Journal of the Hellenic Veterinary Medical Society

Vol 76, No 4 (2025)

Concentration of heavy metals in the blood plasma of grazing-cattle of the smelter in Mitrovica

I Krasniqi-Cakaj, B Krasniqi, F Gavazaj, B Kurtaj-Bajrami, Q Selimi

doi: [10.12681/jhvms.40809](https://doi.org/10.12681/jhvms.40809)

J HELLENIC VET MED SOC 2025, 76 (4): 9991-10002
ΠΕΚΕ 2025, 76 (4): 9991-10002

Research article
Ερευνητικό άρθρο

Concentration of heavy metals in the blood plasma of grazing-cattle of the smelter in Mitrovica

I. Krasniqi-Cakaj,[1] B. Krasniqi,[1] F. Gavazaj,[1*] B. Kurtaj-Bajrami,[1] Q. Selimi[2]

[1]College of Medical Sciences "Alma Mater Europaea Campus College "REZONANCA", Prishtina, 10000 Kosovo.

[2]University "Isa Boletini"- Mitrovica Str. Ukshin Kovacica, 40000 Mitrovica, Kosovo.

ABSTRACT: The accumulation of heavy metals in animals depends on the concentration in the soil, pasture, duration of exposure and the way of absorption of metals into the blood and other tissues. The aim of the research is to identify the level of lead (Pb), and some heavy metals in the blood plasma of cattle, as a bio-indicator of exposure to pollutants and determine the toxic index of pollution. The determination of the level of metals was carried out in 73 samples of blood plasma of cattle, in 4 locations at different distances from the smelter (2km, 3km, 5km and 40km - controls). The results show the highest level of heavy metal exposure in: location 1 Pb is (0.571 ppm), Cu (1.327 ppm) and Fe (6.376 ppm), while in the control location, there is a lower level of concentration in Pb (0.264 ppm), Cu (0.568 ppm) and Fe (2.363 ppm), compared to the others. The average values plasma Fe in location 1 (6.376 ppm) and 2 (5.946 ppm), is relatively small (Dif.=0.43 ppm), in location 3 there is a moderate decrease compared to the others and the control. Location 1 consistently shows the highest HPI values for all three heavy metals in bovine plasma, indicating higher exposure to pollutants (Pb, Cu and Fe). There are significant differences in the levels of Cu contamination between locations (F statistic = 11.214, p < 0.0001). The results of the research, for the first time in blood plasma, confirm the existence of heavy metal concentrations, even two decades after the closure of the smelter. Heavy metal pollution, especially lead (Pb), in environments with long-term-chronic exposure, endangers the health of animals and humans, which situation must be continuously researched and analyzed.

Keyword: Heavy metals; cattle; plasma; biomarkers; smelter; environmental pollution

Correspondence author:
Fahri GAVAZAJ,
College of Medical Sciences "Alma Mater Europaea Campus College "REZONANCA", Prishtina, 10000 Kosovo
E-mail address: fahri.gavazaj@rezonanca-rks.com

Date of submission: **17-3-2025**
Date of acceptance: **18-11-2025**

INTRODUCTION

The presence of heavy metals in different forms in the environment determines the level of pollution in the environmental and trophic chain, therefore in various studies cattle is considered as a possible biomarker in environmental pollution (Bischoff K., et al., 2014; Perillo, L. et al. 2021).

Degraded soils and metal deposits from long-term mining activity present a particular risk to grazing animals, as metal concentrations can be high both on the soil surface and in grass. Cd, and Pb have been found to be two, three and five times higher in the soil surface than in the subsoil around an abandoned Pb-Zn mine. Monitoring environmental pollution from mines that historically have produced metals, serves to determine the degree of risks derived from pollutants in the biotic and abiotic system (Arvay J et al., 2014; Luo X,et al., 2023).

Heavy metals can be transferred from the soil through pasture grass to domestic animals and cause poisoning of animals and their products, while the use of such products leads to bioaccumulation in human tissues and can cause cancer and non-cancerous diseases (Castro-González N.P et al., 2021; Karimi A et al., 2020; Mohammadi A.A et al., 2019). Due to bioaccumulation, it poses a real threat to organisms, (Al-Obaidi R.G.F et al., 2022), in a series of physiological and biochemical processes, which are determined by the absorbed dose, the route and duration of exposure, whether acute or chronic (Zadnik T, 2010; Tahir I and Alkheraije K.A, 2023). In the Mitrovica district, despite the closure of the smelter, the level of heavy metals in the environment continues to be high, worrying and dangerous for the environment and living organisms (Aliu M et al., 2016; Çarkaj L et al., 2022). Chronic exposure of cattle to toxic metals can cause embryotoxicity, disturbances in spermatogenesis and oocyte development, therefore it is important to continuously monitor environmental pollution with toxic metals (Wrzecinska M et al., 2021).

A study in New Zealand, for the diagnosis of the state of copper (Cu) concentration in the blood of cattle and the activity of ceruloplasmin (CP) in serum and plasma, has estimated that the concentrations of Cu in the serum of cattle are on average about 3 mol /L (190 mg/L) lower than plasma concentrations, indicating that serum Cu concentration is not a suitable surrogate for plasma Cu concentration in determining blood Cu status, as individual variability in loss of Cu during coagulation is very large. In this study, CP activity, both in serum and plasma, was a suitable surrogate for detecting Cu status in bovine blood (Laven R.A and Smith S.L, 2009). Ceruloplasmin (CP) is generally considered to be the major Cu-containing component in blood, as it accounts for 70-90% of total plasma Cu. Studies have shown that metabolic diseases are closely related to systemic inflammation, oxidative stress, and disorders of copper and iron metabolism, CP has protective and diagnostic effects in metabolic diseases (Liu Z et al., 2022). Plasma and serum studies are suitable for the determination of most essential and toxic elements in the blood of livestock (Luna D et al., 2019).

The city of Mitrovica for decades (1933-2000), due to its proximity to the smelter in the past, was one of the cities with the highest degree of heavy metal pollution, especially lead. It was one of the most polluted cities in the northern part of Kosovo and had the largest metallurgical complex of the "Trepça" mine in Europe and in this location there was an industrial complex such as: the lead and zinc smelter, the nitrogen fertilizer production factory, the batteries, zinc and sulfuric acid electrolysis plant. The surrounding area was quite polluted, including residential areas, and presented a chronic risk to the health of biota and human population (Šajn R et al., 2013; Memishi Sh.F.,et al. 2020; Xing W,et al., 2020). An environmental audit ordered by the Kosovo authorities warned that the foundry should be closed as an "unacceptable" source of air pollution with metals, especially lead, therefore the "Trepça" foundry was closed in 2000. An early study in 1968 showed that sheep's milk in the vicinity of the "Trepça" smelter contained on average about 132 γ per 100 g of lead (Pb), a much higher amount than in other areas. The lambs showed paralysis of the limbs and tongue, temporary diarrhoea, anemia and general weakness. No marked lead poisoning was observed in cattle, although their milk in this area showed approximately the same concentrations as that of sheep (Stamatovic S and Milic D, 1968). Recent studies have shown that the content of lead in the soil, grass, and blood and milk in grazing cows has been progressively reduced in the vicinity of the "Trepça" smelter.

