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Multi Element Analysis of Cow Milk: Geographical Origin Determination and Potential Health Risk Assessment

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***Abstract*— *Milk consumption rendering the proof of geographical provenance is a vital issue in food and consumer protection. The present study deals with discrimination of Bangladeshi cow milk according to their region of production using multi element compositions. The concentration of Pb, Cd, Cr, Cu, Co, Ni, As, Mn, Zn, Fe, Ca, Mg, Na and K in fresh cow milk from four climatic zones (South-Central, South-Western, Western and North-Western) were measured by flame and graphite furnace atomic-absorption spectroscopy (FAAS and GFAAS). The reliability of measured analytical data were validated by certified reference milk powder (IAEA-153). Statistical Canonical discriminate analysis indicated clear regional individuality based on some element that can contribute to protect geographical indication. The correlation coefficient matrix showed positive correlation among metals except copper. In addition, health risk assessment by the calculated values of daily intake of analyzed metals, health risk index and hazard index were lower than reference values, indicating that the consumers are not likely to be metal toxicity due to milk consumption from four studied region.*

Keywords*— *Cow's milk, Trace elements, Daily Metal Intake, Health risk assessment.

I. INTRODUCTION

Food authentication validates that a food is in compliance with its label information which may include the food origin and production method. It is an emerging field in recent year because of increasing public awareness about food safety and quality aspects and also essential to prevent mislabeling and adulteration problems. Declaration of basic information such as name of food, ingredients, net content and origin in value added food products is of particular interest since these products are often target of fraudulent labeling. Proof of provenance is highly encouraged as an emerging criterion in the context of food safety and quality and protection of the consumer's right in accordance with national and international regulation and guidelines. Milk is a high value-added product, rich source of natural nutrients for human being which contains more than twenty different trace elements. Many of them are essential trace elements and play important roles in biological systems in human body [1]. Some of them form an integral part of several enzymes. Although they are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element to element and from species to species. Concentration of trace elements in milk is influenced by genetics of individual animal species, lactation stage, farm management (nutritional regime) and environment factors such as season, farm location, and nature of soil, fertilizer application and industrial activities [2]. Industrial and other anthropogenic activities could also be influenced the levels of toxic elements such as Cd, Ni, Co, Pb, Cr, Hg in milk, through the food chain [3,4]. Toxic metals can be transferred from contaminated soil to plants and grass, causing accumulation of toxic metals in grazing ruminants, particularly in cow milk [5]. Recently, metal residues in milk are of particular concern because milk is largely consumed by infants and children. To determine the concentrations of metals in milk could be an important indicator of milk hygiene status as well as an indicator of the degree of pollution in environment where the

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cattle raise. Milk as led to its high consumption worldwide, but increased demand has also made it prone to fraudulent activity. Typically, milk is adulterated either for financial gain or due to poor hygiene conditions of processing, storage, transportation, and marketing. Milk adulteration has been widely reported in developing countries. Milk is most susceptible to be mislabeled and their country of origin can sometimes be in question. In this regard, the use of multi elemental analysis could be a promising tool for origin authentication purposes. Elemental profiling has also been applied for authenticating the origin of different foods such as onion [6,7], tea [8], and tomato [9]. Multi elemental analysis has also been used for instance cow vs buffalo milk [10].

Bangladesh has immense prospects of expanding the dairy sector. People in Bangladesh usually consumed cow's milk buy directly from nearby farms or from market as both liquid and powder form. Milk is produced all over the country. The main milk pocket areas are situated in West and North western part of Bangladesh. Milk production was 6.9 million tons in 2013-14. Thus, there is a production deficit of 7.12 million tons [11,12]. Growth in demands for milk and dairy products 10 % per year and growth of local production is 7-9% per year [13]. So there is a good opportunity for the local dairy industries to contribute to this gap. Despite holding great potential for the sustainable development of the country the dairy industry is facing different challenges in ensuring food safety aspects such as fraud and adulteration, provenance and authentication, toxic metal and microbial contamination, fake labelling etc. Addition of water in liquid milk is a fairly common practice in Bangladesh. In most cases contaminated water from nearby water bodies is used to add and thus milk becomes contaminated especially with heavy metals. Sometimes dubious milk traders increased milk supply by mixing low quality powder milk in liquid milk and selling those as fresh cow milk [14]. Jolly et al. [15] studied the level of heavy metals (Cr, Ni, As, Cd, Hg, Pb, Mn, Cu, Zn and Fe) in commercial powder milk and fresh cow's milk collected from different farms from Dhaka and Barisal district of Bangladesh. They found that powder milk showed significantly higher concentration of heavy metals compared to liquid milk samples and metal pollution index showed heavy metal contamination in powder milk. Muhib et al.[16] investigated a total of 90 samples (33 packaged cow milk , 30 dairy farm milk and 27 milk from small household farmers) from different areas of Savar, Dhaka, Bangladesh to analyse the content of selected metals (Cd,Cr, Pb,Mn, Cu and Fe). The research revealed that Cr exceeded the highest Estimated Daily Intake (EDI) rate (for brand cow milk: 0.413 mg/day, dairy farm cow milk: 0.243 mg/day, domestic cow milk: 0. 352 mg/day)out of the six metals. Kabir et al.[17] studied heavy metal presence in cow milk of different dairy farms near Karnafuli paper mills, Chittagong, Bangladesh.

