Open Veterinary Journal, (2023), Vol. 13(12): 1729-1737
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: 10.5455/OVJ.2023.v13.i12.21

Submitted: 05/10/2023 Accepted: 19/12/2023 Published: 31/12/2023

Detection and health risk assessment of toxic heavy metals in chilled and frozen meat collected from Sharkia province in Egypt

Nashwa H. Mohamed[1,2], Galal Yahya[3,4], Rasha M. El Bayoumi[2], Mohamed A.M. Hussein[2], Simona Cavalu[5], Hesham Dahshan[6], Abdullah F. Alsayeqh[7], Wageh S. Darwish[2*] and Elshimaa A.A. Nasr[8]

[1]*Hospitals of Zagazig University, Zagazig, Egypt*
[2]*Food Hygiene, Safety, and Technology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[3]*Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*
[4]*Department of Molecular Genetics, Faculty of Biology, Technical University of Kaiserslautern, Kaiserslautern, Germany*
[5]*Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania*
[6]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[7]*Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia*
[8]*Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

Abstract
Background: The consumption of meat is a fundamental aspect of global diets, providing essential nutrients and proteins vital for human nutrition. However, ensuring the safety of meat products has become progressively challenging due to potential contamination by toxic heavy metals (HMs) and pathogenic microorganisms.
Aim: This study focuses on assessing the prevalence of Lead (Pb), Mercury (Hg), Arsenic (As), and Cadmium (Cd), in chilled and frozen meat in Sharkia Governorate, Egypt.
Methods: A total of 30 samples, comprising 15 chilled and 15 frozen beef samples, were collected from various marketing stores in Sharkia. Analysis of toxic metals was conducted via atomic absorption spectrophotometer (AAS) following wet digestion.
Results: The average levels (mg/kg) in chilled meat samples were found to be 0.64 ± 0.14 for Pb, undetectable for Hg, 0.02 ± 0.14 for Cd, and 4.66 ± 0.57 for As. In frozen samples, the average concentrations were 0.89 ± 0.21 for Pb, 0.08 ± 0.03 for Hg, 0.02 ± 0.004 Cd, and 5.32 ± 0.59 for As. Generally, the levels of HMs in frozen meat samples were observed to be higher than in chilled samples. Importantly, the levels of Pb were higher than maximum residual concentrations [maximum permissible limit (MPL)] in 53.3% of the chilled and 66.6% of the frozen, Cd levels in chilled and frozen were within the permissible concentrations in all samples, Hg was not identified in all the chilled and in 67% of frozen samples, and As levels were higher than the permissible levels in all samples chilled and frozen. The assessment of human health risk for adults revealed an estimated daily intake (EDI) value of beef meat below the threshold of the oral reference dose (RFD) for all analyzed metals except for As, where 46.7% of chilled samples and 60% of frozen samples exceeded the RFD. Furthermore, both the Hazard Quotient (THQ) for As and Hazard index (HI) for all the analyzed metals were above 1 in 33.3% of chilled samples and 46.7% of frozen samples.
Conclusion: This indicates the remarkable adverse effects on human health associated with the consumption of meat with elevated levels of HMs, emphasizing the need for stringent quality control measures within the food industry.
Keywords: Food safety, Meat, Heavy metals, Estimated daily intake, Hazard index.

Introduction

Chemical elements that have a density higher than 5 g/cm^3 are classified as heavy metals (HMs) (Jaishankar *et al.*, 2014). While trace amounts of these micronutrients—Zn, Fe, Cu, Mn, and Co—are necessary to sustain regular physiological processes, excessive or extended consumption of them can have negative health effects (Renwick, 2006). Traces of toxic HMs and metalloids (THMs) can lead to organ toxicity and other negative health effects. These relatively dense elements are not beneficial to the body (Järup, 2003; Tangahu *et al.*, 2011;

***Corresponding Author:** Wageh S. Darwish. Food Hygiene, Safety, and Technology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
Email: *wagehdarwish@gmail.com*

Tchounwou *et al*., 2012; Jaishankar *et al*., 2014; Jan *et al*., 2015; Kim *et al*., 2015). The World Health Organization (WHO) lists THMs as serious public health problems. In addition, the majority of THMs are classified as category 1 carcinogens (Kim *et al*., 2015; Pollution Control Handbook for Oil and Gas Engineering, 2016). Examples of THMs include As, Cd, Pb, and Hg. The harmful effects of THMs depend on a number of variables, such as the dosage, the mode of exposure, the age of the animals or people exposed, and their level of nutrition (Pamphlett *et al*., 2018; Vicas *et al*., 2021). The usage of THMs in industry, agriculture, medicine, technology, and other anthropogenic contexts can contaminate the environment with these hazardous metals. Thus, there is a chance that humans and food-producing animals (FPAs) will be exposed to this, especially if it moves up the food chain (Tchounwou *et al*., 2012). THM-contaminated pasturelands, water outlets, and the careless use of acaricides are entry points for THMs into the edible tissues of FPAs (Adamse *et al*., 2017). Metals can get into meat from processes done after the animal is killed or from the animal itself. The spread of this kind of contamination can happen in a number of ways, such as through the use of cooking equipment, raw ingredients in food preparation, adding spices, or even components in food packaging. Metals may enter meat products through several different routes, which may have an effect on the product's safety and quality.

THM accumulation in meat poses a risk to health because of its toxicity, even in tiny amounts. For example, Pb, Cd, and Hg have no physiological purpose and are associated with neural system illnesses, bone diseases, and organ dysfunctions (Abou-Arab, 2001; El Nemr *et al*., 2016; Bawuro *et al*., 2018; Chen *et al*., 2019; El-Said *et al*., 2021; Charkiewicz *et al*., 2023).

