



Full Length Article

Assessment of Cadmium, Lead and Copper in Milk and Associated Risks to Consumer Health

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Abstract

Heavy metal contamination is a rising issue in food safety and quality due to its negative effects on human health. Increased urbanization and industrialization have led to heavy metals entering the food chain through natural (High backyard) and anthropogenic sources (mineral fertilizers, industries, and polluted water). These sources directly or indirectly contaminated human food through air, water and soil uptake which cause various diseases like kidney damage, cardiovascular problems, stomach disease, lungs and carcinogenic in large exposure. The present study was conducted to measure the total concentration of cadmium (Cd), lead (Pb) and copper (Cu) in three milk sources *i.e.*, Brands, Dairy and Vendors through nitric acid (HNO₃) digestion method using atomic absorption spectroscopy. Samples were collected from Raiwand, a region of Punjab, Pakistan. Results were analyzed with the permissible limits of heavy metals as per WHO-FAO regulations and the impacts of these trace heavy metals on the Target Hazard Quotient (THQ) and overall Health index (HI). Among the three milk sources studied, vendor milk showed higher levels of Cd, Pb and Cu contamination than the other samples. More than 80% of tested milk samples Cd, Pb, and Cu samples lie beyond the prescribed permissible limits in all sources. Still, there is a negligible effect on consumer health as per the defined THQ and HI as it's below the defined permissible limit by the Environmental Protection Agency (EPA) of ≤ 1.0 . The children are supposed to be more affected by consuming vendors' milk as significantly different from THQ and HI as compared to adults. Overall reduction was observed in the study metals concentration as compared to the previous studies in Pakistan and worldwide after the implication of Food Department (PFA) and Pakistan Standards and Quality Control Authority (PSQCA) regulations.

Keywords: Milk; Heavy metals Contamination; Health Risk; EPA; WHO; PSQCA

Introduction

Trade, market accessibility, productivity, human health, means of subsistence, and eventually, economic growth can all be impacted by food safety. The relationship between food safety, health, nutrition, food security, market access, trade, socioeconomic impact, gender and youth is amply demonstrated by the literature. Public health initiatives are helping to raise consumer knowledge of food safety, and African consumers are starting to demand guarantees of safe meals, especially in urban and affluent areas (Leone *et al.* 2022). Mammary glands in female animals secrete milky liquid called milk, which is used as nourishment for young mammals. Furthermore, one of the most important commodities consumed worldwide is milk production. 852

million tons of milk was produced in 2019, up 1.4% from the previous estimate, according to the Food and Agriculture Organization of the United Nations (FAO). The nutritional advantages of milk drinking, including its high protein, mineral, fat, and sugar content, are closely correlated with its high consumption. Dairy products and milk are essential to a healthy, well-balanced diet. Consuming enough milk and dairy products, especially as a child, may improve health and lower the chance of developing osteoporosis, hypertension, and obesity as an adult (Sipple *et al.* 2020). In contrast, some research revealed that the accumulation of heavy metals, pesticides, and drug residues in milk was linked to an increased risk of a number of ailments, including cancer, kidney problems, joint discomfort and cardiovascular disease (Oz *et al.* 2021).

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Metallic elements that have a density 5 times higher than of water are referred to as heavy metals. Assuming that toxicity and heaviness are related, metalloids that can cause toxicity at low exposure levels, like arsenic (As), are also considered heavy metals (Anyanwu *et al.* 2018). Heavy metals, including lead (Pb), mercury (Hg), zinc (Zn), chromium (Cr), cadmium (Cd), nickel (Ni), copper (Cu), zinc (Zn) and mercury (Hg), are known to be harmful to aquatic ecosystems and physiologically unnecessary (Wu *et al.* 2021). Hazardous metals like heavy metals are frequently linked to anthropogenic sources, which include but are not limited to the nonferrous metallurgical industry, mining, mineral processing, electroplating, leather tanning and chemical industries. The degree of toxicity displayed by these dangerous substances depends on how much and how long living things are exposed to them (Sarker *et al.* 2023).

Pb is ranked second on the Substance Priority List 2022 of the Agency for Toxic Substances and Disease Registry (ATSDR) and is one of the 10 compounds of public health concern according to the World Health Organization (WHO) based on toxicity and potential for human exposure. Even though lead (Pb) is found naturally in the Earth's crust, Pb is released into the air, soil and/or water by human activities such as mining, ores-melting, burning coal and the battery business. Pb causes delayed onset of diseases such as obesity, infertility, cancer, metabolic alteration, autoimmune disorder, mental disease, cardiovascular and neurodegenerative disorders. It is also linked to early life effects such as preterm birth, in utero growth restriction, decreased birth weight, birth defects and cognitive impairment (Kushwaha *et al.* 2018; Briseño-Bugarín *et al.* 2024). It is well known that different parts of the human body contain different amounts of Cd. According to recent epidemiological research, exposure to Cd may also be linked to certain malignancies, including those of the bladder, kidney, pancreas, breast, and prostate. Amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Alzheimer's disease (AD), Parkinsonism and Parkinson's disease (PD), Huntington's disease (HD), and other diseases involving the central nervous system (CNS) may be influenced by it, as well as the decline in cognitive and behavioural abilities (Charkiewicz *et al.* 2023). Similar negative health effects, including as liver damage, kidney failure, neurological abnormalities, and immune system suppression, can result with prolonged exposure to Cu (Covre *et al.* 2022). This research has been focussed to determine the amounts of trace heavy metals (Pb, Cd and Cu) in different milk samples taken from brands, dairy and vendors milk from Riwand region of Pakistan in order to do the human health risk assessment of these milk samples in terms of Pb, Cd, and Cu. WHO/FAO and EPA regulation limits were used to evaluate the fitness criteria and associated health risk to consumers. This study comprised of the following objectives (i) determination of Cd, Cu and Cu total contents in different milk samples (ii) evaluate associated health risk assessment on consumer health by THQ and HI

Table 1: Permissible limits of Pb, Cd and Cu in milk as given by international standards

