



Heavy metal accumulation and physiological responses in *Passer domesticus* from industrial areas of Kosovo

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ABSTRACT

The aim of this study was to provide an integrated evaluation of the effects of chronic exposure to heavy metals in the house sparrow (*Passer domesticus*) by linking tissue bioaccumulation with hematological, biochemical, and reproductive alterations. The study was conducted in two industrial areas of Kosovo (Mitrovica and Drenas) and one rural reference site. The concentrations of heavy metals (Pb, Cd, Cu, Ni, and Zn) were determined in soft and hard tissues using the ICP-OES method. Hematological parameters (RBC, WBC, hematocrit and hemoglobin), plasma enzyme activities (AST, ALT, and ALP), as well as total plasma proteins were analyzed. Histological examination of testes was performed to evaluate spermatogenesis. Results showed significantly higher concentrations of Pb, Cd and Ni in the tissues of birds from industrial areas compared with the reference site, indicating increased metal burden. These changes were associated with decreased erythrocyte number and hematocrit values, increased AST activity, and alterations in biochemical parameters. Histological analysis revealed structural disturbances in seminiferous tubules, reduction of germinal layers, and decreased spermatogenic elements, indicating impaired reproductive function. These findings demonstrate that heavy metal bioaccumulation in *Passer domesticus* is associated with alterations in hematological, biochemical, and reproductive systems, supporting its use as a biological indicator of long-term industrial pollution.

Keywords: heavy metals, bioaccumulation, hematological parameters, biochemical biomarkers, testicular histopathology, *Passer domesticus*.

INTRODUCTION

Heavy metals, such as lead (Pb), cadmium (Cd), nickel (Ni), copper (Cu), and zinc (Zn) are important environmental contaminants in industrial regions due to their persistence and capacity to accumulate in biological systems (Agency for Toxic Substances and Disease Registry, 2008). Their presence in the environment is mainly associated with mining, metallurgical processes, industrial emissions, and fossil fuel combustion (Imeri et al., 2025). Lead (Pb) and nickel (Ni) are among the most relevant toxic metals in industrial environments due to their ability to accumulate in tissues and interfere with physiological processes. In avian species, chronic exposure to these elements has been associated with alterations in hematological parameters, enzymatic activity, and metabolic

regulation, even at sublethal concentrations. These disturbances may not immediately result in mortality, but can lead to progressive impairment of systemic function over time. The biological impact of heavy metals cannot be fully understood through environmental measurements alone. Chemical analyses of soil or water provide information about external contamination, but do not necessarily reflect the biologically available fraction or internal accumulation. For this reason, the use of living organisms as bioindicators represents an effective approach in environmental toxicology,

Biological monitoring allows simultaneous evaluation of exposure, bioavailability, and physiological response (Roux and Marra, 2007). Studies in avian species have demonstrated positive correlations between environmental metal contamination and accumulation in tissues, highlighting

the value of birds as ecological sentinels (Roux and Marra, 2007). Birds are particularly suitable for biomonitoring because of their high metabolic rate, ecological adaptability, and sensitivity to environmental change. Moreover, their position within food webs may facilitate the integration of contamination from multiple sources. The house sparrow (*Passer domesticus*) is especially appropriate as a biological model due to its close association with human settlements, relatively limited home range, and constant exposure to urban and industrial environments. These characteristics enable site-specific evaluation of environmental contamination.

Bioaccumulation constitutes a key mechanism linking environmental contamination to biological effects. The concentration of metals within tissues reflects not only exposure intensity but also absorption, distribution, and retention within the organism. Different tissues may show distinct accumulation patterns depending on their physiological role and metabolic activity. Studies have demonstrated that metals can accumulate in various organs of wildlife, including lungs and heart, and that such accumulation may correspond with systemic alterations (Swaileh et al., 2006). The evaluation of metal concentrations in both soft tissues and more stable tissues, therefore, provides insight into both recent and cumulative exposure. However, the presence of metals in tissues does not automatically indicate functional impairment. For this reason, systemic biomarkers are essential for understanding biological consequences. Hematological parameters, such as erythrocyte count (RBC), leukocyte count (WBC), hematocrit, and hemoglobin concentration, are widely used indicators of physiological status in birds. Hematology plays a crucial role in the assessment of avian health, particularly because clinical signs in birds are often subtle or delayed (Scope et al., 2002). Heavy metal exposure, especially lead intoxication, has been associated with alterations in erythropoiesis and reductions in hematological indices, reflecting interference with normal blood formation processes.

In addition to hematological evaluation, biochemical markers provide information on internal organ integrity. Plasma enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are commonly used indicators of tissue injury in avian species. Elevated AST activity has been associated with hepatic and muscular damage, while alterations in alkaline phosphatase (ALP) and total plasma proteins

may reflect metabolic imbalance or physiological stress. Because birds may mask clinical disease, biochemical profiling offers a sensitive method for detecting subclinical toxicity (Scope et al., 2002). Beyond systemic indicators, reproductive tissues represent highly sensitive targets of environmental contaminants. Heavy metals may interfere with endocrine regulation and cellular differentiation within gonadal tissue. Structural disruption of seminiferous tubules and impairment of spermatogenesis have been described as potential consequences of chronic toxic exposure. Such alterations are ecologically significant because they may compromise reproductive success even when overt mortality is absent. Therefore, histological examination of testicular tissue provides valuable information on the sublethal and long-term biological impact.

Industrial regions in Kosovo, particularly the areas surrounding metallurgical complexes in Mitrovica and Drenas, have historically been associated with elevated environmental metal concentrations. Previous analyses of soil and surrounding environments have documented increased levels of lead and other metals in these regions (Imeri et al., 2019; Elezaj et al., 2013), indicating persistent contamination. Given the ecological characteristics of *Passer domesticus*, this species offers a suitable model for evaluating internal accumulation and biological response under chronic industrial exposure.

