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Trace element concentrations (Fe, Cu, Zn) in the organs of wild boar (*Sus scrofa*) across regions of Central and Eastern Slovakia

Vladimír Hisira[1,A-B,D], Lenka Lešková[2,B], Vladimír Petrovič[3,F], Marian Kadaši[1,A], Simona Mekková[1,B], Veronika Glembová[1,B-C], František Zigo[4,E-F] ✉

[1] *Clinic of Ruminants, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic*
[2] *Department of Public Veterinary Medicine and Animal Welfare, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic*
[3] *Department of Pharmacology and Toxicology, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic*
[4] *Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. The study deals with the accumulation of iron (Fe), copper (Cu), and zinc (Zn) in the organs (kidney, liver, and muscle) of wild boar from four districts of Slovakia with different levels of industrial activity, mining, and agriculture, while also evaluating the concentrations of these trace elements in different age and sex categories of the animals.

Materials and Method. Samples of internal organs and muscle were collected from game (n=147) hunted during individual or group hunts on State or private hunting grounds in the regions of Rimavská Sobota, Revúca, Rožňava, and Košice, located in the southern part of central and eastern Slovakia.

Results. Mean kidney Fe concentration varied from 72.65–115.3 mg·kg^{-1} w.w. Hepatic Fe levels fluctuated from 206.5–288.7 mg·kg^{-1} w.w., and the amount of Fe in muscle tissue ranged from 42.44–70.03 mg·kg^{-1} w.w. Renal Cu values were 3.81–5.24 mg·kg^{-1} w.w., liver Cu values 2.80–3.65 mg·kg^{-1} w.w., and muscle tissue Cu values were 1.52–2.47 mg·kg^{-1} w.w. Mean contents of Zn in kidney moved within the range 27.54–30.72 mg·kg^{-1} w.w., in liver 37.36–42.84 mg·kg^{-1} w.w., and in muscle tissue 28.10–36.06 mg·kg^{-1} w.w. Concentrations of the given elements in different age groups were compared, where these values increased proportionally with age. No significant differences were found between genders.

Conclusions. Based on the obtained results, the presence of the given trace elements in the environment in the counties of central and eastern Slovakia varies significantly, with the highest probability attributable to the mining of industrial minerals and the varying intensities of industrial development in the surveyed areas.

Key words

liver, muscle, kidney, wild boar, Slovakia, trace element

INTRODUCTION

Wild boar are widely distributed across Slovakia and are well adapted to local environmental conditions. Wild species are valuable bioindicators for assessing environmental contamination and serve as suitable model organisms for studying the accumulation of minerals, including macrominerals, trace elements, and heavy metals, in living systems [1].

The accumulation of these elements is closely associated with their presence in soil and feed within the food chain [2]. The soil–plant–animal system is highly complex, and interactions among its components must be considered alongside the geochemical characteristics of soils, which may predispose certain regions to deficiencies or toxicities in specific trace elements. Under such conditions, clinical or subclinical manifestations may occur, often leading to adverse physiological effects. The influence of geochemical imbalances, particularly low trace element availability, on animal health has been documented in both older and more recent studies [3].

Trace elements such as copper, iron, manganese, selenium, zinc, chromium, fluorine, iodine, and molybdenum are essential for normal biological functions. Trace metals cannot be synthesized by the organism and must be obtained through diet. They play key roles in metabolic and physiological processes, including growth and development, serving as enzyme cofactors and regulators of hormone synthesis and function [4]. Copper is involved in enzymatic reactions, iron metabolism, connective tissue formation, and antioxidant defence systems. Iron is a key component of haemoglobin and myoglobin and is essential for oxygen transport and cellular respiration. Zinc acts as a structural or catalytic component of more than 300 enzymes and is important for immune function, growth, and hormonal regulation [5]. However, both deficiency and excessive intake may result in adverse effects, including accumulation and toxicity, often linked to

✉ Address for correspondence: František Zigo, Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Komenského 73, 04001 Košice, Slovak Republic
E-mail: frantisek.zigo@uvlf.sk

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environmental burdens driven by anthropogenic activities such as industrial emissions, mining, and agriculture [6]. Although these elements are indispensable for normal biological function, they may become toxic at excessive concentrations. Copper toxicity is primarily associated with hepatic accumulation, leading to oxidative stress, cellular damage, and, in severe cases, haemolysis [7]. Iron overload can promote the formation of reactive oxygen species via Fenton-type reactions, leading to oxidative damage to proteins, lipids, and DNA, particularly in the liver [8]. Excess zinc intake may disrupt the homeostasis of other essential metals, especially copper and iron, by interfering with their absorption and metabolism, ultimately leading to secondary deficiencies and physiological disturbances [9]. Assessment of an organism's mineral status, therefore, commonly involves determining trace element concentrations in biological fluids (e.g., blood serum, plasma) and tissues [10].

OBJECTIVE

The aim of this study is to evaluate the accumulation of iron, copper, and zinc in selected internal organs (liver, kidney, and muscle) of wild boar from four districts of central and eastern Slovakia. The study is also focused on comparing the concentrations of these trace elements across age groups and between sexes to identify potential biological and environmental factors influencing their distribution. In addition, relationships among metal concentrations in individual tissues were assessed, and differences in accumulation patterns among the organs examined were evaluated. The results were further interpreted in the context of environmental contamination and the physiological roles of the trace elements studied.