Mislabeling and adulteration are other serious issues in the dairy industry in Bangladesh; these not only cause major financial losses but also pose a significant risk to human health. Generally, high quality products from certain geographical locations can be sold at premium prices. Nonetheless some lower quality products are nowadays mislabeled and sold at higher prices, resulting in an unfair food market. But, in recent years consumers have begun to notice the geographical origin of their product due to its relation with quality and authenticity of the food product. So, the geographical origin of food product plays a vital role in analyzing the quality of food. Moreover, authenticity and detection of adulteration are the increasing challenges for researchers, consumers, industries, and regulatory agencies in Bangladesh. But to the best of our knowledge there has not been any report of identifying the geographical origin of milk in Bangladesh. Considering the importance of milk authenticity the present work intended to provide an extensive overview for discriminating the milk according to geographical regions (South-Central, South-Western, Western and North-Western) in Bangladesh based on elemental composition. Though government and commercial dairy enterprises collect milk from smallholders from across the country and then process and distribute the milk to all major cities in the country hence it is vital to know the quality of the milk along with the product origin. Thus, the levels of various essential and toxic trace elements have also been investigated for determining the daily intake of metals (DIM), health risk index (HRI) and hazard index (HI).

II. MATERIALS AND METHODS

Sampling sites

Bangladesh is located in subtropical monsoon region having seven distinct climatic zones. In this study, fresh cow milk was collected from different dairy farm of four climatic zones: South-Central (n= 12), South-Western (n=10), Western (n=9) and North-Western (n=6) shown in Fig.1.

Elemental analysis of milk

Instrumental,Chemicals, and Sample digestion

A Varian AA240FS Atomic Absorption Spectrometer (AAS) was used for the determination of Pb, Cd, Cr, Cu, Co, Ni, Mn, Zn, Fe, Ca, Mg, Na, K. Only, As was determined using

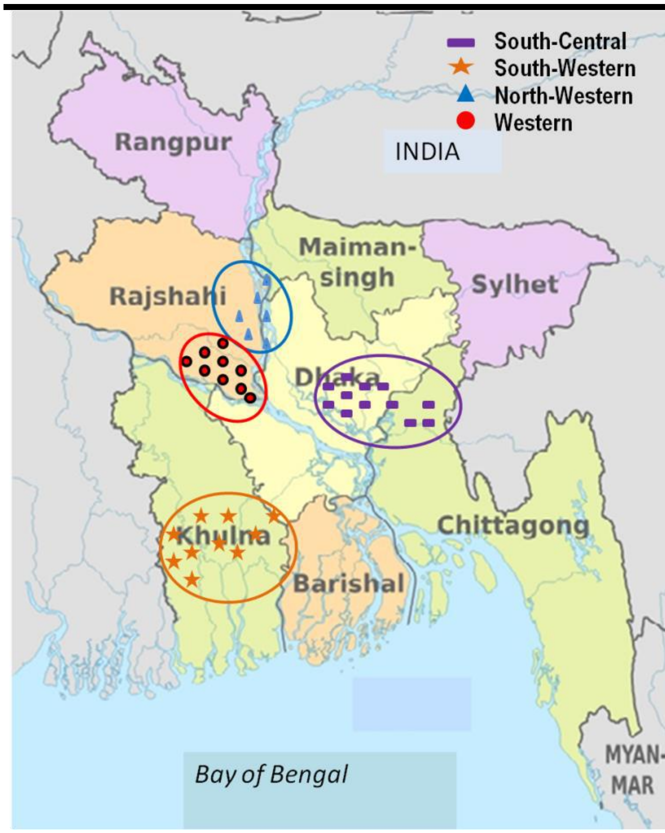

Fig. 1 Map showing sampling areas of four climatic regions of Bangladesh

hydride generation AAS (HG-AAS) techniques using a Varian AA240 equipped with hydride vapor generator (VGA 77). The purity of argon and acetylene gases was 99.999% and 99.99% respectively. Hollow cathode lamps were used for the rest of the metals analyzed. Individual standard solution (Spectropure, USA) of target element was supplied by Varian Inc, USA with highest purity level (99.98%).

