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Quantification of Heavy Metals in Ready-To-Drink Milk Tea Samples Collected from Markets of Multan, Pakistan

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ABSTRACT

This study investigates the quantification of heavy metals in milk tea prepared in various restaurants in Multan, Pakistan, assessing the associated health risks and performing a proximate analysis of its key components. A total of 75 tea samples were collected from lower-, middle-, and upper-class restaurants and analyzed for heavy metal content using Atomic Absorption Spectroscopy (AAS) after a rigorous sample preparation and wet digestion process. Toxic metals, including arsenic, lead, and cadmium, were evaluated alongside essential minerals such as iron, zinc, copper, and cobalt. Results revealed arsenic concentrations exceeding WHO permissible limits in 65% of water samples, while lead and cadmium levels surpassed thresholds in 40% and 38% of cases, respectively. Additionally, proximate analyses of black tea and milk were conducted to measure moisture, protein, fat, fiber, and ash contents using established AOAC methods. Statistical analyses (ANOVA and LSD) highlighted significant differences in heavy metal concentrations across the sampled restaurant classes, with lower-class establishments showing the highest contamination levels. Heavy metal contamination poses a serious risk to food safety and public health. This study measured arsenic, cadmium, cobalt, copper, iron, lead, and zinc in milk, tea, water, and sugar from restaurants of different classes. Contamination was highest in lower-class restaurants due to inadequate facilities, while upper-class establishments had the lowest levels. Middle-class restaurants showed moderate contamination, with higher iron and zinc in milk. The findings highlight the health risks of heavy metal exposure and the urgent need for stricter contamination controls to improve food safety standards in Pakistan.

INTRODUCTION

Food has been central to human survival, serving as a primary energy source and the cornerstone of various biological processes since the earliest days of civilization. The relationship between humans and food spans centuries, evolving with advances in science and technology. Early humans relied on minimal scientific knowledge to consume raw plants and animals, but the progression of scientific understanding led to innovations in food processing and preservation (Kadac-Czapska et al., 2022). Despite these advancements, food safety remains a critical issue, as contamination from hazardous substances such as heavy metals, fertilizers, and microbial agents continues to pose significant health risks (Suhani et al., 2021).

Heavy metal contamination in food is especially concerning due to its multiple sources, including environmental pollution, industrial waste, and agricultural practices. These metals are broadly categorized into essential metals—such as iron (Fe), zinc (Zn), and copper (Cu)—which are required in trace amounts for biological functions, and non-essential metals—such as lead (Pb), cadmium (Cd), and mercury (Hg)—which are toxic even at low concentrations (Shahjahan et al., 2022). While essential metals play crucial roles in human metabolism, their excessive accumulation can lead to toxicity. Non-essential heavy metals, on the other hand, are associated with severe health issues, including oxidative stress, genotoxicity, organ dysfunction, and cancers (Fu & Xi, 2020). Studies have shown that exposure to heavy metals can impair cognitive development, cause chronic diseases, and result in poor health outcomes, particularly among vulnerable populations like children (Farkhondeh et al., 2021).

Heavy metals enter food at various stages, from cultivation to processing and storage. Contamination sources include polluted soil and water as well as industrial byproducts. This global issue has been observed in widely consumed products such as tea, dairy, and grains, with significant geographical variations.

For instance, tea—a widely consumed beverage—has been reported to contain metals like arsenic (As), cadmium (Cd), and lead (Pb) due to soil contamination and packaging materials (Zhang et al., 2021). Studies in Pakistan, China, and Nigeria have quantified the levels of these metals in tea, linking them to potential health risks despite regulatory thresholds (Idrees et al., 2020; Ozukwe et al., 2023).

Recognizing the health risks of heavy metal exposure, global and regional regulatory authorities such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Punjab Food Authority (PFA) have established permissible limits for heavy metals in food. For example, the PFA mandates that arsenic in milk should not exceed 0.1 mg/kg, with corresponding limits for lead (0.02 mg/kg), mercury (1.0 mg/kg), and cadmium (1.5 mg/kg) (Punjab Food Authority, 2018). However, ensuring compliance with these limits remains challenging due to varying environmental and industrial conditions.

The significance of mitigating heavy metal contamination in food goes beyond its health impacts. It also affects food quality, consumer trust, and the global supply chain. This review synthesizes current research on the sources, mechanisms, and health impacts of heavy metal contamination in food, focusing on regulatory measures and potential solutions. By providing a comprehensive overview, this article aims to contribute to enhancing food safety and protecting public health worldwide.

