

Determination of Arsenic, Lead and Cadmium in Buffalo Milk in Faridabad District of Haryana using ICP-MS

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Brief Report

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ABSTRACT

Milk has largely been considered as a whole food due to the presence of wide variety of macro and micro nutrients. Milk is heavily consumed all over the world, but it is more vulnerable to contamination and most common among them is heavy metal contamination. Increased environmental pollution can lead to the deposition of heavy metals in fodder and drinking water for the milk producing animals. To analyse the content of heavy metals, the milk samples were tested from different areas of the Faridabad district of Haryana India for Arsenic, Lead and Cadmium deposition.

- Milk is nutrition dense food but is more prone to contamination.
- Increased environmental pollution and careless handling of milk can lead to accumulation of heavy metal toxins.
- Heavy metals are linked with serious health issues for humans.
- Milk samples were analysed to identify toxins including Arsenic, Cadmium and Lead.

INTRODUCTION

Across the world milk has been recognised as an important constituent of the daily diet. Milk is a complex mixture of carbohydrates, protein, fats and various vitamins and minerals. The composition of milk varies depending upon genes, breed, species, lactation period and age. (Food Safety and Standards Authority Of India 2007). Milk constitute of 3.3% of the total protein, all the essential amino acids and are present in two forms which have different physical and chemical properties, casein and whey are the two main types of protein found in milk with a content of 80% and 20% respectively (Handbook of Milk Composition 1995). There are approximately 400 different fatty acids present in milk fat making it a complex mixture and are present in the form of an emulsion consisting of triglycerides (98%), diacylglycerols(2%), phospholipids(1%), cholesterol(0.5%) and free fatty acids (0.1%) making the milk fats insoluble in water or any other aqueous liquids. (Handbook of Milk Composition 1995). Sugar in milk is present in the form of a disaccharide known as lactose. Glucose and galactose combine with the help of 1-4 glycosidic linkage and form lactose which is the major source of energy in milk ^[1]. About 8% of recommended daily intake of thiamine is obtained through milk, 35% of recommended intake of riboflavin, 17% of RDA of pantothenic acid and 47% of daily reference intake of vitamin B12 can be obtained by consumption of 250 ml of milk (Handbook of Milk Composition 1995). The vitamin A content of milk can vary according to the fat content of milk. vitamin E and vitamin K are in very minimal amounts in milk (FAO AND WHO,2002). As far as minerals are considered 70% of dietary calcium is derived from milk making it difficult to meet daily calcium requirements without its consumption (Canaby- Rocella and Mellemab, 2010). Approximately 16-20% of daily reference intake of magnesium can be obtained through milk. Phosphorus performs various biological functions in human body and occurs as organic and inorganic phosphate in all body tissues. 30-45% of total phosphorus intake is obtained through milk. Heavy metals are any metallic matter that have a volume more than 6 grams per cubic meter and a very high density. If present in large quantities in any food product, they can lead to central nervous system disorders, affects body's immunity (ability to fight against diseases), organ failures, certain cancers and also reduced enzymatic activity. If not toxicity, heavy metals if once enter the body can accumulate in the cellular structure altering the normal functioning of cells and can lead to bioaccumulation in body organs. Kidney cannot excrete heavy metals beyond a certain capacity and over exposure of these heavy metals can over burden and lead to a potential renal damage. Arsenic(As), Lead(Pb), Cadmium(Cd), Mercury(Hg) are some examples of heavy metals and can cause serious health damage. India is one of the most polluted countries in the world according to the World Health Organisation. Due to the increased industrial activity, construction work and vehicle pollution the environment has been exposed to heavy metals ^[2]. A reduced air

quality due to the accumulation of heavy metals can enter the food chain. Through rainfall these heavy metal pollutants get deposited on the soil, thus making their way into the crops and. Also, an increased incidence of water pollution due to industrial dump has rapidly increased heavy metal pollution in water.