Supra pure HNO_3 and all other Chemicals was purchased from E. Merck, Germany and all solutions were prepared using deionized water (18 MΩ/ cm) produced using an E-pure system (Thermo Scientific, USA). Milk samples were mixed with concentrated HNO_3 in a XP vessel, then digested in a microwave accelerator reaction system (MARS'5, CEM Corporation, USA) following US EPA procedure 3051A. After digestion, the solution was made up to 10 mL using ultra-pure water following AAS measurement for metal estimation.

Calibration and Accuracy measurement

The method was validated for linearity range, detection and quantification limits, accuracy, and precision. Instrument detection limit (IDL) at 99% confidence limit is calculated using following formula: IDL = t (0.01,df) s; Where, df= degree of freedom, s= standards deviation and t= critical value for the student t-Test at 99% confidence level. Here, t value is 2.821 (for df 9). Therefore, IDL = 2.821*s; Instrument Quantification limit (IQL) = IDL*10; Method detection limit (MDL) = 4* IDL; and Limit of Quantification (LOQ) =10* IDL.

The linearity range for each metal was estimated by five points calibration curve using different concentration of stock standards. The calibration points for Pb, Cr, Cd, Cu, Co, K, Mn, Ni, Zn, Mg, and Na were ranged from 0.1 to 1 mg/L; whereas for As, Ca, and Fe that was from 1.0 to 10 mg/L. The accuracy of the method was evaluated by analyzing a certified reference milk material IAEA153, provided by International atomic Energy Agency. Mean recoveries of the analyzed metals were between 92.09% to 99.89%, indicated accuracy between certified and measured values (Table 1).

Table 1: Method validation by Reference material (IAEA 153)

Heavy metals	Certified value (mg/kg)	Measured value (mg/kg)	Mean Recovery (%)
Ca	12870	12555±1130	97.55
Cu	0.57	0.56±0.04	98.25
Fe	2.53	2.33±0.21	92.09
K	17620	17600±1584	99.89
Mg	1060	1020±82	96.23
Mn	0.19	0.18±0.01	94.74
Na	4180	4030±363	96.41
Zn	39.56	37.95±3.03	95.94

Health Risk Assessment

The Daily intake of metal (DIM) was calculated by the following equation:

DIM = (C metal × D food intake)/ B average weight; where C_{metal}, $D_{food\ intake}$, and $B_{average\ weight}$ represent the metal concentrations in milk (mg/L), daily intake of milk (L) and average body weight respectively. The average milk intake

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of population of Bangladesh is 30.56 ml/person/day [18], the average body weight ($B_{average\ weight}$) is 70 kg for adults [19].

The Health Risk Index (HRI) of analyzed metals was calculated by the following equation [20]: HRI= DIM/ Rf_D(safe limit of a metal per day having no hazardous effect during life time). Oral Reference Doses used for calculating HRI for Cr, Ni, Cu, Pb, Cd, Mn and Zn were followed by USEPA IRIS [21], for Co followed by Food and Nutritional Board (2004) and for Fe followed by Friberg et al.[22]. To evaluate the overall health risk through more than one heavy metal of an individual food item, the Hazard Index (HI) was developed [23] as the sum of the hazard quotients (Health Risk Index):

$$HI= \sum HQ = HQ_{Cr}+ HQ_{Co}+ HQ_{Cd}+ HQ_{Pb}+ HQ_{Ni} +HQ_{Mn} +HQ_{Zn} +HQ_{Cu} +HQ_{Fe}$$

Like HRI, HI<1 indicates that the predicted exposure is unlikely to pose potential health risks.

