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Heavy metals accumulation in plankton and water of four aquatic complexes from Danube Delta area

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Abstract. The aim of the present study is to determine the accumulation of heavy metals in plankton collected from four aquatic complexes: Somova-Parcheş, Şontea-Fortuna, Holbina-Dunavăţ (with the highest concentrations of heavy metals) and Matiţa-Merhei, from the Danube Delta area, one of the most important Biodiversity Reserve in Europe. In order to achieve this study, the concentrations of six heavy metals were taken into account: cadmium, chromium, nickel, lead, manganese, and zinc. The study was conducted during a five year period 2007-2011 and the statistical analysis were based on mean concentrations, expressed in µg/g. Samples were digested at the Anton Paar microwave oven and analyzed by mass spectrometry with inductively coupled plasma (ICPMS). High values of bioconcentration factor in plankton (BCF) have been found for cadmium; expressed as a ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment. Also, the results show that plankton has a very high potential of heavy metals accumulation, most significant variation of heavy metals concentrations in plankton is depend on the aquatic complexes position from the Danube River and also on the influence of anthropogenic sources.
Keywords: nature reserve, aquatic ecosystem, bioconcentration, biodiversity, water pollution.

Rezumat. Scopul acestui studiu este de a determina acumularea metalelor grele în planctonul colectat din patru complexe acvatice, anume: Somova-Parcheş, Şontea-Fortuna, Holbina-Dunavăţ (cu cele mai mari concentraţii de metale grele) şi Matiţa-Merhei, din zona Deltei Dunării, una dintre cele mai importante Rezervaţii ale Biodiversităţii din Europa. Pentru a realiza acest studiu, au fost luate în considerare concentraţiile a şase metale grele: cadmiu, crom, nickel, plumb, mangan şi zinc. Studiul a fost realizat în decursul a cinci ani 2007-2011. Pentru analizele statistice s-au folosit concentraţii medii, exprimate în µg/g. Probele au fost mineralizate la cuptorul cu microunde Anton Paar şi analizate prin spectrometrie de masă cu plasmă cuplată inductiv (ICPMS). Valori mari ale factorului de bioconcentrare (BCF) în plancton au fost găsite pentru cadmiu; exprimat ca raport dintre concentraţia unei substanţe chimice în interiorul unui organism şi concentraţia acesteia în mediul înconjurător. De asemenea, rezultatele arată că planctonul are un potenţial foarte ridicat de acumulare a metalelor grele, variaţii semnificative ale concentraţiilor de metale grele în plancton depinzând de poziţia complexului acvatic faţă de Dunăre, precum şi de influenţa surselor antropice.
Cuvinte cheie: rezervaţie naturală, ecosistem acvatic, bioconcentrare, biodiversitate, poluarea apelor.

Introduction. It is known that metals are natural components of aquatic ecosystems where they are found in relatively low concentrations. The main sources of microelements, heavy metals in natural waters are related to natural processes and human impact. Heavy metals and many of the majority of industrial pollutants cannot be degraded and, therefore, they are found as accumulations in water, soil, bottom sediments and living organisms. Also, they are considered to be one of the most important species of aquatic ecosystems pollutants due to their environmental persistence and tendency to be concentrated in aquatic organisms.

In addition, heavy metals present cumulative toxic effects at very low concentration on the aquatic organisms including plankton, aquatic plants, invertebrates and vertebrates (Schüürmann & Markert 1998). Heavy metals enter in the natural aquatic environment, by processes of erosion and dissolution of rocks or anthropogenic by accidental pollution or improper treatment of industrial and domestic wastewater (Agbozu et al 2007). Typical results of the human activities have as result in an increased levels of heavy metals present in freshwater and among these: cadmium, chromium, nickel, lead, manganese, and zinc are most specific (Farkas et al 2001). There is a need to quantify also the external influences from the Danube situated close to study area, which have a significant impact on these sensitive ecosystems and analyze, especially, heavy metals accumulation in water, plankton and sediment (Iticescu et al 2013), because health of aquatic organisms is directly influenced by heavy metal pollution and affected by their tendency to bioconcentration in different tissue types (plant, animal or human) (Popek et al 2008, 2009; Mudgal et al 2010a,b; Petrescu-Mag & Petrescu-Mag 2010; Petrescu-Mag et al 2010; Oroian et al 2013; Ndome et al 2014; Riani et al 2014; Ernawati 2014; Petrovici & Pacioglu 2010).

