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Original Research Article

ASSESSMENT OF ENVIRONMENTAL POLLUTION WITH METALS IN SOME INDUSTRIAL REGIONS OF KOSOVO USING CHICKEN (*Gallus gallus domesticus*) BREAST FEATHERS

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Abstract: The aim of this study was to assess the presence of metals in three regions of Kosovo using chicken (*Gallus gallus domesticus*) breast feathers collected from the industrial regions of Mitrovica and Obiliq and the non-industrial region of Dragash. This study was carried out from September to November 2016, and feathers were collected from 90 individual domestic chickens housed as free range. The concentrations of metals in the chicken feathers were determined with atomic absorption spectrometry (AAS). The range of average measured concentrations of metals (μg g^{-1}) in examined regions were: Zn 109-131, Mn 6.17-31.30, Cu 22.1-27.2, Cr 5.09-19.0, Ni 12.3-15.8, Pb <0.0945-15.5, Cd 11.1-12.3 and As <0.099-7.44. The highest average levels of metals were determined in regions (μg g^{-1}): Dragash: Zn 131, Cu 27.2, Mn 31.3, Cr 19.0, Ni 15.8; Mitrovica As 7.44, Cd 12.3, Pb 15.5. High statistically significant differences ($p<0.001$) were found between the three regions for Pb, As, Mn, and Cr content. There were no significant differences ($p>0.05$) between the Mitrovica and Obiliq regions in terms of Zn content, Mitrovica and Dragash in terms of Cu or between the Obiliq and Dragash regions in terms of Cd content. These results should concern the environmental agencies in Kosovo and encourage them to take concrete steps by periodically checking these pollutants in these two industrial regions. Based on our results, we recommend that programmes for monitoring environmental pollution from metals could use chicken feathers as an important and valuable test material.

Key words: trace metals; AAS; chicken feathers; environmental pollution; Kosovo

Introduction

In recent years, the increase in industrial activities has had an impact on environmental pollution, which has shifted the equilibrium of the global ecosystem (1, 2). Among the pollutants that contribute to this problem are non-essential metals, such as Pb, Hg, Cd, and As (3).

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Non-essential metals are likely to enter a living organism through the food chain (4). These metals can cause various health problems in the organism by weakening the immune system, causing oxidative stress (5), effecting the blood biochemical parameters, reducing reproduction capabilities, reducing body weight, increasing heart diseases, and leading to the manifestation of different mutations and neoplasms (6, 7, 8, 9, 10).

Bioindication and biomonitoring are forms of monitoring of environmental pollution that help to identify sources of essential and non-

essential metals as well as their effects on the living world (11, 12). Using these methods, several authors have investigated various flora and fauna such as plants (13), insects (14), and fish (15, 16) that could serve as bioindicators (17). Birds have been recognized since the 1960s as potential bioindicators of environmental pollution (18). With the aim of measuring environmental pollution from metals and their effects on living organisms, researchers have used different materials taken from birds, such as blood, lungs, liver, musculature, and the gizzard (19, 20, 21, 22, 23). Fairly reliable results for the measurement of environmental contamination with metals have been obtained using avian feathers (24, 25, 26, 27). Specifically, metals present in the feathers reflect their concentration in the circulating blood during the 2-3 weeks of feather formation, which in turn represents both exposure and mobilization from internal tissues (28, 29). Thus, feathers serve as an archive of metal exposure during feather formation (30).

Avian feathers have been confirmed to be a suitable material for measuring effective contamination of metals in the environment (31). The concentration of metals in feathers has been shown to be much higher than that in other organs because feathers contain the protein keratin, which is a protein that metals tend to bind. Also, feathers are considered one of the best ways to eliminate metals from a chicken's body (32, 31, 33).

