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Assessment of Cadmium and Mercury Contamination of Milk and Dairy Products in Vietnam

Kien Xuan Nguyen; PhD[1], Chuyen Van Nguyen; PhD[2], Trang Thi Thu Nguyen; MSc[2], Ha Thi Nhi Tran; PhD[3], Dien Duc Nguyen; MSc[2], Ba Van Nguyen; PhD[4], Anh Tuan Le; MSc[2], Minh Duc Tong; MSc[2], Kha Van Tran; MSc[2], Truong Thi Hoang; MSc[2], Trung Hoang Nguyen; MSc[2], Thien Duc Nguyen; BSc[2], Hang Thi Dieu Dinh; PhD[5], Hung Van Mai; PhD[6] & Phuong Minh Nguyen; PhD*[7]

[1] Department of Military Medical Command and Organization, Vietnam Military Medical University, Ha Noi city, Vietnam; [2] Department of Military Hygiene, Vietnam Military Medical University, Ha Noi city, Vietnam; [3] Hanoi Department of Health, Ha Noi city, Vietnam; [4] Military Hospital 103, Vietnam Military Medical University, Ha Noi city, Vietnam; [5] Hai Duong Medical Technical University, Hai Duong city, Vietnam; [6] Faculty of Education, VNU University of Education, Ha Noi city, Vietnam; [7] Department of Military Occupational Medicine, Vietnam Military Medical University, Ha Noi city, Vietnam.

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***Corresponding author:**
phuongk21@gmail.com
Department of Military Occupational Medicine, Vietnam Military Medical University, 160 Phung Hung road, Ha Dong distric, Ha Noi city, Vietnam.

Postal code: 12108
Tel: +84 912052887

ABSTRACT

Background: Milk and dairy products are nutritious and can play a significant role in a healthy diet. The safety of milk decreases with increase in concentration of Cadmium (Cd) and Mercury (Hg). The aim is to evaluate the status of Cd and Hg contamination regarding milk and dairy products in some provinces and cities of Vietnam. **Methods:** 367 samples of liquid milk, yogurt, cheese, milk cake and milk powder were collected in six large cities and provinces of Vietnam, then Cd and Hg levels were quantitatively analyzed. Samples were digested before analysis to remove organic compounds, and the heavy metal concentrations were determined by atomic absorption spectrophotometry. **Results:** The average concentrations of Cd and Hg in liquid milk were 64.55 and 29.99 ppb; in yogurt, 49.09 and 24.72 ppb; in cheese, 115.81 and 33.75 ppb; in milk cake, 84.44 and 18.08 ppb; and in milk powder, 61.78 and 43.21 ppb, respectively. **Conclusion:** Hg concentrations in 19.3% of liquid milk, 9.49% of yogurt, 14.29% of cheese, 9.09% of milk cake, and 22% of milk powder were higher than the maximum permitted levels according to national regulations. In contrast, Cd concentrations of all the samples were less than the maximum permitted levels so as not to affect the health of consumers of milk and dairy products produced and processed in Vietnam.

Keywords: *Heavy metals; Cadmium; Mercury; Milk; Dairy products.*

Introduction

Milk and dairy products are part of a balanced diet because milk is a food with high nutritional value whose protein contains all the essential amino acids required by humans, and also supplies lipids with a high biological value. Milk provides vitamins and minerals in a balanced ratio, so for all these reasons, the consumption of milk and dairy products is increasing. In Vietnam, milk has been included in school food program to improve the stature of Vietnamese children and increase interest in the quality of milk. Milk and dairy products can be contaminated with heavy metals, making them hazardous to health. Cd and Hg are two chemical contaminants deserving

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particular attention because of their toxicity and bioaccumulation in food chain. Environmentalists have pointed out that Cd and Hg are considered toxic because they are harmful to human and animal health (Eleboudy *et al.*, 2017). In Vietnam, the maximum concentrations of Cd and Hg in food have been prescribed by a National Technical Regulation as 1000 and 50 ppb, respectively, for liquid milk, yogurt, cheese, milk cake (ingredients include liquid milk, butter, sugar) and milk powder (Vietnam Ministry of Health, 2011).