MATERIAL AND METHOD

The purpose of recent study was to evaluate the quantification of heavy metals. Current study was performed in the Department of Food Safety and Quality Management, FFSN, Bahauddin Zakariya University, Multan

Sampling Plan

Seventy-five tea samples were collected from upper, middle, and lower-class restaurants in Multan and stored in polythene envelopes to avoid contamination. The samples were then transported to the Department of Food Safety and Quality Management, BZU, Multan, for analysis under proper storage conditions.

Sample Preparation

Sample's weight was correctly measured by electronic balance and the weight of resistant foil was measured to escape any error. The weight of sample was 0.5 gram that was taken & measured accurately.

Digestion Procedure

Wet Digestion Process

Each flask was filled with 0.5 grams of sample. Subsequently, 10ml of nitric acid and 5ml of perchloric acid were added to each flask. After that, aluminum foil was used to cover each flask, and they were all left in the dark for the night. All of the flasks were put on a hot plate the following day to aid with digestion. When the contents of the flasks were placed on a hot plate, the liquid inside began to fume and turned orange before a clear solution was produced. According to the study done by (Akinyele and Shokunbi, 2015), flasks were taken off the hot plate when there was just one milliliter of solution remaining. Then the dilution was completed by utilizing the distilled water. 50 ml of diluted solution were filtered to make up the entire volume of samples. For further use, all of the filtered solutions were put into plastic bottles and labeled with numbers ranging from 1-75 for spectroscopy examination.

Spectroscopy Analysis

The first indication of the atomic absorption process was found in 1802, when the sun's spectrum included Fraunhofer lines. It was established in 1953 that the AB may be used as a tool for carrying out quantitative processes. In AB, aspiration was carried out using a flame. Because this method only takes three to ten seconds, it was able to comprehend the concentration of both minor and big elements. It was necessary to have a light source to use this procedure. Specific gas was needed for those specific metals. Because the procedure is mechanized, it has been used to evaluate the metals in pharmaceutical and metallurgical samples.

Principle of ABS

Every atom has a specific number of electrons surrounding its nucleus. An atom can exist in two different states: its ground state and its excited state. In an electrical setup, the ground state is stable, while the excited state is unstable. Because of the instability caused by energy release, atoms in the excited or unstable state were able to return to the ground or stable state. Light of a particular wavelength was absorbed by the element when it was passed, allowing for the quantitative measurement of absorbed light. A lamp with a precise source that could only allow certain light to flow through was used (Beaty and Kerber, 1993).

Standards Preparation

Preparations of solutions were used for the standards of 1000 ppm. Lead, cadmium, arsenic, iron, zinc, copper, and cobalt levels in the samples were analyzed using a strong flame created with the use of acetylene and air. First, a standard approach was used to analyze the blanks, and checked the allowable limits of identification for different kinds of heavy metals. Testing was operated for several metal-testing at atomic absorption spectrophotometers (Moniruzzaman and Gan, 2016).

Proximate Analysis

To assess the moisture content, protein level, fat percentage, fiber analysis, and ash content of milk, and black tea proximate analysis was used.

Protein Analysis

AOAC methodology was applied to explore protein (Thiex, 2009). Three steps were used in influential the protein content: first, samples were used for digestion. Subsequently, 3 grams of the samples were placed in the digestion flask and heated by mantle to turn a light hue by combining H_2SO_4 with the digestion mix. For distillation, NaOH solution (4 percent) boric acid and indicator were used produced NH3 gas. 0.1N H_2SO_4 in burette was used to evaluate amount of N present in the sample.

$$\text{N \% age} = \frac{(0.0014 \times \text{ vol. of } 0.1\text{N H2SO4 } \times \text{ 250ml})}{(\text{Volume of dilute sample} \times \text{ Weight of origional volumel})} \times 100$$

$$\text{Protein \% age} = \text{N\% age} \times 6.25$$

Fat Analysis

The Soxhlet apparatus was used to evaluate the fat percentage in the samples. 2 grams of powdered material were weighed in a pre-weighted paper and placed in a flask containing n-hexane, then hexane used to quantify fat %, by soxhlet chamber. The sample was free of oil after 3–4 hours and 7–8 washings. After oil was extracted, sample was placed in petri dish and placed in desiccator for cooling. Fat percentage was measured by given formula:

$$\text{Fat \% age} = \frac{\text{Weight of defated sample}}{\text{Weight of original sample}} \times 100$$