Concerning environmental pollution with metals, different researchers have analysed different samples in different regions of Kosovo, such as soils (34), earthworms (35), sparrows (36) and the results obtained from these surveys show multiple levels of non-essential metals compared to unpolluted areas. Based on this research, the most polluted region in terms of metals was the Mitrovica region and its surroundings, which is the most polluted region in all of Europe. This result was also confirmed by international agencies (37). Contributing significantly to this pollution in the region of Mitrovica was the Pb and Zn smelter, from which Pb was produced at approximately 730 tons per year at the end of 1990s (38). The Pb and Zn smelter was closed in August 2000 by the NATO military forces (39). However, numerous studies have been carried out in this region after the closure of the foundry, with the results showing alarming levels of metals (Pb, As, Cd, Zn, etc.) and their effects on animals and humans (36, 39). In the Obiliq region, there are two thermal power stations, as well as coal mines, and investigations revealed that in the grey matter that flows from these power plants, the metals were present in very high levels (40). Dragash is a region that does not contain any industrial factory; therefore, we used this region as a control.

Based on the above findings, we conducted a survey to assess the presence of metals in chicken feathers obtained from the following three different regions of Kosovo: Mitrovica, Obiliq and Dragash.

Materials and methods

Study area

The samples were collected from three study regions (areas), including Mitrovica, Obiliq and Dragash, as shown in Figure 1.

The Mitrovica region is located in the northern part of Kosovo, and it has the largest metallurgic and mining complex (Trepca) in Europe for the extraction of Pb, Cd and Zn. Many industrial plants existed in the complex, such as a Pb smelter, fertilizer production plant, refinery, battery factory, Zn electrolysis facility and a sulfuric acid plant. Significant amounts of metal pollutants have been released into the surrounding area, including populated residential areas, and these pollutants have been associated with human health risks (41).

Obiliqi is an industrial region that is located in the centre part of Kosovo. This region contains two thermal power stations, "Kosova A" and "Kosova B", as well as coal mines for coal extraction. Coal contains the metals Pb, Hg, Ni, Cd, As, and others (42), and the combustion from coal-based power plants is one of the most important processes contributing to pollution of the environment and groundwater because of the air ash distribution that contains many metals (43).

Dragash is a non-industrial region and is located in the southern part of Kosovo near Sharr Mountain. Geological analysis has shown that Sharr Mountain is very rich in metals, such as Fe, Cu, Mn, Cr, and Zn (44). These metals can enter living organisms through the food chain (4).

