INVESTIGATION OF HEAVY METALS IN WATER AND DAIRY MILK OF GREATER CHITTAGONG DISTRICT OF BANGLADESH

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Roll No: 0113/04
Registration No: 0135
Session: January-June/2013

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Pharmacology

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December 2014

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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December 2014

Dedication

I dedicate this small piece of work

to my beloved parents and brother

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Acknowledgements

Firstly, I would like to express my deepest sense to ―The Almighty Allah, who enables me to finish off the research work and dissertation successfully for the degree of Master of Science (MS) in Pharmacology under the Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University (CVASU). Secondly, I would like to expresses the first and foremost heartiest appreciation, deepest sense of gratitude and best regards to my supervisor Prof **Dr. Md. Ahasanul Hoque**. It was my immense pleasure and amazing experience to work with him. Without his guidance it would not be possible for me to complete the research and then write up the dissertation successfully. I feel much pleasure to convey my profound thanks to my co-supervisor, **Shamsul Morshed**. Assistant Professor, Department of Applied Chemistry and Chemical Technology, CVASU for his valuable advice, scholastic guidance, inspiration and suggestions during my study period.

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List of abbreviation

Abbreviation	Elaboration
%	Percentage
°C	Degree centigrade
µg	Microgram
µm	Micrometer
CDC	Centers for disease control and prevention
CI	Confidence interval
cm	Centimeter
CMPC	Chittagong metropolitan city
CVASU	Chittagong veterinary and animal sciences university
H_2O_2	Hydrogen per oxide
HCl	Hydrochloric acid
HNO_3	Nitric acid
IDF	International dairy federation
mg/L	Milligram/liter
ml	Milliliter
MS	Master of science
OR	Odds ratio
PRTC	Poultry research training centre
WHO	World health organization

Summary

The presence of heavy metals (copper, lead, nickel etc.) in water as well as dairy milk is a common problem in Bangladesh. No systematic study has previously been attempted for investigation of heavy metals both in water and milk which could pose threats to public health. Therefore, a cross sectional study was conducted on different water sources and dairy milk of greater Chittagong in Bangladesh. The overall objective of the study was to assess the status of the selected heavy metals in water and dairy milk, identify risk factors and evaluate possible public health risk.

A total of 108 water samples (72 tube well and 36 tap water) and 96 dairy milk samples, along with epidemiological and ecological data were obtained from the selected study sites and evaluated the samples obtained by Atomic Absorption Spectrometry during December 2013-May 2014.

The prevalence of heavy metals in tap water was zero for lead, nickel and copper in the present study. The overall prevalence of lead was higher in tube well water (37.5%) than milk (6.3%). The prevalence of lead in tube well water of Chittagong was significantly higher (61.1%) than in Chandpur (13.9%) but lower in dairy milk in Chittagong (4.2%) than in Chandpur (8.3%). The prevalence of nickel was detected both in tube well water and dairy milk at high prevalence (30.6-37.5%) in Chandpur. Contrarily, nickel was detected only in dairy milk at high rate of prevalence (27.1%) in Chittagong.

Around 45% of lead positive water samples (N=56) and 26% of lead positive dairy milk samples (N=51) crossed the reference values (reference values: 0.05 mg/L and 0.49 mg/L for water and dairy milk, respectively). Milk samples obtained from Chittagong crossed the reference value of lead had high frequency (44.8%).

Fisher's exact test followed by a univariable logistic regression was applied to identify potential risk factors associated with lead and nickel in dairy milk. Site, area and industry were identified risk factors for lead in dairy milk (OR= 4.6: Chittagong versus Chandpur; OR=16.7: semi urban versus rural and OR=14.7: semi urban versus urban; OR=4.5: industry present versus industry absent). Area and presence of industry were determined risk factors for nickel in dairy milk (OR=5.4: semi-urban versus rural; OR=2.0: industry versus non-industry).

Thirty one percent of nickel positive tube well water (N=18) also crossed the reference value in this study. Tube well water samples obtained from Chittagong crossed the reference value of lead had higher frequency (95.5%) than in Chandpur (13.9%).

The presence of lead and nickel in tube well water and dairy milk at high concentration that crossed the reference values is certainly posing animal and public health threat such as causing haematotoxicity, neurotoxicity, genotoxicity, reproductive toxicity etc. Therefore, possible techniques to reduce the risk level of heavy metals in tube well water and dairy milk along with public awareness should be sought in the study areas.

Key words: *Lead, Nickel, Copper, Tube well water, Dairy milk, Greater Chittagong.*

Chapter I: Introduction

Presence of heavy metals at variable concentrations in water, dairy milk, meat and vegetables is common all over the world due to their indiscriminate uses as therapeutics, insecticides and other purposes in animal pasture lands, fruits, vegetable fields and plants (Ryser and Sauder, 2006; Cheraghi et al., 2009; Khairiah et al., 2009; Armah et al., 2010; Sollitto et al., 2010; Kumar and Puri, 2012). Heavy metal is also highly prevalent in Bangladesh because of its infrastructural and industrial development, people's unawareness, and lack of proper policy and even execution of current policy to stop using heavy metals for different purposes (Tong et al., 2000). The prevalent areas for heavy metal in this country include greater Noakhali districts, Rajshahi district etc. (Tong et al., 2000; Rasul and Jahan, 2010). Lead, nickel and copper are essential heavy metals to maintain the body metabolism, but they become toxic at high concentrations for animals and humans (Kumahor, 2011). Heavy metals especially lead, copper and nickel have been reported to be present above the maximum permissible limits in tube well water, due to the reason that tube wells are being installed on the banks of sewage drains, resulting in elevated levels of heavy metals in tube well water (Latif et al., 2008).

Lead is found at high concentration in tube well water (Frisbie et al., 2002). Most likely source of exposure of lead is ingestion of contaminated food and drinking water. Potentially high levels of lead may occur in the following industries: lead smelting and refining industries, battery manufacturing plants, steel welding or cutting operations, construction, rubber products, plastics industries, printing industries, firing ranges etc. (CDC, 2007).

A high level of lead has been reported in raw milk and that may be due to the animal ranches around polluted area, in addition to the unhygienic conditions of milkman and the containers for transporting and at vendor shop (Awan et al., 2005).

Nickel concentration in drinking water can be much higher due to pollution of water supply or leaching from nickel-containing pipes (Daldrup et al., 1983; Sunderman et al., 1988). Many types of cement also contain nickel (Friberg, 1948; Aslam, 2010). The higher nickel residues in cattle milk may also be due to industrial effluents rich in nickel which is drained into sewerage water that ultimately increases the nickel uptake to the fodder from the soil (Aslam et al., 2011).

Copper can be released into the environment by both natural sources and human activities; examples of natural sources are wind-blown dust, decaying vegetation, forest fires and sea spray as well as examples of human activities are mining, metal production, wood production and phosphate fertilizer production (Lenntech, 2009). Direct contact of milk with copper bearing metals presented a greater opportunity for contamination of milk (Hankinson, 1975).

Cow’s milk is a valuable source of essential minerals but some toxic elements may be accidently added during handling, processing, and remixing of milk (Lutfullah et al., 2014). The presence of heavy metals in cow’s milk due to exposure of lactating cow to environmental pollution or consumption of feeding stuffs and water (Carl, 1991; Okada et al., 1997). Besides, contamination of cow’s milk can also happen through the feedstuff of animals exposed with heavy metals (Nasreddine and Parent-Massin, 2002).