Cd is present in polluted air because of emissions from industrial activities (World Health Organization, 2007). Cd is also present in polluted water, leading to soil pollution. Soil can also be contaminated by waste from coal-fired factories, and from the manufacture of batteries, metals, and phosphate fertilizers. Therefore, plants and grasses grown in contaminated soil will accumulate heavy metals in all parts of the plant such as roots, stems, leaves and fruit (World Health Organization, 2007). Bioaccumulation rate of heavy metals was notably greater in rice, grass, some legumes, vegetables, and onions compared to other plants (Shokri *et al.*, 2022, World Health Organization, 2007). Animals can also be contaminated with heavy metals through food chain. The accumulation of heavy metals in cows, buffaloes and goats comes mainly from the fodder grown in contaminated soil or irrigated by contaminated water. Heavy metals accumulate in mammals consuming this fodder, so their milk also becomes contaminated with heavy metals (Younus *et al.*, 2016). Humans are then exposed to heavy metals from consuming the meat and milk of these animals, which over a long period can cause cancer, teratogenicity, endocrine disruption and long-term health effects (Worlh Health Organization, 2011). Milk and dairy products are therefore a group of foods at a high risk of heavy metal contamination because of direct contamination with animal milk and the equipment used for its preservation and processing. During the process of cheese production, the acidic environment created during fermentation corrodes instruments and equipment, thus increasing the risk of heavy metal contamination (Dağcilar and Gezer, 2021, Enb *et al.*, 2009, Younus *et al.*, 2016).

Mercury, one of the most toxic heavy metals, is present in the environment due to agricultural activities and industrial emissions. Several studies have reported that the use of traditional methods of fungicidal treatment for cattle can cause their contamination with mercury. The poor quality control of animal food is the main cause of Hg contamination of cattle, and their milk (López-Alonso *et al.*, 2007). Human exposure to Hg at different concentrations will cause damage to nervous system, brain, and kidneys and also visual impairment and memory loss (Singh *et al.*, 2019). The issue of food hygiene and safety is of great interest to consumers. Determining the concentrations of heavy metals in milk and dairy products is important for reassuring consumers about the safety of food products they are buying. Preventive medicine experts have reported that consuming milk contaminated with heavy metals can change immune system and cause cancer (Singh *et al.*, 2019).

In Vietnam, the current situation on the level of contamination of cow's and goat's milk and dairy products by heavy metals has not yet been studied. Therefore, the present study aims to provide an up-to-date assessment of the contamination of milk and dairy products by Cd and Hg in Northern, Central, and Southern Vietnam.

Materials and Methods

Research location

Samples of milk and dairy products were collected in Ha Noi, Hai Phong, Da Nang, Buon Ma Thuot, Ho Chi Minh City, and Can Tho in Vietnam. Heavy metals in samples were analysed in labolatory of Department of Military Hygiene, Vietnam Military Medical University.

Chemicals

Standard solutions of Cd, Hg (1000 ppm) were purchased from Merck (Darmstadt, Germany). Sulfuric acid (H_2SO_4 98%), nitric acid (HNO_3 65%), potassium permanganate ($KMnO_4$), potassium peroxodisulfate ($K_2S_2O_8$), hydroxylammonium

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chloride ($NH_2OH.HCl$), tin (II) chloride dihydrate ($SnCl_2$), hydrogen peroxide (H_2O_2), and perchloric acid ($HClO_4$ 70%) were also purchased from Merck. All of the chemical solutions were freshly prepared each day. The standard curve solutions were diluted with HNO_3 (1%).

Sampling

All the 367 milk samples were collected between July 2019 and June 2020. They included liquid milk, yogurt, cheese, milk powder, milk powder intended for making milk tea and milk cake (ingredients include liquid milk, butter and sugar). They were purchased at stores, markets, and supermarkets in Ha Noi, Hai Phong, Da Nang, Buon Ma Thuot, Ho Chi Minh City and Can Tho. The samples comprised 114 liquid milk, 158 yogurt, 21 cheese, 44 milk cake and 30 milk powder. The milk and dairy products collected came from different brands and were of different flavors. After collection, the samples were stored at between 0 and 4 °C in sample storage boxes and were then immediately sent to the laboratory to be stored at – 20 °C until analysis.

Digestion of milk

The samples of milk and dairy products (1 ml or 1 g) were digested with HNO_3, H_2SO_4 and $HClO_4$ mixed together in the ratio of 10:1:1 with 5 ml H_2O_2 in Multi wave PRO microwave reaction system (Anton Paar GmbH, Graz, Austria.). First, the milk samples were placed into Teflon tubes, and then digestion mixture of HNO_3, H_2SO_4, $HClO_4$, H_2O_2 was added. After gentle shaking for 20 min to allow the digestion reaction to occur, Teflon tubes were placed in multiwave PRO to complete the digestion of the samples (USEPA, 1996). The temperature of the Multiwave PRO was programmed as follows: heating to 170 °C for 5 min and holding for 10 mins; increasing the temperature to 200 °C and holding it for 15 min. Finally, it is cooled to room temperature. After digesting milk, the sample solution should be transparent and free of residues and lipids. After digestion, the samples were diluted before analysis with HNO_3 (1%) to a total volume of 25 ml. The dilution factor for Cd was therefore 25.

