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Accumulation of chromium, cadmium and arsenic in white-tailed sea-eagle feathers (Aves: *Haliaeetus albicilla*) from the Danube Delta Biosphere Reserve and surrounding areas (Romania)

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Abstract: *Haliaeetus albicilla* is a top raptor that can be used as a model for contaminants transfer in trophic chain. In this study we studied the accumulation of three contaminants on feathers from breeding individuals of white-tailed sea-eagle collected between 2012 and 2018 in the Danube Delta Biosphere Reserve and its surroundings. The concentrations of two heavy metals (chromium, cadmium) and one metalloid (arsenic) were determined by inductively coupled mass spectrometry. Chromium concentrations ranged from 0.32 to 1.33 mg/kg for juveniles and between 0.68 to 2.92 mg/kg in the case of adult birds. Cadmium concentrations in juveniles were between 0.007 to 0.098 mg/kg and in adults it was between 0.036 to 0.105 mg/kg. Arsenic concentrations in young birds ranged from 0.17 to 1.80 mg/kg, while in adults it was between 0.57 and 1.97 mg/kg. In general the concentrations of the three elements are smaller in the study area compared with other areas. Our research showed important intraspecific differences. Thus juveniles had smaller concentrations of chromium and arsenic compared to adults. We suggest that the juveniles are better indicators for pollutans in the study area as they are feed from local sources, compared with the adult birds that in the winter period use other areas for hunting.

Key words: Raptor, heavy metals, feathers, wetland

1. Introduction

Until recently, the white-tailed sea-eagle (WtSe) was classified as an endangered species in the Danube Delta Biosphere Reserve (DDBR). Globally, including in Romania, this bird is under strict protection, regulated by the Berne, Bonn and Washington conventions but also by the European Birds Directive. Being the terminus of the Danube River, DDBR gathers pollutants derived from industrial and agricultural activities of 9 countries (Burada et al., 2015). Thus, defining and establishing the chemical stress factors for WtSe are essential in identifying the best protection and conservation measures.

This bird species is located at the end of the food chain, thus it is highly affected by bioacumulation and biomagnification of toxic elements throughout the aquatic ecosystem (Rutkowska et al., 2018; Yilmaz et al., 2007; Zhao et al., 2012). The accumulation of heavy metals in aquatic food chains is the subject of research both nationally and internationally, through the major impact they have on their stability (Battaglia et al., 2005) which can be assessed by biological monitoring. In this aspect, birds and their feathers can act as bioindicators that provide information on the concentrations and on the effects of the micropollutants (Kushwaha, 2016; Rutkowska et al., 2018), as well as the level of local contamination and around nesting sites (Ullah, 2013). There is a growing interest of using raptors as santinel for monitoring persistence substances in the environment in Europe, though there are several constraints for collecting the raptor samples (Dulsat-Masvidal et al., 2021). It should be noted that an international research group has been set up on chemicals stored in predatory feathers (Movalli et al., 2019).

At the organisms level, heavy metals undergo biochemical processes before being distributed and accumulated differently in their organs (liver, kidneys), tissues (muscle, bones, fats), eggs, feathers and excrements (Burger, 1993; Rapôso da Silva et al., 2018). Unlike other classes of pollutants, which can be biodegradable and can be completely destroyed, heavy metals are not, but have the ability to be transformed into more toxic or complex.

Although worldwide there are studies on the use of feathers as a matrix of metal monitoring (Burger, 1993), in the territory of the DDBR, these researches are carried out for the first time in adult and juvenile WtSe feathers. There are several studies on the poisoning of birds with heavy metals and arsenic in Europe (Lebedeva, 1997; Hernández et al., 1999; Berglund et al., 2011; Kitowski et al., 2014). In Romania, several studies have been conducted on the concentrations of heavy metals in abiotic elements, plants, fish and birds (Tudor et al., 2006; Vignati et al., 2013; Ilie et al., 2014; Burada et al., 2014; Burada et al., 2015; Burada et al., 2016; Burada et al., 2017; Marinov et al., 2019), however these are few, so with this

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study we want to initiate research on bird toxicology for better management of bird species.