While previous investigations have examined individual aspects of heavy metal toxicity such as tissue accumulation, hematological parameters, or biochemical alterations, there remains a need for integrated assessment (Elezaj et al., 2001; Katavolos et al., 2007). Environmental contaminants rarely affect a single biological system in isolation. Instead, chronic exposure may generate coordinated responses involving circulatory, metabolic, and reproductive systems simultaneously. An integrative approach allows evaluation of whether elevated tissue metal burden corresponds consistently with functional changes across multiple physiological domains.

The present study aimed to provide a comprehensive multi-system evaluation of heavy metal exposure in *Passer domesticus* inhabiting industrial areas of Kosovo. By combining measurements of bioaccumulation in soft and hard tissues with hematological, biochemical, and histological parameters, this study sought to clarify the relationship between internal metal burden and systemic biological response.

MATERIALS AND METHODS

The design of the study was based on a series of previous investigations, in which various biological aspects of this species were analyzed as a bioindicator of heavy metal pollution in the industrial regions of Kosovo. These studies included analyses of hematological blood parameters (Millaku et al., 2015a), assessment of biochemical alterations and hepatic enzyme activity (Millaku et al., 2015b), histopathological analysis of reproductive tissues (Millaku et al., 2015c), as well as investigations on the use of *Passer domesticus* as a bioindicator of heavy metal pollution in the environment (Millaku et al., 2015d; Millaku et al., 2015e). The results obtained from these studies have contributed to a broader understanding of the impact of industrial pollution on bird populations. The present study aimed to provide a more integrated evaluation of the biological responses of *Passer domesticus* to chronic exposure to heavy metals in the environment. Samples were collected during the same seasonal period in order to minimize the influence of environmental variation on the physiological parameters of the studied organisms. Adult male individuals of *Passer domesticus* were captured from three different locations in Kosovo and classified according to the sampling area: two industrial sites (Mitrovica and Drenas) and one rural reference site (Ujmir). A total of 60 individuals were analyzed, with 20 individuals collected from each locality (Mitrovica, Drenas, and Ujmir). Only male individuals were included in the study in order to ensure consistency in histological and reproductive analyses.

Sampling area

Characteristics of pollution in the city of Mitrovica

Mitrovica is located in the northern part of Kosovo, at the confluence of the Iber and Sitnica rivers. The economic development of this region has historically been closely linked to the metallurgical industry, particularly to the industrial complex “Trepça”. This complex includes an extensive system of mines, flotation facilities, and metallurgical plants for the processing of lead and zinc ores. The study area includes several industrial sites and metallurgical waste deposits located in the region of Mitrovica and Zvečan. The spatial distribution of these industrial zones and the location of the mining waste deposits are presented in Figure 1, which illustrates the main pollution areas within the study region.

As a consequence of industrial activities carried out over several decades, environmental pollution in Mitrovica and the surrounding areas has long been considered a serious ecological problem (Hyseni et al., 2010; Boisa et al., 2013). Metallurgical activities have been a major source of heavy metal dispersion in soil, water, and the atmosphere (Fresse et al., 2004). The main sources of pollution have included the lead smelter in Zvečan, mineral flotation processes, and metal processing plants. During the period of intensive operation of the smelter, considerable amounts of industrial dust containing high concentrations of lead were emitted into the atmosphere. It has been reported that approximately 1,500 tons of dust were released annually from the smelter stack, with lead content reaching up to 60% (Fresse et



Figure 1. View of the study area in Mitrovica

al., 2004). Historical data indicate that in 1989 alone, about 660 tons of lead were emitted into the atmosphere from the industrial activities of the metallurgical complex.

In addition to the direct emissions from the smelter, a significant proportion of pollution also originated from diffuse emissions of metal-rich dust from mining waste deposits and from metal refining processes. Several studies have shown that airborne lead concentrations during this period were far above the permitted air quality limits, in some cases reaching values several tens of times higher than the recommended ones.

Besides atmospheric pollution, high concentrations of lead have also been detected in agricultural products and in various biological organisms in this region. For example, elevated levels have been reported in agricultural crops such as spinach, as well as in animal products such as milk, and in the blood of residents living in this area (Berisha et al., 2000). These data indicate that industrial pollution in Mitrovica has had a significant and long-term impact on the local environment. For this reason, this region is considered a suitable area for studying the biological effects of heavy metals on living organisms.

Characteristics of pollution in the city of Drenas

The city of Drenas is located approximately 20 km west of Pristina, the capital of the Republic of Kosovo. The municipality of Drenas covers an area of about 290 km², representing approximately 2.66% of the total territory of Kosovo. The area includes 42 settlements and is surrounded by the mountain ranges of Berisha, Kasmaqi, Qyqavica, Golesh, and Lipovica (Blinaja). According to recent estimates, the municipality has approximately 73,300 inhabitants, with a population density of about 284 inhabitants per square kilometer.

One of the main sources of industrial influence in this region is the Ferronickel metallurgical complex, located near the city of Drenas in the central part of Kosovo. The Ferronickel plant began operations in 1984, and its activity was temporarily suspended in 1998 during the conflict period in Kosovo. The complex includes several open-pit mines, among which the Dushkaja mine with estimated reserves of about 6.2 million tons of ore, the Suka mine with about 0.8 million tons, and the Gllavica mine with approximately 6.8 million tons of mineral reserves. Currently, Ferronickel is considered one of the most important industrial enterprises in the metallurgical sector

in Kosovo. The metallurgical factory remaining one of the main sources of industrial impact in this region.

In the metallurgical processing of nickel ores, the mineral is treated in rotary kilns and electric furnaces to produce ferronickel, an alloy widely used in the metallurgical industry. The average chemical composition of the ore includes various elements, such as Ni + Co (about 1.2%), Fe (26.0%), SiO₂ (47.0%), CaO (2.5%), Cr₂O₃ (1.2%), and MgO (11.0%). This process also produces a considerable amount of metallurgical slag, which contains components such as SiO₂ (55–57%), MgO (about 10%), total Fe (about 20%), and CaO (about 4%). The production capacity of the Ferronickel metallurgical complex reaches approximately 12,000 tons of nickel per year (Rizaj et al., 2008).

