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Heavy Metal Concentrations with Regard to Inter-Species Variation in Cattle and Buffalo Milk Collected from Different Areas of North Gujarat

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A B S T R A C T

In the present study, heavy metal residual concentrations of cadmium (Cd), lead (Pb) and copper (Cu) in cattle and buffalo milk samples collected from three districts of the Northern part of Gujarat state were determined. A total of 800 samples were collected during the entire study in summer and winter seasons. Heavy metal (Cd, Pb and Cu) residues in milk samples were determined by the Atomic Absorption Spectrometry (AAS) technique. The average values of Cd, Pb and Cu in the milk of cattle were found to be 0.033 ± 0.002, 0.121 ± 0.014 and 0.097 ± 0.012 mg/L, respectively. Similar values for buffalo milk were 0.030 ± 0.002, 0.139 ± 0.015 and 0.168 ± 0.036 mg/L, respectively. The inter-species comparison between cattle and buffalo demonstrated the higher level of Cd, Pb and Cu in buffalo milk. There was non-significant difference in the level of heavy metals in the milk of cattle and buffalo.

Introduction

Food safety and traceability control in the production of dairy animal products has become crucial criteria for the consumers because of the novel requirements of food for disease prevention and health (Poti *et al.,* 2012). Contamination of environment with heavy metals leads to the presence of such toxic compounds in the food of animal origin in varying degree. Heavy metals often termed as ‘toxic metals’ are important environmental contaminant leading to their appearance in milk. This may be through natural or anthropogenic sources. The risk involved is due to their persistence, toxicity and ability to be accumulated into the environment and subsequently into the food chain.

Heavy metals are bio-transferred, bio-accumulated and biomagnified in food chain and food webs. Animals like cattle, buffalo, sheep and goat feed on grasses and plants which may contain pollutants like heavy metals, so that heavy metals are bio-transferred in their bodies and these heavy metals either harm or accumulate in their bodies (Nazir *et al.,* 2015).

The level of heavy metals in the milk of dairy animals directly indicates food safety and hygienic status of food for consumers. It also indirectly reflects degree of environmental contamination where milk is either produced or procured (Licata *et al.,* 2004 and Gonzalez-Montana *et al.*, 2012).

Contamination of milk by toxic metals can be a possible health risk to human population (Caggiano *et al.,* 2005). Pollution of the environment with metals such as lead is a world-wide problem. Lead and cadmium residues in milk are of particular concern because milk is largely consumed by infants and children (Zheng *et al.,* 2007). Lead is well absorbed from milk (Morisson and Quarterman, 1987). Lead is a non-essential element and causes fatal type of disease such as Alzheimer's disease, renal, reproductive, endocrine, nervous disorders and decreases intelligence quotient (IQ) level of children (Ahmad *et al.,* 2011). Cadmium extremely irritates stomach leading to antiperistalsis, vomition, and diarrhoea and may cause death (Bushra *et al.,* 2014). Cadmium is also implicated in high blood pressure, prostate cancer, mutations and fetal death (Pitot and Dragan, 1996). Copper plays a very significant role in growth while overexposure of copper can cause hepatic copper II overload disease, tissue injury, irritation of lungs and hepatic cancer (Bushra *et al.,* 2014).

The northern region of Gujarat state contributes more than 50% share of total milk production of Gujarat. The three districts included in the present study are Banaskantha, Mehsana, Gandhinagar, which contribute nearly 13.19, 7.03 and 3.27% share of total milk production of North Gujarat, respectively. The dairying and animal husbandry are major tools for livelihood and food security. The quality and safe milk production are the backbone of dairy industry for domestic as well as foreign export. Hence the present investigation on monitoring of heavy metals in milk of cattle and buffalo in North Gujarat was planned with objectives to generate data relevant to heavy metals (Cd, Pb and Cu) in cattle and buffalo milk over different areas of North Gujarat and to determine if any inter-species variation exists in this regard.