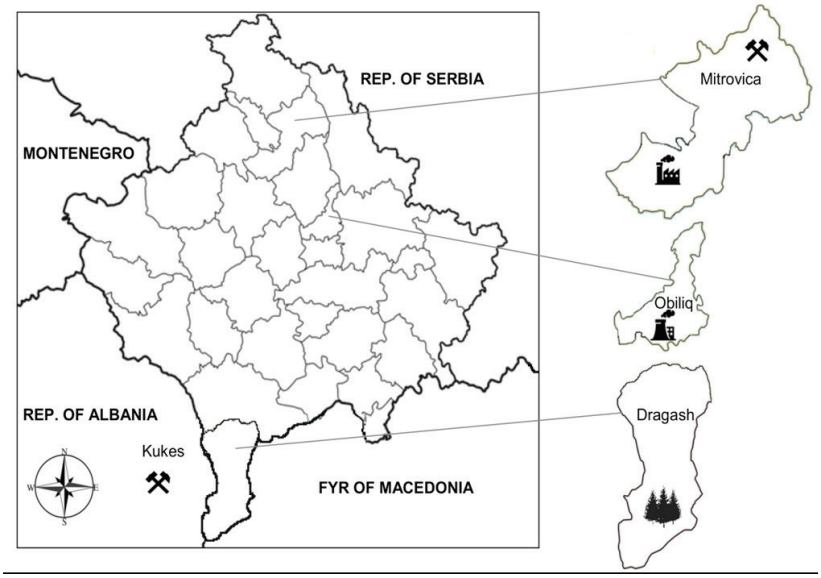

Figure 1: Map of Republic of Kosovo - location of Mitrovica, Obiliq and Dragash

Sampling plan and analytical procedures

The study was performed according to ethical guidelines of the Department of Veterinary Medicine, University of Pristina. In this research backyard chickens *Gallus gallus domesticus* were used, kept in these three regions. Breast feathers were collected by cutting them off with scissors at the skin level for the assessment of metal contamination from chicken which were grown in industrial regions of Mitrovica and Obiliq and the non-industrial region of Dragash. Three farms were sampled in each region and 10 chickens were selected from each farm. The number of chickens was 30 individuals from each region, a total of 90 domestic chickens. All of them were between 1-2 years old, mix breed, females and hatched on selected farms. All collected feathers were dark grey to black. The chickens were kept as free range (out roaming the day and housed at night), and they were fed mostly with maize grains from the same region along with feed they browsed when roaming freely on farm holding. The samples were sent to the laboratory for metal analysis at the Faculty of Agriculture and Veterinary, University of Pristine, Kosovo. Feathers were weighed, stored in plastic bags, labelled for later identification, and kept at –20 °C until they were dissected and analysed.

All feathers were washed three times with acetone followed by deionized water, and then the feathers were dried in an oven at 70 °C for 24 hours. Additionally, all laboratory dishes were cleaned with concentrated sulfuric acid, then washed with distilled water and dried in an oven. The feather samples (0.5 g) were digested with 70 % nitric acid (Sigma Aldrich, Merck, Darmstadt, Germany) and 30 % hydrogen peroxide (Sigma Aldrich, Merck, Darmstadt, Germany) at room temperature overnight. The next day, the samples were completely digested in a digestion unit DK heating digester (VELP Scientifica, Usmate (MB), Italy) at 150 °C for 4 hours until the solutions became clear (45, 46). After cooling, the solution volume was brought up to 50 mL using deionized water. Following digestion, the solutions were filtered through 0.45 µm acid-resistant filter paper Whatman (Sigma Aldrich, Merck, Darmstadt, Germany). The concentrations of metals in chicken breast feathers were measured with an atomic absorption spectrometer (AAS) (MSeries, Thermo, Cambridge, UK) using the flame method. Very low concentrations of Cd and Pb in extracts were determined using a graphite furnace atomic absorption spectrometry (GFAAS), whereas As was determined through hydride generation atomic absorption spectrometry (HGAAS). For quality assurance, internal reference materials (i.e., cow liver and spleen) were used for measuring metals. The recovery rates for the internal reference materials were between 78.5 – 107.3 %, with a low relative standard deviation (RSD) of 13 %. For all analysed metals, the limits of detection (LOD) (based on 3σ of the blank) and the limits of quantification (LOQ) (based on 9σ of the blank) given in Table 1 were calculated based on the DIN-32645 calculation procedure (47).

Table 1: Limits of detection and quantification (µg g-1) for analysed metals for chicken feathers samples at given wavelengths within AAS analysis

Element	Wavelength nm	Limit of detection [3σ]	Limit of quantification [9σ]
		µg g^{-1}	µg g^{-1}
As	193.7	0.033	0.099
Cd	228.8	0.021	0.063
Pb	217	0.0315	0.0945
Ni	232	0.105	0.315
Cr	357.9	0.186	0.558
Mn	279.5	0.063	0.189
Cu	324.8	0.108	0.324
Zn	213.9	0.036	0.108

Table 2: Average concentration value (M) ± standard error of mean (SEM) and concentration range of metals (µg g^{-1}) found in the chicken feathers and the differences in metal concentrations between the three regions of Mitrovica, Obiliq and Dragash