The industrial processes developed in this complex are accompanied by significant emissions of industrial dust and metallurgical waste into the environment. During the technological process, large quantities of industrial slag are also produced, which in most cases are deposited in open areas near the industrial complex. The surface area of mines and open industrial waste deposits has gradually expanded over the years. In 1991, these areas covered approximately 1700 hectares (Shllaku et al., 1992; Ibrahim et al., 2010). During the production and mineral processing processes, gases and particles containing various heavy metals are released into the atmosphere, among which the most significant are nickel, copper, lead and arsenic (Mehmeti et al., 2001). A general view of the Ferronickel industrial area and the surrounding environment is presented in Figure 2, which illustrates the extent of the industrial complex and the main environmental elements around the study area.

Tissue collection and preparation

Following dissection, soft tissues including liver, kidneys, lungs, and heart were excised, rinsed in physiological saline solution to remove surface contaminants, and stored at –20 °C until further chemical analysis. Bone tissues (femur and tibia) were also collected to evaluate cumulative metal deposition in mineralized structures. Testes were carefully removed from male individuals, fixed in 4% buffered formalin, and prepared for histological examination according to standard histological procedures.



Figure 2. View of the study area in Drenas

Determination of heavy metal concentrations

The concentrations of Pb, Cd, Cu, Ni, and Zn in tissue samples were determined using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Prior to analysis, tissue samples were oven-dried to constant weight, homogenized and subjected to acid digestion using concentrated nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) under controlled laboratory conditions. The digested samples were diluted with deionized water and analyzed following instrument calibration and quality control procedures. Metal concentrations were expressed as $\mu\text{g g}^{-1}$ d.w.

Hematological analysis

Hematological parameters, including erythrocyte count (RBC), leukocyte count (WBC), hematocrit (Hct), and hemoglobin concentration (Hgb), were determined using standard laboratory methods. Hemoglobin concentration was measured using the cyanmethemoglobin method, while hematocrit values were determined using the microhematocrit technique. A Neubauer hemocytometer was used for erythrocyte counting. Leukocyte counting was also performed using a Neubauer hemocytometer following standard blood dilution procedures. The evaluation and interpretation of avian hematological values followed established clinical principles for birds (Scope et al., 2002).

Biochemical analysis

Plasma was separated by centrifugation and analyzed for AST, ALT, ALP, as well as total

protein (TP). Enzymatic activities were measured using standard spectrophotometric methods with an automated biochemical analyzer, following manufacturer protocols and laboratory quality control procedures.

Histological evaluation of testes

Formalin-fixed testicular tissues were dehydrated in graded ethanol series, embedded in paraffin, and sectioned at approximately 4–5 μm thickness using a microtome. The sections were stained with hematoxylin-eosin (H&E) and examined under light microscopy. The structural organization of seminiferous tubules, integrity of the germinal epithelium, and the progression of spermatogenesis were evaluated comparatively among study groups.

Statistical analysis

Prior to statistical analysis, the data were tested for normal distribution. Differences among groups were evaluated using Student's t-test and one-way ANOVA, where appropriate. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SigmaStat software.

RESULTS AND DISCUSSION

The results of heavy metal bioaccumulation (lead – Pb, cadmium – Cd, copper – Cu, nickel – Ni and zinc – Zn) in the tissues of the house sparrow (*Passer domesticus*) from the industrial localities of Drenas and Mitrovica, as well as

from the reference locality Ujmirë, are presented in Table 1. All values are expressed as mean ± standard deviation on a dry weight basis.

Overall, the results show that the concentrations of heavy metals in the analyzed tissues of the house sparrow were generally higher in the individuals collected from the polluted sites (Drenas and Mitrovica) compared with those from the reference site (Ujmirë). This indicates a higher

level of exposure of sparrow populations to industrial pollution in these regions. For Pb, the highest concentration was recorded in the tibia tissue from Mitrovica ($511.8 \pm 154.7 \mu\text{g g}^{-1}$), while the lowest concentration was observed in the testes tissue from the reference site Ujmirë ($2.64 \pm 0.97 \mu\text{g g}^{-1}$). In most of the analyzed tissues, the Pb concentrations in the birds from polluted areas were significantly higher compared with the

Table 1. Concentrations of heavy metals (Pb, Cd, Cu, Ni and Zn) in different tissues of house sparrow (*Passer domesticus*) from reference and polluted sites in Kosovo

Tissue	Metal	Reference	Drenas	Mitrovicë
Liver	Pb	8.22±2.5	10.3±5.4	14.8**±4.6
	Cd	1.32±0.1	2.43**±0.7	3.17***±1.3
	Cu	15.2±4.3	25.3**±8.2	23.3**±5.8
	Ni	0.34±0.2	3.97**±1.7	0.93**±0.5
	Zn	62.8±4.9	91.3**±35.2	64.1**±17.1
Kidneys	Pb	32.3±12.6	118.6**±52.2	112.7**±44.1
	Cd	3.12±1.26	20.9**±9.32	17.2**±5.79
	Cu	21.5±6.7	31.3**±8.63	27.1**±4.96
	Ni	6.72±1.13	9.88**±6.04	5.05**±0.64
	Zn	45.8±6.52	62.3**±16.5	85.1**±16.4
Lungs	Pb	3.87±3.84	14.6**±5.92	21.7**±2.3
	Cd	0.61±0.45	2.11**±0.82	2.52**±0.75
	Cu	3.85±0.91	8.26**±5.81	8.57**±5.25
	Ni	1.74±0.53	12.78**±12.3	10.3**±12.9
	Zn	21.01±5.89	69.8**±39.3	83.8**±94.02
Heart	Pb	2.5±0.9	5.96**±2.74	11.09**±6.47
	Cd	0.23±0.17	0.92**±0.29	1.21**±0.61
	Cu	8.87±5.22	19.34**±5.15	23.2**±4.47
	Ni	0.84±0.85	2.48**±2.28	1.28**±0.6
	Zn	30.4±8.92	77.3**±25.9	91.7**±53.9
Testes	Pb	2.64±0.97	38.9**±30.9	34.1**±18.4
	Cd	0.12±0.07	9.07**±6.07	5.88**±2.68
	Cu	0.33±0.11	10.8**±4.59	19.6**±6.24
	Ni	0.09±0.04	9.34**±3.67	11.2**±3.78
	Zn	31.4±4.99	65.1**±21.6	214.3**±85.8
Femur	Pb	17.6±4.57	82.3**±19.7	510.4**±143.1
	Cd	0.22±0.11	0.64**±0.35	0.74**±0.51
	Cu	3.49±1.27	5.21**±2.71	14.3**±7.92
	Ni	6.38±3.93	30.9**±10.9	12.1**±7.76
	Zn	141.2±30.9	214.7**±49.2	234.9**±63.7
Tibia	Pb	13.2±3.91	85.7**±24.7	511.8**±154.7
	Cd	0.28±0.21	0.89**±0.37	0.84**±0.23
	Cu	1.68±1.03	3.18**±1.62	3.74**±2.17
	Ni	4.36±2.35	11.08**±6.01	8.91**±4.96
	Zn	240.8±40.5	298.1**±107.7	385.2**±146.2