Pre-treatment for mercury

Ten ml of the diluted sample after digesting the milk (as above) were put into a flask and then made up to a total volume of 200 ml with HNO_3 (1%). After adding 10 ml of H_2SO_4 (98%) and 5 ml of HNO_3 (65%), the flask was gently shaken. After adding 20 ml of $KMnO_4$ (5%) the flask was gently shaken to allow reaction for 15 min. After adding $K_2S_2O_8$ (5%), the flask was heated in a water bath controlled at 95 °C for 2 h, to avoid any loss of mercury. Following cooling the solution to room temperature, 8 ml of $NH_2OH.HCl$ (10%) were added and the flask gently was shaken. HNO_3 (1%) was added to make up the volume to 250 ml; then,10 ml of $SnCl_2$ was added immediately which was connected to the equipment for pre-treating the mercury.

Analysis of heavy metals

Atomic absorption spectrophotometry (AAS) (model AZ3000, Hitachi Ltd., Tokyo, Japan) was used to quantify heavy metals. Hg was quantified by cold vapor method and Cd by flame method at wavelengths of 253.7 and 228.8 nm, respectively. The limits of detection (LOD) of Hg and Cd were 0.02 and 1 ppb, respectively. A standard curve was prepared using at least 5 points for each element.

Quality control

Blank sample method was used to control the quality of analytes using two blank samples: the first one was prepared at the same time as the digestion of the milk sample to check for contamination, and the second one was prepared when creating the standard curve to check for heavy metal contamination from the chemicals prepared for analysis.

During the digestion of the milk samples and laboratory analysis, contamination by heavy metals in the sample must be avoided to ensure that the analytical results are reliable and of good quality. Laboratory instruments made of metal should be avoided to reduce the possibility of contamination. The laboratory glass instruments and Teflon tubes were washed with soap, rinsed with sulfochromic acid (50 g of $K_2Cr_2O_7$ mixed in 500 ml of H_2SO_4 (98%)) and then rinsed with distilled water and

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deionized water before use to ensure that there was no contamination by heavy metals.

The standard curves for all the analytical indicators were constructed using at least five different concentrations, the minimum value being set as the LOD of the instrument. Standard concentrations for Cd were 0, 1, 5, 10 and 50 ppb, and for Hg, 0, 0.02, 0.04, 0.1 and 2 ppb. The correlation coefficient of 99.99% indicated a highly linear relationship over the concentration range studied.

In order to provide valid and reliable data, the percentage recoveries must be implemented in the analytical process. The percentage recovery was a sample tested as a background. The sample was added with different concentrations of low, medium and high concentrations of the standard solution and the test was performed in triplicate. Spiking level for Cd were 1; 5; 50 ppb and for Hg 0.04; 0.1; 2 ppb. The test results for Cd and Hg in milk samples after adding the spiked sample were calculated (**Table 1**), ranging from 93.0% to 94.02% for Cd, and from 92.5% to 95.5% for Hg. The values of these percentage recoveries indicated that the method was highly accurate and could be used for the analysis of heavy metals.

Table 1. The percentage recoveries of Cadmium and Mercury of the analytical method.

Sample	**Cadmium**			**Mercury**		
Spiking level (ppb)	50	5	1	2	0.1	0.04
Level found (ppb)	47.01	4.67	0.93	191	0.093	0.037
Recovery (%)	94.02	93.52	93.00	95.5	93.0	92.5

Data analysis: The concentrations of heavy metals were calculated as means±SD based on five replicate measurements of Cd and Hg. Data were analyzed by ANOVA using SPSS (version 19.0, IBM Corp., Armonk, NY, USA).

Results

Levels of Cd contamination

Table 2 shows the mean, maximum, and minimum Cd concentrations (ppb) found in the samples of milk and dairy products. The mean concentration of Cd in the samples of cheese was the highest at 115.81 ppb, followed by milk cake and liquid milk at 84.44 and 64.55 ppb, respectively. The average concentration of Cd in milk powder was 61.78 ppb, which was much higher than that of yogurt, 49.09 ppb.