Fiber Analysis

Fiber content was evaluated by acid and base ashing. First, a 0.255 N sulfuric acid solution was used to acid digest a 2-gram material that had been introduced to a beaker. The specimen was cleansed and rinsed with water, mixed with NaOH solution, cooked for 30 minutes, cleaned, and refilled. A crucible was filled, warmed, and soaked, and charred at 550oC for 5-6 hours. The sample was dried & then weighed. After weighing the dry sample, the fiber content was ascertained.

$$\text{Fiber \% age} = \frac{\text{Weight of sample before ashing } - \text{Weight of sample after ashing}}{\text{Weight of original sample}} \times 100$$

Ash Analysis

The AOAC method (2016) was applied to find out the ash % of sample. A 5 g sample was put in crucible and burnt till sample was free from carbon & no black smoke was observed. The crucible sample was put in the furnace at 555°C for burning.

$$\text{Ash \% age} = \frac{\text{Weight of ash content}}{\text{Weight of sample}} \times 100$$

Statistically Analysis

The statistical analysis ANOVA, LSD design, mean, and standard deviation were calculated using the Statistics 8.1 software.

RESULTS AND DISCUSSION

Milk and sugar are key components of milk tea, prepared by steeping tea leaves and flavoring with milk, creamer, or sugar. Heavy metals in water, sugar, black tea, and milk tea pose health risks. This study quantified heavy metals in milk tea from Multan restaurants and performed proximate analysis of milk and black tea (Thiex, 2009; AOAC, 2016).

Quantification of Heavy Metals

Heavy metals in water

The study evaluated toxic metals (lead, cadmium, arsenic) and minerals (iron, zinc, copper, cobalt) in water used for milk tea preparation. Arsenic levels exceeded WHO limits in 65% of samples, ranging from 0.00–0.316 mg/L, consistent with studies by Hussain et al. (2023) and Rasool et al. (2015). Lead levels exceeded permissible limits in 40% of samples (mean 0.0410±0.011 mg/L), aligning with findings by Jehan et al. (2020) and Haq et al. (2011). Cadmium levels (0.001–0.00482 mg/L) were above WHO limits in 38% of samples, similar to Jehan et al. (2020). Copper levels were above NEQS (1993) limits in 35% of samples but within WHO standards, resembling Singh et al. (2014). Iron levels exceeded WHO limits (0.3 mg/L) in 35% of samples, consistent with Rosborg et al. (2005). Zinc levels were within permissible limits, aligning with Jehan et al. (2020).

Table 1

Completely Randomized ANOVA table for heavy metals in water

Heavy metals	Source	DF	SS	MS	F	P
	Treatment	2	0.00163	8.155E-04	63.3	0.0000
As	Error	42	0.00054	1.287E-05	……………..	……………..
	Total	44	0.00217	……………..	……………..	……………..
Cd	Treatment	2	0.11453	0.05727	110	0.0000

	Error	42	0.02181	0.00052	……………..	……………..
	Total	44	0.13635	……………..	……………..	……………..
Co	Treatment	2	0.01190	0.00595	53.5	0.0000
	Error	42	0.00467	0.00011	……………..	……………..
	Total	44	0.01657	……………..	……………..	……………..
Cu	Treatment	2	0.34636	0.17318	111	0.0000
	Error	42	0.06566	0.00156	……………..	……………..
	Total	44	0.41202	……………..	……………..	……………..
Fe	Treatment	2	0.14783	0.07391	792	0.0000
	Error	42	0.00392	0.00009	……………..	……………..
	Total	44	0.15174	……………..	……………..	……………..
Pb	Treatment	2	6.19912	3.09956	37.3	0.0000
	Error	42	3.49150	0.08313	……………..	……………..
	Total	44	9.69062	……………..	……………..	……………..
Zn	Treatment	2	0.00221	0.00110	6.73	0.0029
	Error	42	0.00689	0.00016	……………..	……………..
	Total	44	0.00909	……………..	……………..	……………..

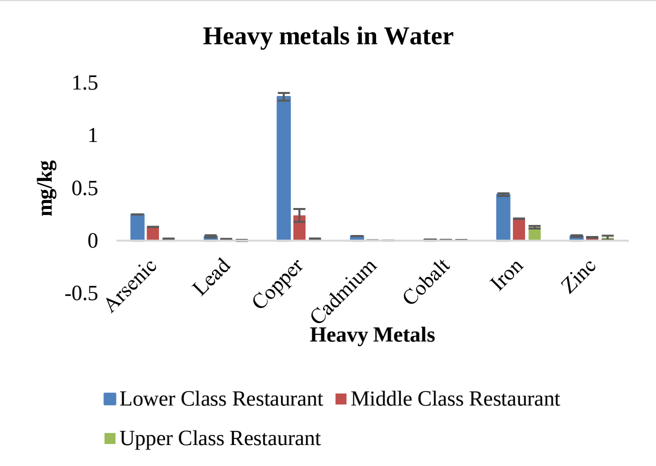
Table 2

Concentration of selected heavy metals and mineral elements in water samples used for the making of milk tea