International food safety regulatory bodies, including those under the WHO (2012), the European Food Safety Authority (EFSA), and the Egyptian Organization for Standards and Quality (EOS, 2023), have set maximum permissible limits (MPLs) for these metals in edible animal products due to worries about food safety and the buildup of THMs. Because THM exposure poses serious health concerns and has negative impacts on human health, it is illegal to exceed certain MPLs in edible tissues.

To determine the possible health concerns linked to THM consumption, it is important to regularly check the amounts of these compounds in food. Methods of risk assessment frequently rely on figuring out the THQ. A THQ score of less than one means that there is no appreciable risk connected to the food (Wehedy *et al*., 2018). Therefore, to determine the levels of HMs (Pb, Cd, Hg, and As) in frozen and cooled meat, our study used atomic absorption spectroscopy. Evaluating the damage to human health posed by these metals' presence in frozen and refrigerated beef samples was the goal.

Materials and Methods

Sample collection

Thirty 10-gm samples of beef (15 chilled and 15 frozen) were haphazardly gathered from various establishments spread across the six main cities of the Sharkia Governorate, Egypt. As soon as feasible, the samples were aseptically transported in an ice box to the Meat Hygiene Laboratory of the Zagazig University Faculty of Veterinary Medicine for analysis.

Preparing the sample

3 ml of 65% nitric acid and 2 ml of 70% perchloric acid were combined with one gram of each sample. This combination was placed in falcon tubes and left to stand at room temperature for the entire night. The tubes were then heated for three hours at 70°C in a water bath with intermittent whirling every thirty minutes. The material was diluted with 20 ml of de-ionized water and filtered once it had cooled to room temperature. The filtrate obtained was kept in glass tubes with caps made of polyethylene films until the HM analysis was completed (Wehedy *et al*., 2018).

Analysis of HMs

A careful blank solution was made for the HM analysis using 10 ml of 65% nitric acid and 2 ml of 70% perchloric acid. Purely specialized atomic absorption spectrophotometer (AAS) standards for Pb, Cd, Hg, and As were used to carefully manufacture standard solutions of these HM. Wet digestion was applied to both the blank and standard solutions, and the results were then diluted with 20 ml of de-ionized water. The Buck Scientific 210VGP AAS, specifically the PerkinElmer model (Spectra-AA10, USA), was used at the Central Laboratory (Faculty of Veterinary Medicine, Zagazig University, Egypt) to analyze the HM content of the digested blank and standard solutions. For Pb, Cd, and As, the analysis process used an air/acetylene flow (5.5/1.11/m) flame; for the detection of Hg, the cold vapor atomic absorption spectroscopy approach was utilized (Wehedy *et al*., 2018). For Pb, Cd, Hg, and As, the limits of detection (LOD) were set at 0.01 μg/g, 0.005, 0.005, and 0.02 μg/g, respectively. THM concentrations were given as μg/g wet weight (ww), which is the same as mg/kg. The Egyptian Organization for Standardization's maximum allowable limits and other pertinent international standards were used to compare the residual metal concentrations in the tested samples.

Evaluation of danger to human health

The United States Environmental Protection Agency's (US EPA, 2014) recommendations were followed in the risk assessment, which was based on the metal amounts found in the meat samples. HM toxicity is unaffected by cooking, and the swallowed dose of the contaminant is thought to be equal to the adsorbed dose (Copat *et al*., 2013). The following metrics were used to construct the health risk assessment of the HM levels in the meat samples under examination: the

Hazard Quotient (THQ), the Hazard index (HI), and the estimated daily intake (EDI).

EDI

Based on the amounts of HMs in muscle and the rate of consumption, the EDI of HMs was calculated. The following formula was used to calculate the metals EDI: EDI = (Cm * FIR)/BW (US EPA, 2014). In this formula, Cm stands for the HM concentration in the sample (mg/kg ww), FIR for the ingestion rate of bovine meat (35.287 g/day) (FAOSTAT, 2023), and BW for the adult Egyptian body weight (70 kg). The measured values were then contrasted with the acceptable daily intakes (TDIs) for the metals (FAO/WHO, 2011).

THQ

THQ was utilized to evaluate the potential health hazards linked to noncarcinogenic toxic metals. According to Wehedy *et al*. (2018), THQ is the ratio of the projected pollutant dose to the reference dose (RFD), which represents the amount of contaminant exposure per day that the population can withstand without suffering a major health risk. A THQ of less than one indicates that there is little chance of the population suffering from discernible negative impacts. The following is the formula provided by Yi *et al*. (2011) to estimate THQ: (EF*ED*FIR*C/RFD*BW*AT) * 10^3 is THQ, where EF stands for exposure frequency (365 days/year), ED for exposure duration (average duration of 70 years), FIR for meat ingestion rate (g/day), C for HM concentration in meat (mg/kg), BW for adult average body weight (70 kg), RFD for oral RFD (mg/kg/day), and AT for average exposure time (365 days/year × exposure years, assuming 70 years).

According to FAO/WHO (2011), the oral RFD for Pb, Cd, Hg, and As are 0.004, 0.001, 0.0016, and 0.04 (mg/kg bw/day), respectively.

HI

HI is a comprehensive metric used to assess the possible harm of any HM to human health. It is computed by adding the HQ for every metal separately, as shown in the following equation:

$$HQPb + HQCd + HQHg + HQAs = HI = \sum HQ$$

When the HI is more than 1, it raises questions about possible negative consequences on human health (Wehedy *et al.*, 2018).

Statistical analysis

Measurement data represent triplicate analysis for each sample and values represent mean±SD. Analysis of variance for each measurement followed by Tuley's HSD test was done where *p*-values less than 0.05 are considered significant.

Ethical approval

Not needed for this study.