A research conducted by Central Water Commission says 42 Indian rivers have at least two toxic heavy metals beyond the permissible limits. Most of the water for irrigation of crops is taken through these waters and deposit deep into the soil leading to increased heavy metal concentration of agricultural land. In the region of national capital around Yamuna river is used as agricultural feed to grow most of the crops supplied to these areas. These crops are later used as fodder for most of the milk producing animals thus making their way into their milk. Also drinking of water containing heavy metals by the animals lead to an accumulation of heavy metals in animals. Organic fertilizers, overuse of pesticides, herbicides and agrochemicals also contribute to heavy metal exposure in animals. Unhygienic handling practices such as unclean metal containers used during processing or transportation can also contribute to accumulation of heavy metals in milk. Cadmium is soft, ductile, bluish white, malleable, and is highly carcinogenic. Cadmium compounds are extremely toxic for animals, plants and human beings. Cadmium is widely distributed in soil, air plants, water and in animal tissues. Contamination in humans can occur mainly through cigarette smoking and tobacco and through ingestion of certain foods mainly meat and milk. Cadmium toxicity can lead to serious pulmonary, renal and bone damage. It is suggested that even low concentrations of cadmium intoxication can alter various metabolic processes in human body.

When lead enters the body through inhalation or ingestion it is distributed to various organs such as liver, kidney, brain and bones. Adults typically absorb up to 20% of ingested inorganic lead after a meal and up to 60-80% on an empty stomach. Children absorb about 50% of ingested lead after a meal (ATSDR 2010) and up to 100% on an empty stomach. Bioaccumulation of lead in human body majorly take place in bones and teeth. The accumulated lead can enter the blood stream during pregnancy and through placenta can enter the foetus causing neurological disorders in the infant. Children and malnourished people are at a higher risk of lead toxicity. Also calcium, iron or zinc deficiency promotes absorption of lead in humans. Lead is associated with central nervous system disorders and lower intelligence quotient. It is a well-known risk factor for cancer, cardiovascular diseases and gout. Arsenic can enter human body through inhalation consumption of contaminated food and water and can also be exposed through dermal route. Inorganic arsenic is considered as a human poison and if ingested in higher doses can lead to death. Immediate exposure can lead diarrhoea, GI haemorrhages and circulatory collapse. Conjunctivitis, redness, pain and swelling are some of the ocular changes observed. Long term exposure of arsenic can cause skin changes like appearance of warts or corns on palms and skin patches or hyperpigmentation and can give rise to skin cancer. Studies in animals show that large doses of inorganic arsenic that cause illness in pregnant females can also cause low birth weight, foetal malformations, and even foetal death as it can easily pass through placenta^[3].

Arsenic toxicity can lead to numerous types of cancer by changing the normal cellular metabolism. Since, heavy metals are highly toxic in nature and most of them are cumulative toxins and once they enter the body can be absorbed by most of the organs. They can affect the functioning of almost all the organs including neurological activities of brain, metabolic reactions of liver, excretion through renal systems and so on. It can be an etiological factor for various types of cancer, cardio-vascular diseases, chronic kidney diseases, liver failure, degeneration of neurons. Now, these effects are life threatening making it extremely important for the concerned authorities and general public to keep monitoring the food that they are eating. Also, authorities responsible for pollution control should be alarmed with these concerns as it is the main cause of bioaccumulation of these pollutants in food. Proper monitoring system is required to control heavy metal environmental pollution to ensure urban food safety (Figure 1).

METHODOLOGY

Study area

Faridabad is one of the most populated and largest city, situated in the state of Haryana. The city was established in the year 1607 and was named after Shaikh Farid. The total area of Faridabad is 742.9 km² with total of 89 sectors out of which 18 sectors fall under the HUDA jurisdiction and rest 71 sectors under MCF. Due to an increased demand Faridabad's dairy industry milk production is establishing in the city. The dairy market in Faridabad is controlled by Haryana Dairy Development Corporation, according to them, the amount of milk to be supplied in 24 hours largely depends on the demand of the consumer^[4].

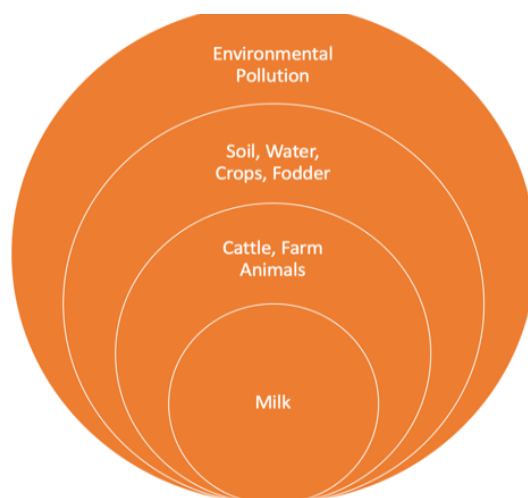
Sample collection

In order to understand the dietary status of milk and associated contaminations in Faridabad district, the entire area was divided into different zones on the basis of survey which was conducted to understand the needs, awareness, volume of milk purchased and behaviour of the consumer (Figure 2).