The levels of lead in the milk of cattle near the smelter are more than twice higher than the standards given by the European Commission Regulation (European Commission) and the area near the smelter still poses a threat to the welfare of cattle and human health (Krasniqi-Cakaj I et al., 2020; Dehari-Zeka M et al., 2020). The areas around the smelters are contaminated with heavy metals, sulfur dioxide, dust, smoke and special metals, which are deposited in the soil and accumulate in plants (Elezaj I.R et al., 2013; Sahiti H et al., 2023).

The aim of the research is to determine the presence and distribution of heavy metals, specifically Pb, Cu and Fe in the blood plasma of grazing cattle, in four locations at different distances from the pollution source (smelter). To contribute with valuable information for environmental monitoring and determination of pollution levels in the environmental and trophic chain, so that decision makers can implement sustainable practices for environmental management.

MATERIALS AND METHODS

Study area

This study was conducted to investigate the concentrations of heavy metals in the blood plasma of grazing cattle in the villages located near the lead and zinc smelter "Trepça" (Zveçan), in Mitrovica, at a distance of 1.0, 2.0 and 5.0 km (contaminated area), and the control area - 40 km from the smelter area (uncontaminated area), fig.1. This area lies around the geographical coordinates: 42 ° 54 N and 20 ° 50 E, with a hilly-mountainous relief rich in important minerals not only in Kosovo, but also in the entire Illyrian Peninsula and beyond.

The lead smelter operated for several decades in Zveçan until it was closed (2002), therefore Zveçan and the city of Mitrovica are hotspots of lead and

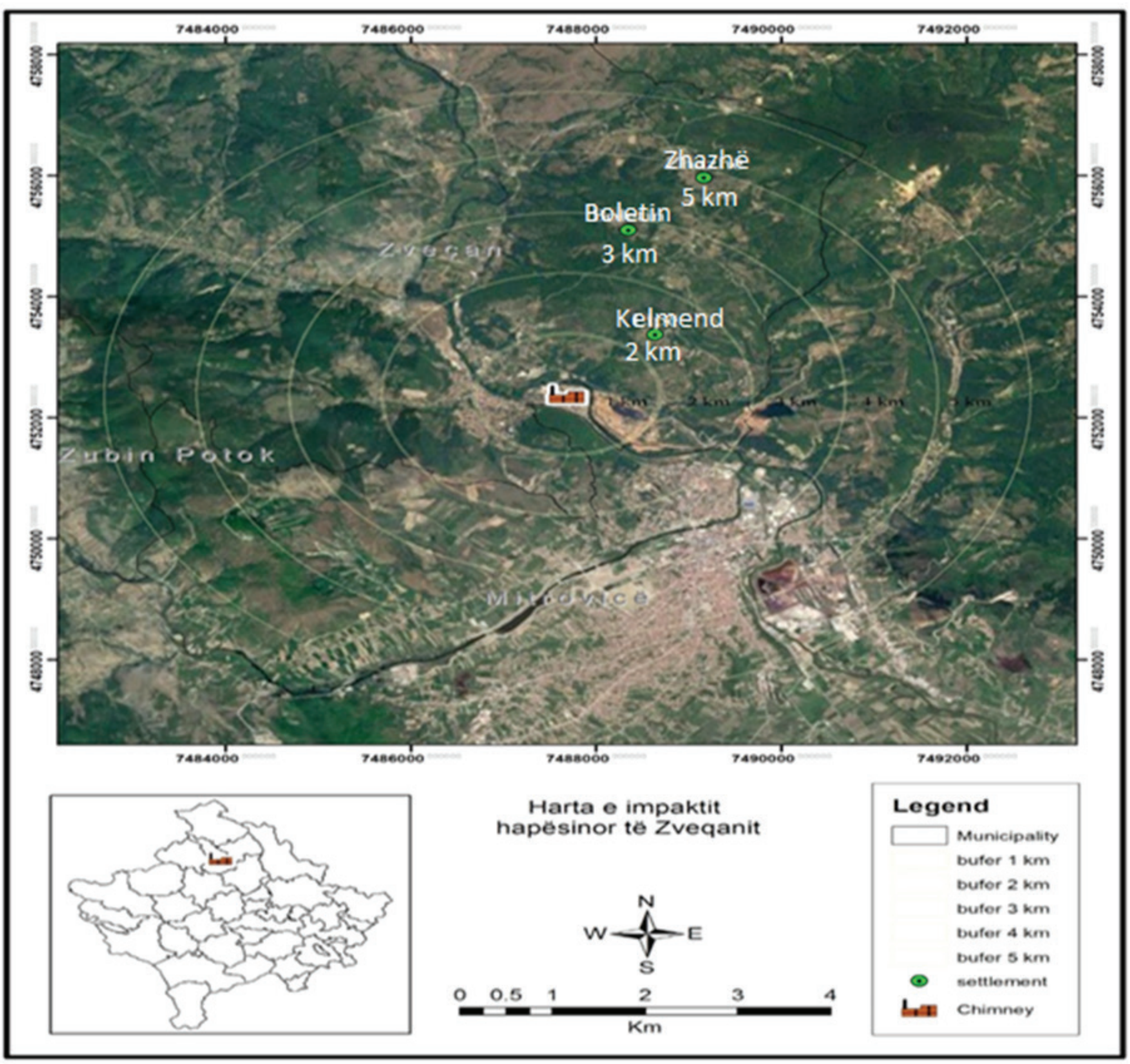


Figure 1. Map of areas affected by pollution in the vicinity of the "Trepça" foundry in Zveçan.

heavy metal pollution. Air emissions of lead have fallen dramatically since the closure of the Zveçan smelter, but their waste still pollutes the air (wind-blown dust), water and land (dust runoff and deposition), so the land around the smelter is still contaminated from deposits of lead and other metals, which is a major source of exposure risk (Kosovo country environmental analysis. MESP, 2016). Various statistical models are used to derive results for pollution levels, dust emissions from contaminated sites and waste dumps, and environmental impacts from lead and zinc waste. This is justified by the results of the 2021 study (in 0-5 cm Fe the value is 24,936 mg/kg), (Kastrati G et al., 2021), and the findings of studies by other authors where the values of heavy metals in the vicinity of the smelter " Trepça", exceed several times the values set according to the European Union average and the New Dutch list up to 152 km2 of the researched area (Aliu M et al., 2016).