Statistical analysis

Statistical software (SPSS 24) was used for data analysis. Three multivariate techniques including hierarchical cluster analysis (HCA), canonical discriminate analysis (CDA) and correlation matrix analysis were carried out using multi element data milk. To investigate similarity and dissimilarity in metal concentration between sampling sites (both spatial and temporal variations), HCA analysis was employed on the metal data treated by Ward's method of linkage with the squared Euclidean distances to determine distance between clusters. CDA was employed by using the standard, stepwise mode to examine the differences between two or more groups with respect to predictor variables (multi-elements) in milk samples. In the current study, sampling sites were treated as spatial grouping, meanwhile metal concentrations were considered as temporal grouping. In discriminant analysis, only a minimal number of selected variables would be sufficient to provide the maximum separation of the data into groups. A correlation closet to 0 means no linear relationship between them at a significant level of $p < 0.05$

Table 2: Concentration of toxic heavy metals and nutritional trace elements (mg/L) in raw milk of different geographic origin in Bangladesh

Element (µg/L)	South-Central (n=12)	South-Western (n=10)	Western (n=9)	North-Western (n=6)
Pb	2.14±0.754	0.79±0.013	0.79±0.097	0.82±0.076
Cd	0.44±0.15	0.09±0.012	0.13±0.076	0.092± 0.07
Cr	0.99±0.029	0.49±0.012	0.49±0.052	0.52±0.02
Cu	0.09±0.008	0.29±0.011	0.21±0.133	0.21±0.04
Co	0.56±0.11	0.39±0.012	0.39±0.015	0.39±0.14
Ni	1.59±0.085	0.39±0.011	0.39±.007	0.39±0.01
As	0.21±0.012	0.02±3.66E-18	0.02±0.001	0.02±0.006
Mn	0.24±0.025	0.14±0.007	0.15±0.01	0.16±0.01
Zn	4.06±1.95	2.30±0.54	2.91±1.36	3.13±0.34
Fe	5.46±0.19	0.48±0.015	0.49±0.014	0.50±0.01
Ca	1335.31±319.11	913.3±41.28	750.78±58.92	715 ±100.8
Mg	249.17±59.43	86.9 ±7.15	84.27±10.14	69.82 ±10.32
Na	468.98±250.67	234.5 ± 28.37	191.33±10.22	208.33±14.61
K	1280.23 ±227.91	876.5 ± 24.49	997.22±80.89	883.67±12.48

III.RESULTS AND DISCUSSION

Trace metal concentrations

Trace metals concentration in milk might be influenced by lactation stage, feed, pollutants present in the environment.Thus, among the multi element compositions of cow's milk examined in this study (Table 2), South-Central zone possess higher concentration (except Cu)compared to the other. Most of the farms of South-Central zone are situated in industrial areas and were exposed to various sources of pollution either directly or indirectly such as discharged wastes into nearby water bodies, locating beside roads with heavy traffic results in pollutant accumulation in farm areas. Similarly, Dhanalakshmi & Gawdaman [24] and Iftikhar et al. [25] also found higher heavy metals concentration in industrial or urban areas compared to those of rural areas.

Discriminant analysis (CDA)

A stepwise canonical discriminant analysis was applied to elemental abundances to discriminate raw milk samples from the four different climatic zones of Bangladesh (Fig.2). Among the 14 elements, Ca, Mg, Na, K, Mn, Zn and Cu were selected for canonical discriminant analysis. Milk samples from South-Central and South-Western were well separated in the graph displaying in the Fig.2A based on macro elements while samples from West and North-West were overlapping. The powerful differentiating dimension was function 1(96.3%) and then function 2(3.7%). Fig 2B showed canonical discrimination according to microelement level of milk samples among the four regions. The Wilks' Lambda test indicated significant contributing variables to the discriminant models at *p-level*<0.001.