Elements	Mitrovica n 30	Obiliq n 30	Dragash n 30	P Value		
	1	2	3	1:2	1:3	2:3
As	7.44±0.33 (6.00-9.99)	1.50±0.01 (1.04-1.88)	<0.099#	0.000***	0.000***	0.000***
Cd	12.30±0.47 (10.10- 15.69)	11.70±0.12 (9.97-11.82)	11.10±0.12 (10.15-11.80)	0.024*	0.043*	0.904
Pb	15.50±0.45 (13.28-17.72)	2.50±0.13 (1.23-2.79)	<0.0945#	0.000***	0.000***	0.000***
Ni	14.30±0.55 (11.80-17.75)	12.30±0.38 (10.42-14.58)	15.80±0.58 (12.73-19.26)	0.003**	0.042*	0.000***
Cr	11.10±0.21 (9.43-12.20)	5.09±0.19 (4.10-6.21)	19.00±1.12 (14.02-27.14)	0.000***	0.000***	0.000***
Mn	15.20±0.53 (12.74-19.40)	6.17±0.20 (4.97-7.52)	31.30±1.47 (21.39-39.54)	0.000***	0.000***	0.000***
Cu	25.00±0.68 (20.73-28.75)	22.10±0.39 (19.86-23.90)	27.20±1.20 (19.60-32.20)	0.001**	0.072	0.001**
Zn	111.00±2.36 (97.70-120.75)	109.10±2.70 (99.58-128.90)	131.00±6.54 (102.00-172.38)	0.613	0.018*	0.001**

Note: *Significance level: p<0.05*, p<0.01**, p<.001***, # LOQ-limit of quantification*

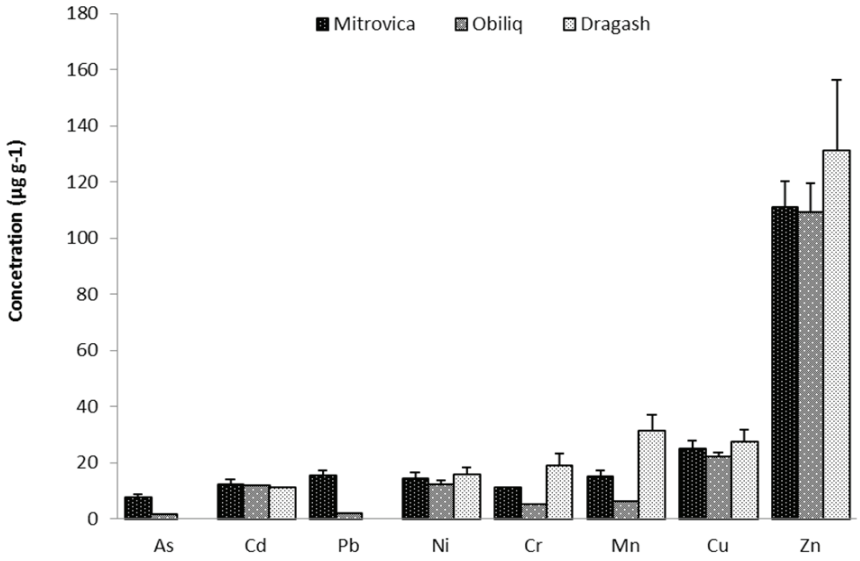

Figure 2: The average and standard deviation of As, Cd, Pb, Ni, Cr, Mn, Cu and Zn in breast chicken feather in the three regions of Kosovo (The sample size is 30 animals for each region presented in the figure)

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, USA) software, version 21. The t-test and analysis of variance (ANOVA) were used to assess the differences in metal concentrations between the three regions. The level of significance for the differences was set at $p<0.05$, $p<0.01$ and $p<0.001$.

Results and discussion

The descriptive statistics for the metal (Pb, As, Cd, Cr, Mn, Ni, Cu and Zn) concentrations in chicken feathers from the three regions (Mitrovica, Obiliq and Dragash) determined in this study are shown in Table 2. In a more comprehensive measurement of the quantity of elements in the chicken feathers, we observed the following alignment for almost all the regions: Zn > Cu > Mn > Ni > Cr > Cd > Pb > As. For better visualization of the differences, the metal concentrations in chicken feathers are presented in Figure 2.