Method of data collection

Sampling of 10 ml of blood was performed by puncture in the jugular vein in all grazing cattle by qualified and experienced personnel (trained phlebotomist-veterinarian), avoiding injury and unnecessary stress to the animals. This study, conducted for the first time, was carried out during the period May-June 2022, where limited conditions existed in these localities in taking the number of samples. 73 blood samples were collected from grazing cattle (Simmental breed), in 3 localities within a radius of 2-3-5 km from the "Trepça" foundry: Kelemnd village – 2 km (there were only 11 cows), Boletin village - 3 km (16 cows), Zazhë village – 5 km (30 cows) and the control village Koliq - 40 km from the foundry – (16 cows). Blood was stored in BD vacationer (LH 170 I.U; UK.). Blood samples were then refrigerated at 4 °C during transport to the laboratory for digestion and analysis. Blood plasma is extracted after centrifugation in tubes for 10 minutes at 3000 rpm/min. After centrifugation, 2.0 ml of clear supernatant (2 ml mass) were taken from each plasma sample, and placed on Teflon plates for mineralization. 5 ml of nitric acid (HNO_3 65%) and 1 ml of hydrogen peroxide (H_2O_2 30%) were added. The Teflons together with the sample were placed in the microwave (Speed wave MWS 3+, Berghof, Germany).

Method of data collection

The specific program (for mineralization of plasma and blood) has been selected, with conditions that are presented according to the standards (2022). After the end of the time, according to the program in the microwave software, the samples were allowed to cool for about 30 minutes at room temperature. Then the samples were filtered in a normal container (50 ml). The normal container is filled with distilled water to the specified volume. The mineralized samples, as such, were read in ICP-OES (Optima 2100 DV ICP-OES Spectrometer, PerkinElmer, USA) for the content of chemical elements as requested, under wavelength conditions presented in the report. The ICP reading was done according to the EPA 6010C: 2007 method.

Statistical analysis

The results of One Way ANOVA and Tukey HSD test (IBM SPSS Statistics 20, IBM Corp., Armonk, NY, USA), show that there is no significant-significant difference in HPI values among the four locations (F statistic = 1.269, p = 0.292). Therefore, the observed differences in HPI values can be attributed to random variability and we do not have sufficient evidence to reject the null hypothesis that there are no significant differences in HPI values among the four locations (Table 5 and Graph 4). The formula used for HPI calculation is as follows:

$$HPI = \frac{\sum_{i=1}^{n}\left(\frac{C_i}{S_i}\right)}{N}$$

Where:

Ci - represents the concentration of the i-th heavy metal,

Si - denotes the standard permissible limit for the i-th heavy metal,

Table 1. Microwave software designed for plasma mineralization according to standards.

Parametrs;	Step I	Step II	Step III	Step IV	Step V
Temperature (^{0}C)	145	170	190	100	100
Pressure (bar)	30	30	30	0	0
Time (minutes)	5	10	15	10	10

N- is the total number of heavy metals under consideration.

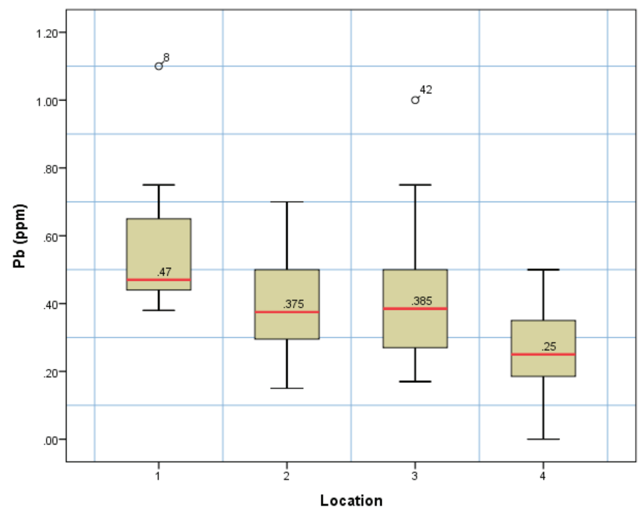
For Pb, the standard permissible limit is > 0.035 ppm :

(CDC-Centers for Disease Control and Prevention, 2018).

For Cu, the standard permissible limit is > 14.95 ppm :

(CDC-Centers for Disease Control and Prevention, 2018).

For Fe, the standard permissible limit is > 4 ppm: (Singhi, S.C.et al. 2003).



Graph 1. Graph for distribution of Pb (Lead) concentration (ppm) in bovine plasma according to locations.

RESULTS

Data for Lead (Pb) Concentration

Research data have shown that the average level of Pb (Lead) in cattle plasma shows the highest level of Pb in location 1- (0.571 ppm/kg), while in location 3 and 2 the average levels of Pb in plasma are approximate (0.405 ppm/kg) and (0.391 ppm/kg), presenting comparable exposure levels. In contrast, the level of Pb in the plasma of cattle at site 4-control shows the lowest level of Pb (0.264 ppm/kg), (Table 2 and Graph 1)

Data for copper (Cu) concentration

The average concentration of Cu (copper) in cattle plasma shows the highest level of Cu, at location 1-2km (1.327 ppm), at location 2; 3km has an average lower Cu concentration (0.803 ppm) compared to site 1, at site 3; 5km Cu (0.631 ppm), and location 4-40km average concentration is (0.568 ppm), presenting comparable exposure levels. As can be seen from the results, these locations seem to have different average levels of Cu among the four locations, where we have a progressive decrease (Table. 3 and Graph. 2).

Data for iron (Fe) concentration

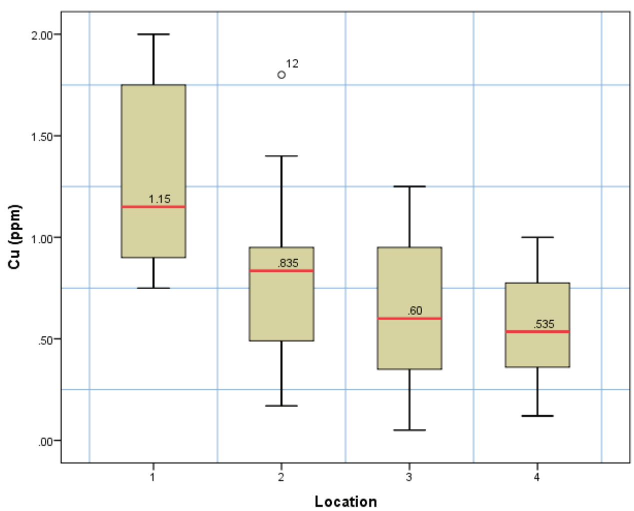
The highest mean concentration of Fe (iron) in the plasma of the cattle population was found at site 1 (6,376 ppm). The difference between the mean concentrations of Fe (Iron) in bovine plasma at site 1 (6.376 ppm) and site 2 (5.946 ppm) is relatively small (Dif.=0.43 ppm), suggesting that both sites have relatively high levels of Fe in bovine plasma. The mean plasma Fe (iron) concentration of cattle at site 3 has a moderate decrease in Fe levels compared to the other two sites, whereas the lowest mean plasma Fe concentration was observed at site 4 (2.363

Table 2. Descriptive data for lead concentration Pb-(ppm) in bovine blood plasma by location.

