ASSESSMENT OF HEAVY METAL LEVELS IN SOIL, GRASS, AND DAIRY PRODUCTS, AND ANOVA ANALYSIS OF OMEGA-3 AND OMEGA-6 FATTY ACIDS IN MEAT CALVES IN KOSOVO

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ABSTRACT

The contemporary landscape, shaped by urbanization and rapid technological advancements, has undergone significant transformations. The discharge of hazardous substances stands out as a primary contributor to heavy metal contamination in our environment. A plethora of research underscores the detrimental impact of heavy metal pollution on ecosystems, posing threats to the survival of vital plant and animal populations. Moreover, heavy metals can potentially infiltrate our food supply, presenting the risk of long-term health consequences when integrated into the food chain. This experimental investigation sheds light on the accumulation of heavy metals in key elements of our ecosystem, including dairy products, soil, grass, and the omega-3 and omega-6 components in meat and dairy samples in Kosovo. Employing the inductively coupled plasma optical emission spectroscopy (ICP-OES) method for heavy metal analysis and gas chromatography (GS-FID) for omega-3 and omega-6 assessments, our study involves metals such as arsenic, cadmium, chromium, copper, iron, mercury, lead, and zinc. The findings suggest that dairy products exhibit an average level of contamination, possibly linked to pollution from coal power plants. However, this revelation does not absolve us from the need for sustained vigilance and ongoing attention to this critical environmental issue. Continued observation and proactive measures remain imperative to mitigate the potential consequences of heavy metal contamination in our ecosystem and safeguard both environmental and human well-being.

Keywords: contamination level, food products, urbanization, ICP OES, GS-FID techniques

INTRODUCTION

The significance of milk and its products in maintaining a well-rounded and nutritious diet is universally acknowledged. Renowned for its rich composition of essential fatty acids, proteins, lactose, vitamins, minerals, and an array of other nutrients, milk stands as a cornerstone of dietary diversity. Beyond its individual nutritional merits, milk serves as a vital component in the creation of various globally consumed dairy products such as cheese, yogurt, and butter. In essence, these dairy staples not only contribute to the palatability of meals but also play a crucial role in meeting diverse nutritional needs worldwide (Górska-Warsewicz H et al., 2019; Thorning TK et al., 2016). The amounts of heavy metals in dairy products depend on several factors, such as fed, the environment in which it was grown, the procedures used to prepare the dairy products, etc. In general, heavy metal pollutants may be present in greater concentrations in dairy products produced from animals that were reared in environments with high levels of heavy metal pollution or fed diets rich in heavy metals. (Aggarwal A, 2022). To lower the danger of exposure to heavy metals from dairy products, it is advisable to choose dairy products made from animals raised in locations with low levels of heavy metal pollution and fed diets low in heavy metals. Eating a balanced, varied diet that includes a variety of foods is also essential to lessen the risk of exposure to heavy metals. Considering the widespread consumption of milk, the potential for contamination to seamlessly infiltrate the human body is a cause for concern (P. Licata et al., 2004; Meshref et al., 2014). Metals like lead, cadmium, arsenic, and mercury have the capacity to accumulate in animal tissues and subsequently find their way into the human system through the intake of dairy products such as milk, cheese, and yogurt. The transfer of these metals from the food chain to our bodies underscores the importance of monitoring and ensuring the safety of dairy products, given their integral role in daily nutrition. Furthermore, this study entailed a comprehensive exploration of the levels of omega-3 and omega-6 fatty acids present in meat composition. Applying the Gas Chromatography with Flame Ionization Detection (GS-FID) technique, we meticulously examined these essential polyunsaturated fats. The assessment of these fatty acids is of paramount importance, offering invaluable insights into the nutritional composition of the foods we consume. A nuanced understanding of omega-3 and omega-6 levels significantly contributes to our grasp of the broader dietary impact, emphasizing its profound implications for human health and well-being. This analytical approach not only allows us to elucidate the intricate relationship between essential fatty acids and the overall quality of our food supply but also plays a pivotal role in addressing the pressing issue of heavy metal pollution. By integrating the study of fatty acid profiles with our investigation into heavy metal contamination, we strive to paint a holistic picture of the intricate dynamics that shape the nutritional and environmental facets of our food system.

