

COMPARATIVE ANALYSIS OF BIOCHEMICAL, TOXIC, AND TRACE ELEMENT PROFILES IN RED DEER (*CERVUS ELAPHUS*) AND ROE DEER (*CAPREOLUS CAPREOLUS*) FROM EASTERN SLOVAKIA

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Abstract: Wild ungulates represent valuable bioindicators of environmental quality due to their exposure to contaminants through diet and habitat. This study assessed concentrations of selected toxic and trace elements and evaluated biochemical parameters in red deer (*Cervus elaphus*, n = 37) and roe deer (*Capreolus capreolus*, n = 8) to determine their health status, characterize environmental conditions, and compare interspecific differences. Blood samples were analysed for elements (As, Cd, Cr, Cu, Mn, Hg, Tl, Zn) using ICP-MS and for biochemical parameters (TP, ABL, GLO, ALT, AST, ALP, GLU, CHOL, TG, CRE, BUN, Ca, P). Cadmium and zinc concentrations were significantly higher in roe deer ($p = 0.012$; $p = 0.018$), while other elements did not differ significantly ($p > 0.05$). Manganese showed high variability in both species. No statistically significant interspecific differences were found in biochemical parameters. Slight elevations in ALT, AST, and ALP were likely associated with capture-and handling-related stress rather than pathological conditions. The animals were kept in a game reserve and supplemented with feed during the winter months, which may contribute to a more stable nutritional status and reduced variability in biochemical profiles. Overall, the low levels of toxic elements and absence of major biochemical alterations suggest a relatively unpolluted environment.

Keywords: red deer, roe deer, trace elements, biochemical parameters

1. INTRODUCTION

Wildlife represents an important link between the environment and humans and plays a key role in monitoring environmental quality. In Central, Eastern and Northern Europe, commonly consumed wild ungulates such as red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), are frequently exposed to toxic metals from various contamination sources and are therefore considered valuable bioindicators of environmental pollution, reflecting local exposure through their diet and habitat (Kierdorf & Kierdorf, 2006; Ramanzin et al., 2010; Cappelli et al., 2020; Abrantes & Vieira-Pinto, 2023; Lénárt et al., 2023). Toxic elements (TEs), including arsenic (As), cadmium (Cd), mercury (Hg), and thallium (Tl), enter organisms from the abiotic environment, accumulate across trophic levels, and bioaccumulate in tissues

and organs. Due to their persistence and long biological half-life, they pose risks to food chains and may induce adverse acute and chronic effects in wildlife and humans (McDowell, 2003; Changfeng et al., 2019; Tajchman et al., 2020; Udom et al., 2025). Their toxicity is mainly associated with interactions with biomolecules, disruption of enzymatic processes, and the induction of oxidative stress through the overproduction of reactive oxygen species and depletion of antioxidants (Jezný et al., 2014; Hazrat et al., 2019; Kim et al., 2019; Wan et al., 2024). Cd is a non-essential, persistent, and highly toxic element capable of mimicking essential metals such as zinc, binding to plasma proteins, and interfering with physiological processes. Its non-biodegradability contributes to bioaccumulation in food chains, posing a risk to both animals and humans (Genchi et al., 2020; Balali-Mood et al., 2021). While

essential elements such as zinc (Zn) and copper (Cu) are required for physiological functions, their excess may lead to toxicity, whereas Cd, Hg, and As are harmful even at low concentrations (Hazrat et al., 2019; Wan et al., 2024). Zn plays an important role in enzymatic processes and metabolism, whereas manganese (Mn), absorbed in the gastrointestinal tract and regulated via the hepatobiliary system, may cause neurological disorders upon chronic exposure (Terrin et al., 2015; Evans & Masullo, 2023). Biochemical markers are widely used to evaluate the physiological condition and health status of game populations (Žele & Vengušt, 2012). They provide insight into organ function, particularly the liver, and reflect metabolic processes (Gowda et al., 2009). The combined assessment of toxic and trace elements together with biochemical parameters allows for a more comprehensive evaluation of environmental contamination and its biological effects on wildlife (Cygan-Szczegielniak, 2021; Hunchak, 2024). In wild ungulates, such as red deer and roe deer, selected biochemical parameters including: total proteins (TP), albumin (ALB), globulin (GLOB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), the AST/ALT ratio, glucose (GLU), cholesterol (CHOL), triglycerides (TG), creatinine (CREA), urea (BUN), calcium (Ca), and phosphorus (P) are commonly used to characterize metabolic processes and overall physiological condition (Barić et al., 2011; Karpiński et al., 2023). Alterations in these parameters may indicate disturbances associated with exposure to toxic elements. As these species are part of the human food chain, such assessments are relevant not only for wildlife monitoring but also for evaluating potential risks to food safety (Lazăr et al., 2025).

