REPORT ON THE ACCUMULATION OF HEAVY METALS IN THE FEATHERS OF SOME WETLAND BIRDS IN THE DANUBE DELTA (ROMANIA)

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An assessment was carried out in the Danube Delta Biosphere Reserve (Romania, 2015) regarding heavy metal residues in the feathers of native wild bird species that depend on wetlands for all or part of their life cycle. The concentrations of trace elements (As, Cr, Mn, Ni, Pb and Zn) were determined in the feathers of Ferruginous Duck, White Stork, Common Little Bittern, Little Egret, Great White Pelican, Pygmy Cormorant, Common Tern and Western Marsh-harrier. Arsenic concentrations varied between 1.610 mg/kg (Ferruginous Duck) and 3.437 mg/kg (Great White Pelican). The highest chromium concentrations were found in Pygmy Cormorant (11.063 mg/kg) and Great White Pelican (12.368 mg/kg); the highest zinc concentration was found in Ferruginous Duck (163.237 mg/kg). The highest concentrations of manganese and nickel were found in the White Stork (66.541 mg/kg – Mn; 6.276 mg/kg – Ni), Western Marsh-harrier (59.037 mg/kg; 4.075 mg/kg) and Little Egret (64.347 mg/kg; 5.900 mg/kg). The concentration of lead showed values between 3.474 mg/kg (Western Marsh-harrier) and 0.426 mg/kg (Ferruginous Duck), while the other studied species had concentrations up to 0.9 mg/kg. Laboratory analyses revealed important interspecific differences and a high capacity of waterbirds for storing heavy metals in plumage.

Keywords: heavy metals, feathers, birds, Danube Delta, Romania.

INTRODUCTION

At the European level, a series of natural and anthropic limiting factors affect wild bird species, most of which are protected by a number of European and international directives and conventions. Heavy metals are among these limiting factors. There have been several studies regarding heavy metal bird poisoning in Europe (Lebedeva *et al*., 1997; Hernandez *et al*., 1999; Berglund *et al*., 2011; Kitowski *et al*., 2014).

In Romania, there have been several studies regarding heavy metal concentrations in abiotic elements, plants and fish (Dinescu *et al*., 2004; Tudor *et al*., 2006; Vignati *et al*., 2013; Ilie *et al*., 2014; Ionescu *et al*., 2015; Burada *et al*., 2014; 2015; 2016; 2017; Ștefănuţ *et al*., 2018). However, studies focusing on bird species are lacking (Fasolă-Mătăsaru *et al*., 2017). In order to evaluate the degree of heavy metal accumulation in bird species of Community interest from the Danube Delta Biosphere Reserve, a non-invasive protocol was applied. According to literature, this type of monitoring can show annual variations in heavy metal concentrations due to the fact that these substances are integrated into new plumage during the moulting period (Frantz *et al*., 2012; Gushit *et al*., 2016).

The present study was carried out during a colonial waterbird breeding census and the monitoring of bird species of Community interest from the Danube Delta Biosphere Reserve on the Romanian territory.

MATERIAL AND METHODS

All the samples were collected (May to August, 2015) in the Danube Delta Biosphere Reserve [DDBR, Gâștescu & Știucă (2008)] and its immediate vicinity, in the eastern part of Romania. Sampling locations included several reas: Murighiol, Uzlina Lake, Nufăru, Maliuc fishpond, Sălcioara fishpond, Nebunu Lake, Fortuna Lake and Draghilea channel (Fig. 1).