In general, the results from our study showed that there was a difference in the metal concentration in chicken feathers between the three studied regions. Among the metals included in the study, higher significant differences in the concentration of metals between the three regions were observed for Pb, As, Cr, and Mn ($p<0.001$).

Concentration profiles and risk to the avian fauna

Lead

Significantly higher average Pb concentration was observed in the Mitrovica region (15.5 µg g^{-1}) compared with the Obiliq region (2.5 µg g^{-1}), whereas the concentration of Pb in the Dragash region was at a minimum level, below the LOQ value. This high variation in the Pb concentration shows the real differences in environmental pollution between the three regions. The occurrence of this variation between the regions can be explained due to the presence of the large metallurgic and mining complex (Trepca) in the Mitrovica region, which is also considered to be the main source of environmental pollution. High concentrations of Pb in chicken feathers have also been reported by other authors from different countries around the world, including South Korea (48) at 3.32 – 20.8 µg g^{-1}, China (46) at 3.95 – 13.8 µg g^{-1}, and Nigeria (49) at 0.64 – 1.62 µg g^{-1}. The study conducted by Salwa et al. (50) found that the concentration of Pb in poultry feathers was 0.869 µg g^{-1}. Although feathers are connected to the blood circulation, Pb accumulates in the keratin protein of the feathers (51). A high level of Pb was also found in human blood samples that were collected for research in the Mitrovica region (52). Indeed, the concentration of Pb in the blood of humans living in the Mitrovica region was shown to be at a relatively alarming level (3.8 µg dL^{-1}) (53).

Arsenic

The highest average As concentration was detected in the Mitrovica region (7.44 μg g^{-1}), followed by the Obiliq region (1.5 μg g^{-1}), and no measurable level was observed for the Dragash region. Industrial development, such as mines, is considered to be the major source of air pollution from As (54). Given that a mining complex is situated in the Mitrovica region, the presence of a higher concentration of As was expected for this region compared to the Obiliq and Dragash regions. Other authors have reported similar levels, including values from Belgium (55) at 0.88 – 23.35 μg g^{-1}, Pakistan (56) at 19 – 21.4 μg g^{-1}, and Malaysia (50) up to 0.472 μg g^{-1}. According to Stafilov et al. (57), the concentration of As in soil from the Mitrovica region was 55 μg g^{-1}.

Chromium

The highest average concentration of Cr was found in the Dragash region (19.0 μg g^{-1}), followed by Mitrovica (11.1 μg g^{-1}) and the Obiliq region (5.09 μg g^{-1}). The differences in the Cr concentration between the regions were found to be statistically significant ($p<0.001$). The significantly higher concentration of Cr in the chicken feathers from the region of Dragash could be related to the fact that this region is located near the city of Kukes in Albania, which is considered to be the region with the largest Cr mine in Europe (58, 59). Similar results for the Cr concentration in feathers have been reported by other studies in different countries around the world, including Malaysia (50) at 2.66 – 4.85 μg g^{-1} and Pakistan (56) at 19 – 21.1 μg g^{-1}.

Manganese

The results of this study showed that the Dragash region also had the highest average Mn concentration in chicken feathers (31.3 μg g^{-1}) compared to Mitrovica (15.2 μg g^{-1}) and Obiliq (6.17 μg g^{-1}). The Dragash region is very rich in minerals containing Mn (60). Similar concentrations have been found in the feathers of birds in other studies, including 7.21 – 65.98 μg g^{-1} (50) and 15.3 – 26.9 μg g^{-1} (31). The Mn concentration in the feathers could be related to the concentrations of ingested contaminants as well as to the contents of contaminants in the air (61).