The prevalence of lead, nickel and copper in water has been reported to be 100% each in India and in Bangladesh (Ahmad and Goni, 2010) and all of the samples crossed the reference values (lead: 0.05 mg/L, nickel: 0.01 mg/L and copper: 0.005 mg/L). The other investigation has also been reported the prevalence of nickel of 100% in water of which 30% samples crossed the reference value (0.01 mg/L) in India (Reddy et al., 2012). The prevalence of copper of 96% in water has been documented in Korea (Jung, 2001).

The prevalence estimate of copper in milk have been recorded to be 100% of which 68-100% crossed the reference value (0.10 mg/L) in Bangladesh and India (Giri et al., 2011). The prevalence of lead ranging from 0-100% has been reported in milk, whereas the zero prevalence of nickel has been published in milk in India (Giri et al., 2011). However, the prevalence of lead has been reported to be 88% in milk in Bangladesh (Shahriar et al., 2014) and all samples maintained below the reference value.

The prevalence of copper has been estimated as 62% in milk and of the prevalence, 60% crossed the reference value (0.10 mg/L), whereas the prevalence of lead has been estimated 100% in dairy milk in Nigeria (Lawal et al., 2006) and most of the samples crossed the reference.

The aforementioned prevalence estimates of different heavy metals in water and milk have been produced by the sporadic and unstructured studies and moreover, no study has previously been conducted in Chittagong region. Hence, to address the limitations of those studies the present cross-sectional study has been performed in Greater Chittagong.

City and industrial areas and agricultural activities (such as heavy metals used as therapeutics, fertilizers) have been determined as risk factors for the presence of heavy metals at high concentration in water (Pais, 1992; Ahmad, 2002; Konuspayeva et al., 2009). Transhumance along roads and/or motorways, fodder contamination, climatic factors, such as gusty winds, and the use of pesticide compounds, contaminated pasture with industrial effluents and mining area may be associated with the presence of lead in dairy milk (Jigam et al., 2011). Factors associated with the presence of heavy metals in water and dairy milk have not previously been attempted to explore in this region of Bangladesh. Therefore, the present study has been justified to conduct determining risk factors that influence the prevalence of heavy metals in water and dairy milk.

Animal health and public health have been reported to be at risk due to high concentration of heavy metals (lead, nickel and copper) exposed through water and milk (Olivares and Uauy, 1996; Patrick, 2006; Das and Büchner, 2007; Stern, 2010; Kianoush et al., 2013). Animal health risks include anemia, encephalopathy, foot drop/wrist drop (palsy), nephropathy etc due to lead at high concentration; nephrotoxic, hepatotoxic, neurotoxic etc. due to nickel; homeostasis, hepatomegaly etc. due to copper at high concentration. Public health risk constitutes encephalopathy, nephropathy, hypertension etc. due to lead at high concentration; immunotoxic, nephrotoxic, hepatotoxic etc. due to nickel; wilson disease, cirrhosis of the liver, tachycardia etc due to copper at high concentration. No investigation has earlier been carried out to assess the health risk due to exposure of heavy metals through drinking water and milk in Greater Chittagong. The present study has therefore been conducted to assess health risk in the study areas.

With the aforementioned background the present investigation has been performed with the following objectives:

1.1 Objectives

- To estimate the prevalence of lead, nickel and copper in water and milk of dairy cattle in Greater Chittagong
- To determine risk factors associated with the prevalence of the selected heavy metals in water and dairy milk in Greater Chittagong

1.2 Anticipated outcomes

- Estimated the prevalence of lead, nickel and copper in water and dairy milk in Greater Chittagong
- Identified risk factors associated with the prevalence of the selected heavy metals in water and dairy milk in Greater Chittagong
- Recommended animal health and public health risk according to the level of concentration of the selected heavy metals obtained in water and dairy milk in Greater Chittagong

Chapter II: Review of Literature

Pertinent literatures on heavy metals (copper, lead and nickel) in water and dairy milk have been reviewed and identified scientific gaps in this chapter.

2.1 Heavy metals

Heavy metals can be defined as a subset of elements that exhibit metallic properties such as hard, opaque, shiny, and having good electrical as well as thermal conductivity. It comprises the transition metals (zinc, mercury etc.), some metalloids (arsenic, boron etc.), lanthanides (lanthanum, cerium etc.), and actinides (actinium, protactinium etc.). Heavy metal is also defined as those having a specific density of more than 5 g/cm^3 (Suciu et al., 2008). They can also be chemical elements with the density greater than 4 g/cm^3 found in all kinds of soils, rocks and water in terrestrial and freshwater ecosystem (Adelekan and Abegunde, 2011).

2.2 Sources of heavy metals

Largely, within the European community eleven elements of highest concern are arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin and thallium. Metals occur naturally in our environment, but rarely at toxic levels, especially in the Earth's crust, where they contribute to the balance of the planet (Harikumar et al., 2010; Obodai et al., 2011). Generally, metals enter the aquatic environment through atmospheric deposition, erosion of geological milieu or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining waste (Aderinola et al., 2009; Adelekan and Abegunde, 2011; Obodai et al., 2011). In addition, metals, naturally get to waters by chemical weathering of minerals and soil leaching (El-Bouraie et al., 2010). This is how tube well and tap water can be contaminated with heavy metals through anthropogenic and natural activities. The sources of groundwater contaminants are presented in **Table 2.1**.

Table 2.1: Sources of groundwater contaminants (in particular heavy metals) (Barcelona et al., 1990).

Category 1: Sources designed to discharge substances 1. Sub-surface percolation from septic tanks/cesspools 2. Injection wells Hazardous waste Nonhazardous waste (e.g., brine disposal) Non-waste (e.g., solution mining) 3. Land application Waste water (spray irrigation) Waste water by-products (bio-solids) Hazardous waste Nonhazardous waste	**Category 3:** Sources designed to retain substances during transport or transmission 1. Pipelines Hazardous waste Nonhazardous waste Non waste 2. Materials transport and transfer operations Hazardous waste Nonhazardous waste Non waste
Category 2: Sources designed to store, treat, and/ or dispose of substances; discharge through unplanned release 1. Landfills Industrial hazardous waste Industrial nonhazardous waste 2. Open dumps, including illegal dumping 3. Residential disposal 4. Surface impoundments Hazardous waste Nonhazardous waste 5. Materials stockpiles (non waste) 6. Graveyards 7. Animal burial 8. Above-ground storage tanks Hazardous waste Nonhazardous waste Non waste 9. Underground storage tanks Hazardous waste Nonhazardous waste Non waste 10. Containers Hazardous waste Nonhazardous waste Non waste 11. Open burning and detonation sites 12. Radioactive disposal sites	**Category 4:** Sources discharging substances as a result of other planned activities 1. Irrigation practices 2. Pesticide applications 3. Fertilizer applications 4. Animal feeding applications 5. De-icing salt applications 6. Urban run-off 7. Percolation of atmospheric pollutants 8. Mining and mine drainage Surface mine related Underground mine related **Category 5:** Sources providing conduit or inducing discharge through altered flow patterns 1. Production wells Oil and gas wells Geothermal and heat recovery wells Water supply wells 2. Other wells Monitoring wells Exploration wells 3. Construction excavation **Category 6:** Naturally occurring sources whose discharge is created and/or exacerbated by human activity 1. Groundwater–surface-water interactions 2. Natural leaching 3. Salt water intrusion

Increased road way traffic has caused a significant increases the toxic heavy metals in the environmental and affected all the global air, water and food (Zamir and Hussain, 2001) which in turn affect animals.

Food stuffs grown on contaminated soil or irrigated with impure water accumulate metal contents and are a big source of heavy metals exposure to the animals and humans (Ward and Savage, 1994). In animal body, metals enter through animal's feeds, green fodder, drinking water and pharmaceutical medicines etc. Other sources are accidental access to limed field, mineral supplements with high content of trace metal and licking of painted surfaced containing metallic pigments (Raikwar et al., 2008).