Table 2. Concentration of Cadmium (ppb) and percentage exceeding the maximum permitted levels (QVCN - National Technical Regulation of Vietnam) in samples of milk and dairy products on sale in Vietnam.

Products	**n**	**Min**	**Max**	**Mean±SD**	**Percentage exceeding QCVN Limit**	**QCVN Limit (μg/kg or μg/l)**
Cadmium						
Liquid milk	114	0	858.50	64.55 ± 142.38	0	1000
Yogurt	158	0	895.00	49.09 ± 123.25	0	1000
Cheese	21	0	881.00	115.81 ± 224.76	0	1000
Milk cake	44	0	540.00	84.44 ± 126.67	0	1000
Milk powder	30	0	655.00	61.78 ± 159.11	0	1000
Mercury						
Liquid milk	114	0	112.50	29.99 ± 26.07	19.30	50
Yogurt	158	0	83.33	24.72 ± 22.23	9.49	50
Cheese	21	0	112.50	33.75 ± 26.72	14.29	50
Milk cake	44	0	93.75	18.08 ± 23.21	9.09	50
Milk powder	30	0	137.50	43.21 ± 31.76	22.00	50

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The range of concentrations regarding Cd in all the samples of milk and dairy products was 0 to 895.00 with average concentration of Cd which was 62.99 ppb. The number of samples of milk and dairy products contaminated with Cd was 139 out of 367, a percentage of 37.87%, of which 0% exceeded the maximum permitted levels (**Table 2**).

Levels of Hg contamination

Concentrations of Hg in the samples of liquid milk, yogurt, cheese, milk cake and milk powder shown in **Table 2** were relatively similar. The highest average concentration of Hg of 43.21 ppb belonged to milk powder, with high concentrations found in full cream milk and the milk powder used for tea, where one sample had a concentration of 137.5 ppb. The average concentration of Hg in cheese of 33.75 ppb was almost twice the one in milk cake of 18.08 ppb. Similarly, the average concentration of Hg in liquid milk of 29.99 ppb was slightly higher than that in yogurt of 24.72 ppb. The maximum permitted levels for Hg in milk and dairy products are 50 ppb (Vietnam Ministry of Health, 2011). The percentage of samples exceeding the permitted level for milk powder was 22.00%, which was significantly higher than that for cheese, 14.29%. The percentages exceeding the permitted level for yogurt and milk cake were similar, 9.49% and 9.09%, respectively. Liquid milk had the second greatest percentage of samples exceeding the maximum permitted level, 19.30% (**Table 2**).

The range of concentrations of Hg in all the samples of the milk and dairy products was 0 to 137.5 ppb. The average concentration of Hg in milk and dairy products was 27.59 ppb. The number of samples of milk and dairy products contaminated with Hg was 271 out of 367, a percentage of 73.84%, of which 13.62% exceeded the maximum permitted levels (**Table 3**).

Table 3. Concentration (ppb) of heavy metals in milk and dairy products on sale in Vietnam compared with the maximum permitted levels.

Metal	Min	Max	Mean±SD	Number contaminated	Percentage contaminated	Percentage exceeding QCVN Limit
Cadmium	0	895.50	62.99±140.45	139	37.87	0
Mercury	0	137.50	27.59±25.35	271	73.84	13.62

Contamination of 1 or 2 heavy metals (Cd and Hg) in milk and dairy products

The number of samples contaminated with one heavy metal, 210, was the largest category, accounting for 57.22% of the total; 57 samples were not contaminated with either of the two heavy metals under study, or the level of contamination was less than the limit of detection of the instrument; and 100 samples were contaminated with both Cd and Hg, accounting for 27.25% of the total (**Table 4**).

Table 4. Percentage of the total number of samples contaminated with 0, 1 or 2 heavy metals.

	n	%
Not contaminated with Cd or Hg	57	15.53
Contaminated with either Cd or Hg	210	57.22
Contaminated with both Cd and Hg	100	27.25

Discussion

Comparison of the levels of Cd and Hg contamination of milk and dairy products in Vietnam with those produced elsewhere

This assessment of the actual Cd contamination of milk and dairy products in Vietnam had certain similarities and differences compared with similar studies in other parts of the world. The analytical results in **Table 2** can be compared with similar samples in Italy where Caggiano reported that the average concentration of Cd in milk and dairy products was 60±20 ppb, a similar value to the present study (Caggiano *et al.*, 2005). Similarly, the concentration of Cd in cheese in the present study was similar to that reported by Bakircioglu *et al.* of 120 ppb (Bakircioglu *et al.*, 2011). The Cd concentrations reported in liquid milk and cheese sampled in Egypt, Croatia, Iran, and Pakistan were