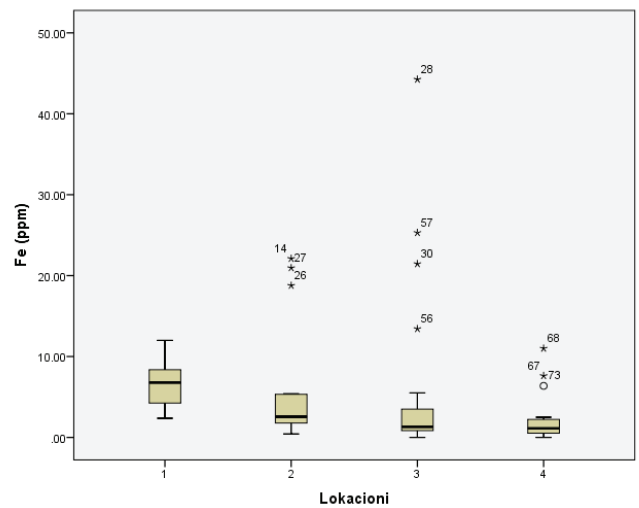
Location	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
1	11	0.571	0.212	0.064	0.428	0.713	0.38	1.10
2	16	0.391	0.158	0.040	0.307	0.476	0.15	0.70
3	30	0.405	0.181	0.033	0.337	0.472	0.17	1.00
4	16	0.264	0.138	0.035	0.191	0.338	0.00	0.50
Total	73	0.396	0.193	0.023	0.351	0.441	0.00	1.10

Table 3. Descriptive data for Cu (copper) concentration (ppm) in cattle plasma by location.

Location	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
1	11	1.327	0.472	0.142	1.010	1.644	0.75	2.00
2	16	0.803	0.413	0.103	0.582	1.023	0.17	1.80
3	30	0.631	0.350	0.064	0.500	0.762	0.05	1.25
4	16	0.568	0.272	0.068	0.423	0.713	0.12	1.00
Total	73	0.760	0.443	0.052	0.656	0.863	0.05	2.00



Graph 2. Graph for distribution of Cu (Copper) concentration (ppm) in cattle plasma by location.



Graph 3. Box plot for distribution of Fe (Iron) concentration (ppm) in bovine plasma by location.

Table 4. Descriptive data for Fe (iron) concentration (ppm) in bovine plasma by location.

Location	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
1	11	6.376	2.954	0.891	4.392	8.361	2.37	12.00
2	16	5.946	7.440	1.860	1.982	9.911	0.43	22.10
3	30	4.900	9.530	1.740	1.342	8.459	0.00	44.25
4	16	2.363	3.157	0.789	.680	4.045	0.00	11.00
Total	73	4.796	7.308	0.855	3.091	6.501	0.00	44.25

ppm). This indicates that site 4 has the lowest mean Fe levels among the four sites (Table 4 and Graph. 3).

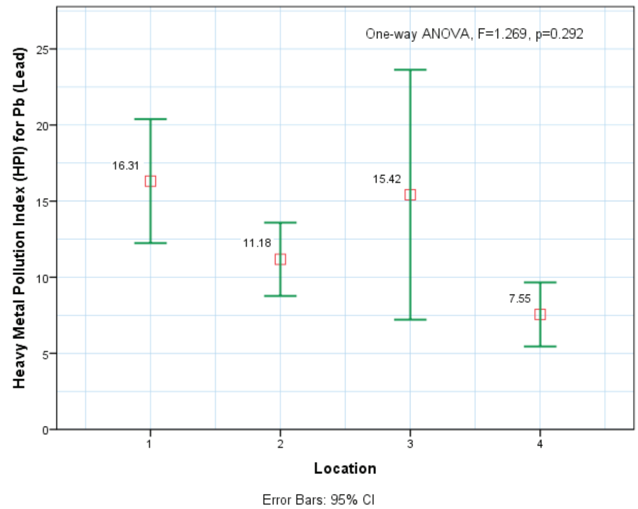
Heavy Metal Pollution Index (HPI)

Heavy Metal Pollution Index (HPI) for Pb (Lead)

Location 1 has the highest Heavy Metal Pollution Index (HPI) value for Pb (Lead) (16.31 ppm), indicating a relatively higher level of Pb pollution in bovine plasma compared to the other locations. Location 3 also exhibits a high HPI value (15.42 ppm), which is close to the HPI of Location 1, suggesting a relatively higher level of Pb pollution similar to Location 1. On the other hand, Location 2 has a moderate level of Pb pollution in comparison to the other locations, with an HPI value of 11.18 ppm. Meanwhile, Location 4 has the lowest level of Pb pollution in bovine plasma compared to the other three locations, with an HPI value of 7.55 ppm.

The results of the One-way ANOVA tests indicate that there is no significant difference in HPI values among the four locations (F-statistic = 1.269, p = 0.292). Therefore, the observed differences in the HPI values could be attributed to random variability, and we do not have enough evidence to reject the null hypothesis that there are no significant differences in HPI values among the four locations (Table 5 and Graph. 4).

The data of our study show that the Heavy Metal



Graph 4. Heavy Metal Pollution Index (HPI) for Pb (Lead) by location.

Pollution Index (HPI) for Pb (Lead) (16.31 ppm), in location 1- has the highest value indicating a relatively higher level of Pb pollution in bovine plasma compared to other locations. Location 3 also exhibits a high HPI value (15.42 ppm), which is close to the HPI value of location 1-, accounting for a relatively higher level of similar Pb contamination in the two locations. On the other hand, site 2 has a moderate level of Pb contamination compared to other sites, with an HPI value of 11.18 ppm. Mean while, site

Table 5. Heavy Metal Pollution Index (HPI) for Pb (Lead), Cu (Copper) and Fe (Iron) by location.