The presence of heavy metals in cow's milk due to exposure of lactating cow to environmental pollution or consumption of feeding stuffs and water (Carl, 1991; Okada et al., 1997). Contamination of milk by heavy metals arises from modern agricultural practices, industrial pollutants in the environment, animal feeds and use of sewage sludge in agriculture (Kira and Maihara, 2007; Birghila et al., 2008; Jigam et al., 2011).

2.2.1 Sources of lead

Lead is a ubiquitous harmful metals presence in the food chain due to the result of the water pollution (Pavlovic et al., 2004).Source of lead in drinking water is mainly though the lead pipes, or tin solders and brass fixture materials (Schock, 1990). Besides, phosphate rock is an important source of lead. These rocks are mainly used in fertilizers preparations. In addition, this phosphate rock is also used in detergent manufacturing and used in livestock and poultry feed preparation (Stoica et al., 1997). So, phosphate rocks are found to be an important sources of lead in water which ultimately enters in food chain (Javed et al., 2009).

The food chain is an important source of lead accumulation, especially for plants grown on polluted soils. Significant amounts of lead can be transferred from contaminated soil to plants and grass, causing accumulation of these potentially toxic metals in grazing ruminants, particularly in cattle (López et al., 2003; Miranda et al., 2005). More to the point, lead toxicity is frequently observed in farm animals, especially in those grazing on pasture in the vicinity of metallurgic complexes and also close to busy roads. Species susceptibility to lead has been described in cattle, particularly the young ones (Šmirjákova et al., 2005). Besides, lead contents increased in cow milk after oral intake of large quantities of lead through forages (Marshall et al., 1998). Moreover, a high level of lead in raw milk is due to the animal ranches around polluted area, in addition to the unhygienic conditions of milkman and the containers for transporting and at vendor shop (Awan et al., 2005).

2.2.2 Sources of nickel

The primary natural source of nickel in drinking water is leaching from ultramafic rocks and the soil derived from these rocks. It is found primarily combined with oxygen (oxides) or sulfide. Nickel compounds are also used for nickel plating, to color ceramics, to make some batteries and as catalyst in different chemical processes. These may be the possible means for water contamination (Klan et al., 2011). Small amount of nickel is found in water and milk (Aslam, 2010). Nickel concentration in drinking water can be much higher due to pollution of water supply or leaching from nickel-containing pipes (Daldrup et al., 1983; Sunderman et al., 1988).

Nickel toxicity in animals and plants can occur by the intake of polluted water. The main factor which contributes in water pollution is acid rain which has a tendency to mobilize nickel from soil and increase nickel concentration in groundwater (Aslam, 2010).

The higher nickel residues in milk of cattle may also be due to industrial effluents rich in nickel which is drained into sewerage water that ultimately increases the nickel uptake to the fodder from the soil (Aslam et al., 2011).

2.2.3 Sources of copper

Copper is a very common substance that occurs naturally in the water and spreads via water through natural phenomena like widely using of copper by humans, instance applying in the industries as well as in agriculture (Lenntech, 2009). Copper can leach into water primarily from pipes, but fixtures and faucets (brass), and fittings can also be a source. The amount of copper in water also depends on the types and amounts of minerals in the water, how long the water stays in the pipes, the amount of wear in the pipes, the water's acidity and its temperature (Nathan et al., 2014). Most copper compounds settle and bound to water and occur in the environment after release through application in agriculture (Lenntech, 2009).

Copper can be released into the environment by both natural sources and human activities; examples of natural sources are wind-blown dust, decaying vegetation, forest fires and sea spray and examples of human activities are mining, metal production, wood production and phosphate fertilizer production (Lenntech, 2009).

2.3 Prevalence of lead, nickel and copper in water and dairy milk

The prevalence of lead, nickel and copper in water has been reported to be 100% each in India and in Bangladesh (Ahmad and Goni, 2010) and all of the samples crossed the reference values (lead: 0.05 mg/L, nickel: 0.01 mg/L and copper: 0.005 mg/L). Another investigation has also been reported the prevalence of nickel of 100% in water of which 30% samples crossed the reference value (0.01 mg/L) in India (Reddy et al., 2012). The prevalence of copper of 96% in water has been documented in Korea (Jung, 2001).

The prevalence estimate of copper in milk have been recorded to be 100% of which 68-100% crossed the reference value (0.10 mg/L) in Bangladesh and India (Giri et al., 2011). The prevalence of lead ranging from 0-100% has been reported in milk, whereas the zero prevalence of nickel has also been published in milk in India (Giri et al., 2011). However, the prevalence of lead has been reported to be 88% in milk in Bangladesh (Shahriar et al., 2014) and all samples maintained below the reference value.

The prevalence of copper has been estimated 62% in milk and of the prevalence, 60% crossed the reference value (0.10 mg/L), whereas the prevalence of lead has been estimated 100% in dairy milk in Nigeria (Lawal et al., 2006) and most of the samples crossed the reference value.

The aforementioned prevalence estimates of different heavy metals in water and milk have been produced by the sporadic and unstructured studies and no study has previously been conducted in Chittagong region. Hence, to address the gaps of those studies the present cross-sectional study has been performed in Greater Chittagong.

2.4 Risk factors for the presence of lead, nickel and copper in water and dairy milk

City and industrial areas and agricultural activities (such as heavy metals used as therapeutics, fertilizers) have been determined as risk factors for the presence of heavy metals at high concentration in water (Pais, 1992; Ahmad, 2002; Konuspayeva et al., 2009). Transhumance along roads and/or motorways, fodder contamination, climatic factors, such as gusty winds, and the use of pesticide compounds, contaminated pasture with industrial effluents and mining area may be associated with the presence of lead in dairy milk (Jigam et al., 2011). Factors associated with the presence of heavy metals in water and dairy milk have not previously been attempted to explore in Bangladesh. Therefore, the present study has been justified to conduct determining risk factors that influence high prevalence of heavy metals in water and dairy milk.

2.5 Animal and public health risk due to lead, nickel and copper

Animal health and public health have been reported to be at high risk due to high concentration of heavy metals (lead, nickel and copper) exposed through water and milk (Olivares and Uauy, 1996; Patrick, 2006; Das and Büchner, 2007; Stern, 2010; Kianoush et al., 2013). Health risks due to lead, nickel, copper etc have been summarized in **Table 2.2** (Harris, 1983; Olivares and Uauy, 1996; Perazella, 1996; Pizarro et al., 1999; Zacarías et al., 2001; Araya et al., 2003; Patrick, 2006; Das et al., 2008; Stern, 2010; Kianoush et al., 2013).

Table 2.2: Animal and public health risk due to exposure of heavy metals

Heavy metals	**Animal: Conc. of permissible limit (mg/L)**	**Effect**	**Human: Conc. of permissible limit**	**Effect**
Lead	0.05–1	Anemia, encephalopathy, foot drop/wrist drop (palsy), nephropathy etc.	10 µg/dL	Encephalopathy, nausea, vomiting, nephropathy, hypertension etc.
Mercury	0.5-0.1	Stomatitis, nausea, nephrotic syndrome, neurasthenia, tremor etc.	0.7–42 $\mu g/m^3$	Diarrhea, fever, vomiting etc.
Arsenic	0.8-0.11	Hypopigmentation, hyperkeratosis etc.	0.01 mg/L	Nausea, vomiting, painful neuropathy, diarrhoea, encephalopathy, arrhythmia etc.
Nickel	Below detection limits	Haematotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic, immunotoxic etc.	0.2 $\mu g/m^3$	Immunotoxic, genotoxic, pulmonary toxic, nephrotoxic, hepatotoxic, neurotoxic, haematotoxic etc.
Copper	30	Homeostasis, hepatomegaly etc.	70 - 150 µg/dL	Nausea, vomiting, diarrhoea, difficulty in respiration, hemolytic anemia, hematuria, liver/ kidney failure etc.

