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Assessment of Lead and Zinc Contamination in Commercial Sausages with Different Meat Content in Iran

Saeid Vahedi [a], Zohre Farahmandkia [b], Hassan Hassanzadazar [c], Fatemeh Masaebi [b], Mohammad Reza Mehrasbi [b] *

a. Social Determinants of Health Research Center, Health and Metabolic Diseases Research Institute, Zanjan University of Medical Sciences, Zanjan, Iran.
b. Health and Environment Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
c. Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran.

***Corresponding author:** Department of Environmental Health, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran. Postal Code: 4515786349. E-mail: zmehr@zums.ac.ir

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ABSTRACT

Background: Lead and zinc are heavy metals of concern due to their potential adverse health effects. Human exposure through the consumption of contaminated food products has increasingly raised public health concerns. This study, conducted in 2025, aimed to assess the concentrations of lead and zinc in various types of sausages available in the Iranian market, considering different commercial brands and meat contents.
Methods: A total of 99 sausage samples were collected from retail outlets across Iran, representing 11 popular brands with varying meat percentages. Lead and zinc concentrations were determined using acid digestion followed by Plasma-Mass Spectrometry (ICP-MS). The limits of detection (LOD) and quantification (LOQ) values were 0.07 mg/kg and 0.24 mg/kg for lead, and 0.0008 mg/kg and 0.0027 mg/kg for zinc, respectively. One-way analysis of variance (ANOVA) was used to assess significant differences in mean heavy metal concentrations among brands and meat content categories.
Results: The mean concentrations of lead and zinc in the analyzed samples were 28.39 ± 19.00 µg/kg and 11.69 ± 5.04 mg/kg, respectively. Lead concentrations ranged from 0.10 µg/kg to 81.85 µg/kg, while zinc concentrations varied from 0.01 mg/kg to 25.11 mg/kg. Zinc levels increased significantly in sausages containing 80% meat or more, whereas lead levels showed no significant variation with meat percentage ($p > 0.05$).
Conclusion: According to Codex Alimentarius (CXS 193-1995), all analyzed samples contained lead and zinc levels below the maximum permissible limits (100 µg/kg and 50 mg/kg, respectively). These findings provide useful information on the safety and quality of sausage products in the Iranian market and may contribute to improved food safety monitoring and public health protection.

1. Introduction

Food safety is one of the most important factors affecting human health (Borchers et al., 2010). Unfortunately, millions of people worldwide fall ill and hundreds of thousands die every year due to the consumption of unsafe food (Fung et al., 2018). Exporting safe food products can also contribute to a country's economic growth (Golovin et al., 2020). With improvements in living standards across different societies, enhancing food safety has become one of the key demands of modern consumers (Friel & Ford, 2015). Today, consumers are increasingly interested in purchasing a variety of