MATERIAL AND METHOD

Samples collection and instrumentation

Dairy products

Nine samples were carefully obtained from home farms in the defined zones, including three samples of milk, three of cream, and three of cheese. Every sample went through a rigorous collection process, guaranteeing data reliability by doing three duplicates. The sampling sites were carefully selected from several districts in Kastriot, an area renowned for its environmental difficulties, primarily because of its proximity to the power plants of Kosova. The digestion of dairy products was conducted using the Microwave Oven BS EN13805, guaranteeing a comprehensive and uniform approach. We completed the examination of heavy metal contents in our samples using the ICP-OES technique (EPA 6010C method) simultaneously. ICP-OES model utilized for the analysis was the PerkinElmer Optima 8200. This method is known for its robustness and ability to provide rigorous results. In addition, the GC-FID instrument was used to evaluate the levels of omega-3 and omega-6 fatty acids in the samples. This provided important information for the overall nutritional evaluation of the gathered dairy products. This rigorous process highlights the necessity of acquiring precise and thorough understanding of the quality and safety of these dairy products, particularly in a location renowned for its industrial operations and possible sources of contamination.

Soil and grass samples

Systematic collection of soil and grass samples was conducted from grazing areas frequently utilized by cattle. The sample procedure entailed the retrieval of roughly 2 kg of soil per location utilizing a 30 cm depth probe. The soil samples were carefully packaged in labeled plastic containers and quickly transferred to the laboratory for subsequent examination. After a period of drying, the soil samples were crushed to facilitate the precise measurement of heavy metal concentrations. The grass samples, meticulously collected following the elimination of solid debris, were diligently stored in pristine plastic bags. After being gathered, around 0.5 grams of the grass samples underwent a thorough treatment procedure in a microwave system, employing a mixture of nitric acid and hydrogen peroxide. This stringent methodology guarantees the maintenance of sample integrity while simplifying the subsequent examination of heavy metal concentrations. The methodical gathering and analysis of these soil and grass samples are crucial stages in our endeavor to thoroughly evaluate the ecological consequences and possible levels of pollutants in regions where cattle feed.

Meat Sampling

Meat samples were rigorously chosen from calves aged 6 months and 1 year. Each piece, weighing around 100 grams, consisted of meat parts such as beefsteak, rump steak, round steak, lungs, and liver. These selected samples provide a thorough assessment of various cuts within this specific age group, providing vital information about their culinary/nutritional characteristics. The Gas Chromatography with Flame Ionization Detection (GC-FID) technique was utilized to examine fatty acids. This exact methodology efficiently segregates and measures different fatty acids in the samples, yielding comprehensive data on their composition and concentration. The samples underwent treatment with a combination of hexane, methoxide in methanol, and a centrifuge separation.

RESULTS AND DISCUSSIONS

Kosova A sampling site

Figure 1 shows the concentration of heavy metals in this sampling site A. The results are presented in mg kg^{-1}.
The results obtained from our analysis indicate significant variations in lead (Pb) concentrations among dairy samples. Yogurt displayed a concentration of 1.364 mg kg-1, cream had 0.0069 mg kg-1, and cheese showed a concentration of 0.071 mg kg-1. Zinc (Zn) concentrations ranged from 0.209 mg kg-1 in yogurt to 0.199 mg kg-1 in cheese, with cream having a higher concentration of 1.222 mg kg-1. Chromium (Cr) was present in yogurt (0.419 mg kg-1), cream (0.218 mg kg-1), and cheese (0.215 mg kg-1). Cadmium (Cd) concentrations were around 0.194 mg kg-1 in yogurt, 0.216 mg kg-1 in cream, and 0.269 mg kg-1 in cheese. Additionally, trace amounts of arsenic (As) were detected: 0.01 mg kg-1 in yogurt, 0.24 mg kg-1 in cream, and traces (0.011 mg kg-1) in cheese. Copper (Cu) and iron (Fe) were also present, with yogurt containing 0.034 mg kg-1 Cu, cream having 0.002 mg kg-1 Cu, and cheese with 0.004 mg kg-1 Cu. Iron concentrations were 3.38 mg kg-1 in yogurt, 4.51 mg kg-1 in cream, and 3.2 mg kg-1 in cheese. Mercury (Hg) concentrations in the dairy products were found to be below 0.001 mg kg 1.

