SPECTROSCOPIC INVESTIGATION OF TOXIC HEAVY METALS IN DIFFERENT MILK AND MILK PRODUCTS: A COMPARATIVE STUDY

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Roll No.: 0119/07

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MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND SYMBOLS USED

Symbols	Elaboration
°C	Degree centigrade
µg	Microgram
mg/L	Milligram per liter
mg/kg	Milligram per kilogram
µg /dl	Microgram per deciliter
gm	Gram
Mm	Micrometer
Ppm	Parts per million
Cd	Cadmium
Pb	Lead
Ni	Nickel
Cr	Chromium

Symbols	Elaboration
IDF	International Dairy Federation
ATSDR	Agency for Toxic Substances and Disease Registry
IPCS	International Program on Chemical safety
IARC	International Agency for Research on Cancer
Fig.	Figure
FAO	Food and Agriculture Organization
WHO	World Health Organization
%	Percentage

ABSTRACT

The balanced nutritional food in the human diet is referred to as milk. Milk contains a wide variety of macro and micronutrients, yet it is possible for milk and milk products to be contaminated with heavy metals. The goal of the current inquiry was to identify the levels of lead, chromium, cadmium, and nickel in market pasteurized milk, raw fresh milk, ghee, yogurt, and powdered milk from various brands. A sum of five fresh milk trial selected from dairy farms at Khulsi, Chattogram. Five different brand powdered milk samples, three different brand ghee and yogurt samples and five different brand market pasteurized milk trial selected from Chattogram were get hold of inspection. In raw, fresh milk, concentrations of lead (Pb), chromium (Cr), cadmium (Cd), and nickel (Ni) were determined to be 0.126 mg/L, 0.193 mg/L, 0.022 mg/L, and 0.159 mg/L, respectively. Lead (Pb), Chromium (Cr), Cadmium (Cd), and Nickel (Ni) concentrations in brand-name market pasteurized milk were 0.223 mg/L, 0.177 mg/L, 0.035 mg/L, and 0.291 mg/L, respectively. Lead (Pb), Chromium (Cr), Cadmium (Cd), and Nickel (Ni) values in powder milk samples from various brands were 0.189 mg/L, 0.154 mg/L, 0.040 mg/L, and 0.280 mg/L, respectively. Additionally, the amounts of lead (Pb), chromium (Cr), cadmium (Cd), and nickel (Ni) were 0.010 mg/kg, 0.385 mg/kg, 0.027 mg/kg, and 0.029 mg/kg respectively, in samples of yogurt from various brands. Additionally, the amounts of Lead (Pb), Chromium (Cr), Cadmium (Ca), and Nickel (Ni) in various brands of ghee samples were 0.130 mg/kg, 0.237 mg/kg, 0.167 mg/kg, and 0.167 mg/kg respectively. The levels of the four heavy metals were compared between several dairy farms in Chattogram, Bangladesh as well as between various brands. The levels of those four heavy metals within most of the samples were found within permitted range of different approved authorities. But in some samples those limit exceeded the limits which can cause health risk to humans. The way of their entry to the human food chain is through the contaminated milk and powder milk due to the presence of industries and highway roads near the dairy farms, animal feeds contaminated with heavy metals and processing steps.

Keywords: Milk, contamination, atomic absorption spectrophotometer, heavy metals.

Chapter 1 Introduction

Contamination of the environment with heavy metals is a major concern because of their toxicity and bio-accumulative ability. Industrialization and traffic lead to increased concentrations of heavy metals (Mazej et al., 2010).Through dietary and non-dietary exposure, heavy metals enter living things where they collect and last a very long period. These are beginning by entering the food stuff and after that, it moves on to the tissue (Baykov et al., 1996). Among the principal hazardous metals that build up in food chains and have a cumulative effect are lead, chromium, cadmium, mercury, and arsenic (Cunningham and Saigo, 1997). The amount of heavy metal that accumulates relies on both the metal's properties and the target organ. Because they are retained in living tissues, heavy metals frequently have physiologically harmful effects.

