

Original Research

Concentration of Trace Elements in Raptors from Three Regions of Slovakia, Central Europe

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Abstract

Slovakia situated in the territory of Central Europe has a rich mining and industrial history with a strong pollutive impact on the environment, with heavy metals and metalloids as the most often analysed pollutants. Despite a relatively advanced toxic elements research, none enquiry has been performed in birds of prey, the most suitable bioindicators, in Slovakia yet. Regarding the lack of information, the presented study represents the first integrated results of the contamination status of raptor species with selected trace elements (arsenic, cadmium, chromium, copper, mercury, manganese and lead). In all, livers and muscles of 80 individuals across twelve species were collected and analysed using atomic absorption spectroscopy. Within the study, copper was the element detected with the highest concentrations, in several individuals overlapping the toxic threshold. Additionally, the results disclosed high hepatic lead concentrations, pointed to the probability of acute poisoning through lead shot ingestions, in three birds of prey. Subsequent comparative analyses revealed significantly higher concentrations of mercury in surveyed Spiš and Tatry region when compared with the other two sampling regions. Also, the highest average and median values concerning liver concentrations of cadmium and manganese and muscle concentrations of chromium were measured in samples originated from this region. The whole territory of the Spiš and Tatry region is covered by national parks and protected areas, and several European Important Bird Areas occurred there. Together with rich mining and smelting history and also the existence of natural geochemical anomalies, toxic elements biomonitoring should be continued in this area.

Finally, it can be concluded that the first oriented research in Slovakia showed that the concentration of monitored metals in the liver and muscles of birds of prey did not exceed the established tolerable

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levels, except for copper, cadmium, and lead, for which concentrations exceeding defined toxic thresholds were occasionally found and cases of intoxication them with were reported.

Keywords: heavy metals, trace elements, raptors, pollution, bioindicators

Introduction

The environmental contamination with toxic elements, especially heavy metals and metalloids, is mainly caused by anthropogenic activities, expanding chemical industry and technical advances, but also by natural phenomena such as weathering or volcanic eruptions. However, it is estimated that at least 60 out of 118 naturally occurring elements were introduced into biogeochemical cycles on a larger scale as the results of human activity rather than natural causes [1].

The territory of Slovakia is characterised by numerous metal ore deposits and accordingly by a rich mining history that has flown into the formation of many heavy metal loaded habitats. Primary trace elements habitats are created especially at ore bodies and veins, secondary heavy metal enriched habitats are formed from mine heaps, slag wastes and in the surroundings of smelters [2].

According to geological researches, mankind currently witnesses the sixth species extinction which is accompanied not only by climate change but also by anthropogenic emission of mercury and other heavy metals [3]. The potential impact of accumulated toxic elements on humans and animals health could be monitored through the sentinel organism, especially birds that are in a majority of developed EU countries most commonly reported as victims of the poisoning. Above all, birds of prey, usually standing at the top of the food chain, long-lived, territorial, and with well-known biology, are the most convenient indicators of environmental pollution [4,5].

In Slovakia, 40 Important Bird Areas (IBA) have been identified, of which 39 have been proposed to be designated a Special Protection Area (SPA). The total area of all 40 IBAs is 13 840.85 km², which makes 28.2% of the area of Slovakia. The largest IBA, covered 1280 km², is situated in the Volovské Vrchy Mountains belonging to the Slovenské Rudohorie Mountains on the border of Gemer and Spiš regions [6]. Both regions were included as sampling areas in the here presented study. In this area, several raptors species, in particular owls, are regularly observed, including Ural owl (*Strix uralensis*), tawny owl (*Strix aluco*) and eagle owl (*Bubo bubo*). In our study, strigiform birds with nocturnal activity are represented by four species: long-eared owl (*Asio otus*), eagle owl, tawny owl and Ural owl. So far, 12 species of nocturnal raptors - owls have been recorded in Slovakia, 10 of which are breeders. The most frequently occurring species are the long-eared owl and tawny owl with more than 2 500 breeding pairs, followed by the Ural owl with

1 400-2 500 breeding pairs and of eagle owl with only 300-400 pairs recorded [7]. Other significant IBAs are situated in the lowland area of the south-eastern part of Slovakia in Dolný Zemplín, the region also included in our study as a sampling locality. It is the most important breeding and migrating site for waterbirds in Slovakia. Additionally, in this area the largest national population of the imperial eagle (*Aquila heliaca*) is inhabited [6]. Regarding diurnal raptor species, 33 of them have been recorded in Slovak territory so far. Eighteen of them are regular breeders and only eight species including northern goshawk (*Accipiter gentilis*), common buzzard (*Buteo buteo*), common kestrel (*Falco tinnucullus*) and imperial eagle (*A. heliaca*) listed in our set of samples are breeding residents. The rest of the raptor species examined within our study, concretely long-legged buzzard (*Buteo rufinus*) and rough-legged buzzard (*Buteo lagopus*), are migrants, either visiting Slovakia during the breeding season or just occurring on migration [7]. *A. heliaca* together with saker falcon (*Falco cherrug*) are the most endangered raptor species not only in Slovakia but also worldwide. In the territory of Slovakia about 40 nestling couples of both species have been observed. The main threatening factors of these species represent electrocution, bird crimes including poisoning and illegal shooting, disturbance and logging in breeding territories, loss and degradation of breeding and feeding habitats [7]. Common buzzard, *Buteo buteo*, was the most prevalent species examined within our study. It is the most common raptor species not only in Slovakia but also in Europe. In Slovakia, it nests in different types of habitats except for areas with an altitude over 1 300 m. The number of breeding pairs varies between 5 000 and 7 000 [7, 8]. Two relative species, *B. rufinus* and *B. lagopus*, are considered wintering species, but in the context of global climate changes it is assumed that *B. rufinus* could also nest in the territory of Slovakia. Small rodents predominate in the food of *Buteo* species and the size of their population influences also buzzards' abundance during the individual years [8]. The second most numerous species of birds of prey in Slovakia, with a count of 5 000-7 000 breeding pairs, is the common kestrel (*Falco tinnunculus*) widely distributed in the whole territory. The other two species of falconiformes, Eurasian hobby (*F. subbuteo*) and saker falcon (*F. cherrug*) are much less abundant with around 600 and 40 nesting pairs. Whereas *F. subbuteo* occurred in all Slovak regions, *F. cherrug* inhabited only southern areas [7, 8]. The last of the species examined in our study, northern goshawk (*Accipiter gentilis*) is also a relatively common bird of prey nesting in Slovakia,