Note: Asterisks indicate significant differences compared with the reference site (Ujmir): *p < 0.05, **p < 0.01, ***p < 0.001.

reference group. For Cd, the highest value was recorded in the kidney tissue from Drenas ($20.9 \pm 9.32 \mu\text{g g}^{-1}$), whereas the lowest value was observed in the testes from the reference population in Ujmirë ($0.12 \pm 0.07 \mu\text{g g}^{-1}$). In general, cadmium accumulation was markedly higher in kidney tissue compared with other organs. Cu showed the highest concentration in the kidneys of birds from Drenas ($31.3 \pm 8.63 \mu\text{g g}^{-1}$), while the lowest concentration was recorded in the testes from the reference locality ($0.33 \pm 0.11 \mu\text{g g}^{-1}$).

In all analyzed tissues, Cu concentrations were higher in birds from polluted sites compared with the control group. For Ni, the highest concentration was observed in the femur tissue from Drenas ($30.9 \pm 10.9 \mu\text{g g}^{-1}$), while the lowest value was detected in the testes from the birds collected in Ujmirë ($0.09 \pm 0.04 \mu\text{g g}^{-1}$). Nickel concentrations were generally higher in bone tissues than in soft tissues. For Zn, the highest concentration was recorded in the tibia tissue from Mitrovica ($385.2 \pm 146.2 \mu\text{g g}^{-1}$), whereas the lowest value was observed in the testes from the reference population ($31.4 \pm 4.99 \mu\text{g g}^{-1}$). In most tissues, Zn concentrations were higher in the individuals from polluted sites compared with those from the reference locality. Analysis of metal distribution among tissues indicates that bone tissues (tibia and femur) exhibited the highest accumulation levels for some metals, particularly Pb and Zn. In contrast, cadmium and copper showed relatively higher concentrations in soft tissues, especially in the kidneys and liver. Nickel concentrations were also higher in bone tissues, indicating a tendency for deposition in skeletal structures.

Overall, the results demonstrate a heterogeneous distribution of heavy metals in the analyzed tissues of *Passer domesticus*, where bone tissues act as major storage sites for certain metals such as Pb and Zn, while soft tissues, particularly the kidneys, represent important organs for the accumulation of Cd and Cu.

The concentrations of heavy metals in the tissues (lungs and heart) of the house sparrow (*Passer domesticus*) are presented in Table 1. All results are expressed on a dry weight basis. It was observed that different tissues of the house sparrow showed variation in the mean concentrations of metals. In general, the concentrations of heavy metals, such as Pb, Cd, Cu, Ni and Zn were higher in the tissues of sparrows from polluted sites (Mitrovica and Drenas) compared with those from the reference site (Ujmir village).

In the lungs of sparrows from polluted sites, the highest values of heavy metals were recorded as follows: Pb concentration $21.764 \pm 2.309 \mu\text{g/g d.w.}$; Cd concentration $2.519 \pm 0.758 \mu\text{g/g d.w.}$; Cu concentration $8.574 \pm 5.258 \mu\text{g/g d.w.}$; Ni concentration $12.784 \pm 12.338 \mu\text{g/g d.w.}$; and Zn concentration $83.832 \pm 94.029 \mu\text{g/g d.w.}$ In the heart tissue of sparrows from polluted sites, the highest values of heavy metals were: Pb concentration $11.09 \pm 6.479 \mu\text{g/g d.w.}$; Cd concentration $1.214 \pm 0.617 \mu\text{g/g d.w.}$; Cu concentration $23.213 \pm 4.47 \mu\text{g/g d.w.}$; Ni concentration $2.481 \pm 2.289 \mu\text{g/g d.w.}$; and Zn concentration $91.72 \pm 53.903 \mu\text{g/g d.w.}$

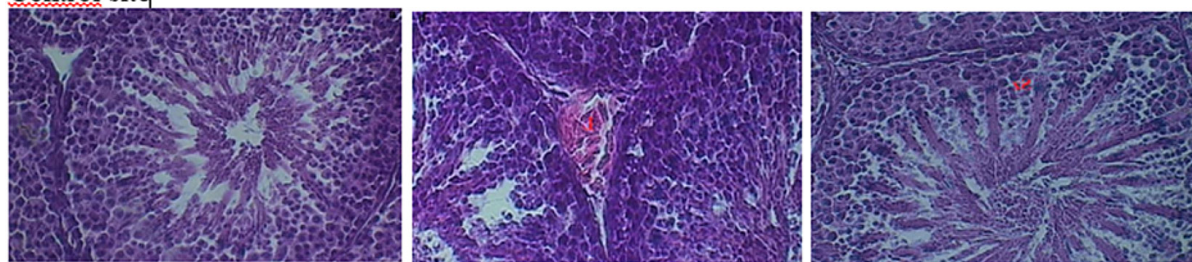
The lowest Pb concentration was recorded as $2.5 \pm 0.901 \mu\text{g/g d.w.}$ in the heart of the house sparrows from the reference site. The lowest Cd concentration was $0.228 \pm 0.179 \mu\text{g/g d.w.}$ in the heart of sparrows from the reference site. The lowest Cu concentration was $3.852 \pm 0.906 \mu\text{g/g d.w.}$ in the lungs of the house sparrows from the reference site. The lowest Ni concentration was $0.8 \pm 0.355 \mu\text{g/g d.w.}$ in the heart of the sparrows from the reference site. The lowest Zn concentration was $21.016 \pm 5.89 \mu\text{g/g d.w.}$ in the lungs of the sparrows from the reference site.