The aim of this study is to determine the concentrations of selected toxic and trace elements and to evaluate selected biochemical parameters in red deer and roe deer, recognized as bioindicators of environmental quality, in order to assess the health status of these populations, characterize the environmental conditions in which they live, and compare the observed parameters between the two species.

2. MATERIALS AND METHODS

The experimental group consisted of farmed (semi-free-ranging) males of red deer (*Cervus elaphus*) (n = 37) and roe deer (*Capreolus capreolus*) (n = 8) from a game reserve located in the eastern part of the Slovak Republic. The animals had unrestricted access to natural water sources and were fed hay during the winter season. All individuals showed no

clinical signs of disease. All blood samples were collected during the non-reproductive season, from immobilized animals using standard veterinary procedures. Blood was obtained from the jugular vein by a veterinarian using sterile needles and syringes. For trace and toxic element analysis, blood samples (6 mL) were collected from a subset of animals (red deer, n = 15; roe deer, n = 8) into trace element-certified tubes (royal blue top, BD Vacutainer® tubes; Becton Dickinson, USA) to minimize contamination. The concentrations of As, Cd, Cr, Cu, Mn, Hg, Tl, and Zn were determined by inductively coupled plasma mass spectrometry (ICP-MS) in an accredited diagnostic laboratory (Laboklin, Germany). For biochemical analyses (8.5 mL) blood samples were collected into serum separator tubes (Serum Separator Tubes – SST™ II Advance, BD Diagnostics, USA; e.g., BD Vacutainer® SST) and were centrifuged within 2 hours after blood collection. The serum was then separated and stored frozen at –20 °C until analysis. The following biochemical parameters were determined: total protein (TP), albumin (ALB), and globulin (GLOB) as indicators of protein profile; alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the AST/ALT ratio to assess enzymatic activity; glucose (GLU), cholesterol (CHOL), and triglycerides (TG) as metabolic parameters; creatinine (CREA) and urea (BUN) as renal function indicators; and calcium (Ca) and phosphorus (P) representing mineral status. These biochemical indicators were measured using an automated analyser (COBAS® Integra 400 Plus, Roche Diagnostics, Switzerland).

2.1. Statistical methods

Descriptive statistical analyses were performed for all analysed trace and toxic elements and biochemical parameters to characterize data distribution and variability. Descriptive statistics included mean, median, minimum, maximum, standard deviation (SD), standard error of the mean (SEM), interquartile range (IQR), coefficient of variation (CV), and 95% confidence interval (CI). Normality was assessed using the Shapiro–Wilk test. As several variables were not normally distributed, non-parametric methods were applied. Differences between red deer and roe deer were evaluated using the two-tailed Mann–Whitney U test, and results are presented as U statistics and corresponding p-values. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software Inc., San Diego, CA, USA).

3. RESULTS AND DISCUSSION

Descriptive statistics for trace and toxic elements are summarised in Table 1, whereas biochemical parameters are presented in Tables 2-7.

3.1. Toxic elements

Arsenic concentrations ranged from 0.10 to 1.02 µg/L. Comparison between roe deer (n = 8) and red deer (n = 15) using the Mann–Whitney U test revealed no statistically significant difference between the groups (U = 73.5, p = 0.40). Although mean concentrations were higher in roe deer, this difference was not statistically significant and may be attributed to within-group variability, with all values remaining within the reference range. Cadmium concentrations exhibited high variability (CV = 131.4%) and were significantly higher in roe deer compared to red deer (U = 18.0, p = 0.012). Hg concentrations were predominantly below 0.60 µg/L, corresponding to the method's limit of quantification (LOQ). Due to the high proportion of values below this threshold, statistical comparison between the two species was not considered appropriate. Similarly, no statistically significant

differences in Tl concentrations were observed between roe deer and red deer (p > 0.05).

