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Determination of lead and cadmium level in milk of dairy animals in Mehsana district of Gujarat using ICP-AES

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ABSTRACT
The present study was undertaken to observe levels of two heavy metals viz. cadmium and lead in milk of dairy animals using Inductively Coupled Plasma Atomic Emissions Spectroscopy. A total of 30 milk samples were collected from cattle and buffaloes reared in peri-urban area of Mehsana district of Gujarat State, and analyzed for concentrations of cadmium and lead. Results showed traces of cadmium in 24 samples and lead in all 30 samples, however, concentration of these heavy metals were below maximum permissible levels as recommended by Food Safety and Standards Authority of India.
Keywords: *Cadmium, Lead, Milk Level, ICP-AES, Gujarat.*

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INTRODUCTION

The whole world is facing challenge of environmental pollution due to never ending race of industrialization and urbanization. The impact of environmental pollution on safety and quality of foods of livestock origin especially, dairy animals are not uncommon. Technological progress, various industrial activities, and increased road-way traffic have caused significant increase in heavy metals in environment and affects air, soil, water and consequently, food [1]. The high level of heavy metal residues in milk is of great significance from public health point of view. Lead (Pb) and Cadmium (Cd) are amongst the elements that have caused the most concern in terms of adverse effects on human health. This is because they are readily transferred through food chains and are not known to serve any essential biological function. Lead and Cadmium residues in milk are also of particular concern as milk is largely consumed by infants and children [2]. Accumulation of lead produces damaging effects in the hematopoetical, hematic, renal, gastrointestinal systems. It induces reduced cognitive development and intellectual performance in children, increased blood pressure, and cardiovascular diseases in adult as well as liver and kidney dysfunction [3, 4]. Cadmium enters into human and animal body through food chain and can cause serious diseases. It is implicated in high blood pressure, prostate cancer, mutations and fetal (embryonic) death [5, 6, and 7].
Thus, contamination of milk with heavy metals imposes risk on consumer's health. Additionally, presence of toxic metals in milk also constitutes a trade barrier for dairy industries because of technological risk involved in processing and packaging. Hence, the present study was planned to investigate the levels of cadmium and lead in milk of dairy animals in peri-urban area of Mehsana district of Gujarat.

MATERIALS AND METHODS

A total of 30 milk samples (fresh and raw) were collected from dairy cattle (5) and buffaloes (25) from farmers' door-step in the peri-urban area of Mehsana district of North Gujarat. The milk sample were collected in the 15 ml centrifuge tube and then kept in ice jacketed thermo cool box. Samples were transferred and stored at – 4^{o}C temperature until analysis.

Digestion of the samples

The method prescribed by [8] was followed with some minor modifications. Five ml of raw milk sample was measured into a beaker and topped with 5 ml distilled water. Exact 5 ml of 0.1 M trichloroacetic acid was added to the sample to precipitate the proteins in the milk. The samples were then centrifuged at a speed of 4000 RPM for 20 minutes. The aqueous fraction was separated by decanting. The aqueous part of the sample was then poured into the porcelain crucibles. Crucible content was ashed in a muffle furnace for three hours at 50^{o}C. The ash was then dissolved and reconstituted in 15 ml of 6 % nitric acid solution. The dissolved sample was then filtered with filter paper (Whatman Paper No. 41). It was then poured into dry and clean centrifuge for temporary storage until assay was done. The final make up of 15 ml were done for each sample before analysis.

Preparation of standard metal ion solution:

Stock solution (100 mg/l) of both cadmium and lead were prepared using appropriate metal salt of AR grade quality in dil. hydrochloric acid. The working standards of these solutions were prepared by appropriate dilutions in distilled water.

Instrumentation

The samples were analyzed on Inductively Coupled Plasma Atomic Emissions Spectroscopy (ICP-AES, Model iCAP 6000, Thermo fisher). Instrument configuration and general experimental conditions are summarized in Table 1. For each sample three determinations were performed and average results were reported. Detection limits of the Cd and Pb studied in milk samples (Table 2) were determined from the standard addition curves of each element in different samples. It was based on the usual definition as the concentration of the analyte yielding a signal equivalent to three times the standard deviation of the blank signal. The detection limits of the present method are good and permit the determination of the elements in milk at background concentrations.