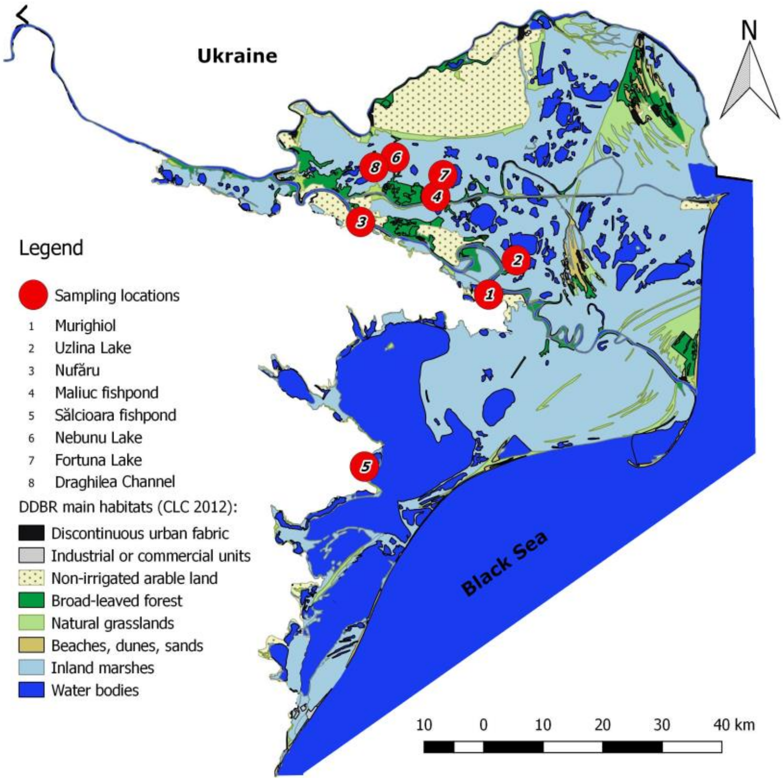

Fig. 1. Locations of sample collection in the Danube Delta Biosphere Reserve.

We sampled moulted flight feathers in feeding and roosting areas, as well as feathers collected from dead individuals (approximately 5 g of feathers per individual). All the samples were primary flight feathers (remiges), as it has been shown that different feathers accumulate trace elements in different concentrations (Guo *et al*., 2001). The concentration of trace elements (As, Cr, Mn, Ni, Pb and Zn) was determined for Ferruginous Duck – *Aythya nyroca* (1 individual), White Stork – *Ciconia ciconia* (5 individuals), Common Little Bittern – *Ixobrychus minutus* (1 individual), Little Egret – *Egretta garzetta* (2 individuals), Great White Pelican – *Pelecanus onocrotalus* (1 individual), Pygmy Cormorant – *Phalacrocorax pygmeus* (1 individual), Common Tern – *Sterna hirundo* (3 individuals) and Western Marsh-harrier – *Circus aeruginosus* (1 individual).

Sample analysis was conducted by acid mineralization, necessary for trace elements determination, with the help of an Anton Paar Multiwave 3000 microwave oven. The trace element content was analysed using the ICP-MS Elan DRC-e, which is applicable for the determination of small concentrations of a large number of elements. Approximately 1–2 g of each sample was weighed and 5 ml HNO_3 and 2 ml of H_2O_2 were added. After a short pre-reaction time (15 minutes), the recipients were closed with special caps and placed in the protection layer with secured cap, after which it was introduced into the rotor. The power of the device was gradually raised to 600 W over a period of 5.5 minutes and maintained at this level for 4.5 minutes. In the last stage, the power was raised to 1000 W and maintained for 10 minutes. The total mineralization time was 20 minutes, while the total cooling time was 20–25 minutes.

Following the completing of the mineralization program and the cooling time, the quartz recipients were opened, and the contents transferred into 50-ml volumetric flasks that were then filled to the mark with acidified water.

The content of heavy metals was analysed with the help of *Elan DRC-e ICP-MS* applicable in the determination of small concentrations of a large number of elements. Trace elements are nebulized and transformed into aerosols which are then carried by argon into the plasma torch. The resulting ions are trained in plasma and introduced via an interface into a mass spectrometer according to SR EN ISO 17294-2, 2005 (****2005).

RESULTS AND DISCUSSION

We analysed a total of 15 individual samples from eight locations, belonging to eight species (Table 1). The highest concentrations were recorded for Zn, with values between 12,307 to 158,237 mg/kg.