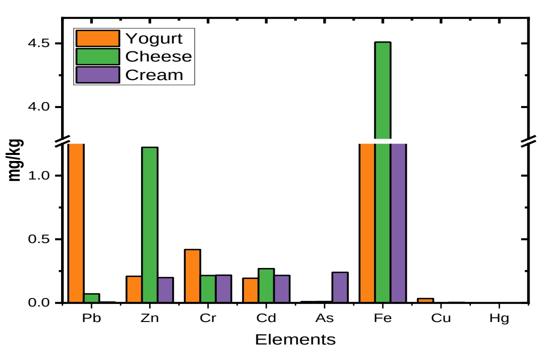

Figure 1. The concentration of elements in dairy products in Kosova A (mg kg ^{1}).

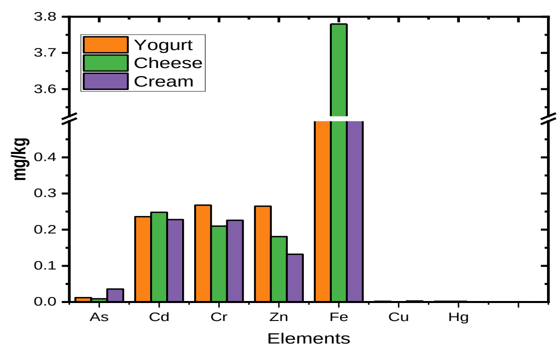

Figure 2. The concentration of elements in dairy products in Sampling site Q (mg kg ^{1}).

Q sampling site

Figure 2 shows the concentration of heavy metals in this sampling site and Figure 4 shows the sum of the elements in yogurt, cream, and cheese samples. The results are presented in mg kg $^{1.}$
The concentrations of heavy metals in dairy products are also presented at sampling site Q. According to the results, the Pb concentration varies from 0.093 mg kg^{-1} in yogurt, 0.0097 mg kg^{-1} in cream, and 0.0072 mg kg^{-1} in cheese. Regarding the concentration of Zn in the yogurt sample, we have a slightly higher concentration than in the Kosovo A sample, here it is 0.265 mg kg-1, while in cheese is 0.181 mg kg-1, and in cream also lower than in the previous sampling site, so it is 0.132 mg kg-1, where something like that is expected because near Kosovo A are also the coal ashes (Morina et al., 2012). Chromium is also recorded in our dairy samples. It ranges from 0.268 mg kg-1 in the yogurt sample, 0.226 mg kg-1 in the cream sample, and 0.21 mg kg-1 in the cheese one. The concentration of Cd is 0.236 mg kg-1 in yogurt, 0.228 mg kg-1 in cream, and in the cheese sample, it is 0.248 mg kg-1. As was also detected in our samples, 0.012 mg kg-1 in the yogurt sample, 0.036 mg kg-1 in the cream sample, and 0.009 mg kg-1 in the cheese sample. Similarly, copper (Cu) and iron (Fe) were identified in our samples. The yogurt sample exhibited a Cu concentration of 0.003 mg kg-1, the cream sample had 0.003 mg kg-1 Cu, and the cheese sample contained 0.001 mg kg-1 Cu. In terms of iron (Fe), the yogurt sample recorded a concentration of 2.53 mg kg-1, the

cream sample measured 2.99 mg kg-1, and the cheese sample registered 3.78 mg kg-1. It's worth noting that the concentration of mercury (Hg) in our dairy products remains below 0.002 mg kg 1.

Sampling point H

The following figures show the study area (H Sampling point) of our samples. Figure 3 shows the concentration of heavy metals in this sampling site and Figure 4 shows the sum of the elements in yogurt, cream, and cheese samples. The results are presented in mg kg 1.