In the lungs, the mean concentrations of Pb, Cd, and Zn showed highly significant differences ($p < 0.001$) between the polluted sites and the reference site, whereas the mean concentrations of Cu and Ni showed significant differences ($p < 0.01$) between these sites. In the heart tissue, the mean concentrations of Pb, Cd, Cu, and Zn showed highly significant differences ($p < 0.001$) between the polluted sites and the reference site, while the mean concentration of Ni showed a significant difference ($p < 0.01$) between these sites.

To evaluate the biological impact of exposure to pollution in industrial areas, a histological analysis of the testes of *Passer domesticus* captured in Mitrovica and Drenas was performed, while the individuals from the reference area of Ujmir served as the control group (Figure 3).

Histological preparations revealed clear differences between the birds from polluted areas and those from the reference site. In the testes of the birds from Mitrovica, noticeable alterations in the structure of the seminiferous tubules were observed. In several preparations, necrotic processes of the testicular tissue were evident, accompanied by enlargement of the interstitial spaces and desquamation of the spermatogenic epithelium. In some cases, the seminiferous tubules contained only a small number of

Control site

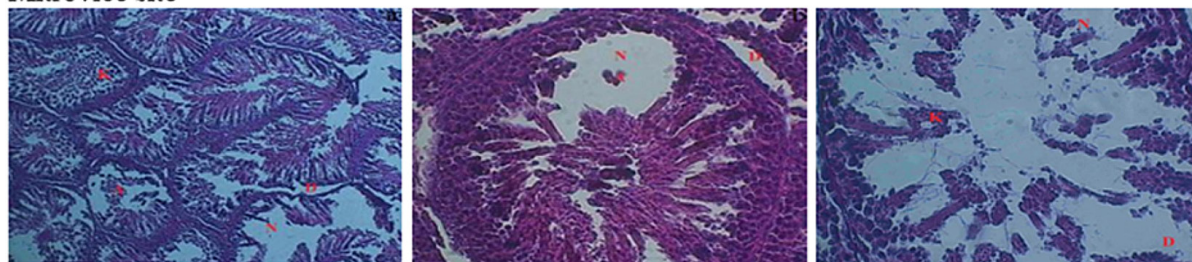


a

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Mitrovica site

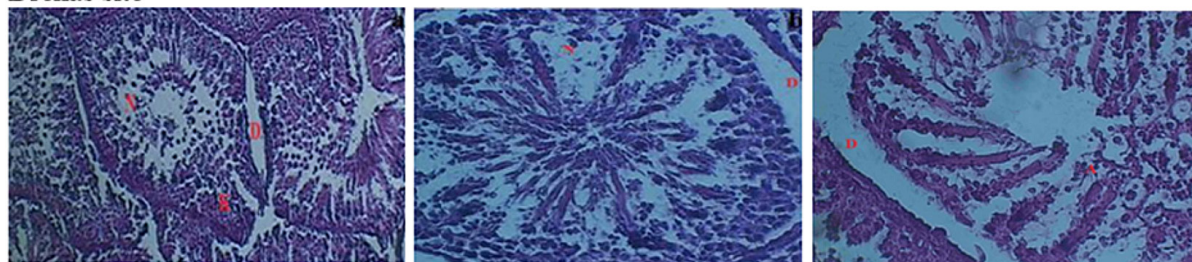


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Drenas site



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Figure 3. Microscopic appearance of the testes of the house sparrow (*Passer domesticus*) from three localities in Kosovo (May): (a–c) control site, Ujmir, showing normal testicular structure with Leydig cells (L) and spermatozoa (Sz); (d–f) Mitrovica site, showing histopathological alterations including necrosis (N), desquamation of the spermatogenic epithelium (D), apoptosis (A) and karyopyknosis (K); (g–i) Drenas site, showing similar pathological changes such as necrosis (N), desquamation of the spermatogenic epithelium (D), apoptosis (A) and karyopyknosis (K)

spermatozoa, indicating disturbances in the process of spermatogenesis. Changes in germinal cells were also observed, including apoptosis and karyopyknosis, which are characteristic features of cellular damage (Figure 3).

Similar alterations were also observed in the testes of the birds captured in Drenas. Microscopic analysis revealed the presence of necrosis and desquamation of the spermatogenic epithelium in several seminiferous tubules. In some preparations, a reduction in the number of germinal cells and disturbances in the normal organization of seminiferous tubule structures were also noted (Figure 3).

In contrast to these findings, the testes of the birds from the reference area (Ujmir) exhibited a normal histological structure. The seminiferous tubules were normally organized and contained

well-formed layers of spermatogenic cells, with numerous spermatozoa present in the lumen. In this tissue, a large number of Leydig cells were also observed in the interstitial spaces, which is characteristic of normal testicular function (Figure 3).

Overall, the histological results indicate that the testes of the birds from polluted areas show degenerative and necrotic changes in the germinal epithelium, whereas in the birds from the reference area, the structure of this tissue remains preserved. These alterations include necrosis, desquamation of the spermatogenic epithelium, apoptosis, and karyopyknosis of germinal and Sertoli cells, suggesting a possible effect of chronic exposure to pollution on the reproductive function of this species.

To evaluate the effect of exposure to industrial pollution on the metabolism of *Passer*

domesticus, several biochemical blood parameters were analyzed, including the activity of ALT, AST, ALP, and the concentration of total plasma proteins (PT). The mean values of these parameters for the birds captured in polluted areas (Drenas and Mitrovica) and in the reference area (Ujmirë) are presented in Figure 4.