The samples were collected from ZONE A, B, C keeping in mind the population density and distribution/circulation of milk by unorganized sector i.e private gawallas. A total of 15 samples in sterilized glass bottles were collected. The samples were labelled as A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, B6, C1, C2, C3, C4. Then the samples were taken in an ice box to avoid milk spoilage to the lab for analysis. Their 50 ml of milk from each sample was extracted for digestion and analysis.

Instrumentation

The heavy metal analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS). It is an equipment used for isotopic and multielement analysis and provides highly accurate result due to its high metal sensitivity. The technique is less



HOW HEAVY METALS MAKE THEIR WAY IN MILK

Figure 1. Graphical abstract.

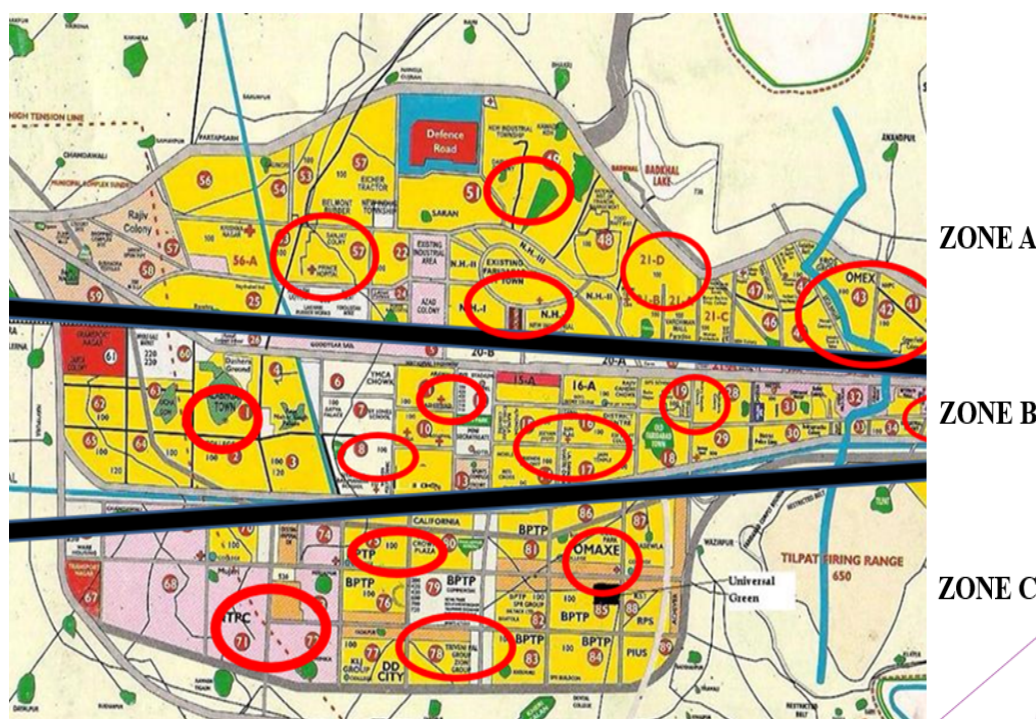


Figure 2. Population Density and Distribution/Circulation of Milk by Unorganized Sector.

cumbersome as it does not require complicated pre-treatment procedures. It is a highly specific and reactive to minor trace elements. Firstly, the samples were pre-treated and turned into an aqueous solution and then they are vaporized with the help of a nebulizer. In the second place, the samples were introduced to the plasma which consists of positively charged argons and negatively charged electrons in order to positively charge the metallic ions, this step is essential as negatively charged particles cannot be trace using this procedure. The positively charged ions pass through the quadruple mass analyser where compounds of the samples are separated depending upon their mass to charge ratio^[5]. Lastly the special mass detector is able to detect the ions and provides result on a computer monitor attached to it (Table 1).

RESULT AND DISCUSSIONS

To estimate the amounts of heavy metals present in milk sample analysis was conducted for Arsenic, Lead and Cadmium. The milk samples were identified with the concentrations of arsenic, lead and cadmium from the samples taken from district of Faridabad (Tables 2,3).