Plankton has a major role in the food chain of aquatic environment like the rest of plants and animal organism and represents the main food source from the entire food chain circle with indirect influence on human population which is exploiting water resources for food. The plankton (phytoplankton and zooplankton) has also the tendency to bioconcentrate heavy metals and it is found in all types of aquatic ecosystems, the degree of bioconcentration depending of species and seasonality (Roy et al 2010). Bioconcentration is a process whereby a substance is taken and up concentrated by living organisms from their environment and diet and stored in the body. It includes the effect on an organism's internal concentration as a result of the organism taking up a chemical via the respiratory surface and skin (uptake), moving it internally (distribution), changing it (metabolism) and returning it to the environment (elimination).

Because water quality depending directly on development of industries (Tampus et al 2014) sustainable water management first of all should include beside other aspects political commitment and a steady legal background (Cojocariu et al 2011; Ndome et al 2014).

The purpose of this study is to determine the heavy metals (Cd, Cr, Ni, Pb, Mn, Zn) amount accumulated in water and plankton from four important aquatic complexes (Somova-Parcheş, Şontea-Fortuna, Holbina-Dunavăţ and Matiţa-Merhei) considered protected areas from Danube Delta and to assess the bioconcentration factors from plankton which is used as a indicator in determining the heavy metal trace from aquatic ecosystems.

Another goal is to compare the specific accumulation of heavy metals in biological systems within different aquatic ecosystems situated in the same area but influenced by different positions within the Danube Delta, different parameters, properties and behavior.

Material and Method

Study was conducted in the Danube Delta Biosphere Reserve (DDBR), which extends between the arms of Chilia in the north (117 km to Black Sea), Tulcea (19 km) and Sfântu Gheorghe (109 km to Black Sea) (Gâştescu & Stiucă 2008) (Figure 1). Therefore, four representative aquatic complexes in Danube Delta Biosphere Reserve were selected, respectively: Somova-Parcheş (pre-deltaic area, west part of DDBR), Şontea-Fortuna (between the arms of Chilia and Sulina), Holbina-Dunavăţ (ecological restoration complex located in southern area) and Matiţa-Merhei (in the north area of DDBR).

Sampling and preservation of water and plankton. The water samples were collected according to standard SR ISO 5667/1998. To determine the metal content, 500 mL water was collected and preserved with 2.5 mL of concentrated nitric acid in non-filtered form.

To evaluate the concentrations of heavy metals in plankton, the representative samples were obtained by filtration of 500 L of surface water, through plankton net (35 mm mesh size). The filtrated material was fixed with nitric acid (concentration 65%). Plankton samples were prepared for digestion using Buchner funnel on a filter (1 μm), conditioned to a constant mass (St-Cyr et al 1997).

Figure 1. Map of Danube Delta Biosphere Reserve.