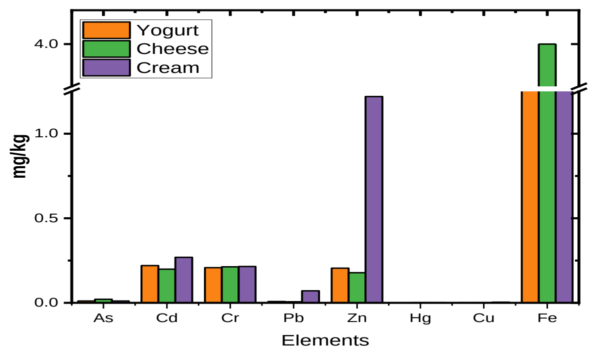

Figure 3. The concentration of elements in dairy products in Q sampling point (mg kg 1).

The arsenic levels exhibited remarkable consistency among the three dairy products, with both yogurt and cheese showcasing an arsenic concentration of 0.011 mg kg-1, while cream displayed a slightly higher concentration of 0.021 mg kg-1. Despite this slight variance, these levels remain notably low and fall within acceptable limits for this element. Cadmium concentrations, on the other hand, displayed variability across the dairy products. Cheese recorded the highest concentration at 0.269 mg kg-1, followed by yogurt at 0.22 mg kg-1, and cream at 0.199 mg kg-1. Considering cadmium's classification as a toxic heavy metal based on health data (Goyer, R. A. et al., 2010), monitoring its presence in food products becomes imperative to ensure adherence to established safety thresholds. The chromium levels within dairy products exhibited homogeneity, ranging from 0.208 mg kg-1 in yogurt to 0.215 mg kg-1 in cheese, with cream registering 0.213 mg kg-1. These concentrations, falling within the low range, align with the essential nature of chromium, a trace element vital for bodily functions (Vincent, J. B et al., 2019). Lead concentrations in dairy products demonstrated significant variation, with cheese presenting the highest level at 0.071 mg kg-1, followed by cream at 0.0066 mg kg-1, and yogurt at 0.0077 mg kg-1. It is imperative to emphasize that, despite these relatively low levels, efforts should be made to minimize its presence in food products. Zinc levels-maintained consistency across dairy products, ranging from 0.178 mg kg-1 in cream to 0.205 mg kg-1 in yogurt. Zinc, an essential nutrient found in various food sources, underscores the nutritional balance of these dairy products (Hess SY et al., 2009). Mercury levels remained uniformly low across all dairy products, with concentrations consistently below 0.001 mg kg 1. This favorable outcome aligns with the imperative to prevent the presence of mercury, a toxic metal, in food products. Copper concentrations in cheese were recorded at 0.004 mg kg-1, followed by cream and yogurt, both at 0.001 mg kg-1. While copper is an essential element, its potential harm at excessive levels underscores the importance of prudent monitoring (Turnlund, J. R. et al., 1998). If we examine the results obtained from the figures provided above (Figures 1-6), a consistent trend emerges where the highest concentrations of heavy metals are observed in the samples collected around Kosovo A. This outcome is not surprising, considering that this sampling point includes an ash dump, which presents a heightened risk of pollution. The existence of higher heavy metal concentrations close to the ash dump fits predictions and raises the possibility that the concentrations and pollution sources are related. Similar results are also found in various papers (Kabala, C., & Singh, B. R. 2001; Zhuang, P et al., 2009; Jusufi et al., 2016; Jusufi et al., 2017). In our endeavor to monitor metal pollution, we broadened our analysis to encompass soil and grass samples sourced from the grazing areas of the nourished cows. The results highlight a higher concentration of metals in the grass samples compared to those identified in the dairy products. The following figure (Figure 4) shows the presence of the results recorded in grass samples in three sampling sites analyzed, while Figure 5, the results presented in the soil samples. The data is in mg/kg.

The heavy metal analysis of grass and soil samples from three distinct sites (Kosova A, H, and Q) reveals notable variations in metal concentrations. Grass samples show differing levels of lead, copper, nickel, and zinc across the sites, indicating spatial heterogeneity in metal accumulation. In soil samples, lead, copper, nickel, and zinc concentrations exhibit similar variability, with specific sites demonstrating higher levels. If we compare the results of soil with the Dutch Standards, we see that in all cases the maximum concentration of metals is lower than this standard for soil samples, except that in some cases, we have a higher concentration of nickel and copper, but this concentration in soil samples is not consistent in dairy products measured.