The results indicate that the activity of plasma enzymes differs markedly between the studied localities. The mean ALT activity was 68.7 U/L in the birds from the reference area (Ujmirë), while higher values were recorded in the birds from the polluted areas, reaching 180.7 U/L in Drenas and 175.1 U/L in Mitrovica. More pronounced differences were observed for AST activity. In the birds from the reference site, the mean AST value was 257.2 U/L, whereas substantially higher values were recorded in the polluted areas, with 705.6 U/L in Drenas and 811.7 U/L in Mitrovica. A similar trend was observed for ALP. In the reference area, the mean activity was 163.7 U/L, while in the birds from Drenas and Mitrovica the recorded values were 246.1 U/L and 230.6 U/L, respectively.

The concentration of total plasma proteins (PT) showed a slight decreasing trend in birds from polluted areas. In the birds from the reference locality, the mean value was 42.5 g/L, whereas in the birds from Drenas and Mitrovica the values were 39.6 g/L and 33.9 g/L, respectively.

Overall, the results indicate that the activity of several plasma enzymes, particularly AST and ALP, is noticeably higher in the birds captured in

industrial areas, while the concentration of total plasma proteins shows a slight decreasing tendency. These differences are clearly reflected in the graphical distribution presented in Figure 4.

To evaluate the effect of industrial pollution on the physiological condition of *Passer domesticus*, several hematological blood parameters were analyzed, including erythrocyte count (Er), leukocyte count (Le), hematocrit (Hct), and hemoglobin concentration (Hb). The mean values of these parameters for the birds captured in polluted industrial areas (Drenas and Mitrovica) and in the reference site (Ujmir) are presented in Table 2.

Table 2. Hematological parameters in the house sparrow (*Passer domesticus*) from the control site (Ujmir) and polluted sites (Drenas and Mitrovica)

Blood parameters	Reference site	Polluted sites	
	Ujmirë	Drenas	Mitrovicë
Er (x10 ¹² /L)	3.48 ± 0.5	2.61'' ± 0.7	2.83' ± 0.6
Le (x10 ⁹ /L)	5.2 ± 1.3	7.9' ± 2.2	8.2' ± 3.2
Hct (%)	45.3 ± 3.2	30.4'' ± 3.4	31.3'' ± 4.9
Hb (g/L)	191.2 ± 17.4	156.7'' ± 20.8	184.1 ± 23.5

Note: Er – erythrocytes; Le – leukocytes; Hct – hematocrit; Hb – hemoglobin. *p < 0.05, **p < 0.01 indicate statistically significant differences compared with the control site.

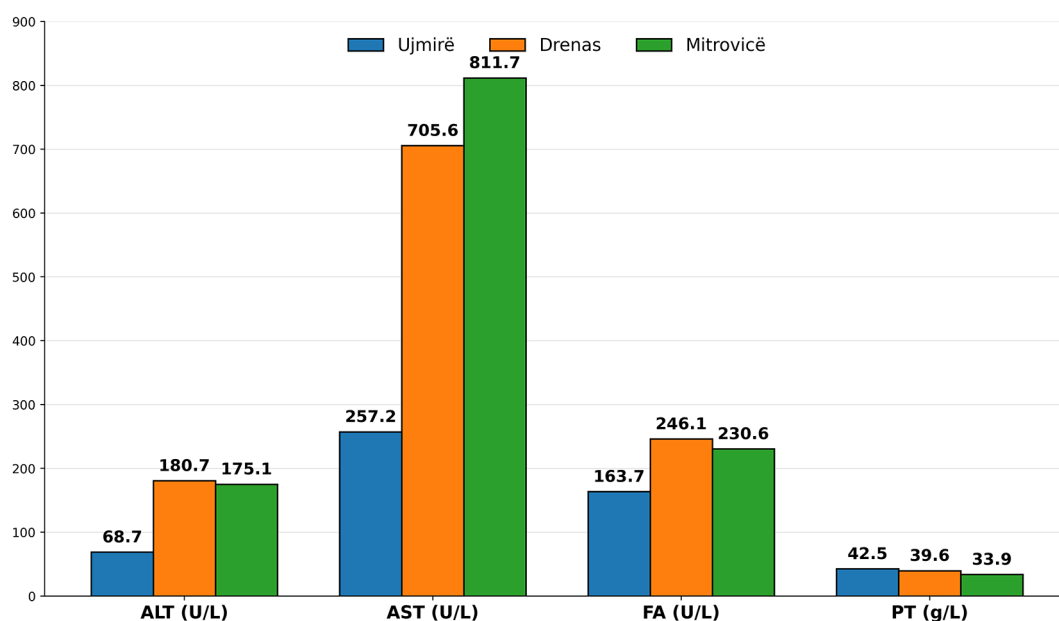


Figure 4. Biochemical parameters in *Passer domesticus* from Ujmir (control site), Mitrovica and Drenas: ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; TP – total protein

The results indicate noticeable differences between the birds from the reference site and those from polluted areas. The mean erythrocyte count (Er) in the birds from the reference site was $3.48 \pm 0.5 \times 10^{12}/L$, while lower values were recorded in the birds from polluted sites, specifically $2.61 \pm 0.7 \times 10^{12}/L$ in Drenas and $2.83 \pm 0.6 \times 10^{12}/L$ in Mitrovica. These results indicate a decrease in erythrocyte number in the birds exposed to industrial pollution.

In contrast, leukocyte counts (Le) were higher in the birds from polluted sites. The mean leukocyte count in the reference site was $5.2 \pm 1.3 \times 10^9/L$, while higher values were recorded in birds from polluted areas, reaching $7.9 \pm 2.2 \times 10^9/L$ in Drenas and $8.2 \pm 3.2 \times 10^9/L$ in Mitrovica.

Hematocrit (Hct) also showed noticeable differences between the localities. In the birds from the reference site, the mean value was $45.3 \pm 3.2 \%$, whereas in the birds from polluted areas, the values were lower, specifically $30.4 \pm 3.4 \%$ in Drenas and $31.3 \pm 4.9 \%$ in Mitrovica.

