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Elemental content in the tissues of the song thrush *Turdus philomelos* I. Accumulation of macro- and microminerals in internal organs and tissues

M. HAAS and M. KOČVARA

Institute of High Mountain Biology, Žilina University,
Tatranská Javorina 7, SK-059 56, Slovak Republic;
e-mail: martina.haas@uniza.sk

Abstract. The study provides a basic picture of the level of accumulation of metallic elements in the tissues of the song thrush *Turdus philomelos*. Between 1995 and 2022, thrush carcasses were randomly collected on roads in the High Tatras region (Slovakia). Elements in heart, liver, kidney, pectoral, femoral uscle and femur tissue were analysed by X-ray fluorescence spectrometry from dead individuals. The highest measured values (> 1000 ppm) were found for the elements P, S, Cl, K, Ca and Fe. The trend of accumulation of these elements is approximately the same in internal organs. The content of other elements (Cr, Mn, Cu, Zn, Se, Rb, Mo, Ba, Pb, Hg) was an order of magnitude lower (< 200 ppm) in organs and tissues, and the trend of accumulation in individual organs is variable. We also focused on the correlation between individual elements and tissues. Evaluation in relation to other factors such as gender, location and season will be the subject of an upcoming study.

Key words: accumulation of elements, internal organs, song trush, High Tatras

Introduction

Chemical elements that are part of abiotic and biotic components of the environment come from natural sources. Their natural quantities and cycling can be disturbed by natural events (volcanic activity, floods, forest fires), or due to human activities (industrial and agricultural emissions). A disproportionate increase in the concentration of an element in the atmosphere, lithosphere, and hydrosphere can result in contamination of the environment, which can be reflected in changes to ecosystems. These changes are progressively manifested from the lowest levels of trophic chains. The transfer of elements difficult to degrade from biological structures leads to biomagnification, (i.e., an increase in the concentration of elements in higher trophic levels), particularly in the case of heavy metals that are biologically non-degradable, such as mercury (Lv and Liu 2019) Although some of the potentially toxic elements (e.g., Cu, Zn, Ni) are important components of biological structures (Azimi *et al.* 2003, 2005; Pacyna and Pacyna 2001; van der Gon *et al.* 2007; Engwa *et al.* 2019), their elevated levels have negative effects on the functioning of organisms (Mukhtar *et al.* 2020). The toxicity of these compounds is determined by various parameters, including chemical species, route of exposure, dose, as well as gender, age, genetics, and nutritional status of those exposed (Kaur and Sharma 2022).

In addition to organically bound biogenic elements (hydrogen, carbon, nitrogen, and oxygen), there are about 20 inorganic mineral elements that are considered essential for animal life. These essential mineral elements are classified into two main groups according to their concentration in the animal's body; macro-elements (calcium, manganese, sodium, potassium, phosphorus, chlorine and sulphur), and micro-elements (iron, zinc, manganese, copper, iodine, cobalt, nickel, fluorine, vanadium, chromium, molybdenum, selenium, tin, silicon).

Minerals and trace elements have many functions in the body; they are essential components of skeletal structures, play a key role in maintaining osmotic pressure (and thus regulating the exchange of water and solutes in the body), serve as structural components of soft tissues, are essential for nerve transmission and muscle contraction, play a fundamental role in the acid-base balance of the body (and thus regulate the pH of blood and other body fluids), and serve as essential components of many enzymes, vitamins, hormones and respiratory pigments, or as cofactors of metabolism, catalysts and activators of enzymes.

Among the elements, heavy metals form an exclusive group, which are known for their toxic and degrading effects on organisms, even at low concentrations. Most commonly, heavy metals are defined as metals with high densities, atomic weights, or atomic numbers (Kaur and Sharma 2021). The criteria used vary depending on the author and context (Pourret *et al.* 2021). This group also includes biologically irreplaceable trace elements (e.g., Cu, Zn, Mn, Co, Cr, Fe, Mg, Mo, Ni, etc.), which are required for various biochemical and physiological functions (WHO/FAO/IAEA 1996), as well as other essential chemical elements (Ba, Hg, Cd, Pb, Cd, etc.) that do not have established biological functions (Chang *et al.* 2018).

The toxicity of heavy metals increases when they enter the environment and bind to environmental elements such as water, soil, or air. The heavy metals with the most potential for contamination include mercury, cadmium, arsenic, chromium, nickel, copper, and lead (Kaur and Sharma 2021). Harmful heavy metals including iron, mercury, and lead deteriorate soil quality and agricultural yields. Long-term exposure to heavy metals like zinc, lead, copper, nickel, and cadmium can damage both plant and animal organisms. Heavy metals accumulate in the kidneys, liver, brain, bones, heart, and other vital organs, resulting in improper function.

Sentinel species are most used to investigate the process of incorporation of harmful elements in food chains, where direct toxic metal loads can be detected, and environmental health can be monitored through these bioindicators (Matsinos and Wolf 2003; Hazen *et al.* 2019; Paiva *et al.* 2015). Analysis of environmental contamination using living organisms versus abiotic factors is a preferable method because it can provide accurate information on the bioavailability and bio-transfer of heavy metal pollutants (Markowski *et al.* 2013; Bauerová *et al.* 2017).

Environmental contaminants and the movement of pollutants through ecosystems, as well as their harmful effects on organisms, are a common subject of study in avian ecotoxicology (García-Fernández 2014). Birds are often used as bioindicators of pollution, because they are a well-studied group of animals biologically, ecologically, and ethologically, with precise system classification (Padoa-Schioppa *et al.* 2006; Martínez 2012). They inhabit diverse habitats and fill diverse feeding niches. Foraging and seasonal migrations represent a complex way of life. Through food and water intake, toxic elements enter the birds' bodies, where they subsequently bioaccumulate in various tissues (Dolan *et al.* 2017; Vizuete *et al.* 2018).

Environmental pollutants have been studied in various tissues of birds, including feathers, preen oil, eggshells, liver, kidney, blood, pectoral, and pelvic muscles (Sani *et al.* 2020; Castro *et al.* 2011; Frantz *et al.* 2012). The accumulation of toxic trace elements in different tissues depends on organ physiology, including detoxification and excretion potential as well as distance from the source, type, and extent of exposure (Abbasi *et al.* 2015).

The accumulation of toxic metals in tissues provides information about the environmental situation (Dmowski 1999). Toxicological studies have analyzed common internal organs such as liver and kidney for this purpose (Swaileh and Sansur 2006; Kitowski *et al.* 2017; Mukhtar *et al.* 2020; Zaman *et al.* 2022). Other tissues, such as skeleton, muscle (Zhang and Ma 2011; Mukhtar *et al.* 2020), eggs (Espín *et al.* 2016; Ding *et al.* 2019), and tissues collected in a non-invasive manner such as feathers and excreta have also been the subject of previous studies (Dauwe *et al.* 2000; Adout *et al.* 2007; Costa *et al.* 2013; Markowski *et al.* 2013). The chemical nature of the contaminant also plays a large role in preferential bioaccumulation in different tissues (Dolan *et al.* 2017; Castro *et al.* 2011). In addition to individual organ intoxication, the negative effects of heavy metals in birds include population declines, avian mortality, reduced disease resistance, and reduced egg production (e.g., Scheuhammer 1987; Richard *et al.* 2021; Pandiyan *et al.* 2022).

