Assessment of contamination of milk and milk products with heavy metals

Manju Singh, Suvartan Ranvir, Rajan Sharma, Kamal Gandhi and Bimlesh Mann

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Abstract: Milk and milk products are a significant part of healthy diet. However, contamination of milk can be dangerous and detrimental for the health of consumers. The safety of milk and milk products decreases with increasing concentration of toxic compounds and environmental pollutants. The study was aimed to investigate the contents of some heavy metals in 30 samples of commercially available milk and milk products (dahi, cheese, paneer, khoa and milk powder) collected from five different vendors, from Karnal district of Haryana (India) to know whether the intakes comply with the permissible levels for toxic elements. Mean concentration of all the heavy metals analyzed showed a range of 3.38-26.4, 3.16- 13.89, 3.77- 21.04 and 3.34- 10.68 ppb for cadmium, lead, arsenic and mercury, respectively. Results indicated that the content of cadmium was maximum in all the samples except for paneer, where mercury concentration was maximum.

Keywords: Cheese, Dahi, Khoa and milk powder, Heavy metals, Milk, Paneer, Safety

Introduction

The continuous urbanization as well as industrial development has led to increase in the accumulation of xenobiotics especially heavy metals. The contamination of which has been a big human health issue particularly, if transmitting into human body via food chain. Metals having density more than 5 g /cm³ are called heavy metals (Sani, 2011). Heavy metals are cumulative poisons and are toxic even at very low concentrations. These are introduced to our body through food ingestion and inhalation. The two main aspects of toxicity of these metals are: (a) they have no known metabolic function, but when present in the body, they disrupt normal cellular processes, leading to toxicity in a number of organs, and (b) the potential to accumulate in biological tissues, a process known as bioaccumulation (Al-Maylay et al. 2014). Bioaccumulation of toxic heavy metals, like lead, cadmium, arsenic, mercury etc. in milk and milk products is a matter of great concern. The main sensitive target organs of heavy metals are soft tissue, such as kidneys, liver and the central nervous system. Heavy metal accumulation in dairy animals adversely affects their health and milk production. The heavy metal contaminants enter animal systems due to pollution of air, water, soil, and consumption of contaminated feed; improper manufacturing practices and use of contaminated equipments also contribute to the contamination of milk with heavy metals. (Yuzbasi et al. 2003; Caggiano et al. 2005). The metals like lead, cadmium, mercury, arsenic are particularly undesirable. Lead and cadmium are the major environmental pollutants, as these can easily enter into body through food chain. Both of them do not have any biological functions; rather they produce many harmful effects in animals (Swarup et al. 2006; Patra et al. 2008; Smitha et al. 2010). Copper and zinc can also be toxic when ingested in excessive amounts and deficiency of these metals can cause impairment of many biological activities (Sharma and Agarwal, 2005). When released into open area, some heavy metals can enter into human and animal body.

Because of their potential in poisoning environment and food, scientists feel obligated to work on this subject. Milk and most of the dairy products are likely to be exposed to heavy metal contamination. These products are important parts of human diet. Previous investigations revealed that a number of heavy metals in different dairy products might pose a serious health risk for humans and animals. Therefore, it is essential to monitor milk and milk products for the presence of heavy metals regularly. Heavy metals as such are not part of composition of milk but they can occur in milk because of human activities such as...
industrial and agricultural processes, which resulted in an increase in concentration of heavy metals in air, water, and soil. Subsequently, these metals are taken up by plants and animals, and find their ways to food chain (Athar and Ahmad., 2002). Lead finds its way in food systems through petroleum industry, batteries, ammunition and X-ray shielding devices. When human beings are exposed to it, it causes kidney and brain damages, miscarriage in pregnant woman and infertility (Assi et al. 2016). The accumulation of toxic metals such as cadmium and Lead in ruminant animals mostly cow, buffalo and goat is mainly from fodder, which grows on contaminated soil. Such accumulation of heavy metals not only have pernicious effect on the cattle, but also on the population who is consuming such contaminated milk and meat (Mata et al. 1995). Cadmium (Cd) is present in soil; it comes into food system through the wastes of coal, batteries and metal industries and through phosphate fertilizers. Limited exposure to Cd causes diarrhea, stomach irritation, whereas long-term exposure causes kidney and lungs damage (Mata et al. 1995). Mercury (Hg) combines with other elements and forms organic and inorganic mercury compounds which are used in thermometer, dental fillings, light bulb etc. Exposure of mercury in varying levels causes damage to nervous system, brain and kidney. It also reduces vision and causes memory problems (Hutton and Symon, 1986). Arsenic is a heavy metal mainly originated from volcanic activities, forest fires and from paint soap and dyes industry. Exposure to it causes vomiting, nausea and longer level of exposure may lead to death (Saha et al. 1999). Due to the toxic effect of heavy metals on human health, various regulatory bodies have prescribed limit for them. Nowadays, people are more concerned about food safety. Hence, it is important to monitor the level of these heavy metals and other contaminants in milk and milk products so that consumer could get wholesome and safe product. Supporters of milk argue that the concentration of chemical contamination is so low that it does not pose a serious threat to health. Scientific evidence repudiates this logic. Though not widely recognized, heavy metal contaminants present in milk cause alteration of the body’s immune system and thus have damaging effect. The single molecules of toxins in the body cause many diseases such as cancer. The distribution of heavy metals in bovine milk and milk products has not been studied adequately. The present study might provide important information regarding the content of heavy metals in different milk and milk products.