No investigation has earlier been carried out to assess the health risk due to exposure of heavy metals through drinking water and milk in Greater Chittagong. The present study has therefore been conducted to assess health risk in the study areas.

2.6 Conclusion

In conclusion sources of heavy metals (in particular lead, nickel and copper), prevalence of the selected heavy metals along with risk factors and health risk have been discussed and assessed the justifications to conduct the current investigation of the selected heavy metals in water and dairy milk in Greater Chittagong (Chittagong metropolitan and Chandpur).

Chapter III: Materials and Methods

3.1 Description of study sites

Different water sources (tube wells and tap water) and selected dairy farms belonging to Chittagong Metropolitan City (CMPC) of Chittagong district and Shaharasti as well as Hagigang upazilla of Chandpur district were considered for the current study. Plate industry, pipe industry etc. are located within CMPC, which are likely sources of heavy metal discharged directly or indirectly in different water sources (Khan et al., 2011; Molla et al., 2014). Chittagong-Dhaka highway has also passed through CMPC; therefore huge number of traffics runs through daily, increase the chance of heavy metals (such as lead) contamination in the air which in turn, could affect animals nearby through air-borne transmission of heavy metals or some other alternative ways (Voutsa and Samara, 2002; Ny and Lee, 2010). Chandpur district as a whole is considered as one of the heavy metal contaminant areas in Bangladesh because of the existence of geothermal environments of volcanic deposits, geothermal systems and basin-fill deposits of alluvial lacustrine origin (Huq et al., 2006).

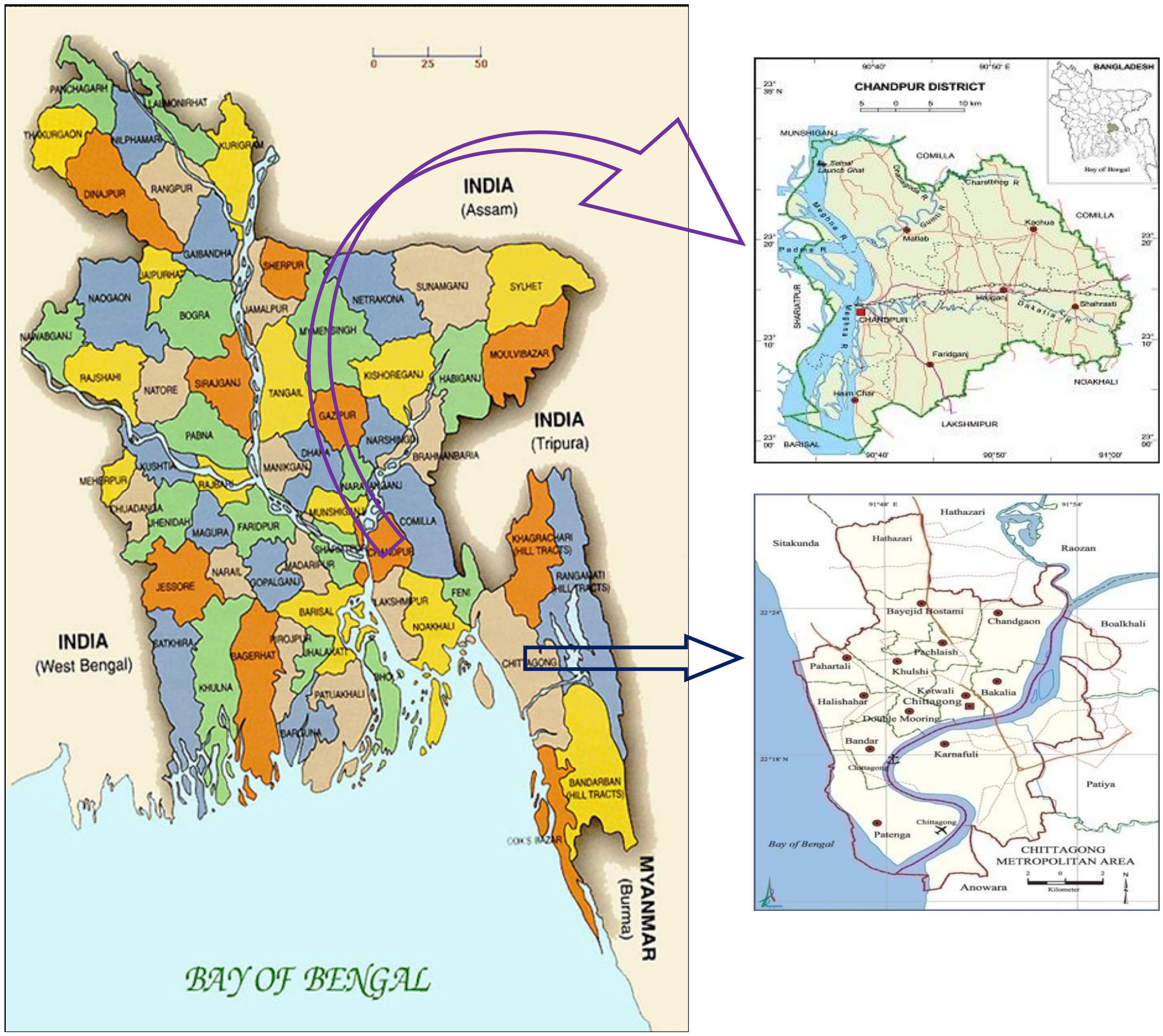

Figure 1: Study sites locating in the map of Bangladesh

3.2 Study design and period

A cross-sectional study was conducted on different water sources and selected dairy farms milk in the study sites in order to investigate heavy metals during December 2013-May 2014.

Table 3.1: Sampling of water and dairy milk

Sites	Sub-sites	Sampled no of Tube wells (N)	Sampled no of Tap (N)	Pooled milk samples (N)
Chittagong Metropolitan	Potenga	5 (15)	5 (25)	6 (20)
	Madam Bibir Hat	4 (4)	5 (22)	5 (5)
	Balur Math	4 (4)	4 (18)	4 (15)
	Sagorika	3 (3)	3 (15)	5
	Karnel Hat	2 (2)	6 (6)	6 (20)
	Pahartali bazaar area	9 (9)	5 (5)	10
	Lalbag	6 (6)	3 (15)	8
	Kodomrasul	3 (11)	5 (26)	4 (12)
Total		**36 (54)**	**36 (132)**	**48 (95)**
Chandpur	Shaharasti	21 (4305)	-	21 (545)
	Hagigang	15 (2980)	-	27 (672)
Total		**36 (7285)**		**48 (1217)**

N=Total no. of tube wells/Taps/Farms

A total 108 water samples (N=7339) and 96 pooled dairy milk samples (N=1312) dairy farms) were obtained from the study sites (See details in **Table 3.1**). A random sampling technique was performed for each type of samples. Sample size was calculated using the following set of criteria:

For Chittagong: Water samples were taken from 20-30% of tube well (N=54) and tap water (N=132), whereas pooled milk samples were taken from 30% of dairy farms (N=95).

For Chandpur: Water samples were taken from 0.5% of tube well (N=7285), whereas pooled milk samples were taken from 4% of dairy farms (N=1217). This sampling strategy was applied because Chandpur region is considered as one of the hotspots for heavy metals other than the financial constraint of the project.