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products derived from the same food source, and meat products such as sausages, hot dogs, and hamburgers are no exception (Nam et al., 2010). Their pleasant taste, ease of preparation, and lower cost compared to fresh meat have contributed to a growing trend in their consumption (Leroy & Degreef, 2015). Toxic heavy metals, including lead, mercury, arsenic, chromium, and nickel are characterized by high density and toxic properties, and they can accumulate in body tissues (Balali-Mood et al., 2021; Farahmandkia et al., 2023; Farahmandkia et al., 2025). Exposure to these metals can have serious effects on human health, including neurological problems, behavioral disorders, kidney damage, reduced intelligence in children, and increased blood pressure in adults (Jaishankar et al., 2014). Therefore, research on the removal or reduction of heavy metals from food is essential for maintaining public health. The meat used in the production of processed meat products may contain certain levels of heavy metals (Hwang et al., 2011). In addition, other ingredients such as salt, spices, water, oil, and milk powder that are added during production may also introduce heavy metals into the final product. Heavy metal contamination in food matrices is a complex issue originating from diverse pathways. While contamination is frequently attributed to environmental reservoirs, such as contaminated soil and irrigation water, mounting evidence highlights that food processing operations constitute a significant, and often underestimated, source of metal entry into the final product (Alloway, 2012). This processing-related ingress can occur through the introduction of contaminated ingredients-including salt, spices, water, oils, and milk powder-added during formulation. Furthermore, specific packaging and processing technologies introduce distinct risks. For instance, elevated concentrations of tin in canned commodities frequently indicate intense migration of metals from the container lining, potentially introducing associated toxic elements such as cadmium or lead. Moreover, the industrial manufacturing of heavily processed meats involves extensive and prolonged contact with metallic machinery, presenting an additional route for elemental transfer (Olmedo et al., 2024). For specialists in the food industry, attention is directed toward those elements utilized in high volumes during production activities that subsequently accumulate in the environment, posing a significant hazard due to their inherent biological activity and toxicological profiles. In this context, cadmium, lead, zinc, and copper are of primary concern, as they exhibit pronounced toxicological properties even at the lowest exposure thresholds (Fedoseeva et al., 2023). Compounding these issues, metals can also leach from distribution infrastructure, such as piping and storage tanks, directly into processing water, leading to subsequent transfer into the food product (WHO, 2011). Thus, measuring heavy metal concentrations in meat products is necessary to ensure food safety and protect consumer health (Islam et al., 2022). Lead is a heavy and toxic metal recognized as a hazardous substance due to its specific characteristics, including high density and its ability to accumulate in living tissues (Kumar et al., 2020). The health effects of lead include damage to the nervous and digestive systems-manifesting as abdominal pain, nausea, vomiting, diarrhea, and liver damage-as well as kidney damage, cardiovascular problems such as high blood pressure, and an increased risk of stroke and heart attack. It can also affect the reproductive systems of both men and women, as well as the skin and immune system (M. S. Collin et al., 2022). Zinc plays a role in many biochemical and physiological processes in the body and acts as a powerful antioxidant (Parveen et al., 2017). Zinc plays an important role in strengthening the immune system, promoting wound healing, influencing taste and smell, and participating in the metabolism of carbohydrates, fats, and proteins. However, excessive zinc intake can lead to side effects such as nausea, vomiting, diarrhea, and digestive disorders, and it may interfere with the absorption of other minerals such as copper (Saghaleini et al., 2018). Heavy metals can enter the environment from both natural and anthropogenic sources. Natural sources include geological processes, volcanic activity, and rock erosion, while anthropogenic sources include industrial activities, agriculture, and the use of products containing heavy metals (Lafta et al., 2024). According to the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995), the maximum permissible levels for lead and zinc in meat products are 100 µg/kg and 50 mg/kg, respectively (Codex Alimentarius Commission, 1995). Effective management and control of heavy metals through appropriate measures can help protect human health and the environment (Ahmad et al., 2021). The primary objectives of this investigation were twofold. Firstly, this study aimed to quantitatively assess and compare the quality profiles of commercially available sausage products sourced from various manufacturers. Secondly, the research sought to ascertain the degree of heavy metal contamination in these products by rigorously benchmarking their measured concentrations against established national Iranian and relevant international food safety standards. Furthermore, recognizing the existing paucity of precise epidemiological data regarding the initial contamination load of meat and ancillary raw materials utilized in sausage processing, this study was designed to contribute foundational knowledge. Specifically, an ancillary objective was to establish a preliminary contamination baseline by analyzing the heavy metal content across sausage formulations containing disparate percentages of meat, thereby offering insight into the initial contamination contribution originating from primary ingredients.

2. Materials and Methods

Nitric acid (HNO_3, 68%; Ariyachem Co., Iran) and a Borna hot plate heater (Teb Azma Co., Iran) were utilized for sample digestion. The concentrations of heavy metals were quantified using an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, USA). The instrument was operated under the following conditions: RF power, 1200 W; auxiliary gas flow, 1-2 L/min; solution uptake rate, 1 mL/min; and mass range, 7-238 amu. The instrument operates at a unit mass resolution of approximately 1 amu. An electron multiplier (EM) detector was employed for signal detection. The dynamic range of the instrument spanned 0.001 µg/kg to 1000 ppm. Argon plasma gas flow was maintained at 17 L/min, abundance sensitivity ranged from 10^6 to 10^8, and the scanning rate was 3000 amu/s.