3.2. Trace elements

Trace element concentrations were generally more variable in roe deer than in red deer (Table 1). No statistically significant difference in Cr concentrations was observed between the two species (U = 33.0, p = 0.21). Mn concentrations exhibited high variability in both species. In red deer, concentrations ranged from 5.83 to 303.3 µg/L (CV = 131.5%; median = 26.3 µg/L), whereas in roe deer they ranged from 2.48 to 620.3 µg/L (CV = 113.1%; median = 101.4 µg/L). The distribution of Mn concentrations deviated significantly from normality in red deer (Shapiro–Wilk test, W = 0.697, p = 0.0002), whereas no deviation from normality was observed in roe deer (W = 0.848, p = 0.091). Comparison using the Mann–Whitney U test revealed no statistically significant difference between the species (U = 36.0, p = 0.129). Cu concentrations exhibited moderate variability but did not differ significantly between the species (U = 43.0, p = 0.42). In contrast, Zn concentrations were significantly higher in roe deer than in red deer (U = 20.0, p = 0.018).

Table 1. Descriptive statistics of trace and toxic element concentrations in blood samples of red deer (*Cervus elaphus*, n = 15) and roe deer (*Capreolus capreolus*, n = 8).

Parameter	Red deer (n = 15) Mean ± SD	Roe deer (n = 8) Mean ± SD	Red deer Median (Q1–Q3)	Roe deer Median (Q1–Q3)	Red deer Min–Max	Roe deer Min–Max	Red deer CV (%)	Roe deer CV (%)
Arsenic (µg/L)	0.43±0.29	0.71±0.61	0.34 (0.20–0.72)	0.44 (0.25–1.20)	0.10-1.02	0.17-1.85	66.6	85.5
Cadmium (µg/L)	0.45±0.59	11.92±23.83	0.20 (0.07–0.53)	2.24 (0.64–2.69)	0.05-1.72	0.06-69.60	131.4	199.9
Chromium (µg/L)	0.13±0.21	0.16±0.13	0.05 (0.05–0.06)	0.09 (0.06–0.29)	0.05-0.75	0.05-0.36	157.1	81.2
Copper (µmol/L)	20.04±5.70	22.28 ±3.08	19.0 (16.5–21.8)	17.4 (14.9–24.9)	13.0-33.6	14.0-53.0	28.4	58.7
Manganese (µg/L)	63.31±83.2	195.0±20.5	26.3 (14.6–81.1)	101.4 (30.9–63.8)	5.83-303.3	2.48-620.3	131.5	113.1
Mercury (µg/L)	0.60±0.00	0.57 ±0.10	0.60 (0.60–0.60)	0.60 (0.60–0.60)	<0.60-0.60	0.33-0.60	0.0	16.9
Thallium (µg/L)	0.056±0.01	0.16±0.20	0.05 (0.05–0.05)	0.05 (0.05–0.29)	0.05-0.10	0.05-0.60	28.5	126.1
Zinc (µmol/L)	14.54±6.16	28.78±27.05	13.4 (11.3–17.6)	22.65 (10.1–32.2)	6.6-30.2	9.2-91.9	42.4	94.0

Notes: values are presented as mean ± standard deviation (SD), median (Q1–Q3), and minimum–maximum (Min–Max). CV = coefficient of variation. Concentrations are expressed in µg/L for arsenic, cadmium, chromium, manganese, mercury, and thallium, and in µmol/L for copper and zinc. Mercury values below the limit of detection (<0.6 µg/L) were treated as 0.60 µg/L for statistical purposes. n = number of samples.

3.3. Biochemical parameters

Protein profile parameters were comparable between red deer and roe deer (Table 2). In red deer, the median TP concentration was 64.4 g/L (Q1–Q3: 52.2–69.7), while in roe deer it was 63.55 g/L (47.98–73.73). ALB concentrations were slightly higher in red deer (median 24.8 g/L; 21.6–27.4) compared to roe deer (23.10 g/L; 18.08–29.15), whereas GLOB levels were similar between species, with medians of 37.7 g/L (30.7–43.1) and 36.05 g/L (29.90–47.65), respectively. Statistical comparison did not reveal any significant interspecific differences. TP concentrations did not differ between the two species ($U = 141.0$, $p = 0.847$), with a negligible effect size ($r = 0.03$) (Table 2). Similarly, ALB levels showed no significant difference ($U = 123.5$, $p = 0.476$). GLOB concentrations were also comparable between species ($U = 147.0$, $p = 0.988$).