MATERIALS AND METHOD

Study area. The study was conducted in four districts of central and eastern Slovakia with varying levels of industrial and agricultural activity: 1) Rimavská Sobota (RS) district – characterized as an agricultural region with minimal industrial influence; 2) Revúca (RA) district – associated with mining activities, particularly the extraction and processing of iron ore and magnesite; 3) Rožňava (RV) district – with a historical legacy of mining and metallurgical processing of iron ore; 4) Košice (KE) district – a major industrial centre, with extensive iron-processing facilities and a history of magnesite production (Fig. 1).

Sample collection. Samples of liver, kidney, and muscle tissue were collected from wild boar (*Sus scrofa*) of different age categories immediately after hunting and evisceration. Sampling was conducted in hunting grounds within the selected districts (RS, n = 37; RA, n = 57; RV, n = 23; KE, n = 30), yielding a total of 147 individuals. Each animal was classified according to age and sex at the time of sampling (Tab. 1). Tissue samples were stored at –20 °C until laboratory analysis.

Sample preparation and elemental analysis. Samples were processed in the laboratory using microwave-assisted wet digestion. Tissue samples (kidney, liver, and muscle) weighing

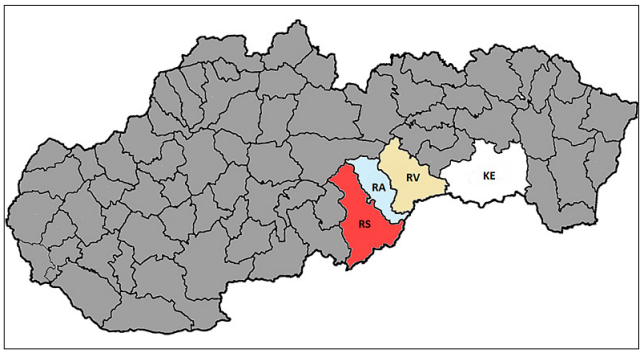

Figure 1. Map of the Slovak Republic showing the geographical location of the four study districts (RS – Rimavská Sobota, RA – Revúca, RV – Rožňava, KE – Košice).

Table 1. Number of animals within each age class and number of males and females

District	Age categories			Gender		Totally
	0.5	0.5–1.5	over 1.5	male	female	
Rimavska Sobota (RS)	14	7	16	14	23	**37**
Revuca (RA)	14	17	26	25	32	**57**
Roznava (RV)	12	6	5	17	6	**23**
Košice (KE)	9	9	12	13	17	**30**

1.3–1.8 g were placed into digestion vessels. Subsequently, 6 mL of nitric acid (HNO_3, 65%, analytical grade; Merck KGaA, Germany) and 2 mL of hydrogen peroxide (H_2O_2, 30%, analytical grade; microChem, Slovakia) were added to each sample.

Digestion was performed using a Multiwave laboratory system (Milestone, Italy; Microwave Digestion System with MDR Technology, MLS-1200 Mega). The mineralisation programme was carried out under the following conditions: a maximum pressure of 2 bar, a maximum internal temperature of 100 °C, a maximum external temperature of 30 °C, and ventilation for 1 min. The microwave programme consisted of five steps: 250 W for 1 min, 0 W for 30 s, 300 W for 5 min, 600 W for 5 min, and 0 W for 30 s.

After digestion, samples were quantitatively transferred into 25 mL volumetric flasks and diluted to volume with deionized water. Each sample was digested once.

Concentrations of Cu, Fe, and Zn were determined by flame atomic absorption spectrometry (FAAS) using an AAnalyst instrument (PerkinElmer, USA) and expressed on wet weight basis (w.w.). Measurements were performed at wavelengths of 328 nm for Cu, 247.7 nm for Fe, and 213.9 nm for Zn. A standard PerkinElmer burner (10 cm, N040–0103) with an air–acetylene flame was used, where acetylene served as the fuel gas and compressed air as the oxidant.

External calibration was performed using mixed standard solutions (0.20 $mg \cdot L^{-1}$) prepared from certified reference standards (Fluka Analytical, Sigma-Aldrich, Switzerland) for Cu, Fe, and Zn. Each sample was analyzed in duplicate. Analytical performance parameters were as follows: for Cu, LOD 0.02931 $mg \cdot L^{-1}$, LOQ 0.08793 $mg \cdot L^{-1}$, CV 2.27%, and R^2 = 0.9992; for Fe, LOD 0.06022 $mg \cdot L^{-1}$, LOQ 0.18066 $mg \cdot L^{-1}$, CV 5.03%, and R^2 = 0.9986; for Zn, LOD 0.03615 $mg \cdot L^{-1}$, LOQ 0.10845 $mg \cdot L^{-1}$, CV 2.95%, and R^2 = 0.9994.