International standards	Pb	Cd	Cu	References
WHO/FAO	-	0.02 mg/L	0.4 mg/L	(Luqman <i>et al.</i> 2023)
European Commission	20 µg/L	20 µg/L	-	(Hasanvand <i>et al.</i> 2024)
Australia and New Zealand	10-20 µg/L	2.6 µg/L	-	
China	50 µg/L	No limit is set	-	
India	20 µg/L	No limit is set	-	

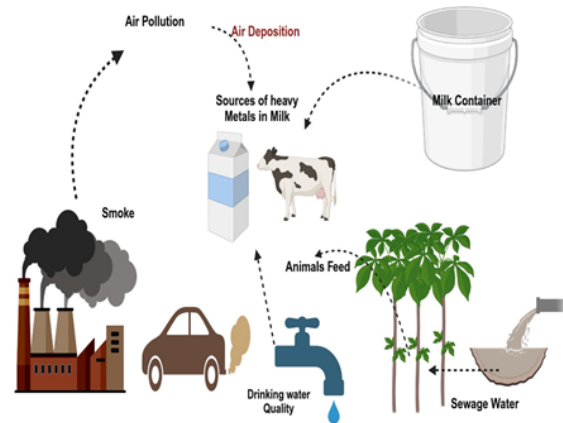


Fig. 1: Sources of heavy metals contamination in milk

value. The permissible limits for Pb, Cd and Cu as set by different international standards are given in Table 1. Different international standards have different range of permissible limits but in this study, we followed the permissible limits of WHO/FAO. Different sources of heavy metals contamination in milk have been described in Fig. 1.

Materials and Methods

Sampling sites and collection of samples

The present study investigated the trace metal contents in different types of milk used/drunk by people in Riwand, Lahore, Pakistan. Samples from different vendors/Shops 1- Raw Milk (Local Raw Milk Seller), 2- Brand Milk (Packing Milk of Different Companies) 3- Milk from Dairies (directly take samples from dairies in the surrounding area). Sampling was done in three locations *i.e.*, (Lahore to Riwand Road, Riwand to Kasur Road and Manga Road) for Vendors/Shops and dairies. Samples of brand Milk was taken from Stores (250 mL tetra pack). Fifteen samples of each source (vendors & dairy) were collected in pre-washed sampling bags, put in Ice cube boxes and immediately shifted to the laboratory, stored at -20 degrees Celsius for further analysis.

Equipment and reagents

Atomic absorption Spectrophotometer (AAS) (Shimadzu,

Japan), Calibrated by an ISO/IEC17025-2017-certified Laboratory, was used to determine the concentrations of Cd, Pd and Cu in the milk samples. Nitric Acid (HNO₃ 65%), hydrogen peroxide (H₂O₂ 35%) and HPLC-grade water were used in sample preparation. All the chemicals used were purchased from CRM-Accredited Supplier (Merck and Sigma-Aldrich). The Reference samples for milk GBW10115 (10 Element Milk Powder) was used to verify method while equipment accuracy by Recovery Test.

Sample preparation and calibration

Present study was designed in University of Agriculture Faisalabad (UAF), Pakistan and all experimental work was done in Tara Group (Pvt. Ltd.) and imperial crop Sciences Laboratory (ISO/IEC Certified Lab.). Heavy metals like Cd, Pb and Cu was determined in milk samples using AAS. Two (2 g) wet samples were added to the digestion Tube, (6 mL Nitric Acid + 1 mL H₂O₂) and digested sample using a hot plate as used by Muhib *et al.* (2016). Standard solution of each metal (Cd, Pb and Cu) was prepared at different concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5 and 5 mg/L from Sigma-Aldrich (USA) Certified Reference Material and regression line (R²) passed for more than 0.995. AAS spectral lines were set to 228.67, 324.57 and 217.35 for Cadmium (Cd), Copper (Cu) and Lead (Pb) respectively. Certified reference material was used to determine the method accuracy, recovery and MDL for testing method and equipment, results of Recovery and MDL were discussed in Table 2. The recovery % of CRM was measured using CRM of Cd, Pb and Cu by Spiked Concentration or Concentration Measured (CM) *i.e.*, Known ppm) and Concentration measured by equipment (CE) as by Equation 1:

$$\text{Recovery}\% = \frac{CE}{CM} \times 100 \quad (\text{Eq.1})$$

Health risk assessment

Human health risk assessment due to the consumption of heavy metals contaminated milk of different sources (Dairy, Brands and Vendors) intake through food chain measured by THQ and sum of individual THQ to measure HI as used by Yan *et al.* (2022). THQ and health index (HI) of non-carcinogenic health risk assessment by drinking heavy metals contaminated milks of sources samples was calculated for adults and children by Equations (Eq. 2 & Eq. 3). The Environment Protection Agency (EPA) guidelines and limits for daily metals intake permissible values used to calculate THQ and HI for non-carcinogenic health risk associated to drinking heavy metals contaminated milk for long time and its exposure to health of adults and children. THQ is defined and calculate using the following equation (Eq. 2).

$$THQ = \frac{EF \times ED \times W_{milk} \times C_{metal}}{RFD \times W_{body} \times TA} \quad (\text{Eq.2})$$

Herein, the description of equation as,

EF = the time of exposure and frequency (exposure

Table 2: Instrument and Method Calibration

CRM (Spiked)	Observed	Recovery	SD ±	MDL (Minimum
Sigma-Aldrich salts	(Average n-3)	(%)	(n-3)	Detection Limit)
*Cd (1 ppm) (CdCl ₂)	0.998	99.8	0.01	0.003
≥ 99)				
*Pb (1 ppm) (PbCl ₂)	0.992	99.2	0.006	0.001
≥ 99)				
*Cu (1 ppm) (CuSO ₄)	1.001	100.1	0.002	0.005
≥ 99)				
Reference Milk (10	----	----	-----	-----
Element)				
**Cd (0.205 ± 0.01)	0.207	100.98	0.002	-----
ppm				
**Pb (0.214 ± 0.01)	0.211	98.60	0.004	-----
ppm				
**Cu (2.15 ± 0.09)	2.14	99.53	0.007	-----
ppm				

*CRM solution of metal (Cd, Pb and Cu) Sigma Aldrich, USA

**Reference Milk (GBW10115, 10 Element Milk Powder) used to verify method, equipment Accuracy by Recovery Test)

frequency, 365 days a year).