In this study, the song thrush (*Turdus philomelos*) was chosen as the subject species. It is a common species inhabiting the foothills of Slovakia, where the research was conducted, and its ecological, physiological, and morphological characteristics are well known (Robinson *et al.* 2004).

The genus *Turdus* has a cosmopolitan distribution, with 65 known extant species occurring in South America, Central and North America, Africa, and Eurasia; one species (*T. merula*) has been introduced to Australia (Voelker *et al.* 2007). The species breeds in most of Europe (although not in most of Iberia, lowland Italy, or southern Greece). Birds from Scandinavia, Eastern Europe, and Russia migrate to wintering areas around the Mediterranean, North Africa, and the Middle East, but few birds in the more temperate west of the breeding range leave their breeding areas (Clement and Hathway 2010). Most of the population is migratory, and winters in small numbers in Slovakia. It commonly breeds throughout Slovakia, with the highest breeding sites recorded in the Tatra Mountains at 1680 m a.s.l. (Šťastný and Krištín 2021). It occurs in all habitats, including coniferous and deciduous forests, as well as habitats associated with humans, such as gardens and parks (Danko *et al.* 2002).

The aim of this study is to investigate the rate of metal accumulation in the internal organs of the song thrush. Carcasses of dead birds found on roads due to being hit by cars, or near buildings when they died by hitting a window, were used. The carcasses were collected between 1995 and 2022, from the High and Low Tatras. Levels of metallic elements (phosphorus, sulphur, chlorine, potassium, calcium, chromium, manganese, iron, copper, zinc, rubidium, molybdenum, barium, lead, and mercury) were detected in tissues of internal organs (heart, liver, kidneys), in muscle tissue (pectoral and femoral muscles), and in bone (femur). Mercury levels were also determined in the tail feathers. In this study, we will focus on the accumulation of metallic elements in the tissues analysed, and their evaluation in relation with other factors such as gender or location will be the subject of a subsequent study.

Material and Methods

Research location

Individuals that died because of a collision with a car or hitting a window at the Institute of High Mountain Biology (IHMB; N 49.2678175°; E 20.1432986°) in Tatranská Javorina (Slovakia), were used in this research. Birds that died as a result of collision with a car were found on the following roads: road 539 near the village of Štôla (N 49.0913317°; E 20.1414197°); road 540 from the village of Veľká Lomnica (N 49.1192931°,E 20.3526094°) in the direction - Stará Lesná - Tatranská Lomnica (N 49.1658472°; E 20.2790739°); road 537 in the direction Nová Polianka (N 49.1183203°; E 20.1517989°) - Tatranské Matliare (N 49.1775389°; E 20.2940356°); road 66 - Tatranská Kotlina (N 49.2263017°; E 20.3221019) - Ždiar (N 49.2698083°; E 20.2652392°) - Podspády

(N 49.2805942°; E 20.1755375°) - border crossing (Podspády - Jurgów); and on road 3078 in the direction from Podspády - Tatranská Javorina - Lysá Poľana (N 49.2633806°; E 20.1165394°). A group of 12 dead birds found at Chopok (April 2013, north part; N 48.9441158°; E 19.5902914°), was also included in the research. These birds died because of a sudden change in weather conditions during spring migration. Birds were collected throughout 1995-2022, between March and October and stored in freezer boxes (-18 °C) at the IHMB. In total, 52 individuals represented the set of investigated birds.

Laboratory analysis

If possible, wing length, tarsometatarsal length, and tail length were measured prior to necropsy. Autopsies were performed according to standard protocols. After necropsy, internal tissues (heart, liver, kidney), pectoral and thigh muscles, and femur were collected. As far as possible, a tail feather was taken from each individual to determine mercury levels. Tissues were dried separately in an IF 160 Plus laboratory incubator (Memmert, Germany) at 50°C and 30% air circulation for 24 h before further analysis. After drying, the samples were homogenised in a cryomill (Retsch, Germany).

Analysis of metals by X-ray fluorescence spectrometry

The presence of metallic elements in tissues was detected through röntgene fluorescence spectrometry, using a DELTA handheld XRF analyzer (Olympus, Innov-X Systems, USA). The homogenized sample was squeezed to cover the entire surface of the vial, which was placed in a closed Delta XRF work box (Olympus, Innov-x Systems, USA), an accessory of the device. The spectrometer was calibrated using the NCS ZC 71001 certified bovine liver reference standard. Samples were analysed for 240 seconds in three 80-second measurements. The elements measured were P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, and Pb. Only elements that were regularly measured above the detection limit were included in the statistical analysis.

Direct analysis of mercury in samples

Mercury levels in individual tissue and feather samples were determined using a DMA-80 direct mercury analyser (Milestone, Italy). The method for determination of Hg in the sample uses the principles of thermal decomposition, amalgamation, and atomic absorption. The analysis of the sample in the instrument itself does not require any sample preparation.

Statistical analysis

Elemental levels were measured in 35 heart samples, 45 liver samples, 7 kidney samples, 50 pectoral muscle samples, 48 thigh muscle samples, 47 femur samples and 47 feather samples. The measured levels of the elements (phosphorus, sulphur, chlorine, calcium, potassium, iron, chromium, manganese, copper, zinc, selenium, rubidium, molybdenum, barium, lead) that were routinely measured above the detection limit were statistically evaluated. Mercury levels, which were measured using a direct mercury analyzer, are also included in the overall comparisons. Statistical processing of the results was performed using Statistica 12 (StatSoft, Inc.). Homogeneity of variance and normality were confirmed by the Shapiro-Wilk test; however, the data tested did not have a normal distribution. Descriptive statistical methods were used to calculate the mean values of the elements for each organ. Tissues and mean levels of analyzed elements were compared using the Kruskal-Wallis H test, and pairwise differences between two tissues were tested using the Mann-Whitney U test. The confidence interval was 95% ($p < 0.05$).

Results

Content of metal elements and their distribution in tissues

The measured values of elements detected by X-ray fluorescence spectrometry were regularly above the detection limit, including: P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, Rb, Mo, Sb, Ba, and Pb. Mercury values were determined by direct analysis. The proportional representation of each detected element in internal organs and tissues (heart, liver, kidney, pectoral and femoral muscle, femur) is shown in Fig. 1.

The highest measured values (> 1000 ppm) were found for phosphorus, sulphur, chlorine, potassium, calcium, and iron. The trend of accumulation of these elements (Fig. 2) was approximately the same in the internal organs. In the thigh muscle, there were significantly higher values of calcium (M-W U (1; 97) = 606; p = 0.00004), sulfur (M-W U (1; 97) = 759 ; p = 0.002675), and chlorine (M-W U (1; 97) = 704; p = 0.000674), compared to pectoral muscle.

Levels of other elements in organs and tissues were an order of magnitude lower (< 200 ppm) and the trend of accumulation in individual organs is variable (Fig. 3). In thigh muscle, significantly higher levels of chromium (M-W U (1; 97) = 683; p = 0.000381), manganese (M-W U (1; 97) = 873; p = 0.029242), zinc (M-W U (1; 97) = 213; p = 0.00000), and barium (M-W U (1; 95) = 842.5; p = 0.033496) were found compared to the pectoral muscle. Conversely, significantly higher levels of copper (M-W U (1; 78) = 184; p = 0.00000) were found in the pectoral muscles.