Food Type	Sampling Class	Arsenic	Pb	Cu	Cd	Co	Fe	Zn
Water	Lower Class Restaurant	0.249± 0.00608^{a}	0.0410± 0.0105^{b}	1.3673± 0.0365^{a}	0.00437± 0.001^{b}	0.00810± 0.00107^{a}	0.4377± 0.01246^{a}	0.0435± 0.0080^{a}
	Middle Class Restaurant	0.131± 0.00115^{b}	0.0102± 0.0068^{b}	0.2399± 0.0613^{b}	0.00265± 0.00071^{c}	0.00682± 0.00092^{b}	0.2091± 0.0011^{b}	0.0316± 0.0034^{b}
	Upper class Restaurant	0.0120± 0.00063^{b}	0.0019± 0.0051^{a}	0.01653± 0.0437^{c}	0.001405± 0.000326^{a}	0.00419± 0.00116^{c}	0.1279± 0.0121^{c}	0.0269± 0.02041^{b}

Figure 1

Heavy metals in water



Heavy metals in black tea

The study quantified three heavy metals (lead, cadmium, arsenic) and four trace minerals (iron, zinc, copper, cobalt) in black tea used for milk tea preparation. Maximum arsenic (0.1607±0.042 mg/kg), lead (1.2727±0.039 mg/kg), and copper (2.1467±0.198 mg/kg) levels were detected in lower-class restaurant samples. Cadmium and cobalt were highest in lower-class samples (0.753±0.0821 mg/kg and 1.3993±0.305 mg/kg, respectively) and lowest in upper-class samples. Iron (0.8627±0.075 mg/kg) and zinc (3.0767±0.517 mg/kg) levels were also highest in lower-class samples. Arsenic levels exceeded the permissible limit (0.001 mg/kg; Patra et al., 2005) in 68% of samples, resembling Shekoohiyan et al. (2012). Lead exceeded WHO's limit (0.5 mg/kg) in 45% of samples, with results aligning with Shekoohiyan et al. (2012) and Zazouli et al. (2010). Cadmium levels exceeded permissible limits (0.5 mg/kg) in 35% of samples, with findings consistent with Sultana et al. (2023). Copper levels exceeded limits in 37% of samples, supporting findings by Record et al. (1996). Trace elements in black tea were often above allowable limits, posing health risks.

Table 3

Completely Randomized ANOVA table for heavy metals in black tea

Heavy metals	Source	DF	SS	MS	F	P
As	Treatment	2	0.07332	0.03666	20.3	0.0000
	Error	42	0.07586	0.00181	………………..	………………..
	Total	44	0.14918	………………..	………………..	………………..
Cd	Treatment	2	0.01177	0.00589	4.11	0.0234
	Error	42	0.06013	0.00143	………………..	………………..
	Total	44	0.07190	………………..	………………..	………………..
Co	Treatment	2	4.32108	2.16054	57.5	0.0000
	Error	42	1.57789	0.03757	………………..	………………..
	Total	44	5.89898	………………..	………………..	………………..
Cu	Treatment	2	19.8010	9.90049	417	0.0000

	Error	42	0.9971	0.02374	………………..	………………..
	Total	44	20.7981	………………..	………………..	………………..
Fe	Treatment	2	0.93438	0.46719	190	0.0000
	Error	42	0.10309	0.00245	………………..	………………..
	Total	44	1.03747	………………..	………………..	………………..
Pb	Treatment	2	8.12993	4.06496	581	0.0000
	Error	42	0.29388	0.00700	………………..	………………..
	Total	44	8.42380	………………..	………………..	………………..
Zn	Treatment	2	29.0390	14.5195	152	0.0000
	Error	42	4.0008	0.0953	………………..	………………..
	Total	44	33.0398	………………..	………………..	………………..