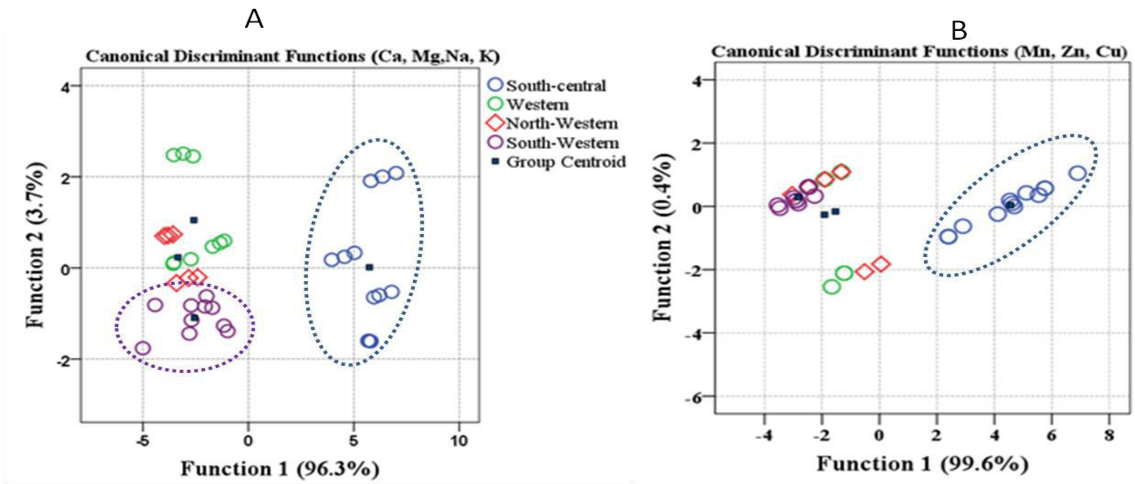

Fig. 2. Canonical Discrimination among geographic regions using significant multi element parameters

Hierarchical Cluster and Correlation analysis

Hierarchical Cluster Analysis (Ward's method) was used to show group distribution according to variables (14 elements) with significant differences (p<0.05).The Squared Euclidian distance and coefficient of similarity were used to group the cases in clusters in term of their similarity. The nearest distance suggested the highest degree of relationship, therefore, those objects are considered to belong to the same group. Fig. 3 revealed the dendogram representing clusters of the observations

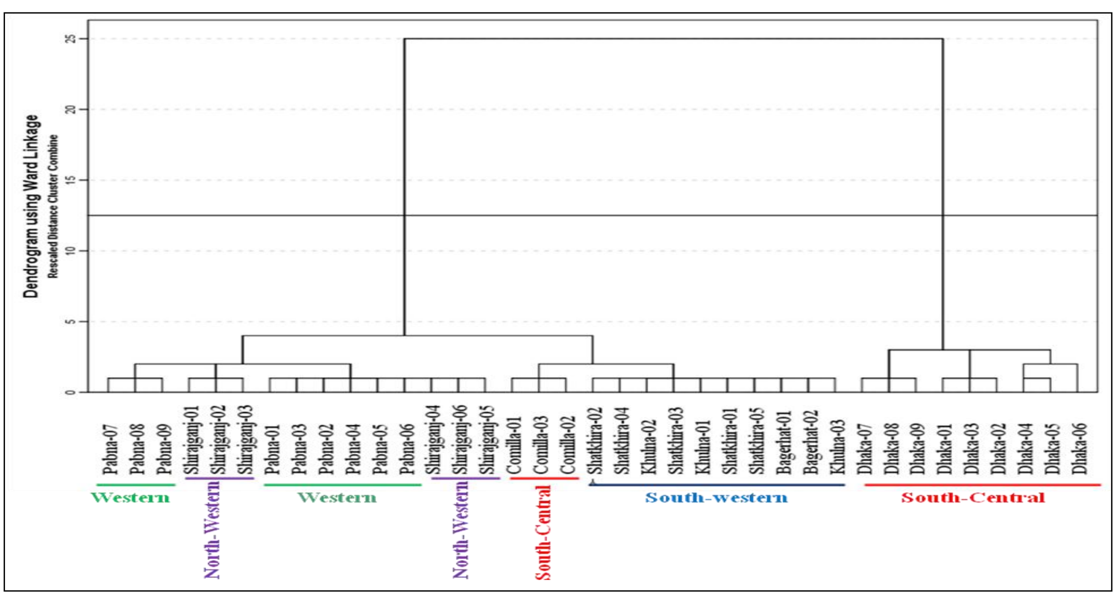

Fig. 3 Dendogram using elements in raw milk

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corresponding to each geographical origin of the milk samples. The first cluster was comprised of Western (n=9), North-Western (n=6), South-Western (n=10) and three sample of South-Central. The second cluster was composed of nine samples of South-Central. The cluster analysis byusing 14 elements revealed that HCA did not enable for a well determination of the geographical origin of cow milk.

The Pearson's correlation coefficient matrix (Table 3) revealed inter-metallic association at the differentsignificant levels. All metals showed positive and strong significant correlation with other metals except Copper.