Statistical analysis. Data were analyzed using Microsoft Excel (Microsoft Corp., USA). Results were presented as mean

± standard error of the mean (SEM). Differences in trace element concentrations among tissues and age categories were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Differences between the sexes were assessed using an independent-samples t-test. Statistical significance was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS

Trace element concentrations in tissues. Mean iron (Fe) values ranged from 72.65–115.3 mg·kg^{-1} w.w. in the kidneys, from 206.5–288.7 mg·kg^{-1} w.w. in the liver, and from 42.44–70.03 mg·kg^{-1} w.w. in muscle tissue. The renal Fe content was significantly elevated in wild boar from the RV and RA mining-impacted areas compared with the KE site ($p < 0.05$ and $p < 0.001$, respectively). In hepatic tissue, the RA area showed higher Fe accumulation than the RS and KE areas ($p < 0.05$ and $p < 0.01$, respectively), while specimens from RV also differed significantly from those collected at KE ($p < 0.05$). Moreover, individuals originating from RS exhibited greater Fe levels in muscle tissue than those from KE ($p < 0.05$) (Tab. 2).

Mean copper (Cu) concentrations ranged from 3.81–5.24 mg·kg^{-1} w.w. in the kidneys, from 2.80–3.65 mg·kg^{-1} w.w.

Table 2. Iron concentration in visceral organs and muscle of wild boar from Slovakia

	x ± S.E.M min. max.	0.5 min. max.	0.5–1.5 min. max.	over 1.5 min. max.	Female min. max.	Male min. max.
			Renal iron concentration (mg.kg^{-1} w.w.)			
RS (n 37)	93.98 ± 6.8 21.8 188.84	77.8 ± 7.66 21.88 120.31	103.4 ± 14.98 51.55 188.84	113.1 ± 14.24 55.73 173.93	93.82 ± 8.042 21.88 173.93	94.31 ± 13.20 42.42 188.84
RA (n 57)	115.3 ± 5.276^{a} 43.17 194.9	86.26 ± 9.69^{A} 43.17 140.4	107.7 ± 8.36 54.02 178.3	129.1 ± 7.11 ^{c,A} 86.4 194.9	117.9 ± 7.341^{d} 43.17 194.9	112.2 ± 7.678^{e} 54.02 178.68
RV (n 23)	100.9 ± 8.011^{b} 52.18 187.77	79.93 ± 6.575^{B,C} 52.18 128.8	123.7 ± 19.71^{B} 78.4 171.87	132.6 ± 15.20^{C} 106.4 187.77	102.6 ± 9.255 52.18 187.77	93.68 ± 12.00 53.65 128.8
KE (n 30)	72.65 ± 4.616^{a,b} 24.05 139.25	65.20 ± 6.489 35.36 88	69.37 ± 4.343 56 81.83	87.13 ± 10.85^{c} 64.46 139.25	70.06 ± 7.523^{d} 35.3 139.25	71.01 ± 3.142^{e} 50.42 83.15
			Hepatic iron concentration (mg.kg^{-1} w.w.)			
	x ± S.E.M min. max.	0.5 min. max.	0.5–1.5 min. max.	over 1.5 min. max.	Female min. max.	Male min. max.
RS (n 37)	229.8 ± 12.49^{f} 92.41 439.39	216.0 ± 19.25 92.41 306.4	246.5 ± 19.39 161.05 308.74	235.2 ± 23.13^{i} 122.86 439.39	228.5 ± 13.41 92.41 308.74	233.9 ± 24.07 95.28 439.39
RA (n 57)	275.8 ± 13.48^{f,g} 90.21 460.9	223.3 ± 33.26 90.21 318.2	255.5 ± 23.17 103.97 365.6	302.2 ± 16.57 128.79 460.9	273.2 ± 19.75 90.21 423.34	279.6 ± 17.13 127.8 460.9
RV (n 23)	288.7 ± 17.23^{h} 169.43 454.54	245.1 ± 15.09^{D} 169.43 330.53	299.8 ± 24.93 273.82 373.8	367.1 ± 34.22^{i,j,D} 324.6 454.54	302.2 ± 20.56^{k} 179.04 454.54	250.9 ± 27.34 169.43 330.53
KE (n 30)	206.5 ± 15.59^{g,h} 103.08 388.15	171.8 ± 18.32 103.08 249.29	234.8 ± 19.05 160.68 280.99	231.2 ± 33.96^{j} 103.08 388.15	198.3 ± 19.80^{k} 103.08 281.49	224.7 ± 23.94 160.68 388.15
			Muscle iron concentration (mg.kg^{-1} w.w.)			
	x ± S.E.M min. max.	0.5 min. max.	0.5–1.5 min. max.	over 1.5 min. max.	Female min. max.	Male min. max.
RS (n 37)	70.03 ± 3.538^{l} 31.63 106.79	66.81 ± 4.905^{m,n} 31.63 90.16	80.59 ± 6.01^{o} 56.5 100.09	84.76 ± 5.368 69.09 106.79	69.74 ± 4.204^{r} 31.63 101.19	70.53 ± 6.624 33.84 106.79
RA (n 20) live (n 57)	61.07 ± 5.853 21.45 110.01	43.03 ± 6.753 21.45 62.44	63.13 ± 9.841 25.82 87.45	76.64 ± 8.774 62.22 110.01	71.70 ± 9.289 40.00 110.01	54.70 ± 7.11 21.45 87.45
RV (n 23)	56.08 ± 6.347 20.77 111.72	44.62 ± 6.417^{m,E,F} 20.77 96	52.52 ± 5.040^{E} 43.59 63.97	107.2 ± 13.97^{p,F} 61.42 148.59	63.79 ± 8.842 20.77 148.59	48.37 ± 12.05 31.58 96.00
KE (n 30)	42.44 ± 4.981^{l} 13.73 94.67	21.45 ± 4.652^{n,G} 11.61 45.17	43.96 ± 6.654^{o} 22.58 62.86	54.94 ± 9.945^{p,G} 24.67 94.67	37.08 ± 8.107^{r} 11.78 93.33	47.90 ± 6.054 11.60 94.67