Location	Heave metal	N	Minimum	Maximum	HPI	Std. Deviation	One-way ANOVA F-test	Sig.
1	Pb	11	10.86	31.43	16.31	6.06	1.269	0.292
2		16	4.29	20.00	11.18	4.52		
3		30	4.86	128.57	15.42	21.98		
4		16	0.00	14.29	7.55	3.94		
1	Cu	11	0.05	0.13	0.089	0.03	11.214	<0.0001
2		16	0.01	0.12	0.054	0.03		
3		30	0.003	0.08	0.042	0.02		
4		16	0.008	0.07	0.038	0.02		
1	Fe	11	0.59	3.00	1.59	0.74	0.893	0.449
2		16	0.11	5.53	1.49	1.86		
3		30	0.00	11.06	1.23	2.38		
4		16	0.00	2.75	0.59	0.79		

4-control resulted in the lowest level of Pb contamination in bovine plasma compared to the other three sites, with an HPI value of 7.55 ppm.

Heavy Metal Pollution Index (HPI) for Cu (Copper)

Location 1 has the highest HPI value for Cu (Copper) (0.089 ppm), indicating a relatively higher level of Cu contamination in bovine plasma compared to the other locations. Site 2 has an average level of Cu contamination compared to other sites, with an HPI value of 0.054 ppm. Location 3 has an HPI value of 0.042 ppm, indicating a relatively lower level of Cu contamination compared to locations 1 and 2. Location 4 has the lowest HPI value (0.038 ppm), suggesting the lowest level of Cu contamination in bovine plasma at the four sites. Results of one-way ANOVA tests suggest that there are indeed significant differences in Cu contamination levels between sites (F statistic = 11.214, $p < 0.0001$), (Graph. 5). With such a small <p value, we reject the null hypothesis that there are no significant differences in HPI values between the four locations. Tukey HSD test results highlight distinct levels of Cu contamination at site 1 compared to the other three sites.

Heavy Metal Pollution Index (HPI) for Fe (Iron)

The Heavy Metal Pollution Index (HPI) value for Fe (Iron) in bovine plasma shows variations among the four locations. Location 1 and Location 2 have relatively higher Fe pollution levels (1.59 ppm and 1.49 ppm, respectively), while Location 3 and Location 4 have lower Fe pollution levels (1.23 ppm and 0.598 ppm, respectively).

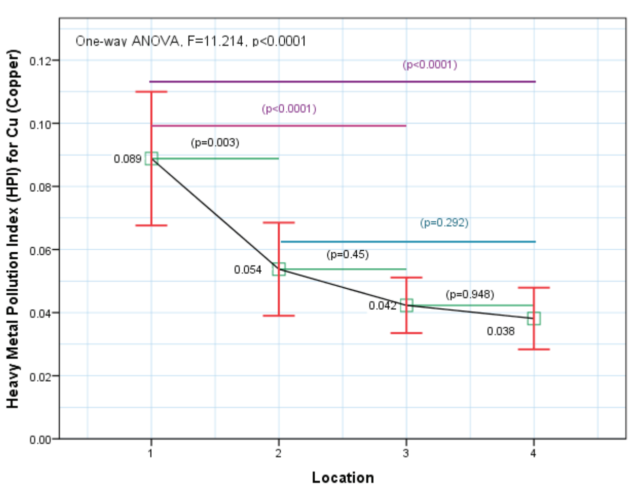
However, the One-way ANOVA results suggest that these differences in HPI values are not statistically significant (F-statistic = 0.893, p = 0.449). This indicates that the observed variations in Fe pollution levels among the locations could be attributed to random variability, and we do not have enough evidence to reject the null hypothesis of no significant differences in HPI values among the four locations (Table 4 and Graph. 6).

The results show changes in the Heavy Metal Pollution Index (HPI) for Pb, Cu and Fe in bovine plasma in the three polluted locations, compared to the control location. Location 1 consistently shows the highest HPI values for all three heavy metals in bovine plasma, indicating higher exposure to contaminants (Pb, Cu and Fe). There are significant differences in Cu contamination levels between sites (F statistic = 11.214, $p < 0.0001$).

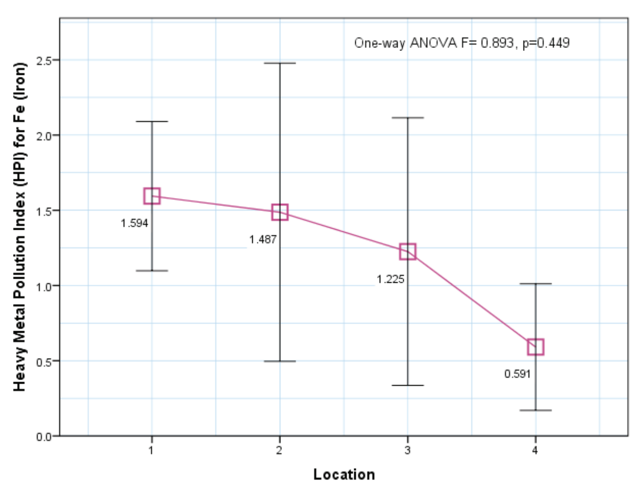
DISCUSSION

Comparison of study results for Pb (Lead) with other research.

Our results show a progressive reduction of lead level in blood plasma (Loc.1; 0.571ppm. Loc.2; 0.391 ppm; Loc.3: 0.405ppm and Loc.4 control; 0.264 ppm-respectively), in grazing cattle in near the foundry “Trepça” at a distance of 2, 3, 5 km. Similar to our study, the results found in the study done a few years ago (2020-2022), in determining



Graph 5. Heavy metal pollution index (HPI) for Cu (copper) by location.



Graph 6. Heavy Metal Pollution Index (HPI) for Fe (Iron) by location.

the level of Pb in the blood of cattle in the villages near the “Trepça” smelter, showed a progressive decrease of Pb in the total blood. The level of lead in the blood (BLL), in locations at a distance of at a distance of 2, 3, 5 km, from the source of pollution, is significantly higher and a significant value ($P<0.001$; 0.0403±0.0277; 0.0268±0.0113; 0.0248±0.0168; 0.0128±0.0041 mg/kg-respectively), compared to the control site at a distance of 40 km. Also the activity values of the blood ALAD enzyme in Kelmend and Boletin is also yesterday lower ($P<0.01$; 3.7±1.3; 4.6±1.6; U/LE, respectively), compared to ALAD enzyme activity (6.0±2.9 U/LE), in the blood of cows that graze in the control area-Koliq, and there is a negative and significant correlation between Pb in blood and ALAD activity ($r=-0.812$; $P<0.004$), in pasture cattle, in Kelmend 2km away from the smelter, (Krasniqi-Cakaj I. et al., 2020). This is also compared with research on the effects of chronic environmental exposure to lead (Pb) on the blood lead level (BLL), (24 ± 11.8, 12 ± 4.5, 11 ± 5.4 and 8.0 ± 2.8 µg/L) of residents living in three villages (Kelmend, Boletin and Zhazhë- at a distance of 2, 3, 5 km), and exposure levels in residents living near a zinc (Zn) smelter, and identification of factors affecting exposure (Dehari-Zeka M. et al., 2020; Jo H. J et al., 2021).