The bioavailability of these substances, which is reflected by the metal's properties, dietary information, and the age of the organisms, is related to the uptake of heavy metals by living things. Milk is a very nutrient-dense food that is part of the human food supply cycle. It is a good way to get vitamins, minerals, and other nutrients that are good for your health (Hashish et al., 2012). Milks are easily digested and include a sizable amount of nutrients necessary for the healthy development and upkeep of bodily tissue. Investigations into metal contamination of foods, particularly milk, which is an essential component of peoples' diets, especially children's diets, are increasing as a result of global environmental pollution with trace elements (IDF, 1991).The Industrializations extraction and dissemination of mineral compounds from their natural reserves have accompanied progress across the planet. Numerous minerals, particularly trace elements, have experienced chemical transformations due to technical operations, which have caused them to eventually pass into the water and air where they are disseminated and then enter the food chain. Heavy metal compounds may be ingested by cattle from a variety of sources; metal traces may accumulate in their milk and flesh (Nisianakis et al., 2009).

The diet often contains some trace elements that are essential for healthy human health. Although humans need the trace elements iron (Fe) and copper (Cu), all metals are hazardous in higher doses (Chronopoulos et al., 1997; Lane and Morel, 2009). Other hazardous heavy metals including nickel (Ni), cadmium (Cd), lead (Pb), and chromium (Cr) can accumulate over time in cattle and result in life-threatening sickness. Some substances that are hazardous in general are advantageous to some creatures or situations (Lane et al., 2005). Human poisoning from heavy metals can be either acute or chronic. There have been numerous reports of heavy metal intake worldwide through the food chain (Muchuweti et al., 2006). In addition to lowering energy levels and harming important organs including the liver, kidneys, lungs, and central nervous system, heavy metal toxicity can also cause damage to the blood's chemical makeup.

Several heavy metals found in milk samples, including Fe, Mn, Zn, and Cu, are extremely good for human health while Cr, Cd, Pb, and Ni are very bad for human health and have a detrimental impact on human health. Milk and dairy products (such as yogurt, ghee, etc.) have a number of questionable impacts on human health, including cholesterol metabolism, immune system regulation, diarrhea, eradication of Helicobacter pylori, antimicrobial, anti-mutagenic, anti-cancer, and antioxidant action (Hernandez et al., 2014). Despite having many positive advantages, customers would still be exposed to harmful health impacts because milk and dairy products may include chemicals and pollutants (Licata et. al., 2004). In general, heavy metals are very scarce in milk and dairy products. Any food's quality can be determined by the level of heavy metal contamination, and inadequate environmental pollution control, processing conditions, cleanliness, and husbandry can have an impact on the quality of milk products (Capcarova et al., 2017). Arsenic, lead, cadmium, chromium, copper, and zinc are a few possible danger factors found in milk (Li et. al., 2005).

In order to demonstrate the accumulation behavior of heavy metals from milk to milk products and the impact of heavy metal absorption from various dairy products, we investigated the concentrations of Pb, Cr, Cd, and Ni in both raw milk and milk products (yoghurt, ghee, etc.) within different dairy farms and products from different brands.

1.1 Objectives:

- To determine whether raw milk, pasteurized milk, powdered milk, and milk products contain heavy metals (Lead, Chromium, Cadmium, and Nickel).
- To determine the level of heavy metals in raw milk in order to research the quality parameters for milk products.
- To compare the result between the samples that is prepared by wet digestion procedure and dry ashing technique.

Chapter 2 Review of Literature

Relevant literatures on heavy metals (cadmium, lead, chromium, nickel) in different milk and milk products have been discussed and reviewed in this chapter.

2.1 Heavy metal

Metals with relatively high densities, atomic weights, or atomic numbers are typically referred to as heavy metals. It is a group of elements that display metallic characteristics like hardness, opacity, shine, and good electrical and thermal conductivity. It consists of lanthanides (lanthanum, cerium, etc.), actinides, some metalloids (arsenic, boron, etc.), transition metals (zinc, mercury, etc.), and some metalloids (actinium, protactinium etc.). A heavy metal is also one that has a particular metal density more than 5 g/cm^3 (Suciu et al., 2008). In terrestrial and freshwater ecosystems, they can also be chemical elements having a density more than 4 g/cm^3 that are present in a variety of soils, rocks, and water (Adelekan and Abegunde, 2011).