Materials and Methods

Reagents

All the reagents -Nitric acid (65%), Hydrochloric acid (37%), Sulphuric acid (98%), Potassium Iodide, Palladium Nitrate, Sodium Borohydride, Standards of all the heavy metals (1000 ppm) of Cadmium, Lead, Mercury and Arsenic was procured from Sigma-Aldrich, St. Louis Missouri, USA. (Sigma). All sample preparations and measurements were carried out using double deionized water (Cascada water purifier, UK) having conductance 0.055 μS/cm2.

Equipment and apparatus

Analytical balance (Mettler Toledo) with the precision of ± 0.001g, Borosilicate volumetric flasks (25, 50, 100 mL), measuring cylinders, pipettes, micro pipettes, muffle furnace, hot plate, desiccator, quartz crucible, atomic absorption spectrophotometer (AA-7000, Shimadzu) equipped with GFA-7000A, a graphite furnace atomization unit and HVG-1, which allow a high sensitive analysis of inorganic elements by electro-thermal atomic absorption spectrometry (the furnace method) and hydride vapour atomic absorption spectrometry was used. For lead and cadmium hollow cathode lamp with air-argon flame was used to conduct spectral analysis using high-density graphite tubes. The LOD (BCG-D2) for lead, cadmium and arsenic was 0.05-0.2, 0.004-0.02 and 0.08-0.3 ppb; the LOQ (BCG-D2) was 0.2-0.6, 0.01-0.06 and 0.3-1.0 ppb, respectively.

Sample collection

Samples of dahi, cheese, paneer, khoa and milk powder were collected from five different local vendors with three repetitions from Karnal city, Haryana (India), to investigate the effect of environmental pollution on the heavy metal content of raw milk.

Ashing and digestion of milk fractions for Lead, Cadmium and Arsenic

All the samples were ashed according to AOAC (2002) using microprocessor controlled muffle furnace (Matrix Scientific Instruments, New Delhi). Five grams of milk / milk product was charred in silica crucibles (50 ml) at a temperature less than 100°C. The charred samples were cooled to room temperature and ashed in muffle furnace at 550°C for 6 h, until white or grey ash was obtained. All the crucibles were cooled in desiccators. Ash was diluted with 3 to 4 ml of 30% nitric acid in 50 ml volumetric flask and volume was made up to mark with double distilled water. All the samples were filtered using Whatman filter paper no. 41.

Sample Pre-treatment for reduction of Arsenic

In a 25 ml volumetric flask, 2 ml of sample was taken and this 2 ml of hydrochloric acid (37%) and 1.25 ml of 20% potassium iodide solution were added and volume was made to 25 ml for the standard solution / unknown samples. The mixture was kept undisturbed for 30 min. Then filtered the sample using Whatman filter paper no.41 and made the volume up to the mark using 50 ml volumetric flask.