ED = life period of human to exposure like average life (70 years).

W milk = average daily milk intake (0.127 kg/day) for children and (0.066 kg/day for adults) defined by FAO, food and agriculture organization.

C Metal (mg/kg) = concentration of metals measured in milk.

W Body = average body weight (kg) for children average weight considers 30 kg and for adults 60 kg.

RFD = References dose per day of toxic metals defined by EPA, lead 0.004 mg/kg, cadmium 0.001 mg/kg and copper 0.04 mg/kg of body weight daily.

The value of (THQ) less than or equal (≤ 1) has no or less effects as non-carcinogenic health risk, and more than ≥ 1 value more has negative impact on humans as non-carcinogenic risk to human health. Target hazard quotient of individual heavy metals calculated and sum of all used to calculated health index impact overall using equation (Eq. 3).

$$HI = \sum \{THQ_{Cu} + THQ_{Pb} + THQ_{Cd} + \dots\} \quad (\text{Eq.3})$$

Similar to THQ the value of health index (HI) less than or equal (≤ 1) has no or less effects as non-carcinogenic health risk, and more than ≥ 1 value more has negative impact on humans as non-carcinogenic risk to human health.

Data analysis

Heavy metal sample was analyzed in triplicates and average results of triplicate used for data analysis. Results were analyzed for descriptive analysis using Excel-2021, Origin-2021 and SPSS Tools of Heavy metals concentration in different milk samples and its impact as THQ and HI.