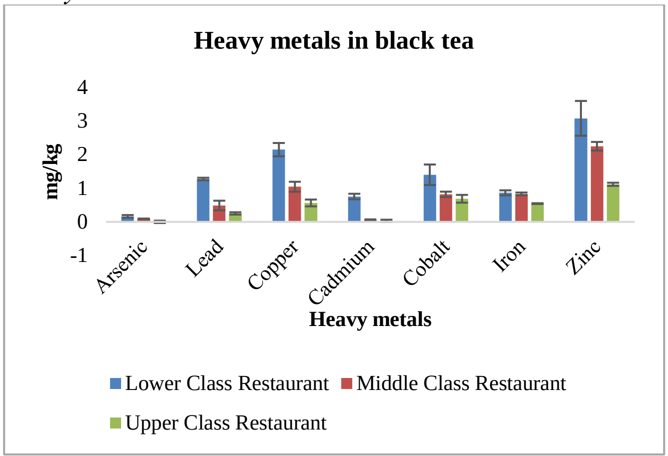
Table 4

Concentration of selected heavy metals and mineral elements in black tea samples used for the making of milk tea

Food Type	Sampling Class	Arsenic	Pb	Cu	Cd	Co	Fe	Zn
Black tea	Lower Class Restaurant	0.1607± 0.0415^{a}	1.2727± 0.0385^{a}	2.1467± 0.1975^{a}	0.753± 0.0821^{a}	1.3993± 0.3050^{a}	0.8627± 0.0748^{a}	3.0767± 0.5169^{a}
	Middle Class Restaurant	0.0912± 0.0072^{b}	0.4867± 0.1423^{b}	1.0433± 0.1484^{b}	0.0487± 0.0247^{ab}	0.8180± 0.0796^{b}	0.8309± 0.0404^{a}	2.2473± 0.1282^{b}
	Upper-class Restaurant	0.00101± 0.0354^{b}	0.2514± 0.0347^{c}	0.5620± 0.1010^{c}	0.0311± 0.03280^{b}	0.6860± 0.1156^{b}	0.5423± 0.01196^{b}	1.1167± 0.0467^{c}

Figure 2

Heavy metals in black tea



Heavy metals in milk

The study quantified arsenic, lead, cadmium, copper, cobalt, iron, and zinc in milk used for milk tea. Arsenic levels ranged from 0.00–0.0812 mg/L, with 63% samples exceeding the Codex limit (0.05 mg/L). Lead levels (0.01–0.527 mg/L) exceeded the WHO limit (0.02 mg/L) in 44% of samples, with maximum levels in lower-class restaurant milk. Cadmium and cobalt were highest in lower-class samples (0.1649±0.0376 mg/L). Copper exceeded WHO's limit (2.0 mg/L) in 36% of samples. Iron (0.8033±0.214 mg/L) and zinc (0.9333±0.175 mg/L) peaked in middle-class samples. Results align with previous studies on milk contamination.

Table 5

Completely Randomized ANOVA table for heavy metals in milk

Heavy metals	Source	DF	SS	MS	F	P
As	Treatment	2	0.01690	0.00845	4.33	0.0195
	Error	42	0.08195	0.00195	……………..	……………..
	Total	44	0.09886	……………..	……………..	……………..
Cd	Treatment	2	0.17656	0.08828	167	0.0000
	Error	42	0.02220	0.00053	……………..	……………..
	Total	44	0.19876	……………..	……………..	……………..
Co	Treatment	2	0.00134	6.717E-04	1.90	0.1627
	Error	42	0.01487	3.541E-04	……………..	……………..
	Total	44	0.01622	……………..	……………..	……………..
Cu	Treatment	2	8.39374	4.19687	113	0.0000
	Error	42	1.55989	0.03714	……………..	……………..
	Total	44	9.95363	……………..	……………..	……………..
Fe	Treatment	2	1.82326	0.91163	54.2	0.0000
	Error	42	0.70626	0.01682	……………..	……………..
	Total	44	2.52951	……………..	……………..	……………..
Pb	Treatment	2	1.33015	0.66508	6600	0.0000
	Error	42	0.00423	0.00010	……………..	……………..
	Total	44	1.33438	……………..	……………..	……………..
Zn	Treatment	2	0.34263	0.17131	4.94	0.0119
	Error	42	1.45731	0.03470	……………..	……………..
	Total	44	1.79994	……………..	……………..	……………..