Sample preparation for Mercury

2 ml of nitric acid and 3 ml of H2SO4 were added to 5 g sample placed in the digestion tube. Samples were heated at 120°C in the
heating block for 5 min. The temperature was controlled to prevent losses of mercury. After cooling, 9 ml of nitric acid and 1 ml H\textsubscript{2}O\textsubscript{2} (20%) were added and heated for 8 min. Then again, 19 ml H\textsubscript{2}O\textsubscript{2} was added and heated at 200°C for 5 min. After cooling, the digestion products were transferred to 50 ml volumetric flasks and the volume was made up to the mark with double distilled water and filtered with Whatman 41 filter paper (Antunovic et al. 2005). Cadmium and lead were estimated using Graphite Furnace Atomic Absorption Spectrometry (GFAAS), arsenic using Hydride Vapour Atomic Absorption Spectrometry (HVAAS) and mercury using Cold Vapour Atomic Absorption Spectrometry (CVAAS).

**Statistical Analysis**

The concentrations of all the metals were reported as the mean ± S.D. Data obtained were tested by ANOVA (one-way ANOVA randomized complete blocks). Differences and similarities between the means of metal contents of different milk products were achieved by using the Duncan Multiple Range test. Each metal was analyzed at least three times for each product. Ash content was reported as mean of six determinations.

**Results and Discussion**

The recovery of the heavy metals such as cadmium, mercury, lead and arsenic in different milk samples were collected from LRC, NDRI Karnal after spiking them by 50, 100 and 150% of the observed level in unspiked milk was calculated. Recoveries were found to be between 85 and 98% for all the metals.

<table>
<thead>
<tr>
<th>Type of</th>
<th>Type of Heavy Metal</th>
<th>Lead</th>
<th>Cadmium</th>
<th>Mercury</th>
<th>Arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>Mean ± S.D.</td>
<td>6.10±1.5</td>
<td>11.17±0.78</td>
<td>6.04±0.97</td>
<td>7.13±1.53</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4.55-8.16</td>
<td>9.96-11.89</td>
<td>4.88-7.23</td>
<td>4.87-8.94</td>
</tr>
<tr>
<td>Paneer</td>
<td>Mean ± S.D.</td>
<td>5.32±0.92</td>
<td>5.07±2.5</td>
<td>7.15±1.76</td>
<td>6.13±1.87</td>
</tr>
<tr>
<td>Dahi</td>
<td>Mean ± S.D.</td>
<td>6.51±2.39</td>
<td>9.95±1.41</td>
<td>6.23±1.46</td>
<td>7.98±2.62</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4.12-9.79</td>
<td>7.84-11.50</td>
<td>4.04-8.04</td>
<td>4.05-11.32</td>
</tr>
<tr>
<td>Cheese</td>
<td>Mean ± S.D.</td>
<td>6.09±2.9</td>
<td>10.06±0.87</td>
<td>6.60±1.58</td>
<td>6.02±1.06</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3.16-10.93</td>
<td>9.16-10.99</td>
<td>4.87-8.68</td>
<td>5.01-7.73</td>
</tr>
<tr>
<td>Khoa</td>
<td>Mean ± S.D.</td>
<td>12.84±2.38</td>
<td>20.14±4.04</td>
<td>8.98±1.35</td>
<td>18.13±2.64</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>Mean ± S.D.</td>
<td>4.43±0.51</td>
<td>9.34±1.03</td>
<td>4.75±0.83</td>
<td>8.18±1.08</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3.99-5.01</td>
<td>7.73-10.2</td>
<td>3.34-5.55</td>
<td>6.70-9.70</td>
</tr>
</tbody>
</table>

**Table 1** FSSAI limits for different heavy metals in milk

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Parts per million by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>2.5</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.1</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.5</td>
</tr>
<tr>
<td>Mercury</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2 Concentration (ppb) of heavy metals in milk and milk products procured from five vendors

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Type of Heavy Metal</th>
<th>Lead</th>
<th>Cadmium</th>
<th>Mercury</th>
<th>Arsenic</th>
</tr>
</thead>
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<td>Cheese</td>
<td>Mean ± S.D.</td>
<td>6.09±2.9</td>
<td>10.06±0.87</td>
<td>6.60±1.58</td>
<td>6.02±1.06</td>
</tr>
<tr>
<td>Khoa</td>
<td>Mean ± S.D.</td>
<td>12.84±2.38</td>
<td>20.14±4.04</td>
<td>8.98±1.35</td>
<td>18.13±2.64</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>Mean ± S.D.</td>
<td>4.43±0.51</td>
<td>9.34±1.03</td>
<td>4.75±0.83</td>
<td>8.18±1.08</td>
</tr>
</tbody>
</table>