$\bar{x}$ – average value; SEM – standard error of the mean; min. – minimum value; max. – maximum value; 0.5 – half-year-old wild boar; 0.5–1.5 – wild boar aged from 0.5 – 1.5 years; >1.5 – wild boar older than 1.5 years; *p* – significance of differences between group means: b, c, e, f, h, i, k, l, m, o, p, r, A, B, C, E – $p < 0.05$; d, g, j, D – $p < 0.01$; a, n, F, G – $p < 0.001$

in the liver, and from 1.52–2.47 mg·kg^{-1} w.w. in muscle tissue (Tab. 3). Mean renal Cu concentrations were significantly higher in animals from the RA district compared with those from the KE and RS districts ($p < 0.01$). For hepatic Cu, significant site-related variation was limited to the comparison between the RS and RA districts, with higher values recorded in animals from RA ($p < 0.05$). In muscle tissue, the highest Cu concentrations were found in wild boar from the RA district, which were significantly higher than those observed in animals from the RV and RS districts ($p < 0.01$ and $p < 0.001$, respectively). Furthermore, samples of wild boar from RV contained more Cu than those from KE ($p < 0.001$).

Mean zinc (Zn) concentrations ranged from 27.54–30.72 mg·kg^{-1} w.w. in the kidneys, from 37.36–42.84 mg·kg^{-1} w.w. in the liver, and from 28.10–36.06 mg·kg^{-1} w.w. in muscle tissue (Tab. 4). Overall, mean Zn concentrations in wild boar organs were comparable among the studied districts, with no statistically significant differences detected

Age-related differences. Trace element concentrations increased with age across all tissues. In kidney, average Fe concentrations ranged from 65.20–132.6 mg·kg^{-1} w.w. depending on age group and district (Tab. 2), hepatic Fe concentrations ranged from 171.8–367.1 mg·kg^{-1} w.w., and muscle Fe concentrations from 21.45–107.2 mg·kg^{-1} w.w. Statistically significant differences among age groups were observed primarily for iron. These differences were detected in kidney (RA – $p < 0.05$; RV – $p < 0.05$), liver (RV – $p < 0.001$), and muscle tissue (RV – $p < 0.001$; KE – $p < 0.001$) (Tab. 2). Inter-district comparisons revealed significant differences in muscle Fe concentrations between RS–RV ($p < 0.05$) and RS–KE ($p < 0.001$) in the youngest age group (0.6 years), and between RS–KE ($p < 0.05$) in the 0.6–1.5-year group. In the oldest group (>1.5 years), significant differences were found in kidney (RA–KE – $p < 0.05$), liver (RS–RV – $p < 0.05$; RV–KE – $p < 0.01$), and muscle (RV–KE – $p < 0.05$) (Tab. 2).

The average copper concentrations ranged from 2.94–6.35 mg·kg^{-1} w.w. in kidney, 2.02–3.98 mg·kg^{-1} w.w. in liver, and 1.34–2.96 mg·kg^{-1} w.w. in muscle tissue (Tab. 3). Similarly to Fe, Cu concentrations increased with age: renal Cu (RV – $p < 0.05$), muscle Cu (RA – $p < 0.01$), with the highest values observed in mining regions. Comparisons among districts revealed significant differences in renal Cu concentrations between RA–KE ($p < 0.05$) in the youngest age group (0.6 years), and between RA–KE ($p < 0.05$) in the 0.6–1.5-year group. In the oldest group (>1.5 years), no significant differences were found (Tab. 3).

Average zinc concentrations in the kidney ranged from 23.73–36.75 mg·kg^{-1} w.w., in the liver from 23.12–48.52 mg·kg^{-1} w.w., and in the muscle tissue from 20.6–41.49 mg·kg^{-1} w.w. (Tab. 4). Zn levels also showed an increasing trend with age: kidney, liver of wild boars in RS ($p < 0.05$, 0.01) and liver and muscle in KE ($p < 0.05$). Age-specific inter-district comparisons showed significant differences in hepatic Zn concentrations between the RA and KE districts in intermediate (0.5–1.5-year) age groups ($p < 0.05$) and in muscle Zn concentrations ($p < 0.05$). However, no significant differences among districts were detected in the oldest age category (>1.5 years) (Tab. 3).

Sex-related differences. In males, mean renal Fe concentrations ranged from 71.01–112.2 mg·kg^{-1} w.w., hepatic Fe from 224.7–279.6 mg·kg^{-1} w.w., and muscle Fe from 47.90–70.53 mg·kg^{-1} w.w. In females, mean renal Fe ranged from 70.06–117.9 mg·kg^{-1} w.w., hepatic Fe from 198.3–302.3 mg·kg^{-1} w.w., and muscle Fe from 37.08–71.70 mg·kg^{-1} w.w. (Tab. 2).