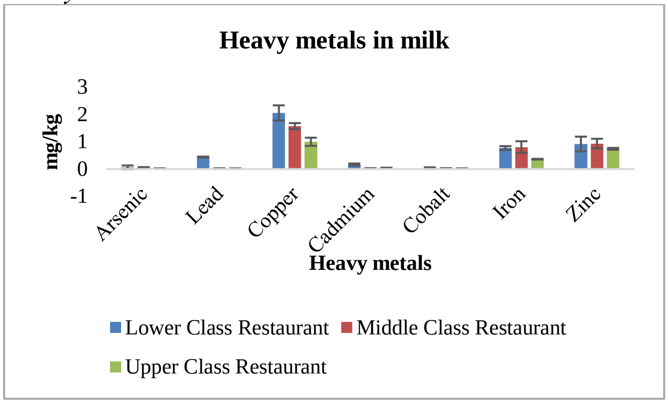
Table 6

Concentration of selected heavy metals and mineral elements in milk samples used for the making of milk tea

Food Type	Sampling Class	Arsenic	Pb	Cu	Cd	Co	Fe	Zn
Milk	Lower Class Restaurant	0.0615± 0.0704^{a}	0.4415± 0.0144^{a}	2.0560± 0.2772^{a}	0.1649± 0.0376^{a}	0.0318± 0.0314^{a}	0.7720± 0.0674^{a}	0.9176± 0.2691^{a}
	Middle Class Restaurant	0.0479± 0.0329^{b}	0.0196± 0.00816^{b}	1.5720± 0.1114^{b}	0.0263± 0.0055^{b}	0.0261± 0.0052^{a}	0.8033± 0.2139^{a}	0.9333± 0.1748^{a}
	Upper class Restaurant	0.0171± 0.0080^{b}	0.0175± 0.0073^{c}	0.9993± 0.1489^{c}	0.0387± 0.0119^{b}	0.0185± 0.0072^{a}	0.3615± 0.0128^{b}	0.7409± 0.0336^{b}

Figure 3

Heavy metals in milk



Heavy metals in sugar

The study quantified arsenic, lead, cadmium, copper, cobalt, iron, and zinc in sugar used for milk tea. Arsenic levels ranged from 0.003–0.215 mg/kg, with 61% exceeding the WHO limit (0.1 mg/kg). Lead levels ranged from 0.04–0.617 mg/kg, with 37% surpassing the WHO limit (0.5 mg/kg). Copper, cobalt, iron, and zinc were highest in sugar from lower-class restaurants but mostly within permissible limits. Results align partially with previous studies, highlighting contamination concerns in lower-class samples.

Table 7

Completely Randomized ANOVA table for heavy metals in sugar

Heavy metals	Source	DF	SS	MS	F	P
As	Treatment	2	0.00086	4.313E-04	6.09	0.0048
	Error	42	0.00298	7.087E-05	……………..	……………..
	Total	44	0.00384	……………..	……………..	……………..
Cd	Treatment	2	0.00520	0.00260	85.1	0.0000
	Error	42	0.00128	0.00003	……………..	……………..
	Total	44	0.00649	……………..	……………..	……………..
Co	Treatment	2	0.02165	0.01082	12.8	0.0000
	Error	42	0.03555	0.00085	……………..	……………..
	Total	44	0.05719	……………..	……………..	……………..
Cu	Treatment	2	2.01707	1.00853	1194	0.0000
	Error	42	0.03546	0.00084	……………..	……………..
	Total	44	2.05253	……………..	……………..	……………..
Fe	Treatment	2	1.94497	0.97249	60.4	0.0000
	Error	42	0.67571	0.01609	……………..	……………..
	Total	44	2.62068	……………..	……………..	……………..
Pb	Treatment	2	0.05619	0.02810	0.80	0.4571
	Error	42	1.47939	0.03522	……………..	……………..
	Total	44	1.53558	……………..	……………..	……………..
Zn	Treatment	2	2.47681	1.23841	54.4	0.0000
	Error	42	0.95667	0.02278	……………..	……………..
	Total	44	3.43348	……………..	……………..	……………..

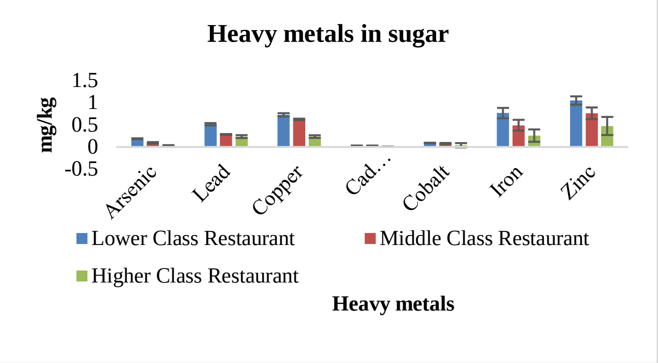
Table 8

Concentration of selected heavy metals and mineral elements in sugar samples used for the making of milk tea