Statistical comparison using the Mann–Whitney U test did not reveal any significant interspecific differences in enzymatic activity (Table 3). ALT activity did not differ between species ($U = 146.5$, $p = 0.976$, $r < 0.01$), indicating a negligible effect size.

Similarly, no significant differences were observed for AST ($U = 117.0$, $p = 0.362$, $r = 0.14$), the AST/ALT ratio ($U = 125.0$, $p = 0.501$, $r = 0.10$), ALP ($U = 116.0$, $p = 0.350$, $r = 0.14$), with consistently small effect sizes across all parameters (Table 4).

Triglyceride concentrations did not differ between species ($U = 147.0$, $p = 0.988$, $r < 0.01$), indicating a negligible effect size (Tables 5,6). Similarly, CHOL levels showed no significant difference ($U = 136.0$, $p = 0.733$, $r = 0.05$). GLU concentrations did not differ significantly between species ($U = 109.0$, $p = 0.253$), although a small effect size was observed ($r = 0.17$). CRE concentrations were comparable between red deer and roe deer, with no statistically significant difference observed ($U = 146.0$, $p = 0.965$), and a negligible effect size ($r = 0.01$). Variability was moderate in both species, slightly higher in red deer. Similarly, urea concentrations did not differ significantly between species ($U = 110.0$, $p = 0.262$).

Table 2. Descriptive statistics and interspecific comparison of protein parameters in red deer (*Cervus elaphus*, $n = 37$) and roe deer (*Capreolus capreolus*, $n = 8$).

Parameter	Red deer ($n = 37$) Mean $\pm$ SD	Roe deer ($n = 8$) Mean $\pm$ SD	Red deer Median (Q1–Q3)	Roe deer Median (Q1–Q3)	Red deer Min–Max	Roe deer Min–Max	Red deer CV (%)	Roe deer CV (%)
Total protein (g/L)	62.90 $\pm$ 11.74	61.25 $\pm$ 14.27	64.4 (52.2–69.7)	63.55 (47.98–73.73)	38.5–92.9	39.8–81.1	18.7	23.3
Albumin (g/L)	25.42 $\pm$ 5.79	23.83 $\pm$ 6.72	24.8 (21.6–27.4)	23.10 (18.08–29.15)	17.4–42.1	15.6–35.7	22.8	28.2
Globulin (g/L)	37.48 $\pm$ 9.75	37.48 $\pm$ 9.58	37.7 (30.7–43.1)	36.05 (29.90–47.65)	20.2–65.8	24.4–50.9	26.0	25.6

Notes: data are presented as mean $\pm$ standard deviation (SD) and median (Q1–Q3). CV, coefficient of variation; U, Mann–Whitney U statistic. Statistical significance was set at $p < 0.05$.

Table 3. Comparison of enzymatic parameters between red deer (*Cervus elaphus*, $n=37$) and roe deer (*Capreolus capreolus*, $n=8$).

Parameter	Red deer ($n=37$) Mean $\pm$ SD	Roe deer ($n=8$) Mean $\pm$ SD	Red deer Median (Q1–Q3)	Roe deer Median (Q1–Q3)	Red deer CV (%)	Roe deer CV (%)	U	p-value
ALT (U/L)	95.03 $\pm$ 176.20	76.38 $\pm$ 93.54	40.0 (27.0–78.5)	43.0 (28.5–87.75)	185.44	122.48	146.5	0.976
AST (U/L)	858.8 $\pm$ 587.5	634.8 $\pm$ 543.4	728.0 (353.5–600.0)	525.5 (177.3–092.0)	68.41	85.61	117.0	0.362
AST/ALT	10.22 $\pm$ 9.43	12.31 $\pm$ 10.44	8.26 (0.0–15.75)	11.75 (1.78–21.81)	92.28	84.80	125.0	0.501
ALP (U/L)	295.1 $\pm$ 426.5	139.3 $\pm$ 112.4	139.0 (78.5–300.0)	108.5 (48.75–244.0)	144.53	80.72	116.0	0.350

Notes: data are presented as mean $\pm$ SD and median (Q1–Q3). ALT, alanine aminotransferase; AST, aspartate aminotransferase; AST/ALT ratio; ALP, alkaline phosphatase; U, Mann–Whitney U statistic; CV, coefficient of variation. $p < 0.05$ was considered statistically significant.