2.1 Sampling Method

In this study, 99 sausage samples representing 11 popular brands with varying meat percentages were randomly collected from retail outlets across Iran. For each sample, portions were taken from the initial, middle, and final sections of the product, homogenized, and accurately weighed using an analytical balance in the laboratory. The prepared samples were then packaged, labeled, and stored at -18 °C until further analysis. The required sample size was calculated using Daniel's formula: $N = Z^2 \times S^2 / D^2$

2.2 Digestion Method

Sample digestion was performed following the general procedure described in U.S. EPA Method 3050B (US EPA, 1996), with minor adaptations for processed meat matrices. Approximately 2.0 g of each homogenized sausage sample was weighed into an acid-washed Falcon tube. To each tube, 5 mL of concentrated nitric acid (HNO_3) was added, and the samples were left to pre-digest for 24 hours at room temperature to ensure adequate acid penetration and preliminary oxidation of organic matter. After the pre-digestion period, the Falcon tubes were placed in a beaker filled with distilled water, forming a controlled water bath system, and then heated on a hot plate at approximately 95 °C for 30 minutes to achieve complete dissolution of the sample matrix and release of trace elements (Mehrasebi et al., 2023). The digested solution was subsequently filtered through Whatman No. 42 filter paper to remove any undigested residues. The clear filtrate was diluted to a final volume of 15 mL with distilled water, transferred into acid-washed polyethylene vials, and stored at 4 °C before instrumental analysis. Quantitative determination of heavy metals was performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in accordance with the quality control recommendations outlined in EPA Method 3050B.

For the analysis of the samples, an Agilent 7900 ICP-MS equipped with an EM detector was used. The instrument was fitted with an autosampler and operated with high-purity argon gas (99.9%) as the carrier gas. The Dbi of the instrument was 1 mL, and its mass range was 260 amu. After obtaining the instrumental data, the dilution factor was calculated by dividing the final volume by the sample weight using the following formula:

$$Dilution\ factor = \frac{V(cc)}{m(gr)}$$

2.3 Method Validation and Quality Control

Instrumental calibration for Inductively Coupled ICP-MS was established using a series of multi-element standard solutions (Merck, Darmstadt, Germany) prepared in the appropriate acid matrix. Linearity was confirmed across the anticipated concentration range, with all target analytes demonstrating correlation coefficients (R^2) > 0.999 (R^2 = 1.0 for Pb and R^2 = 0.999 for Zn). Instrument performance was monitored by analyzing Continuing Calibration Verification (CCV) standards after every tenth sample injection.

To evaluate the accuracy and precision of the digestion and analytical procedure for the sausage matrix, quality control parameters were assessed using method blanks, duplicate analyses, and matrix spike recovery tests conducted with each analytical batch.

The LOD and LOQ were determined based on the calibration curve characteristics. Specifically, the LOD was calculated as 3times the ratio of the standard deviation of the blank signal (SD) to the slope of the calibration curve (S) (LOD = 3.3 × SD/S), and the LOQ was calculated as 10times this ratio (LOQ = 10 × SD/S). The established performance metrics for the target analytes were:

Lead (Pb): LOD = 0.07 mg/kg and LOQ = 0.24 mg/kg.

Zinc (Zn): LOD = 0.0008 mg/kg and LOQ = 0.0027 mg/kg.

Precision was assessed via the relative standard deviation (%RSD) of replicate measurements, which consistently remained below 5 %. Accuracy was confirmed through matrix spike recoveries, yielding values of 105-107% for lead and 91-95% for zinc (Belouafa et al., 2017). These recovery ranges fall within the acceptable limits (85 %–115 %) stipulated by EPA Method 3050B quality control guidelines (US EPA, 1996). Furthermore, to mitigate contamination risks, all acid-washed glassware and plasticware were subjected to a final soak in in 10 % HNO_3 for 24 hours before being rinsed with deionized water before sample handling.

2.4 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics® version 25 (IBM Corp, Armonk, NY, USA). Descriptive statistics, including the mean, median, standard deviation (SD), and interquartile range (IQR), were calculated to summarize the distribution of the data. The Kolmogorov-Smirnov test was utilized to evaluate the normality of the data distribution. For comparing metal concentrations across different sample groups, the ANOVA was employed. Statistical significance was established at a threshold of p < 0.05. Where significant differences were identified by the ANOVA, Tukey's Honestly Significant Difference (HSD) post hoc test was subsequently applied to determine specific pair-wise mean differences among the sausage sample groups.

