN BLACK SEA COAST

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ABSTRACT

The paper presents contaminants bioaccumulation and discuss their biomagnification in species belonging to five different trophic levels in the pelagic area of the Romanian Black Sea, namely plankton, 11 species of fish and dolphin.

The biomagnification of total polynuclear aromatic hydrocarbons was not observed in the studied trophic chain. The biomagnification process of organochlorine pesticides was observed at trophic level 3 and at trophic level 5, while biomagnification of polychlorinated biphenyls was highlighted at trophic levels 2, 3, and especially 5. Heavy metals, except for nickel, showed significantly higher levels in planktonic organisms, compared to other trophic levels. The biomagnification process of copper, lead, nickel and chromium was observed at trophic level 3, copper and chromium, and trophic level 5, while cadmium biomagnification was not observed.

Key-Words: Black Sea, hazardous substances, biota, pelagic trophic chain

AIMS AND BACKGROUND

The aim of the paper is to evaluate the level of hazardous substances in the biota belonging to the main links of the pelagic trophic chain of the Black Sea ecosystem in order to understand the way these contaminants are accumulated and transferred from one trophic level to another.

The anthropogenic impact on the marine ecosystem results in overexploitation, habitat destruction, increased frequency of disease, extermination and replacement of some species. Although the effects of contaminants are less obvious than those previously mentioned, their study is equally important for

understanding and managing human interactions with the marine ecosystem (Hylland & Vethaak, 2011).

Marine organisms take and accumulate contaminants through bioconcentration, biomagnification and bioaccumulation. Bioaccumulation is defined as taking a contaminant from an abiotic environment (bioconcentration) and / or food (biomagnification), i.e. from all possible sources (Gray, 2002).

The study of contaminants bioaccumulation in biota is an essential part of various programs that assess the chemical status of the marine environment. Monitoring of pollutant concentrations in the biota can be used to identify sources of pollution, to carry environmental risk assessment, or to verify compliance with the maximum limits permitted by current legislation for both environment and human consumption.

EXPERIMENTAL

Biota samples (N = 51) were collected along the Romanian seaside between Sulina and Vama Veche, on the 5-30m bathymetric strip (Fig. 1). The organisms, namely plankton, 11 species of fish and dolphin, were collected from the pelagic area of the Romanian seashore in 2016 - 2017 (N = 27) and 2012 - 2013 (N = 24) period. Organisms frozen on bord were defrozen in the laboratory and muscle tissue from dolphin and fish was sampled. Plankton samples were obtained by filtering 1.5 – 2 L of sea water. Samples were freeze-dried and subsequently analysed for heavy metals, polyaromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs). The results were expressed to wet weight.

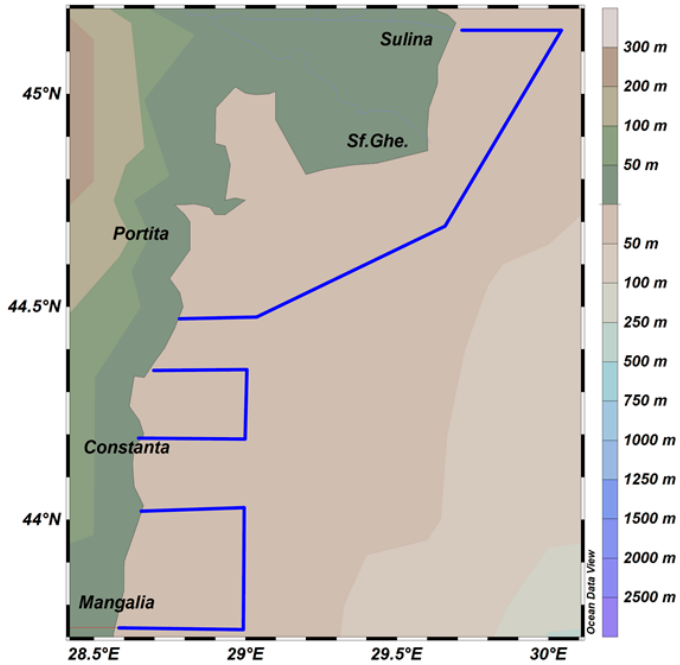

Fig. 1. Biological sampling areas at the Romanian Black Sea coast.

Metal analyses were performed according to IAEA-MEL (IAEA-MEL, 1999). 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 polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polynuclear aromatic hydrocarbons (PAHs) analysis the freeze-dried samples were homogenized and about 2 g of the dried tissue were used for analysis. Internal standards, 2, 4, 5-Trichlorobiphenyl (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 (IAEA-MEL, 1995).

RESULTS AND DISCUSSIONS

Marine ecosystems, including the Black Sea, are biological networks where the success of species depends on numerous biological interactions by which energy is transferred from primary producers (the basis of the food chain) through intermediate consumers to the upper link, predators and back on the detrimental path of decomposition. The aggregate effect of these interactions is the functioning of the ecosystem, which includes nutrient recycling, primary and secondary productivity, through which coastal and marine ecosystems offer natural benefits (Doney et al., 2012). Thus, in marine ecosystems predators eat a variety of food, the size of prey being smaller than those of the predator.