Sample preparation and instruments. The mineralization stage was made differently depending on the type of sample in Anton Paar oven.
Water samples. 25 mL of sample and 5 mL HNO_3 were introduced in quartz vessels of Anton Paar oven. After a short pre-reaction time (10 min), the vessels were hermetically sealed using a special device which is inserted into a protective sheath, covered and then placed properly in the rotor. The energy rises to 1200 W in 5.5 minutes and is maintained at this power for 4.5 minutes. The total mineralization process lasts 10 minutes and the total cooling time is 30-35 minutes. After completing the program and the cooling time, vessels are opened and the content is removed in balloons of 50 mL and brought to the mark with bi-distilled water.
Plankton samples. In quartz vessels was weighed 1-2 g of plankton, and then was added 5 mL HNO_3 and 2 mL H_2O_2. After a short time of pre-reaction (15 min) the vessels are closed with special lids, place in the sheath with secured protective cap, and then place the rotor properly. The energy slowly rises at 600 W in 5.5 minutes and maintained at this power for 4.5 minutes, then rises to 1000 W and maintained this power for 10 minutes. The total mineralization time is 20 minutes and the total cooling time is 20-25 minutes. After completing the program and the cooling time, the quartz vessels are opened and the content is removed in balloons of 50 mL and brought to the mark with acidified water. The heavy metals concentrations from plankton were expressed in μg/g dry matter (SR EN ISO 17294-2 2005).
Calibration solutions. For all the heavy metals are made the flow charts and the calibration curves using the Perkin Elmer Pure Plus Atomic Spectroscopy Standard, certified reference material 10 μg/mL, Multi-element ICP-MS. The calibration curves are made in five linear points using the excel interface. The coefficient R2, for the calibration curves takes values between 0.9994-0.9997 which represent a very good correlation between the intensity and the standard concentration.

The heavy metals concentrations were analyzed using the ICP MS Elan DRC-e. Inductively coupled plasma-mass spectrometry which is useful to analyze the small concentrations of a large number of elements. The optimization solution used, was ELAN 6100Setup /Stab./Masscal. Solution. The optimization solution contains the elements:

Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, U. The labeled concentrations are 10 ppb for each element (SR EN ISO 17294-2 2005).

Bioconcentration factor (BCF). The bioconcentration level depends on the nature of the chemical compound, species, duration of exposure, concentration in water and its accumulation level in plankton (Ivanciuc et al 2006). In relation to the bioconcentration factor definition, bioaccumulation factor represents the ratio between the element concentration in tissue and in the water.

Results and Discussion

Heavy metals accumulation in water samples. In accordance with the Water Framework Directive (no.2000/60/C.E.), transposed into Romanian legislation by Order M.M.G.A. no.161/2006, the concentrations of all six heavy metals concentrations analyzed (Cd, Mn, Pb, Ni, Cr, Zn) from water samples, have been reported to class quality II (good ecological status). Except for zinc, in each complex were registered excess of heavy metals concentrations (Table 1).