Results

In terms of routine updates for the safety of the marketed chilled and frozen meat products, we decided to analyze the traces of HMs Pb, Cd, Hg, and As and assess that they are within the permissible legal limits, we collected 30 samples of beef meat products (15 chilled and 15 frozen from the same store) marketed in six different cities of Sharkia governorate (Fig. 1), which ranks the third most populous among Egypt's governorates, positioned in the northern region of the country, it holds this significant status in terms of population density.

Levels of HMs in meat

Across the 15 chilled meat samples, notable variations were observed in the concentrations (μg/g w.w.) of HMs (Table 1). Pb concentrations ranged between 0.28 and 1.5, indicating considerable variability among the samples. Pb was undetectable in four samples (5, 6, 8, and 10C), while 53.3% of the samples exceeded the permissible level. Hg was absent in all samples. Cd levels ranged from 0.01 to 0.07, a range that did not significantly exceed the permissible limit. Five samples were devoid of Cd (2, 4, 6, 9, and 10C). However, As levels varied from 1.49 to 8.36, surpassing the permissible limit significantly.

Examining the HM levels in the frozen samples (Table 2), the concentrations were generally higher compared to the chilled samples, particularly for As, Pb, and Hg. Pb concentrations ranged from 0.04% to 2.6% and 66.6% of the samples were above the permissible MPL, while Hg was detected in five samples with concentrations ranging from 0.09% to 0.45% and 33.3% of the samples exceeding the acceptable levels. Cd levels remained within permissible limits, ranging

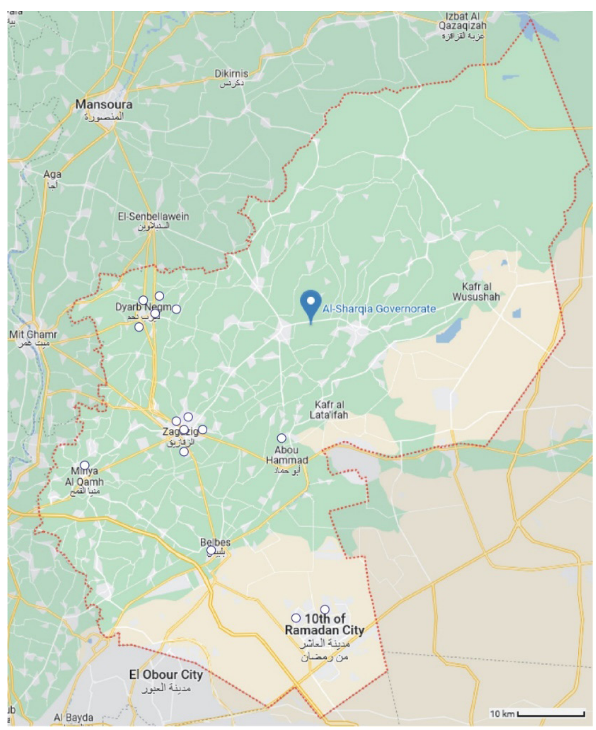

Fig. 1. Declarative map of Sharkia province in Egypt and distribution of sample collection.

Table 1. Elemental concentrations (μg/g ww) of Pb, Cd, Hg, and As in the examined chilled meat samples.

Chilled sample No.	Pb	Hg	Cd	As
1C	**0.72 ± 0.12 [a]**	0.00	0.03 ± 0.01 [a]	**1.49 ± 0.25 [a]**
2C	**1.18 ± 0.16 [b]**	0.00	0.00	**5.1 ± 0.57 [c]**
3C	**1.29 ± 0.16 [b]**	0.00	0.04 ± 0.01 [a]	**4.05 ± 0.5 [c]**
4C	0.28 ± 0.12 [a]	0.00	0.00	**3.1 ± 0.35 [b]**
5C	0.00	0.00	0.01 ± 0.005 [a]	**3.66 ± 0.32 [b]**
6C	0.00	0.00	0.00	**7.06 ± 0.57 [c]**
7C	0.32 ± 0.12 [a]	0.00	0.02 ± 0.005 [a]	**5.29 ± 0.57 [b]**
8C	0.00	0.00	0.06 ± 0.03 [a]	**6.24 ± 0.57 [c]**
9C	**0.84 ± 0.14 [b]**	0.00	0.00	**8.42 ± 0.57 [c]**
10C	0.00	0.00	0.00	**5.76 ± 0.57 [c]**
11C	**1.5 ± 0.16 [b]**	0.00	0.03 ± 0.01 [a]	**8.36 ± 0.57 [c]**
12C	**1.33 ± 0.12 [b]**	0.00	0.07 ± 0.04 [a]	**2.33 ± 0.27 [b]**
13C	**0.58 ± 0.11 [a]**	0.00	0.03 ± 0.01 [a]	**1.57 ± 0.17 [a]**
14C	**1.25 ± 0.14 [b]**	0.00	0.03 ± 0.01 [a]	**3.35 ± 0.37 [b]**
15C	0.35 ± 0.12 [a]	0.00	0.03 ± 0.01 [a]	**4.1 ± 0.42 [c]**

Values in bold exceed the maximum permissible limits. Data are expressed as mean ± standard deviation (SD). Values not sharing the same superscript (lowercase alphabets, a, b, c) in the same column differ significantly at $P < 0.05$.

Table 2. Frozen meat heavy metal levels (μg/g w.w.).