Correlation between different tissues and elements

Phosphorus (P) levels were highest in thigh bones and lowest in muscle tissue. Within organs, phosphorus levels were varied (K-W H (5; 227) = 125.3939; p = 0.0000) and did not differ between kidney and heart and between pectoral and femoral muscles (Table 1).

The lowest *sulphur* (S) values were measured in bones and the highest were measured in kidneys and other internal organs; these differences were significant (K-W H (5; 228) = 75.1651; p = 0.0000). Sulphur levels did not differ between liver and heart tissue, or between liver and muscle tissues (Table 1).

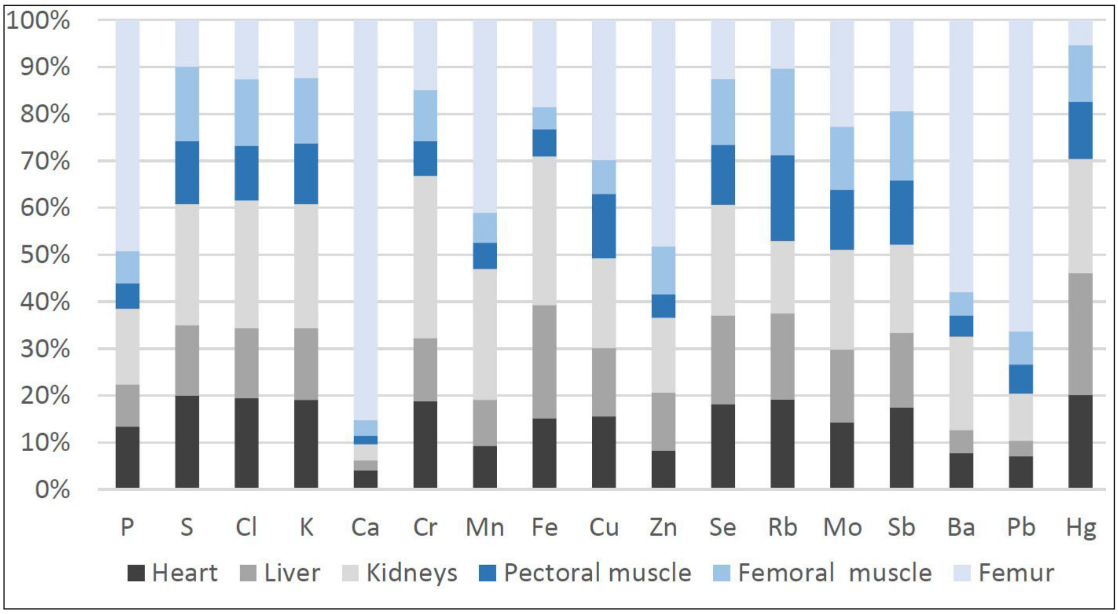

Fig. 1. Proportional element concentrations among internal organs, muscles, and bone of song thrush.

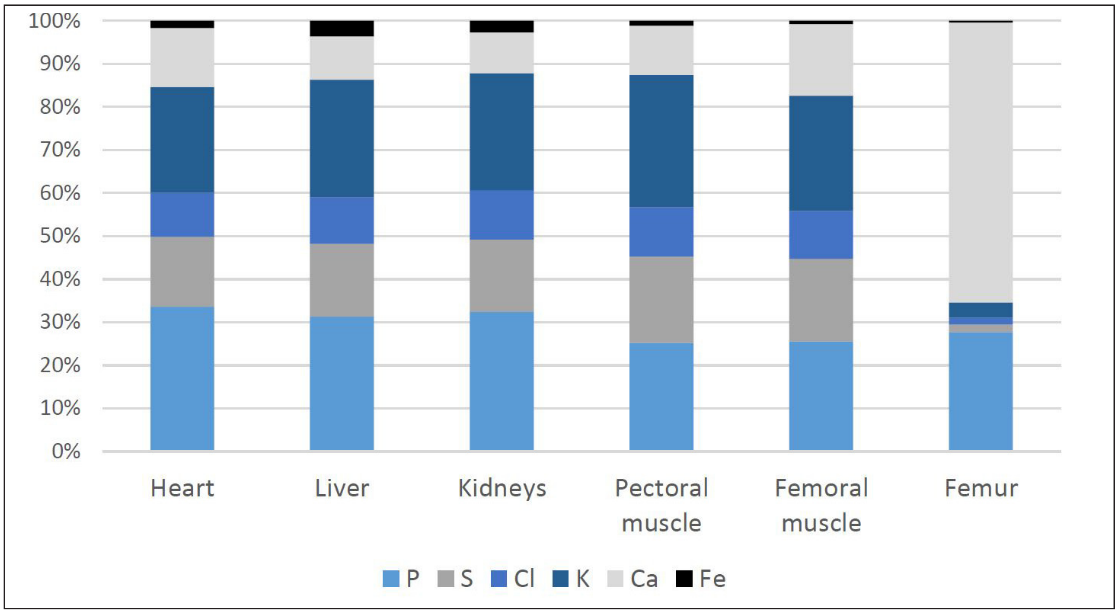

Fig. 2. Trend in accumulation of elements with highest measured concentrations in internal organs and muscles and bone.

Differences in *chlorine* (Cl) accumulation in individual tissues were significant (K-W H (5; 228) = 37.4814; p = 0.00000). The highest chlorine levels were measured in internal tissues such as kidney, heart, and liver, and the lowest in bone. Chlorine levels did not differ between kidney and heart, femoral muscle and liver, femoral bone and liver, or between femoral bone and muscles (pectoral, femoral) (Table 1).

Potassium (K) accumulation in individual tissues (K-W H (5; 228) = 39.5962; p = 0.00000). The lowest potassium levels were found in bone and the highest in tissues of internal organs, especially kidney. Potassium levels did not differ between heart and kidney, between femoral muscles and liver, femoral muscle and pectoral muscle and between femoral bone and muscles (Table 1).

Calcium (Ca) is a major element in bone tissue (highest measured Ca values), therefore, we assessed accumulation both in bone tissue and without it. In both cases, differences in element accumulation between tissues were significant (with bone: K-W H (5; 227) = 117.5013; p = 0.0000; without bone: K-W H (4; 183) = 26.95600; p = 0.0000). Values in pectoral muscles were the lowest and differed significantly between and internal organs (heart, liver, kidney), as well as between pectoral and femoral muscles. Predictably, differences were significant between bone and the other tissues analysed (Table 1).

Iron (Fe) levels in tissue varied significantly (K-W H (5; 228) = 124.2566; p = 0.0000). The highest iron levels were found in the kidney and liver; these values were not significantly different (Table 1). The lowest values were measured in muscle tissues, which were also not significantly different. The differences between bone tissue, heart, and liver values were also insignificant.