Hemoglobin concentration (Hb) in the blood also varied among the localities. The birds from the reference site showed a mean value of $191.2 \pm 17.4 \text{ g/L}$, while lower values were recorded in birds from polluted areas, specifically $156.7 \pm 20.8 \text{ g/L}$ in Drenas and $184.1 \pm 23.5 \text{ g/L}$ in Mitrovica.

Overall, the results indicate that the birds from industrial areas are characterized by a decrease in erythrocyte number, hematocrit, and hemoglobin concentration, while the leukocyte count shows an increase compared with the birds from the reference site. These differences are clearly reflected in the numerical data presented in Table 2.

In this study, the heavy metal concentrations in *Passer domesticus* were higher at the polluted sites (Mitrovica and Drenas) than at the reference site (Ujmir), confirming chronic exposure of birds to industrial sources. In particular, mineralized tissues (tibia and femur) were identified as key deposition sites for Pb and Zn, which is consistent with the evidence that Pb preferentially accumulates in physiologically less active tissues such as bone (Selimi et al., 2001).

Differences in Pb accumulation among bones have also been reported in *Aythya marila*, where Pb levels were influenced by age and sex, whereas Cu and Ni did not show a clear age dependence (Szefer and Falandtsh, 1986). In *Passer domesticus*, the elevated Pb levels in Mitrovica can be explained largely by dietary exposure through ingestion of contaminated food collected from soil,

pavements, waste containers, and surface water. Small grit particles used for mechanical digestion in the gizzard may represent an additional Pb source. Respiratory uptake may also contribute in birds due to their high oxygen demand during flight, and higher respiratory Pb uptake has been reported in the urban pigeons compared with rural populations. However, considering the ecology of house sparrows (short lifespan, limited home range) and the closure of the Mitrovica lead - zinc smelter since 2000, gastrointestinal uptake likely represents the dominant exposure route, while airborne exposure may have decreased.

High Zn concentrations in bone (femur and tibia) are consistent with the reports from urban pigeons and other bird species, including regional studies from Mitrovica and surrounding areas (Elezaj et al., 2001; 2013). Co-accumulation of Pb and Zn in bone has been described in urban pigeon populations in London and Mitrovica (Selimi, 2001). The Pb – Zn relationship is complex because Zn is considered antagonistic to Pb toxicity and may influence Pb metabolism, including reactivation of enzymes involved in heme synthesis. This interaction may partly explain the positive Pb – Zn correlations observed in some tissues.

In Drenas, elevated Ni concentrations in both soft and hard tissues are expected given the influence of nickel-processing industry and the documented metal burden in this region (Boisa et al., 2013; Šajin et al., 2013; Shehu et al., 2011). Nickel is readily transferred through the food chain and is associated with potential carcinogenic and hepatotoxic effects, although critical toxic thresholds for wild birds remain insufficiently defined. Comparisons with other studies suggest that the obtained values are broadly consistent with the reports from different bird species in contaminated environments (Barbieri et al., 2007), supporting substantial chronic exposure, particularly at the Drenas site.

Cd, a highly toxic element, tends to accumulate mainly in kidneys and liver through binding to metallothionein, with kidneys considered the critical target organ in chronic Cd exposure. The Cd concentrations measured in the kidneys from Drenas and Mitrovica exceed the threshold proposed for wild birds, indicating increased biological risk. The obtained findings agree with the reports of Cd accumulation in the urban bird populations from the region and from other polluted environments (Elezaj et al., 2013). The

positive Cd – Zn relationships observed in some tissues may reflect antagonistic interactions, where increased Zn availability can mitigate Cd toxicity, including effects on reproductive tissues (Selimi, 2001).

Regarding Cu, although it is an essential element regulated by homeostatic mechanisms, higher Cu levels were recorded at polluted sites, consistent with the studies reporting increased Cu in urban environments without necessarily indicating overt toxicity (Swaileh et al., 2006; Barbieri et al., 2007). Importantly, acute Cu poisoning reported in the literature is associated with much higher hepatic concentrations than those observed here, suggesting that the obtained values more likely represent chronic environmental exposure rather than acute intoxication.

Overall, these results support the use of *Passer domesticus* as a reliable biomonitor of metal pollution. The accumulation profile varies by metal and tissue type, reflecting differences in uptake pathways, internal transport and retention, and interactions between essential and toxic elements.

The histopathological alterations observed in the testes of *Passer domesticus* from the polluted sites, particularly in Mitrovica, included degeneration of the seminiferous epithelium, atrophy of seminiferous tubules, desquamation of spermatogenic cells, karyopyknosis, and necrosis of the tubular epithelium. These findings indicate impaired spermatogenesis and structural damage to the reproductive tissue associated with environmental contamination.

Similar alterations have been reported in experimental and field studies. Degenerative changes in testicular tissue following exposure to heavy metals have been described in laboratory rodents and wild animals exposed to industrial pollution around the Trepça smelter. Comparable findings have also been reported in the urban pigeons (*Columba livia*) from polluted environments (Selimi, 2001). Experimental studies further confirm that lead exposure can cause structural alterations in seminiferous tubules, including reduction of cellular components, separation of the basal membrane, and degeneration of spermatogenic cells.

Lead exposure has also been associated with increased apoptosis in germ cells within seminiferous tubules, resulting in reduced spermatogenic activity. Similar reproductive alterations have been reported in birds exposed to lead intoxication. In addition, cadmium accumulation in reproductive tissues has been shown to induce

comparable histopathological damage in testes, including degeneration of spermatogenic cells and disruption of seminiferous tubules.

These findings suggest that chronic exposure to heavy metals, particularly Pb and Cd, may negatively affect reproductive structures and spermatogenesis in *Passer domesticus*, leading to potential reductions in reproductive capacity in the populations inhabiting polluted environments.