Mean copper concentrations in males ranged from 3.72–4.83 mg·kg^{-1} w.w. in kidney, 2.45–3.54 mg·kg^{-1} w.w. in liver, and 1.42–2.31 mg·kg^{-1} w.w. in muscle. In females, mean Cu concentrations ranged from 3.90–5.56 mg·kg^{-1} w.w. in kidney, 2.73–3.75 mg·kg^{-1} w.w. in liver, and 1.47–2.54 mg·kg^{-1} w.w. in muscle (Tab. 3).

Mean zinc concentrations in males ranged from 27.93–31.46 mg·kg^{-1} w.w. in kidney, 35.46–48.96 mg·kg^{-1} w.w. in liver, and 25.98–37.15 mg·kg^{-1} w.w. in muscle. In females, Zn concentrations ranged from 27.92–32.0 mg·kg^{-1} w.w. in kidney, 33.01–41.7 mg·kg^{-1} w.w. in liver, and 21.06–35.45 mg·kg^{-1} w.w. in muscle (Tab. 4).

Sex-related differences in trace element concentrations were generally less pronounced. Overall, no statistically significant differences in trace element concentrations were observed between males and females in the same district. However, significant differences were detected in the kidneys at the RA and KE in both sexes ($p < 0.05$), whereas in the liver and muscle, differences were found only among females from the RV and KE sites and the RS and KE sites, respectively ($p < 0.05$) (Tab. 2). A significant difference in hepatic copper concentration was identified between females from the RA district and males from the RV district, with higher values recorded in females from RA ($p < 0.05$) (Tab. 3). Regarding zinc concentrations, differences were detected only between females from the RS and RA sites, with females from RS showing higher values ($p < 0.05$) (Tab. 4).

DISCUSSION

The present study investigated the accumulation patterns of iron (Fe), copper (Cu), and zinc (Zn) in liver, kidney, and muscle tissues of wild boar (*Sus scrofa*) from four Slovak regions with differing levels of industrial and mining activity. The results clearly demonstrate that both environmental exposure and biological factors such as age significantly influence trace element distribution, whereas sex appears to have a negligible effect.

Tissue-specific accumulation patterns. The highest concentrations of Fe and Cu were consistently observed in liver and kidney tissues, consistent with their physiological roles in metabolism, detoxification, and storage. The liver is the primary organ involved in iron homeostasis, including ferritin storage and heme metabolism, while the kidneys contribute to the filtration and excretion of trace elements. Similar distribution patterns have been reported in wild boar populations across Europe, confirming that internal organs, particularly liver and kidney, are more reliable indicators of environmental exposure than muscle tissue [11]. Muscle tissue exhibited the lowest concentrations of Fe and Cu, reflecting its limited role in trace element storage. This pattern is consistent with findings in other free-ranging ungulates, where muscle typically reflects only background exposure levels rather than accumulation [12]. Zinc showed a more homogeneous distribution across tissues, as expected given its essential physiological function and robust homeostatic regulation.

Table 3. Copper concentration in visceral organs and muscle of wild boar from Slovakia