The findings of various studies show that plasma and serum samples seem suitable and interchangeable for the determination of most essential and toxic elements in cattle blood (Luna D et al., 2019). Another study comparable to our study, presenting the concentration of lead (Pb) 0.489 µg/mL (or pmm), in the serum of pet dogs, can justify the data comparable to our work in Loc.3: 0.405ppm. This study was conducted to assess the degree of exposure to heavy metals of pets in a polluted urban area of northwestern Poland, including Pb as a toxicant, and the degree of environmental metal poisoning (Tomza-Marciniak A et al., 2011).

Comparison of study results for Cu (Copper) with other research.

The average concentration of Cu (copper) in the plasma of cattle shows the highest level of Cu at the 1-2km location (1.327 ppm), at the 2-3km location there is a lower average concentration of Cu (0.803 ppm), at the 3-5km location Cu (0.631 ppm), and even lower at the 4-40km location (0.568 ppm). To evaluate our data, the results made in Spain (2019) can be used, where the data on the state of copper (Cu) concentration in the blood of cattle in the plasma show a higher concentration of Cu of 965 ± 36 ug/ L or 0.965± 36 ppm in plasma (Luna D et al., 2019). This study serves to compare the approximate data of our research to determine the state of copper (Cu) concentration: 1.327ppm; 0.803 ppm and 0.631 ppm, in the plasma of cattle in three locations near the smelter.

The study done in Ragusa - Italy, for the analysis of heavy metals in milk and serum of cows at different distances of the farms from the pollution area, for the values of copper (Cu) in the serum shows the following values: 2.36±0.64 mg/L (3.7km) and 3.06 ±2.29 mg/L (6km), (Monteverde V et al., 2022), which values are consistent with the data of our study for the concentration of copper (Cu) in plasma 1.327ppm and 0.803ppm in two locations near the source of pollution (2km and 3km).

Similar to our study, such research of heavy metals in serum, milk and plasma of animals, shows that they are an important bioindicator to detect the exposure of environmental pollutants, in monitoring and maintaining the hygienic state of the environment and the health of animals (Pilarczyk R., 2013).

Comparison of study results for Fe (Iron) with other research.

The highest Fe (iron) concentration in the plasma of the cattle population was found in site 1 (6,376 ppm). The difference between the mean Fe (iron) concentrations in the plasma of cattle in site 1 (6,376 ppm) and site 2 (5,946 ppm) is relatively small (Dif.=0.43 ppm), suggesting that both sites have relatively high Fe levels in the plasma of cattle. The mean Fe concentration in the plasma of cattle in site 3 has a moderate decrease (4,900 ppm), while the lowest mean Fe concentration in plasma was observed in site 4 (2,363 ppm). The study conducted in Ragusa, for the determination of heavy metal levels in milk and serum, is comparable to our study for the serum level. The Fe concentration was higher in serum in group 1; (20.71mg/l), at a distance of 3.7 km from an industrial area and in group 2 the Fe concentration has a lower value (15.48 mg/l), at a distance of 6 km from a chemical fertilizer factory (Monteverde V et al., 2022).

Our results of Fe in plasma (Loc.1: 6.376 ppm. Loc.2: 5.946 ppm. Loc.3: 4.900 ppm), can be compared with the data presented with the level of iron (Fe) in serum, finding a progressive decrease of exposure and source of pollution. Various studies have proven that the concentration of Fe in plasma is lower than in serum (Killilea D.W et al., 2017), (the opposite of the concentration of Cu in plasma and serum), the study conducted in Galicia-Spain confirms this, presenting the concentration of Fe in plasma with the following data: 91.5± 8.2ug/L (ppm) and serum 95.8± 13.1ug/L (ppm), (Luna D et al., 2019).

Heavy Metal Pollution Index (HPI) for Pb (Lead)

Some heavy metals and their properties and toxicity have been known for thousands of years. Furthermore, their use in industry represents a major source of environmental and occupational pollution, with potential exposures arising from natural sources (e.g., through contamination of food and water), industrial processes, and commercial products (Uugwanga, M.N., et al 2021; Machado, C. C. et al., 2023;).

Our study data show that the Heavy Metal Pollution Index (HPI) for Pb (Lead) (16.31 ppm), at location 1- has the highest value indicating a relatively higher level of Pb contamination in cattle plasma compared to other locations. The results of the study according to the Heavy Metal Pollution Index (HPI), show that we still have values of the occurrence of these contaminants even after a long time of closure of the smelter. These data justify our investigation according to a study of blood lead values >0.35 ppm diagnosed during lead poisoning in cattle (Cowan V, et al., 2016). Lead poisoning should be <0.35 ppm, because the physiological level of Pb in animal blood is up to 0.1-0.24 ppm, and >0.35 ppm is a diagnostic symptom of bovine Pb poisoning (Arslan H.H et al., 2009). According to the CDC, the updated blood lead reference value to <3.5 µg/dL = 0.035 ppm, in human blood (CDC-Centers for Disease Control and Prevention, 2018), indicates that the higher the blood lead concentration, the higher the percentage distributed in plasma, because blood lead levels (BLL) increase plasma levels more (ATSDR, Health Consultation 2023).

Heavy Metal Pollution Index (HPI) for Cu (Copper)

The results of the Tukey HSD test show that there are significant differences in the Heavy Metal Pollution Index (HPI) for Cu (copper) between site 1 and each of the other three sites. This indicates that the levels of Cu contamination in location 1 are significantly different from those in location 2, location 3 and 4. However, the test did not find any significant difference in HPI values between location 2 and others (Loc. 3 and 4), and between site 3 and 4. This suggests that Cu contamination levels at site 2 are not significantly different from those at sites 3 and 4. According to the authors' estimates, toxic values of Cu in plasma are 0.9 to 1.1 µg/mL, or > 1.2 µg/mL, and toxic values >14.95 ppm according to CDC (CDC-Centers for Disease Control and Prevention, 2018). The data of our study do not show the same values for toxic level with Cu with 0.089ppm and 0.0540 ppm and can be compared with a reference study 0.7 and 0.9 µg/mL (ppm), while other studies suggest that plasma Cu values of 0.5 µg/mL or less are indicative of low Cu stores in the liver (Claypool D. W et al., 1975). Copper availability in feed is a critical factor that can influence whether livestock develop copper poisoning (Otter, A., et al.. 2023).