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lower than those reported in the present study by some authors (Ismail *et al.*, 2015, Meshref *et al.*, 2014, Pavlović *et al.*, 2004). Licata *et al.* reported that Cd concentrations of 47 milk samples collected in Calabria region of Italy were less than the limit of detection of the instrument (Licata *et al.*, 2012). Póti *et al.* also reported the concentration of Cd in milk in Hungary as 12±1 ppb (Póti *et al.*, 2012). Both of these results were much lower than the results of the present study. Similarly, Beikzadeh *et al.* reported that Cd contamination in the samples of liquid milk and yogurt in Tabriz was 2.343 to 6.070 ppb and 3.143 to 8.830 ppb, respectively, much lower than those determined in the present study which were 64.55 and 49.09 ppb, respectively (Beikzadeh *et al.*, 2019). Therefore, compared with Cd levels permitted by national regulation of Vietnam (Vietnam Ministry of Health, 2011), these two products could be considered safer than other products.

Cd is commonly found in natural environment, air, water, and soil of industrial zones. Animals can be contaminated by Cd through soil, water, and fodder grown in contaminated soil leading to contaminated milk. Cd is also present in phosphate fertilizers. Therefore, cattle and their milk are contaminated with Cd through consuming fodder and drinking water originating in contaminated areas, which increases the risk of Cd contaminating milk (Singh *et al.*, 2019). High-temperature food preservation methods such as pasteurization and ultra-high temperature (UHT) treatment used in dairy processing do not affect the concentration of Cd in milk (Beikzadeh *et al.*, 2019). The average concentration of Cd in cheese was 115.80 ppb in this study, which was higher than that in other products, possibly because of the acidic environment created during fermentation. This process could corrode equipment and instruments, leading to Cd contaminating the cheese samples: high concentrations in cheese were thus probably caused by a poorly controlled production process.

Data on the levels of mercury contaminating milk in Vietnam are still limited; therefore, the results of the present study were compared with those from other regions of the world. The results of this study on average concentration of Hg in milk and dairy products were similar to those of Rezaei *et al.* who reported that Hg levels in liquid milk, cheese ,and yogurt were 21.16±2.65 ppb, 27.27±2.65 and 23.81±2.65 ppb, respectively (Rezaei *et al.*, 2014). Miedico *et al.* pointed out that the concentrations of Hg in all the milk samples studied in Italy were less than the LOD of the analytical equipment used and much less than the results of the present study (Miedico *et al.*, 2016). The results of Rey-Crespo *et al.* on the levels of contamination of organic milk by heavy metals in Spain (Rey-Crespo *et al.*, 2013), and those of Caggiano *et al.* on milk and dairy products in Southern Italy, were much less than the results of present study (Caggiano *et al.*, 2005). Similarly, the results on cheese, yogurt, and liquid milk samples in the present study were slightly higher than those of Dağcilar and Gezer, who reported Hg levels of 15.00±9.00, 6.00±4.00 and 3.66±3.21 ppb, respectively (Dağcilar and Gezer, 2021).

Differences between the results of the present study and other studies were probably caused by different levels of pollution in each region. Hg in emissions, waste, and wastewater in industrial zones, and in the soil can contaminate the food consumed by animals, which, in turn, contaminates their milk and those consuming it.

Some milk samples exhibited high concentrations of mercury, possibly caused by contaminated food and water. The present study did not collect the information on the level of Hg contaminating water, air, soil, and animal food on dairy farms. The contamination of water by Hg may be caused by natural processes from the earth's crust, by human industrial activities and by its presence in fungicides. Hg can also enter food chain and human body, with long-term exposure leading to chronic poisoning. Mercury is a volatile metal and is easily absorbed through skin and lungs (De la Cueva *et al.*, 2021) and has been shown to be harmful to various organs of the human body. It not only damages genes but also the brain and kidneys and can be a carcinogen (Singh *et al.*, 2019).

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Conformity of levels of Cd and Hg contamination of Vietnamese milk and dairy products to national regulations

Regarding Cd contamination, no samples of milk and dairy products exceeded the maximum permitted levels (**Table 2**). However, regarding Hg contamination, all the five products investigated included some samples which exceeded the maximum permitted levels. While milk powder exhibited the greatest number of samples exceeding the maximum permitted levels, milk cake exhibited the smallest number.