Results

Concentration of Pb, Cd and Cu in milk samples

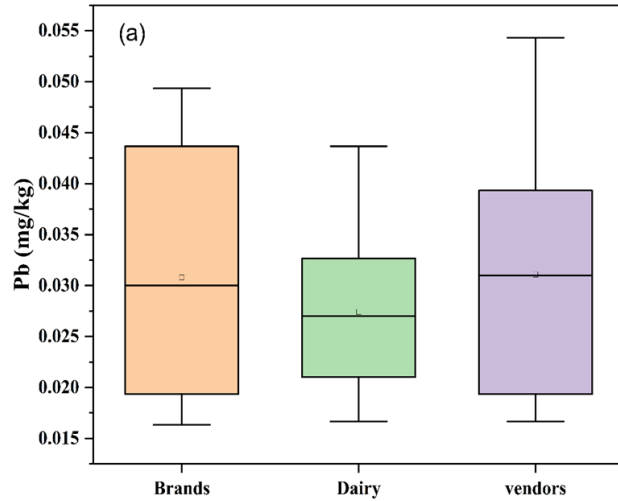


Fig. 2a: Amount of Pb (mg/kg) in milk samples

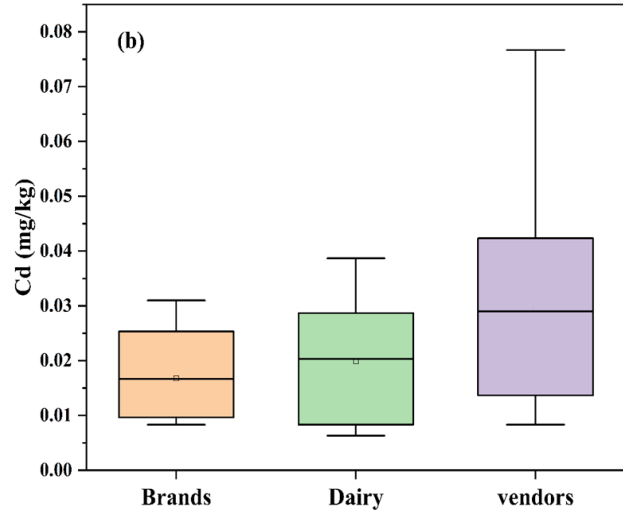


Fig. 2b: Amount of Cd (mg/kg) in milk samples

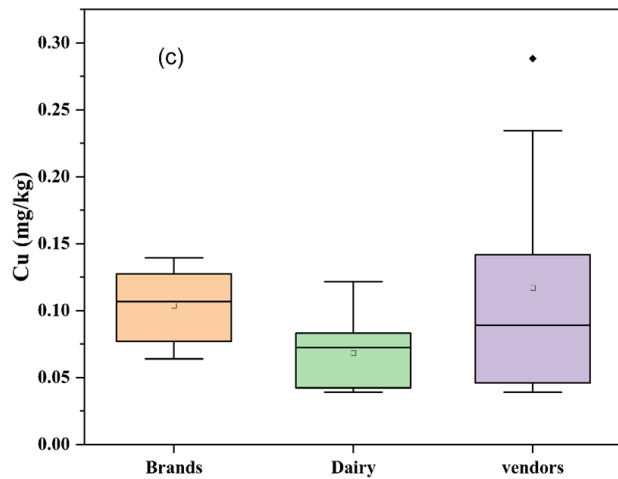


Fig. 2c: Amount of Cu (mg/kg) in milk samples

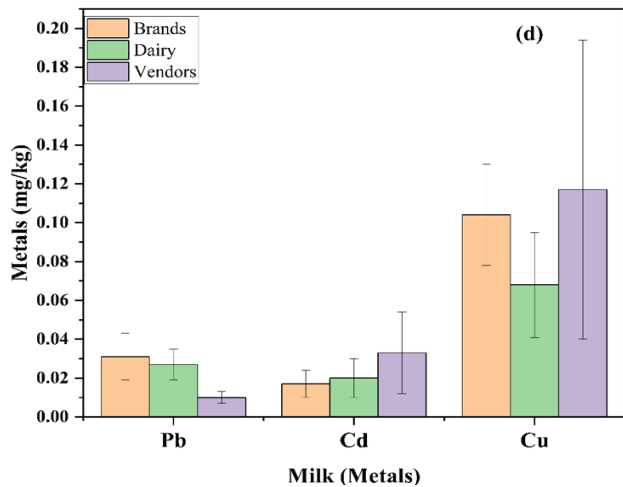


Fig. 2d: Comparison of Cd, Cu, and Pb concentrations (mg/kg) in milk samples

The concentrations (mg/kg) of heavy metals (Pb, Cd and Cu) determined in brands, dairy and vendors milk samples are shown in Fig. 2a–c. The comparison of their concentrations is shown in Fig. 2d. The amount of Pb was ranged 0.017–0.049, 0.017–0.039 and 0.017–0.054 mg per 1 L of milk in brands, dairy and vendors milk samples respectively. The amount of Cd was ranged 0.008–0.031, 0.006–0.039 and 0.008–0.077 in brands, dairy and vendors milk, respectively. Similarly, the amount of Cu was ranged from 0.064 to 0.139 in brand milk, 0.039–0.122 in dairy milk and 0.039–0.288 in vendors milk samples. The vendors milk samples showed more pollution of Pb, Cd and Cu as compared to the other milk samples under study.

Target Hazard Quotient (THQ)

THQ is a risk assessment metric that is used to analyze the

possible health risks associated with consuming food, water, or other sources that may expose one to hazardous substances like chemicals or heavy metals. When assessing the likelihood of side effects that are not cancer-causing, the THQ is very helpful (Su *et al.* 2021). The health risk due to the contamination of Pb, Cd and Cu in brands, dairy and vendor milk samples is shown by THQ in Fig. 3 (for adults) and Fig. 4 (for children). The Cd contamination pose more health risk in all the 3 brands, dairy and vendors milk samples as compared to the Pb and Cu by keeping in view the amounts detected of all 3 heavy metals and their concerned health risks.

Health Index (HI)

The HI affected by consuming milk of brand, dairy and vendors in adults and children has been shown in Fig. 5 which

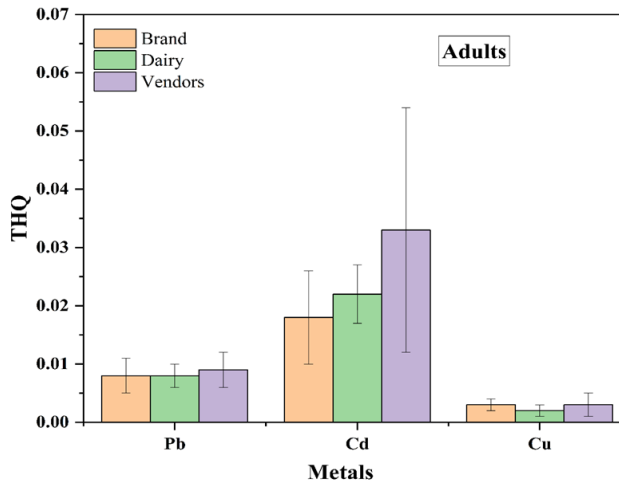


Fig. 3: Target Hazard Quotient (THQ) in adults

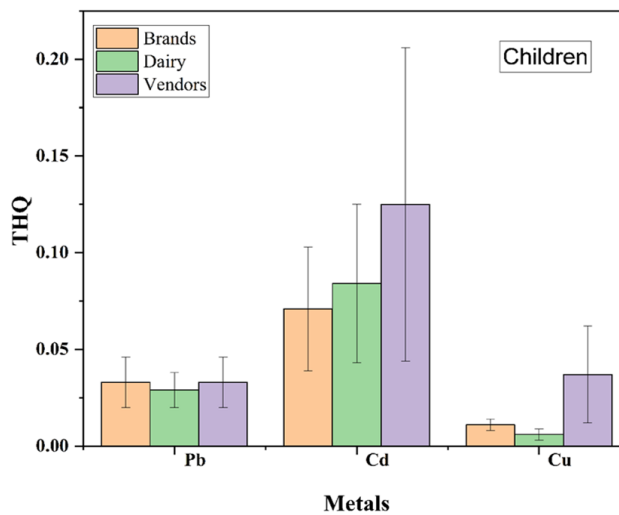


Fig. 4: Target Hazard Quotient (THQ) in children

shows the vendors milk affected more HI in both adults and children. Milk from vendors is more prone to contamination, has less sanitation, and has poor quality control.

Discussion

As compared to all the 3 heavy metals under investigation, Cu showed more contamination in milk samples. It's possible that Cu contaminated milk samples came into touch with Cu-made tools and utensils. If milk comes into contact with copper or copper-alloy equipment (such as pipes, containers or utensils) during the stages of milking, processing, and storage, it may leach copper ions into the milk, causing contamination (Norouzirad *et al.* 2018). The likelihood of copper contamination can be raised by neglecting to maintain pasteurizers, storage tanks or milking machines that use copper or copper-alloy components (Mahmoudi *et al.* 2017). Cows that drink more copper-rich feed or water may pass on this excess copper to their

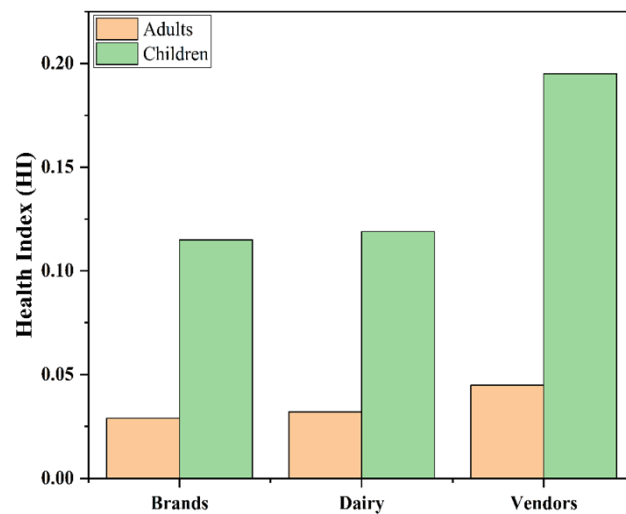


Fig. 5: Health Index (HI) affected by consuming milk of brand, dairy, and vendors in adults and children

offspring. This may occur as a result of using copper-based supplements or because feed crops are contaminated by copper-containing soil or water sources (Tahir *et al.* 2017). Exposure to the environment can lead to copper pollution, especially in regions where copper is abundant in the groundwater or soil. Plants and water can absorb copper, which can then build up in animal products like milk (Luqman *et al.* 2023). Copper contamination of dairy animals' feed can also result from the use of copper-based fertilizers and pesticides in agriculture, and this contamination can then show up in the animals' milk (Abedi *et al.* 2020).