For ethical reasons, it is recommended to use feathers as a nondestructive and noninvasive tool for biomonitoring metal contamination in birds (Ullah, 2013). Another advantages of this approach are that the metals are stored in feathers only while they develop and grow and that after collecting the samples, the concentration of the metals does not change under variable storage conditions, over long periods of time (Burger, 1993).

The study of metal accumulation in the feathers of juvenile birds can be an instrument of measure for the level of pollution in the environment surrounding the nesting area, where the food source is located. By its part, feathers of adult bird may reflect exposure at other times of the year and in other areas than those used for reproduction (Kim and Koo, 2007).

The aim of this study is to show the degree of accumulation of these three pollutants with a high degree of toxicity in WtSe feathers and to validate the hypothesis that the values will differ from juveniles to adults.

2. Materials and methods

2.1. Study site

The study area primarily included the entire territory of the DDBR. We also took into account the forests near the Reserve, as the birds nesting there but hunt in the DDBR.

DDBR consists of complexes of river branches, canals, lakes, wetlands, abandoned or used fishing facilities, swamps and marshes, etc., with variable water levels. Of these, approximately 199,600 ha are reed habitats. Nonfloodable areas form approx. 16.8% of the total, and the tree vegetation, a vital element for nesting, occupies around 260 km² (Hanganu et al., 2002). We proceeded to analyze a batch of biological samples consisting of WtSe feathers, in order to highlight the presence of two heavy metals – chromium, cadmium and metalloid (arsenic).

The research on the content of heavy metals in the WtSe feathers was carried out between 2012 and 2018 and were based on 54 feather samples, belonging to both adult and juvenile specimens were collected. The maps were made using the QGIS program. Garmin 62S GPS was used to accurately locate the WtSe samples. Feather samples were collected from 20 locations (Figure 1; Table 1).

The biological material is represented by sheaded feather that were collected from the ground from 20 locations (Figure 1; Table 1): 17 nests used by the WtSe in the DDBR, 2 nests in the surrounding areas, respectively in the Babadag and Agighiol forests and one feather from an active territory in Tichilesti area.