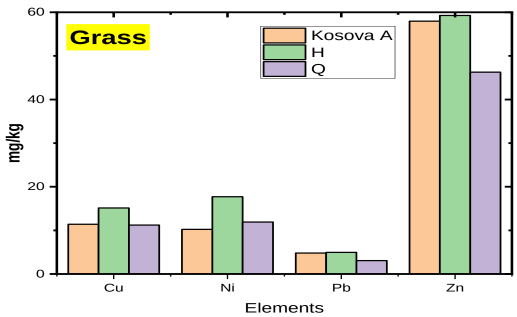

Figure 4. The concentration of elements in grass in the sampling sites (mg/kg).

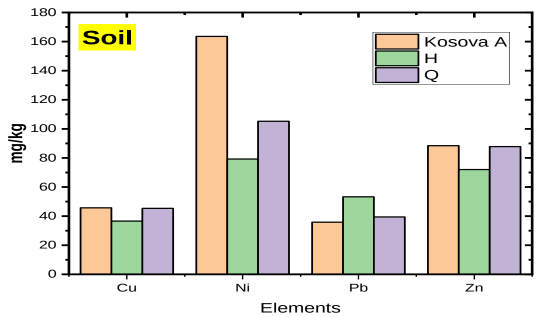

Figure 5. The concentration of elements in soil in the sampling sites (mg/kg).

Data on heavy metal concentrations (Cu, Ni, Pb, Zn) in grass and soil samples from three locations (Kosova A, H, Q) were subjected to PCA. The PCA results, including principal components and explained variance ratios, were obtained using the scikit-learn library in Python. The PCA results (Table 1) indicate that PC1 is the predominant source of variation, capturing 96.03% of the total variance. The loading values of PC1 suggest a strong influence of Cu, Ni, and Pb concentrations, particularly in grass samples. PC2, although contributing a smaller proportion (3.33%), highlights additional nuances in the dataset, with Zn concentrations playing a more prominent role.

Table 1. Principal Components and Loading Values.

	PC1	**PC2**
Cu_grass	−58.49	6.6
Cu_soil	−6.28	0.08
Ni_grass	−58.06	3.76
Ni_soil	131.1	18.22
Pb_grass	−71.65	12.93
Pb_soil	−10.65	−16.61
Zn_grass	12.06	−10.30
Zn_soil	61.97	−14.69

The dominance of PC1 suggests a commonality in the factors influencing heavy metal concentrations in both grass and soil samples. The strong loading values for Cu, Ni, and Pb in PC1 imply a shared source or environmental process contributing to their variations. PC2, while less influential, provides additional information, potentially indicating secondary sources or localized influences, particularly in Zn concentrations. The PCA results underscore the utility of this analytical approach in identifying key patterns in heavy metal concentrations. The strong explanatory power of PC1 highlights the importance of Cu, Ni, and Pb in driving variations across locations, while PC2 adds further nuance to the understanding of the dataset. These findings contribute valuable insights for future studies aimed at elucidating the sources and dynamics of heavy metal contamination in environmental samples. This interlinkage of the heavy metal's concentration in soil and grass samples is clearly seen from dendogram analysis presented in Figure 6.

This indicates that environmental factors specific to each location play a crucial role in shaping the heavy metal concentration profiles in both grass and soil. This analysis serves as a powerful tool for unraveling intricate relationships within environmental datasets. The clustering patterns and dissimilarity heights offer insights into the factors influencing heavy metal concentrations, paving the way for informed environmental management. The correlation analysis of heavy metal concentrations in grass and soil samples as presented in Figure 7 illuminates intriguing connections between different elements.

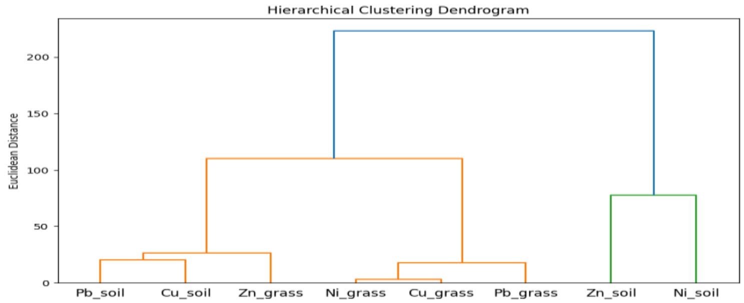

Figure 6. Dendogram analysis of heavy metals in soil and grass samples.