Materials and Methods

Reagents

The trichloroacetic acid and nitric acid (SDFL, India, Extra pure grade) were procured and used for the analysis of heavy metals. The de-ionized water was prepared using Ultra Water Purifier (SG, Germany). All glassware was thoroughly cleaned with a detergent solution, rinsed with metal free water and soaked overnight or longer in a covered acid bath containing 6% HNO_3 solution.

Collection of samples

Milk samples from cattle and buffalo were collected from different regions of North Gujarat having distinct climatic and geographic characteristics. Three different districts i.e. Gandhinagar, Mehsana and Banaskantha were chosen for sample collection. The five villages were selected within each designated area of sample collection.

The total number of samples collected for the entire experiment was 800. The area-wise and species-wise details of sample collection are shown in Table 1. The samples were collected during morning milking hour directly by milking of animals at the collection point/ farmers' door-step. About 10 ml of milk was collected in a clean and dry centrifuge tube. The samples were placed immediately in an ice jacket box packed with an ice pack and ice cubes. On reaching the laboratory (same day), the samples were transferred to deep freeze at – 20 º C until analyzed by AAS.

Standard preparation in AAS analysis

The standards of each element were procured from ECIL, India and used for the AAS analysis. The stock solution of 100 mg/L was prepared and used to prepare working standards of 0.1, 0.2, 0.4, 0.8, 1.0 and 10.00 mg/L using 6% HNO_3. The calibration curve was prepared by plotting known concentration *versus* absorbance values and used to estimate the concentration of toxic metals from unknown samples.

Sample digestion

Five ml of milk sample was measured in a beaker and topped to 5 ml with distilled water (this was done to dilute the milk since it was too thick for analysis in its undiluted form). Exact 5 ml of 0.1M 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 then separated by decanting and poured into the porcelain crucibles having a mark for easy identification. Crucible content was ashed in a muffle furnace for three hours at 500 ºC. 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 tubes (Prior washed with de-ionized water) for temporary storage until assay was done. The final make-up of 15 ml was done for each sample before analysis (Patel *et al.,* 2017).

Analytical procedure for AAS

Heavy metal (Cd, Pb and Cu) residues in milk were determined by the AAS (Atomic Absorption Spectrometry). Atomic Absorption Spectrophotometer (ECIL, Electronics Corporation of India Limited, Model AAS4141) equipped with hydride generator was used for determination of heavy metals. The hollow-cathode lamps for Cd, Pb and Cu (Photron) were employed as the radiation source. The fuel used was a mixture of air and acetylene. Digested samples were analyzed using flame atomic absorption spectrophotometer. Standard dilutions for each metal were prepared in five different concentrations from their respective stock solution (100 mg/L) to obtain a calibration curve. All the measurements were run in triplicate for the samples and standard solutions. The instrumental conditions during the analysis are listed in Table 2.

Quality assurance

Appropriate quality assurance procedures were taken to ensure the reliability of the results. In addition, samples were carefully handled to avoid contamination. Moreover, the glassware was properly cleaned and reagents used were of analytical grades. The de-ionized water was used throughout the study. Each time standards were run before the sample run in the AAS.

Statistical analysis

The significance of the result difference between cattle and buffalo were tested using student's 't' test. The impacts of species variation on the concentration of heavy metal in milk were tested using SPSS 16.0 for Windows® and GraphPad PRISM® (version 7.00).

Results and Discussion

Interspecies comparison of mean ± SE values of Cd, Pb and Cu (mg/L) in cattle and buffalo milk collected from all four areas is summarized in Table 3. There was non-significant difference in the average concentrations of Cd, Pb and Cu in cattle and buffalo milk. Interspecies comparison of a range of Cd, Pb and Cu (mg/L) in cattle and buffalo milk collected from all four areas is presented in Table 4. The interspecies differences were evident in minimum and maximum values of all respective metals between cattle and buffalo milk collected from all four areas. The highest concentration of Cu in buffalo (8.959 mg/L) and cattle milk (2.370 mg/L) were observed.

Summary of interspecies comparison of cattle and buffalo milk samples with respect to presence of heavy metals and comparison with MRLs of respective metals are presented in Table 5. The results of the study were direct indicator of food safety and quality of milk. No samples of cow and buffalo milk were found to contain heavy metals (Cd, Pb and Cu) above MRLs.