Nickel

The average concentration of Ni in chicken feathers in the Mitrovica region was found to be 14.3 μg g^{-1}. The highest average concentration of Ni observed in the Dragash region was 15.8 μg g^{-1}, while the lowest concentration was found in the Obiliq region at 12.3 μg g^{-1}. High statistically significant differences were observed when the Obiliq and Dragash regions were compared ($p<0.001$). These differences were less dramatic when Mitrovica to Obiliq ($p<0.01$) and Mitrovica to the Dragash region ($p<0.05$) were compared. From the results of Honda et al. (62), it is known that Ni is related to the pigment of chicken feathers, which makes sense because most of the chickens used for our analysis had a black feather colour. The average level of the Ni concentration in feathers shows a wide range depending on the country in which the study was done. For example, a study conducted by Salwa et al. (50) found the average concentration of Ni to be 1.9 – 2.7 μg g^{-1}. In contrast, a higher Ni concentration was found in the study conducted by Malik and Zeb (31) in Pakistan, at 7.8 – 9.0 μg g^{-1}, whereas an extremely high concentration of Ni was observed in the study of Abdullah et al. (56) in Pakistan, at 41.6 – 84.8 μg g^{-1}.

Copper

Regarding the Cu concentration in the chicken feathers, average concentrations of 25.0 μg g^{-1} for Mitrovica, 22.1 μg g^{-1} for Obiliq and 27.2 μg g^{-1} for the Dragash region were determined. Statistically significant differences between regions Mitrovica and Obiliq as well between Obiliq and Dragash regions were found ($p<0.01$), while no difference was found between the Mitrovica and the Dragash regions ($p>0.05$). The Dragash region is located in the Sharr Mountain area, which contains the metals Cr and Cu (44). Our study results were comparable to study results obtained by other authors, including levels of 10.4 μg g^{-1} (48), 17.0 μg g^{-1} (63), and 6.60 μg g^{-1} (50). In the study conducted by Dauwe et al. (51) in Belgium, higher Cu concentration in poultry feathers of 69 – 88 μg g^{-1} were reported. In contrast, Malik and Zeb (31) in Pakistan found a lower amount of Cu in poultry feathers at 3.7 – 4 μg g^{-1}.

Cadmium

Cadmium is a non-essential metal for living organisms (64). In our study, the average concentrations of Cd in chicken feathers were as follows: 12.3 μg g^{-1} in Mitrovica, 11.7 μg g^{-1} in Obiliq and 11.1 μg g^{-1} in the Dragash region. Significant differences in Cd levels were determined between the regions of Mitrovica and Obiliq and of Mitrovica and Dragash ($p<0.05$). However, there were no significant difference between Obiliq and Dragash regions ($p>0.05$). The results regarding the presence of Cd in feathers are similar to results from researchers in other countries, such as Belgium (51) and Pakistan (56). Our results from the Mitrovica region were also comparable to the results of Zogaj and Düring (65), who confirmed the presence of Cd in various samples from soil, plants, etc.

Zinc

Zinc is an essential metal (66), and the average concentration of Zn in chicken feathers in the Mitrovica region was 111.0 μg g^{-1}. A similar concentration was also observed in the Obiliq region at 109.0 μg g^{-1}, while a substantially higher concentration was found in the Dragash region at 131.0 μg g^{-1}. The presence of Zn in the chicken feathers in the Dragash region was higher compared to the other regions, in which more non-essential metals (Pb, As, etc.) were present.

The average concentration of Zn was shown to be significantly different between the Obiliq and Dragash regions ($p<0.01$). Statistically significant differences were also found between the Mitrovica and Dragash regions ($p<0.05$), whereas the Zn concentration in chicken feathers did not differ between the Mitrovica and Obiliq regions ($p>0.05$). Comparable results were obtained in the study conducted by Salwa et al. (50) who studied the Zn concentration in chicken feathers in 2012 in Malesia (104.0 μg g^{-1}). The Zn concentration in the feathers of other birds in a study by Jungsoo and Tae-Hoe (48) in Korea, varied between 67.9 – 103 μg g^{-1}, while the study results presented by Malik and Zeb (31) from Pakistan, showed a concentration of Zn in the range of 133.8 – 155.2 μg g^{-1}. A considerably greater concentration of Zn in bird feathers was found in the results presented by Abdullah et al. (56) in Pakistan at 226 – 529 μg g^{-1}.