Table 1

Trace element concentrations for sampled feathers

Prelevation place	**Species**	**Values (mg/Kg)**					
		As	**Cr**	**Mn**	**Ni**	**Pb**	**Zn**
Murighiol	*Ciconia ciconia*	2.856	3.948	108.827	7.868	2.007	130.485
Nufăru	*Ciconia ciconia*	2.534	1.2	153.43	6.508	0.74	143.761
Maliuc fishpond	*Ciconia ciconia*	1.625	0.683	37.714	5.208	0.672	146.654
Murighiol	*Ciconia ciconia*	3.12	2.924	16.31	6.018	3.019	136.401
Murighiol	*Ciconia ciconia*	2.659	3.607	16.424	5.78	1.31	145.925
Draghilea Channel	*Phalacrocorax pygmeus*	1.589	4.063	22.258	1.694	0.608	12.572
Murighiol	*Sterna hirundo*	3.226	2.803	14.109	1.445	3.337	20.402
Murighiol	*Sterna hirundo*	2.776	2.257	41.063	2.557	1.803	18.849
Murighiol	*Sterna hirundo*	1.796	1.926	15.895	1.855	1.697	12.307
Uzlina Lake	*Circus aeruginosus*	2.924	4.619	59.037	4.075	3.474	157.986
Nebunu Lake	*Egretta garzetta*	3.584	7.542	79.688	4.585	0.966	119.627
Nebunu Lake	*Egretta garzetta*	1.709	0.848	49.005	7.215	1.408	119.307
Maliuc fishpond	*Ixobrychus minutus*	2.239	6.815	23.418	1.905	1.263	93.527
Fortuna Lake	*Pelecanus onocrotalus*	3.437	12.368	25.215	2.789	1.558	136.357
Salcioara fishpond	*Aythya nyroca*	1.61	4.58	18.431	1.391	0.426	158.237
Average		**2.5122**	**4.012**	**45.38**	**4.059**	**1.61**	**103.49**
Max		**3.584**	**12.368**	**153.43**	**7.868**	**3.474**	**158.23**
Min		**1.589**	**0.683**	**14.109**	**1.391**	**0.426**	**12.307**

All of the six studied elements (As, Cr, Mn, Ni, Pb and Zn) have bioaccumulative and toxic effects on the organism (Hill, 2010). It should be noted that the accumulation of heavy metals (internally, from food and water) occurred during the period of growth of feathers, for about 1–3 months during the previous year, very likely in the Danube Delta (all birds were adults).

The studied birds either moult after the breeding period, as in the case of *Aythya nyroca* (Cramp & Simmons, 1977), or during the breeding period until the start of migration, for the other species. However, in the case of large species, the moulting of flight feathers can occur over longer time periods (Ramirez & Panuccio, 2019), and the accumulation of these elements could have occurred outside the DDBR.

Also, the question of the origins of these trace elements should be treated with care, as it is very likely that the samples suffered from external contamination, either by atmospheric deposition or by contact with water, as has been shown in the case of Ni, Pb and Zn (Dauwe *et al*., 2003). In this situation the origin of the contaminants can also be from outside of the DDBR, as all the sampled species belong to species that have migratory populations.

Moreover, some species (the Great White Pelican at least) can frequently feed at great distances from the colony during the nesting period and, again, the accumulation (from food, water and atmospheric deposition) of these elements have occurred outside the DDBR. A comparison of our results with previous studies regarding background concentrations of DDBR ecosystems (water and sediments, Burada *et al.*, 2014; Ibram *et al.*, 2019) showed our values to be lower.

There are also differences ($p < 0.05$) between trace element concentrations found in bird feathers and those found in aquatic macroinvertebrates (Ibram *et al.*, 2019) and fish (Burada *et al.*, 2017). However, these comparisons must be regarded with caution, since the sampling periods were different and samples were collected from different places in the DDBR. Thus, we cannot make definitive conclusions of this kind regarding bioaccumulation phenomena for the studied elements.