2.2 Types of heavy metal

Heavy metal can be divided into two categories:

- Essential metals and
- Non-essential metals

Co, Cr, Cu, Zn, Fe, and Ni are essential metals, while Pb, Hg, As, and Cd are non-essential. A lack of critical components leads to faulty biological function; an abundance of important elements can be harmful. When ingested, non-essential metals can potentially have harmful effects (Uluozlu et al., 2009).

2.3 Properties of heavy metal

Heavy metal shows different physical and chemical properties those are:

Table 2.1: Heavy metal properties (Xu et al., 2014)

bodily characteristics	
Density	typically more
Hardness	most are quite hard
Thermal expantivity	usually lower
Melting point	minimal to extreme
Tensile strength	generally higher
Chemical properties	
Periodic table location	group 3 through 16 contains almost all of them.
Abundance in Earth's crust	less abundant
Main occurrence (or source)	*lithophilesor chalcophiles*(Au is a siderophile)
Reactivity	less reactive
Sulfides	insoluble Extremely
Hydroxides	usually insoluble
Salts	the majority of colorful solutions originate in water
Complexes	colored mostly

2.4 Influence of heavy metals on human and environmental pollution

When taken in excess of the bio-recommended levels, heavy metals turn hazardous to humans. This is due to the fact that when taken in excess, heavy metals are not digested by the organs and instead build up in the soft tissues, harming the body and generating biotoxic consequences. Effects could be neurotoxic, toxic (acute, chronic, or sub-chronic), carcinogenic, teratogenic, or mutagenic in origin. The following have been reported as general symptoms of cadmium, lead, arsenic, mercury, zinc, copper, and aluminum poisoning: gastrointestinal (GI) disorders, diarrhoea stomatitis, tremor, hemoglobinuria causing a rust-red color to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled. Individual metals exhibit specific symptoms of their (McCluggage et al., 1991).

In agricultural, manufacturing, pharmaceutical, industrial, or residential environments, they may enter people's bodies through food, drink, air, or skin absorption. Although heavy metals are naturally present in the ground, human-caused activities lead them to concentrate. Metals are present in our surroundings naturally, although they are rarely in hazardous quantities, especially in the crust of the globe where they help maintain the planet's equilibrium. Common causes include lead-acid batteries, fertilizers, paints, treated wood, outdated water supply infrastructure, mining and industrial waste, automobile emissions, and micro plastics in the world's oceans (Howell et al., 1985).