**Lead**

The lead content (ppb) ranged from 4.55-8.16, 3.97-6.28, 4.12-9.79, 3.16-10.93, 11.69-13.89 and 3.99-5.01 with a mean value of 6.15±1.5, 5.32±0.92, 6.51±2.39, 6.09±2.9, 12.84±2.38 and 4.43±0.51, respectively was observed for the samples of milk, paneer, dahi, cheese, khoa and milk powder, respectively (Table 2). It was found that the maximum lead content was in first and fifth vendor milk samples whereas it was similar in second, third and fourth vendor samples (Fig. 1). *Paneer* samples of the third vendor were found to have minimum lead content as compared to other vendors (Fig. 2). The maximum lead content was observed in *dahi* samples collected from the fifth vendor whereas minimum was in fourth vendor’s samples (Fig. 3). Almost similar lead content was found in all the samples of *khoa* (Fig. 4). Maximum lead content was present in third vendor’s *cheese* sample and minimum in that from the fourth vendor’s (Fig. 5). The lead content was found highest in first and fifth vendor’s milk powder samples whereas it was similar in second, third and fourth vendors’ samples (Fig. 6). The higher content of lead in milk/milk products might be due to high exposure of soil and water to the sources of lead near the hazardous waste sites (Wani et al. 2015; Tchounwou et al. 2012).

**Cadmium**

The cadmium content (ppb) ranged from 9.96-11.89, 3.38-9.53, 7.84-11.5, 9.16-10.99, 16.91-26.41 and 7.73-10.02 with a mean value of 11.17±0.78, 5.07±2.5, 9.95±1.41, 10.06±0.87, 20.14±4.04 and 9.34±1.03, respectively was observed for the samples of milk,
The cadmium content was maximum in third and fifth vendors’ milk samples, while the minimum was in first vendor’s milk sample (Fig. 1). The paneer samples showed maximum cadmium content in fifth vendor’s samples and minimum content in first and fourth vendors’ samples (Fig. 2). The cadmium content in dahi samples was observed in the order of vendor 5 > 3 > 4 > 2 > 1 (Fig. 3). The maximum cadmium content found in fourth vendor’s khoa samples, whereas similar content was observed in first, second and third vendor’s khoa samples (Fig. 4). Fourth and fifth vendors’ cheese samples showed maximum cadmium content, while the minimum content was in first and third vendor’s sample (Fig. 5). Cadmium content was almost similar in milk powder samples of first, second and fourth vendors and was minimum in third and fifth vendors’ samples (Fig. 6). The higher content of cadmium in milk/milk products might be due to high exposure of...
soil and water to the sources of cadmium near the hazardous waste sites (Wani et al. 2015; Tchounwou et al. 2012).

Mercury

The mercury content (ppb) ranged from 4.88-7.23, 4.23-8.53, 4.04-8.04, 4.87-8.68, 7.46-10.68 and 3.34-5.55 with a mean value 6.04±0.97, 7.15±1.76, 6.23±1.46, 6.60±1.58, 8.98±1.35 and 4.75±0.83, respectively was observed for the samples of milk, paneer, dahi, cheese, khoa and milk powder, respectively (Table 2). Mercury content was maximum in first and fifth vendors’ milk samples, whereas it was minimum in third vendor’s milk sample (Fig. 1). Mercury content in the paneer samples was observed to be maximum in third and fifth vendors’ samples (Fig. 2). In fifth vendor’s dahi samples, mercury content was maximum, while the minimum content was observed in third vendor’s samples (Fig. 3). The mercury content was in the order 4 >3>1>5>2 for khoa samples (Fig. 4). Mercury content was maximum in fourth vendor’s cheese sample, while the minimum content was in second and fifth vendors’ samples (Fig. 5). Second vendor’s milk powder sample had higher mercury content, whereas minimum content was in fourth vendor’s sample (Fig. 6). Higher content of mercury might be due to more exposure of water to the sources of mercury. Mercury enters water as a natural process of off-gassing from
the earth’s crust and also through industrial pollution (Dopp et al. 2004). Also the algae and bacteria methylate the mercury entering the waterways (Sanfeliu et al. 2003).