but its abundance is declining. It mainly preys on birds and it is considered the most important regulator of corvids. In the past it was significantly hunted, it is also harmed by forestry activities and chemization of the environment [8].

However, despite being the most suitable biomonitors and sentinels of environmental contamination, birds of prey are strictly protected in many European countries and sample collection can be quite difficult. Concretely, in Slovakia, despite relatively advanced heavy metals biomonitoring and research, none enquiry has been performed in raptors. Regarding the lack of information from this geographic region, the aim of our study was to obtain the first integrated results of contamination status of several birds of prey with selected trace elements, arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn) and lead (Pb), and to determine its dependency on sampling regions with their environmental peculiarities.

Material and Methods

Sample Collection and Preparation

A total of 80 individuals across twelve species of birds of prey were collected during the study. Five species ranged to the orders Accipitiformes [*Accipiter gentilis* (n = 2), *Aquila heliaca* (n = 4), *Buteo buteo* (n = 47), *Buteo lagopus* (n = 3), *Buteo rufinus* (n = 3)], three to the Falconiformes: [*Falco cherrug* (n = 1), *Falco subbuteo* (n = 2), *Falco tinnunculus* (n = 2)], and four species were classified to the order Strigiformes [*Asio otus* (n = 3), *Bubo bubo* (n = 2), *Strix aluco* (n = 1), *Strix uralensis* (n = 10)]. All birds integrated into the study were free-living and died naturally or as a result of injury or disease. Found carcasses were referring to several state organisations (Raptor Protection of Slovakia, Museum of the Tatra National Park, Clinic for birds and exotic animals of the University of veterinary medicine and pharmacy in Košice, Rehabilitation station in ZOO Bojnice, Košice Airport Bioprotection, State Nature Conservancy of the Slovak Republic-Slovenský kras National Park, Slovenský raj National Park, Pieniny National Park, Regional Conservation Centre in Prešov and Vihorlat protected area) and consequently submitted at Institute of Parasitology SAS for examination.

During the autopsy, from each cadaver, the liver and muscle tissues from the *musculus pectoralis* were taken and stored separately in plastic bags at -20°C till further analyses.

Sampling Areas

The majority of the examined animals were collected on the territory of different districts in the eastern part of Slovakia. For results interpretation 73 from 80 examined individuals were categorized

into three different regions according to their origin: 38 were included to Region I (Abov and Gemer with districts Košice and Rožňava), 15 fell into Region II (Dolný Zemplín including districts Trebišov, Michalovce, and Sobrance) and 20 individuals came from Region III (Spiš and Tatry with districts Spišská Nová Ves, Stará Ľubovňa, Kežmarok, Poprad and Liptovský Mikuláš) (Fig. 1). The remaining seven birds of prey were originated in other parts of Slovakia and were not geographically analyzed but were included in the analysis related to species.

Region I includes two informal regions, the Abov and Gemer situated in the south of eastern Slovakia. The Abov region lies in the municipality of Košice, the second largest city of Slovakia, and its surroundings. The core of the Abov region is the L-shaped Košická kotlina basine surrounded by Slanské Vrchy Mountains, Volovské Vrchy Mountains, and the south-western part of the region is bounded by karstic landscape with plateaux of Slovak Karst [9]. In Košice, the main source of environmental pollution represents an old residue of long-term magnesite industry and metallurgy in the U.S. Steel Company [10]. District Rožňava located in Gemer region is situated to the west from Košice between the slopes of the Slovenské Rudohorie Mountains and National Park of Slovak Karst. It is a large forested mountain area with prevailing beech forests in the eastern parts and the spruce forests in the west. Due to their location in the Carpathian Mountains close to warmer lowlands, there are a high variety and richness of bird species. The territory is characterized by the 700-old mining history of gold, silver, and iron.

Region II, Dolný Zemplín, is situated in the south-eastern part of Slovakia and it is bound to Ukraine in the east and Hungary in the south. The large part of the region is covered by Eastern Slovak Lowland, the second-largest lowland in Slovakia with an area of 4000 square kilometers, which comprises rivers, canals, marshes, wet meadows, floodplain forests, and abandoned agricultural land. This area is considered the most important breeding and migrating site for waterbirds in Slovakia. Dolný Zemplín is a mainly rural region, in the districts Michalovce and Trebišov most of the agricultural production is concentrated [9]. From an environmental point of view, Vojany thermal power station, a major source of fly ash in Slovakia, is situated in the south of this region [10].