Heavy Metal Pollution Index (HPI) for Fe (Iron)

Peak serum iron levels <350 µg/dL (3.5ppm) are associated with minimal toxicity. Levels between 350-500 µg/dL (3.5-5.0ppm) are associated with moderate toxicity. Levels >500 µg/dL (5.0ppm) are associated with severe toxicity. Serum iron levels peak 2 to 4 hours after ingestion. Approximately 10% of iron is absorbed from the intestines and then bound to transferrin. Normal serum iron levels range from 50 to 150 µg/dL (0.5 -1.50 ppm). When iron levels increase after significant ingestion, transferrin becomes saturated with Fe, excess Fe will circulate in the blood as free iron, which is directly toxic to target organs.

According to the research results, we have an exponential decrease in the values for the heavy metal pollution index of Pb (lead), Cu (copper) and Fe (iron), depending on the distance of chronic exposure in the villages, Kelmend 2km. > Boletin 3km > Zhazhë 5km> than the control of Koliq 40 km. The time frame of the study may overlook temporal changes in heavy metal concentrations, requiring extended monitoring for accuracy. This study is in agreement with the study carried out in the blood, milk and urine of cows (Castro-Gonzalez, N.P, 2021), which proves the presence of heavy metals that determines the degree of pollution of the environmental and trophic chain with which we can consider cattle as a biomarker of environmental pollution.

Focusing on Pb, Cu, and Fe, the study may not cover all potential pollutants. Future research should broaden the scope to include a comprehensive array of contaminants. Additional research, including epidemiological studies, is essential to establish potential health risks from observed heavy metal levels.

Limitations of the study

Although this study provides useful information for the first time on the concentration of heavy metals in the plasma and blood of cattle and the degree of toxicity of animals after a long period of exposure, it remains to periodically investigate the level of heavy metals and monitor the health of the population. Periodic studies should be carried out in these localities near the smelter and the degree of environmental toxicity should be measured. Despite the limitations and other difficulties, it is necessary to work with the population, to increase their awareness of the importance of research, to cooperate with interested parties for the analysis and dissemination of data.

CONCLUSIONS

The research data realized for the first time in these locations in the blood plasma of cattle can serve as a control indicator to detect the toxic risks even after several decades of the closure of the smelter, related to the presence of heavy metals in tissues and consequences for animal and human health. Based on these results, the level of lead (Pb) in the plasma showed a progressive decrease moving away from the smelter and was lower in the control group. The average concentration of Cu (copper) in cattle plasma shows the highest level of Cu in location 1, 2 and 3, while the control location presents comparable levels of exposure. The highest average concentration of Fe (iron) in the plasma of the cattle population was found in location 1. The difference in concentrations of Fe (Iron) in the plasma of cattle in location 1 and 2 is small (Dif. = 0.43 ppm), suggesting that they have high levels of Fe in bovine plasma, site 3 has a moderate decrease in Fe compared to the other two, while site 4 has the lowest mean Fe levels compared to the three sites.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGMENTS

Special thanks to the College of Medical Sciences “ALMA MATER EUROPAEA, CAMPUS “REZONANCA”, co-authors for providing language assistance, writing assistance, and analysis in the paper summary.

REFERENCES

Arvay J et al., 2014. Contamination of wild-grown edible mushrooms by heavy metals in a former mercury-mining area J. Environ. Sci. Health, B 49 (11):815-27. doi: 10.1080/03601234.2014.938550.

Aliu M et al., 2016. Spatial distribution of lead in soils of Pb-Zn mining and smelting area of the Mitrovica Region, Republic of Kosovo. J Environ Sci Health A 51:588–595. DOI: 10.1080/10934529.2016.1141628.

Al-Obaidi R.G.F et al., 2022. Evaluation of Heavy Metal Pollution in the Blood Serum of Industrial Workers, Mosul, Iraq. Revista Bionatura. Volume. 7 (2) 4. DOI. 10.21931/RB/2022.07.02.4.

ATSDR 2023 Health Consultation.

Arslan H.H. et al., 2009. Evaluation of the Relationship of Blood Heavy Metal, Trace Element Levels and Antioxidative Metabolism in Cattle Which Are Living Near The Trunk Roads. Kafkas Univ. Vet. Fak .Derg. 17 (Suppl A): 77-82. DOI:10.9775/kvfd.2010.3433

Bąkowska M et al., 2010. Heavy Metals and Other Elements in Serum of Cattle from Organic and Conventional Farms. Biological Trace Element Research 143(2):863-70. DOI 10.1007/s12011-010-8910-z

Bischoff K et al., 2014. Lead excretion in milk of accidentally exposed dairy cattle. Food Additives & Contaminants: Part A. Taylor & Francis.31(5), 839-884. DOI: 10.1080/19440049.2014.888787

CDC (Centers for Disease Control and Prevention) Centers for Disease Control and Prevention; 2018.

Castro-González, N.P et al., 2021. Heavy metals in blood, milk and cow’s urine reared in irrigated areas with wastewater. Journal List Heliyon v.7(4). e06693. PMC8079447. DOI:10.1016/j. Helion.

Claypool D. W., et al., 1975. Relationship between the level of copper in the blood plasma and liver of cattle. J. Anim. Sci. 41:911-914. DOI: 10.2527/jas1975.413911x

Cowan V, Blakley B. (2016). Acute lead poisoning in western Canadian cattle—a 16-year retrospective study of diagnostic case records. Can Vet J. (2016) 57:421 PMCID: PMC4790235 PMID: 27041761.

Çarkaj L et al., 2022. Roman Snail (Helix pomatia L.) as Bioindicator of Heavy Metals Pollution in Mitrovica Town, Kosovo. Ecol. Eng, 3, 64-71. doi.org/10.12912/27197050/147149.

Dehari-Zeka M, et al., 2020. Blood lead level (BLL), δ-aminolevulinic acid dehydratase activity (ALAD), hemoglobin (Hb) and hematocrit (hct) in primary school-children and adult residents living in smelter rural areas in Kosovo. J Environ Sci Health A Tox Hazard Subst Environ Eng.;55(10):1179-1187. doi: 10.1080/10934529.2020.1780851.

Donald S et al., 2002. The relationship between lead in plasma and whole blood in women. E. Health. Perspect., 110 (3): 263–268. doi: 10.1289/ehp.02110263

Elezaj I.R et al., 2013. Metal Accumulation, Blood δ-Aminolevulinic Acid Dehydratase Activity and Micronucleated Erithrocytes of Feral pigeons (Columba livia) Living Near Former Lead-Zinc Smelter “Trepça”- Kosovo. E3S web Conference, 1, 34001.