3. Results and Discussion

The mean concentrations of lead and zinc in all samples were 39.28 ± 19.00 µg/kg and 11.69 ± 5.04 mg/kg, respectively, with the highest concentrations of lead and zinc being 81.85 µg/kg and 25.11 mg/kg, respectively. According to Codex Alimentarius standard (CXS 193-1995) and the Iranian national tolerance standards, the maximum permissible levels for lead and zinc are 100 mg/kg and 50 mg/kg, respectively (Codex Alimentarius Commission, 1995; Iran National Standards Organization, 2021). Comparison with these standards shows that the concentrations of both metals in all samples are below the maximum permissible limits. In this study, the Kolmogorov-Smirnov test indicated that the data followed a normal distribution.

The mean concentrations of lead and zinc across different brands and meat percentages are presented in Tables 1-5 and Figures 1-2. Among the brands, samples from Brand A and Brand C had the highest and lowest lead concentrations, with means of 52.11 ± 16.95 µg/kg and 11.47 ± 5.07 µg/kg, respectively. For zinc, samples from Brand G and Brand B had the highest and lowest concentrations, with means of 16.79 ± 4.77 mg/kg and 7.83 ± 2.63 mg/kg, respectively.

Table 1. Descriptive statistics of lead based on sausage brands (µg/kg)

Brand	Mean ± SD	Median (IQR)	Min	Max
A	52.11 ± 16.95 [c]	51.96 (29.80)	28.20	77.35
B	30.04 ± 11.29 [abc]	28.47 (19.08)	15.60	49.50
C	11.47 ± 5.07 [a]	12.65 (6.13)	1.19	17.86
D	12.73 ± 8.12 [ab]	11.84 (14.28)	0.10	24.71
E	18.11 ± 11.83 [ab]	17.39 (21.39)	0.10	36.00
F	28.15 ± 17.31 [abc]	22.62 (32.03)	9.56	57.49
G	25.57 ± 15.77 [ab]	22.40 (21.86)	2.08	55.40
H	35.48 ± 17.07 [abc]	40.67 (23.88)	0.10	55.17
I	34.35 ± 15.16 [abc]	33.37 (23.64)	7.16	55.50
J	25.12 ± 20.79 [ab]	31.97 (43.27)	0.10	51.10
K	37.28 ± 26.62 [bc]	41.02 (43.33)	0.10	81.85

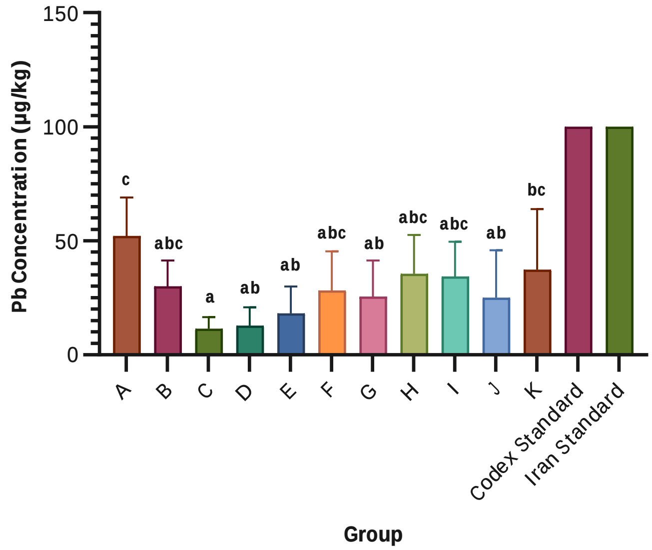
* Means sharing the same letters in each column are not significantly different according to Tukey's test ($p > 0.05$).

Table 2. Descriptive statistics of zinc based on sausage brands (mg/kg)

Brand	Mean ± SD	Median (IQR)	Min	Max
A	10.53 ± 2.14 [ab]	10.39 (3.03)	7.93	14.94
B	7.83 ± 2.63 [a]	7.44 (4.63)	3.50	10.71
C	8.61 ± 7.77 [a]	4.41 (15.00)	1.51	20.34
D	11.44 ± 5.13 [ab]	12.24 (9.58)	4.20	19.03
E	14.35 ± 5.58 [ab]	11.34 (10.11)	9.72	24.00
F	11.44 ± 2.23 [ab]	12.94 (4.02)	8.05	13.37
G	16.79 ± 4.77 [b]	13.88 (8.21)	13.27	25.11
H	9.72 ± 2.96 [a]	10.45 (3.73)	4.58	14.40
I	9.32 ± 0.86 [a]	9.46 (1.59)	8.13	10.55
J	14.35 ± 3.18 [ab]	(54.6) 03.15	9.87	31.18
K	13.87 ± 7.08 [ab]	17.59 (11.26)	0.01	20.04