Region III for the purpose of our study represents an informal historical regions Spiš and Tatry neighbouring Poland in the north and bounded with river Váh in the west and Slovenské Rudohorie Mountains in the south. The core of the Spiš region is formed by the basins of the rivers Hornád and Poprad. More than 40% of the region territory is covered by forests [9]. In the past, the Spiš region was characterized by the strongly developed mining activities and long-termed production of copper, mercury, manganese, lead, and zinc [10]. Nowadays,



Fig. 1. Sampling areas of examined raptors species. Region I (Abov and Gemer); Region II (Dolný Zemplín); Region III (Spiš and Tatry).

Tatry and Spiš are mainly tourist regions with three national parks situated on their territories.

According to environmental regionalization of Slovakia (ERS) conducted on information on the status of the environment in the SR over the last 20 years, Slovak territory is divided into three types of environmental quality. Regarding sampling regions, only Tatry area from Region III was classified as a region of 1st environmental quality (regions with the non-disturbed environment). On the other hand, Spiš area from the same sampling region was included to the 2nd environmental quality (regions with the moderately disturbed environment) with several zones (e.g Slovenské Rudohorie Mountains) ranked as regions of 3rd environmental quality (regions with the heavily disturbed environment). All districts from Region I (Abov and Gemer) and Region II (Dolný Zemplín) were included in the 2nd or 3rd environmental quality [11].

Samples Analyses

In the frame of the present study, liver and muscle tissues from birds of prey were analysed for seven trace elements (arsenic – As, cadmium – Cd, chromium – Cr, copper – Cu, mercury – Hg, manganese – Mn and lead – Pb).

Each sample (0.5-1.0 g) was digested in a Microwave Digestion System Ethos 1 (Milestone S.r.l.) using 30% hydrogen peroxide and 65 % nitric acid (Suprapur, Merck) in a 1:7 v/v. The digestion was held for 30 minutes, where the maximum temperature 200°C was reached in 15 minutes and was held to the end of the program next 15 minutes. After the digestion, samples were diluted with ultra-pure water (Milli-Q, Merck) and analysed for the elements concentration using electrothermal atomic absorption spectrophotometer (ZEEnit 700P, Analytik Jena AG). For detection of mercury concentration hydride generation atomic absorption spectroscopy was used (ZEEnit 700P, Analytik Jena AG).

Along with samples following Certified Reference Materials (CRM) were prepared to ensure the validity and accuracy of the analysis. For liver samples as CRM was used Bovine Liver BCR – 185R (European Commission – Joint Research Centre, Institute for Reference Materials and Measurements), and for muscle samples as CRM Chicken Meat GBW10018 (Institute of Geophysical and Geochemical Exploration, Langfang, China) was used. All concentrations of monitored elements are expressed in ppb (parts per billion; ng/g) on a wet weight basis (ww). In discussion, when comparing our data with the data expressed per dry weight basis (dw), we used a conversion factor of

3.0 for both liver and muscle to calculate the corresponding concentrations according to recommendation [12].

The data obtained within the study were put through descriptive statistical analyses. Data from all elements analysed except As in muscle are included in Table 1. It is since all arsenic values in muscle are below the limit of detection (LOD). Data for Cu and Mn for both organs, Cr for muscle, Hg for muscle only in region III and Cd for liver were without non-detects. The rest

of the data includes both detected and non-detected values, (so-called censored data) and must be analyzed concerning the amount of data below the detection limits. If non-detects were recorded between under 50%, non-detects were replaced with 0 and Aitchison's method was used for mean and variance calculation. In the rest of the censored data with clearly more than 50% non-detects, the only number of non-detects, median, and range of data are presented. Guidelines of the Environmental Protection Agency were used [13].

Table 1. Trace elements concentrations (ng/g; ppb ww) in livers and muscles of examined birds of prey in three different regions of Slovakia. If more than 50% non-detects, only number of non-detects, median and range of data are presented. Limit of detection (LOD) for each elements measured in liver and muscle.

	Region I (n = 38)		Region II (n = 15)		Region III (n = 20)		LOD (ng/g)	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
As								
Number of detects	3	-	1	-	1	-	5.052	5.967
Median	Nd	-	Nd	-	Nd	-		
Range	Nd-32	-	Nd-1	-	Nd-30	-		
Cd								
Number of detects	38	10	15	5	20	9	1.252	2.211
Average	234	-	171	-	318	-		
Median	124	Nd	101	Nd	174	Nd		
Sd	329	-	223	-	340	-		
Range	0-1844	Nd-78	7-797	Nd-142	7-975	Nd-296		
Cr								
Number of detects	9	38	8	15	4	20	0.975	1.004
Average	-	84	18	60	-	111		
Median	Nd	52	5	48	Nd	56		
Sd	-	142	27	38	-	179		
Range	Nd-94	0-879	Nd-98	14-135	Nd-168	4-823		
Cu								
Number of detects	38	38	15	15	20	20	2.188	9.697
Average	5972	4716	7040	3929	9116	4605		
Median	5264	3498	4625	3714	7919	3294		
Sd	3720	4286	6093	2372	5399	3808		
Range	2521-25791	1303-26247	2037-23438	1670-10772	2990-21676	1718-19395		
Hg								
Number of detects	30	37	11	13	17	20	0.173	0.670
Average	95	90	42	128	325	78		
Median	53b	68	23b	123	167a	67		
Sd	136	71	75	100	336	63		
Range	Nd-565	Nd-314	Nd-278	Nd-323	Nd-1135	10-269		

Table 1. Continued.