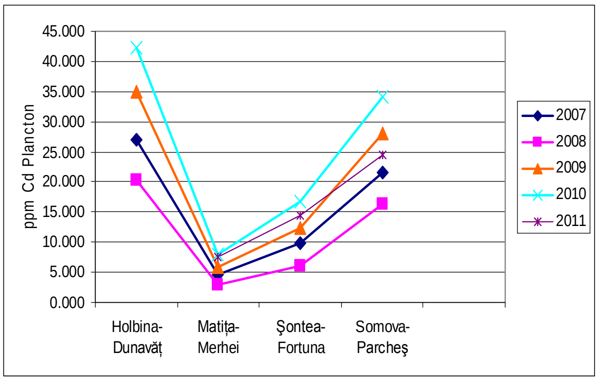
Table 1

Multi-annual concentrations of heavy metals in water samples

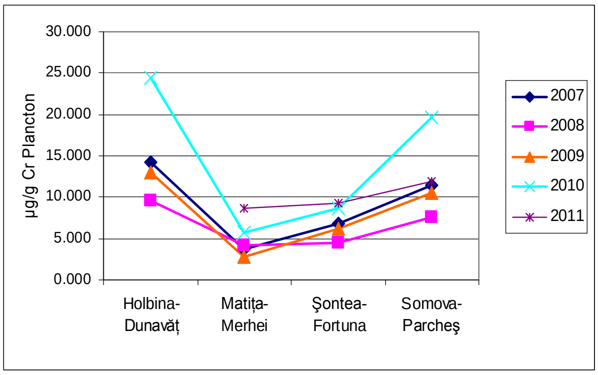
		2007	***2008***	***2009***	***2010***	***2011***
Holbina - Dunavaț	Cd µg/L	8.30	8.67	9.13	10.47	11.05
	Mn µg/L	152.97	210.48	92.32	147.85	136.83
	Pb µg/L	42.61	48.04	32.52	35.99	39.37
	Ni µg/L	86.18	38.16	41.10	98.55	54.95
	Cr µg/L	81.24	72.56	71.81	55.39	73.12
	Zn µg/L	333.78	249.58	310.79	312.02	209.12
Șontea – Fortuna	Cd µg/L	7.11	6.33	9.33	9.50	8.00
	Mn µg/L	241.72	171.17	54.67	156.67	125.50
	Pb µg/L	21.39	34.67	28.22	11.33	17.00
	Ni µg/L	104.28	31.67	89.67	92.33	41.17
	Cr µg/L	26.00	32.67	35.00	56.67	47.33
	Zn µg/L	144.56	124.50	178.89	197.33	172.00
Matița – Merhei	Cd µg/L	7.80	6.27	7.69	10.09	7.28
	Mn µg/L	199.82	251.74	88.38	172.17	161.24
	Pb µg/L	24.33	36.02	29.35	23.21	32.11
	Ni µg/L	67.97	27.15	39.50	88.82	85.38
	Cr µg/L	28.59	47.67	47.67	61.27	41.72
	Zn µg/L	230.59	165.54	187.15	182.27	161.40
Somova - Parcheș	Cd µg/L	5.82	5.80	9.26	6.60	4.21
	Mn µg/L	154.80	135.50	96.45	139.50	121.45
	Pb µg/L	9.29	8.23	6.81	7.31	6.93
	Ni µg/L	64.05	47.90	35.85	48.70	47.80
	Cr µg/L	56.80	28.65	29.40	56.23	58.50
	Zn µg/L	175.95	167.95	128.35	125.58	150.60

Results revealed significant variations in the concentrations of heavy metals in the whole period studied, depending on water intake of the Danube (it is practically the end zone after the 9 countries and several industrial and agricultural pollution sources) and industrial area near Tulcea City (Somova-Parcheş), and the anthropic impacts with influences on Holbina-Dunavat complex. Moreover, it can be observed a downward trend in concentrations of Pb and Zn in Somova-Parcheş complex, possibly due to reduced emissions from industrial activity near Tulcea City. Also, manganese concentrations were decreased in 2009, most likely due to low rainfall, which reduced the amount of pollutants entrained in water from the soil surface. Cadmium registered significant variations over the period studied, most likely due to the significant contribution of the Danube water.

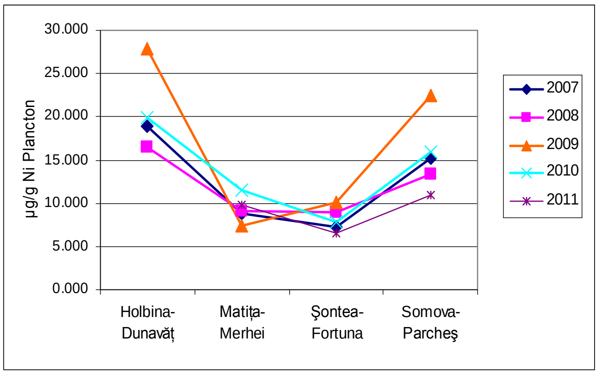
Heavy metals accumulation in plankton. The dynamics of heavy metals concentrations from plankton samples from the four aquatic complexes is represented by the average of the annual samplings that include three (one per spring, summer and autumn season) it is shown in figure 2.