Jo H. J et al., 2021.Chronic exposure to lead and cadmium in residents living near a zinc smelter. Int J Environ Res Public Health. PubMed. 18(4): 1731. doi.org/10.3390/ijerph18041731

Krasniqi-Cakaj I et al., 2020. Relationship between lead (Pb) concentration in soil, grass, blood, milk and δ-aminolevulinic acid dehydratase (ALAD) activity, hemoglobin (Hb) and hematocrit (Hct) in grazing cows from vicinity of smelter “trepça” in Kosovo. J Chem Heal Risks. 10(3):203–212. DOI:10.22034/jchr.2020.1897429.1123

Kosovo country environmental analysis. MESP, 2016.

Kastrati G et al., 2021. Investigation of concentration and distribution of elements in three environmental compartments in the region of Mitrovica, Kosovo: Soil, honey and bee pollen. Int. J. Environ. Res. Public Health 2021, 18, 2269. DOI:10.3390/ijerph18052269

Killilea D.W et al., 2017. Identification of a Hemolysis Threshold That Increases Plasma and Serum Zinc Concentration. J. Nutr. 147:1218–1225. DOI: 10.3945/jn.116.247171

Karimi A et al., 2020. Assessment of human health risks and pollution index for heavy metals in farmlands irrigated by effluents of stabilization ponds. Environ. Sci. Pollut. Res. Int. PubMed. 2020;27(10):10317–10327. DOI: 10.1007/s11356-020-07642-6.

Liu Z et al., 2022. Molecular Functions of Ceruloplasmin in Metabolic Disease Pathology. Diabetes Metab Syndr Obes. 2022 Mar 3;15:695-711. doi: 10.2147/DMSO.S346648.

Luo X,et al., 2023. Soil heavy metal pollution from Pb/Zn smelting regions in China and the remediation potential of biomineralization. J Environ Sci (China).. Epub 2022 Jan 25. PMID: 36375948. DOI: 10.1016/j.jes.2022.01.029

Laven R.A and Smith S.L, 2009. Copper deficiency in sheep: An assessment of the relationship between concentrations of copper in serum and plasma. N. Z. Vet. J. 56:334–338. DOI:10.1080/00480169.2008.36856

Luna D et al., 2019. Determination of essential and toxic elements in cattle blood: Serum vs plasma. Animals 2019. PubMed. 9(7), 465. DOI:10.3390/ani9070465.

Monteverde V et al., 2022. Heavy Metal Levels in Milk and Serum of Dairy Cows from Different Farms Located near an Industrial Are. Animals (Basel). 12(19): 2574. doi: 10.3390/ani12192574.

Mohammadi A.A et al., 2019. Carcinogenic and non-carcinogenic health risk assessment of heavy metals in drinking water of Khorramabad, Iran. Methods X. PubMed .6:1642–1651. DOI: 10.1016/j.mex.2019.07.017 .

Memishi Sh.F.,et al. 2020. Chronic Efects of Lead Exposure on Oxida-tive Stress Biomarkers in Feral Pigeon (Columba livia) from Smelter Area in Kosovo. Jordan Journal of Biological Sciences, 13. Supplementary Issue. DOI:10.12912/27197050/147149.

Machado, C. C; et al., 2023. Clinical and Forensic Signs Resulting from Exposure to Heavy Metals and Other Chemical Elements of the Periodic Table. J. Clin. Med. 2023, 12, 2591. doi.org/10.3390/jcm12072591.

Otter, A. et al .,2023. A form of hepatogenous copper poisoning in fattening cattle associated with the ingestion of mouldy straw. Veterinary Record Case Reports, e592. DOI:10.1002/vrc2.592

Perillo L et al., 2021. Quantification of Some Heavy Metals in Hair of Dairy Cows Housed in Different Areas from Sicily as a Bioindicator of Environmental Exposure-A Preliminary Study Animals (Basel) Europe PubMed Central. 11(8):2268. DOI: 10.3390/ani11082268

Pilarczyk R, 2013.Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and HolsteinFriesian cows from organic farm. Environ. Monit. Assess. PMID: 23572404. 185(10): 8383–8392. DOI: 10.1007/s10661-013-3180-9

Stamatovic S and Milic D, 1968. Problems of air pollution in Yugoslavia. Proccedings of the First European Congress on the Influence of Air Pollution on Plants and Animals. Wageningen, Aprill 22 to 27, 255-257.

Sahiti H et al., 2023. Bioaccumulation and distribution of Pb, Ni, Zn and Fe in stinging nettle (Urtica dioica) tissues and heavy metal-contamination assessment in the industrial zone of smelter Ferronikeli (Drenas-Kosovo). J Environ Sci Health A Tox Hazard Subst Environ Eng. Taylor & Francis. 58(9):805-810. DOI: 10.1080/10934529.2023.2236535.

Singhi S.C et al., 2003. Acute iron poisoning: clinical picture, intensive care needs and outcome. Indian Pediatr. PubMed. 40(12):1177-1182. PMID: 14722368.

Šajn R et al., 2013 Heavy metal contamination of topsoil around a lead and zinc smelter in Mitrovicë, Kosovë. Journal of Geochemical Exploration. Elsevier, Volume 134, Pages 1-16134, 1-16. DOI:10.1016/j.gexplo.2013.06.018

Tomza-Marciniak A et al. 2012. Lead, Cadmium and Other Metals in Serum of Pet Dogs from an Urban Area of NW Poland Biol Trace Elem Res. PubMed.149:345–351. DOI:10.1007/s12011-012-9433-6.

Tahir I et al., 2023. A review of important heavy metals toxicity with special emphasis on nephrotoxicity and its management in cattle. Front Vet Sci. PubMed. 10:1149720. doi.org/10.3389/fvets.2023.1149720

Uugwanga, M.N., et al 2021. Heavy metal pollution index of surface and groundwater from around an abandoned mine site, Klein Aub. /doi.org/10.1016/j.pce.2021.103067

Wrzecinska M et al., 2021. Disorders of the Reproductive Health of Cattle as a Response to Exposure to Toxic Metals. Biology (Basel). PubMed. 10(9): 882. DOI: 10.3390/biology10090882.

Xing W,et al., 2020. Cadmium, copper, lead and zinc accumulation in wild plant species near a lead smelter. Ecotoxicol Environ Saf. 2020 Jul 15;198:110683. doi: 10.1016/j.ecoenv.2020.110683. Epub 2020 Apr 30. PMID: 32361499. DOI: 10.1016/j.ecoenv.2020.110683.

Zadnik T, 2010. Monitoring of lead in topsoil, forage, blood, liver, and kidneys in cows in a lead polluted area in Slovenia (1975-2002) and a case of lead poisoning in 1993. International Journal of Chemical Engineering. Volume 2010, Article ID 940206. 9 pages10, 1-9. DOI:10.1155/2010/940206