3.3 Collection, preservation and transportation of field samples and recording data

Fifty ml water (tube well/tab) and 15 ml pooled milk samples were collected and kept in a standard volumetric flask with the unique identification number. Obtained samples were then transferred through ice eskie to the laboratory of Chittagong Veterinary and Animal Sciences University and stored in -20°C at Poultry Research Training Centre (PRTC) laboratory until laboratory testing being performed (Ahmad and Goni, 2010). Required epidemiological and ecological data were recorded in structured record keeping sheet through physical inspection and personal interview. The data including site, sub site, breed of the cow in the farm, average age of the cow in the farm, types of surrounding industry, types of water, farm ecology etc. **(Appendix I)** were collected.

3.4 Diagnostic evaluation

3.4.1 Processing of samples

Water sample: Amount of 50 ml of water was digested with 10 ml of concentrated HNO_3 at 80°C until the solution became transparent. The solution was filtrated through Whatman filter paper (0.45 μm pore size). Then the filtrate was diluted to 50 ml with deionized water into the standard volumetric flask. Twelve ml sample was then taken into the falcon tube from the standard volumetric flask containing filtrated sample. Processed sample was then analyzed by Atomic Absorption Spectrometry (ZEEnit 700) (Ahmad and Goni, 2010).

Milk sample: A 10 ml of raw milk was taken in a tube and then 10 ml of 65% HNO_3, 2 ml of 30% H_2O_2 and 20 ml of concentrated HCl respectively were added to it. After that deionized water was added up to 50 ml and kept overnight in digestion chamber containing 80-90°C temperature at PRTC lab of CVASU. After 24 hours digestion, this sample was filtrated through Whatman filter paper and poured on standard volumetric flask. Then 12 ml sample was taken into the felcon tube from the standard volumetric flask containing filtrated sample and used for evaluation by Atomic Absorption Spectrometry (ZEEnit 700) (Rosas et al., 1999).

3.4.2 Atomic Absorption Spectrometry (ZEEnit 700)

Samples were evaluated by Atomic Absorption Spectrometry as per protocol described by Ahmad and Goni (2010) for water samples and Rosas et al. (1999) for milk samples. Approximately 12 ml of processed samples (water or milk) were taken in a cup suitable to fit into the sample tray of the Atomic Absorption Spectrometry (ZEEnit 700). On starting the machine around 2-3 ml processed sample were automatically drawn for a single metal detection (lead/nickel/copper). The sample solution was aspirated by a pneumatic analytical nebulizer and transformed into an aerosol, which was then introduced into a spray chamber, where the sample was mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (less than 10 μm) entered into the flame. This conditioning process was responsible that only about 5% of the aspirated sample solution reached the flame.

On top of the spray chamber was a burner head that produced a flame that was laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passed through this flame at its longest axis, and the flame gas flow-rates was adjusted to generate the highest concentration of free atoms. The burner height was also adjusted, so that the radiation beam passed through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

The processes in a flame include the following stages:

- **Desoluation (drying):** The solvent was evaporated and the dry sample nano-particles were remained.
- **Vaporization (transfer to the gaseous phase):** The solid particles were converted into gaseous molecules.
- **Atomization:** The molecules were dissociated into free atoms.
- **Ionization:** Depending on the ionization potential of the analyte atoms and the energy available in a particular flame, atoms in part converted to gaseous ions.

In flame Atomic Absorption Spectrometry a steady-state signal was generated during the time when the sample was aspirated and read the results on the screen of the machine. Concentration of metals was expressed in average mg/L^{-1}.

3.5 Data analysis

Field and laboratory data were entered into the Microsoft Excel 2007 programme. Data were cleaned and checked for integrity in MS Excel 2007 before exporting to STATA-11 (StataCrop, 4905, Lakeway Drive, College station, Texas 77845, USA) for analysis. Descriptive statistics (including percentage, frequency number and mean) were performed to express individual results of copper, lead and nickel in water and milk. To compare the results of prevalence of heavy metal between/among different factors Fisher's exact test was applied. The level of significant was set at 0.05.

A univariate logistic regression was used to identify the potential risk factors associated with the binary response variable of lead in dairy milk (yes/no)/ nickel in tube well water (yes/no). Categorical factors of site (Chittagong/Chandpur), area (semi-urban/urban/rural), presence of industry (yes/no), breed of dairy cows in the farm (Holstein Frisian Cross/Jersey Cross) and average age of cows in the farm (up to 4 years/more) were used for the logisitic regression analysis. The results of logistic regression analysis were presented in Odds Ratio (OR) and 95% confidence interval.

Chapter IV: Results

4.1 Prevalence of heavy metals in tube well and tap water in Chittagong and Chandpur

The overall prevalence of heavy metal in tube well water was 80.6% (N=72) of which lead contributed 37.5%; nickel contributed 15.3%; both lead and nickel contributed 26.4% and a combined category of lead, nickel and copper contributed 1.4%. The overall prevalence of heavy metal in tube well water was significantly higher in Chittagong (61.1%) than that of Chandpur (100%) (p<0.001) **(Table 4.1).**

Lead was only detected in tube well water in both study sites, whereas both nickel and copper were only detected in Chandpur. The prevalence was significantly higher in Chittagong (61.1%) than that of Chandpur (13.9%) (p<0.001) **(Table 4.1).**

Among the positive samples (58, 80.6%) 96.6% crossed the reference values (44.8% lead, 31% nickel, 19% lead and nickel, and 1.7% lead, nickel and copper). The prevalence of lead (crossed the reference value) was significantly greater in Chittagong (95.5%) than that of Chandpur (13.9%) (p<0.001). However, other metals that crossed the reference values were detected in Chandpur. The summary estimates of concentration of metals (mg/L) that crossed the reference values were average 0.45 (0.05-1.22) for lead, 0.45 (0.07-0.65) for nickel and 0.01 for copper **(Table 4.2).**

No positive samples for the selected metals were found in any of the tape water samples of Chittagong (N=36).

Table 4.1: Prevalence of metals in tube well water of Chittagong and Chandpur (*Positive samples having any value of the selected metals were included to estimate the prevalence of corresponding metals*)

	N=72	**Chittagong (36)**	**Chandpur (36)**	
Metal types	**n (%)**	**n (%)**	**n (%)**	**p (Fisher's exact) (2-tailed)**
Lead	27 (37.5)	22 (61.1)	5 (13.9)	<0.001
Nickel	11 (15.3)	0	11 (30.6)	
Lead and nickel	19 (26.4)	0	19 (52.8)	
Lead, nickel and copper	1 (1.4)	0	1 (2.7)	
Total	**58 (80.6)**	**22 (61.1)**	**36 (100)**	**<0.001**

Table 4.2: Prevalence of metals in tube well water samples of Chittagong and Chandpur (*Positive samples that crossed the reference values of the selected metals were included to estimate the prevalence of corresponding metals*)

	N=58	**Chittagong (22)**	**Chandpur (36)**	
Metal types	**n (%)**	**n (%)**	**n (%)**	**p (Fisher's exact) (2-tailed)**
Lead	26 (44.8)	21 (95.5)	5 (13.9)	<0.001
Nickel	18 (31.0)	0	18 (50.0)	
Lead and nickel	11 (19.0)	0	11 (30.6)	
Nickel and copper	1 (1.7)	0	1 (2.7)	
Total	**56 (96.6)**	**21 (95.5)**	**35 (97.2)**	**1.00**

4.2 Prevalence of heavy metals in dairy milk in Chittagong and Chandpur

The overall prevalence estimate of heavy metals in dairy milk was 53.1% (N=96) (6.3% lead, 32.3% nickel, 1.1% lead and copper, 10.4% lead and nickel, 2.1% copper and nickel, 1.1% lead, nickel and copper). The overall prevalence was statistically equal between Chittagong (60.4%) and Chandpur (45.8%) (*p*=0.219). No difference of prevalence were observed for lead and nickel between study sites (4.2% versus 8.3%; *p*=0.677). Other metals were only detected in Chittagong (2.1% lead and copper, 20.8% lead and nickel, 4.2% copper and nickel, 2.1% lead, nickel and copper **(Table 4.3).**