**Arsenic**

The arsenic content (ppb) ranged from 4.87-8.94, 3.77-8.98, 4.05-11.32, 5.01-7.73, 14.61-21.04 and 6.7-9.7 with a mean value 7.13±1.15, 6.13±1.87, 7.98±2.62, 6.02±1.06, 18.13±2.64 and 8.18±1.08, respectively was observed for the samples of milk, paneer, dahi, cheese, khoa and milk powder, respectively (Table 2). The highest content of arsenic was found in third vendor’s milk samples, whereas lowest in fifth vendor’s milk samples (Fig. 1). The arsenic content in paneer samples of third vendor was observed to be maximum, while minimum content in fifth vendor’s sample and approximately similar content in first and second vendor’s paneer samples (Fig. 2). The vendor order of arsenic content in dahi samples was 5>2>4>1>3 (Fig. 3). Maximum concentration of arsenic content was observed in fourth vendor’s samples of khoa, whereas minimum content in third vendor’s khoa samples (Fig. 4). In cheese samples, arsenic content was

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**Fig. 5** Heavy metal concentration in cheese procured from five vendors

**Fig. 6** Heavy metal concentration in milk powder procured from five vendors
highest in third vendor’s samples, whereas the lowest was in fifth vendor’s samples (Fig. 5). Arsenic content was found to be highest in third vendor’s milk powder (Fig. 6). The cause of higher content of arsenic in milk/milk products can due to use of arsenic containing compounds, which are used in veterinary medicine for the eradication of tapeworms in cattle (Tchounwou et al. 2012).

The lead and cadmium contents in all the samples were lower than that reported in literature (Tripathi et al. 1999; Licata et al. 2004). Presence of lead in milk and milk products could be due to various factors like transhumance along roads, fodder contamination, climatic factors like winds and use of chemical fertilizers. Cadmium concentration in milk and milk products varies largely depending on the type of food and cadmium load in the food production environment (Olsson et al. 2002). The presence of cadmium in samples could be due to fodder contamination through soil. Cadmium is also the major impurity present in phosphate fertilizers. A recent study suggested that the main input of cadmium to animal feed in milch animals is feeding crops in heavy metal premixes, fish meal, and minerals like limestone and phosphate (Tu et al. 2007). Arsenic has been the only human carcinogen with registered evidence of carcinogenic risk by both inhalation and ingestion (Bhattacharya et al. 2007) and related with lung, liver, and skin bladder cancer in human beings (Kapaj et al. 2006). There are limited data on mercury residues in milk in comparison with other heavy metals, especially lead and cadmium. The most important anthropogenic sources of mercury pollution in the environment are mining and combustion, agricultural materials, industrial and urban discharges (Zhang and Wong, 2007). Mercury is one of the most toxic heavy metals in our environment. Studies have suggested that fungicidal treatments with organo mercurial seed dressings have traditionally been a source of mercury for farm animals, whilst the recent practice of adding fishmeal to feed is the main source of mercury for livestock (López-Alonso et al. 2007).

Cadmium was found maximum in fifth vendor’s milk, paneer and dahi; fourth vendor’s cheese, khoa and second vendor’s milk powder. Lead was maximum in fifth vendor’s milk/milk products except cheese. Lead was observed to be maximum in third vendor’s cheese. Arsenic was maximum in third vendor’s milk, paneer, cheese and milk powder; fifth vendor’s dahi and fourth vendor’s khoa. Mercury was maximum in fifth vendor’s milk, paneer and dahi; fourth vendor’s cheese, khoa and second vendor’s milk powder. Milk/milk products procured from fifth vendor were found to have maximum content of heavy metals, while the first vendor’s milk/milk products had least content of heavy metals. Milk and milk products get contaminated with heavy metals either by the consumption of the contaminated feed/water by the animals or through the utensils used for transportation (Chandrakar et al. 2018). Differences observed may be due to procurement of milk from areas lying near industrial units by the fifth vendor, whereas the first vendor likely procured milk from areas away from industrial pollution.

Conclusions

The information about the presence of heavy metals in market milk products is not available, which is essential for policymaking, standard formation and to take any corrective action, if any. Our data has been recorded in a region with lesser environmental pollution. Studies needed to evaluate the content of heavy metals on large number of milk/milk products to confirm less toxicological risks in this region. Among all the heavy metals studied, cadmium concentration was maximum in all the milk products except paneer, wherein the mercury concentration was maximum. The study has indicated that heavy metal contamination in milk and milk products was much below the MRL prescribed by FSSAI, therefore milk and milk products are safe for human consumption. Further investigation is required to identify the cause of elevated levels of cadmium in milk and milk products.

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References