The pelagic area comprises the organisms of the water column that are the basis of most marine trophic chains and contains most of the fishery resources. Contaminants were analyzed in species belonging to five different trophic levels in the pelagic area of the Romanian Black Sea, ie plankton belonging to the basic level - 1, sprat (*Sprattus sprattus* Linnaeus, 1758), anchovy (*Engraulis encrasicolus* Linnaeus, 1758), whiting (*Merlangius merlangus euxinus* Linnaeus, 1758), planktonophagous species belonging to the trophic level 2, Caspian shad (*Alosa caspia* Eichwald,1838), golden grey mullet (*Liza aurata* Risso, 1810), Danube shad (*Alosa immaculata* Bennett,1835), feeding on different species of small fish belong to trophic level 3, horse mackerel (*Trachurus mediterraneus ponticus* Aleev,1956), bluefish (*Pomatomus saltatrix* Linnaeus,1766), garfish (*Belone belone euxini* Günther, 1866), rapacious fish - level 4 feeding on fish belonging to trophic levels 2 and 3, Atlantic bonito (*Sarda sarda* Bloch, 1793), and dolphins (*Tursiops truncatus* ssp. *ponticus* Barabasch, 1940), belonging to level 5, respectively top predators.

Between these species, the relationships are complex, being actually a trophic network (Figure 2).

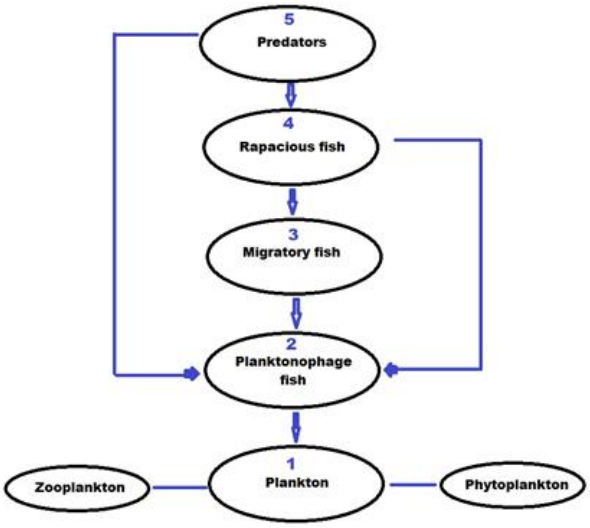

Fig. 2. The pelagic trophic network - the Romanian Black Sea coast.

Polynuclear Aromatic Hydrocarbons

Polynuclear aromatic hydrocarbons are omnipresent contaminants detected in a wide range of both biotic and abiotic environmental matrices, generated primarily by incomplete combustion of organic materials. There are two major types of polynuclear aromatic sources are formed whenever organic substances are exposed to high temperatures under low or even anoxic oxygen conditions (petroleum and coal heat treatment, fuel combustion in cars and trucks, incomplete combustion wood and fuel in heating systems). Petrogenic PAHs come from natural sources and form during the maturing of crude oil. The number and position of the aromatic rings in the molecule determines the physical and chemical properties, the mode of reaction to the environmental factors and their interaction with the organisms.

Concentrations of polynuclear aromatic hydrocarbons varied widely from the detection limit to concentrations of 0.49 μg / g wet tissue. For certain compounds, namely naphthalene, phenanthrene and anthracene, concentrations of 8.06 μg / g wet tissue (phenanthrene) and even higher, respectively 45.86 μg / g wet tissue (naphthalene) and 55.06 μg / g wet tissue (anthracene) were measured (Table 1). These compounds are, moreover, the dominant compounds. This dominance of low molecular weight hydrocarbons and, in particular, naphthalene has also been reported in other marine ecosystems (Dhananjayan & Muralidharan, 2012; Ke et al., 2017). Low molecular weight aromatic hydrocarbons are generally present at higher concentrations in fish tissues because petrogenic PAHs, which have a low molecular weight, appear to be highly bioavailable compared to pyrogenic PAHs of molecular weight which are often associated with particles and are less available for absorption in organisms (Hylland, 2006).

Table 1. Variation of polynuclear aromatic hydrocarbons concentrations in pelagic organisms at the Romanian Black Sea coast.

Polynuclear Aromatic Hydrocarbons (PAHs) [µg/g]*	Average	Minimum	Maximum	Std. Dev.
Naphtalene	1.451	0.0001**	45.867	7.750
Acenaphthylene	0.010	0.0001	0.124	0.027
Acenaphthene	0.013	0.0001	0.136	0.033
Fluorene	0.031	0.0001	0.365	0.080
Phenanthrene	0.580	0.0001	8.059	1.731
Anthracene	2.387	0.0001	55.063	9.793
Fluoranthene	0.014	0.0001	0.215	0.044
Pyrene	0.015	0.0001	0.233	0.047
Benzo(a)anthracene	0.009	0.0001	0.137	0.025
Chrysene	0.011	0.0001	0.201	0.036
Benzo(b)fluoranthene	0.012	0.0001	0.234	0.042
Benzo(k)fluoranthene	0.006	0.0001	0.092	0.017
Benzo(a)pyrene	0.007	0.0001	0.095	0.018
Benzo(g,h,i)perylene	0.008	0.0001	0.103	0.024
Dibenzo(a,h)anthracene	0.006	0.0001	0.106	0.019
Indeno(1,2,3-c,d)pyrene	0.011	0.0001	0.161	0.032
PAHs	4.758	0.0001	106.880	18.450

*wet weight; ** DL=0.0001µg/g

The absence or low concentration of certain polynuclear aromatic hydrocarbons in fish samples can be attributed to rapid biotransformation (Deb et al., 2000). The accumulation and metabolism of polynuclear aromatic hydrocarbons in fish may be influenced by various factors such as route and duration of exposure, tissue lipid content, environmental factors, species differences, age and sex, and exposure to other xenobiotics (Varanasi et al., 1987).