The concentration values for arsenic varied between 1.610 mg/kg for the Ferrouginous Duck and 3.437 mg/kg for the Great White Pelican. In general, the arsenic concentrations show little variation ($S^2 = 0.496$) for the studied species (Fig. 2). The differences between values may be caused by the trophic factor or phenological characteristics of each species. However, when concentrations have low variation, we can consider that these are caused by the natural level of arsenic in the environment.

Comparing the levels of arsenic found in birds with the ones found in fish in the DDBR (Burada *et al.*, 2017), we found higher concentrations in our samples, which can be an indicator of a bioaccumulation process via the trophic pyramid.

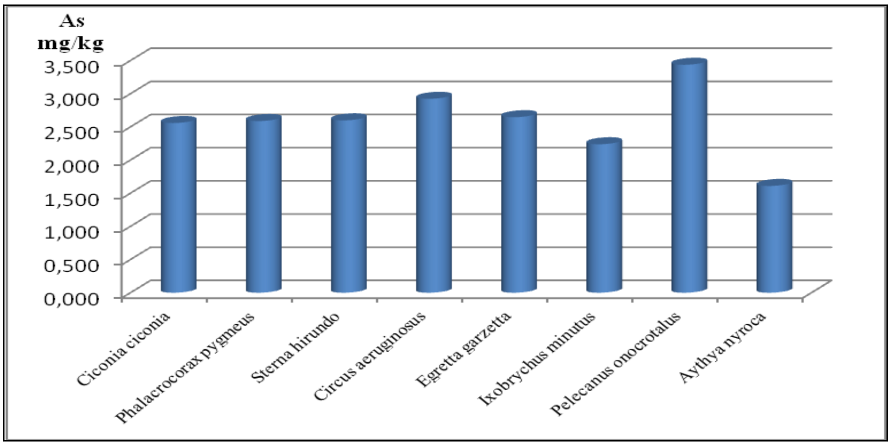

Fig. 2. Arsenic concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

The graphical representation of chromium concentrations (Fig. 3) shows a significant difference of accumulation capacities of this heavy metal between the Pygmy Cormorant – 11.063 mg/kg and the Great White Pelican – 12.368 mg/kg, and other species. These differences are most likely linked to the size of fish that are consumed by different species of birds (including benthic fish that are in close contact with the substrate). The differences between these concentrations can also be linked to the feeding areas, as well as with the different migration periods, different stopover sites and especially with wintering areas.

The minimum values, up to 2.472 mg/kg, were identified for White Storks, a species that frequently consumes its prey from dry habitats, especially amphibians, snakes and lizards, and much less fish (unlike fish, many amphibians and reptiles spend less time or none at all in water).

For some species, the variation of chromium shows no clear pattern. In the case of the Common Tern, a fish eating bird, chromium was found in smaller concentrations than in the Ferruginous Duck which is a species that predominantly feeds on plants of aquatic origin and rarely eats invertebrates (Kiss *et al*., 1980; Kiss *et al*., 1984; Kiss *et al*., 1986; Ciochia, 1992).

The values of chromium in birds were smaller than those found in sediments (Ibram *et al*., 2019), though larger than the average concentrations found in macroinvertebrates and in most of the studied fish (Burada *et al*., 2017; Ibram *et al*., 2019).