Our results showed lesser amounts of heavy metals (Pb, Cd and Cu) determined in milk samples in all the brands, dairy and vendors milk samples as compared to the similar research conducted in the region of Pakistan in 2015 (Akhtar *et al.* 2015). It showed the positive effect of regulatory bodies *i.e.*, Pakistan Standards and Quality Control Authority (PSQCA) and Punjab Food Authority (PFA) in implementation of food laws and regulations to milk and dairy products in Pakistan.

Compared to Cu and Pb, Cd is more dangerous to health since it can be harmful even at low exposure levels (Zafarzadeh *et al.* 2022). It builds up in the body over time, particularly in the liver and kidneys, and with extended exposure, it can cause serious health problems. Although Pb and Cu are harmful as well, it usually takes larger exposure levels to have comparable long-term effects (Jaafarzadeh *et al.* 2023). Since cadmium has a lengthy biological half-life, especially in the kidneys, it remains in the body for many years after it first enters. Even with a minimal daily consumption, continuous low-level exposure can cause a progressive buildup that can lead to toxicity. While Pb and Cu also bioaccumulate, they are often eliminated more quickly than cadmium (Elafify *et al.* 2023).

Unlike Cu, a vital trace element needed for regular

biological functions like red blood cell production and enzyme activity, Cd has no known biological role in the human body. Pb damages several organ systems, especially the nervous system, but at comparable exposure levels, its effects—particularly in cases of milk contamination might not be as bad as those of cadmium (Chirinos-Peinado *et al.* 2022).

Our results are in agreement with Muhib *et al.* (2016) in which the Cd showed more THQ values as compared to the Pb and Cu THQ values.

As per our results, THQ values of heavy metals are higher in children than adults. Children are particularly susceptible to the harmful effects of heavy metals because of a number of physiological and behavioural characteristics. Individual body weight is taken into account in THQ computations. Children's bodies are more susceptible to the effects of the same quantity of pollutant taken as those of adults since they weigh substantially less than adults. Because they are growing and have faster metabolic rates than adults, children require more food and water per unit of body weight. A youngster, for instance, might take more milk, fruits, or other foods in proportion to their body size than an adult does. This implies that children might consume higher concentrations of pollutants in relation to their smaller size, which would raise the contaminant's estimated daily intake (EDI) and raise the THQ (Bortey-Sam *et al.* 2015; Gao *et al.* 2020; Almeida *et al.* 2022).

The Hazard/Health Index, or HI for short, is a technique for risk assessment that is used to determine the total possible health risk that results from exposure to various hazardous substances like heavy metals by eating, breathing or coming into contact with the skin. In research on food safety and the environment, it is frequently used to assess whether exposure to many pollutants at once is a serious risk to one's health (Yan *et al.* 2022). Pb, Cd, Cu and other heavy metals can have a substantial impact on the HI, particularly when ingested through milk. The individual THQs for each heavy metal are summed to determine the HI when the milk contains multiple heavy metals (Feizi *et al.* 2022).

Vendor milk can get contaminated since it is frequently gathered and kept using antiquated or non-standard techniques. For instance, heavy metals may seep into milk if it is kept in contaminated areas or kept in copper or galvanized vessels (Năstăsescu *et al.* 2020). Contamination levels may rise much more if these containers are not properly maintained or cleaned. The risk of metal contamination in branded and dairy milk is greatly decreased by the use of food-grade materials or stainless steel during collection and processing (Miclean *et al.* 2019). Vendors occasionally dilute milk with water, which can introduce pathogens, particularly if the water isn't fit for human consumption. Heavy metals including Pb, Cd, Cu, and Hg may be present in contaminated water, and when consumed, they can all raise the HI level. Dairy and branded

milk is usually inspected for adulteration, filtered and pasteurized, reducing the possibility of waterborne pathogens, heavy metals, and other chemical contaminants (Zhou *et al.* 2019).

When made a comparison of the present results with previous studies, it is revealed that the most prevalent industrial metal that can contaminate the air, water, soil, and food chain is Pb (Raikwar *et al.* 2008). Anthropogenic processes like mining, smelting and refining are the cause of the widespread presence of lead in the environment. The remobilization of historical sources, such as lead in soil, sediment, and water from mining regions, as well as natural processes including volcanic activity, geochemical weathering, and sea spray emissions, are additional sources of lead in the environment. According to estimates from the Institute for Health Metrics and Evaluation, lead exposure caused 24.4 million Disability-Adjusted Life Years (DALYs) and 1.06 million deaths in 2017. Dietary lead exposure disrupts the circulatory and neurological systems and has an impact on a number of other body organs (Malhat *et al.* 2012). Additionally, it might result in anemia, spontaneous absorption, elevated blood pressure, renal failure, behavioral abnormalities, neurological illnesses, joint weakness and a decrease in IQ (Neto *et al.* 2019). As indicated in Table 3, the concentration of Pb in raw milk in Pakistan reported in 2011 was 23.2 mg/kg which was too high but in 2015, it was 0.014 mg/kg and in 2017, it was only 0.014–0.033 mg/kg. This decrease in Pb concentration indicates the positive role of regulatory authorities in Pakistan.