The accumulation of polynuclear aromatic hydrocarbons occurs in all marine organisms. However, tissue concentrations are very variable, depending on their concentrations in the environment, the level and time of exposure, and the ability of species to metabolize these compounds (Meador et al., 1995).

As primary producer (phytoplankton) and consumer (zooplankton) in the marine ecosystem, plankton is the early stage of the bioaccumulation of many chemicals in the environment, especially persistent organic substances, such as polynuclear aromatic hydrocarbons.

The highest concentrations of polynuclear aromatic hydrocarbons, with a mean of approximately 100 times higher than in the other levels, were determined in the plankton samples, while the top predators had the lowest concentrations (Fig. 3). Large concentrations of polynuclear aromatic hydrocarbons in plankton have also been reported in other studies. Although they were not considered bioaccumulative in many organisms, polynuclear aromatic hydrocarbons had the highest concentrations in particulate organic matter (West et al., 2011).

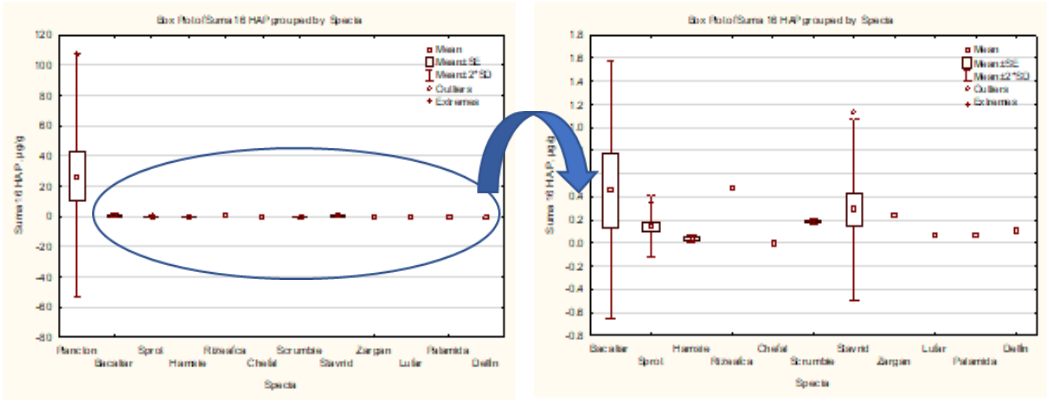

Fig. 3. Variation of PAHs concentrations in pelagic species at different trophic levels at the Romanian Black Sea coast.

Most vertebrates rapidly metabolize and eliminate polynuclear aromatic hydrocarbons. Tissue concentrations of polynuclear aromatic hydrocarbons do not increase (biomagnification) from the lowest to the highest levels of trophic chains (Hylland, 2006).

As contaminant concentrations vary with species and age groups within the same species, the biomagnification process is a less common phenomenon in marine trophic chains (Gray, 2002).

Considering the total concentration of polynuclear aromatic hydrocarbons (sum of 16 PAHs) we can not speak of biomagnification in the studied trophic chain, biomagnification factor (BMF) values being all subunits (Fig. 4).

The analysis of individual compounds highlighted the biomagnification of high molecular mass compounds at the trophic level 3 where BMF values reached 13.2 for chrysene (Fig. 5).

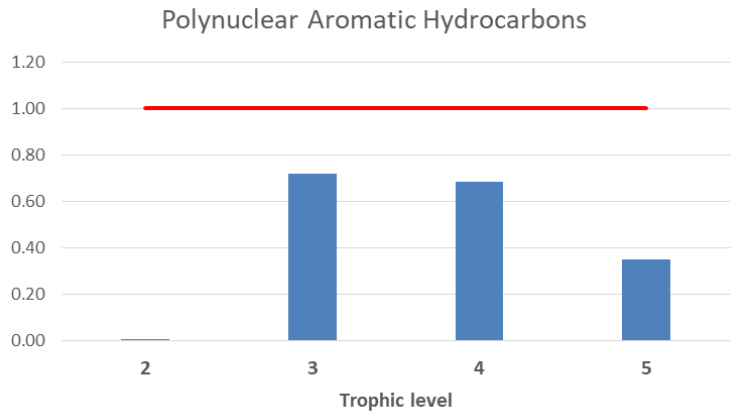

Fig. 4. Biomagnification factor (BMF) of total polynuclear aromatic hydrocarbons in the trophic levels of the pelagic chain at the Romanian Black Sea coast.