Frozen sample No.	Pb	Hg	Cd	As
1F	**2.29 ± 0.21 [b]**	0.00	0.04 ± 0.004 [a]	**7.65 ± 0.59 [c]**
2F	**0.94 ± 0.13 [b]**	0.00	0.00	**3.73 ± 0.46 [b]**
3F	**2.6 ± 0.21 [b]**	0.00	0.04 ± 0.004 [a]	**11.34 ± 0.67 [c]**
4F	**1.27 ± 0.16 [b]**	**0.26 ± 0.03 [b]**	0.04 ± 0.004 [a]	**6.67 ± 0.38 [b]**
5F	**1.13 ± 0.15 [b]**	**0.18 ± 0.03 [b]**	0.04 ± 0.004 [a]	**6.06 ± 0.38 [b]**
6F	**0.5 ± 0.12 [a]**	0.00	0.00	**4.64 ± 0.34 [b]**
7F	0.00	0.00	0.00	**3.91 ± 0.3 [b]**
8F	0.00	**0.45 ± 0.04 [c]**	0.02 ± 0.002 [a]	**5.15 ± 0.44 [b]**
9F	0.04 ± 0.01 [a]	0.00	0.00	**7.23 ± 0.59 [c]**
10F	0.00	0.00	0.03 ± 0.002 [a]	**4.68 ± 0.34 [b]**
11F	**1.28 ± 0.16 [b]**	0.00	0.01 ± 0.002 [a]	**3.34 ± 0.38 [b]**
12F	**0.94 ± 0.11 [b]**	0.00	0.02 ± 0.001 [a]	**2.94 ± 0.21 [b]**
13F	0.16 ± 0.08 [a]	**0.15 ± 0.01 [b]**	0.00	**5.97 ± 0.48 [b]**
14F	**1.2 ± 0.12 [b]**	0.00	0.00	**4.13 ± 0.29 [b]**
15F	**0.98 ± 0.1 [b]**	**0.09 ± 0.01 [a]**	0.03 ± 0.002 [a]	**2.39 ± 0.27 [b]**

Values in bold exceed the maximum permissible limits. Data are expressed as mean ± standard deviation (SD). Values not sharing the same superscript (lowercase alphabets, a, b, c) in the same column differ significantly at $P < 0.05$.

from 0.01 to 0.04, whereas As concentrations were notably higher, ranging from 2.39 to 11.34.

EDI of HMs

The EDI of Pb, Hg, Cd, and As for each sample (chilled or frozen) in this study was calculated. For chilled samples the EDI of Pb, Cd, Hg, and As ranged from 0.1411 to 0.7562, 0.0000, 0.0101 to 0.0353, and 0.7511 to 4.2445 μg/kg BW/day, respectively (Table 3). For frozen samples, samples the EDI of Pb, Cd, Hg, and As ranged from 0.0807 to 1.310, 0.0454 to 0.2268, 0.0050 to 0.0202, and 1.2048 to 5.7165, respectively (Table 3). In comparison to the metals' tolerable daily intake (TDI) of 3.57, 0.71, 1.00, and 2.1 for Pb, Hg, Cd, and As respectively, our analysis revealed that seven chilled samples and nine frozen samples exceeded the tolerable daily intake for As, while they remained within acceptable limits for the other three HMs.

THQ and HI

Finally, we conducted a health risk assessment by evaluating the noncarcinogenic effects of HMs present in the meat samples. The THQ represents the relationship between the determined pollutant dose and the RFD. The RFD values for Pb, Cd, Hg, and As are 0.004, 0.001, 0.0016, and 0.0003 (µg/g bw/day), respectively. The RFD is the daily exposure to a contaminant that the population can experience over a lifetime without significant hazards. In all samples, both chilled and frozen, except for sample 8F, the THQ levels for Pb, Cd, and Hg were within acceptable limits. However, for As, levels exceeded the accepted standards in four chilled and six frozen meat samples (Table 4). The HI, which represents the summation of HQs for all metals (ΣHQ), is used to assess the potential health risks. As anticipated, samples with high THQ for arsenic exceeded the acceptable HI limit, both in chilled and frozen samples (Table 4).

Discussion

Meat is a rich source of essential nutrients such as protein, iron, B vitamins, and various minerals crucial for the body's well-being. In the global diet, meat is a staple food in numerous populations, and its consumption is expected to rise, especially in developing countries, over the next decade (Smith *et al.*, 2022). Contamination of meat with HMs (metals with atomic number higher than 20) represents a serious health concern. HMs (Pb, Cd, Hg, and As), while existing in the soil, have unknown biological functions for plants or animals (Lasat, 1999). HMs when present in meat, can pose serious health risks upon consumption, leading to potential toxicity, and various health hazards in humans. Consequently, monitoring and ensuring the safety of meat products is crucial to safeguard public health. EFSA had set maximum residual concentrations (MPL) for Pb (0.1 ppm), Cd (0.05 ppm), Hg (0.01 ppm), and As (0.01 ppm) (EC, 2006). Numerous studies have been directed to assess the THMs levels in the edible tissues of FPAs and fish in Egypt (Dorne *et al.*, 2011; Alturiqi and Albedair, 2012; Ahmed *et al.*, 2017; Darwish *et al.*, 2018; Darwish *et al.*, 2019; Sallam *et al.*, 2019; Abd-Elghany *et al.*, 2020; Ezedom *et al.*, 2020; Kamaly and Sharkawy, 2023). Our research focused on assessing the concentration of HMs in both chilled and frozen meat available in the Sharkia province, an Egyptian governorate. We collected 30 random samples of chilled and frozen beef from various retail stores across Sharkia. Upon analysis, we observed that over 50% of the chilled samples surpassed the maximum allowable levels of Pb. Meanwhile, Cd levels fell within the acceptable range, and Hg was not detected. However, remarkably, all samples exceeded the acceptable limits

Table 3. EDI of different metals for each sample compared to the tolerable daily intake (TDIs).