Cd is one of the most hazardous industrial and environmental heavy metals because of its lengthy half-life (15–30 years) and several harmful effects on human health, including teratogenic, carcinogenic, hepatotoxic, nephrotoxic, skeletal, and reproductive impacts (Zhong *et al.* 2018), it can bioaccumulate in a variety of tissues, including the kidneys and liver, which exacerbates its harmful effects on human health. Mining and smelting activities are two examples of industrial emission sources that frequently combine with cadmium exposure. Electroplating, pigments, and especially plastics, plastic stabilizers, and Ni-Cd rechargeable batteries are the main industrial applications for Cd (Yan *et al.* 2022). As indicated in Table 3, the concentration of Cd in raw milk in Pakistan reported in 2011 was 0.17 mg/kg, in 2015 it was 0.001 mg/kg and in 2017, similar concentrations were observed *i.e.*, 0.001–0.004 that were very low as compared to concentrations reported in 2011.

Although Cu is necessary for healthy human growth but its excessive use can have harmful consequences on health, particularly Wilson's disease, which is characterized by a ceruloplasmin deficit (Lawal *et al.* 2010). As indicated in Table 3, the concentration of Cu in raw milk in Pakistan reported in 2015 was 0.04–0.09 mg/kg, it was 0.04–0.09 mg/kg in 2017 and in our current study carried out in 2024

Table 3: Investigations of heavy metals concentrations across different regions of the world and in Pakistan

Country	Samples	Concerned metals under study	Methods employed	Results	References
Turkey	Milk and yogurt	Pb	Solid phase extraction	15-61 ng/mL for milk; 21-42 ng/mL for yogurt	(Cigdem <i>et al.</i> 2013)
Korea	Milk and yogurt	Pb, Cd and Cu	ICP-MS	The level of toxic trace elements, including Cu, Cd and Pb, was very low and there was no threat to consumers.	(Khan <i>et al.</i> 2014)
Croatia	Raw cow's milk	Pb and Cd	Electrothermal atomic absorption	Pb = 0.27 mg/kg; Cd = 0.037 mg/kg	(Pavlovic <i>et al.</i> 2004)
Italy	Milk and dairy products	Pb and Cd	GF-AAS	The high concentration of heavy metals in milk and dairy products is less significant than for age specimens.	(Caggiano <i>et al.</i> 2005)
Romania	Milk products	Pb and Cd	ICP-AES	Detection limit was range from 0.4 to 7.03 ng/g	(Birghila <i>et al.</i> 2008)
Hungary	Milk of ewes	Pb and Cd	ICP-OES	The concentrations of lead and cadmium were 0.023 mg and 0.012 mg per kg of milk.	(Póti <i>et al.</i> 2012)
Spain	Organic milk	Pb, Cd and Cu	ICP-MS	Toxic metal in milk were in general very low and no statistically significant differences were observed between organic and conventional milk.	(Rey-Crespo <i>et al.</i> 2013)
Canada	Raw cow milk	Pb, Cd and Cu	ICP-MS	Mean concentrations of Pb, Cd and Cu were > 0.002, > 0.001 and 0.03–0.1 mg/kg.	(Zwierzchowski and Ametaj 2019)
China	Raw cow milk	Pb and Cd	ICP-MS	Mean concentrations of Pb and Cd were 0.0014 and 0.0001 mg/kg.	(Zhou <i>et al.</i> 2019)
Pakistan	Raw cow milk	Pb and Cd	AAS	Mean concentrations of Pb and Cd were 23.2 and 0.17 mg/kg.	(Aslam <i>et al.</i> 2011)
Pakistan	Raw cow milk	Pb, Cd and Cu	AAS	Mean concentrations of Pb, Cd, and Cu were 0.014, 0.001 and 0.738 mg/kg.	(Ismail <i>et al.</i> 2015)
Pakistan	Raw cow milk	Pb, Cd and Cu	AAS	Mean concentrations of Pb, Cd and Cu were 0.014–0.033, 0.001–0.004 and 0.04–0.09 mg/kg.	(Ismail <i>et al.</i> 2017)
Pakistan	Raw cow milk	Pb, Cd and Cu	AAS	Mean concentrations of Pb, Cd and Cu were 0.017-0.054, 0.008-0.077 and 0.039-0.288 mg/kg	Current study

and the lowest concentration detected was 0.039 mg/kg. This decrease in Cu concentrations indicates the positive role of regulatory authorities in Pakistan. better results are expected in the coming years.

Conclusion

In comparison to the other heavy metals, copper showed higher levels of contamination in all sources studied, which may be due to the use of copper-based utensils, processing equipment, and feeding animals with copper-contaminated forage as copper-based fungicide is mostly sprayed in agriculture fields and water. In brand and dairy milk samples, the levels of heavy metals (Cd, Pb and Cu) contamination are slightly above the permissible limits set by WHO applied in Pakistan by PFA (Punjab Food Authority) and PSQCA (Pakistan Standard and Quality Control Authority). This is attributed to their hygienic environment and the implementation of good manufacturing practices (GMPs) in their production units. In vendor milk samples, the levels of contamination are also below permissible limits; however, these levels are higher compared to those in brand and dairy milk samples, indicating a greater risk associated with this source. Our investigation identified no risk to the public from consuming milk from all three sources, but it would be advisable to follow strict protocols in milk production and sales to ensure food safety and quality. From a future perspective, it is recommended to adopt proper hygienic measures and safety protocols in milk production and sales. Use forage with low or minimum mineral fertilizers, avoid open grazing near industrial discharge and use proper covered containers in case of vendors selling milk in main roads with heavy traffic. This study just measured the

concentration of heavy metals in milk, in future some studies on sources like forage, water and storage tank may also be conducted to know about the sources and chances to elimination of these heavy metals in milk. Our investigation focused only on lead (Pb), cadmium (Cd) and copper (Cu) and if other heavy metals are considered, contamination levels may exceed permissible limits, posing a risk to the safety of milk sold in Pakistan.