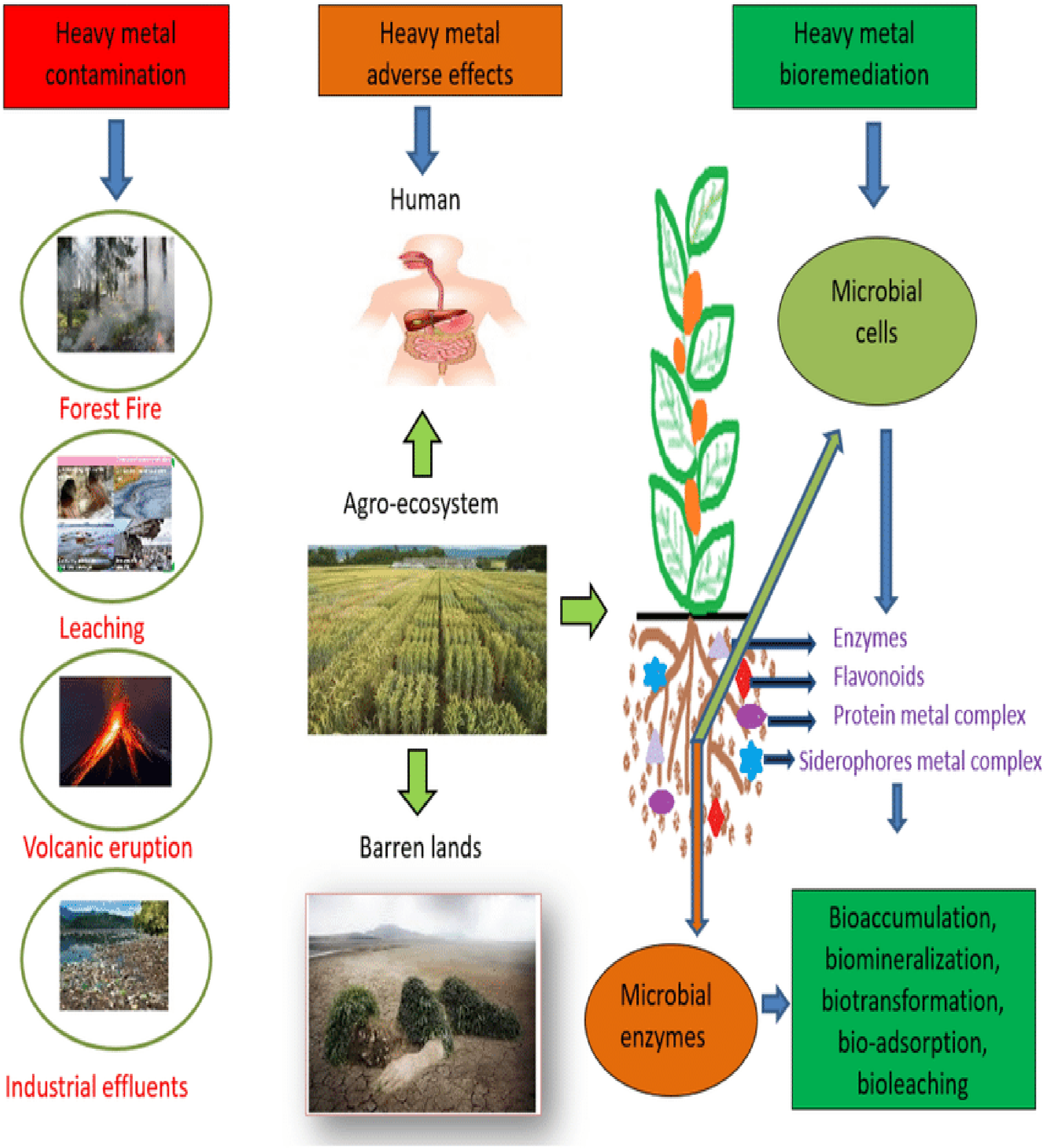

Figure 2.1: Main channels for metalloid, heavy metal and human exposure (Verma et. al., 2021)

Adults frequently experience industrial exposure, but children typically experience it through ingestion (Roberts et al., 1999). Normal hand-to-mouth activity among children may reduce harmful levels (i.e. coming in contact with polluted soil or eating objects that are not food such as dirt or paint chips). Less frequent exposure pathways include undergoing radiological treatment, administering incorrect doses or monitoring patients receiving intravenous (parenteral) nourishment, having a thermometer break, or attempting suicide or homicide (Lupton et al., 1985; Smith et. al., 1997).