	x ± S.E.M min. max.	0.5 min. max.	0.5–1.5 min. max.	over 1.5 min. max.	female min. max.	Male min. max.
			Renal copper concentration (mg.kg^{-1} w.w.)			
RS (n 37)	3.95 ± 0.234^{a,b} 1.82 7.89	3.49 ± 0.302 1.84 5.68	3.66 ± 0.610 1.82 6.93	4.53 ± 0.357 2.77 7.89	3.90 ± 0.331^{d} 1.82 7.89	4.03 ± 0.306 2.16 6.84
RA (n 57)	5.24 ± 0.251^{a} 1.85 9.96	4.58 ± 0.492^{c} 1.85 7.86	5.03 ± 0.354 3.10 9.31	5.89 ± 0.41 2.53 9.69	5.56 ± 0.365^{d} 1.85 9.96	4.83 ± 0.32 2.54 7.30
RV (n 23)	4.74 ± 0.297^{b} 2.54 7.26	4.19 ± 0.385^{A} 2.54 6.36	4.50 ± 0.454 3.00 6.36	6.35 ± 0.322^{A} 5.79 7.26	4.83 ± 0.339 2.54 7.26	4.29 ± 0.767 2.79 6.18
KE (n 30)	3.807 ± 0.312 1.24 7.57	2.94 ± 0.377^{c} 1.24 4.59	3.55 ± 0.349 1.93 5.77	4.719 ± 0.4954 2.39 7.57	3.91 ± 0.492 2.25 7.57	3.72 ± 0.416 1.24 7.43
			Hepatic copper concentration (mg.kg^{-1} w.w.)			
RS (n 37)	2.80 ± 0.140^{e} 1.22 5.99	2.41 ± 0.170 1.22 3.22	2.66 ± 0.147 2.27 3.22	3.24 ± 0.264 2.53 5.99	2.73 ± 0.169^{f} 1.22 4.41	2.93 ± 0.252 1.69 5.99
RA (n 57)	3.65 ± 0.175^{e} 1.69 6.10	3.17 ± 0.239 2.51 4.81	3.36 ± 0.185 1.69 5.08	3.98 ± 0.186 2.10 6.10	3.75 ± 0.181^{f} 1.69 6.10	3.54 ± 0.176^{g} 2.10 5.55
RV (n 23)	2.99 ± 0.202 1.36 5.36	2.49 ± 0.156 1.36 3.28	3.56 ± 0.470 1.83 4.63	3.71 ± 0.523 2.27 5.36	3.12 ± 0.232 1.79 5.36	2.45 ± 0.333^{g} 1.36 3.01
KE (n 30)	3.09 ± 0.24 1.24 7.72	2.89 ± 0.317 1.68 3.68	2.02 ± 0.262 1.24 2.78	3.86 ± 0.578 2.14 7.72	3.33 ± 0.403 1.24 7.72	2.81 ± 0.22 1.68 4.5
			Muscle copper concentration (mg.kg^{-1} w.w.)			
RS (n 37)	1.63 ± 0.116^{h} 0.47 3.83	1.34 ± 0.125 0.47 2.27	1.67 ± 0.149 0.99 2.35	1.94 ± 0.253 1.14 3.83	1.52 ± 0.105 0.48 2.35	1.60 ± 0.218 0.47 3.83
RA (n 57)	2.47 ± 0.157^{h,i} 1.4 3.77	1.9 ± 0.206^{B} 1.4 2.45	2.45 ± 0.174^{k} 1.91 2.90	2.96 ± 0.218^{B} 2.26 3.77	2.54 ± 0.208 1.49 3.26	2.31 ± 0.274 1.40 3.77
RV (n 23)	1.73 ± 0.102^{i,j} 0.91 2.61	1.54 ± 0.119 0.91 2.37	1.89 ± 0.263 0.99 2.79	2.02 ± 0.171 1.46 2.61	1.83 ± 0.106 0.95 2.79	1.42 ± 0.242 0.91 2.23
KE (n 30)	1.52 ± 0.132^{j} 0.45 3.74	1.34 ± 0.120 0.51 1.94	1.52 ± 0.2133^{k} 0.45 2.56	2.06 ± 0.291 0.76 3.74	1.47 ± 0.199 0.45 3.74	1.59 ± 0.174 0.72 2.86

$\bar{x}$ – average value; SEM – standard error of the mean; min. – minimum value; max. – maximum value; 0.5 – half-year-old wild boar; 0.5–1.5 – wild boar aged from 0.5 – 1.5 years; >1.5 – wild boar older than 1.5 years; *p* – significance of differences between group means: c, e, A, k – $p < 0.05$; a, b, d, f, g, i, B – $p < 0.01$; h, j – $p < 0.001$

Zinc is tightly controlled through intestinal absorption and biliary excretion, preventing excessive accumulation under normal environmental conditions [13].

Environmental influence and regional differences. A major finding of this study is the elevated concentration of Fe and Cu in wild boar from the mining regions of Revúca and Rožňava. These areas have a long history of iron ore and magnesite extraction and processing, which has contributed to persistent soil contamination. Heavy metals in soils may remain elevated for decades after mining activities cease because of their low mobility and strong binding to soil particles. Consequently, they enter the food chain via ingestion of plants and soil, particularly in omnivorous species such as wild boar [6]. Recent studies have shown that post-mining landscapes in Europe still represent significant sources of trace metal exposure for wildlife [5, 17]. The observed spatial differences support the concept that wild boar reflect local geochemical conditions and anthropogenic impacts. Their rooting and foraging behaviour increases direct contact with contaminated soil, making them particularly sensitive bioindicators of environmental pollution [14].

Age-dependent bioaccumulation. A clear age-related increase in Fe, Cu, and Zn concentrations was observed across all tissues, with the highest levels recorded in the oldest age group (>1.5 years). This pattern is consistent with bioaccumulation processes, where trace elements progressively accumulate in tissues over time due to continuous exposure and incomplete elimination. Iron showed the most pronounced age dependency, particularly in liver and kidney tissues. This may be attributed to its high biological turnover and storage capacity in ferritin complexes. Copper also increased with age, although to a lesser extent, reflecting its tighter physiological regulation. Zinc displayed the weakest age-related trend, consistent

Table 4. Zinc concentration in visceral organs and muscle of wild boar from Slovakia