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Author contributions

Sofia Badar: Study design, Data collection, Formal analysis, Writing-original draft. Muhammad Arshad: Technical guidance, Methodology, Data curation, Writing-review & editing. Syed Ali Hassan: Writing-original draft, Data curation, Writing-review & editing. Laiba Badar: Sample collection, Testing assistance, Sample preparations.

Declaration of Interest

The authors declare that they have no known competing interests that could affect the work reported in this paper.

Data Availability

Data will be made available upon request.

Ethical Approval

This work does not involve experiments on animals or

humans.

Funding Source

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References

- Abedi AS, E Nasser, F Esfarjani, F Mohammadi-Nasrabadi, M Hashemi Moosavi, H Hoseini (2020). A systematic review and meta-analysis of lead and cadmium concentrations in cow milk in Iran and human health risk assessment. *Environ Sci Pollut Res* 27:10147–10159
- Akhtar S, T Ismail, M Riaz, M Shahbaz, K Amin (2015). Minerals and heavy metals in raw and ultra heat treated commercial milks in Pakistan. *Intl J Food Allied Sci* 1:18–24
- Almeida CCD, D dos, S Baiao, P Rodrigues, TD Saint’Pierre, RA Hauser-Davis, KC Leandro, VMF Paschoalin, MPD Costa, CA Conte-Junior (2022). Toxic metals and metalloids in infant formulas marketed in Brazil and child health risks according to the target hazard quotients and target cancer risk. *Intl J Environ Res Publ Health* 19:11178–11191
- Anyanwu BO, AN Ezeji, ZN Igweze, OE Orisakwe (2018). Heavy metal mixture exposure and effects in developing nations: An update. *Toxics* 6:65–96
- Aslam B, I Javed, FH Khan, ZU Rahman (2011). Uptake of heavy metal residues from sewerage sludge in the milk of goat and cattle during summer season. *Pak Vet J* 31:70–75
- Birghila S, S Dobrin, G Stanciu, A Soceanu (2008). Determination of major and minor elements in milk Through ICP-AES. *Environ Eng Manage J* 7:805–808
- Bortey-Sam N, SMM Nakayama, Y Ikenaka, O Akoto, E Baidoo, YB Yohannes, H Mizukawa, M Ishizuka (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 Saf* 111:160–167
- Briseno-Bugarin J, X Araujo-Padilla, VM Escot-Espinoza, J Cardoso-Ortiz, JAFDL Torre, A Lopez-Luna (2024). Lead (Pb) Pollution in Soil: A systematic review and meta-analysis of contamination grade and health risk in Mexico. *Environments* 11:43–58
- Caggiano R, S Sabia, M D’Emilio, M Macchiato, A Anastasio, M Ragosta, S Paino (2005). Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy. *Environ Res* 99:48–57
- Charkiewicz AE, WJ Omeljaniuk, K Nowak, M Garley, J Nikliński (2023). Cadmium Toxicity and Health Effects- A Brief Summary. *Molecules* 28:6620–6635
- Chirinos-Peinado D, J Castro-Bedriñana, E Rios-Rios, G Mamani-Gamarra, E Quijada-Caro, A Huacho-Jurado, W Nuñez-Rojas (2022). Lead and cadmium bioaccumulation in fresh cow’s milk in an intermediate area of the central Andes of Peru and risk to human health. *Toxics* 10:317–331
- Cigdem E, BF Senkal, M Yaman (2013). Determination of lead in milk and yoghurt samples by solid phase extraction using a novel aminothiazole-polymeric resin. *Food Chem* 137:55–61
- Covre WP, SJ Ramos, WVDS Pereira, ESD Souza, GC Martins, OMM Teixeira, CBD Amarante, YN Dias, AR Fernandes (2022). Impact of copper mining wastes in the Amazon: Properties and risks to environment and human health. *J Hazard Mater* 421:126688
- Elafify M, M EL-Toukhy, KI Sallam, NM Sadoma, SM Abd-Elghany, A Abdelkhalek, AH El-Baz (2023). Heavy metal residues in milk and some dairy products with insight into their health risk assessment and the role of *Lactobacillus rhamnosus* in reducing the lead and cadmium load in cheese. *Food Chem Adv* 2:100261
- Feizi R, F Hamidi, N Jaafarzadeh, M Ghahrchi, A zafarzadeh (2022). Determination and health risk assessment of heavy metals (Pb, Cd, Cu and Zn) in different brands of pasteurized milk. *Intl J Environ Anal Chem* 102:6892–6903
- Gao Y, X Li, J Dong, Y Cao, T Li, HW Mielke (2020). Snack foods and lead ingestion risks for school aged children: A comparative evaluation of potentially toxic metals and children’s exposure response of blood lead, copper and zinc levels. *Chemosphere* 261:127547–127558
- Hasanvand S, Z Hashami, M Zarei, S Merati, M Bashiry, R Nag (2024). Is the milk we drink safe from elevated concentrations of prioritised heavy metals/metalloids? – A global systematic review and meta-analysis followed by a cursory risk assessment reporting. *Sci Total Environ* 948:175011–175026
- Ismail A, M Riaz, S Akhtar, A Farooq, MA Shahzad, A Mujtaba (2017). Intake of heavy metals through milk and toxicity assessment. *Pak J Zool* 49:1413–1419
- Ismail A, M Riaz, S Akhtar, T Ismail, Z Ahmad, MS Hashmi (2015). Estimated daily intake and health risk of heavy metals by consumption of milk. *Food Addit Contam B Surveill* 8:260–265
- Jaafarzadeh N, K Tari, MR Samarghandi, MP Fard, S Jorfi, R Feizi, M Mehrbakhsh (2023). Non-carcinogenic risk assessment of cadmium and lead in raw milk from dairy production farms in Iran, using Monte Carlo simulation approach. *J Food Compos Anal* 115:104864
- Khan N, IS Jeong, IM Hwang, JS Kim, SH Choi, EY Nho, JY Choi, KS Park, KS Kim (2014). Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). *Food Chem* 147:220–224
- Kushwaha A, N Hans, S Kumar, R Rani (2018). A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. *Ecotoxicol Environ Saf* 147:1035–1045
- Lawal A, S Mohammed, D Damisa (2010). Assessment of levels of copper, cadmium and lead in secretion of mammary gland of cows grazed on open fields. *Sci World J* 1:7–10
- Leone C, H Thippareddi, C Ndiaye, I Niang, Y Diallo, M Singh (2022). Safety and Quality of Milk and Milk Products in Senegal - A Review. *Foods* 11:3479–3492
- Luqman M, MUF Awan, SH Khan, R Ahmed, HH Yang, MR Goraya (2023). Wastewater driven trace element transfer up the food chain in peri-urban agricultural lands of Lahore, Pakistan. *Agric Water Manag* 289:108509
- Mahmoudi R, AA Sari, S Valizadeh, R Kiani (2017). Lead and Cadmium Contamination in Raw Milk and Some of the Dairy Products of Hamadan Province in 2013-2014. *J Health* 8:27–34
- Malhat F, M Hagag, A Saber, AE Fayz (2012). Contamination of cows milk by heavy metal in Egypt. *Bull Environ Contam Toxicol* 88:611–613
- Miclean M, O Cadar, EA Levei, R Roman, A Ozunu, L Levei (2019). Metal (Pb, Cu, Cd and Zn) transfer along food chain and health risk assessment through raw milk consumption from free-range cows. *Intl J Environ Res Publ Health* 16:40–64
- Muhib MI, MAZ Chowdhury, NJ Easha, MM Rahman, M Shammii, Z Fardous, ML Bari, MK Uddin, M Kurasaki, MK Alam (2016). Investigation of heavy metal contents in cow milk samples from area of Dhaka, Bangladesh. *Intl J Food Contam* 3:1–10
- Năstăsescu V, M Mititelu, M Goumenou, AO Docea, E Renieri, DI Udeanu, E Oprea, AL Arsene, CE Dinu-Pirvu, M Ghica (2020). Heavy metal and pesticide levels in dairy products: Evaluation of human health risk. *Food Chem Toxicol* 146:111844
- Neto MCDV, TBC Silva, VED Araújo, SVCD Souza (2019). Lead contamination in food consumed and produced in Brazil: Systematic review and meta-analysis. *Food Res Int* 126:108671–108709
- Norouzrad R, JR González-Montaña, F Martínez-Pastor, H Hosseini, A Shahrouzian, M Khabazkhoob, FA Malayeri, HM Bandani, M Paknejad, B Foroughi-nia, AF Moghaddam (2018). Lead and cadmium levels in raw bovine milk and dietary risk assessment in areas near petroleum extraction industries. *Sci Total Environ* 635:308–314
- Oz F, E Oz, E Aoudeh, AMA El-Aty, M Zeng, T Varzakas (2021). Is ultra-high temperature processed milk safe in terms of heterocyclic aromatic amines? *Foods* 10:1247–1259
- Pavlovic I, M Sikiric, JL Havranek, N Plavljanić, N Brajenovic (2004).