Strong positive correlations, particularly in copper, suggest shared environmental influences. Moderate associations in nickel and lead hint at varying contributing factors, while positive links in zinc concentrations underline interconnected dynamics. These findings emphasize the importance of considering multiple metals concurrently in environmental assessments, offering valuable insights for targeted monitoring and management strategies.

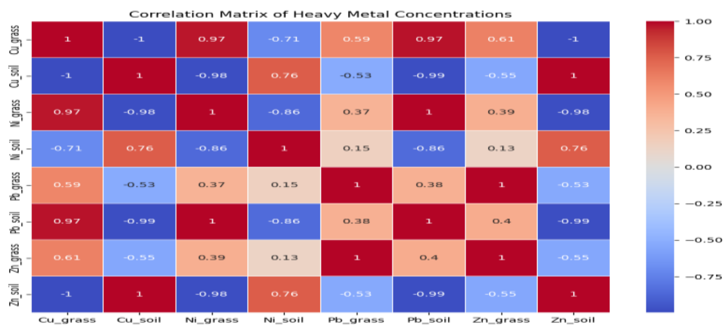

Figure 7. Correlation (heatmap) between the heavy metal concentrations in soil/grass samples.

This investigation delves into the critical examination of omega-3 and omega-6 fatty acid levels within meat samples, pivotal components essential for human well-being. Beyond their integral role in fundamental physiological processes such as brain development, immune system modulation, and cardiovascular health, maintaining a balanced dietary ratio of these fatty acids is paramount. The study, inspired by the works of DiNicolantonio JJ (2021) and Dimitra Karageorgou (2023), aims to shed light on the composition of these essential fatty acids in meat. Emphasizing the significance of informed dietary choices, the research underscores the importance of achieving an optimal balance to promote overall health and mitigate potential risks associated with fatty acid imbalances, including chronic illnesses and inflammation (Poli et al. 2023). A total of four calves were used as sampling points for the omega 3 and omega 6 samples: two Limousin calves aged 5 and 12 months, and two Simmental calves with the same age range. The following table (Table 2) shows the summary of the results of omega 3 and omega 6 in different parts of the body of calves' meat.

Table 2. The presence of omega 3 and omega 6 in meat samples.

	Omega 3	**Omega 6**	**Omega 6/3**
1 Lungs	7.39	19.13	2.59
1 Round steak	6.63	28.99	4.37
1 Liver	2.71	8.05	2.97
1 Rump steak	1.97	14.69	7.46
1 Beef Steak	2.98	15.89	5.33
2 Round Steak	3.35	29.93	8.93
2 Liver	0.99	3.11	3.14
2 Lungs	0	3.48	-
2 Rump steak	3.4	14.79	4.35
2 Beef Steak	0.87	3.92	4.51

The results present a comprehensive overview of omega-3 and omega-6 fatty acid levels, along with their respective ratios, in various meat samples. Notably, lungs and round steak from the first set (Samples 1) exhibit higher omega-3 concentrations (7.39 and 6.63, respectively), while liver and rump steak in the same set show relatively lower values. The omega-6 content is particularly pronounced in round steak (28.99) and rump steak (14.69), leading to higher omega-6/3 ratios (4.37 and 7.46). In the second set (Samples 2), round steak stands out with both elevated omega-6 levels (29.93) and a notably high omega-6/3 ratio (8.93). The variations in omega-3 and omega-6 levels across these samples highlight the importance of assessing both individual fatty acid concentrations and their ratios for a comprehensive understanding of the nutritional composition in different cuts of meat.