	x±S.E.M min. max.	0.5 min. max.	0.5–1.5 min. max.	over 1.5 min. max.	Female min. max.	Male min. max.
			Renal zinc concentration (mg.kg-1 w.w.)			
RS (n 37)	27.54 ± 1.620 17.97 65.89	23.73 ± 0.999[a] 17.97 30.73	25.27 ± 1.139[b] 20.81 29.38	36.75 ± 4.45[a,b] 22.74 65.89	27.92 ± 2.802 18.37 39.96	31.46 ± 3.896 17.97 65.89
RA (n 17)	30.72 ± 2.28 18.93 35.06	24.36 ± 1.887 18.93 27.04	30.50 ± 2.331 23.81 34.48	34.50 ± 4.146 25.97 35.06	32.00 ± 3.647 18.93 58.18	28.81 ± 1.805 23.81 34.90
RV (n 23)	29.04 ± 1.512 22.20 55.34	27.24 ± 1.03 22.20 35.22	26.81 ± 1.246 22.52 30.21	35.61 ± 5.501 25.61 55.34	28.74 ± 1.913 22.52 55.34	30.07 ± 1.636 25.88 35.22
KE (n 25)	29.37 ± 2.193 20.06 60.03	29.75 ± 3.042 20.06 42.79	25.08 ± 1.808 20.36 33.53	34.79 ± 6.692 22.56 60.03	31.29 ± 3.799 20.09 60.03	27.93 ± 1.917 20.06 42.79
			Hepatic zinc concentration (mg.kg^{-1} w.w.)			
RS (n 37)	39.19 ± 1.779 21.64 61.39	33.81 ± 2.346[c] 21.64 46.46	40.89 ± 3.486 29.88 56.81	45.74 ± 3.375[c] 31.12 61.39	38.34 ± 2.340 24.56 61.39	40.46 ± 2.803 21.64 56.81
RA (n 17)	40.33 ± 4.25 21.32 75.37	23.12 ± 1.12 21.32 26.3	46.13 ± 5.393[A] 27.53 57.18	47.13 ± 8.021 29.21 75.37	33.01 ± 4.451 21.32 57.18	48.69 ± 6.484 23.02 75.37
RV (n 23)	42.84 ± 3.45 24.01 67.01	38.42 ± 2.694 24.01 51.63	35.20 ± 3.348 29.99 44.81	48.52 ± 6.022 29.48 67.01	41.70 ± 2.7 24.01 67.01	35.46 ± 4.489 24.02 48.41
KE (n 25)	37.36 ± 2.293 21.10 59.66	34.96 ± 1.607[d] 28.1 41.35	28.50 ± 2.108[A,e] 21.1 32.58	46.44 ± 4.221[d,e] 32.92 59.66	35.88 ± 2.608 27.24 51.95	39.32 ± 3.677 21.10 59.66
			Muscle zinc concentration (mg.kg-1 w.w.)			
RS (n 36)	36.06 ± 2.092 17.55 60.09	32.09 ± 2.478 17.55 48.38	37.09 ± 4.78[B] 19.52 53.47	40.94 ± 4.769 21.99 60.09	35.45 ± 2.525[C] 18.79 58.99	37.15 ± 3.808 17.55 60.09
RA (n 17)	28.41 ± 2.74 14.84 53.85	24.51 ± 2.740 14.84 31.49	29.58 ± 4.46 18.56 40.14	31.14 ± 6.738 19.05 53.85	21.06 ± 1.573[C] 14.84 27.27	34.85 ± 3.69 21.45 53.85
RV (n 23)	28.10 ± 1.796 16.28 52.87	26.89 ± 2.138 16.28 38.37	25.16 ± 1.805 18.71 29.15	35.40 ± 5.861 27.77 52.87	28.76 ± 2.237 16.28 52.87	25.98 ± 2.495 20.69 34.12
KE (n 25)	30.94 ± 3.451 15.65 68.99	23.76 ± 2.125[f] 15.65 29.62	20.60 ± 1.638[B,g] 16.28 26.36	41.49 ± 5.883[f,g] 24.14 68.99	27.58 ± 2.567 19.26 40.92	35.29 ± 4.956 15.56 68.99

$\bar{x}$ – average value; SEM – standard error of the mean; min. – minimum value; max. – maximum value; 0.5 – half-year-old wild boar; 0.5–1.5 – wild boar aged from 0.5 – 1.5 years; >1.5 – wild boar older than 1.5 years; *p* – significance of differences between group means: b, c, d, e, f, g, h, A, B,– $p < 0.05$; a¸ C – $p < 0.01$

with its strong homeostatic control mechanisms. Similar age-dependent accumulation patterns have been reported in wild boar and other wild mammals, confirming that long-lived individuals tend to reflect cumulative environmental exposure more accurately than younger animals [1].

Sex-related variability. No statistically significant differences between males and females were observed for any of the studied elements. Although slight numerical differences were present in some tissues, these did not indicate consistent sex-specific accumulation patterns. This finding is consistent with previous studies suggesting that sex has a minor influence on trace element bioaccumulation compared to environmental exposure and age [7]. Differences in metabolism, hormonal status, or feeding behaviour between sexes in wild populations are generally insufficient to produce significant variations in trace metal burdens [15].

Ecotoxicological implications. From an ecotoxicological perspective, the results confirm that wild boar is a suitable sentinel species for monitoring environmental contamination by trace metals. Its omnivorous diet, wide home range, and close interaction with soil make it highly sensitive to spatial variation in contamination levels.

Although the concentrations observed in this study do not indicate acute toxicity, the elevated levels in mining regions suggest long-term exposure to anthropogenic pollution sources. Chronic exposure to elevated Fe and Cu levels may not necessarily result in immediate toxic effects due to physiological regulation, but may contribute to subtle biochemical alterations and oxidative stress responses, especially when combined with other contaminants such as Cd or Pb [16, 31]. Recent research has emphasized the importance of using wild boar as a biomonitoring tool not only for assessing ecosystem health, but also for food safety, given the increasing consumption of game meat in Europe [16].