Mn							1.681	0.732
Number of detects	38	38	15	15	20	20		
Average	4317	237	3965	209	5323	236		
Median	4209	226	3797	222	4942	217		
Sd	1572	102	1454	74	2045	71		
Range	1476-8049	74-705	1984-7837	73-341	2381-9188	159-417		
Pb							5.996	6.352
Number of detects	6	3	2	2	5	2		
Average	-	-	-	218	-	2226		
Median	Nd	Nd	Nd	Nd	Nd	Nd		
Range	Nd-13269	Nd-969	Nd-35452	Nd-333	Nd-13198	Nd-4441		

Ppb ww – parts per billion on a wet weight basis; nd – not detectable

To compare differences among regions only data without non-detects values and those censored data with less than 50% non-detects (Liver: Cd, Cu, Hg, Mn; muscle: Cr, Cu, Hg, Mn) was done by the nonparametric Kruskal-Wallis ANOVA with a subsequent post-hoc test using software Statistica [14]. Only significant differences are presented by boxplots.

Results and Discussion

Heavy metals, both essential and nonessential, and metalloids, are the most often analysed pollutants in wildlife. Wild animals chronically bioaccumulate trace elements generally without visible reaction to them, which would allow these organisms to be considered as suitable bioindicators [12]. Within the wildlife, avifauna, especially inland bird species, is the most intensively observed group of animals included in pollution testing and used as sentinels of environmental and human health [15]. Primarily birds of prey, situated at the top of the food chain, are at a higher risk of heavy metals accumulation in their tissues than other avian species [5].

Outgoing from numerous experimental and clinical studies, it is unambiguous that different trace elements are preferably deposited in different tissues at different rates. Although the liver and kidneys are the most frequently selected samples for biomonitoring of heavy metals and metalloids, muscles represent also an important source of information taking into account that various toxic elements are transferred between animals from different trophic levels via food chains. This is especially important in the case of mercury [16, 17]. Besides soft tissues some toxic elements, for instance, lead, are predominantly measured in bones [18]. On the other hand, metal concentrations in feathers are highly variable and depend on bird species and age, and for that reason some researcher state that feather samples

have only low priority for ecotoxicological studies [19, 20].

In our, here presented study, trace element concentrations, including arsenic, cadmium, chromium, copper, mercury, manganese, and lead, were evaluated in livers and muscles in twelve species of birds of prey. The results expressed as average, median, standard deviation, and minimum and range values, are presented within the context of sampling regions (Table 1) and examined raptor species (Table 2). For four species (*F. cherug*, *F. subbuteo*, *B. bubo* and *S. aluco*), with the occurrence of one or two investigated individuals, only concentrations of tested elements without calculations are presented in Table 3.

Copper (Cu)

Within our study, copper was detected with the highest concentrations and ranged between 2 037 and 25 791 ppb ww in liver samples, and from 1 303 to 26 247 ppb ww in muscles (Table 1). Copper, an essential mineral element widely distributed in nature, is an important part of enzymes and proteins, and inevitable for the energy metabolism and defense system of animals. On the contrary, copper is used very often in agriculture as a component of pesticides and may be extremely toxic when ingested. The main anthropogenic Cu-sources compromise pyrometallurgical industry, coal and oil combustion and steel and iron manufacturing [21]. Anyway, copper poisoning occurs especially in small ruminants and it is very rare in birds. For instance, the maximum tolerable level of copper set by the National Research Council Committee (USA) is 100-250 mg/kg in poultry, but only 15 mg/kg in sheep [22]. No exact data are available on the toxicity of Cu in avian wildlife, but according to US Department of the Interior Guidelines, no-effect level of Cu in bird liver has been stated at <60 mg/kg (60 000 ppb dw) [23]. In our study, the

Table 2. Trace elements concentrations (ng/g; ppb ww) in livers and muscles of six birds of prey species examined within the study.

	<i>Aquila heliaca</i> (n = 4)		<i>Buteo buteo</i> (n = 47)		<i>Buteo lagopus</i> (n = 3)		<i>Buteo rufinus</i> (n = 3)		<i>Asio otus</i> (n = 3)		<i>Strix uralensis</i> (n = 10)	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
As												
Number of detects	-	-	4	-	1	-	1	-	-	-	1	-
Median	-	-	nd	-	nd	-	nd	-	-	-	nd	-
Range	-	-	nd-32	-	nd-5	-	nd-31	-	-	-	nd-30	-
Cd												
Number of detects	1	-	29	2	1	-	-	-	-	-	9	4
Average	-	-	209	-	-	-	-	-	-	-	678	-
Median	nd	-	132	nd	nd	-	-	-	-	-	663	nd
Sd	-	-	298	-	-	-	-	-	-	-	509	-
Range	nd-108	-	nd-1499	nd-296	nd-91	-	-	-	-	-	nd-1844	nd-167
Cr												
Number of detects	1	4	2	37	-	2	-	2	-	1	-	8
Average	-	68	-	95	-	43	-	106	-	-	-	88
Median	nd	57	nd	55	-	49	-	63	-	nd	-	94
Sd	-	34	-	170	-	40	-	132	-	-	-	61
Range	nd-94	40-118	nd-104	nd-879	-	nd-79	-	nd-253	-	nd-39	-	nd-170
Cu												
Number of detects	4	4	47	47	3	3	3	3	3	3	10	10
Average	9788	4697	5324	4129	5290	3213	7361	3576	9876	5763	9195	2107
Median	10435	4901	4859	3426	4506	3044	8687	3476	5266	6211	6982	1772
Sd	5674	1604	2221	2042	2175	621	3883	426	9160	1844	6549	1232
Range	3623-14658	2601-6384	2037-12516	1373-13725	3616-7748	2695-3902	2990-10408	3208-4043	3937-20424	3736-7342	3284-23483	1327-5535

Table 2. Continued.