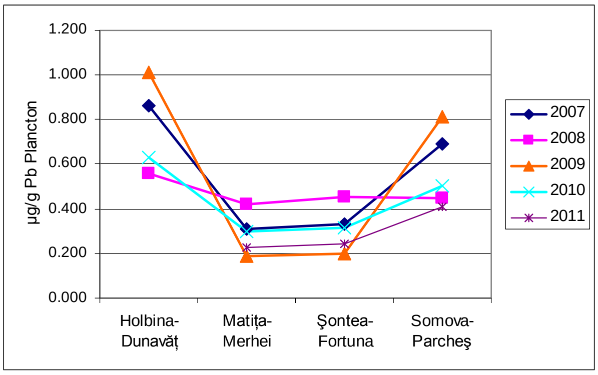
2.a. Cadmium accumulation in plankton



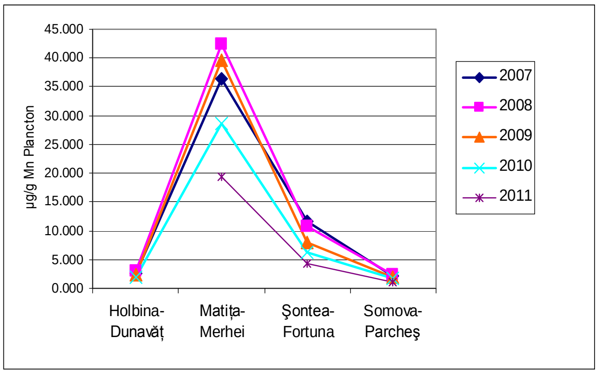
2.b. Chromium accumulation in plankton

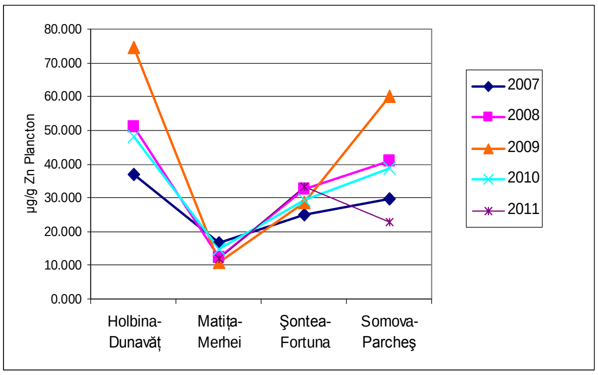


2.c. Nickel accumulation in plankton



2.d. Lead accumulation in plankton collected





2.e. Manganese accumulation in plankton

2.f. Zinc accumulation in plankton

Figure 2. Heavy metals accumulation in plankton collected from the four studied aquatic complexes: Somova-Parcheş, Şontea-Fortuna, Holbina-Dunavăţ and Matiţa-Merhei.

It was observed that phytoplankton from Somova-Parches aquatic complexes, Sontea-Fortuna, Holbina-Dunavat and Matita-Merhei is generally dominated by diatoms, especially in the spring season and the flowering water with diatoms is dominated by a wide range of taxa. Cyanobacteria grow in abundance especially after June. Blooms of cyanobacteria and diatoms are not unique mutual. Other taxa (Cryptophyte and Chrysophyte) are much less abundant. Zooplankton is also rich and is represented mainly by rotifers and copepod crustaceans and cladocerans. Common phytoplankton species were diatoms species: *Asterionella sp.*, *Synedra acus*, *S. ulna*, *Fragilaria sp.*, *Melosira sp.*, followed by common cloroficee: *Scenedesmus quadricauda*, *Pediastrum duplex* and cianoficee: *Anabaena sp.*, *Oscillatoria sp.* Common zooplankton species were: *Daphnia sp.*, *Eurycercus sp.*, *Simocephalus sp.*, *Sida sp.*, *Diaphanosoma sp.*, *Holopedium sp.*, *Leptodora sp.*, *Bosmina sp.* (cladocerans).

Copepods were encountered throughout the study in all stages of development. Numerical phytoplankton growth entails also an increasing number of zooplankton populations; such rotifers thrive early in the same time with diatoms and cladocerans and copepods later, soon after the emergence and development of blue-green algae.

The results showed significant differences in the metal concentrations in plankton from different aquatic complexes. For Cd, the concentration varied between 2.874 µg/g (2008) in aquatic complex Matita-Merhei and 42.356 µg/g (2010) in aquatic complex Holbina - Dunavat (Table 1.a.), similar results being reported by Atici et al 2010.