* Means sharing the same letters in each column are not significantly different according to Tukey's test ($p > 0.05$)

ANOVA Results showed significant differences among all samples, including comparisons between brands and different meat percentages. When comparing different brands, significant differences were observed for both lead and zinc concentrations ($p < 0.05$). Post-hoc tests indicated that the mean lead concentration in Brand A products was significantly higher than in Brands C, D, E, G, and J ($p < 0.001$). The mean lead concentration in Brand C products was significantly lower than in Brands H and K ($p < 0.001$). No significant differences were observed among the other brands regarding lead concentration. For zinc, the mean concentration in Brands B, C, and H products was significantly lower than in Brand G products ($p < 0.001$).



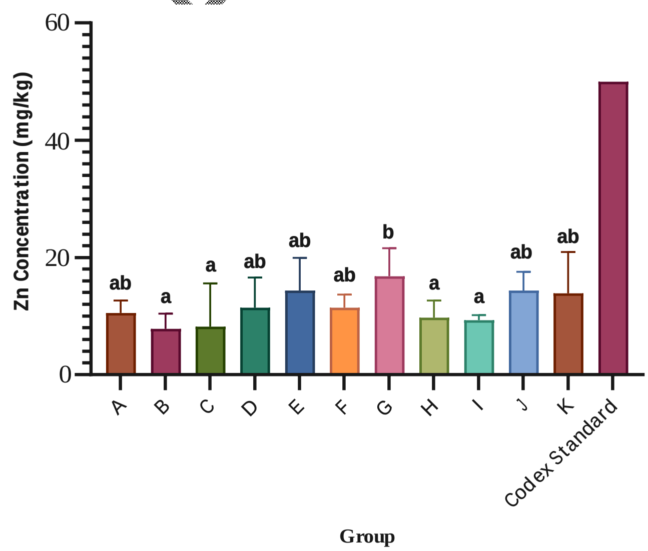


Figure 1. Comparison of the mean concentrations of lead and zinc across brands with the Codex standard and the maximum tolerance of the Iranian National Standard
* Columns sharing the same letters are not significantly different from each other according to Tukey's test ($p > 0.05$).

To compare the meat percentages of the samples in the statistical analysis, samples containing 40-55% meat were classified as low meat percentage, 60-70% as medium meat percentage, and 80-90% as high meat percentage. The mean heavy metal concentrations in samples with low, medium, and high meat percentages were 25.54 ± 18.30, 29.46 ± 19.01, and 30.13 ± 19.83 µg/kg for lead, and 8.97 ± 3.81, 10.96 ± 4.16, and 14.77 ± 5.12 mg/kg for zinc, respectively.

The ANOVA results showed no significant difference in the mean lead concentration with increasing meat percentage in the samples ($p > 0.05$). In contrast, a significant increase in the mean zinc concentration was observed in samples containing 80% meat or more ($p < 0.05$). This finding has not been reported in previous studies, and it is possible that elevated zinc levels in animal feed and supplements contribute to zinc contamination in meat. Because meat is a major raw material in sausage production, products with higher meat percentages exhibit significantly higher zinc concentrations.

Table 3. Descriptive statistics of lead based on meat percentage in sausages (µg/kg)

Meat Percentages	Mean ± SD	Median (IQR)	Min	Max
Low (40-55%)	25.54 ± 18.30 [a]	25.40 (24.99)	0.10	81.85
Medium (60-70%)	29.46 ± 19.01 [a]	32.21 (32.51)	0.10	57.49
High (80-90%)	30.13 ± 19.83 [a]	24.99 (29.90)	0.10	77.35

* Means sharing the same letters in each column are not significantly different according to Tukey's test ($p > 0.05$).

Table 4. Descriptive statistics of zinc based on meat percentage in sausages (mg/kg)

Meat Percentages	Mean ± SD	Median (IQR)	Min	Max
Low (40-55%)	8.97 ± 3.81 [a]	9.87 (4.57)	0.01	13.71
Medium (60-70%)	10.96 ± 4.16 [a]	10.55 (7.30)	4.32	18.22
High (80-90%)	14.77 ± 5.12 [b]	14.10 (8.68)	8.05	25.11

* Means sharing the same letters in each column are not significantly different according to Tukey's test ($p > 0.05$).