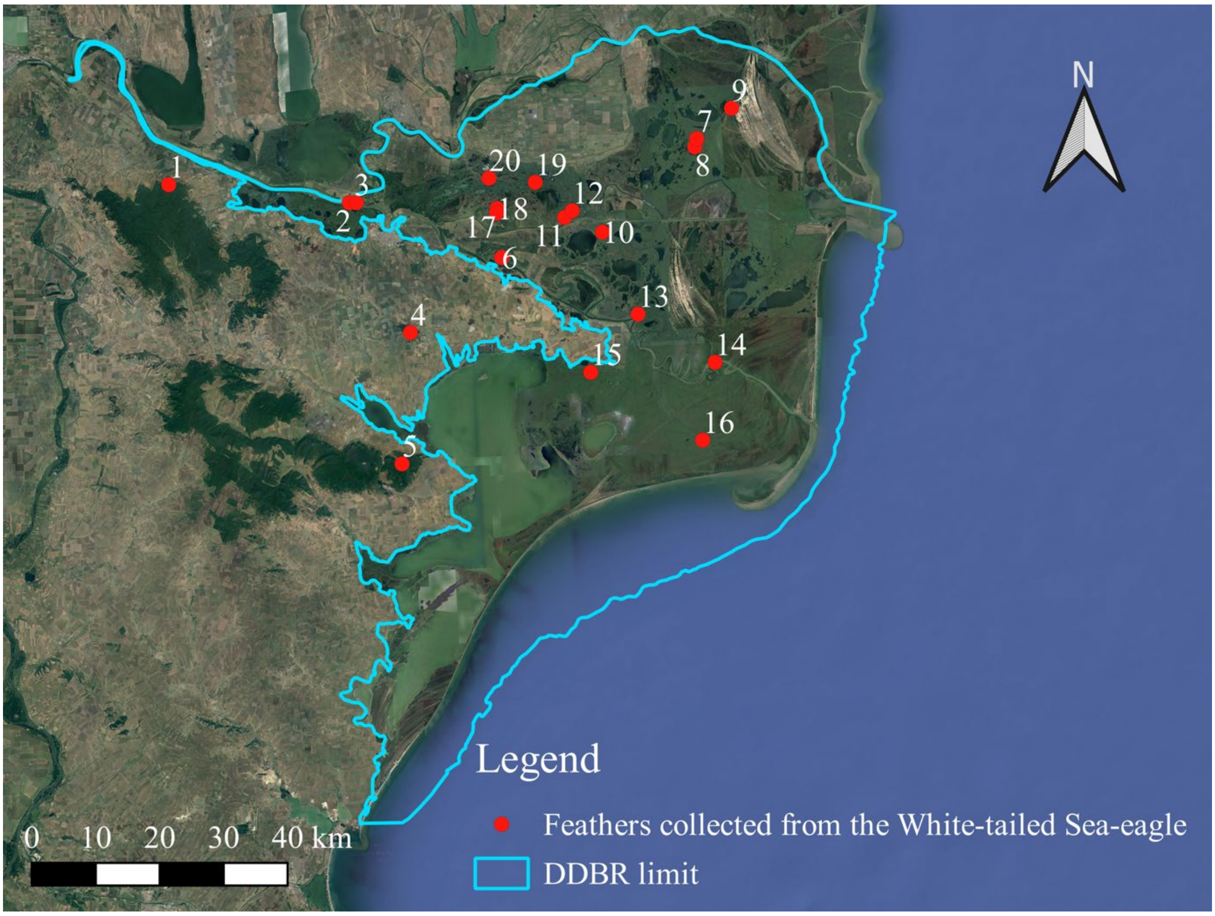

Figure 1. Geographical distribution of sampling points for WtSe (*Haliaeetus albicilla*) from DDBR and the surrounding areas.

Table 1. Spatial arrangement of samples and their distribution by age groups.

Collection date	Sampling point code	Sampling point location	Number of feather samples		
			Adult	Juvenile	Total
24.06.2018	S1	Tichilesti - Isaccea	1		1
09.07.2018	S2	Ceatal Chilia 1		1	1
20.05.2018; 09.07.2018	S3	Ceatal Chilia 2 (Mihailescu)		2	2
31.10.2017; 14.05.2018	S4	Mandra forest (Agighiol)	1	2	3
14.05.2018; 01.06.2018	S5	Babadag forest, Dealul Ascutit		3	3
12.04.2017; 08.06.2018	S6	Baltenii de Jos	2	1	3
24.09.2016	S7	Letieni channel		23	23
14.06.2018	S8	Sfistofca channel	1		1
11.08.2017	S9	Letea Poala Baltii 2		1	1
12.04.2017	S10	Gorgova Est	1		1
09.04.2012	S11	Iulia Mile 22-1	1		1
27.04.2017	S12	Rotund lake	1		1
04.04.2018	S13	Taranova channel	1		1
08.06.2018	S14	Sf. Gheorghe Branch (Km 23-2)	1	1	2
05.11.2017	S15	Lipovenilor channel	1		1
09.05.2017	S16	Crasnicol channel	1		1
01.11.2016	S17	Papadia forest	4		4
28.04.2017	S18	Papadia Noua 2 channel	1		1
08.06.2018	S19	Sontea channel (Marele S)	2		2
14.06.2017	S20	Nebunu Lake	1		1
			20	34	54

For the determination of metals, samples of feathers have been transported in plastic bags at ambient temperature. In the laboratory, they have been stored at room temperature, in the dark, as long as tissue or blood debris has been removed, (Rudnick et al, 2009; Espins et al, 2014), in plastic labeled bags. The feathered samples were washed with bidistiled water and acetone to remove the external contamination. (Esins et al, 2014; Rapôso da Silva et al., 2017). After the pretreatment stage, the feathers were dried in the oven at 60 °C, for 48 h, brought to constant mass and cut into small 1 mm pieces in order to expose as much of the wedge as possible to the extraction process. (Rapôso da Silva et al., 2017). The metals are extracted using 5 mL of HNO_3 and 2 mL of H_2O_2. The detection limit is 0.02 µg/L. Using certified reference material, the degree of recovery was 95%.