Among positive samples (51, 53.1%) 27.5% crossed the threshold values (25.5% lead and 2.0% lead and copper) and all of these samples obtained from Chittagong. The summary estimates of concentration of lead and copper (mg/L) were average 0.24 (0.08-0.44) and 0.11, respectively **(Table 4.4).**

Table 4.3: Prevalence of metals in dairy milk of Chittagong and Chandpur (*Positive samples having any value of the selected metals were included to estimate prevalence of corresponding metals*)

	N=96	**Chittagong (48)**	**Chandpur (48)**
Metal types	**n (%)**	**n (%)**	**n (%)**
Lead	6 (6.3)	2 (4.2)	4 (8.3)
Nickel	31 (32.3)	13 (27.1)	18 (37.5)
Lead and copper	1 (1.1)	1 (2.1)	0
Lead and nickel	10 (10.4)	10 (20.8)	0
Copper and nickel	2 (2.1)	2 (4.2)	0
Lead, nickel and copper	1 (1.1)	1 (2.1)	0
Total	**51 (53.1)**	**29 (60.4)**	**22 (45.8)**

Table 4.4: Prevalence of metals in dairy milk of Chittagong and Chandpur (*Positive samples that crossed the reference values of the selected metals were included to estimate prevalence of corresponding metals*)

	N=51	**Chittagong (29)**	**Chandpur (22)**	
Metal types	**n (%)**	**n (%)**	**n (%)**	**Average concentration (mg/L)**
Lead	13 (25.5)	13 (44.8)	0	0.24 (0.08-0.44)
Lead and copper	1 (2.0)	1 (3.4)	0	0.11 (Copper)
Total	**14 (27.5)**	**14 (48.3)**	**0**	

4.3 Risk factor analysis for the prevalence of lead and nickel in dairy milk

Site (Chittagong/Chandpur), area (semi-urban/urban/rural) and presence of industry (yes/no) were tested to assess their effect on the occurrence of lead (yes/no) and nickel (yes/no) in tube well water, however none of factors was identified as a risk factor.

Site, area, presence of industry, breed of dairy cattle (Holstein Frisian cross/Jersey cross) and age (up to 4 years/more than 4 years) were evaluated to examine their effect on the occurrence of lead (yes/no) and nickel (yes/no). Site, area and industry were identified risk factors for the occurrence of lead, whereas area and industry were identified risk factors for the occurrence of nickel **(Table: 4.5).**

The prevalence of lead in dairy milk in Chittagong was significantly higher (29.2%) than that of Chandpur (8.3%) (p=0.017; OR 4.6; 95% CI 1.4-15.0). The prevalence of lead in dairy milk obtained from farms of semi-urban areas were significantly greater (57.1%) than the prevalence in either rural (7.4%) or urban (8.3%) areas (p<0.001; OR 16.7; CI 3.1-89.4: rural as referral; OR 14.7; CI 3.8-57.0: urban as referral). The prevalence of lead in dairy milk collected from the farms having nearby industry (16.7-31%) was significantly higher than that of the farms having no industry (8.3%) (*p*=0.018; OR 4.5; CI 1.4-15.0: no industry as referral) **(Table: 4.5).**

Table 4.5: Univariate analyses between a binary response variable of metal (Lead/Nickel) and the selected risk factors

		Lead		**2-tailed *p* (Fisher's Exact test)**	**Nickel**		**2-tailed *p* (Fisher's Exact test)**
Factor	**Class**	**Yes (%)**	**No**		**Yes (%)**	**No**	
Site	Chittagong	14 (29.2)	34	0.017	26 (54.1)	22	0.151
	Chandpur	4 (8.3)	44		18 (37.5)	30	
Area	Urban	4 (8.3)	44	<0.001	18 (37.5)	30	0.007
	Semi-urban	12 (57.1)	9		16 (76.2)	5	
	Rural	2 (7.4)	25		10 (37.0)	17	
Industry	Iron	1 (16.7)	5	0.018	1 (16.7)	5	0.039
	Plate/and Pipe	13 (31.0)	29		25 (59.5)	17	
	None	4 (8.3)	44		18 (37.5)	30	
Breed	Holstein Frisian cross	8 (23.5)	26	0.419	19 (55.9)	15	0.199
	Jersey cross	10 (16.1)	52		25 (40.3)	37	
Age (year)	Up to 4	9 (20.5)	35	0.795	17 (38.6)	27	0.222
	More than 4	9 (17.3)	43		27 (51.9)	25	

The prevalence estimate of nickel in dairy milk was significantly greater in semi-urban area (76.2%) than the prevalence in urban (37.5%) or rural (37.0%) areas (p=0.007; OR: urban versus rural 1.0; 95% CI 0.4-2.7; OR: semi-urban versus rural 5.4; 95% CI 1.5-19.4). The prevalence of nickel was higher in dairy milk obtained from farms close to industry (plate/and pipe) (59.5%) than that of no-industry (37.5%) (p=0.039; OR: industry versus non-industry 2.0; 95% CI 0.9-4.4) **(Table: 4.5).**

Chapter V: Discussion

Heavy metals existed in different water sources, milk and vegetables at high concentration is serious animal health and public health risk. This chapter has discussed the main findings of the present study under the two broad headings as follows:

5.1 Discussion on prevalence of selected heavy metals in water and dairy milk

The overall prevalence of lead was significantly higher in tube well water (37.5%) than in dairy milk (6.3%) in the present study which coincide to some extent with earlier studies (Jung, 2001; Shahriar et al., 2014). These studies reported the prevalence of lead was 29% in water and 0% in dairy milk.

Interestingly, the prevalence of lead in tube well water of Chittagong was significantly higher (61.1%), but lower in dairy milk (4.2%). High prevalence of lead in tube well water may be due to a random discharge of waste water of workshops and small industrial units to the environment inside the Chittagong metro city as well as abundance of huge traffics which could pollute water sources within the city (Pirsaheb et al., 2013).

Nickel was detected both in tube well water and dairy milk in Chandpur at prevalence of 30.6-37.5%. Contrarily, nickel was detected only in dairy milk in Chittagong at prevalence of 27.1%. High prevalence of nickel both in water and dairy milk in Chandpur could be due to the wide use of this element as fertilizers, plating, color ceramics, making some batteries, as catalyst in different chemical processes etc. which can contaminate environment and water sources which in turn affect dairy cattle in that region. This explanation is supported by many other studies (Klan et al., 2011; Pirsaheb et al., 2013). The same reason can be applied on why nickel was more prevalent in dairy milk in Chittagong.

Around 45% lead positive water samples (N=56) and 26% lead positive milk samples (N=51) crossed the reference values in the present study (0.05 mg/L for water according to WHO and 0.49 mg/L for milk according to IDF) (Giri et al., 2011; Kumar and Puri,

2012) which have clear indication of public health threat. Lead crossing threshold concentration causes health effect of hypertension, decrement of renal function, adverse reproductive outcome, hampering cognitive development, hampering mental development etc. (Byers and Lord, 1943; Petit and Alfano, 1979; Grant and Davis, 1989; Tong et al., 2000; Kosnett, 2009). Boiling temperature can neither destroy the lead nor reduce the level of concentration (Curtis, 1998; Han et al., 2006). Therefore, in order to reduce lead concentration sieving drinking water and dairy milk in the study areas should be performed before human consumption (Srinivasan and Sorial, 2009).