Chilled sample No.	Pb	Hg	Cd	As	Frozen sample No.	Pb	Hg	Cd	As
1C	0.3630	0.0000	0.0151	0.7511	**1F**	1.1544	0.0000	0.0202	**3.8564**
2C	0.5948	0.0000	0.0000	**2.5709**	**2F**	0.4739	0.0000	0.0000	1.8803
3C	0.6503	0.0000	0.0202	2.0416	**3F**	1.3107	0.0000	0.0202	**5.7165**
4C	0.1411	0.0000	0.0000	1.5627	**4F**	0.6402	0.1311	0.0202	**3.3623**
5C	0.0000	0.0000	0.0050	1.8450	**5F**	0.5696	0.0907	0.0202	**3.0548**
6C	0.0000	0.0000	0.0000	**3.5589**	**6F**	0.2521	0.0000	0.0000	**2.3390**
7C	0.1613	0.0000	0.0101	**2.6667**	**7F**	0.0000	0.0000	0.0000	1.9710
8C	0.0000	0.0000	0.0302	**3.1456**	**8F**	0.0000	0.2268	0.0101	**2.5961**
9C	0.4234	0.0000	0.0000	**4.2445**	**9F**	0.0202	0.0000	0.0000	**3.6446**
10C	0.0000	0.0000	0.0000	**2.9036**	**10F**	0.0000	0.0000	0.0151	**2.3592**
11C	0.7562	0.0000	0.0151	**4.2143**	**11F**	0.6452	0.0000	0.0050	1.6837
12C	0.6705	0.0000	0.0353	1.1746	**12F**	0.4739	0.0000	0.0101	1.4821
13C	0.2521	0.0000	0.0151	0.7914	**13F**	0.0807	0.0756	0.0000	**3.0095**
14C	0.6301	0.0000	0.0151	1.6887	**14F**	0.6049	0.0000	0.0000	2.0819
15C	0.1764	0.0000	0.0151	2.0668	**15F**	0.4940	0.0454	0.0151	1.2048
TDIs	**3.57**	**0.71**	**1.00**	**2.1**					

Values in bold significantly exceed the maximum permissible TDIs ($P < 0.05$).

Table 4. THQ and HI of different metals from consumption of the examined samples.

Chilled sample No.	Pb	Hg	Cd	As	HI	Frozen sample No.	Pb	Hg	Cd	As	HI
1C	0.0907	0.0000	0.0151	0.2504	0.3562	**1F**	0.2886	0.0000	0.0202	**1.2855**	**1.5942**
2C	0.1487	0.0000	0.0000	0.8570	**1.0057**	**2F**	0.1185	0.0000	0.0000	0.6268	0.7452
3C	0.1626	0.0000	0.0202	0.6805	0.8633	**3F**	0.3277	0.0000	0.0202	**1.9055**	**2.2533**
4C	0.0353	0.0000	0.0000	0.5209	0.5562	**4F**	0.1601	0.8192	0.0202	**1.1208**	**2.1202**
5C	0.0000	0.0000	0.0050	0.6150	0.6200	**5F**	0.1424	0.5671	0.0202	**1.0183**	**1.7480**
6C	0.0000	0.0000	0.0000	**1.1863**	**1.1863**	**6F**	0.0630	0.0000	0.0000	0.7797	0.8427
7C	0.0403	0.0000	0.0101	0.8889	0.9393	**7F**	0.0000	0.0000	0.0000	0.6570	0.6570
8C	0.0000	0.0000	0.0302	**1.0485**	**1.0788**	**8F**	0.0000	**1.4178**	0.0101	0.8654	**2.2932**
9C	0.1059	0.0000	0.0000	**1.4148**	**1.5207**	**9F**	0.0050	0.0000	0.0000	**1.2149**	**1.2199**
10C	0.0000	0.0000	0.0000	0.9679	0.9679	**10F**	0.0000	0.0000	0.0151	0.7864	0.8015
11C	0.1890	0.0000	0.0151	**1.4048**	**1.6089**	**11F**	0.1613	0.0000	0.0050	0.5612	0.7276
12C	0.1676	0.0000	0.0353	0.3915	0.5944	**12F**	0.1185	0.0000	0.0101	0.4940	0.6226
13C	0.0630	0.0000	0.0151	0.2638	0.3419	**13F**	0.0202	0.4726	0.0000	**1.0032**	**1.4959**
14C	0.1575	0.0000	0.0151	0.5629	0.7356	**14F**	0.1512	0.0000	0.0000	0.6940	0.8452
15C	0.0441	0.0000	0.0151	0.6889	0.7482	**15F**	0.1235	0.2836	0.0151	0.4016	0.8238

Values in bold exceed the recommended limits.

for As. The frozen samples exhibited higher levels of all HMs, with approximately one-third of the samples showing traces of Hg.

In a prior investigation assessing HMs levels in meat served at Zagazig University Hospitals, it was found that 66.7%, 58.8%, and 80% of the samples examined exceeded the MPL for Pb, Cd, and As, respectively (Wehedy *et al.*, 2018). In a separate investigation analyzing HM levels in beef carcasses at Beni-Suef abattoir in Egypt, it was observed that the average residual levels of HMs did not surpass the permissible limits. However, higher concentrations were accumulated in the kidneys in comparison to the liver and muscle tissues (Khalafalla *et al.*, 2011).

In our investigation, we identified Pb concentrations measuring 0.64 ± 0.14 mg/kg in chilled beef and 0.89 ± 0.21 mg/kg in frozen beef. Various studies have reported Pb concentrations in muscle, liver, and kidney samples of edible tissues from diverse animal species in various countries, ranging between 0.008 and 5.48 mg/kg (Liu, 2003; Sedki *et al.*, 2003; Alturiqi and Albedair, 2012; Alkmim Filho *et al.*, 2014; Bazargani-Gilani *et al.*, 2016; Hashemi, 2018), the levels observed in our beef samples align with or slightly exceed some of these reported values. In our findings, Cd concentrations were recorded at 0.02 ± 0.14 mg/kg in chilled beef and 0.02 ± 0.004 mg/kg in frozen beef, which are within permissible limits. Other studies have indicated Cd levels in muscles and organs ranging from 0.005 to 1.25 mg/kg (Onianwa *et al.*, 2000; Sedki *et al.*, 2003; Waegeneers *et al.*, 2009; Ihedioha and Okoye, 2013; Bortey-Sam, *et al.*, 2015; López-Alonso *et al.*, 2016). Generally, the Cd levels in our beef samples fall within or below these reported values.