The quality of milk needs special attention to make it acceptable widely without imposing any risk on consumers' health. Once heavy metals get absorbed, accumulate in the body, even for the whole span of life (Bernard, 2008). Even at low concentrations, certain heavy metal can adversely affect a number of metabolic processes in the animal body.

Being hazardous and toxic, the presence of heavy metals in the foods of livestock origin especially dairy milk needs to be evaluated and its safety must be established with respect to quality and quantity of metal contaminants. Further, such study indirectly reflects the levels of contamination and pollution of environmental resources as well as their link to the health status of dairy animals. Looking at all these important aspects, the present study to monitor the levels of heavy metals and to study inter-species variation in the milk of cattle and buffalo of North Gujarat were planned and executed.

The inter-species variation, when compared, higher levels (mg/L) of Cd (0.030 ± 0.002), Pb (0.139 ± 0.015) and Cu (0.168 ± 0.036) in buffalo milk were observed than their respective values, Cd (0.033 ± 0.002), Pb (0.121± 0.014) and Cu (0.097 ± 0.012) in cattle milk in present investigation. This might be due to non-selective grazing habit of buffalo species and can graze at any contaminated vegetation. While cattle being highly selective in grazing habits, is likely to avoid the grazing or feeding on contaminated food sources. The interspecies variation in the level of heavy metals might be due to variation in feeding/ grazing habits, disposition characteristics of particular species with respect to toxic metals and intensity of exposure in terms of duration and amount. Contradictory results were reported by Birghila *et al.*, (2008) for Cd, Pb and Cu in cow milk in Romania; Qin *et al.*, (2009) for Cd in cattle milk in China and Japan; Aslam *et al.*, (2011) for Cd and Pb in cow milk in Pakistan and Abdulkhaliq *et al.*, (2012) for Cu and Pb in cattle milk in Palestine, as compared to findings of present study. Furthermore, lower concentrations of Cd and Pb in cattle milk (Tona *et al.*, 2013) were also reported in Nigeria.

The variations in the concentration of heavy metals observed in present study in comparison to other reported studies might be due to the difference in levels of exposure, intensity of contamination of natural resources and feeding/grazing patterns over study areas involved in other reports.

The average concentration of lead in present study ranged from 0.001 to 2.019 mg/L in cattle milk and 0.001 to 2.442 mg/L in buffalo milk with a mean value of 0.121 and 0.139 mg/L, respectively. Extremely higher average value of Pb concentration in cattle milk (18.870 mg/L) was observed in Faislabad area of Pakistan (Javed *et al.*, 2009).

Table.1 Species and area wise details of sample collection

Sr. No.	District	Species		Total no. of samples collected
		Cattle	**Buffalo**	
1	Mehsana	116	284	400
2	Gandhinagar	135	65	200
3	Banaskantha	59	141	200
	Total	310	490	800

Table.2 Instrumental condition for analysis

Sr. No.	Parameters	Cd	Pb	Cu
1	Wavelength (nm)	228.8	217	324.8
2	Slit width (nm)	0.5	1.0	0.5
3	Light source	HCL-Hollow Cathode Lamp		
4	Flame type	Air/C_2H_2		
5	Current(ma)	3.5	10	5
6	AAS Technique	Flame		

Table.3 Interspecies comparison of mean ± SE values of Cd, Pb and Cu (mg/L) in cattle and buffalo milk collected from all four areas

Residue	Cattle	Buffalo
Cd	0.033 ± 0.002	0.030 ± 0.002
Pb	0.121 ± 0.014	0.139 ± 0.015
Cu	0.097 ± 0.012	0.168 ± 0.036

* = Significantly ($p \leq 0.05$) different from their respective values

Table.4 Interspecies comparison of range of Cd, Pb and Cu (mg/L) in cattle and buffalo milk collected from all four areas

Residue	Cattle (Range)	Buffalo(Range)
Cd	0.001-0.080	0.001-0.088
Pb	0.001-2.019	0.001-2.442
Cu	0.001-2.370	0.001-8.959