2.2. Statistical analysis

For the statistical analysis, the total dataset of 54 samples was utilized in the hypothesis testing of different values in adults and juveniles (except in the case of Cr, where only 52 values were considered).

Differences in Cr levels were checked with Welch's two sample t-test as both groups are normal (Shapiro-Wilk normality test: p-value > 0.05) but heteroscedastic (Levene's test for homogeneity of variance, p-value<0.001).

The concentration of As in the samples from juveniles was not normal so to compare them with the adults the nonparametric Mann-Whitney U test was utilized. In the case of Cd, values from juveniles and adults were also lacking in normality so the same test was used for the comparison.

To check the differences between concentrations of these three elements in juveniles a Kruskal-Wallis rank sum test was run and Wilcoxon signed rank exact test for paired samples with Bonferroni correction of p-values was used as the post hoc test.

3. Results

The analysis of chromium shows significatively higher concentration of this metal in adults (1.54 ± 0.69 mg/kg) than in juveniles (0.74 ± 0.21 mg/kg) ($t = 4.7837$, $df = 18.627$, $p < 0.001$).

Thus, in the case of juvenile specimens, the values vary between 0.32 mg/Kg for the samples taken from location S7 and 1.33 mg/Kg for the samples taken from location S9 (Figure 2).

In the case of the feathers that came from adult bırds, the minimum value of Cr was 0.68 mg/kg at location S17 near Păpădia forest. From this location we have also obtained the maximum value, 2.92 mg/kg, proving that the samples were belonging to both adults of the breeding pair.

Regarding the arsenic, the distribution of the concentrations was also significatively higher in adults (1.16 ± 0.37 mg/kg) than in juveniles (0.63 ± 0.36 mg/kg) ($W = 589.5$, $p < 0.001$) (Figure 3).

The concentrations of arsenic in juvenile feathers ranges from 0.17 mg/kg for biological material taken from location

S4, and 1.80 mg/kg for the biological material taken from location S3. In the case of adult specimens, the variation of arsenic concentrations was between 0.57 mg/kg at S15 and 1.97 mg/kg at S17.

Last, the distribution of the concentrations of cadmium in adult feathers (0.06 ± 0.03) was not so significatively different than in juvenile feathers (0.08 ± 0,02) (W = 510.5, $0.05 > p > 0.001$). The values of cadmium concentration in feathers from juveniles varies from 0.007 mg/kg in S7 sampling point, to 0.098 mg/kg also in S7 (Figure 4).

The adult specimens presented values in the range of 0.036 mg/kg for the samples taken from location S13, and 0.105 mg/kg for those taken from location S17.

The concentrations of these three contaminants vary within the samples from juveniles (Kruskal-Wallis chi-squared = 69.45, df = 2, $p < 0.001$). Level of cadmium is significantly lower than the other elements level ($p < 0.001$) but there are not differences between chromium and arsenic ($p > 0.05$) (Figure 5).