These results support the concept that non-essential metals can have high affinity for sulfhydryl groups and can bind to reduced cysteines in peptides and proteins (67, 68, 69, 70). The current findings also support the data obtained by other researchers related to environmental pollution with metals and their effects on living organisms in the regions of Mitrovica and Obiliq (40, 65, 71).

Conclusions

Our investigation detected high levels of metals in chicken feathers in three studied regions of Kosovo (Mitrovica, Obiliq and Dragash). The outcomes of this research clearly show that the region most polluted by metals was the Mitrovica region, with Pb as the primary metal followed by Cd and As. In the Mitrovica region, Zn was identified as the primary non-essential metal. After the Mitrovica region, the most polluted region was Obiliq, where the primary metal was Cd followed by Pb and As. In Obiliq, we also found higher concentrations of Zn than other non-essential metals. Dragash was the region with the lowest levels of non-essential metals, and among the essential metals, Zn was found at the highest concentrations followed by Mn, Cu, Cr and Ni. The most polluted region was Mitrovica, followed by Obiliq, and the least polluted region was Dragash.

Using avian feathers to determine the degree of environmental pollution is a reliable approach for biomonitoring, as shown by numerous researchers from different countries. Based on our results, monitoring of metals in the feathers of chickens can serve as a valuable method for the evaluation of the environmental pollution. Conducting similar surveys in the future could contribute towards the management of environmental pollution, which would promote the health of all living organisms as well as overall public health.

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OCENA ONESNAŽENOSTI OKOLJA S KOVINAMI NA OSNOVI PREISKAVE PRSNIH PERES PIŠČANCEV (*Gallus gallus domesticus*) V NEKATERIH INDUSTRIJSKIH PODROČJIH KOSOVA

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Povzetek: Namen raziskave je bil oceniti prisotnost kovin v treh področjih Kosova s pomočjo piščančjega (*Gallus gallus domesticus*) perja, vzorčenega v industrijskih področjih Mitrovica in Obiliq ter neindustrijski regiji Dragaš. Raziskava je bila izvedena od septembra do novembra 2016, perje pa je bilo odvzeto 90 piščancem v prosti dvoriščni reji. Koncentracije kovin v piščančjih peresih so bile določene z atomsko absorpcijsko spektrometrijo (AAS). Razpon povprečnih izmerjenih koncentracij kovin (µg/g perja) v preiskovanih območjih je bil: Zn 109-131, Mn 6,17-31,30, Cu 22,1-27,2, Cr 5,09-19,0, Ni 12,3-15,8, Pb <0,0945-15,5, Cd 11,1-12,3 in As <0,09-7,44. Najvišje povprečne koncentracije kovin (µg/g) so bile ugotovljene v področjih Dragaš: Zn 131, Cu 27,2, Mn 31,3, Cr 19,0, Ni 15,8 in Mitrovica: As 7,44, Cd 12,3, Pb 15,5. Statistično pomembne razlike ($p < 0,001$) med tremi področji so bile ugotovljene za vsebnost Pb, As, Mn in Cr. Statistično pomembnih razlik nismo ugotovili v vsebnosti Zn med Mitrovico in Obiliqom, v vsebnosti Cu med Mitrovico in Dragašem ter v vsebnosti Cd med Mitrovico in Dragašem. Rezultati raziskave bi morali biti zaskrbljujoči za okolijske agencije na Kosovu in bi jih morali spodbuditi, da sprejmejo konkretne ukrepe, predvsem občasno preverjanje omenjenih onesnaževal v teh industrijskih področjih. Na osnovi naših rezultatov priporočamo spremljanje onesnaženosti okolja s kovinami z uporabo piščančjega perja kot primernega in uporabnega preskusnega materiala.

Ključne besede: kovine v sledovih; AAS; piščančje perje; onesnaženost okolja; Kosovo