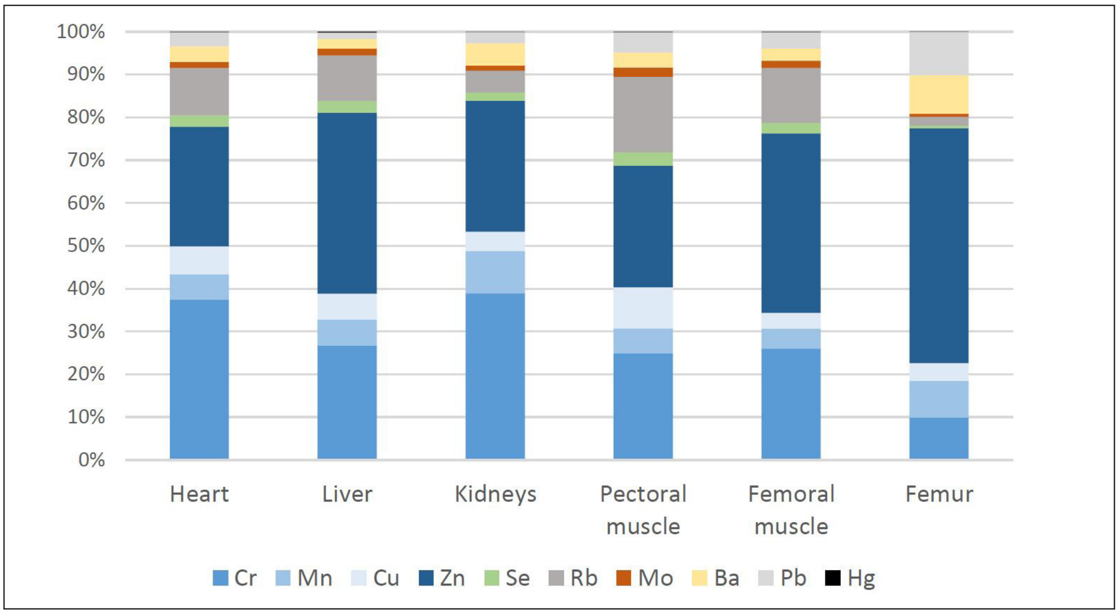

Fig. 3. Accumulation of elements with lower measured concentrations in internal organs and muscles and bone.

Chromium (Cr) accumulation differed significantly between individual tissues (K-W H (5; 228) = 60.5434; p = 0.0000). The highest levels were recorded in kidney and visceral tissues. Levels differed significantly except for between liver and femoral muscles (Table 1).

Manganese (Mn) accumulation in individual tissues differed significantly (K-W H (5; 228) = 117.5377; p = 0.0000). Muscle tissues showed the lowest values of this element; the highest values were found in bone. Values did not differ between femoral muscle tissue and liver tissue. Accumulation in other organs differed significantly from each other (Table 1).

Copper (Cu) accumulation differed significantly in individual tissues (K-W H (5; 164) = 44.4947; p = 0.00000). The highest values were recorded in bone, and these values differed significantly from levels in liver and muscle tissue (Table 1). Copper levels in femoral muscle tissue were significantly different from those in viscera and pectoral muscle tissue, and also levels significantly differed between liver and heart.

Zinc (Zn) accumulation differed significantly in individual tissues (K-W H (5; 227) = 156.7165; p = 0.0000). The highest amount of Zn was measured in bone tissue. Zinc levels did not differ significantly between femoral muscle and liver tissue (Table 1), while other element concentrations varied.

Selenium (Se) primarily accumulated in internal organs (kidney, liver, heart), and differential accumulation in tissues was statistically significant (K-W H (5; 126) = 3.1492; p = 0.0103). Accumulation differed between pectoral muscle, liver and kidney (Table 1).

Rubidium (Rb) preferentially accumulates in visceral tissues, and also in muscles, and its levels differed between the tissues analyzed (K-W H (5; 228) = 64.1; p = 0.0000). The lowest values were measured in bone tissue and differed significantly from other tissues (Table 1).

Molybdenum (Mo) accumulation differed between individual tissues (K-W H (5; 228) = 97.5683; p = 0.0000). The highest values of molybdenum were recorded in kidneys. Levels of molybdenum in liver and heart did not differ, nor did levels differ between femoral muscle and heart, femoral muscle and pectoral muscle, or between bone and kidney (Table 1).

The accumulation of *barium* (Ba) in individual tissues differed significantly (K-W H (5; 205) = 82.22; p = 0.0000). The highest values were measured in bone and kidneys. The levels did not differ between liver and muscles.

Although *lead* (Pb) levels were below the detection limit in many samples, we decided to further evaluate given its status as a toxic heavy metal. Of the values obtained, the highest levels were measured in bone tissue, which is also the most important organ for Pb accumulation (Table 1). Values in other tissues differed significantly (K-W H (5; 71) = 34.3826; p = 0.00000). The measured values in bone were significantly different from other tissues analyzed, except kidneys (Table 1). When comparing individual tissues without bone, we found no significant differences in Pb accumulation (K-W H (4; 27) = 4.280643; p = 0.3694).

Mercury (Hg) accumulation varied in individual tissues (K-W H (5; 231) = 79.9061; p = 0.0000). Of the tissues studied, mercury accumulated the most in the liver and kidneys. Mercury was also measured in feathers, which is common. These levels were the highest and also significantly different from those in other tissues (Table 1). Mercury levels did not differ significantly between internal organs, and also between muscles (Table 1).

Discussion

Minerals are chemical elements present in animal organisms in minor amounts, which are part of body structures and are necessary for the proper functioning of metabolic processes in the body. They enter the body through food. Macro-minerals provide essential ions in body fluids and form the major structural components of the body (Brinkman *et al.* 2018). Macro-minerals include cal-

Element	Tissue	n	Mean (ppm)	SE	Heart	Liver	Kidn.	PM	FM	Fem.
P	Heart	34	21262.90	4217.933						
	Liver	45	14338.60	2001.997	*					
	Kidneys	7	25716.00	3711.072	-	*				
	PM	49	8645.00	1463.045	*	*	*			
	FM	47	10869.70	2283.981	*	*	*	-		
	Femur	45	78540.60	6069.7	*	*	*	*	*	
S	Heart	34	10281.82	538.743						
	Liver	45	7718.44	612.141	*					
	Kidneys	7	13293.00	2055.161	-	*				
	PM	50	6913.16	395.103	*	-	*			
	FM	47	8177.36	357.281	*	-	*	*		
	Femur	45	5101.40	342.36	*	*	*	*	*	
Cl	Heart	34	6502.03	498.409						
	Liver	45	4980.71	436.539	*					
	Kidneys	7	9094.14	2030.353	-	*				
	PM	50	3917.31	289.121	*	*	*			
	FM	47	4726.43	238.168	*	-	*	*		
	Femur	45	4224.40	252.08	*	-	*	-	-	
K	Heart	34	15540.21	911.793						
	Liver	45	12479.67	799.216	*					
	Kidneys	7	21561.43	3800.387	-	*				
	PM	50	10547.58	691.361	*	*	*			
	FM	47	11381.26	561.237	*	-	*	-		
	Femur	45	10017.40	626.37	*	*	*	-	-	
Ca	Heart	34	8680.26	5116.48						
	Liver	45	4594.76	1258.743	-					
	Kidneys	7	7442.43	3360.9	-	-				
	PM	50	3907.29	2252.205	*	*	*			
	FM	47	7084.94	3268.75	-	-	-	*		
	Femur	44	184068.40	12021.45	*	*	*	*	*	
Fe	Heart	34	1055.38	122.573						
	Liver	45	1678.62	302.001	*					
	Kidneys	7	2204.57	577.179	*	-				
	PM	50	406.28	51.787	*	*	*			
	FM	47	328.49	33.371	*	*	*	-		
	Femur	45	1288.80	136.96	-	-	*	*	*	
Cr	Heart	34	101.62	9.343						
	Liver	45	72.38	15.992	*					
	Kidneys	7	186.71	36.065	*	*				
	PM	50	40.38	5.302	*	*	*			
	FM	47	58.55	5.982	*	-	*	*		
	Femur	45	80.40	8.6	*	*	*	*	*	
Mn	Heart	34	15.72	4.596						
	Liver	45	16.41	6.115	*					
	Kidneys	7	47.11	28.969	*	*				
	PM	50	9.55	2.104	*	*	*			
	FM	47	10.67	2.348	*	-	*	*		
	Femur	45	69.40	6.010	*	*	*	*	*	