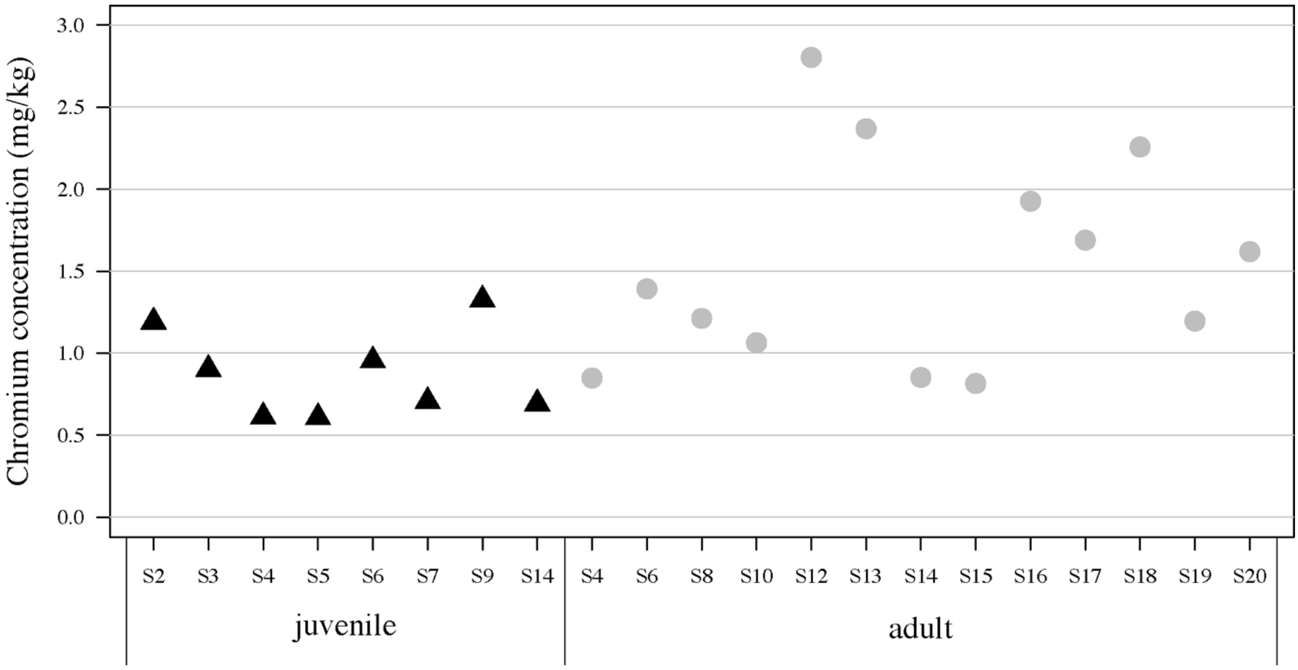

Figure 2. Accumulation level of chromium identified in the feathers sorted by age. Average values for each sampling point are showed when there were multiple samples available.