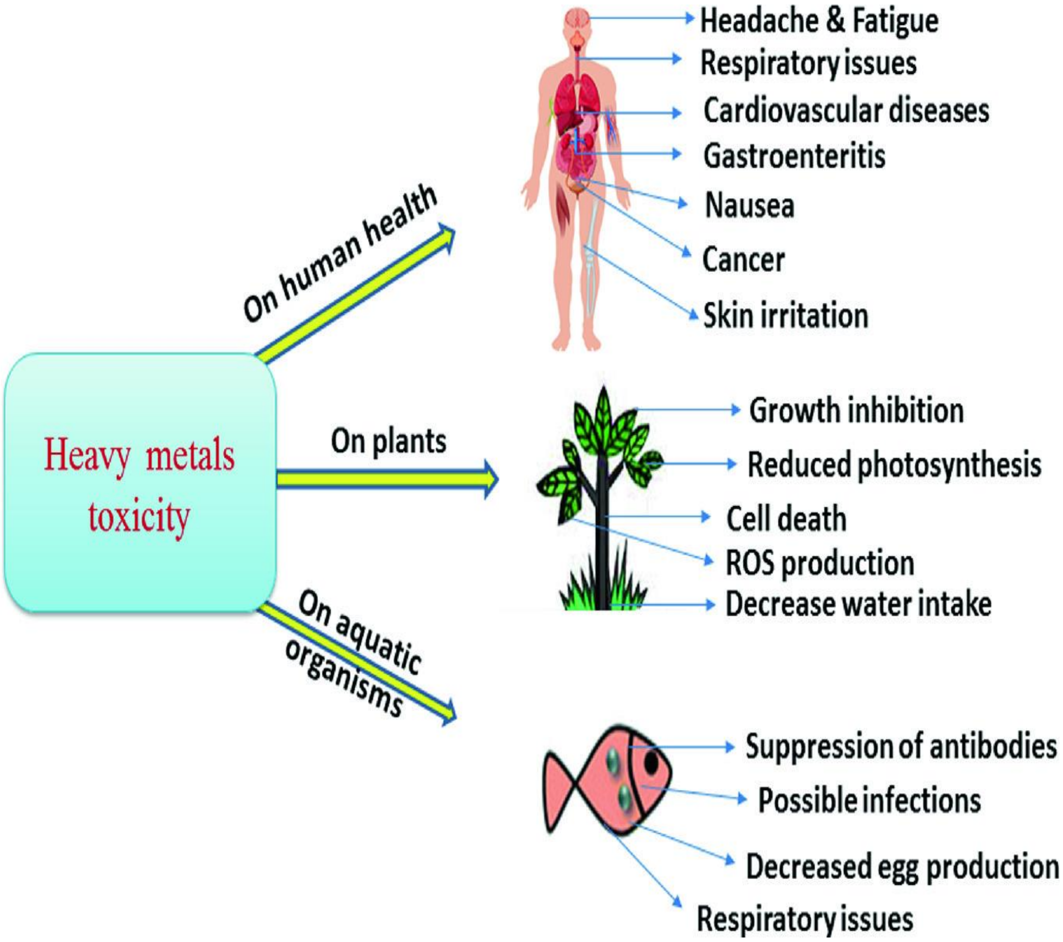

Figure 2.2: Environmental effects of heavy metals (Vijaya et al., 2014).

After being released into the environment, heavy metals can linger in streams for decades or even millennia at quantities dangerous to human health. There are several ways to rid the environment of these types of contaminants, but they are typically more expensive and difficult to employ effectively. To extract or eliminate inert metals and metal contaminants from contaminated soil and water in the present day, phytoremediation is an efficient and reasonably priced technical option. This technology is possibly economical and favorable to the environment (Bieby et al., 2011).

To allow the best biological performance for the majority of species, it is important to remember that the concentrations of particular metals in living tissues must be kept very low and should be maintained within appropriate limits (Misra and Mani, 2009). Due to their toxicity, persistence, and non-biodegradable qualities in the environment, heavy metals are regarded severe pollutants and pose a threat to humans and other biological life forms (Adeleken and Abegunde, 2011).

A single pollution could lead to a long-term exposure of human, microbial, animal, flora, and other edaphic communities to heavy metals, according to Adeleken and Abegumde's (2011) observation that heavy metals have poor environmental mobility. The issue of this atmospheric heavy metal pollution won't go away overnight, it might be asserted. However, it will continue to be a series of large industrial activities for many generations and is projected to grow quickly going forward. Compiling historical and current catalogs of atmospheric heavy metal content is a crucial task in this regard (Shrivastav et al., 2001).

Figure 2.3: Neurotoxic effects of heavy metal (jarup et al., 2003)

2.5 Concentration of heavy metals in milk

Public health is thought to be more affected by the global milk contamination caused by xenobiotic chemicals and environmental contaminants including toxic metals, mycotoxin, and dioxin that are fed to cattle (Seyed and Ebrahim, 2012). Consumption of this tainted milk serves as an additional source of exposure to heavy metals (Ruqia et al., 2015). Industrial or domestic effluents, combustion, bushfires, the breakdown of chemical fertilizers and pesticides, among other things, are the main sources of metal exposure for humans (Degnon et al., 2012). Overexposure to heavy metals can result in a variety of adverse health effects, including cancer (Bushra et al., 2014), hepatotoxicity, neurotoxicity, vomiting, a decline in intelligence quotient (IQ), Alzheimer's disease, behavioral disorders, and more (Ahmad et al., 2011). In nature, heavy metals cannot degrade; therefore, they accumulate in food chains through bio-transformation, bio-accumulation, and biomagnification (Aslam et al., 2011). Because the lipophilic contaminants will make their way into the permanent fat molecules, where heavy metals cannot be removed easily, complete eradication or prevention of chemical contaminants from milk is not possible (Girma et al., 2014).