Hg												
	1	3	37	37	1	2	3	3	2	3	3	9
Number of detects	1	3	37	37	1	2	3	3	2	3	3	9
Average	-	63	90	82	-	51	421	131	38	61	289	135
Median	nd	81	44	69	nd	54	109	92	35	58	213	120
Sd	-	43	151	74	-	50	620	91	40	9	248	102
Range	nd-21	nd-90	nd-679	nd-326	nd-67	nd-100	19-1135	65-235	nd-79	53-71	nd-810	nd-296
Mn												
Number of detects	4	4	47	47	3	3	3	3	3	3	3	10
Average	3226	248	4309	249	3255	192	6271	251	3858	225	4353	145
Median	3288	238	4226	263	3652	213	7242	292	3464	226	3756	145
Sd	1001	84	1482	94	953	73	3506	75	1595	11	1602	37
Range	2168-4161	164-351	1476-7837	139-758	2167-3944	110-252	2381-9188	165-296	2497-5614	214-235	2382-7501	85-209
Pb												
Number of detects	2	1	2	3	-	-	1	-	-	-	1	-
Median	nd	nd	nd	nd	-	-	nd	-	-	-	nd	-
Range	nd-35452	nd-656	nd-13269	nd-4441	-	-	nd-639	-	-	-	nd-564	-

ppb ww – parts per billion on a wet weight basis; nd – not detectable

highest content of Cu, overlapping this toxic threshold (20 000 ppb ww after conversion), was detected in muscle tissue of *F. tinnunculus* from Košice (Region II) with concentration 26 247 ppb ww (78 741 ppb dw after conversion) and livers of *B. bubo* (25 791 ppb ww) from Region II, *S. uralensis* (23 483 ppb ww) from Region I, and *A. gentilis* and *S. aluco* from Region III (21 676 and 20 424 ppb ww) (Tables 2 and 3). Based on results of several scientific studies, it was found that the average concentration of Cu in liver samples of birds of prey from Europe is circa 17 mg/kg dw (5 666 ppb ww) with higher concentrations (more than 39 mg/kg dw) measured in common buzzards from Sicily [21]. A relatively high concentration of Cu in Slovak raptors is probably related to an occurrence of natural mineral deposits and long history of copper mining in Slovakia territory. At present, there are sixteen reserved deposits with reported Cu content, occurring mainly in Gemer and Spiš regions (regions II and III in our study) [24]. In these areas value of Cu concentration in the soil exceeds 150 mg/kg [25].

Manganese (Mn)

Manganese was the second-most detected element, though with high differences of concentrations recorded in livers' samples (1 476-9 188 ppb ww) and muscles (73-705 ppb ww) (Table 1). Mn is essential for lipid metabolism, growth and reproduction system. On the other hand, during the last decades, a dramatic increase of Mn compounds in the atmosphere was measured. Most Mn emissions have a connection to the production of iron and steel. In Slovakia, the main source of Mn in the soil is U.S. Steel Company in the Košice region [11]. According to a review study, Mn concentration in European birds of prey varies between 5.3 to more than 12.0 mg/kg dw (1 766 and 4 000 ppb ww after conversion) in the liver and 0.5 to 3.5 mg/kg dw (166 and 1 166 ppb ww after conversion) in muscles [26]. The maximum tolerable concentration of manganese in animals' diet is high. Manganese concentration in the liver above 70 ppm dw is considered as poisoning, but there are practically no studies that reported acute toxicosis [22].

Mercury (Hg)

The third metal found within the present study in the livers and muscles of birds of prey was mercury. Mercury together with cadmium and lead belongs to the group of particularly toxic metals. It is estimated that during the last 200 years, the concentration of Hg in nature has increased at least three times. Moreover, Hg released from natural and anthropogenic sources circulates in nature for a long time [27]. Proposed indicative liver Hg concentration associated with mortality in birds is 20 mg/kg ww (20 000 ppb ww) and with effect on reproductive system 2 mg/kg ww (2 000 ppb ww) [28]. Within our study, Hg was detected

with the highest concentration in *S. uralensis* (Ural owl), with a median value of 213 ppb ww in livers and 120 ppb ww in muscles (Table 2). Comparing our results with other European researches, Kitowski et al. (2016) in Poland revealed a significantly higher average hepatic concentration of mercury in sparrowhawks (666 ppb ww), with the highest individual level of 3 996 ppb ww, if compared with kestrels (mean value 213 ppb ww) and common buzzards (211 ppb ww) [29]. The authors, based on Redundancy analysis, showed that higher concentrations of mercury in livers of sparrowhawks are strongly dependent on the consummation of small granivorous birds (such as passerines) feeding on grains illegally treated with Hg-based fungicide what was confirmed also in monitoring carried out in Hungary [30]. Another study conducted in Eastern Poland revealed Hg concentration in livers of white-tailed eagles (*Haliaeetus albicilla*) with mean values of 300 and 466 ppb ww in the northern and southern region, respectively [31]. In another study, carried out in Denmark, sparrowhawks and white-tailed eagles showed significantly higher mercury concentrations in their livers (average values of 1 280 and 1 210 ppb ww) than other raptor species included in the research [32]. The highest individual level of mercury in our study (1 135 ppb ww) was measured in the liver of *B. rufinus* (long-legged buzzard) from a locality situated in the Slovak-Polish border and belonged in Region III (Table 2). Subsequent comparative analyses revealed significantly higher concentrations of mercury in surveyed Region III when compared with the other two sampling regions (Table 1). The territory of the Spiš region was evaluated as a region with extremely polluted soils [25]. It was an important gold and mercury mining area for centuries and it can be also the reason for the higher Hg concentration in some samples [33]. Recent monitoring confirmed also strong contamination of surface waters in Spiš with several metals including mercury, copper and lead [34]. Moreover, in this region besides anthropogenic influences also geochemical anomalies are conducive to higher content of heavy metals [25, 33].