While our study did not detect Hg in most of the samples, we detected significant levels of As, in line with previous research (Chen *et al.*, 2013; Liang *et al.*, 2019). These levels surpass acceptable limits and pose increased health hazards associated with consuming such meat. these levels are not acceptable and increase the health hazards of consuming this meat.

The assessment of element intake and its contribution to toxic values revealed that seven chilled and nine frozen samples surpassed the permissible daily intake for As, consequently elevating both the THQ and the overall HI. This increase caused five chilled and seven frozen samples to surpass the acceptable HI limit. Prolonged exposure to Pb is associated with several health issues including hemolytic anemia, atherosclerosis, osteoporosis, liver apoptosis, ovarian atrophy, pathological organ changes, central nervous system (CNS) impairment, and renal toxicity (Adeyemi *et al.*, 2009; Shaheen *et al.*, 2016; Wehedy *et al.*, 2018), while continued exposure to As over time leads to inflammatory, degenerative, and neoplastic alterations in various systems within the body, including the cardiovascular, nervous, reproductive, respiratory, and hematopoietic systems (Faires, 2004; Azevedo *et al.*, 2018; Živkov Baloš *et al.*, 2019; Okoye *et al.*, 2022).

Conclusion and recommendations

This study underscores the presence of concerning levels of HMs, specifically Pb, As, and to a lesser extent, Hg, in both chilled and frozen meat samples collected from Sharkia Governorate, Egypt. While Cd levels remained within permissible limits in all samples, Lead concentrations surpassed maximum residual concentrations in a substantial percentage of both chilled and frozen samples. Arsenic levels were consistently above permissible limits in all samples, indicating a significant concern for consumer safety.

Notably, frozen meat exhibited higher concentrations of HMs compared to chilled meat, signifying potential differences in preservation methods impacting metal accumulation. The absence of Hg in all chilled samples and a majority of frozen samples contrasts with elevated Pb and As levels found across both types of meat. While Pb, Cd, and Hg levels in the meat samples generally pose minimal noncarcinogenic risks, the exceeding levels of As raise substantial concerns regarding potential health hazards. Addressing As contamination in meat products is imperative to mitigate health risks and ensure the safety of consumers. Our study came out with a subset of recommendations to uphold meat safety.

- Regulatory Measures: Urgent measures should be taken to address the elevated levels of Pb and As, which exceed permissible limits in the majority of the meat samples. Reassessing and reinforcing regulatory standards regarding HM concentrations in meat products is crucial to safeguard consumer health.
- Quality Control and Monitoring: Implement rigorous quality control measures along the production and distribution chain to monitor HM levels in meat. Regular testing for toxic metals should be mandatory to ensure compliance with safety standards.
- Public Awareness and Education: Educate consumers about the potential risks associated with consuming meat containing elevated levels of HM. Encourage informed choices and promote awareness regarding food safety practices.
- Preservation Techniques: Investigate and optimize preservation methods to mitigate HM accumulation in meat products. Assess the impact of different storage and processing techniques on reducing metal concentrations.
- Further Research: Conduct broader studies to explore the sources of HM contamination in meat products within the region. Investigate environmental factors, agricultural practices, and potential sources of contamination along the supply chain.
- Addressing the issue of HM contamination in meat is critical to ensuring food safety standards are met and safeguarding the health of consumers. Collaborative efforts between regulatory bodies, producers, and consumers are imperative to mitigate the risks associated with elevated HM levels in meat products.

Acknowledgments

The authors would like to thank the facilities and technical support provided by their respective universities and institutes.

Authors' contribution

Formal analysis, NHM, GY; investigation, WSD, MAMH, NHM, EAN, HE; resources, NHM, SC, and AFA; conceptualization, WSD and MAMH; methodology, NHM; software, GY; validation, NHM., RMEB, and GY; GY, WSD, MAMH, data curation; writing—preparation of the initial draft; writing—review and editing; NHM, GY, RMEB, SC, WSD, EAN, and MAMH; visualization; GY; supervision; WSD and MAMH; project administration; WSD and MAMH; funding acquisition, SC, AFA.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

There was no outside support for this study.

Data availability

Upon request, the respective authors can provide the data used in this work.

References

Abd-Elghany, S.M., Mohammed, M.A., Abdelkhalek, A., Saad, F.S.S. and Sallam, K.I. 2020. Health risk assessment of exposure to heavy metals from sheep meat and offal in Kuwait. J. Food Prot. 83(3), 503–510.

Abou-Arab, A.A. 2001. Heavy metal contents in Egyptian meat and the role of detergent washing on their levels. Food Chem. Toxicol. 39(6), 593–599.

Adamse, P., van der Fels-Klerx, H.J.I. and Jong, J. de. 2017. Cadmium, lead, mercury and arsenic in animal feed and feed materials—trend analysis of monitoring results. Food Addit. Contam. Part A, 34(8), 1298–1311.

Adeyemi, O., Ajayi, J.O., Olajuyin, A.M., Oloyede, O.B., Oladiji, A.T. and Oluba, O.M. 2009. Toxicological evaluation of the effect of water contaminated with lead, phenol and benzene on liver, kidney and colon of Albino rats. Food Chem. Toxicol., 47(4), 885–887.

Ahmed, A.M., Hamed, D.M. and Elsharawy, N.T. 2017. Evaluation of some heavy metals residues in batteries and deep litter rearing systems in Japanese quail meat and offal in Egypt. Vet. World 10(2), 262–269.