Table 3: Correlation coefficients between milk elements

	Pb	Cd	Cr	Cu	Co	Ni	As	Mn	Zn	Fe	Ca	Mg	Na	K
Pb	1													
Cd	.922**	1												
Cr	.811**	.673**	1											
Cu	-.634**	-.507**	-.765**	1										
Co	.960**	.892**	.826**	-.667**	1									
Ni	.825**	.691**	.987**	-.765**	.857**	1								
As	.775**	.653**	.979**	-.737**	.797**	.966**	1							
Mn	.655**	.543**	.939**	-.686**	.676**	.925**	.940**	1						
Zn	.653**	.596**	.572**	-.459**	.495**	.525**	.541**	.536**	1					
Fe	.816**	.682**	.990**	-.765**	.841**	.998**	.972**	.936**	.538**	1				
Ca	.881**	.771**	.820**	-.626**	.801**	.814**	.802**	.711**	.746**	.819**	1			
Mg	.853**	.791**	.709**	-.564**	.739**	.692**	.708**	.620**	.806**	.704**	.942**	1		
Na	.719**	.627**	.674**	-.510**	.613**	.637**	.686**	.622**	.782**	.657**	.868**	.895**	1	
K	.858**	.806**	.807**	-.661**	.800**	.786**	.809**	.748**	.710**	.802**	.853**	.926**	.824**	1

** Correlation is significant at the 0.01 level (2-tailed).

Health Risk Assessment

The estimated daily intakes of metals for adults (Table 4) through milk consumption were less than tolerable daily intake limit set by several authorities [21, 26-28]. Muhib et al.[16] found that DIM values of Cd, Pb, Mn, Cu and Fe for adult in raw milk (collected from one area of South Central region of Bangladesh) were within permissible limits which agreed well with our findings. The present study revealed that the DIM values were below the recommended values, indicating that the dweller in the four studied regions are not likely to be exposed to significant

Table 4: Estimated Average Daily intake of metal (DIM) by consumption of milk from different climatic regions of Bangladesh

Element	DIM (mg/person/day)				Oral reference doses (mg/kg/day)	References
	South-Central	South-Western	West	North-West		
Pb	0.001085	0.000829	0.000349	0.000349	0.004	US EPA IRIS, 2006
Cd	0.000137	5.24E-05	4.37E-05	4.37E-05	0.001	
Cr	0.000463	4.37E-05	0.000218	0.000218	1.5	
Cu	1.83E-05	4.37E-06	0.000131	0.000131	0.04	
Zn	0.001854	0.000974	0.001268	0.001366	0.30	
Mn	0.000349	6.55E-05	0.000349	6.55E-05	0.033	
Ni	0.00074	0.000175	0.000175	0.000175	0.02	
Co	0.000298	0.000174629	0.000175	0.000175	3.01	Food and Nutritional Board, 2004
As	4.37E-05	8.73E-06	4.37E-05	8.73E-06	0.0003	US EPA,1998
Fe	0.002832	0.000218286	0.000218	0.000218	40	FAO/WHO, 2002

health risk due to milk consumption. Thus the values for health risk index and hazard index (Table 5), that have been recognized as a useful indicator for assessing risks associated with the consumption of metal contaminated food, recognized the milk of 4 studied regions of Bangladesh as safe for human consumption. This result corroborates that of Jolly et al. [15] who also found lower values for Cr, Ni, Mn, Cu, Zn and Fe in fresh cow's milk (collected from Dhaka and Barisal district in Bangladesh).

Table 5: Health Risk Index (HRI) and Hazard Index (HI) for heavy metals in raw milk from different climatic regions

Element	Health Risk Index (HRI)				Safe limit (USEPA, 2002)	References
	South-Central	South-Western	West	North-West		
Pb	0.233293	0.041474	0.087314	0.087314	≥ 1	(USEPA, 2002)
Cd	0.130971	0.002619	0.043657	0.043657		
Cr	0.000298	0.002183	0.000146	0.000146		
Cu	0.000109	0.000218	0.003274	0.003274		
Zn	0.006734	0.048678	0.004225	0.004555		
Mn	0.003076	0.003274	0.001984	0.001984		
Ni	0.036672	0.008731	0.008731	0.008731		
Co	8.68429E-05	0.008731429	5.80161E-05	5.80161E-05		
As	0.014552	0.000437	0.00291	0.00291		
Fe	6.11609E-05	0.010914286	5.45714E-06	5.45714E-06		
Hazard Index (HI)						
	0.378034	0.127261	0.132572	0.13752	≥ 1	(USEPA, 1989)

IV.CONCLUSION

The chemo metric approach using elemental profile of raw milk indicated clear discrimination among the selected regions of Bangladesh. This might due to geographical difference and the feeding practices of the animals. Moreover, the values for daily intake of the metals investigated were less than the safe limit as defined by recognized authorities and thus the results revealed no association of health risk and/or hazard with the consumption of cow milk in Bangladesh.

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