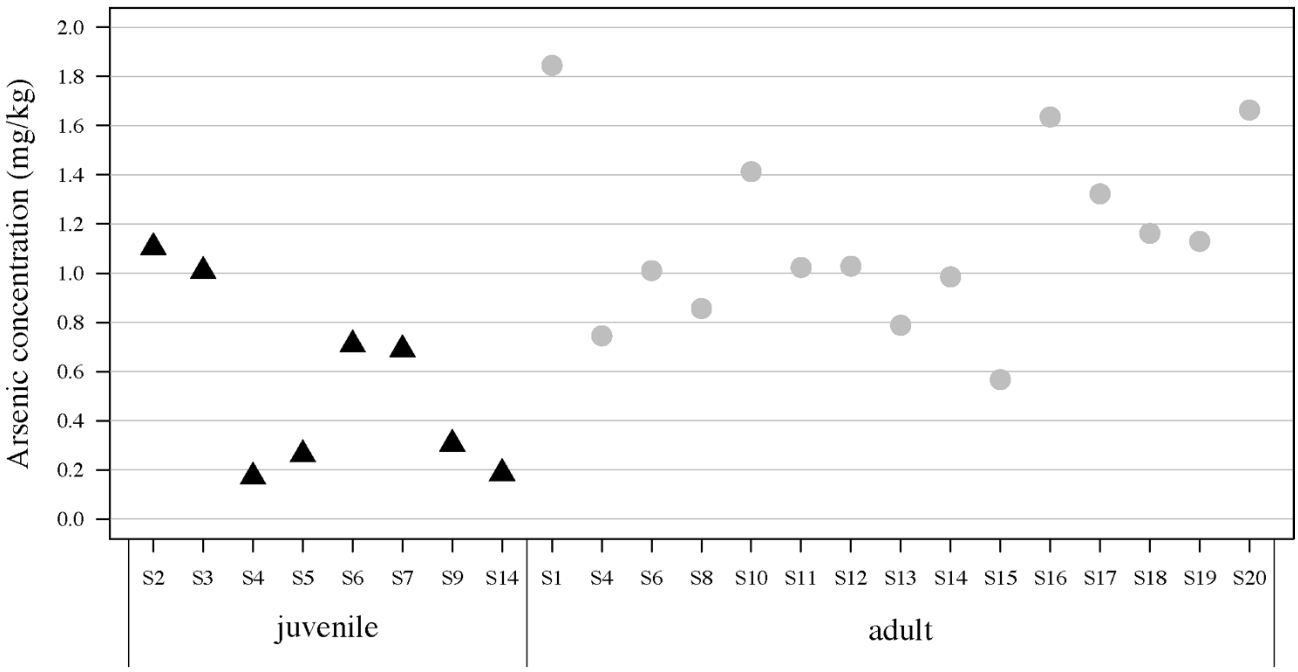

Figure 3. Accumulation level of arsenic identified in the feathers sorted by age. Average values for each sampling point are showed when there were multiple samples available.

4. Discussion

The 2 ways of penetration of contaminants are ingestion of prey and direct inhalation (Sánchez-Virosta et al., 2015). Top predators are often the most affected by the bioamplification of heavy metals and metalloids along the food chain, and their plumage reflects the concentration of toxic elements to which they are exposed during the growth of feathers. When the feather is directly connected to the blood vessels, the elements are incorporated into the structure of the keratin (Dauwe et al., 2003), where they are stored until the molting time. The surface of the feathers can also retain contaminant elements through preening oil and through contact with environmental air, dust and water (Jaspers et al., 2019).

The studies regarding the pollutans accumulation in raptors provide a good background for comparrisons across the spatial and temporal variability. Thus, cadmium concentrations were higher in Poland (Kitowski et al., 2017), Greenland (Krone et al., 2004) and Finland (Krone et al 2006) than in the DDBR while the chromium values are lower in Poland (Kitowski et al., 2017). These comparisons must however be regarded with caution, as the sampling tissues were different, most of these studies using liver tissues. One example of the differences regarding the sampling tissues values is provided by a study in Hokkaido (Hisato et al., 2000) where the cadmium concentrations fom kidneys and liver were higher, while those from muscle tissues were similar with our values.

There are, however, few studies that used feathers as sampling tissue. In one of these, focused on a similar species – *Halliaetus leucocephalus* in Alaska (Burger et al., 2009), the