Cu	Heart	32	17.64	1.779						
	Liver	43	16.51	3.183	*					
	Kidneys	7	21.71	7.552	-	-				
	PM	49	15.52	0.829	-	-	-			
	FM	29	8.27	1.098	*	*	*	*		
	Femur	4	33.8	8.94	-	*	-	*	*	
Zn	Heart	34	75.79	14.749						
	Liver	45	114.44	21.191	*					
	Kidneys	7	146.57	33.741	*	*				
	PM	50	46.05	7.714	*	*	*			
	FM	47	94.36	10.748	*	-	*	*		
	Femur	44	444.9	25.79	*	*	*	*	*	
Se	Heart	20	7.23	0.947						
	Liver	40	7.52	0.731	-					
	Kidneys	7	9.36	2.151	-	-				
	PM	33	5.11	0.324	-	*	*			
	FM	29	5.57	0.453	-	-	-	-		
	Femur	3	5	0.06	-	-	-	-	-	
Rb	Heart	34	29.88	2.614						
	Liver	45	28.75	1.817	-					
	Kidneys	7	24.1	1.427	-	-				
	PM	50	28.56	1.527	-	-	-			
	FM	47	28.93	1.875	-	-	-	-		
	Femur	45	16.1	0.86	*	*	*	*	*	
Mo	Heart	34	3.95	0.154						
	Liver	45	4.32	0.162	-					
	Kidneys	7	5.91	1.058	*	*				
	PM	50	3.54	0.13	*	*	*			
	FM	47	3.76	0.136	-	*	*	-		
	Femur	45	6.3	0.23	*	*	-	*	*	
Ba	Heart	34	9.69	4.535						
	Liver	45	6.16	1.586	*					
	Kidneys	7	24.79	17.567	*	*				
	PM	48	5.64	1.937	*	-	*			
	FM	47	6.3	1.94	*	-	*	*		
	Femur	24	72.4	8.05	*	*	*	*	*	
Pb	Heart	5	8.84	4.748						
	Liver	10	4.02	1.802	-					
	Kidneys	2	12.5	5.5	-	-				
	PM	5	7.7	3.358	-	-	-			
	FM	5	8.7	2.731	-	*	-	-		
	Femur	44	82.4	21.84	*	*	-	*	*	
Hg mg/kg	Heart	35	0.38	0.051						
	Liver	45	0.49	0.063	-					
	Kidneys	7	0.46	0.113	-	-				
	PM	50	0.23	0.03	*	*	*			
	FM	48	0.23	0.024	*	*	*	-		
	Femur	46	0.1	0.02	*	*	*	*	*	
	Feather	47	1.568943	0.233665	*	*	*	*	*	*

Table 1. Average values of measured elements (in ppm) in tissues with differences for individual pairs of tissues calculated according to the Mann-Whitney U test (* significant value $p < 0.05$) PM – femoral muscle, FM – femoral muscle. Mercury was evaluated separately due to the use of a different analytical method.

cium, phosphorus, magnesium, sulphur, and electrolytes (sodium, potassium, chloride). In our research, the highest levels of calcium, phosphorus, sulphur, chlorine, potassium, and iron were found in the analysed tissues of the thrush. Although magnesium belongs to this group of macro-minerals, its levels were lower than the measured values for iron, which is classified as a micromineral. Microminerals are elements found in smaller quantities compared to macro-minerals. However, they also fulfill many functions in the physiology of animals. Microminerals discussed in our study include manganese, zinc, iron, copper, selenium, iodine, cobalt molybdenum, and chromium.

Phosphorus

Phosphorus (P) plays a vital role in multiple biological processes, especially for bone mineralization (Kiefer-Hecker *et al.* 2018; Rao *et al.* 2019). It has an indispensable function in nucleic acid synthesis, energy metabolism, muscle function, enzyme activity, lipid metabolism, and bone mineralization (Berndt and Kumar 2009). Living cells use phosphate to transport cellular energy with adenosine triphosphate (ATP), which is essential for every cellular process that uses energy. Phospholipids are a major structural components of all cell membranes. Calcium phosphate salts help in the strengthening of bones. Phosphorus deficiency can lead to bone pathology and clinical disease (Amanzadeh and Reilly 2006; Tiosano and Hochberg 2009). Elevated levels of phosphorus in the diet can lead to adverse effects on bone, kidney, and heart health (Calvo and Uribarri 2013).

The highest amounts of phosphorus in our results were measured in bone. Bone is the main storage organ of phosphorus, accounting for 85% of its total content in the body (Cook *et al.* 1937; Heaney 2012; Fukumoto 2014). The results of a study by Stępniowska *et al.* (2020), report multifold higher P values in bone than in breast/pectoral muscle or liver, which is consistent with our results.

Among the internal organs, the highest values of P were measured in the kidneys, likely related to their function in regulating serum phosphorus concentration (Nadkarni and Uribarri 2014) and removing excess phosphorus from the blood. Higher blood phosphorus levels are usually the result of consuming copious amounts of dietary phosphorus from a diet deficient in dietary calcium. When kidney function decreases, additional phosphorus accumulates in the blood. When there is too much phosphorus in the blood (hyperphosphatemia), calcium is lost from the bones and stored in the tissues. This can lead to weakened bones and cardiovascular disease.

Sulphur

Elemental sulfur (S) is the 8th most common nutrient among the 14 essential elements required by living organisms (Park and Park 2017). It is an essential component of amino acids cysteine and methionine, and is present in all polypeptides, proteins, and enzymes that contain these amino acids. Sulfur is a component of the major building proteins (e.g., collagen) that make up hair, muscle, connective tissue, and skin. Sulfur is a natural antibiotic (Park and Park 2017) that can perform detoxification by combining with toxins or heavy metals in the liver (Parcell 2002). Sulfur toxicity in poultry causes non-specific pathologies such as poor growth performance, impaired ash deposition in bones, impaired ovarian function, and wet waste problems. High dietary sulfur content has been reported to have adverse effects on hemogram and leukogram values in poultry (Alam and Anjum 2003). The main sources of sulfur in the diet are sulfur-containing amino acids and inorganic sulfate.