About 44.8% milk sample collected from different farms of Chittagong region had higher concentration of lead (average 0.24 mg/L) and this result is similar to the findings of De Castro et al. (2010) in Brazil and higher than Shahriar et al. (2014) in Bangladesh but lower than the concentration of Farid et al. (2004) in Saudi Arabia (average 3.5 mg/L) and concentration of Malhat et al. (2012) in Egypt (1.9 mg/L). Although the findings in this study are far below the level reported by Vidal et al. (2004), Jigam et al. (2011) and Ogabiela et al. (2011) but lead is a toxic material and associated with traffic pollution.

Thirty one percent nickel positive water also crossed the threshold value (0.01 mg/L) which is also indicative of public health risk, can cause haematotoxicity, immunotoxicity, neurotoxicity, genotoxicity, reproductive toxicity, pulmonary toxicity, nephrotoxicity, hepatotoxicity etc. (Chen et al., 2003; Das and Büchner, 2007; Das et al., 2008; Xu et al., 2010; Pari and Amudha, 2011).

5.2 Discussion on risk factors associated with the prevalence of lead and nickel in dairy milk

Area was identified as risk factor for the presence of lead in dairy milk where semi-urban area had the highest prevalence and this can be because of the presence of different kind of industries and use of different fertilizers in the agriculture field. This finding corresponds to many previous investigations (Jigam et al., 2011; Dizaji et al., 2012; Elatrash and Atoweir, 2014).

Area and industry were significant risk factors for the prevalence of nickel in milk. Nickel was highly prevalent in dairy milk obtained from farms of semi-urban area and farms located close to plate/and pipe industry. Reasons behind may be that sewerage water in the study area is pumped out of sewerage drains for irrigation purposes and there is no alternate source of water for both crops and animals (Javed et al., 2009). Besides, the contamination of pastures by the toxic metal nickel from the nearby industry and the accumulation of those metals in grazing animals can also be the other reason. Moreover, the passage of heavy metals is through the soil–feed/grass–cow–milk chain. So the high concentration of the metals in the soil will lead to appearance in the milk in more quantities as compared to the other area (Giri et al., 2011). These results are in agreement with other studies (Martin and Coughtrey, 1982; Javed et al., 2009; Giri et al., 2011).

Limitations of the present study

- The present study was a cross sectional study and covered only limited sites and limited number heavy metals as well as limited sample size obtained from Chandpur because of overall financial constraints
- As Atomic absorption spectrometry (ZEEnit 700) was newly used for the study at CVASU, there might have occurred some diagnostic uncertainties, even though the study followed a standard diagnostic protocol.

Chapter VI: Conclusion and Recommendation

The prevalence of lead was significantly higher in tube well water (37.5%) than in dairy milk (6.3%). Nickel was detected both in tube well water and dairy milk at high prevalence in Chandpur (30.6-37.5%) but found only in dairy milk at high prevalence in Chittagong (27.1%). Approximately quarter to fifty percent positive samples (lead/nickel) in tube well water and dairy milk crossed the reference values, suggesting a certain public health risk of causing of neurotoxicity, hypertension, nephrotoxicity, hepatotoxicity etc. for children and adult as well. Moreover, prolong accumulation of these lead and nickel may cause various chronic diseases in animal and public health. Therefore, needful techniques to reduce the risk level of heavy metals in tube well water and dairy milk along with public awareness through motivation and education should be sought in future. As an immediate suggestion the drinking water of the area should be filtered by the quality control agencies to subside the risk concentration of lead and nickel. Some other recommendations include detoxication and treating both the industrial and domestic effluents that are intended to be used for agricultural purposes. Further investigation of heavy metals on air, soil and grasses in Chittagong and Chandpur is also suggested.

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Appendix I: Questionnaire of investigation of heavy metals in water and dairy milk in Greater Chittagong

A. **Name of the study site:** 1=Chittagong; 2=Chandpur

B. **Sub site:**

C. **Contact address of the farm / location:**

D. **Sample details:**

   I) Sample ID:

   II) Sample types: Milk / Water (1=Tube well water; 2=Tap water)

   III) Sample quantity:

E. **Farm location :** 1=Urban; 2=Semi Urban; 3=Rural

F. **Types of surrounding industry:** 1=Iron; 2=Plate; 3=Pipe; 4= Plate and Pipe; 5=None

G. **Types of breed:** 1=HF x Deshi; 2=Jersey x Deshi; 3=HF x Shahiwal;
4= HF x Pabna; 5=Deshi

H. **Average age of the cow (year) :**

Signature of collector

Appendix II: Spreadsheet of positive water samples of tube well of Chandpur and Chittagong

Site	Sample Id	Copper(mg/L)		Lead(mg/L)		Nickel(mg/L)	
		Tested value	Ref. value	Tested value	Ref. value	Tested value	Ref. value
Chandpur	1	-	0.005[a]	0.0667	0.05[b]	-	0.01[c]
	2	-	0.005[a]	0.0405	0.05[b]	-	0.01[c]
	3	-	0.005[a]	0.1119	0.05[b]	-	0.01[c]
	4	-	0.005[a]	0.0601	0.05[b]	-	0.01[c]
	5	-	0.005[a]	0.0768	0.05[b]	-	0.01[c]
	6	-	0.005[a]	0.1436	0.05[b]	0.0097	0.01[c]
	7	-	0.005[a]	0.1301	0.05[b]	0.0675	0.01[c]
	8	-	0.005[a]	0.0844	0.05[b]	0.1292	0.01[c]
	9	-	0.005[a]	0.1018	0.05[b]	0.2072	0.01[c]
	10	--	0.005[a]	0.1266	0.05[b]	0.2657	0.01[c]
	11	-	0.005[a]	0.067	0.05[b]	0.2576	0.01[c]
	12	-	0.005[a]	0.0716	0.05[b]	0.2653	0.01[c]
	13	-	0.005[a]	0.0869	0.05[b]	0.3067	0.01[c]
	14	-	0.005[a]	0.1016	0.05[b]	0.36	0.01[c]
	15	-	0.005[a]	0.077	0.05[b]	0.4287	0.01[c]
	16	-	0.005[a]	0.0497	0.05[b]	0.4914	0.01[c]
	17	-	0.005[a]	0.0796	0.05[b]	0.5115	0.01[c]
	18	-	0.005[a]	0.024	0.05[b]	0.5456	0.01[c]
	19	-	0.005[a]	0.0236	0.05[b]	0.518	0.01[c]
	20	-	0.005[a]	0.01	0.05[b]	0.4865	0.01[c]
	21	-	0.005[a]	0.0237	0.05[b]	0.4539	0.01[c]
	22	-	0.005[a]	-	0.05[b]	0.4391	0.01[c]
	23	-	0.005[a]	-	0.05[b]	0.4377	0.01[c]
	24	-	0.005[a]	0.0087	0.05[b]	0.4963	0.01[c]
	25	-	0.005[a]	0.0162	0.05[b]	0.5557	0.01[c]
	26	-	0.005[a]	-	0.05[b]	0.5943	0.01[c]
	27	-	0.005[a]	-	0.05[b]	0.625	0.01[c]
	28	-	0.005[a]	-	0.05[b]	0.6224	0.01[c]
	29	-	0.005[a]	-	0.05[b]	0.5799	0.01[c]
	30	-	0.005[a]	-	0.05[b]	0.5309	0.01[c]
	31	-	0.005[a]	-	0.05[b]	0.5063	0.01[c]
	32	-	0.005[a]	-	0.05[b]	0.4964	0.01[c]
	33	-	0.005[a]	0.0048	0.05[b]	0.5139	0.01[c]
	34	-	0.005[a]		0.05[b]	0.5665	0.01[c]
	35	-	0.005[a]		0.05[b]	0.6354	0.01[c]
	36	0.0118	0.005[a]	0.0212	0.05[b]	0.6503	0.01[c]