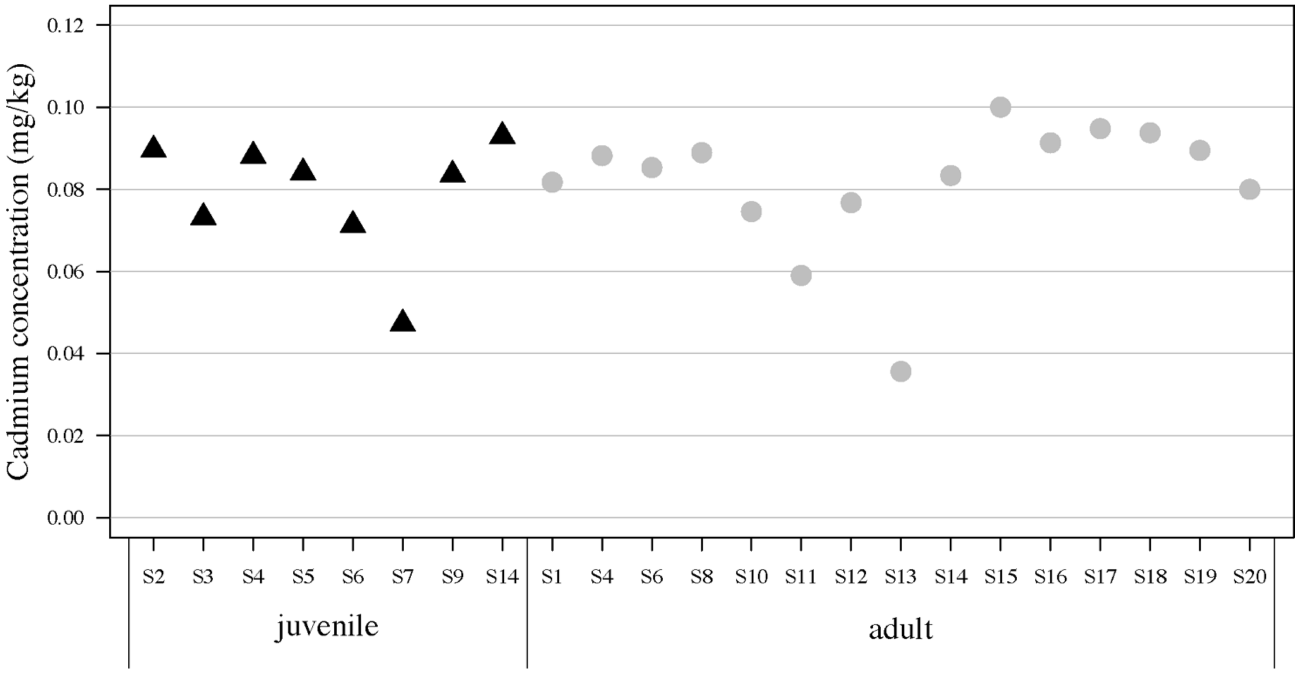

Figure 4. Accumulation level of cadmium identified in the feathers sorted by age.

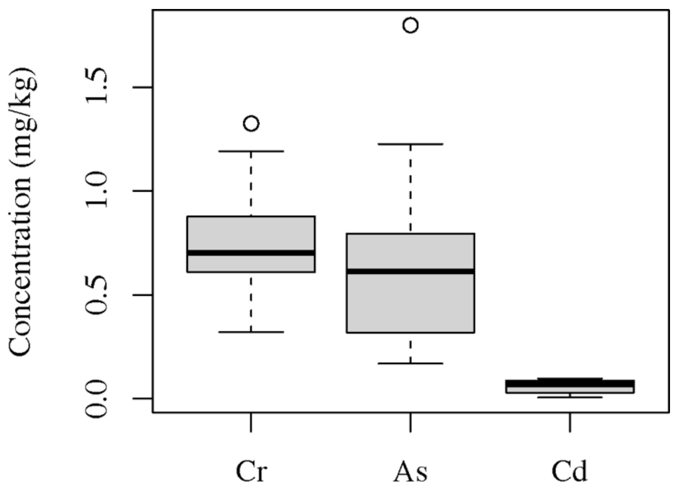

Figure 5. Boxplot (median, variability and outliers can be observed) of the values observed for the three elements in the samples coming from juvenile specimens.

concentration of arsenic from feathers were higher than our values. The authors found no difference regarding the concentrations of cadmium and chromium between adults and juveniles.

We found smaller concentrations of chromium and arsenic in juveniles compared with adults, while cadmium levels were similar. The levels of cadmium are low comparative with other studies. Thus presume that there are no local sources of contamination with cadmium. Nevertheless, other studies did find differences between juveniles and adults regarding cadmium concentrations (Kitowski et al., 2017).

The differences could be caused by a lower level of contamination of the local food source in the study area when the chicks are developing (the WtSe hunts only in close home range in this period) compared to adjacents areas where nonsedentary adults feed outside the breeding period (Alexe et al., 2019). This is also supported by larger variances in the contaminant levels detected in adult feathers.

Limitations of this study could affect the conclusions in the sense that we do not know exactly the time interval in which the analyzed feathers were in direct connection with the blood vessels in each case but in general the bioaccumulation period is shorter in juveniles than in their long-lived parents. Said so, the concentration of these elements in the feathers from juveniles (Figure 5) seems to be more representative of the actual contaminant levels of the DDBR.

The main food source of WtSe in DDBR is the ichthyofauna followed by the avifauna (Alexe, 2019), which suggests the fish population as primary source of contamination with heavy metals, which in turn can indicate the degree of pollution of the aquatic ecosystem.

The degree of contamination showed variations even in the case of the same element that could be generated by instraspecific factors like different age groups and sexes, the experience and predation skills of each individual, the distances between sampling sites (generally nests) to the pollution sources, the feeding radius of the individuals, and their trophobiological preferences.

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