Alkmim Filho, J.F., Germano, A., Dibai, W.L.S., Vargas, E.A. and Melo, M.M. 2014. Heavy metals investigation in bovine tissues in Brazil. Food Sci. Technol., 34(1), 110–115.

Alturiqi, A.S. and Albedair, L.A. 2012. Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. Egyptian J. Aquat. Res. 38(1), 45–49.

Azevedo, L.S., Pestana, I.A., Meneguelli-Souza, A.C., Ramos, B., Pessanha, D.R. and Caldas, D. 2018.

Risk of exposure to total and inorganic arsenic by meat intake among different age groups from Brazil: a probabilistic assessment. Environ. Sci. Pollut. Res. Inter. 25(35), 35471–35478.
Bawuro, A.A., Voegborlo, R.B. and Adimado, A.A. 2018. Bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa State, Nigeria. J. Environ. Public Health 2018, 1854892.
Bazargani-Gilani, B., Pajohi-Alamoti, M., Bahari, A. and Sari, A.A. (2016) Heavy metals and trace elements in the Livers and Kidneys of Slaughtered Cattle, Sheep and Goats. Iran J. Toxicol. 10(6), 7–13.
Bortey-Sam, N., Nakayama, S.M.M., Akoto, O., Ikenaka, Y., Fobil, J.N. and Baidoo, E. 2015 Accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa Area in Ghana, and Associated Human Health Risks. Inter. J. Environ. Res. Public Health 12(8), 8811–8827.
Bortey-Sam, N., Nakayama, S.M.M., Ikenaka, Y., Akoto, O., Baidoo, E. and Yohannes, Y.B. 2015. Human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana: estimation of the daily intakes and target hazard quotients (THQs). Ecotoxicol. Environ Safety 111, 160–167.
Charkiewicz, A.E., Omeljaniuk, W.J., Nowak, K., Garley, M. and Nikliński, J. 2023. Cadmium toxicity and health effects-a brief summary. Molecules (Basel, Switzerland), 28(18), 6620.
Chen, Q.Y., DesMarais, T. and Costa, M. 2019. Metals and mechanisms of Carcinogenesis. Ann. Rev. Pharmacol. Toxicol. 59, 537–554.
Chen, S.-S., Lin, Y.-W., Kao, Y.-M. and Shih, Y.-C. 2013. Trace elements and heavy metals in poultry and livestock meat in Taiwan. Food Addit. Contam. Part B, Surveill. 6(4), 231–236.
Cheremisinoff, N.P., 2016. Pollution control handbook for oil and gas engineering, Hoboken, New Jersey: John Wiley and Sons, Inc.
Copat, C., Conti, G.O., Signorelli, C., Marmiroli, S., Sciacca, S. and Vinceti, M. 2013. Risk assessment for metals and PAHs by Mediterranean seafood. Food Nutr. Sci. 4(7), 10–13.
Darwish, W.S., Atia, A.S., Khedr, M.H.E. and Eldin, W.F.S. 2018. Metal contamination in quail meat: residues, sources, molecular biomarkers, and human health risk assessment. Environ. Sci. Pollut. Res. 25(20), 20106–20115.
Darwish, W.S., Chiba, H., Elhelaly, A.E. and Hui, S.-P. 2019. Estimation of cadmium content in Egyptian foodstuffs: health risk assessment, biological responses of human HepG2 cells to food-relevant concentrations of cadmium, and protection trials using rosmarinic and ascorbic acids. Environ. Sci. Pollut. Res. 26(15), 15443–15457.
Dorne, J.-L.C.M., Kass, G.E.N., Bordajandi, L.R., Amzal, B., Bertelsen, U. and Castoldi, A.F. 2011. Human risk assessment of heavy metals: principles and applications. Metal Ions Life Sci. 8, 27–60.
Egyptian Organization for Standardization (EOS.7136). 2010. Maximum level for certain contaminants in food stuffs. ESNO7136/2010 Cairo, Egypt. 2010: https://www.eos.org.eg/en/standard/12561 accessed on 17 November 2023.
El Nemr, A., El-Said, G.F., Ragab, S., Khaled, A. and El-Sikaily, A. 2016. The distribution, contamination and risk assessment of heavy metals in sediment and shellfish from the Red Sea coast, Egypt. Chemosphere 165, 369–380.
El-Said, G.F., El-Sadaawy, M.M., Shobier, A.H. and Ramadan, S.E. 2021. Human health implication of major and trace elements present in commercial crustaceans of a traditional Seafood marketing Region, Egypt. Biol. Trace Elem. Res. 199(1), 315–328.
European Commission (EC) 2006. Commission of the European Communities. Commission Regulation (EC) No. 1881. Setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Comm. 2006, 364, 24.
Evaluation of certain food additives and contaminants: seventy-third [73rd] report of the Joint FAO/WHO Expert Committee on Food Additives 2011.
Ezedom, T., Asagba, S. and Tonukari, N.J., 2020. Toxicological effects of the concurrent administration of cadmium and arsenic through the food chain on the liver and kidney of rats. J. Basic Appl. Zool. 81(1), 1–12.
Faires, M.C., 2004. Inorganic arsenic toxicosis in a beef herd. Can. Vet. J. 45(4), 329–331.
FAOSTAT 2023. Available from: https://www.fao.org/faostat/en/#home [Accessed 17 November 2023].
Hashemi, M., 2018. Heavy metal concentrations in bovine tissues (muscle, liver and kidney) and their relationship with heavy metal contents in consumed feed. Ecotoxicol. Environ. Saf. 154, 263–267.
Ihedioha, J.N. and Okoye, C.O.B. 2013. Dietary intake and health risk assessment of lead and cadmium via consumption of cow meat for an urban population in Enugu State, Nigeria. Ecotoxicol. Environ. Saf. 93, 101–106.
Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N. 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscipl. Toxicol. 7(2), 60–72.
Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi, I. and Haq, Q.M.R. 2015. Heavy metals and Human health: mechanistic insight into toxicity and counter defense system of Antioxidants. Inter. J. Mol. Sci., 16(12), 29592–29630.
Järup, L., 2003. Hazards of heavy metal contamination. Bri. Med. Bull. 68, 167–182.