Table 4. Results of Mann–Whitney U test and effect size analysis for selected biochemical parameters in red deer (*Cervus elaphus*, n=37) and roe deer (*Capreolus capreolus*, n=8).

Parameter	p-value	Significance	Z (approx.)	r	Effect size
Total protein (g/L)	0.8470	ns	−0.19	0.03	negligible
Albumin (g/L)	0.4761	ns	−0.71	0.11	small
Globulin (g/L)	0.9882	ns	−0.02	0.00	negligible
ALT (U/L)	0.9763	ns	−0.03	<0.01	negligible
AST (U/L)	0.3626	ns	−0.91	0.14	small
AST/ALT ratio	0.5010	ns	−0.67	0.10	small
ALP (U/L)	0.3497	ns	−0.94	0.14	small

Notes: effect size (r) was calculated as $Z/\sqrt{N}$. Interpretation: negligible (<0.10), small (0.10–0.30), moderate (0.30–0.50), large (>0.50), ns = not significant.

Table 5. Comparison of metabolic parameters between red deer (*Cervus elaphus* n=37) and roe deer (*Capreolus capreolus*, n=8).

Parameter	Red deer (n=37) Mean ± SD	Roe deer (n=8) Mean ± SD	Red deer Median (Q1– Q3)	Roe deer Median (Q1– Q3)	Red deer CV (%)	Roe deer CV (%)	U	p-value
Triglycerides (mmol/L)	2.51 ± 2.37	2.70 ± 2.82	1.73 (0.93–3.24)	1.78 (0.78–3.59)	94.33	104.28	147.0	0.9882
Cholesterol (mmol/L)	4.14 ± 3.20	3.75 ± 2.68	3.10 (1.87–5.43)	2.73 (2.46–3.82)	77.35	71.43	136.0	0.7328
Glucose (mmol/L)	8.00 ± 3.63	9.13 ± 11.08	7.54 (5.33–9.73)	5.36 (3.33–8.39)	45.35	121.46	109.0	0.2530

Notes: Data are presented as mean ± standard deviation (SD), median (Q1–Q3), and coefficient of variation (CV, %). Between-group comparisons were performed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

Table 6. Comparison of renal parameters between red deer (*Cervus elaphus*, n=37) and roe deer (*Capreolus capreolus*, n=8).

Parameter	Red deer (n=37) Mean ± SD	Roe deer (n=8) Mean ± SD	Red deer Median (Q1–Q3)	Roe deer Median (Q1–Q3)	Red deer CV (%)	Roe deer CV (%)	U	p-value
Creatinine (μmol/L)	140.51 ± 70.79	128.88 ± 42.36	121.0 (102.0–142.0)	115.5 (105.0–175.0)	50.38	32.87	146.0	0.9645
Urea BUN (mmol/L)	7.71 ± 2.19	9.19 ± 6.08	8.23 (6.26–9.38)	8.19 (6.55–12.08)	28.41	66.16	110.0	0.2621

Notes: data are presented as mean ± standard deviation (SD) and median (Q1–Q3). CREA, creatinine; urea (BUN), blood urea nitrogen; CV, coefficient of variation; U, Mann–Whitney U statistic; Statistical significance was set at $p < 0.05$.

Calcium concentrations did not differ between species ($U = 141.5$, $p = 0.859$, $r = 0.03$), indicating a negligible effect size (Table 7). Similarly, phosphorus concentrations showed no statistically significant difference ($U = 98.5$, $p = 0.145$), although a small effect size was observed ($r = 0.22$).