Anova test analysis

The statistical analysis of heavy metal concentrations between soil and grass samples was carried out using one-way ANOVA. The results show significant differences in the concentrations of most heavy metals between the two sample types, with p-values below 0.05, indicating meaningful variations. For aluminum, the concentration in soil (Kosova A: 31,607 mg/kg, H: 17,839 mg/kg, Q: 19,234 mg/kg) was significantly higher compared to grass (Kosova A: 517.2 mg/kg, H: 1,639 mg/kg, Q: 1,604 mg/kg), with an F-value of 24.29 and a p-value of 0.0079. This suggests a marked difference in aluminum retention between the two mediums. Similarly**,** bismuth concentrations exhibited a substantial difference, with soil values ranging from 0.75 to 1.73 mg/kg and grass values between 6.22 and 7.61 mg/kg. The ANOVA result showed an F-value of 122.63 and a highly significant p-value of 0.00038, indicating a pronounced discrepancy in bismuth accumulation. For copper**,** the soil concentrations (Kosova A: 45.73 mg/kg, H: 36.6 mg/kg, Q: 45.36 mg/kg) were again notably higher than those in grass (Kosova A: 11.41 mg/kg, H: 15.14 mg/kg, Q: 11.22 mg/kg), yielding an F-value of 85.32 and a p-value of 0.00076. This significant difference highlights the variation in copper retention between soil and grass. On the other hand iron as well showed also significant differences, with soil concentrations (Kosova A: 45,243 mg/kg, H: 31,866 mg/kg, Q: 33,725 mg/kg) being much higher than those in grass (Kosova A: 630.2 mg/kg, H: 1,776 mg/kg, Q: 1,567 mg/kg). The F-value was 71.98, with a p-value of 0.0011, indicating a strong disparity in iron accumulation. The nickel content was also significantly different between soil and grass, with F = 16.85 and p = 0.0148. Soil values ranged from 79.27 to 163.55 mg/kg, whereas grass samples contained much lower concentrations (Kosova A: 10.23 mg/kg, H: 17.72 mg/kg, Q: 11.91 mg/kg). Lead concentrations followed a similar pattern, with soil concentrations (Kosova A: 35.82 mg/kg, H: 53.32 mg/kg, Q: 39.47 mg/kg) being higher than those in grass (Kosova A: 4.81 mg/kg, H: 4.95 mg/kg, Q: 3.07 mg/kg), showing an F-value of 51.76 and a p-value of 0.00198, indicative of significant variations.Lastly**,** zinc concentrations in soil (Kosova A: 88.42 mg/kg, H: 72.05 mg/kg, Q: 87.82 mg/kg) were higher compared to grass (Kosova A: 57.97 mg/kg, H: 59.27 mg/kg, Q: 46.27 mg/kg), with an F-value of 17.43 and a p-value of 0.0140, confirming a statistically significant difference in zinc content between the two sample types. The significant p-values obtained for most heavy metals underscore the substantial differences in metal accumulation between soil and grass, likely reflecting differences in environmental exposure, absorption capacity, and mobility of these elements in different matrices. The results highlight the varying behavior of heavy metals in ecosystems, with implications for environmental monitoring and management.

CONCLUSION

- Dairy products might contain heavy metals such as lead, cadmium, arsenic, and mercury, which can be attributed to environmental pollution and contamination in animal feed. The complex interplay of several factors that contribute to heightened levels of heavy metals emphasizes the need of obtaining dairy products from locations with little pollution and feeding animals diets that are low in heavy metals. Overintake of heavy metals has significant health hazards, affecting the central nervous system, kidneys, and liver. In order to reduce these hazards, it is recommended that consumers choose dairy products from animals that are grown in regulated surroundings and are fed diets that are low in heavy metals. In addition, advocating for a varied and well-rounded diet that includes a wide range of food sources can help reduce the risk of heavy metal exposure from a single source.
- When examining the omega-3 and omega-6 levels in meat samples, it is clear that lung and round steak have the greatest quantities of omega-3, whereas liver samples often have lower levels. In contrast, the quantities of omega-6 vary considerably, with round steak exhibiting particularly elevated concentrations. The round steak has a notably high omega-6/omega-3 ratio, which indicates an imbalance between these fatty acids. Given these discoveries, it is advisable for consumers to make well-informed decisions about

their diet by choosing meat products that have a desirable omega-6/omega-3 ratio. These observations emphasize the need of taking into account the heavy metal and fatty acid compositions in different dietary sources, in order to enhance general health and reduce potential health hazards linked to imbalances.

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