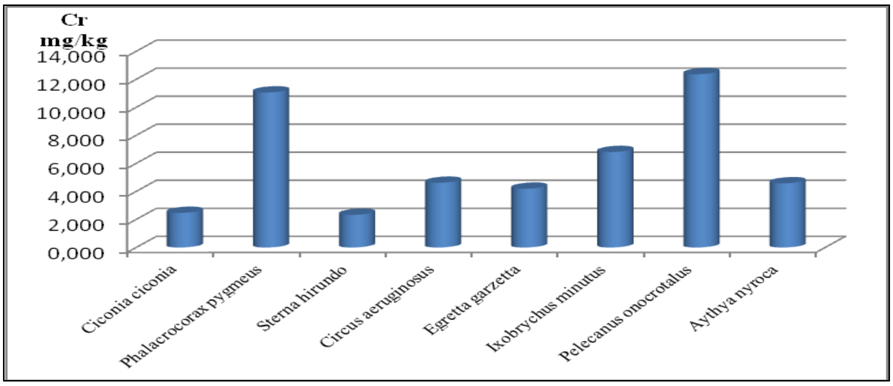

Fig. 3. Chromium concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

Out of all the studied trace elements, manganese was highest in concentration values after zinc. The highest concentrations were recorded for the White Stork (66.541 mg/kg), Western Marsh-harrier (59.037 mg/kg) and Little Egret (64.347 mg/kg) (Fig. 4). For the rest of the species it varied between 18.431 mg/kg and 25.215 mg/kg.

By analysing these differences, we observed that the bird species that feed mainly in the riparian or marsh areas have the highest concentration levels. In contrast, in species that feed in open waters (except the Common Little Bittern), the manganese concentrations were lower.

Manganese is known to be a common element in aquatic ecosystems, occurring in large quantities (Burada, 2015). At concentrations over 0.2 mg/kg, in the presence of oxygen, it precipitates and is deposited in sediments (Allen, 1989), from where it is assimilated in large quantities by aquatic organisms.

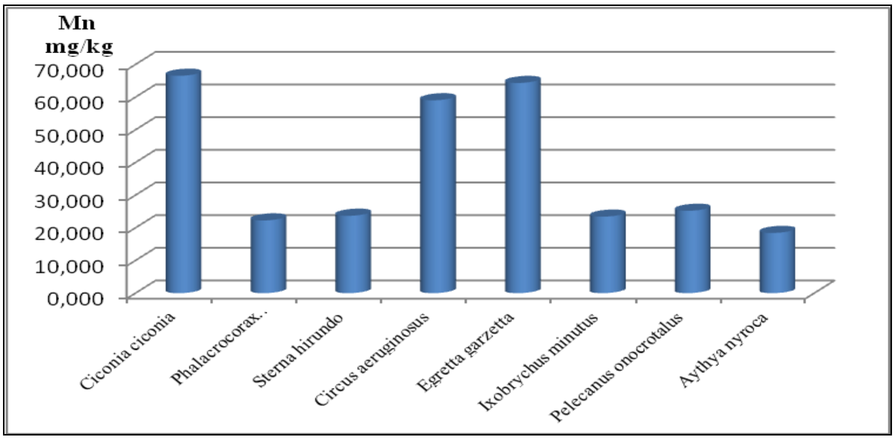

Fig. 4. Manganese concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

In the case of nickel (Fig. 5), much like in the case of manganese, we noted a high accumulation capacity for the species that feed mainly in riparian and marsh areas (White Stork – 6.276 mg/kg, Little Egret – 5.900 mg/kg and Western Marsh-harrier – 4.075 mg/kg). From our results, it seems that both nickel and manganese have a similar concentration distribution among species and it is most likely that they have the same contamination source. The smallest concentration levels of nickel were identified for the Ferruginous Duck (1.391 mg/kg).