* = Significantly ($p \leq 0.05$) different from their respective values

Table.5 Summary of interspecies comparison of cattle and buffalo milk

Residue	Cattle (n=310)			Buffalo (n=490)		
	No. of samples having detectable level	No. of samples having non detectable level	Above MRL	No. of samples having detectable level	No. of samples having non detectable level	Above MRL
Cd	246	64	0	231	169	0
Pb	193	117	0	205	195	0
Cu	198	152	0	141	259	0

MRLs of Cd, Pb and Cu are taken as 1.5, 2.5 and 30 mg/L, respectively as per FSSAI (2011)

The similar higher range of Pb concentration (2.082 – 2.024 mg/L) was reported by Iftikhar *et al.,* (2014) in the Peshawar area of Pakistan in cattle and buffalo milk. The maximum concentration of Pb in buffalo milk in present study observed to be higher than those reported by Nirgude and Bhagure (2015) for buffalo milk in India and Arafa *et al.,* (2014) for cattle and buffalo in Egypt. The maximum concentration of Pb in cow milk was also found to be higher in the present study than those (0.052 mg/L) reported by Dhanalakshmi and Gawdaman (2013) in Tamilnadu. The concentrations range of Pb in present study was observed to be lower than those reported in cows in Iran (Nejatolahi *et al.,* 2014) and, cattle and buffalo in Nigeria (Farid and Baloch, 2012). Higher concentration of Pb in milk was found due to fodder contamination, climatic factors such as wind, use of agro-chemicals and very importantly, high level of contaminants in drinking water. Furthermore, dairy animals graze along rail lines and roadsides contribute the same. Many times, the dairy farms or animal stable are located in close approximation to busy traffic roads and lead which is a fuel additive is emitted from the automobile exhaust to contaminate such environment.

In the present study, the concentration range of Cd in cattle and buffalo milk were observed to 0.001 to 0.080 and 0.001 to 0.088 mg/L with mean concentrations of 0.030 and 0.033 mg/L, respectively. The observed concentration of Cd was found to be lower than those reported in milk of cattle as 1.532 ± 0.124 mg/L by Alani and Al-Azzawi (2015) in raw milk collected from Iraq; 4.16 mg/L by Lyocks *et al.,* (2013) in Kaduna Metro area, Nigeria; 0.416 mg/L by Salah *et al.,* (2012) in Egypt and 0.532 mg/L by Leonidis *et al.,* (2010) in Thessaloniki, capital of Greek Macedonia. According to a study report by Caggiano *et al.,* (2005) from Romania, concentration of Cd in cattle milk samples ranged from 1.000 to 5.000 ppm and lower concentration range of Cd in cattle and buffalo milk were observed in the present study.

Abnormal concentrations of Cu in tissue and blood causes Wilson disease, and acute exposure to Cu cause vomiting, hypertension, and cardiovascular collapse (Ogabiela *et al.,* 2011), and the occurrence of Cu in milk is likely to be influenced by the environment (Qin *et al.,* 2009). The value of Cu concentration ranged from 0.001 to 2.37 mg/L and from 0.001 to 8.959 mg/L with mean concentrations of 0.097 and 0.168 mg/L, respectively in cattle and buffalo milk. The concentration of Cu was lower in cattle milk as compared to buffalo milk. Higher concentrations of Cu in the milk of cow were observed by Alani and Al-Azzawi (2015);

Gebrelibanos *et al.,* (2015); Malhat *et al.,* (2012); Kodrik *et al.,* (2011); EI-Sayed *et al.,* (2011) and AL-Wabel (2008). Similarly, higher concentration of Cu in the milk of both cattle and buffalo (1.692 mg/L) was also reported by Arafa *et al.,* (2014).

The overall summary reflected that no samples collected from any area from any species showed the presence of metals (Cd, Pb and Cu) above recommended MRLs as per FSSAI and the study demonstrated non-significant variation in the levels of all metals under investigation in cattle and buffalo milk.

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