As sulphur is bound in amino acids, its levels in bone tissue are lower than in the other tissues analysed. Our results also confirmed the low values in bone tissue. In bone, sulphur is bound primarily in the form of bone collagen. Another form of organic sulfur is methylsulfonylmethane, which is found in living organisms and has anti-inflammatory and osteogenic properties that make it an excellent material for inducing bone formation and promoting osseointegration (Aljohani 2020).

The highest S values were found in the kidneys. High sulphur values in the kidneys can be explained by the metabolic pathway of sulphur-containing amino acids, in which the kidneys play a key role. In the body, the kidneys are believed to be the third largest producer of H_2S, after the liver and gut (Cao and Bian 2016). Furthermore, kidney homeostasis appears to be impacted by H_2S, with H_2S levels contributing to glomerular filtration rate (GFR), Na^+ excretion, and K^+ excretion (Koning *et al.* 2015). In the liver, sulphur contributes to the digestion and absorption of fats, as it is needed for the formation of bile acids.

Chlorine

Chlorine (Cl) is too reactive to occur as a free element in nature. It occurs primarily as the chloride ion, which is a component of chloride salts. Common chloride minerals include halite (sodium chloride – NaCl), sylvite (potassium chloride – KCl), and carnallite (potassium-magnesium chloride hexahydrate $MgC_{l2}.6H_2O$). Most chloride salts are soluble in water. Its highly reactive properties allow the formation of organic compounds (i.e. containing carbon). There are more than 2000 known organic chlorine compounds. Chlorinated organic compounds are found in almost every class of biomolecule, and in natural products, including alkaloids, terpenes, amino acids, flavonoids, steroids, and fatty acids (Engvild 1986; Wagner *et al.* 2009) These organochlorine compounds include many industrially produced compounds that have toxic effects on the environment, such as vinyl and polyvinyl chlorides (PVCs), pesticides (e.g., DDT), and polychlorinated biphenyls (PCBs).

Chloride is also an essential mineral (Brazin 2006). It is one of three major extracellular anions (with sodium and potassium), and its homeostasis is influenced by renal reabsorption (Selinus *et al.* 2013). As the main electrolyte in the body, it helps in conducting electrical impulses, osmotic pressure, acid-base balance and in conjunction with hydrogen, it forms HCl, which breaks down proteins for absorption of other metallic minerals (Brazin 2006; Ravindran 2013).

Chlorine is found in cells in the extracellular fluids of the body, primarily in the form of sodium chloride and potassium chloride (Costa *et al.* 2012). Thus, as part of the extracellular fluid, its concentrations are higher in visceral tissues (the major metabolic cell mass), as confirmed by the results of our research, where the highest Cl values were measured in internal organs. As part of compounds with other metallic elements, its levels may also increase with the accumulation of the compound in the liver and kidneys (Anderson *et al.* 1989; Kalisińska 2019).

Potassium

Potassium (K) is a major intracellular cation (intracellular K^+) and is present in a wide variety of proteins and enzymes (Vašák and Schnabl 2016; Dietz *et al.* 2023). Potassium levels influence multiple physiological processes (e.g. resting membrane potential of cells, the propagation of action potentials in neuronal, muscular, and cardiac tissue, acid-base homeostasis, fluid and electrolyte balance, and others), and are also important for maintaining homeostasis. 60-75% of total body potassium is found in muscle cells, the remainder in bone, and only 5% is found in extracellular fluid (Rastegar 1990; eClinPath.com 2023). This does not correlate with our results, where K levels were higher in viscera than in muscles, but we also observed the lowest K levels in bone. Emel'ianov *et al.* (1976), report that potassium levels are significantly lower in the heart than in other muscles, which also contradicts our results. Different K concentrations in other muscle types are related to functional specialization of the muscle contractile mechanism (Nesterov 1976). The highest K concentrations were found in kidney tissue in our results, which may be due to the smaller number of samples examined compared to other tissues.

Calcium

Calcium (Ca) is an essential component of bone, cartilage, eggshell formation, blood clotting, muscle contraction, and transmission of nerve impulses. It is also an important co-factor for many enzymes and hormones. In conjunction with phospholipids, Ca plays a key role in the regulation of the permeability of cell membranes and consequently over the uptake of nutrients by the cell. Calcium metabolism in birds is highly efficient and tightly regulated in several tissues, primarily the parathyroid gland, intestines, kidneys, and bones. In total, 99% of the Ca in the body is derived from the skeleton, where, together with phosphorus, it forms hydroxyapatite (Bello *et al.* 2014). Our results confirm that bone tissue is the primary organ in which calcium accumulates.

In the internal organs (heart, kidneys, liver), calcium levels did not differ, but the highest levels were recorded in the heart. Heart muscle contraction is directly determined by the level of calcium elevation during systole (i.e., systolic transient Ca^{2+}) (Eisner 2014). Impaired calcium release causes reduced muscle contraction (systolic dysfunction), and defective calcium removal impedes relaxation (diastolic dysfunction) (Marks 2003). Given the different structure of the heart muscle compared to the other internal organs analyzed, and the key role of calcium for its proper function, a higher calcium content in this organ is expected.

However, Ca levels also differed significantly between the two muscles we analyzed. In the femoral muscle, Ca levels were higher than in the pectoral muscle, which also had the lowest values among all tissues analyzed. The higher Ca values in the femoral muscle compared to the pectoral muscle are confirmed by the study of Flis *et al.* (2020), in common pheasants (*Phasianus colchicus*). This difference is likely due to the different microstructure of muscles with different physiological function. Analysis of muscle-tendon architecture have shown that muscles vary considerably in their design for length change during force generation, due to differing properties of force production and relative fiber strain (the ratio of change in activated length relative to fiber length at rest) (Chen and Kudryashev 2020). Zhang *et al.* (2022) also report that the amount of collagen, intramuscular fat content, and the size and type of muscle fibers may also contribute to the sensitivity of muscle tissue to calcium.

Iron

Iron (Fe) is an essential component of many heme-containing enzymes and proteins, such as hemoglobin, and myoglobin (Kroneck and Torres 2015; Hänsch and Mendel 2009), which participate in the transport, storage, and use of oxygen. Iron is also stored in tissues as ferritin and hemosiderin (Harwey 2008). The largest proportion (60%-70%) of total iron is present in hemoglobin. About 20%-30% is stored as ferritin and hemosiderin (primarily in macrophages and hepatocytes), 3%-7% is present in myoglobin, 1% is present in enzymes, and less than 0.1% is bound to transferrin in plasma (Ponka *et al.* 1998). Chronic ingestion of substantial amounts of absorbable iron in the diet can lead to the storage of iron in the liver in many species (Cork 2000).

Given the significant role of this element in the process of blood formation, high Fe levels are expected in the liver and kidneys (which provide blood filtration), as confirmed by our results. The higher values in the kidneys in our research may be biased by the small number of samples. The highest Fe values in the liver compared to the other tissues analyzed are confirmed by the research of Orlowski *et al.* (2012), who analyzed the elemental content in dead nestlings of *Corvus frugilegus*. Iron homeostasis plays a key role in the efficient functioning of skeletal muscle (Stugiewicz *et al.* 2016). Although our results noted that Fe levels in pectoral and femoral muscles were not significantly different, higher Fe levels were found in the pectoral muscle, related to the physiology and structure of this muscle, which has a dominant role during flight (Biewener 2011).