Table1: ICP-AES operating conditions

Sr. No.	Operating conditions	
1	RF Frequency	27.12 Hz
2	RF Power	2.5 Kw
3	Outer gas flow rate	Ar 17 L/min
4	Intermediate gas flow rate	Ar 1 L/min
5	Carrier gas flow rate	Ar 1 L/min
6	Observation height	18 mm above work coil
7	Plasma's temperature	8000-9000 K

Table2: Detection limits of Cd and Pb for ICP-AES method

Sr. No.	Element	Detection limit (mg/L)
1	Cadmium (Cd)	0.00063
2	Lead (Pb)	0.00703

RESULT

Level of cadmium and lead in individual samples are presented in table 3, whereas, descriptive statistics of Cd and Pb levels and frequency distribution of Cd and Pb residues in milk samples are represented n table 4 and 5, respectively. Table 6 compares interspecies variation in level of two heavy metals. The average concentrations of Pb in milk of cow and buffalo were found to be 0.124 and 0.085 mg/L, respectively. The overall range for Pb was found as 0.012 to 0.442 mg/L. All milk samples showed detectable concentration of Pb. The average concentrations of Cd in milk of cow and buffalo were found to be 0.024 and 0.002 mg/L, respectively. The overall concentration of Cd was found in the range of 0.001 to 0.009 mg/L.

Table 3: Samples showing cadmium and lead concentration in mg/L in cow and buffalo milk collected from peri-urban area of Mehsana District.

Sr. no.	Milk sample ID	Metal concentration in cow and buffalo milk in mg/L	
		Cadmium (N=30)	Lead (N=30)
1	M 1	0.000	0.022
2	M 2	0.005	0.109
3	M 3	0.001	0.027
4	M 4	0.001	0.064
5	M 5	0.005	0.211
6	M 6	0.000	0.025
7	M 7	0.001	0.132
8	M 8	0.001	0.093
9	M 9	0.000	0.020

10	M 10	0.000	0.023
11	M 11	0.003	0.210
12	M 12	0.002	0.075
13	M 13	0.001	0.073
14	M 14	0.001	0.090
15	M 15	0.009	0.356
16	M 16	0.002	0.229
17	M 17	0.001	0.148
18	M 18	0.000	0.024
19	M 19	0.000	0.016
20	M 20	0.009	0.154
21	M 21	0.001	0.032
22	M 22	0.008	0.442
23	M 23	0.001	0.012
24	M 24	0.001	0.038
25	M 25	0.000	0.012
26	M 26	0.000	0.021
27	M 27	0.000	0.017
28	M 28	0.000	0.011
29	M 29	0.005	0.042
30	M 30	0.002	0.026

Table 4: Descriptive statistics of levels of Cadmium and Lead (mg/L) in milk samples (n=30).

Residue	Positive samples		Minimum	Maximum	Average	Mean ± S.E
	No.	%				
Cadmium	24	80	0.001	0.009	0.002	0.002 ±0.001
Lead	30	100	0.011	0.442	0.092	0.092 ± 0.020

Table 5: Frequency distribution of Cadmium and Lead residues in examined milk samples (n=30).

Residue	Permissible limit mg/L (FSSAI, 2011)	Samples within permissible limits		Samples over permissible limit	
		No.	%	No.	%
Cadmium	1.50	30	100	0	0
Lead	2.50	30	100	0	0

Table 6: Interspecies comparative mean ± SE and range of Cadmium and Lead (mg/L) in the milk samples of cow and buffalo.

Residue	Cow milk		Buffalo milk	
	Mean ± SE	Range	Mean ± SE	Range
Cadmium	0.024 ± 0.002	0.000-0.009	0.002 ± 0.001	0.000-0.009
Lead	0.124 ± 0.063	0.016-0.356	0.085 ±0.020	0.011-0.442

Cadmium was not detected in six samples. The maximum permissible limit as per FSSAI for Cd and Pb is 1.50 and 2.50 mg/L, respectively. None of sample was found to contain Cd and Pb above maximum permissible limit. Lead and cadmium are toxic minerals often associated industrial pollution. Additionally, automobile exhaust is also believed to be sources of environmental contamination. Thus, the presence of lead and cadmium in milk is likely to happen in and around urban and industrial area.