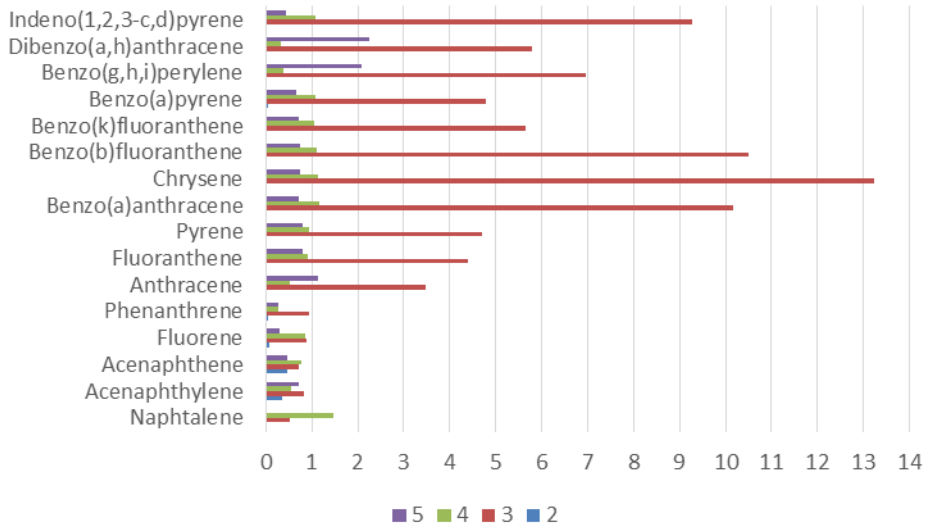

Fig. 5. Biomagnification factor (BMF) of polynuclear aromatic hydrocarbons (individual compounds) in the trophic levels (2-5) of the pelagic chain at the Romanian Black Sea coast.

Organochlorine Pesticides and Polychlorinated Biphenyls

Persistent organic pollutants (POPs) are a matter of global concern. They are highly toxic, long-lasting in the environment and can be transported over long distances away from their area of use, release and emission. Over the last century, large amounts of chlorinated persistent organic pollutants have been produced. Organochlorinated pesticides (OCPs), such as HCH, DDT, dieldrin and clordan, have been widely used in agriculture and sanitation to prevent the spread of malaria and other insect-borne diseases. Polychlorinated biphenyls or PCBs, as they are commonly called, have been used in the industry as plasticizers and dielectric fluids in transformers and capacitors.

Most of the organochlorine pesticides concentrations varied, between "undetected" and 0.3 µg / g wet tissue, but for some compounds were measured grater values (up to 1.64 µg / g wet tissue). The highest values were determined for DDT and its metabolites (Fig. 6).

Polychlorinated biphenyls had most values ranging from "undetected" to 0.17 µg / g wet tissue. For some compounds (PCB 28, PCB 101, PCB 138 and PCB 153), much higher values ranging from 1.8 to 24.55 µg / g wet tissue were measured in 10% of the samples. Higher concentrations were measured for PCBs 28, PCBs 101, PCBs 138 and PCBs 153 (Fig. 7). According to the World Health Organization (2000) (WHO, 2000), PCBs 138 and 153 are the main compounds found in all animal samples. The accumulation of PCB 153 in most species is due to its slow rate of biotransformation and elimination (Dip et al., 2003; Hoekstra et al., 2003).

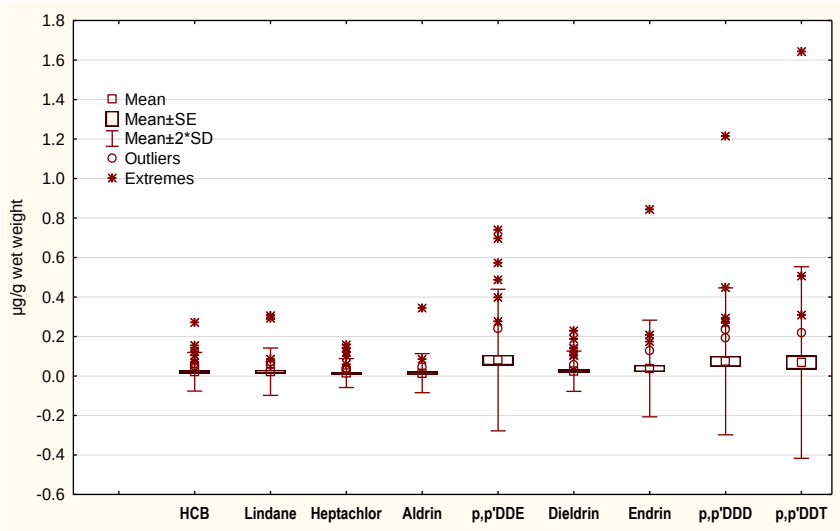

Fig. 6. OCPs variation in pelagic organisms at the Romanian Black coast.

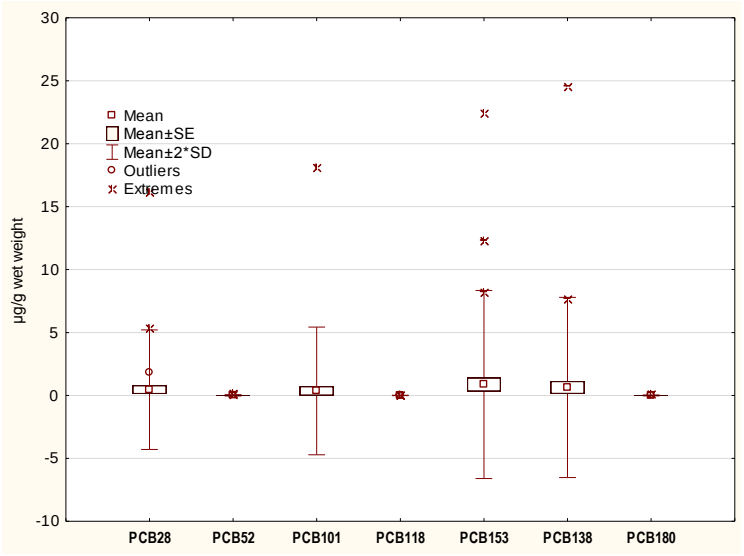

Fig. 7. PCBs variation in pelagic organisms at the Romanian Black coast.