Cadmium (Cd)

Another analyzed element, cadmium, was recorded with higher concentrations in the liver when compared with muscle samples. Generally, cadmium accumulation is greatest in the kidney, followed by the liver. On the other hand, muscles and bones accumulate cadmium only in low levels [22, 35]. The highest average concentration of Cd within our study (318 ppb ww) was measured in livers of raptors from Region III, although the highest individual level of this element (1 844 ppb ww; 5 532 ppb dw after conversion) was found in *Strix uralensis* from Gemer (Region I) and in *Buteo buteo* (1 499 ppb ww) from Levice, the southern part of Central Slovakia (a nondescript region in our study) (Tables 1 and 2). Commonly,

Cd concentration above 3 mg/kg dw (1 000 ppb ww) may indicate increased environmental exposure and considered to be a toxic threshold for birds [36]. From recently published summarized data, cadmium concentrations in livers of terrestrial avian species range widely between less than detection limit and more than 17 000 ppb dw [37]. For instance, in Poland, Kitowski et al. revealed mean concentration of Cd in livers of raptors ranged between 282 and 786 ppb dw with a maximum value of 3 425 ppb dw (1 141 ppb ww after conversion) in one common buzzard. Contrary, in other species of birds of prey much lower cadmium concentrations were measured [29, 31]. Also, in another recent study, Kanstrup et al. (2019) revealed significantly higher cadmium concentrations in livers of common buzzards and tawny owls than in kestrels, goshawks and white tailed eagles [32]. In Galicia, Spain, the mean hepatic concentration of Cd was highest in owls, with mean concentrations moved between 3 381 and 5 516 ppb dw, and the highest rate of 39 890 ppb dw (13 297 ppb ww after conversion) measured in one barn owl (*Tyto alba*), when compared with diurnal raptor species [38]. The most important factor influencing cadmium bioaccumulation in birds' tissues is the level of Cd contamination in their habitat. Higher concentrations of cadmium in the environment are usually recorded around the area with mining and smelting activities. Moreover, this soft metal can enter the environment through other anthropogenic sources – it is used in batteries, plastic stabilizers, fertilizers, etc. [22]. In Slovakia, several biomonitoring surveys focused on heavy metals, including cadmium, in the environment were carried out. According to these studies, high Cd concentrations were found almost all over the territory as a result of geological conditions characterized by the polymetallic ore formation process and metallurgical processing of these ores in the past. Besides, exceeded contents of Cd in the soil and water are the results of long-term phosphoric fertilizer application. As for Cd analysis, its concentrations in burden areas of Eastern Slovakia double exceeded the background values of the cambisols. Except for a few isolated samples, exceeding the content limit of cadmium was revealed in the mountainous area of Spiš and northern Gemer (Region III and I in our study) around the metallurgical and natural geochemical anomalies [10]. Another study, analyzing the soil-plant transfer of heavy metals, confirmed that the highest admissible amount of cadmium in feeds was exceeded in permanent grass of Spiš and the Slovenské Rudohorie Mountains [25]. It corresponds with our findings referring to the highest average hepatic Cd concentration in Region III and also the highest individual concentration in Gemer, Region I.

Chromium (Cr)

Considering chromium, it occurs naturally in trivalent form, which is generally recognized as an

essential mineral, and in the more toxic hexavalent form used in the chemical industry and subsequently contaminating water, air, and soil. In the cells of bird, hexavalent Cr is reduced to trivalent Cr, which produces highly toxic free radicals. Unlike cadmium predominantly concentrated in the avian liver and kidney, the highest percentage of chromium is cumulated in muscles followed by feathers with a much lower amount located in the liver [39]. Also, our results confirmed a much higher concentration of Cr in muscle tissues, ranged from 0 to 879 ppb ww, when compared with liver samples. The highest average Cr concentration, 111 ppb ww (333 ppb dw after conversion), was measured in muscle samples from Region III and the highest individual concentration, 879 ppb ww (2 637 ppb dw after conversion), was recorded in muscle sample of *Buteo buteo* from Region I (Tables 1 and 2). Despite a quite large number of studies that analyzed Cr bioaccumulation in avian species, only seldom birds of preys' muscles were examined [39]. Several studies were performed in the United States on bald eagles (*Haliaeetus leucocephalus*) where Cr cumulation in livers and brains was analyzed with the mean concentration values reached 1280 and 1 470 ppb dw, respectively (427 and 490 ppb ww after conversion) [40]. In Poland, a wider spectrum of raptors species was investigated with Cr concentrations in livers ranged between 300 and 1 220 ppb dw (100 and 406 ppb ww after conversion) [29, 31]. According to our knowledge, no formal reference values of Cr in tissues of wild birds were set. However, according to Wisconsin Veterinary Diagnostic Laboratory (WVDL), the normal values of chromium in avian liver moved between 170 and 1 330 ppb dw (57 and 443 ppb ww) [41].