Cadmium

Cadmium is a cumulative toxin and can get accumulated in cells even if consumed in small quantities (ATSDR). According to the International Dairy Federation cadmium content in milk 0.015 mg/L is considered to be polluted. This tallies that all the samples have cadmium well-below the permissible limits. The possible reasons for cadmium contamination could be due to accumulation

Table 1: Microwave digestion process used for preparation of milk samples for testing.

Sample Name	Volume of Milk (mL)	Conc HCl (mL)	Conc HNO3 (mL)	Internal Temperature Limit (OC)	Max. Microwave Power (Watt)	Max. Pressure (bar)	Time (min)
Sample A1	1	6	2	200	1200	60	30
Sample A2	1	6	2	200	1200	60	30
Sample A3	1	6	2	200	1200	60	30
Sample A4	1	6	2	200	1200	60	30
Sample A5	1	6	2	200	1200	60	30
Sample B1	1	6	2	200	1200	60	30
Sample B2	1	6	2	200	1200	60	30
Sample B3	1	6	2	200	1200	60	30
Sample B4	1	6	2	200	1200	60	30
Sample B5	1	6	2	200	1200	60	30
Sample B6	1	6	2	200	1200	60	30
Sample C1	1	6	2	200	1200	60	30
Sample C2	1	6	2	200	1200	60	30
Sample C3	1	6	2	200	1200	60	30
Sample C4	1	6	2	200	1200	60	30

Table 2: Reference Doses used for Calculation of Target Hazard Quotient According to USEPA.

S.No	Elements	Reference Doses ppm
1	CADMIUM	0.015
2	LEAD	0.02
3	ARSENIC	0.1

Table 3: The amount of lead, cadmium and arsenic detected in milk samples tested using ICP- MS.

S.NO	SAMPLE	CADMIUM (ppm)	LEAD (ppm)	ARSENIC (ppm)
1	Sample A1	0.00019	0.00011	0.00011
2	Sample A2	0.00014	0.00011	0
3	Sample A3	0.00011	0	0
4	Sample A4	0.00011	0	0
5	Sample A5	0.0001	0	0
6	Sample B1	0.00011	0.000061	0
7	Sample B2	0.00011	0.000108	0
8	Sample B3	0.00012	0	0
9	Sample B4	0.00013	0.000224	0
10	Sample B5	0.00016	0.001421	0
11	Sample B6	0.00013	0.000109	0
12	Sample C1	0.000098	0	0
13	Sample C2	0.00011	0	0
14	Sample C3	0.000091	0.000052	0
15	Sample C4	0.00001	0.000359	0

of cadmium in forage or drinking water of the buffalo [6]. Cadmium's maximum risk levels is 0.0001 mg/kg/day depending upon its renal capacity stated in the toxicology report of cadmium given by Agency of Toxic Substances and Disease Registry [7].

Lead

The codex Alimentarius 2015, the maximum permissible limits of lead in milk is 0.02 mg/L according to which, all the values obtained from elemental analysis of milk samples collected from Faridabad have potentially low levels of lead [8]. Samples A3, A4, A5, B3, C1 and C2 depicted no lead contamination. Lead like cadmium are cumulative toxins and once they enter the body have a very low excretion rate (ATSDR) [9].

Arsenic

There is a very limited work on arsenic concentrations in milk although data for arsenic content in sea food is abundantly available. The International Dairy Federation established the maximum limits for arsenic in milk in the year 1986 [10]. The IDF suggests that the arsenic content should not be present in milk in quantities more than 10 ng/g i.e. 0.01 mg/L. In milk samples tested from Faridabad only one sample was found to be contaminated with arsenic (Sample A1). The arsenic contamination could be due to heavy arsenic pollution in the area from where the milk is obtained, or it could be due to arsenic contaminated forage or water. None of the other samples were detected to be contaminated with arsenic [11].

CONCLUSION

This study highlights the necessity of food safety and provides vital data of metal constituents such as cadmium, arsenic and lead. None of the samples crossed the permissible limits of these heavy metals so milk from local sources can be considered safe as far as heavy metal toxicity is considered for these three elements, however bioaccumulation of these heavy metals is still a topic of concern as the data on excretion of these heavy metals from the body is limited thus it needs more elaborative study to find out the rate of health hazards it can cause due to the accumulation of these metals in the cells.

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