In relation to the total content of organochlorine pesticides and the total content of polychlorinated biphenyls, the largest amounts were found in dolphins. In both cases the increasing trend of average concentrations of persistent organic compounds in trophic levels (1-5) is shown (Fig. 8 and Fig.9). The high content of organochlorine pesticides is mainly due to the bioaccumulation of DDT and metabolites, these being the dominant compounds. Similar results have been obtained on other dolphin species. Research on the *Lagenodelphis hosei* (Fraser's dolphin) in the south-west Atlantic Ocean showed that organochlorine pesticides

were dominant, DDT being the most abundant compound (Durante et al., 2016). Similarly, DDT and its metabolites were the most abundant pesticides measured in Chinese white dolphin, followed by the sum of the isomers HCH, endrin, HCB, dieldrin, aldrin, heptachlor (Gui et al., 2014).

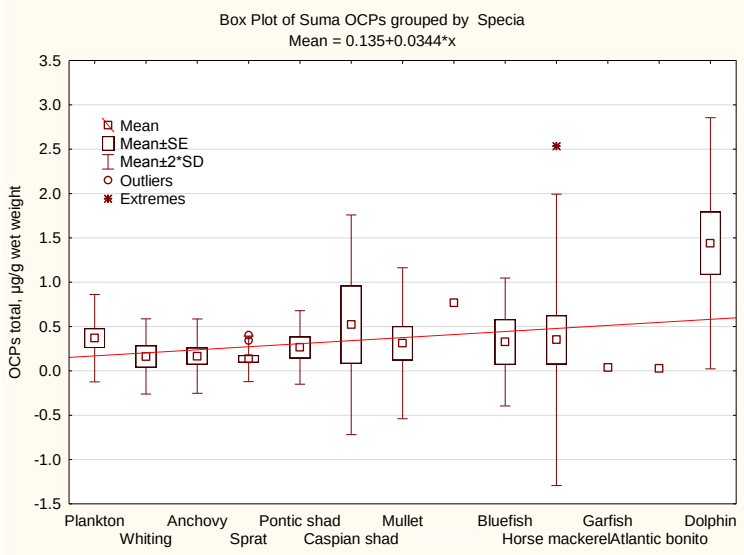

Fig. 8. Variation of OCPs in pelagic species at different trophic levels at the Romanian Black Sea coast.

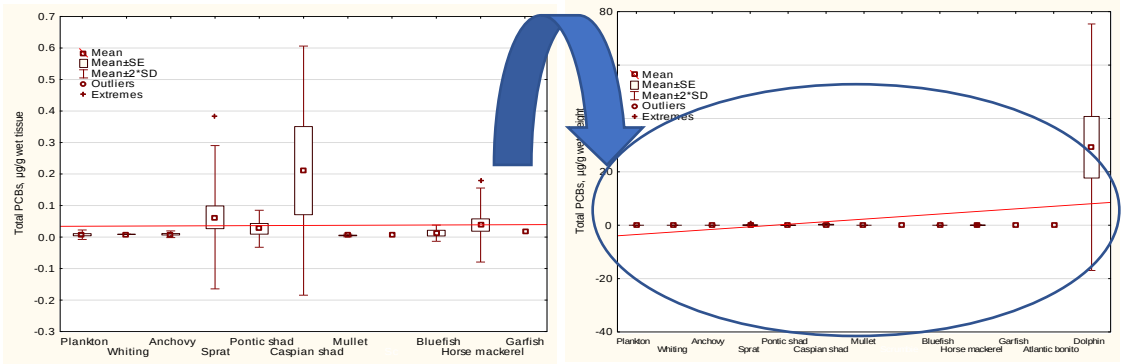

Fig. 9. Variation of PCBs in pelagic species at different trophic levels at the Romanian Black Sea coast.

For this pelagic trophic chain the biomagnification factor had values ranging from 0.57 to 3.60 for organochlorine pesticides and between 0.37 and 724.30 for polychlorinated biphenyls.

The biomagnification process of organochlorine pesticides was observed at trophic level 3 and at trophic level 5 (Fig. 10), while the biomagnification of polychlorinated biphenyls was highlighted at trophic levels 2, 3, and especially 5 (Fig. 11). Marine mammals bioaccumulate and biomagnifies persistent organic pollutants (POPs) through diet (Weisbrod et al., 2001). For instance, PCBs, DDT and HCH concentrations in Chinese white dolphin increased by 99, 212 and 5, respectively, while levels of other organochlorine pesticides increased by 2 to 185 times, indicating a potentially significant biomagnification in the predominant predators (Gui et al., 2014).