The present study provides a comprehensive comparison of selected toxic and trace elements (As, Cd, Cr, Cu, Mn, Hg, Tl, Zn), as well as biochemical profiles (TP, ALB, GLOB, ALP, ALT, AST, GLU, CHOL, TG, CREA, BUN, Ca, P), between two cervid species, red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). In addition to element concentrations, biochemical parameters encompassing protein, enzymatic, metabolic, and mineral profiles were evaluated. Toxicological research on cervids has predominantly focused on the assessment of heavy metal concentrations in

biological matrices. Blood is frequently used as a minimally invasive indicator of recent exposure to environmental contaminants (Baroni et al. 2000; Žele & Vengušt 2012). However, the majority of studies emphasize the analysis of soft tissues, especially the liver, kidneys, and muscles, which are considered key organs for the accumulation and long-term storage of toxic elements (Pokorný & Ribarič-Lasnik 2002; Hermoso de Mendoza García et al., 2011; Durkalec et al. 2015; Baloš et al. 2016).

In addition to internal organs, alternative matrices such as hair have been investigated due to their suitability for non-invasive monitoring and their ability to reflect prolonged exposure to toxic elements (Kucharczak et al. 2006; Herrada et al. 2024). Furthermore, other hard tissues, including bones and teeth, have also been studied for the presence of toxic elements, as they can serve as long-term indicators of

Table 7. Comparison of mineral parameters between red deer (*Cervus elaphus*, n=37) and roe deer (*Capreolus capreolus*, n=8).

Parameter	Red deer (n=37) Mean $\pm$ SD	Roe deer (n=8) Mean $\pm$ SD	Red deer Median (Q1–Q3)	Roe deer Median (Q1–Q3)	Red deer CV (%)	Roe deer CV (%)	U	p-value
Calcium (mmol/L)	1.51 $\pm$ 0.71	1.42 $\pm$ 0.90	1.62 (0.90–1.93)	1.35 (0.69–2.09)	46.76	63.59	141.5	0.8586
Phosphorus (mmol/L)	4.93 $\pm$ 1.36	5.64 $\pm$ 1.46	4.55 (3.92–6.45)	5.73 (4.57–7.00)	27.53	25.90	98.5	0.1446

Notes: data are presented as mean $\pm$ standard deviation (SD), median (Q1–Q3), and coefficient of variation (CV, %). Between-group comparisons were performed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

element accumulation (Demesko et al. 2018). More recently, attention has also been directed towards pathological tissues, including skin tumors, which may provide further insight into the relationship between contaminant burden and disease processes in wild ungulate populations (Matějka Košinová et al. 2024).

Cadmium concentrations exhibited high variability and were significantly higher in roe deer compared to red deer, which is consistent with previous findings indicating that roe deer tend to accumulate Cd at higher rates than red deer (Lazarus et al., 2005; Pokorný, 2000). This observation is further supported by Srebočan et al. (2011), who reported increasing Cd accumulation with age and higher levels in roe deer compared to wild boar.

In contrast, no elevated concentrations of other TEs were detected in either species, with all measured values remaining within reference ranges and without biologically relevant interspecific differences. Among trace elements, elevated Mn concentrations were observed in both red deer and roe deer, while increased Zn levels were detected in roe deer.

The high variability of Mn concentrations observed in the present study is consistent with previous findings indicating that Mn levels may vary considerably depending on environmental conditions, particularly habitat characteristics, vegetation, and local geochemical background (Herrada et al., 2024). Manganese uptake in herbivores is strongly influenced by diet, as it is primarily derived from plant material (Landete-Castillejos et al., 2010), and may also increase with age (Matějka Košinová et al., 2024). Although manganese is an essential trace element, excessive exposure may pose toxicological risks, including inflammatory responses and impaired lung function (Crossgrove & Zheng, 2004; Laur et al., 2020). Higher Mn concentrations observed in the present study are consistent with findings reported by Tajchman et al. (2021), who documented significantly higher levels in farmed deer compared to wild individuals. This pattern may be related to nutritional imbalances, as copper deficiency is known

to be common in farmed red deer and other livestock (Rosef et al., 2004). Overall, differences in trace element concentrations reported across studies likely reflect local environmental conditions, including soil composition, habitat characteristics, and vegetation, which influence dietary intake and element bioavailability (Demesko et al., 2018; Herrada et al., 2024). This is consistent with our results, where high variability in Mn concentrations was observed in both red deer and roe deer populations, without statistically significant interspecific differences. Seasonal supplementary feeding may therefore influence manganese intake and contribute to the variability observed in the present study, as the mineral composition of forage reflects local soil conditions and plant species (Suttle, 2010).