Chromium

Chromium (Cr) is a trace element necessary for human and animal vital activity (Snitynskyĭ *et al.* 1999), though its biological function is still debated (Vincent 2013; Maret 2019; Dallago *et al.* 2016). Chromium plays an important role in the behaviour of insulin, but acute toxicity is known to cause kidney, liver, and blood cell damage (Dayan and Paine 2001), and its carcinogenicity has also been documented (Langard 1990). Additionally, allergic reactions have been described, caused by chromium salts (chromates) (Basketter *et al.* 2000). The toxicity of Cr depends on its valence, with Cr (VI) being more toxic than Cr (III) (Stępniowska *et al.* 2020). In birds, Cr affects physiological processes, improves carbohydrate and lipid metabolism, reduces stress responses, stimulates the immune system and can improve production results and reduce carcass (Sahin *et al.* 2002; Uyanik 2002; Sirirat *et al.* 2012; Khan *et al.* 2015; Ognik *et al.* 2020; Stępniowska *et al.* 2020). Ingested Cr is absorbed by the intestine and bound to plasma proteins, by which it is transported to the liver and other organs (Stępniowska *et al.* 2020). Accumulation of chromium has been shown mostly in the liver, kidneys, and spleen, and less in the heart, muscles, bones, and brain (Uyanik *et al.* 2005; Zha *et al.* 2007; Wang *et al.* 2009). Our results present the highest Cr accumulation in the kidney, heart, and liver, in contrast to the findings of Sirirat *et al.* (2012), who document the highest values in the liver. We also found higher Cr content in bone compared to muscle. Bone is one of the tissues that have been shown to accumulate Cr over an extended of time, versus organs such as heart, pancreas, and brain in which accumulation of the element is relatively short-lived (Lindemann *et al.* 2008). Cr accumulation in bones is due to a similar ionic radius, whereby Cr can sometimes replace Ca in bones (Stępniowska *et al.* 2020).

Manganese

Manganese (Mn) is an essential element in all species and is a critical component in dozens of proteins and enzymes (Erikson and Ascher 2019; Sarnowski and Kellam 2023). It is an enzyme activator for enzymes that ensure the transfer of phosphate groups, a cofactor in macronutrient metabolism, essential for bone formation, and important for defense against free radicals (Lu *et al.* 2007; Suttle 2010). Manganese compounds are less toxic than those of other widespread metals, such as nickel and copper (Hasan 2008), though chronic exposure can induce neurotoxic effects and cause reproductive dysfunction (Adedara *et al.* 2017). In higher vertebrates, excess manganese is accumulated in various tissues (Sarnowski and Kellam 2023), but primarily in bone, with the remainder stored in soft tissues such as liver and kidney (Emsley 2011; Berta *et al.* 2004). This correlates with our results, where Mn accumulated the most in bone and liver tissue. The liver metabolizes Mn, so the levels are often higher in this organ (Kalisińska and Budis 2019).

Copper

Copper (Cu) is an essential component of many oxidation-reduction enzyme systems, and involved in iron metabolism (hemoglobin synthesis, d blood cell formation and maintenance) (Eisler 1998). It is required to produce the pigment melanin (which contributes to skin pigmentation, and bone and connective tissue formation), and to maintain integrity of the myelin sheath of nerve fibers (Stern 2010; Deo *et al.* 2023). Birds are considered relatively resistant to copper (Eisler 1998), but there are frequent reports of copper toxicosis in commercial poultry and wild birds (Lewis *et al.* 2001; Thomas and McGill 2008; Lucia *et al.* 2010). Absorption and accumulation of Cu in organs is highly related to the solubility of the Cu source (Leeson *et al.* 1997; Pesti and Bakalli 1998).

Although our results show the highest Cu concentration in bone, these results are biased by the small sample variation (n = 4). In most of the bones analyzed (total 44), the Cu level was below the detection limit, thus excluding them from statistical evaluation. The low Cu levels in bone compared to internal organs (heart, liver, and kidney) are confirmed in a study by Mukhtar *et al.* (2020). Among the internal organs, the highest values were recorded in kidney, followed by heart, and liver, which is not consistent with results from Zhuang *et al.* (2014), who found the highest values in liver compared to kidney and muscle. Similarly, high proportional distribution of accumulation in heart, liver, and kidney tissue are reported by Dauwe *et al.* (2005) as well as Mukhtar *et al.* (2020).

Zinc

In low concentrations, zinc (Zn) is an essential trace element for maintenance of physiological, metabolic, and biochemical functions in the organism, such as coordination and reduction-oxidation (redox), anion-cation balance, and compartmentalization (Mertz 1981; Prasad 2008; Squadrone *et al.* 2016; Yatoo *et al.* 2013). As a heavy metal, however, it is accumulated in birds through ingested food and respiration (Mukhtar *et al.* 2020). The target organs that are most damaged in zinc intoxication are the pancreas, kidney, liver, and gastrointestinal tract (Puschner *et al.* 1999).

High concentrations of Zn in bones in birds (including passerines) were found by Kim *et al.* (2009), and the same conclusion is supported by our results. In general, zinc accumulates more in calcareous tissues such as bone and eggshell (Sorensen 1991), as opposed to soft tissues (Honda *et al.* 1986). The highest levels of Zn in internal organs (liver, kidney) are confirmed by the results of the study of Lucia *et al.* (2010), however levels and accumulation are different in the analyzed species of waterbirds. Our results confirm the trend of higher accumulation in internal organs compared to muscles.

Selenium

Selenium (Se) is a metalloid trace element that birds need in small amounts for optimum growth and reproduction (Khan *et al.* 2017, 2018), but it can be toxic in large doses (Atroshi 2014). Se is an essential component of the enzyme glutathione peroxidase and serves to protect cellular tissues and membranes against oxidative damage (Surai 2002; Surai and Fisinin 2014). It has also been suggested that selenium participates in the biosynthesis of ubiquinone (coenzyme Q; involved in cellular electron transport), and impacts the absorption and retention of vitamin E (Xia *et al.* 2003). Increasing selenium in feed enhances the ability of the broiler immune system to fight disease (Ibrahim *et al.*

2019; Shojadoost *et al.* 2019). Selenium deficiency has been associated with metabolic problems and compromised growth (Zheng *et al.* 2019). Selenium toxicity depends on its chemical form, with elemental Se posing minor risk to birds because of low bioavailability (Ohlendorf and Heinz 2011). Higher Se levels in tissue are recorded in aquatic and piscivorous birds compared to domestic or terrestrial birds, as food is the main source of Se (Hoffman 2002; Spallholz and Hoffman 2002; Surai *et al.* 2018).