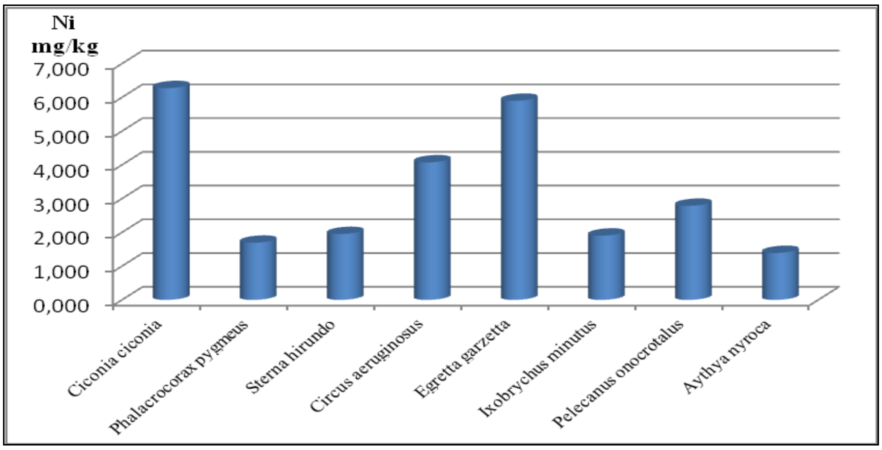

Fig. 5. Nickel concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

The nickel values from sampled feathers are much lower than those found in sediments (Ibram *et al*., 2019), though relatively similar (with the exception of the White Stork, Little Egret and Western Marsh-harrier that showed higher concentrations) to medium concentrations found in aquatic macroinvertebrates and in most fish species (Burada *et al*., 2017; Ibram *et al*., 2019).

Regarding lead, it is important to note that it is an accumulative poison and has no essential biological function, as all of its compounds are toxic. Lead forms strong bonds with enzymes and proteins (e.g. haemoglobin), thus disrupting many metabolic processes (Bolcu & Király, 2012). Lead concentration levels (Fig. 6) varied between 36.474 mg/kg for the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. Overall, the lead concentrations varied only slightly (S^2=0.953).

Considering that the sample size was small and a definitive pattern cannot be observed, preliminary analysis shows that lead levels increase directly in proportion to bioaccumulation along the trophic pyramid. The highest lead concentration was found in the Western Marsh-harrier, a raptor species that feeds mainly on birds and mammals (Ciochia, 1992), species with higher accumulation potentials compared to herbivorous, insectivorous and piscivorous birds.

Thus we can explain a lower lead concentration in the Ferruginous Duck, which is a herbivorous species (Ciochia, 1992). The values of lead concentrations from bird feathers (Fig. 6) were much lower than those from sediments (Ibram *et al*., 2019), but much higher than those found in fish tissues (Ibram *et al*., 2017).

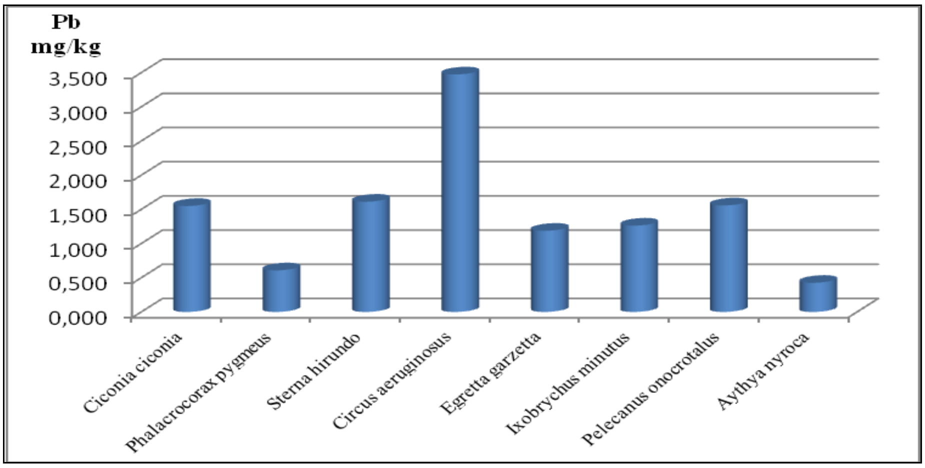

Fig. 6. Lead concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

The zinc concentrations found in bird feathers (Fig. 7) are higher than those from sediments and much higher than those found in aquatic macroinvertebrates (Ibram *et al*., 2019).