Lead (Pb)

Other analysed heavy metal, lead, revealed detectable concentrations only in fourteen livers and eight muscle samples. Liver concentrations of Pb over 6 ppm (6 000 ppb dw; 2 000 ppb ww) are considered as toxic threshold and external signs of poisoning may be present in affected animals. The concentration of Pb in avian liver above 30 ppm (30 000 ppb dw; 10 000 ppb ww) reflects acute poisoning [42, 43]. Lead toxicosis is connected with cardiovascular, haematological and neurodevelopmental signs at low exposure levels, and renal, hepatic and immunological signs at higher doses or long exposure [22]. In our study, the hepatic Pb concentrations over the threshold of acute poisoning (10 000 ppb ww) were measured in three individuals: *Aq. heliaca* from Dolný Zemplín, Region II (35 452 ppb ww), *B. buteo* from Košice belonged to Region I (13 269 ppb ww) and *A. gentilis* from Spiš, Region III (13 198 ppb ww) (Tables 2 and 3). In other two individuals of *B. buteo*, both from Stará Ľubňa district situated on the Slovak-Polish border (Region III), high concentrations of Pb in liver and muscle samples, 3 164 and 4 441 ppb

Table 3. Heavy metals concentrations (ng/g; ppb ww) in livers and muscles of individuals from species *Accipiter gentilis*, *Falco cherrug*, *Falco subbuteo*, *Falco tinnunculus*, *Bubo bubo* and *Strix aluco*.

	<i>Accipiter gentilis</i> (n = 2)		<i>Falco cherrug</i> (n = 1)	<i>Falco subbuteo</i> (n = 2)		<i>Falco tinnunculus</i> (n = 2)		<i>Bubo bubo</i> (n = 2)		<i>Strix aluco</i> (n = 1)
	Individual 1	Individual 2	Individual 1	Individual 1	Individual 2	Individual 1	Individual 2	Individual 1	Individual 2	Individual 1
As										
Liver	nd	nd	nd	nd	nd	nd	nd	nd	nd	69
Muscle	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cd										
Liver	574	184	nd	88	164	354	14	23	211	206
Muscle	6	20	nd	nd	17	nd	nd	nd	nd	nd
Cr										
Liver	nd	nd	nd	nd	50	nd	168	nd	nd	nd
Muscle	29	90	118	51	16	14	43	81	74	87
Cu										
Liver	4995	21676	5364	13444	8306	5472	9445	25791	5324	20424
Muscle	2788	2812	4450	10772	4232	4626	26247	1670	13333	1303
Hg										
Liver	371	357	nd	489	848	74	nd	564	403	42
Muscle	323	314	nd	124	149	54	139	165	173	47
Mn										
Liver	3546	8637	2116	8036	4092	6218	7532	8049	4992	9240
Muscle	203	190	270	341	271	253	227	73	74	163
Pb										
Liver	nd	13198	nd	nd	nd	nd	nd	nd	nd	nd
Muscle	333	nd	nd	nd	nd	nd	nd	nd	nd	nd

ppb ww – parts per billion on a wet weigh basis; nd – not detectable

ww, respectively, were found. A similar study from Eastern Poland revealed in livers of examined *B. buteo* (n = 31) mean lead concentration value of 1 217 ppb dw (406 ppb ww after conversion) with the maximum rate of 15 310 ppb (5 103 ppb ww after conversion) [29]. Another study from Poland focused on the detection of lead in white-tailed eagle, revealed high differences of median Pb liver concentration between the northern and southern area of Eastern Poland with measured values of 36 900 ppb dw (12 300 ppb ww) and 700 ppb dw (233 ppb ww), respectively. Within this study very high individual concentration of Pb, 188 600 ppb dw (62 867 ppb ww after conversion), was confirmed [31]. Kanstrup et al. (2019) found out significantly higher lead concentrations in common buzzards than in kestrels, though an extreme value of lead concentration (149 000 ppb dw; 49 667 ppb ww) was measured in the kestrel shot at the airport

in Jutland, Denmark [32]. Ingested shots through the carrions and games seem to be the main source of lead poisoning in raptors, especially in buzzards and eagles that catch a wide range of prey [44, 45]. Also, the latest European study focused on biomonitoring of terrestrial environment for pollutants confirmed that common buzzard and golden eagle (*Aquila chrysaetos*) proved the most suitable sentinel for lead [46]. Additionally, Monclús et al. (2020) in their research revealed a seasonal peak in blood lead concentrations related to hunting season [47].

Currently, according to Agency for Toxic Substances and Disease Registry (ATSDR) Substance Priority List, lead is considered the second most dangerous environmental poison [48]. Atmospheric lead is a result of industrial and traffic-related emissions. According to the World Health Organisation (WHO), the average concentration of Pb in the air around large European

cities ranged from 0.5 to 3.0 $\mu\text{g}/\text{m}^3$. The most lead-polluted air is found around mines and metal smelters, where it can reach 12 $\mu\text{g}/\text{m}^3$ [49]. In Slovakia, extremely high content of Pb was detected also in soils around agglomeration of Krompachy, Spiš region, as the result of solid pollutants emitted from the chimneys in the pit furnaces [10]. High lead concentrations were measured also in soils of the Volovské Mountains in the border of Gemer and Spiš regions, and an important Pb reserved deposit is situated in Dolný Zemplín [24]. Even though our results disclosed high Pb concentrations in raptors tissues from all sampling regions, the values exceeded 2 000 and even 10 000 ppb ww point to probability of acute poisoning through lead shots ingestion.

Arsenic (As)

Only eight liver samples showed the presence of very small concentrations (from 1 to 69 ppb ww) of metalloid arsenic. Arsenic was detected only in raptors from the genus *Buteo* (*B. buteo*, *B. rufinus*, *B. lagopus*) and *Strix* (*S. uralensis* and *Strix aluco*) (Tables 2 and 3). None of the muscle samples was tested positive for this element. Also, studies across Europe revealed that in most cases concentration of As in animal tissues was lower than the detection limit [50]. Contrary, in birds of prey from Galicia, Northern Spain, high mean levels of hepatic As concentrations were measured and ranged between 1 212 ppb dw (404 ppb ww after conversion) and 6 878 ppb dw (2 293 ppb ww after conversion) [38].