Food Type	Sampling Class	Arsenic	Pb	Cu	Cd	Co	Fe	Zn
Sugar	Lower Class Restaurant	0.1856± 0.0104^{a}	0.514± 0.0254^{a}	0.7261± 0.0368+	0.0285± 0.0046^{a}	0.0905± 0.0052^{a}	0.7653± 0.1170^{a}	1.0480± 0.0948^{a}
	Middle Class Restaurant	0.0932± 0.0163^{b}	0.2816± 0.0101^{a}	0.6227± 0.01616^{b}	0.0250± 0.00771^{a}	0.0767± 0.0133^{a}	0.4900± 0.1229^{b}	0.7607± 0.1319^{b}
	Upper-class Restaurant	0.0287± 0.0120^{b}	0.2349± 0.3242^{a}	0.2343± 0.0303^{c}	0.0041± 0.0033^{b}	0.0387± 0.0483^{b}	0.2567± 0.1395^{c}	0.4733± 0.2048^{c}

Figure 4

Heavy metals in sugar



Arsenic levels ranged from 0.02–0.425 mg/L, with 65% exceeding the WHO limit of 0.2 mg/L. Lead levels ranged from 0.00–0.751 mg/L, with 36% exceeding permissible limits. Copper and cadmium were highest in lower-class restaurant samples, while iron and zinc levels varied across classes. Results highlight contamination concerns, especially in lower-class samples.

Heavy metals in milk tea

The study quantified seven heavy metals and four trace elements in milk tea from different-class restaurants.

Table 9

Completely Randomized ANOVA table for heavy metals in milk tea

Heavy metals	Source	DF	SS	MS	F	P
As	Treatment	2	0.03792	0.01896	0.70	0.5027
	Error	42	1.13900	0.02712	…………….	…………….
	Total	44	1.17692	…………….	…………….	…………….
Cd	Treatment	2	0.22791	0.11396	474	0.0000
	Error	42	0.01010	0.00024	…………….	…………….
	Total	44	0.23802	…………….	…………….	…………….
Co	Treatment	2	0.26736	0.13368	2.29	0.1141
	Error	42	2.45549	0.05846	…………….	…………….
	Total	44	2.72286	…………….	…………….	…………….
Cu	Treatment	2	0.13190	0.06595	24.1	0.0000
	Error	42	0.11497	0.00274	…………….	…………….
	Total	44	0.24687	…………….	…………….	…………….
Fe	Treatment	2	0.06103	0.03052	38.6	0.0000
	Error	42	0.03317	0.00079	…………….	…………….
	Total	44	0.09420	…………….	…………….	…………….
Pb	Treatment	2	1.00890	0.50445	35.9	0.0000
	Error	42	0.58942	0.01403	…………….	…………….
	Total	44	1.59832	…………….	…………….	…………….
Zn	Treatment	2	0.33387	0.16694	2.76	0.0746
	Error	42	2.53729	0.06041	…………….	…………….
	Total	44	2.87116	…………….	…………….	…………….

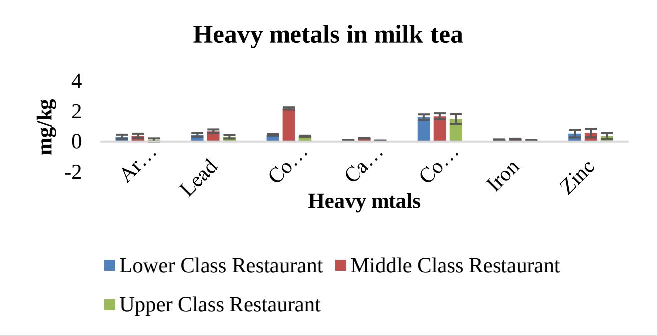
Table 10

Concentration of selected heavy metals and mineral elements in milk tea samples

Food Type	Sampling Class	Arsenic	Pb	Cu	Cd	Co	Fe	Zn
Milk Tea	Lower Class Restaurant	0.3747± 0.1493[a]	0.6813± 0.1224[a]	2.191± 0.0726[a]	0.2247± 0.0186[a]	1.6760± 0.1945[ab]	0.1633± 0.0310[a]	0.5640± 0.2857[a]
	Middle Class Restaurant	0.3227± 0.1362[a]	0.4407± 0.1151[b]	0.4515± 0.05712[a]	0.0849± 0.01864[b]	1.6207± 0.1801[a]	0.1215± 0.0225[b]	0.5300± 0.2535[ab]
	Upper-class Restaurant	0.0987± 0.112[a]	0.3213± 0.1177[c]	0.3530± 0.0399[b]	0.0647± 0.0053[c]	1.4920± 0.3243[b]	0.0732± 0.0300[c]	0.3667± 0.1880[b]