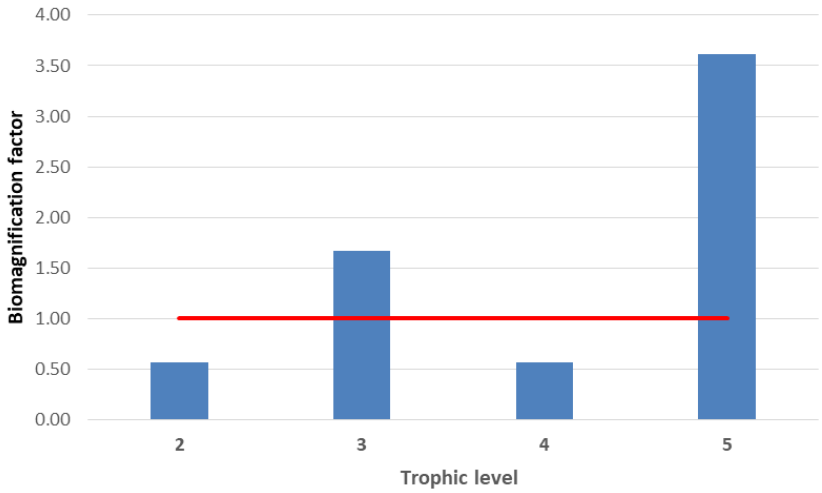

Fig. 10. Biomagnification factor (BMF) of total organochlorine pesticides in different levels of the pelagic trophic chain at the Romanian Black Sea coast.

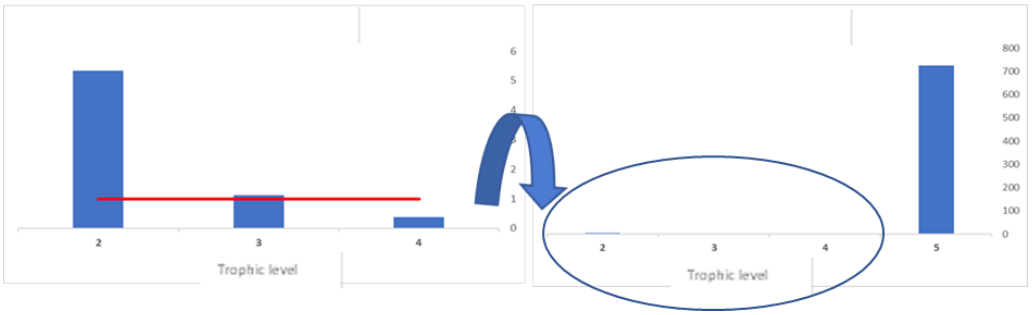

Fig. 11. Biomagnification factor (BMF) of total polychlorinated biphenyls in different levels of the pelagic trophic chain at the Romanian Black Sea coast.

The analysis of the biomagnification process of the individual compounds highlights the differentiated biomagnification of persistent organic pollutants. Larger biomagnification factors correspond to DDT (DDT and metabolites), HCB and endrin between analyzed organochlorine pesticides (Fig.12), and PCBs 28, PCB 101, PCB 138 and PCB 153 (Fig. 13).

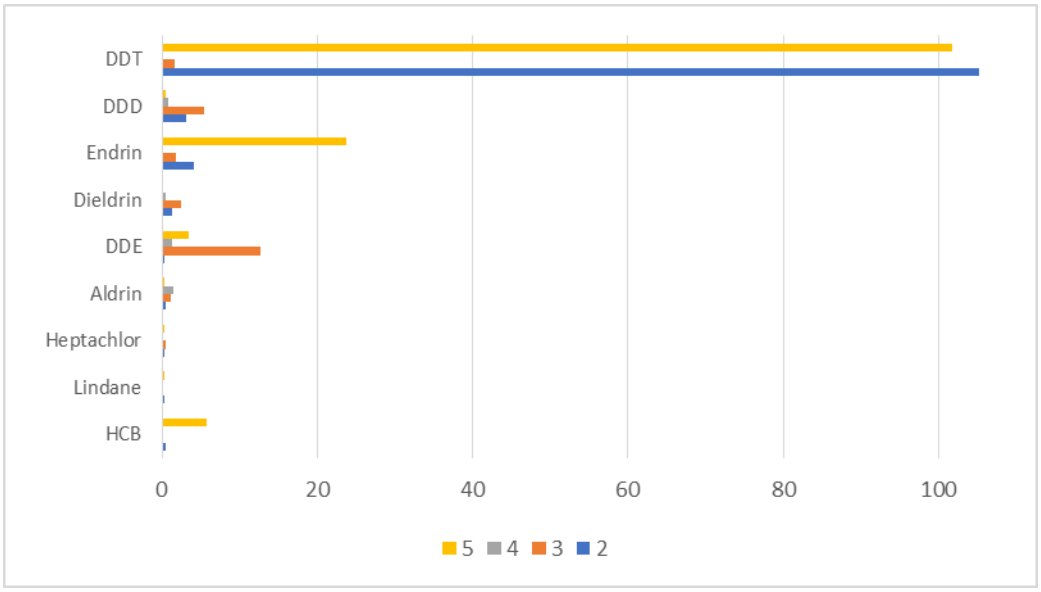

Fig. 12. Biomagnification factor (BMF) of organochlorine pesticides (individual compounds) in levels 2 - 5 of the pelagic trophic chain at the Romanian Black Sea coast.