By analyzing the concentrations of Cr from all the studied complexes is observed that the maximums of 24.362 µg/g were recorded in 2010 in the aquatic complex Holbina-Dunavat followed by complex Somova-Parches 19.568 µg/g in the same year (Figure 2.b.). Sontea-Fortuna and Matita–Merhei have recorded similar values in the range of 3.658 µg/g – 8.621 µg/g (Matita–Marhei) respective 4.535 µg/g – 9.224 µg/g (Sontea–Fortuna).

The highest concentration of Mn values were identified between 2007-2009 (Figure 2.e.), with values ranging between 26.365 µg/g – 29.714 µg/g, in Somova-Parches aquatic complex, possibly caused by the geographical position of complex located in the vicinity of slurry dumps with waste from the aluminum industry, as well as a combination of ferrous alloys, which in recent years has significantly reduced its activity.

Minimum values of Mn were observed in complexes Matita-Merhei 1.104 µg/g and Sontea-Fortuna 2.016 µg/g. The minimum and maximum concentrations of Ni (Figure 2.c.) were registered in 2009, with values between 7.440 µg/g (Matita-Merhei) and 27.890 µg/g (Holbina–Dunavat).

Compared to the rest of analized heavy metals, Pb concentrations (Table 1.d.) recorded the lowest values contained between 0.118 µg/g (Matita-Merhei) si 1.014 µg/g (Holbina-Dunavat). It was observed that Zn (Table 1.f.) showed the highest concentration in plankton samples, its values in all the four complexes analyzed ranging between 74.644 µg/g, (Holbina–Dunavat) and 10.959 µg/g (Matita-Merhei). Analysing the average concentrations of heavy metals in plankton samples from the entire studied period (Figure 2.a–2.f.), it was observed that the higher concentration of heavy metals in aquatic plankton is found in complex Holbina-Dunavat except Mn, followed by complexs Somova-Parches, Matita-Merhei and Sontea-Fortuna, who recorded similar values, except for Zn.

This differentiation between aquatic complexes is most likely caused by geographic location (proximity to different pollution sources) and the significant contribution of the Danube River (the main water supply source).

Bioconcentration factor of heavy metal trace in plankton from the four sites of the Danube Delta. Bioconcentration factor was calculated for all four aquatic complexes, using on the following formula:

$$BCF = \frac{\text{concentration of metal in plankton samples}}{\text{concentration of metal in water samples}}$$

Analyzing bioconcentration factor values calculated (Table 2), study revealed that cadmium present the highest bioconcentration values (5.840 on Somova-Parcheş in 2011), results being comparable to those reported by Hong et al (2005), followed by nickel with values up to 0.679 registered on Holbina-Dunavat in 2009. Zinc values ranged between 0.073 (on Matita-Merhei in 2007 and 2011) and 0.467 (on Somova-Parcheş in

2009), followed closely by chromium values who ranged between 0.058 (on Matita-Merhei in 2009) and 0.440 (on Holbina-Dunavat in 2010). The lowest values of bioconcentration factor were registered by lead with values ranging from 0.007 (Şontea-Fortuna 2009) to 0.120 (Somova-Parcheş 2009), followed by manganese values between 0.006 (Matita-Merhei 2011) and 0.307 (Somova-Parcheş 2009), results comparable with those reported by Chen et al (2000). Moreover, bioconcentration ability of these metals is of particular interest due to their toxicity even at very low concentrations.