Our research confirmed accumulation in internal organs (highest levels in the kidneys). Ohlendorf and Heinz (2011) offer the same conclusion in their study, although they state that a more appropriate organ for diagnosing Se status in birds is the liver. In areas where environmental Se levels are low, concentrations in the kidney are higher than in the liver, but concentrations in these two tissues are similar in birds from high Se areas (Ohlendorf and Heinz 2011). They also report that the Se content in muscle tissue is lower than in internal organs, which is also consistent with our results. This conclusion is explained by the faster accumulation of Se in the liver and slower in the muscles, as well as its elimination from these organs. The relationship of Se accumulation between muscle and liver is subtly more variable than between kidney and liver (Ohlendorf and Heinz (2011). Changes in the increase or decrease of Se in muscle are a response to diet, but take place more slowly (Heinz *et al.* 1990).

Rubidium

Rubidium (Rb) is rarely studied alakali metal, may be an essential ultra-trace element for humans and other organisms (Campbell *et al.* 2005). Despite its higher molecular weight, has several similarities to K^+ (Nyholm and Tyler 2000). In biological systems, it replaces the drosophila in some ways, but has some effects on growth and longevity that prevent complete convertibility (Lombeck *et al.* 1980). Compared to other trace elements, little information is available on the toxicity of Rb and there are no thresholds for its toxicity in birds (Godbless 2020). Substitution of Rb^+ ions with K^+ ions in organisms leads to a similar tendency of treatment, therefore concentrating rubidium in the intracellular fluid (i.e., inside cells) (Relman 1956). The half-life of Rb^+ in animal tissues is reported to be approximately 60 days and is removed from the body by the kidneys in the urine (Relman 1956; Meltzer and Fieve 1975).

The biochemical accumulation of K^+ and Rb^+ in animal tissues is dependent on adenosine 5´-triphosphate (ATP), which is found in the cell membrane of every mammalian cell (Scou 1965). ATP provides energy to drive and support many processes in living cells, such as muscle contraction, nerve impulse propagation, condensate dissolution, and chemical synthesis. When the concentration of Rb^+ in blood plasma increases above its natural level Rb+ competes with K^+ for binding with Na/K-ATPase, leading to increased influx of Rb^+ and decreased K^+ into the cytoplasm, in other words Rb^+ displaces K^+ (Kupriyanov 2013). Our results indicate the highest Rb concentrations in internal organs and in muscle. Since these are organs that need sufficient energy supply in the ATP pool to function, it is likely that Rb levels correlate precisely with the amount of ATP in the tissues.

Molybdenum

Molybdenum (Mo) is an essential element associated with a variety of metalloenzymes and corresponding metabolic functions. It functions as a component of several oxidase enzymes in animals (xanthine oxidase, sulfite oxidase, and aldehyde oxidase) (Johnson *et al.* 1980; Mills *et al.* 1987; Emsley 2011). The main function of molybdoenzymes is catalysis of oxidation and sometimes reduction of certain small molecules in the process of regulating nitrogen, sulfur, and carbon (Kisker *et al.* 1999). Extremely high concentrations of molybdenum can inhibit purine catabolism and other processes. Molybdenum concentrations also affect protein synthesis, metabolism, and growth (Mitchell 2003). Molybdenum prevents plasma proteins from binding to copper and also increases the amount of copper that is excreted in the urine, leading to decreased copper uptake.

Little data is available on the effects of Mo on bird species (Stafford *et al.* 2016). Birds appear to have lower biological requirements for Mo than most mammalian species, but higher resistance to Mo excess (Eisler 1989; Davies *et al.* 1960).

According to the results of the study by Stafford *et al.* (2016), Mo accumulation tendencies are highest in the kidney and liver, an order of magnitude less Mo was accumulated in the femur. The higher accumulation in the kidney is confirmed by our results, but the highest values were found in bone. Multiple factors are hypothesized to influence Mo amounts. The most common include the species and age of the animal; the amount and chemical form of the ingested Mo; and the inorganic sulfate and total sulfur content of the diet and its content of substances such as protein, cysteine, and methionine, capable of oxidation to sulfate in the body (Underwood 1977). Thus, Mo accumulation is influenced by several factors that were not analyzed in this study, which could shed light on Mo accumulation in bone.

Barium

Barium (Ba) is an alkaline heavy metal, which has a variety of uses including in the manufacturing industry and in medicine (most commonly as part of contrast agents in radiology). Barium is chemically similar to magnesium, calcium, and strontium, but even more reactive. The toxicity of barium compounds depends on their solubility. Soluble barium compounds are poisonous. In low doses, barium ions act as a muscle stimulant and higher doses act on the nervous system and cause cardiac irregularities, tremors, weakness, anxiety, shortness of breath and paralysis. The kidney seems to be the most sensitive target organ in rats and mice after long-term exposure to barium chloride in drinking water (Oskarsson 2022).

Our results confirm that Ba levels are the highest in the kidney among the internal organs. However, the highest levels of Ba were recorded in bone, which is probably related to its similar chemical properties to calcium. Barium tends to accumulate in animal tissues, especially in the skeleton, where it inhibits bone mineralisation (Florlanczyk and Taroslawska 2003).

Lead

Lead (Pb) is a heavy metal that has no confirmed biological role and there is no confirmed safe level of exposure to lead (WHO 2018). Lead can form multiply-bonded chains, a property it shares with its lighter homologues in the carbon group. Lead salts are very efficiently absorbed by the body (Emsley 2011). A small amount of lead (1%) is stored in the bones; the remainder is excreted in urine and faeces within a few weeks after exposure. Only about one-third of the lead is excreted by the child. Continuous exposure can lead to bioaccumulation of lead (Collin *et al.* 2022).

Exposure to Pb can cause a variety of sublethal toxic effects up to direct mortality (Kendall *et al.* 1996). The sublethal effects of Pb act on the nervous system, kidneys, liver, intestines, and circulatory system, leading to physiological, biological, and behavioral changes (Scheuhammer 1987; Fisher *et al.* 2006; Cade 2007).

Pb concentrations are generally highest in the blood immediately after absorption, then in the liver and kidneys within days to months, whereas Pb stored in bone can remain for years (Fisher *et al.* 2006; Pain 1996).

Our results confirm the preferential accumulation of Pb in bone; in other tissues, levels were below the detection limit in most samples.

Mecury

Mercury (Hg) is an extremely hazardous heavy metal is considered the most toxic in the environment. Methylmercury contamination of the environment is a globally important problem and birds are useful bioindicators for mercury monitoring programmes. Environmental methylmercury contamination is a globally important problem, and birds are useful bioindicators for mercury monitoring programs; therefore, there are myriad studies on the toxicity and levels of Hg to birds.

High levels of Hg compounds in avian tissues can affect immune, cardiovascular and nervous systems, as well as reproductive capacity (Harada 1995; Morel *et al.* 1998; Tan *et al.* 2009; Fu *et al.* 2010; Tartu *et al.* 2013; Eagles-Smith *et al.* 2016, 2018). Also MeHg is able to cross the blood-brain barrier and can be deposited in eggs (Boening 2000; Frederick and Jayasena 2011).

Hg levels were highest in the tail lips in our results. Feathers are widely used to represent mercury contamination in birds. Of the other tissues analysed, high values were in liver, kidney and heart and the lowest in bone. These results are consistent with the conclusions of other work (e.g. Finley *et al.* 1979).

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