DISCUSSION

Cadmium is regarded as one of the most serious contamination of the modern age. It is absorbed by many plants and sea creatures and because of its toxicity, presents a major problem for foodstuffs. It is now known that the *itai-itai* sickness in Japan was a result of the regular consumption of rice, highly contaminated with cadmium.

The average concentration of cadmium in present study ranged from 0.000 to 0.009 mg/L in cow milk and 0.000 to 0.009 mg/L in buffalo milk with mean value of 0.024 and 0.002 mg/L, respectively.

Presence of cadmium in cow milk samples obtained from different locations in Mumbai city was studied wherein the mean Cd concentration was reported in the range of 0.012 mg/L to 0.013 mg/L [9] which was higher than range found in present study.

Mean Cd concentration was found to be in the range of 0.004 mg/L to 0.018 mg/L in selected milk samples obtained from different regions of Egypt [10] whereas level of cadmium in cow milk (0.001 mg/L to 0.009 mg/L) was found to be lower in present investigation. Range of cadmium concentration in cow milk samples as 0.001 to 0.009 mg/L were reported [11] and these results are similar to present study. Concentrations of cadmium in samples of cow and buffalo milk in industrial and non industrial area of Haryana were determined [12] as 0.008 and 0.006 mg/L, which was lower than the mean value of present investigation. In present study, the overall cadmium concentration was found to be ranging between 0.001 to 0.009 mg/L which was higher than the reported by [13]. According to Indian Dairy Federation standard (1997), the level of 0.006 mg/L of cadmium is regarded as normal for unpolluted milk whereas milk containing 0.015 mg/L of cadmium considered as polluted. In our study, the levels of cadmium were found below the permissible limit (1.5 mg/L) as per FASSAI regulation.

In present study, the minimum to maximum concentration range (overall) of lead milk (cow and buffalo) were observed to 0.011 to 0.442 respectively, with a mean concentration of 0.092 mg/L. The average concentration of lead in present study ranged from 0.016 to 0.356 mg/L in cow milk and 0.011 to 0.442 mg/L in buffalo milk, with mean value of 0.124 and 0.085 mg/L. The presence of lead in all the 30 milk samples might be due to contamination of the soil, water and fodder on which these animals were fed.

Compare to these results, range of higher Pb concentrations in milk of cattle and buffalo were observed by [14], [15], [16] and [17]. However, the concentrations of lead in cow and buffalo milk in present study were also found to be higher than those (0.052 mg/L) reported in Tamilnadu [18].

Lower range level of lead in raw milk as 0.0315-0.0396 mg/L was reported from Tangshan city of China, as compared to present investigation. The concentration range of lead in the present study was observed to be higher than those reported in cow in Iran [20] and, cattle and buffalo in Nigeria [21].

Lead is very toxic and it is potent neurotoxin and has a cumulative effect on the vital organs. The presence of lead in milk samples may be attributable to many factors such as rearing or movement of livestock along road and /or motorways, fodder contamination, climatic factors, such as winds, and the use of lead containing agrochemicals. One of the important sources of lead contamination in milk is water, especially in more contaminated areas, so water testing would be one of the important areas for future study.

In contrary, to these reports, in present study, the lead levels were observed higher than cadmium. This may due to different nature of industrial pollution and contamination of natural resources. The different species of livestock also have varying capacity of metabolism of lead and cadmium. In present investigation, the maximum level of lead and cadmium were observed to be lower than reported toxic levels of both the metals.

CONCLUSION

The concentrations of cadmium detected in 24 samples and lead in all 30 samples were within the permissible limit as per recommended by the FSSAI regulation. It is concluded that the residual concentration of cadmium and lead were found within maximum permissible limit, so milk from such animal are regarded as safe for human consumption with respect to level of cadmium and lead. The conclusion provides important information for food safety and environmental health of area from where samples were collected. Similarly, the result also bears impact on trade quality of raw milk for revenue generation.

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CONFLICT OF INTEREST

Nil

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