- Lead and cadmium levels in raw cow's milk from an industrialised Croatian region determined by electrothermal atomic absorption spectrometry. *Czech J Anim Sci* 49:164–168
- Póti P, F Pajor, Á Bodnár, L Bárdos (2012). Accumulation of some heavy metals (Pd, Cd and Cr) in milk of grazing sheep in North-East Hungary. *J Microbiol* 2:389–394
- Raikwar MK, P Kumar, M Singh, A Singh (2008). Toxic effect of heavy metals in livestock health. *Vet World* 1:28-30
- Rey-Crespo F, M Miranda, M López-Alonso (2013). Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. *Food Chem Toxicol* 55:513–518
- Sarker A, MAA Masud, DM Deepo, K Das, R Nandi, MWR Ansary, ARMT Islam, T Islam (2023). Biological and green remediation of heavy metal contaminated water and soils: A state-of-the-art review. *Chemosphere* 332:138861
- Sipple LR, DM Barbano, MA Drake (2020). Invited review: Maintaining and growing fluid milk consumption by children in school lunch programs in the United States. *J Dairy Sci* 103:7639–7654
- Su C, Y Gao, X Qu, X Zhou, X Yang, S Huang, L Han, N Zheng, J Wang (2021). The Occurrence, pathways and risk assessment of heavy metals in raw milk from industrial areas in China. *Toxics* 9:320-331
- Tahir M, M Iqbal, M Abbas, MA Tahir, A Nazir, DN Iqbal, Q Kanwal, F Hassan, U Younas (2017). Comparative study of heavy metals distribution in soil, forage, blood and milk. *Acta Ecol Sin* 37:207–212
- Wu C, F Li, S Yi, F Ge (2021). Genetically engineered microbial remediation of soils co-contaminated by heavy metals and polycyclic aromatic hydrocarbons: Advances and ecological risk assessment. *J Environ Manage* 296:113185
- Yan M, C Niu, X Li, F Wang, S Jiang, K Li, Z Yao (2022). Heavy metal levels in cow's milk and its health risk assessment: A systematic review of studies in China. *Sci Total Environ* 851:158161
- Zafarzadeh A, Z Bonyadi, K Feyzi (2022). Health risk assessment related to cadmium in dairy products in Gorgan, Iran. *Intl J Environ Anal Chem* 102:4058–4066
- Zhong W, Y Zhang, Z Wu, R Yang, X Chen, J Yang, L Zhu (2018). Health risk assessment of heavy metals in freshwater fish in the central and eastern North China. *Ecotoxicol Environ Saf* 157:343–349
- Zhou X, N Zheng, C Su, J Wang, H Soyeurt (2019). Relationships between Pb, As, Cr and Cd in individual cows' milk and milk composition and heavy metal contents in water, silage and soil. *Environ Pollut* 255:113322
- Zwierzchowski G, BN Ametaj (2019). Mineral elements in the raw milk of several dairy farms in the province of Alberta. *Foods* 8:345-361