Figure 5

Heavy metals in milk tea



Proximate analysis of Black tea

This study analyzed the proximate composition and heavy metal content of black tea, milk, milk tea, sugar, and water. Middle-class restaurant milk showed the highest protein (3.3567±0.154) and ash (1.0727±0.147), while upper-class milk had the highest fiber (6.35±0.0027). Fat content was highest in middle-class samples. Results align partially with studies by Serpen et al. (2012) and Rahman et al. (2019), with notable variations in protein, fiber, and ash content.

Table 11

Completely Randomized ANOVA table for Proximate analysis of Black tea

Proximate	Source	DF	SS	MS	F	P
Protein	Treatment	2	4.11721	2.05861	91.2	0.0000
	Error	42	0.94779	0.02257	………	………
	Total	44	5.06500	………	………	………
Ash	Treatment	2	4.11721	2.05861	91.2	0.0000
	Error	42	0.94779	0.02257	………	………
	Total	44	5.06500	………	………	………
Fiber	Treatment	2	0.44659	0.22330	8.77	0.0007
	Error	42	1.06953	0.02547	………	………
	Total	44	1.51612	………	………	………
Fat	Treatment	2	1.42705	0.71353	12.7	0.0000
	Error	42	2.36283	0.05626	………	………
	Total	44	3.78988	………	………	………

Table 12

Proximate analysis of Black tea

Food Type	Sampling Class	Protein	Ash	Fiber	Fat
Black tea	Lower Class Restaurant	7.0913±0.845^{a}	5.3107±0.147^{bc}	7.6100±0.177^{a}	1.8380±0.294^{a}
	Middle Class Restaurant	5.7753±0.201^{b}	5.6300±0.143^{b}	7.4853±0.115^{ab}	1.6827±0.180^{b}
	Upper-class Restaurant	5.5860±0.404^{bc}	6.0493±0.160^{a}	7.3660±0.178^{ab}	1.4073±0.224^{c}

Proximate Analysis of Milk

Proximate analysis (Table 4.14) revealed middle-class restaurant milk had the highest protein (2.9753±0.243), ash (1.0727±0.147), and fat (3.3567±0.154), while upper-class milk had the highest fiber (6.35±0.0027). Lower-class samples showed the lowest values overall. Previous studies by Kuma et al. (2015) and Dandare et al. (2014) reported higher levels of protein, ash, fiber, and fat than this study.

Table 13

Completely Randomized ANOVA table for Proximate analysis of Milk

Proximate	Source	DF	SS	MS	F	P
Protein	Treatment	2	7.19141	3.59571	105	0.0000
	Error	42	1.43807	0.03424		
	Total	44	8.62948			
Ash	Treatment	2	4.09689	2.04845	67.7	0.0000
	Error	42	1.27139	0.03027		
	Total	44	5.36828			
Fiber	Treatment	2	2.848E-04	1.424E-04	48.6	0.0000
	Error	42	1.230E-04	2.929E-06		
	Total	44	4.078E-04			
Fat	Treatment	2	12.1236	6.06180	219	0.0000
	Error	42	1.1611	0.02765		
	Total	44	13.2847			

Table 14

Proximate Analysis of Milk

Food Type	Sampling Class	Protein	Ash	Fiber	Fat
Milk	Lower Class Restaurant	2.0527±0.173^{c}	0.3533±0.124^{c}	4.84±0.0012^{b}	2.2160±0.186^{b}
	Middle Class Restaurant	2.9753±0.243^{a}	1.0727±0.147^{a}	1.79±0.0004^{bc}	3.3567±0.154^{a}
	Upper class Restaurant	2.2300±0.118^{b}	0.5660±0.232^{b}	6.35±0.0027^{a}	2.3000±0.157^{b}

CONCLUSION

Heavy metal contamination poses a significant threat to food safety and consumer health. This study quantified heavy metals (arsenic, cadmium, cobalt, copper, iron, lead, and zinc) in milk, tea, water, and sugar from restaurants of varying classes. Lower-class restaurants showed the highest contamination levels due to limited food treatment facilities, while upper-class restaurants had the lowest, benefiting from better equipment and practices. Middle-class restaurants showed intermediate levels, with iron and zinc notably higher in their milk samples. These findings highlight the need for improved contamination control in lower-class food establishments to safeguard public health.

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