Lead occurs naturally in the earth, but human activities have significantly contributed to environmental contamination with this metal. Some of the main sources of lead pollution include industrial activities, automobiles, and household products (WHO, 2025). Effective measures can help reduce the negative effects of lead on human health and the environment. These measures include public awareness campaigns, control of contamination sources, water and soil treatment, and the substitution of materials (Chopra & Dhingra, 2021; M. S. Collin et al., 2022; S. Collin et al., 2022; Khan et al., 2021). Processed foods may become contaminated due to the use of contaminated raw materials or packaging containing lead. For example, lead-containing dyes and additives in packaging can lead to food contamination (Karanth et al., 2023). Health risk assessment is a critical component of food safety evaluation, as it provides quantitative insight into how heavy metal contamination influences the quality of meat products, particularly in regions such as the Middle East and Africa, where rapid industrialization and environmental pollution contribute to metal accumulation in livestock. Heavy metals, especially lead, tend to bioaccumulate in animal tissues, thereby posing considerable health risks to consumers. Chronic exposure to such contaminants has been associated with neurotoxicity, carcinogenesis, and various systemic dysfunctions. Conducting comprehensive health risk assessments enables the identification of hazardous concentration thresholds, the establishment of safe consumption limits, and the formulation of effective regulatory measures to safeguard public health. Moreover, comparing measured contamination levels with international safety standards assists authorities in designing targeted interventions to minimize dietary exposure and ensure meat quality and safety (Bright Ichu et al., 2025). Human exposure to heavy metals induces a wide spectrum of adverse health effects, ranging from acute poisoning to long-term, irreversible disorders. Chronic exposure has been linked to carcinogenesis, renal dysfunction, and neurodevelopmental impairment. In children, sustained exposure is associated with impaired growth, decreased intelligence quotient (IQ), and developmental delays. Among adults, heavy metal toxicity has been correlated with deficits in memory performance, slower psychomotor response, general fatigue, and hypertension in men (Al-batti & Al-Enazi, 2025).

Measuring lead concentrations in sausages as a preventive measure can help identify and control food contamination, which is especially important for preventing foodborne illnesses (Nematollahi et al., 2021). Monitoring lead concentrations in sausages provides consumers with accurate information about the quality and safety of food products, helping them make more informed and healthier choices (Shahzad et al., 2023). Increased public awareness of the risks of lead contamination can enhance consumer confidence in food products (Bal-Prylypko et al., 2022).

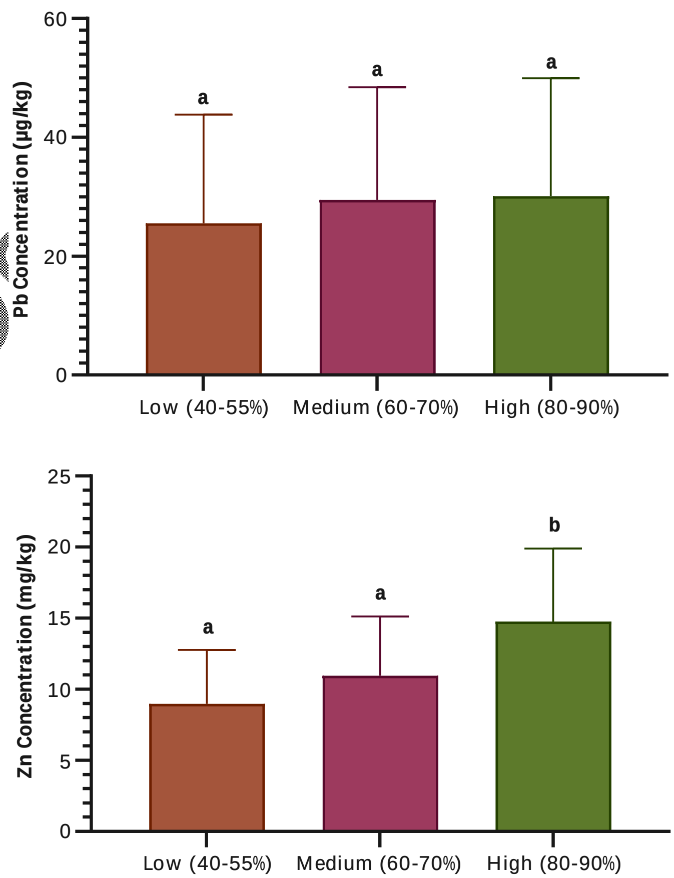


Figure 2. Comparison of lead and zinc mean concentrations by meat percentages
* Columns sharing the same letters are not significantly different from each other according to Tukey's test ($p > 0.05$).