Comparison with European studies. The concentrations of Fe, Cu, and Zn observed in this study fall within the range reported for wild boar in other European regions, including the countries of Central Europe. However, higher values in the mining-impacted areas of Slovakia are consistent with regional studies showing elevated trace metal burdens in wildlife from post-industrial landscapes [12, 17, 20].

Iron. In the present study, the highest mean iron concentration was observed in liver tissue (288.7 mg·kg^{-1}), whereas the lowest level was detected in muscle tissue (42.44 mg·kg^{-1}). A recent Polish study reported significantly higher average iron contents in wild boar livers, ranging from 331.35–535.75 mg·kg^{-1} w.w. [18]. Similarly, a study conducted in Austria reported liver iron concentrations of 177.66 mg·kg^{-1} w.w. and copper levels of 6.66 mg·kg^{-1} w.w. [17]. In Spain, Fe values in wild boar meat ranged between 15–180 mg·kg^{-1} [19], indicating considerable variability among studies. Likewise, Pilarczyk et al. [20] and Tekeli et al. [21] reported lower Fe concentrations of 36.4 and 41.9 mg·kg^{-1}, respectively, whereas Babicz and Kasprzyk [15] and Kalinina et al. [22] documented values exceeding 80 mg·kg^{-1}.

Copper. In the present study, mean copper levels were highest in kidneys (5.24 mg·kg^{-1}) and lowest in muscle tissue (1.52 mg·kg^{-1}). Peak values reached 9.69 mg·kg^{-1} in kidneys, 7.72 mg·kg^{-1} in liver, and 3.74 mg·kg^{-1} in muscle tissue. Overall, Cu levels observed in this study were relatively low compared with those reported in several previous studies. A study conducted in western Slovakia by Gašparík et al. [23] similarly reported the highest Cu accumulation in kidneys, whereas muscle tissue contained the lowest levels. Gašparik et al. also documented significantly lower Cu contents in muscle tissue than in visceral organs, with values reaching 5.57 mg·kg^{-1} in kidneys, 4.28 mg·kg^{-1} in liver, and 1.15 mg·kg^{-1} in muscle tissue. Furthermore, a Swedish study by Malmsten et al. [14] reported a mean kidney Cu level of 6.67 mg·kg^{-1} w.w., with values ranging from 2.54–15.3 mg·kg^{-1} w.w., closely corresponding to the kidney values observed in the present study. Higher hepatic Cu levels were also reported by Diethart et al. [17] in wild boar from Austria, where the mean value reached 6.66 mg·kg^{-1} w.w.

Zinc. Zinc concentrations in the present study were highest in the liver (mean 42.84 mg·kg^{-1}) and lowest in the kidneys (27.51 mg·kg^{-1}), confirming the liver as the primary site of zinc accumulation, followed by muscle tissue. These findings are consistent with previous studies on wild boar, including Vidosavljević et al. [24], who reported Zn contents in wild boar tissues of approximately 36.0 mg·kg^{-1} in the liver and 23.3 mg·kg^{-1} in the kidneys. In addition, Zomborszky et al. [25] analyzed Zn distribution in different muscle types of wild boar and reported values ranging from 35.6–44.8 mg·kg^{-1}, with no significant differences among muscle groups, indicating a relatively uniform distribution of zinc within muscle tissue.

Limitations of the study. A limitation of this study is the focus on only three essential trace elements. Future research should also include such toxic metals as Pb, Cd, and Hg to provide a more complete ecotoxicological assessment. Additionally, incorporating soil and vegetation analyses would enable more precise correlation between environmental contamination and bioaccumulation patterns.

Advanced analytical approaches, such as inductively coupled plasma mass spectrometry (ICP-MS), could also improve sensitivity and enable multi-element profiling at lower detection limits. Long-term monitoring programmes would be beneficial for assessing temporal trends and the effectiveness of environmental remediation efforts in former mining regions.

CONCLUSIONS

The results of this study confirmed that the wild boar (*Sus scrofa*) is a suitable bioindicator of environmental contamination by trace elements, particularly iron, copper, and zinc. The highest concentrations of Fe and Cu were recorded in the liver and kidneys, reflecting their key roles in metabolism and detoxification, while muscle tissue showed the lowest levels, thereby better representing background exposure.

The observed regional differences are consistent with historical mining and metallurgical activities, particularly in the Revúca and Rožňava regions, and suggest possible environmental influence. These findings confirm the long-term impact of industrial activity on soil environments and the subsequent transfer of trace elements into the food chain. In contrast, agricultural areas showed lower concentrations, supporting the importance of regional differences in environmental burden.

Trace element concentrations increased with age, confirming bioaccumulation processes over the animals' lifetime. This trend was most pronounced for iron, less evident for copper, and least variable for zinc, which is strongly regulated by physiological homeostasis. In contrast, no statistically significant differences were found between sexes, indicating that sex is not a decisive factor in the accumulation of the studied trace elements.

From an environmental perspective, the study highlights the importance of historically contaminated areas where residues of mining and industrial activities continue to affect ecosystem quality. Although the measured concentrations do not indicate acute toxic exposure, their cumulative effects may pose risks to wildlife health and the safety of game meat consumption.

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