Higher zinc concentrations observed in roe deer compared to red deer, despite both species inhabiting the same locality, may be attributed to species-specific differences in feeding behaviour and nutrient uptake. The study of Starčević et al. (2025) has shown that mineral composition, including Zn, may vary significantly between cervid species, reflecting differences in diet selection and feeding ecology rather than environmental exposure alone. No statistically significant interspecific differences were detected in the analysed biochemical parameters between red deer and roe deer ($p > 0.05$), which is consistent with previous studies showing substantial overlap in biochemical values in cervids and indicating that these parameters are influenced more by physiological and environmental factors than by species-specific differences (Rosef et al. 2004, Karpiński et al. 2023). Cholesterol levels reported by Lazăr et al. (2025) in free-ranging red deer (119.69 ± 17.55 mg/dL in males; $\approx 3.09 \pm 0.45$ mmol/L) were lower than the values observed in our study. In our dataset, cholesterol concentrations reached 4.14 ± 3.20 mmol/L in red deer and 3.75 ± 2.68 mmol/L in roe deer, indicating only minor interspecific variation. Similarly, blood glucose levels (96.93 ± 12.53 mg/dL; $\approx 5.38 \pm 0.70$ mmol/L) were lower than those observed in our study (8.00 ± 3.63 mmol/L in red deer

and 9.13 ± 11.08 mmol/L in roe deer), confirming relatively stable glycaemia across both species. Serum urea concentrations reported in males (30.42 ± 7.18 mg/dL; $\approx 5.07 \pm 1.20$ mmol/L) were lower than those observed in our study (7.71 ± 2.19 mmol/L in red deer and 9.19 ± 6.08 mmol/L in roe deer), with slightly higher variability observed in roe deer. In contrast, urea concentrations observed in the present study were lower compared to those reported by Karpiński et al. (2023) of free-ranging roe deer (11.2 ± 0.9 mmol/L), which may reflect differences in dietary protein intake, seasonal variation, or hydration status. Physiologically, elevated urea levels are observed during stress, increased catabolism, and post-traumatic conditions (Dziki-Michalska et al., 2024). Creatinine levels in the study by Lazăr et al. (2025) also showed good agreement, with literature values (1.40 ± 0.24 mg/dL; $\approx 123.76 \pm 21.22$ μ mol/L) indicating higher values in red deer (140.51 ± 70.79 μ mol/L) and similar values in roe deer (128.88 ± 42.36 μ mol/L). Elevated ALT, AST, and ALP activities observed in the present study are consistent with previous reports in cervids, where increased enzyme levels were associated with capture-related stress, handling, and immobilization rather than pathological liver or muscular damage (Küker et al., 2015; Hunchak, 2024).

4. CONCLUSIONS

The present study provides a comprehensive comparison of selected toxic and trace elements as well as biochemical profiles between two cervid species, red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). The results indicate that both species inhabiting the studied game reserve exhibit comparable biochemical profiles and generally low burdens of toxic elements, suggesting a relatively unpolluted environment. Although cadmium and zinc concentrations were significantly higher in roe deer, these differences were not reflected in the evaluated biochemical parameters. No statistically significant interspecific differences were detected in the analysed biochemical parameters ($p > 0.05$), which is consistent with previous studies reporting substantial overlap in biochemical values in cervids. This suggests that these parameters are influenced more by physiological and environmental factors than by species-specific differences.

Several limitations should be acknowledged. The relatively small sample size of roe deer (*Capreolus capreolus*, $n = 8$) may have limited the statistical power to detect subtle interspecific differences. Additionally, variability in handling procedures, capture-related stress, seasonal

influences, and individual physiological status may have affected the measured biochemical parameters. The managed conditions of the game reserve, including winter supplementary feeding, may also influence the generalizability of the findings to free-ranging populations.

Despite these limitations, the study provides valuable baseline data on trace element exposure and biochemical profiles in cervids under semi-natural conditions. The findings support the use of red deer and roe deer as bioindicators of environmental quality. Future research should aim to include larger and more balanced sample sizes, particularly for roe deer, and consider additional variables such as age, sex, seasonal variation, and habitat characteristics. Longitudinal studies and more controlled sampling approaches would help to better distinguish between environmental, physiological, and species-specific effects.

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