The results of this study showed that the concentrations of lead and zinc in the samples were within acceptable limits. In samples with meat percentages higher than 80%, the concentration of zinc increased. This increase can be attributed to contamination in animal feed and supplements, leading to the accumulation of zinc in meat, which is the primary component of sausages. The results of this study, along with comparisons to other studies, are presented in Table 5.

Table 5. Comparison of the present study with other studies conducted in Iran and other countries

Number	Study Country	Year	Sample type	Pb (µg/kg)	Zn (mg/kg)	Reference
1	Nigeria - Lafia	2025	Sausages and salami	BDL	-	(Ioryue et al., 2025)
2	Pakistan -Lahore	2023	Sausages and salami	BDL	-	(Shahzad et al., 2023)
3	Malaysia-Pahang	2022	Sausages	97.50	-	(Manan & Ghani, 2022)
4	Southern Italy	2021	Meat Products	310.0	6.21	(Barone et al., 2021)
5	Romania - Iasi	2014	Sausages	820.0	38.40	(Hoha et al., 2014)
6	Iran - Hamedan	2018	Sausages	1880.0	4.61	(Sobhanardakani, 2018)
7	Iran - Tehran	2018	Sausages	48.78	14.65	(Abedi et al., 2018)
8	Iran - Tehran	2011	Sausages	53.50	-	(Abedi et al., 2011)
9	This study	2025	Sausages	28.39	11.69	

* BDL = Below detectable limit

4. Conclusion

In general, all the sausage samples tested had lead and zinc concentrations below the recommended standards. Significant differences in metal concentrations were observed among different brands. When comparing the effect of increasing meat percentage on metal concentrations, only zinc showed a significant increase.

The findings of this investigation underscore the critical necessity of implementing stringent control measures throughout the processed meat supply chain to mitigate heavy metal contamination, specifically focusing on lead. To enhance the food safety profile of processed meat products, manufacturers are strongly advised to implement a rigorous supplier qualification program. This initiative must prioritize raw material sourcing from livestock operations situated significantly distant from known environmental sources of contamination, such as industrial effluent zones and areas proximal to metallurgical or lead mining activities. Furthermore, a prophylactic measure involves the mandated exclusion of any food additives, preservatives, or processing aids suspected of contributing heavy metal residues, with particular emphasis on lead-containing compounds. Equally vital is the reassessment and substitution of packaging materials to eliminate any potential for Pb migration into the final product. We propose that regulatory agencies establish and enforce a comprehensive, routine surveillance and monitoring protocol for heavy metals in commercially available processed meat products. This protocol should incorporate systematic, risk-based sampling programs for marketed sausages, executed by accredited reference laboratories utilizing high-sensitivity instrumentation, such as inductively Coupled ICP-MS, to ensure compliance with established national or international safety thresholds.

Authors' Contributions

Saeid Vahedi: Conceptualization; Methodology; Software; Investigation; Resources; Data curation; Writing-original draft preparation; Writing-review and editing; Visualization. **Zohre Farahmandkia:** Conceptualization; Writing-original Draft preparation; Writing-review and editing. **Hassan Hassanzadazar:** Conceptualization; Writing-review and editing. **Fatemeh Masaebi:** Conceptualization; Software; formal analysis; Writing-review and editing; Visualization. **Mohammad Reza Mehrasbi:** Conceptualization; Validation; Writing-review and editing; Supervision; Project administration; Funding acquisition.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical Considerations

The authors have observed all ethical considerations in this article, including avoiding plagiarism, republishing, data distortion, and data fabrication. (Ethics Number: IR.ZUMS.BLC.1402.070).

Using Artificial Intelligence

Artificial intelligence tools were used solely to improve the grammar, language clarity, and readability of the manuscript. Specifically, a large language model (ChatGPT, OpenAI) was utilized during the manuscript revision stage for linguistic editing purposes only. The AI tool did not contribute to the study design, data collection, data analysis, interpretation of results, or the formulation of conclusions. The authors reviewed and verified all content and take full responsibility for the accuracy, originality, and integrity of the manuscript.

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