Appendix II: Spreadsheet of positive water samples of tube well of Chandpur and Chittagong (continued)

Site	Sample Id	Copper(mg/L)		Lead(mg/L)		Nickel(mg/L)	
		Tested value	**Ref. value**	**Tested value**	**Ref. value**	**Tested value**	**Ref. value**
Chittagong	15	-	0.005[a]	0.0435	0.05[b]	-	0.01[c]
	16	-	0.005[a]	0.1281	0.05[b]	-	0.01[c]
	17	-	0.005[a]	0.1819	0.05[b]	-	0.01[c]
	18	-	0.005[a]	0.2752	0.05[b]	-	0.01[c]
	19	-	0.005[a]	0.3371	0.05[b]	-	0.01[c]
	20	-	0.005[a]	0.3826	0.05[b]	-	0.01[c]
	21	-	0.005[a]	0.4635	0.05[b]	-	0.01[c]
	22	-	0.005[a]	0.5424	0.05[b]	-	0.01[c]
	23	-	0.005[a]	0.5931	0.05[b]	-	0.01[c]
	24	-	0.005[a]	0.6215	0.05[b]	-	0.01[c]
	25	-	0.005[a]	0.7591	0.05[b]	-	0.01[c]
	26	-	0.005[a]	0.7935	0.05[b]	-	0.01[c]
	27	-	0.005[a]	0.821	0.05[b]	-	0.01[c]
	28	-	0.005[a]	0.8481	0.05[b]	-	0.01[c]
	29	-	0.005[a]	0.896	0.05[b]	-	0.01[c]
	30	-	0.005[a]	0.9453	0.05[b]	-	0.01[c]
	31	-	0.005[a]	0.9661	0.05[b]	-	0.01[c]
	32	-	0.005[a]	1.041	0.05[b]	-	0.01[c]
	33	-	0.005[a]	1.034	0.05[b]	-	0.01[c]
	34	-	0.005[a]	1.086	0.05[b]	-	0.01[c]
	35	-	0.005[a]	1.223	0.05[b]	-	0.01[c]
	36	-	0.005[a]	1.101	0.05[b]	-	0.01[c]

Ref. =Reference

b: Kumar and Puri (2012); **a and c** Bhuiyan et al. (2011)

Appendix III: Spreadsheet of positive milk samples of Chandpur and Chittagong

Site	Sample Id	Copper(mg/L)		Lead(mg/L)		Nickel(mg/L)	
		Tested value	Ref. value	Tested value	Ref. value	Tested value	Ref. value
Chandpur	1	-	0.10[a]	0.0757	0.049[b]	-	23.38[c]
	2	-	0.10[a]	0.0402	0.049[b]	-	23.38[c]
	3	-	0.10[a]	0.0161	0.049[b]	-	23.38[c]
	4	-	0.10[a]	0.0178	0.049[b]	-	23.38[c]
	31	-	0.10[a]	-	0.049[b]	0.0663	23.38[c]
	32	-	0.10[a]	-	0.049[b]	0.1303	23.38[c]
	33	-	0.10[a]	-	0.049[b]	0.0543	23.38[c]
	34	-	0.10[a]	-	0.049[b]	0.0752	23.38[c]
	35	-	0.10[a]	-	0.049[b]	0.1038	23.38[c]
	36	-	0.10[a]	-	0.049[b]	0.146	23.38[c]
	37	-	0.10[a]	-	0.049[b]	0.1909	23.38[c]
	38	-	0.10[a]	-	0.049[b]	0.2357	23.38[c]
	39	-	0.10[a]	-	0.049[b]	0.3542	23.38[c]
	40	-	0.10[a]	-	0.049[b]	0.4099	23.38[c]
	41	-	0.10[a]	-	0.049[b]	0.5004	23.38[c]
	42	-	0.10[a]	-	0.049[b]	0.5293	23.38[c]
	43	-	0.10[a]	-	0.049[b]	0.5759	23.38[c]
	44	-	0.10[a]	-	0.049[b]	0.6189	23.38[c]
	45	-	0.10[a]	-	0.049[b]	0.5087	23.38[c]
	46	-	0.10[a]	-	0.049[b]	0.6544	23.38[c]
	47	-	0.10[a]	-	0.049[b]	0.6995	23.38[c]
	48	-	0.10[a]	-	0.049[b]	0.61	23.38[c]

Appendix III: Spreadsheet of positive milk samples of Chandpur and Chittagong (continued)

Site	Sample Id	Copper(mg/L)		Lead(mg/L)		Nickel(mg/L)	
		Tested value	Ref. value	Tested value	Ref. value	Tested value	Ref. value
Chittagong	1	0.0283	0.10[a]	0.1003	0.049[b]	-	23.38[c]
	4	-	0.10[a]	-	0.049[b]	0.269	23.38[c]
	7	-	0.10[a]	-	0.049[b]	0.5914	23.38[c]
	8	-	0.10[a]	-	0.049[b]	0.332	23.38[c]
	9	-	0.10[a]	-	0.049[b]	0.6645	23.38[c]
	10	-	0.10[a]	-	0.049[b]	0.2009	23.38[c]
	11	-	0.10[a]	-	0.049[b]	0.1885	23.38[c]
	16	0.0884	0.10[a]	-	0.049[b]	4.057	23.38[c]
	17	-	0.10[a]	-	0.049[b]	1.447	23.38[c]
	18	-	0.10[a]	-	0.049[b]	1.452	23.38[c]
	19	-	0.10[a]	-	0.049[b]	0.0409	23.38[c]
	20	0.063	0.10[a]	-	0.049[b]	4.247	23.38[c]
	21	-	0.10[a]	-	0.049[b]	0.1332	23.38[c]
	22	-	0.10[a]	-	0.049[b]	1.043	23.38[c]
	27	-	0.10[a]	-	0.049[b]	0.0172	23.38[c]
	33	-	0.10[a]	-	0.049[b]	3.183	23.38[c]
	36	-	0.10[a]	0.0244	0.049[b]	-	23.38[c]
	37	-	0.10[a]	0.0849	0.049[b]	0.2253	23.38[c]
	38	-	0.10[a]	0.038	0.049[b]	0.3818	23.38[c]
	39	-	0.10[a]	0.1042	0.049[b]	0.1982	23.38[c]
	40	-	0.10[a]	0.1491	0.049[b]	1.863	23.38[c]
	41	-	0.10[a]	0.2979	0.049[b]	0.2633	23.38[c]
	42	-	0.10[a]	0.1949	0.049[b]	0.8294	23.38[c]
	43	-	0.10[a]	0.2676	0.049[b]	1.42	23.38[c]
	44	-	0.10[a]	0.2861	0.049[b]	-	23.38[c]
	45	-	0.10[a]	0.3512	0.049[b]	1.2	23.38[c]
	46	-	0.10[a]	0.3651	0.049[b]	1.164	23.38[c]
	47	0.1094	0.10[a]	0.3934	0.049[b]	4.169	23.38[c]
	48	-	0.10[a]	0.4411	0.049[b]	1.14	23.38[c]

Ref. =Reference

a and b: Giri et al. (2011); **c:** Javed et al. (2009).

Brief Biography

Md. Rofy Foysal Talukder passed the Secondary School Certificate Examination in 2003 and then Higher Secondary Certificate Examination in 2005. Mr. Talukder obtained his Doctor of Veterinary Medicine Degree in 2010 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Pharmacology under the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, CVASU. He has immense interest to work in heavy metal detection in water, milk, vegetables etc.