The results of the biochemical analyses showed a significant increase in the activity of aminotransferases (ALT and AST) in the blood plasma of *Passer domesticus* from the polluted sites of Mitrovica and Drenas. Similar increases in these enzymes have been reported in the organisms exposed to heavy metals (Mahmoud et al., 2012; Malik et al., 2004). Elevated ALT activity is commonly associated with liver damage and injury to other tissues, as this enzyme is released into the bloodstream following cellular damage. The high ALT values recorded in this study therefore suggest considerable disturbances in hepatic function. Similarly, increased AST activity may indicate liver damage, but can also be associated with the injury to skeletal muscles, cardiac muscle or other tissues. The elevated AST values observed in the conducted study indicate severe tissue damage, particularly affecting the liver. Increased aminotransferase activity during the exposure to lead has also been reported in experimental studies and under conditions of environmental contamination. In addition, exposure to nickel has been shown to elevate ALT and AST activities in plasma as a consequence of hepatic injury (Sidhu et al., 2004). A significant increase was also observed in the activity of ALP in the plasma of house sparrows from polluted sites. Similar findings have been reported in other studies during the exposure to heavy metals. Alkaline phosphatase is present in several tissues, particularly in bone and the intestinal system, and its increased activity is often associated with alterations in bone metabolism or with pathological processes in the organism (Kopp et al., 2000; Yang et al., 2003).

In the conducted study, a significant decrease in total plasma proteins was also recorded in the sparrows from polluted sites. This reduction may be related to the toxic effects of heavy metals on metabolic processes and liver function. Similar findings have been reported in other studies, where reductions in total protein levels were associated with impaired protein synthesis and

hepatic damage (Sidhu et al., 2004; Kusal et al., 2000). The decrease in total proteins may result from reduced concentrations of albumins and globulins, which play important roles in the transport of various substances and in immune system function (Carlson, 1996). Overall, the observed alterations in biochemical parameters indicate that chronic exposure to heavy metals in the industrial environments of Mitrovica and Drenas has caused significant metabolic and functional disturbances in *Passer domesticus*.

Hematological parameters, such as Hb, Hct, erythrocyte count (Er/RBC) and leukocyte count (Le/WBC) are widely used indicators of health status in both humans and birds, and have been proposed as useful biomarkers for the negative effects of heavy metal exposure in wild birds. In the present study, the evaluation was performed using both an automated analyzer and manual counting, because avian erythrocytes are nucleated and may complicate automated measurements. Both methods showed the same trend of variation among the studied localities, although manual counting resulted in slightly lower absolute values. Such differences between manual and automated methods have also been reported by other authors when comparing these two approaches (Post et al., 2003).

The obtained results indicate a decrease in erythrocyte number and hematocrit values in sparrows from the polluted areas (Mitrovica and Drenas), whereas leukocyte counts were higher compared with the reference site. This pattern is consistent with experimental studies in which exposure to heavy metals (particularly Pb and Cd) is associated with erythropenia/anemia and leukocytosis as a consequence of erythrocyte damage, hemolysis and/or inflammatory processes (Katavolos et al., 2007). During manual evaluation, irregular and immature erythrocytes were also observed, supporting the possibility of toxic effects of metals on hematopoiesis and the stability of red blood cells (Mitchell et al., 2001; Levenwood et al., 2000). The increase in leukocyte number may be related to inflammatory responses and physiological stress, which may be further intensified by impairment of the immune system or opportunistic infections under the conditions of chronic pollution. However, it is also known that capture/handling stress and seasonal factors may influence leukocyte values. In the present study, samples were collected during the same time period in all three localities, and therefore these factors are expected to have affected all groups in a similar manner.

Overall, the hematological alterations observed (decrease in RBC/Hct/Hb and increase in WBC) suggest that chronic exposure to industrial pollution and heavy metals in Mitrovica and Drenas may have caused physiological stress, hematopoietic disturbances and an inflammatory condition in *Passer domesticus*, supporting the use of these parameters as biomarkers for environmental biomonitoring.

CONCLUSIONS

The results obtained in this study indicate that the *Passer domesticus* individuals inhabiting the industrial areas of Mitrovica and Drenas are exposed to elevated levels of environmental contamination by heavy metals. Higher concentrations of Pb, Cd, Cu, Ni, and Zn were consistently detected in the analyzed tissues, suggesting that these birds gradually accumulate metals from their surrounding environment. Among the examined tissues, skeletal structures such as the femur and tibia appear to represent important sites of long-term metal deposition. Because of this property, these tissues may serve as reliable indicators of prolonged environmental exposure. Besides the accumulation in tissues, the findings also revealed noticeable changes in several physiological blood parameters. The birds collected from the industrial areas showed lower erythrocyte numbers and reduced hematocrit values compared with those from the reference locality. Such changes may indicate that chronic exposure to polluted environments can influence hematopoiesis or affect the normal functioning of the circulatory system. At the same time, leukocyte numbers tended to be higher in the birds from the polluted sites, which may reflect a physiological response to environmental stress or to inflammatory processes associated with long-term exposure to contaminants. Alterations were also evident in the biochemical indicators of blood plasma. Increased activity of plasma enzymes, particularly AST and, in some cases, ALT and ALP, may suggest involvement of hepatic and metabolic processes in the organism's response to environmental stressors.

Structural alterations were observed in the seminiferous tubules, including partial desquamation of the spermatogenic epithelium, reduction of germinal cell layers, as well as areas showing necrotic changes and indications of apoptotic processes. These findings suggest that prolonged

exposure to environmental contaminants may interfere with the normal process of spermatogenesis and potentially affect reproductive function. In contrast, the testes collected from the birds in the reference locality displayed normal histological organization and a well-defined progression of spermatogenic stages.

Taken together, this study suggests that the accumulation of heavy metals in *Passer domesticus* is accompanied by changes in several biological systems, including hematological, metabolic and reproductive processes. This indicates that long-term exposure to industrial pollution not only results in metal deposition within tissues but may also lead to functional disturbances in the organism. For this reason, *Passer domesticus* appears to represent a useful biological indicator for monitoring the heavy metal contamination in urban and industrial environments. Combining tissue bioaccumulation data with physiological and histological analyses may provide a more comprehensive understanding of the ecological consequences of environmental pollution and could support the development of long-term monitoring strategies.

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