Generally, As in environment occurs with higher concentration in geographical regions characterized by strong volcanic activity. The Land Use/Land Cover Area Frame Survey (LUCAS) performed in European Union showed that in more than 95 % of topsoil samples As was detected with concentration below 20 mg/kg and a high portion of samples contain As below the limit of detection [33]. In Slovakia, the highest As levels, connected to metal purification industry and fossil fuel combustion, were detected in the Košice region [34].

Regional Differences

Regarding geographical differences, in birds of prey's livers from Region III Kruskal-Wallis Anova ($H(2, N = 73) = 10.06, p = 0.01$) and the subsequent post-hoc test revealed a significantly higher concentration of mercury when compared with Region I ($p = 0.05$) and Region II ($p = 0.05$). Additionally, differences among the three regions were confirmed for copper from the liver ($H(2, N = 73) = 6.85, p = 0.03$), but without significant differences between couples of regions. Also, the highest average and median values concerning liver concentrations of cadmium and manganese and muscle concentrations of chromium were measured in samples originated Region III. Significant differences in the distribution of Hg and Cu in the liver among regions are clear also from Fig. 2.

Region III (Spiš and Tatry region) represents an area located in the north-eastern and north-central parts of

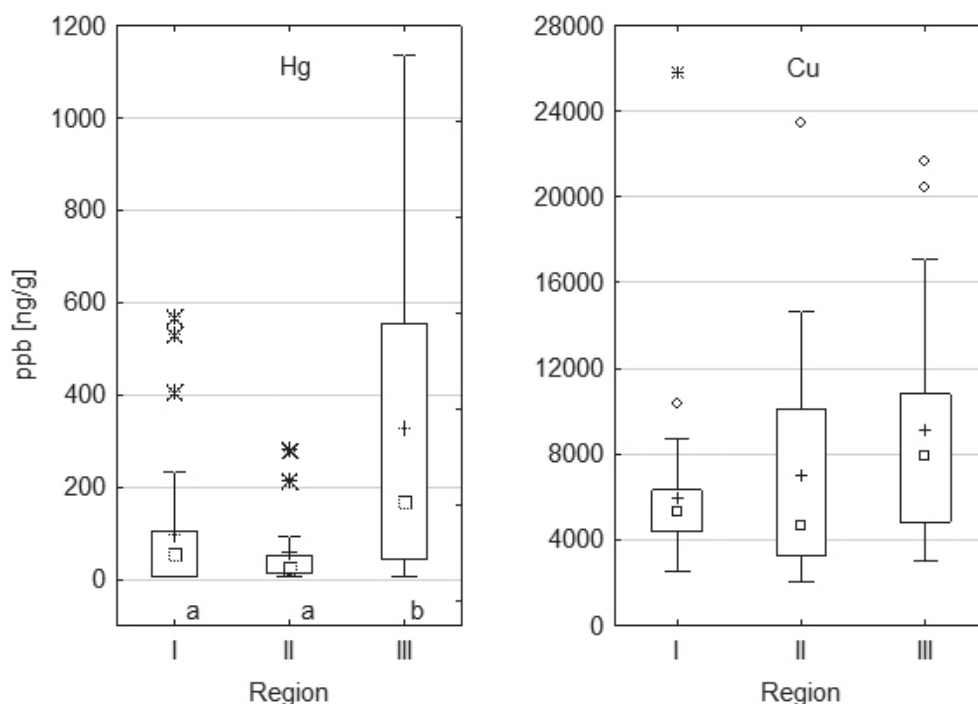


Fig. 2. The boxplots of Hg and Cu distribution in liver samples with significant differences among regions. Significant differences ($p = 0.05$) confirmed by post-hoc test after Kruskal Wallis ANOVA between couples of regions only for Hg are marked by different lowercase letters. Square - median, box - 25%-75%, whiskers - interquartile range, circle - outliers, star - extremes, plus - average.

Slovakia bordering Poland (Fig. 1). Especially in the Spiš region, the most of heavy metal-loaded habitats are located. These habitats were created as a consequence of the long-term history of pyrite, copper, and mercury mining and smelting. The soils of these areas are ranked among the most contaminated with mercury, arsenic, copper, zinc and lead [10]. Besides, the occurrence of natural endogenous geochemical anomalies is particularly prevalent in mountainous zones of this region [25, 33].

Conclusion

Here presented study represents the first published results of toxic elements biomonitoring, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn) and lead (Pb), in birds of prey species from Slovakia. The results revealed that hepatic concentration of Cu and Cd overlapped defined toxic thresholds in several individuals from all sampling regions. Moreover, Pb-analyses confirmed concentration rates suggesting acute poisoning (over 10 000 ppb ww) *Aq. heliaca*, *B. buteo*, and *A. gentilis*, each of them originated in a different region. Subsequent comparative analyses revealed significantly higher concentrations of mercury in surveyed Spiš and Tatry Region when compared with the other two regions. It is worth noting that the whole territory of this region is covered by national parks (High and Low Tatra national parks, Pieniny National Park, and Slovenský raj National Park) and protected areas and several European Important Bird Areas occurred there. The area provides suitable conditions for a high diversity of bird species; it is an important nesting site for forest birds, birds of prey and forest owls. Nevertheless, from a glance at wildlife health and ecology, it is necessary to mention that the whole region is used primarily for tourism and recreational activities. Additionally, the nearly whole area is used for foresting and hunting purposes and a quite large area of military training zone is located here. All these activities lead to an excessive disturbance and threat to the birds. Moreover, considering rich mining and smelting history and also the existence of natural geochemical anomalies, toxic elements biomonitoring should continue in this area.

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Conflict of Interest

The authors declare they have no conflict of interest.

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