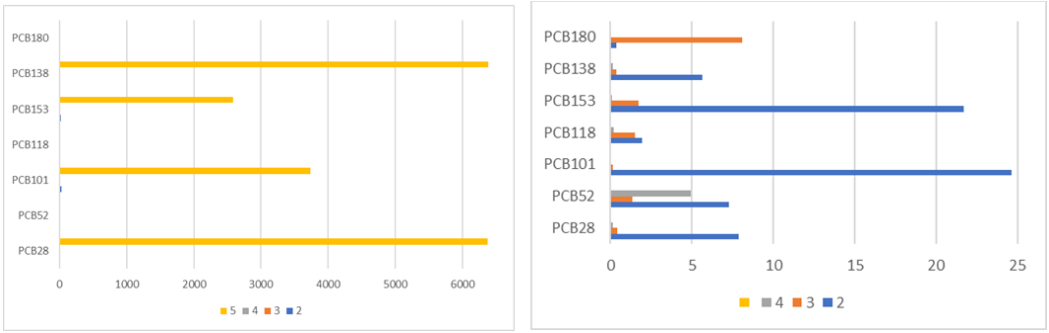

Fig. 13. Biomagnification factor (BMF) of polychlorinated biphenyls (individual compounds) in levels - 5 and 2 - 4 of the pelagic trophic chain at the Romanian Black Sea coast.

Heavy metals

Marine organisms are continually exposed to varying concentrations of metals in marine water, particularly in coastal areas affected by anthropogenic activities, but exhibit a certain selectivity in the accumulation of metals, making a distinction between essential and non-essential metals. Essential metals such as copper, zinc, manganese, iron or cobalt are vital components of many respiratory enzymes and pigments. As a consequence, marine organisms must provide metal tissues in sufficient quantities for metabolic and respiratory needs. The deficiency of these metals, but also the accumulation above certain levels, produces harmful effects (Simkiss & Mason, 1983; White & Rainbow, 1985).

Non-essential metals (lead, arsenic, mercury, cadmium) are very toxic even at very low levels, especially when they accumulate at the level of metabolic sites, interfering with the normal metabolic functions of the essential elements (Depledge & Rainbow, 1990). Thus, although metals are essential components of life, they become detrimental when they are present in excess. Increasing bioavailable levels in the marine environment is a problem for human health and marine ecosystems.

Concentrations of heavy metals in marine organisms samples varied, excluding extreme values, as follow: 2.44 - 24.86 μg / g dry weight copper; 0.08 - 2.97 μg / dry weight cadmium; 0.04 - 8.31 μg / dry weight lead; 0.004 - 36.01 μg / dry weight nickel; 0.004 - 4.74 μg / dry weight chromium. Copper, cadmium, lead and chromium showed significantly higher values in planktonic organisms, compared to the other trophic levels (Fig. 14).

High levels of cadmium have been reported in zooplankton species in the Atlantic, as well as in livers and kidneys of zooplanktonophagous fish (Bustamanet et al., 2003). While organisms at the base of the trophic network, such as unicellular algae, take up predominantly the dissolved metals, organisms located at higher trophic positions are exposed to other sources, such as suspended matter, sediment particles, or feed resources.

Aquatic invertebrates can selectively ingest food particles with the highest nutritional value. It has been shown that the increased presence of algae in suspended or sedimentary particle associations (during phytoplankton flours) almost doubles the efficiency of assimilation of Cd and Zn by planktonivore organisms (Reinfelder et al., 1994). Thus, algal blooms, which contribute to the passage of metals from the water column from the dissolved to the particulate forms, intensify the bioaccumulation processes.

For this pelagic food chain, the biomagnification factor had values ranging from 0.11 to 2.02 copper; 0.07-0.86 cadmium; 0.02 - 4.74 lead; 0.06 - 9.84 nickel; 0.01 - 4.66. The process of biomagnification of copper, lead, nickel and chromium was observed at trophic level 3, copper and chromium, and trophic level 5, while cadmium biomagnification was not highlighted (Fig.15).

Metals biomagnification is defined as an increase of tissue levels at the top of a trophic chain. Theoretically, this occurs when the assimilation efficiency is high and the excretion rate is very low. However, bioaccumulation and biomagnification are not only the result of contaminated food consumption. Tissues are highly specific in their ability to accumulate metals and can also incorporate metals by direct absorption from water or by transfer from other tissues. While taking heavy metals from water through gills is proportional to exposure, the metals taken from fish in food are adjusted so that tissue concentrations are not always proportional to diet levels. Regulatory capacities also differ between different tissues.