The highest values, up to 163.237 mg/kg were observed for the Ferruginous Duck, while lowest, at 93.527 mg/kg, were identified for the Common Little Bittern. These high values recorded for Ferruginous Ducks may be caused by the metabolism of some forms of zinc in aquatic vegetation (the basic food of this species), a hypothesis mentioned by Iordache (2009).

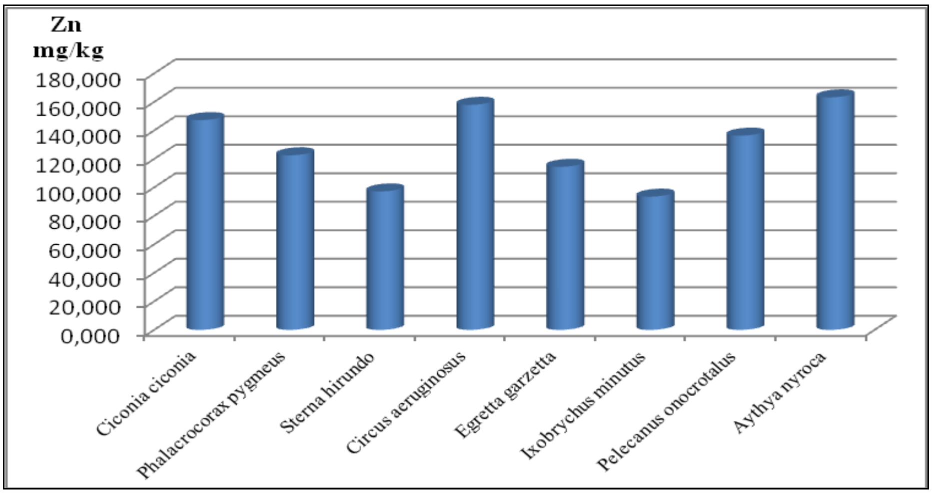

Fig. 7. Zinc concentrations in feathers of bird species
of Community interest in the Danube Delta Biosphere Reserve.

CONCLUSIONS

Our investigations regarding heavy metals in samples collected from eight bird species of Community interest revealed inter-specific differences, most likely caused by differences in diet and other ecological features.

Laboratory analyses revealed that the studied species have a high capacity for accumulation of heavy metals and arsenic in their plumage. However, the values were generally lower than those found by other studies to be present in the sediments and water of the DDBR.

Arsenic concentrations varied between 1.610 mg/kg for *Aythya nyroca* and 3.437 mg/kg for *Pelecanus onocrotalus*, whereas variations were minor in the rest of the studied species.

There was a significant difference in the chromium accumulation capacity in medium-sized piscivorous species compared to large species (*Phalacrocorax pygmeus* – 11.063 mg/kg and *Pelecanus onocrotalus* – 12.368 mg/kg).

Out of all the studied elements (with the exception of zinc), manganese had the highest values. The highest concentrations were identified for species that feed in marshy and riparian areas (*Ciconia ciconia* – 66.541 mg/kg, *Circus aeruginosus* – 59.037 mg/kg and *Egretta garzetta* – 64.347 mg/kg).

In the case of nickel, there was also a similarity in storage capacity among the species that predominantly feed in riparian and marshy areas (*Ciconia ciconia* – 6.276 mg/kg, *Circus aeruginosus* – 4.075 mg/kg and *Egretta garzetta* – 5.900 mg/kg).

The lead concentration range varied between 3.474 mg/kg in the case of the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. The levels of lead increased in proportion to bioaccumulation along the trophic pyramid.

In comparison to background values, zinc concentrations were higher, up to 165.237 mg/kg, as identified in *Aythya nyroca*, while the lowest, 93.527 mg/kg, was found in *Ixobrychus minutus.*

We consider it necessary for the future to repeat these analyses on a larger number of birds. With a larger sample we would be able to analyse individual concentrations of trace elements from different areas of the DDBR. In order to have a better understanding of the feather accumulation phenomenon in DDBR, sampling from bird juvenile specimens is recommended.

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