Kamaly, H.F. and Sharkawy, A.A. 2023. Health risk assessment of metals in chicken meat and liver in Egypt. Environ. Monitor. Assess 195(7), 802.

Khalafalla, F.A., Ali, F.H., Schwagele, F. and Abd-El-Wahab, M.A. 2011. Heavy metal residues in beef carcasses in Beni-Suef abattoir, Egypt. Veter. Ital. 47(3), 351–361.

Kim, H.S., Kim, Y.J. and Seo, Y.R. 2015. An overview of Carcinogenic heavy metal: molecular toxicity mechanism and prevention. J. Cancer Prev. 20(4), 232–240.

Lasat, M.M. 1999. Phytoextraction of metals from Contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J. Hazard. Sub. Res. 2, 1.

Liang, G., Gong, W., Li, B., Zuo, J., Pan, L. and Liu, X. (2019) Analysis of heavy metals in foodstuffs and an assessment of the health risks to the general public via consumption in Beijing, China. Int. J. Environ. Res. Public Health 16, 6.

Liu, Z.P. 2003. Lead poisoning combined with cadmium in sheep and horses in the vicinity of non-ferrous metal smelters. Sci. Total Environ. 309(1–3), 117–126.

López-Alonso, M., Miranda, M., Benedito, J.L., Pereira, V. and García-Vaquero, M., 2016. Essential and toxic trace element concentrations in different commercial veal cuts in Spain. Meat Sci., 121, 47–52.

Okoye, E.A., Bocca, B., Ruggieri, F., Ezejiofor, A.N., Nwaogazie, I.L. and Frazzoli, C. 2022. Arsenic and toxic metals in meat and fish consumed in Niger delta, Nigeria: employing the margin of exposure approach in human health risk assessment. Food Chem. Toxicol. 159, 112767.

Onianwa, P.C., Lawal, J.A., Ogunkeye, A.A. and Orejimi, B.M., 2000. Cadmium and nickel composition of nigerian foods. J. Food Compos Anal. 13(6), 961–969.

Pamphlett, R., Bishop, D.P., Kum Jew, S. and Doble, P.A., 2018. Age-related accumulation of toxic metals in the human locus ceruleus. PloS One 13(9), e0203627.

Renwick, A.G., 2006. Toxicology of micronutrients: adverse effects and uncertainty. J. Nutr. 136(2), 493S–501S.

Sallam, K.I., Abd-Elghany, S.M. and Mohammed, M.A. 2019. Heavy metal residues in some fishes from Manzala Lake, Egypt, and their health-risk assessment. J. Food Sci. 84(7), 1957–1965.

Sedki, A., Lekouch, N., Gamon, S. and Pineau, A. 2003. Toxic and essential trace metals in muscle, liver and kidney of bovines from a polluted area of Morocco. Sci. Total Environ. 317(1–3), 201–205.

Shaheen, N., Ahmed, M.K., Islam, M.S., Habibullah-Al-Mamun, M., Tukun, A.B. and Islam, S. 2016. Health risk assessment of trace elements via dietary intake of 'non-piscine protein source' foodstuffs (meat, milk and egg) in Bangladesh. Environ. Sci. Pollut. Res. Int. 23(8), 7794–7806.

Smith, N.W., Fletcher, A.J., Hill, J.P. and McNabb, W.C. 2022. Modeling the contribution of meat to global nutrient availability. Front. Nutr. 9, 766796.

Tangahu, B.V., Sheikh Abdullah, S.R., Basri, H., Idris, M., Anuar, N. and Mukhlisin, M. 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. International J. Chem. Engin. 2011, 1–31.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. 2012. Heavy metal toxicity and the environment. Exper. Suppl. 101, 133–164.

US EPA, 2014. Reference Dose (RfD): Description and use in health risk assessments | US EPA. Available from: https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments (Accessed 17 November 2023).

Vicas, S.I., Laslo, V., Timar, A.V., Balta, C., Herman, H., Ciceu, A., Gharbia, S., Rosu, M., Mladin, B., Chiana, L., Prokisch, J., Puschita, M., Miutescu, E., Cavalu, S., Cotoraci, C. and Hermenean, A. 2021. Nano selenium-enriched probiotics as functional food products against cadmium liver toxicity. Materials (Basel) 14(9), 2257.

Waegeneers, N., Pizzolon, J.-C., Hoenig, M. and Temmerman, L. de, 2009. Accumulation of trace elements in cattle from rural and industrial areas in Belgium. Food Addit. Contam. Part A 26(3), 326–332.

Wehedy, S.E.E., Darwish, W.S., Tharwat, A.E. and Hafez, A.E. 2018. Estimation and health risk assessment of toxic metals and antibiotic residues in meats served at hospitals in Egypt. J. Vet. Sci. Technol. 9, 2.

World Health Organization. Programme of Food Safety and Food Aid and Global Environment Monitoring System. Food Contamination and Monitoring Programme, 2012. GEMS/Food regional diets : regional per capita consumption of raw and semi-processed agricultural commodities/ prepared by the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food). WHO/ FSF/FOS/98.3. Available from: https://iris.who.int/handle/10665/63989?andlocale-attribute=es.

Yi, Y., Yang, Z. and Zhang, S. 2011. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environ. Pollut. (Barking, Essex: 1987), 159(10), 2575–2585.

Živkov Baloš, M., Jakšić, S. and Ljubojević Pelić, D. 2019. The role, importance and toxicity of arsenic in poultry nutrition. World's Poult. Sci. J. 75(3), 375–386.