The concentrations of Cd in milk determined in the present study were generally low (**Table 2**). These results were similar to those of Rey-Crespo *et al.*, who investigated the levels of trace elements and heavy metals in samples of organic milk in Spain, most of which (84%) had a Cd concentration of less than the limit of detection regarding the analytical instrument used (0.012 ppb) with that of the remaining samples being < 1 ppb, a very low level (Rey-Crespo *et al.*, 2013). When analyzing the concentration of Cd in milk and dairy products in the present study, no samples exceeded the maximum permitted levels. Another reference value for Cd levels in milk is Codex standard (Codex Alimentarius Commission) most recently revised in 2019 (World Health Organization/Food Agriculture Organization, 2019). WHO/FAO recommends a TDI (Total Daily Intake) for Cd in food, drinking water, and air of 25 µg/kg of body weight/day. This means that a daily intake of less than 1.5 mg for 60 kg person would not affect their health. In the present study, the average concentration of Cd in milk and dairy products was between 49.09 and 115.80 ppb (**Table 2**). However, milk is not the only source of Cd contamination: consumers can also be contaminated with Cd through their usual meals and drinking water. Therefore, the effects of Cd on health cannot be conclusively determined.

The adult TDI for mercury is recommended to be less than 4 µg/kg of body weight/day for food, drinking water, and air, with a maximum daily intake for a 60 kg person of 240 µg. The average concentrations of Hg determined in the present study ranged from 18.08 to 43.21 ppb (Table 2). This indicated that the health of an individual only consuming the milk contaminated with Hg would not be affected. However, a comprehensive evaluation of exposure to Cd and Hg through food, drinking water, and air would be required to assess the actual risk to consumers.

The average concentration of Cd in milk and dairy products, which was 62.99 ppb, was twice as high as that of Hg, 27.59 ppb (**Table 3**). Although the average concentration of Cd was higher than that of Hg, no milk samples had a Cd concentration exceeding the maximum permitted levels. The concentrations of mercury in the samples of liquid milk, yogurt, cheese, milk cake and milk powder shown in Table 3 indicated that most of the milk samples were contaminated with mercury, 13.62% of which exceeded the maximum permitted levels. Samples with high concentrations of mercury included samples of milk powder, full cream milk, and milk powder used to make milk tea, where one sample had a concentration of 137.5 ppb. Therefore, consuming a large amount of milk tea during the day could cause Hg poisoning because of its relatively high concentration. Similarly, the number of samples of milk and dairy products contaminated with Cd was 139 out of 367, 0% of which exceeded the maximum permitted levels. This suggested that consuming milk contaminated with Cd would have adverse effects on consumer health.

This was the first study to assess the current state of heavy metal contamination in milk and dairy products in Vietnam based on 367 samples. One limitation of this study was that it could not assess the health risks of the heavy metals. Further studies are therefore needed to investigate Food Ingestion Rate (FIR) per day, Target Hazard Quotient (THQ), Hazard Index (HI), and the effect of heavy metals in milk on human health.

Conclusion

This study has determined the concentrations of Cd and Hg in samples of liquid milk, yogurt, cheese, milk cake, and milk powder using atomic

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absorption spectrophotometry. The results showed that the concentrations of Cd were safe for consumers because they were all less than the maximum permitted level, meaning that Cd toxicity of milk was low. These results were similar to those reported by other authors studying Cd concentration in milk and dairy products in different regions of the world. However, the concentration of Hg in some samples of milk and dairy products exceeded the permitted level, possibly because of high concentrations of Hg in water and animal food. Therefore, control measures must be enforced to reduce these sources of contamination. Further studies should investigate where heavy metal contamination occurs during the stages of producing milk and dairy products, whether it is from the milk of cattle or during food processing and preservation processes.

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Author's contribution

Minh Nguyen P, Xuan Nguyen K, and Van Nguyen C designed the study; Thi Thu Nguyen T, Ha Thi Nhi Tran, Xuan Nguyen K, Duc Nguyen D, Thi Hoang T, Van Tran K, Duc Nguyen T, Thi Dieu Dinh H, and Van Mai H collected the samples and conducted the experimental work; Van Nguyen B, Dưc Tong M, Hoang Nguyen T, and Tuan Le A analyzed the data, Minh Nguyen P and Van Nguyen C wrote the manuscript. All the authors revised and approved the final manuscript

Conflicts of interest

The author(s) declared no conflict of interests.

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