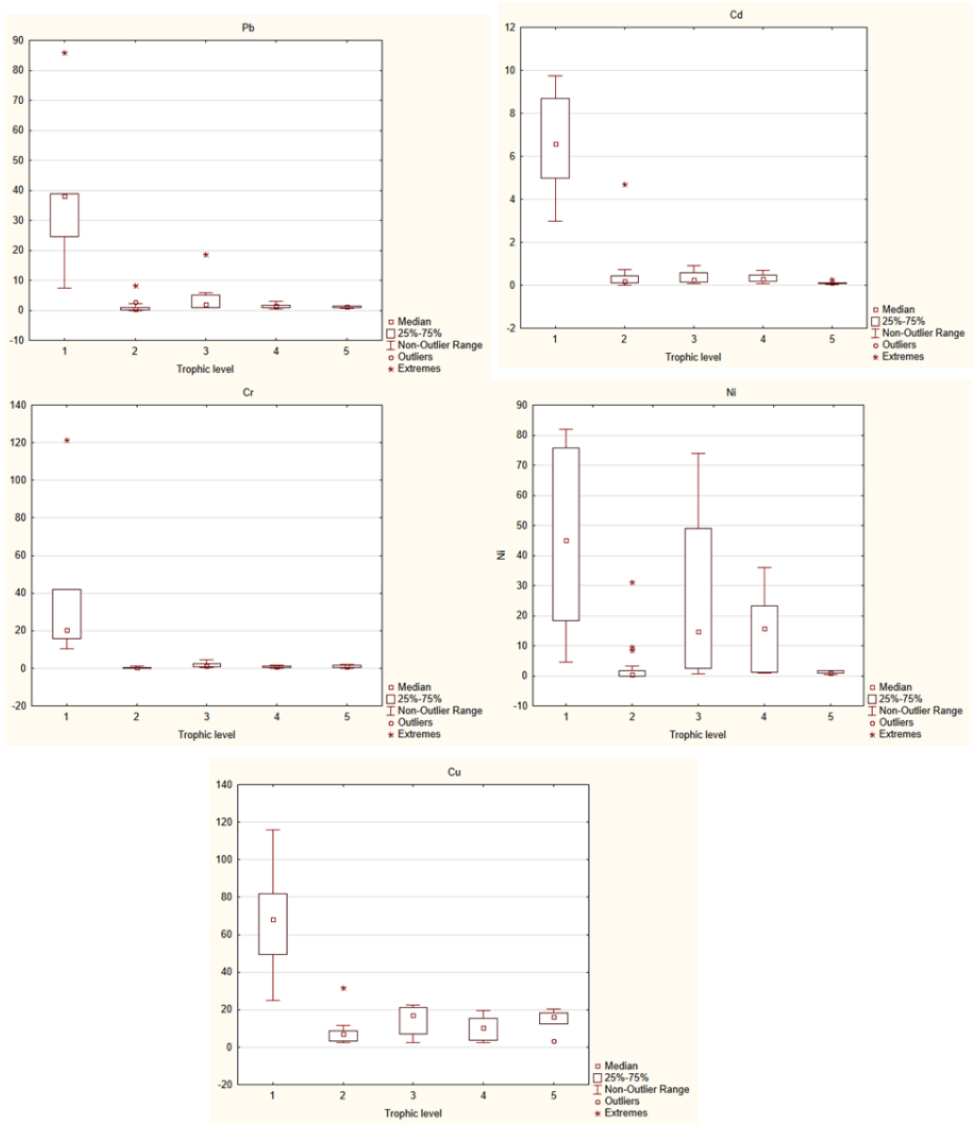

Fig. 14. Variation of heavy metals in different trophic levels at the Romanian Black Sea coast.

In fish, metals can accumulate in certain organs, such as the liver, as a result of exposure but the concentrations of most metals in the muscles (except Hg and Se) are regulated to very low levels. While biomagnification of Hg in predatory fish and marine mammals has been highlighted, it is considered that most other metals do not undergo significant biomagnification processes (Bernhard, 1982).

Literature confirms that the values of certain metals decrease to the upper levels of the trophic chain, the concentrations in *Merluccius merluccius* and *Sarda sarda* being lower than in shrimp (*Parapenaeus longirostris*), which is the food for the two species fish (Bernhard, 1982).

Fig. 15. Biomagnification factor (BMF) of heavy metals (individual compounds) in levels 1 - 5 of the pelagic trophic chain at the Romanian Black Sea coast.

Besides to dietary intake, pelagic organisms are less exposed to high concentrations of metals in the environment, compared to benthic organisms that are in direct contact with sedimentary particles and interstitial water. For example, fish species from the Atlantic have highlighted the importance of trophic and ecological characters, benthic fish exhibiting concentrations of metals in muscles and liver greater than pelagic fish species in the same area (Marcovecchio, 2004).

It should not be forgotten that the bioaccumulation of metals depends not only on biological factors (age, size, reproductive cycle, physiological state), but also on the hydrochemical conditions of the environment, which influence the metals' specificity and indispensability (Marcovecchio, 2004; CIESM, 2002).

CONCLUSIONS

Analysis of contaminants level in the biota, in the main links of a pelagic trophic chain of the Black Sea ecosystem, highlighted the pollutants that represent a significant risk for this ecosystem.

Although not bioaccumulative in many organisms, polynuclear aromatic hydrocarbons had the highest concentrations in particulate organic matter and phytoplankton, respectively. Considering the total concentration of polynuclear aromatic hydrocarbons (sum of 16 PAHs) we can not speak of biomagnification in the studied trophic chain, probably, because most vertebrates metabolize and relatively quickly eliminate polynuclear aromatic hydrocarbons.

Total organochlorinated pesticides and total polychlorinated biphenyls had an increasing trend of their average concentrations in trophic levels (1-5). The highest concentrations were observed in dolphin. The dominant compounds were DDT and its metabolites PCB 28, PCB 101, PCB 138 and PCB 153.

The biomagnification process of organochlorine pesticides was observed at trophic level 3 and at trophic level 5, while biomagnification of polychlorinated biphenyls was highlighted at trophic levels 2, 3, and especially 5.

Heavy metals, except for nickel, showed significantly higher levels in planktonic organisms, compared to other trophic levels. The biomagnification process of copper, lead, nickel and chromium was observed at trophic level 3, copper and chromium, and trophic level 5, while cadmium biomagnification was not observed.

Acknowledgements

This research was partially financed by the Ministry of Research and Innovation, through the Programme NUCLEU, contract no. 35 N / 15.03.2016 "BLUE GROWTH" ORIENTED RESEARCH AIMING AT DEMONSTRATING THE BLACK SEA OPPORTUNITIES AND ITS VALUATION - PROMARE.

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