Table 2

The bioconcentration factor (BCF) values for plankton samples during 2007-2011

		2007	***2008***	***2009***	***2010***	***2011***
Holbina - Dunavaț	BCF_{Cd}	2.946	2.334	4.204	4.044	2.095
	BCF_{Mn}	0.174	0.131	0.182	0.440	0.154
	BCF_{Pb}	0.020	0.012	0.031	0.017	0.019
	BCF_{Ni}	0.220	0.434	0.679	0.202	0.317
	BCF_{Cr}	0.075	0.051	0.085	0.042	0.031
	BCF_{Zn}	0.111	0.204	0.240	0.154	0.212
Șontea – Fortuna	BCF_{Cd}	1.370	0.953	1.319	1.765	1.807
	BCF_{Mn}	0.259	0.139	0.177	0.152	0.195
	BCF_{Pb}	0.016	0.013	0.007	0.028	0.014
	BCF_{Ni}	0.070	0.284	0.113	0.085	0.159
	BCF_{Cr}	0.010	0.018	0.043	0.013	0.020
	BCF_{Zn}	0.173	0.262	0.159	0.149	0.194
Matița – Merhei	BCF_{Cd}	0.595	0.458	0.762	0.791	1.042
	BCF_{Mn}	0.128	0.087	0.058	0.093	0.207
	BCF_{Pb}	0.013	0.012	0.006	0.013	0.007
	BCF_{Ni}	0.131	0.336	0.188	0.129	0.116
	BCF_{Cr}	0.011	0.010	0.021	0.010	0.006
	BCF_{Zn}	0.073	0.075	0.059	0.082	0.073
Somova - Parcheș	BCF_{Cd}	3.715	2.803	3.028	5.155	5.840
	BCF_{Mn}	0.200	0.267	0.356	0.348	0.202
	BCF_{Pb}	0.075	0.054	0.120	0.069	0.059
	BCF_{Ni}	0.238	0.278	0.625	0.328	0.228
	BCF_{Cr}	0.170	0.242	0.307	0.134	0.069
	BCF_{Zn}	0.169	0.244	0.467	0.307	0.150

After analyzing the bioconcentration factor of the four studied aquatic complexes shows that in the period studied (2007-2011), the aquatic complex Somova-Parcheş highlights great ability to bioconcentrate, followed by aquatic complex Holbina-Dunav. The lowest bioconcentration factor was recorded in the aquatic complex Sontea-Fortuna, followed by the values found in complex Matita-Merhei. The bioconcentration factor values can be correlated with the Danube influence and the industrial polluter influence on Somova-Parcheş complex, and for the other three complexes the results can be correlated with distance from the Danube, which means a decrease of heavy metals influence in this areas.

Conclusions. The content of heavy metals in studied plankton varies significantly from one aquatic complex to another depending on the level of concentrations of heavy metals in water. More than that in the results obtained by calculating the bioconcentration factor (BCF), was came to the conclusion that the plankton present a great capacity for accumulation of cadmium and zinc in the conditions and influences of the Danube Delta Biosphere Reserve.

Analyzing the results it was observed that evolution of heavy metal bioconcentration in plankton samples during 2007-2011 can be characterized by an upward trend in the following order: BCF_{Cd} > BCF_{Ni} > BCF_{Cr} > BCF_{Zn} > BCF_{Mn}> BCF_{Pb} except for complex Sontea - Fortuna which follow a successive increase after the model: BCF_{Cd} > BCF_{Zn} > BCF_{Cr} > BC_{Ni} > BCF_{Pb}> BCF_{Mn}. This is a previously trend, but we must consider that changes in concentrations of heavy metals in studied plankton can be also correlated with extreme hydrological events like floods and flash floods that have occurred over the monitored years. Furthermore the results are comparable with the study of Hong et al (2005).

Considering the results, the bioconcentration factor can be used as a tool to describe the bioconcentration of heavy metals in the polluted aquatic environment. Investigations regarding the bioconcentration of heavy metals in plankton of four aquatic complexes (Somova-Parcheş, Şontea-Fortuna, Holbina-Dunavăţ and Matita-Merhei) were performed for the first time; therefore, the results presented could be very useful for checking the health of similar water bodies. It is well known that the ecological status of many rivers is strongly affected by human activities and different types of pollution might cause environmental risk for an aquatic ecosystem Nabekova et al (2004), therefore, these results can be considered elementary information for more detailed basis of future investigations on these aquatic complexes.

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