In less developed nations like Bangladesh, the topic of milk contamination with heavy metals is little studied (Islam et al., 2015; Shahriar et al., 2014). Islam et al. (2015) discovered that milk intake during the research period of 2018 polluted the food chain in the vicinity of Chittagong city in Bangladesh with the elements Cr, Ni, Cu, As, Cd, and Pb. In addition, Bangladesh consumes very little milk (39.2 ml/day), compared to the 250 ml/day recommended intake (Islam et al., 2015). The yearly milk production was 1.74 million tons in 2001 and 2.28 million tons in 2007, according to a prior survey (HIES, 2011; BER, 2007). According to Jamal and Fuad (2013) estimation, milk output will rise to 4.55 million tons in the 2015–16 fiscal years. Additionally, it is believed that the country's consumer population would eventually suffer serious health risks from drinking contaminated milk and milk products due to the scenario of rising milk output.

One of the most significant problems in developing nations is the poisoning of food products by metals and other poisons. Numerous studies have been done on health risks all over the world, including those on arsenic in rice grown in Sri Lanka (Channa et al., 2015), trace metals and alfatoxin in cassava flour in west Africa (Hayford et al., 2016), metal-contaminated mushrooms in Ethiopia (Medhanye et al., 2016), as well as the health risks associated with soil and food contamination in China and India (Khan et al., 2008). (Sridhara Chary et al., 2008). However, it has been noted that poor nations, particularly Bangladesh, place less attention on consumers' long-term exposure to heavy metals from drinking cow's milk. The study has a substantial impact on Bangladesh's public health threat when taking into account the aforementioned problems.

2.6 Challenges with Pollution

Given that milk is a high supply of protein, fat, and important minerals, it is regarded to be almost a complete food (Enb et al., 2009). Along with salt, chlorine, a variety of micro elements, and even heavy metals, cow milk also contains several key elements including calcium, potassium, phosphorus, and magnesium. Metal concentrations in the air, water, and soil have increased as a result of expanded industrial and agricultural processes. Plants absorb these metals, which then build up in their tissues. The tissues and milk of animals who graze on such contaminated plants and drink from such polluted streams likewise collect such metals (Yahaya et al., 2010). A significant portion of these metals ingested by plants and animals then enter the food chain.

Concern over the intake of hazardous metals by humans has grown as a result of this pollution's constant escalation. Metals can enter the body by eating, inhalation, or skin absorption (Ogabiela et al., 2010; Ahmad, 2002). Consumption through ingestion is influenced by eating habits. Cow milk, a crucial food item ingested by people, is one of the main sources of (Farid et al., 2004). According to reports, the primary components of milk have a fairly consistent composition and go through periodic changes based on the lactation stage, the quality of the diet, and external factors, particularly chemical contaminants (Farid et al., 2004; Dobrzanski et al., 2005).

The amount of metals in cow milk has recently received a lot of attention, especially in industrialized and polluted regions of developed and emerging nations worldwide because cows that graze freely on open fields are seen to be bio-indicators of environmental pollution (Korenekovg et al., 2002; LI-Quang et al., 2009). The level of metal in uncontaminated milk is typically minimal, but by breathing contaminated air, eating contaminated food, and absorbing it through the skin, many hazardous substances, including metals and metalloids, build up throughout the food chain (Samaghail et al., 2008). Metal toxicity is dependent on a number of variables, including the specific metal in question, the dose absorbed, and the age of the individual in question.

For instance, children are vulnerable to the effect of lead exposure since they absorb many times the proportion consumed compared to adults and even brief exposure may influence developmental processes (Samara and Richard, 2009).

Because milk is mostly ingested by newborns and children, lead, cadmium, and mercury residue in milk is a serious concern. Although some metals are important industrial materials and serve a variety of biochemical purposes in all living things, their potential toxicity to people and animals is a cause for concern. As a result, it's important to keep an eye on and manage their presence in food.

The assessment of environmental quality and risk to human health are both aided by the measurement of metal levels (Farid et al., 2004; Samaghail et al., 2008). Numerous publications found heavy metals in milk and linked their presence in milk and dairy products to the production process, nursing cows' exposure to environmental pollution, their ingestion of tainted feed and water, and other factors. In the human diet, cow milk is a good source of both micro and macronutrients, and the location of the cows affects the concentration of these nutrients (Dobrzanski et al., 2005). Lead and cadmium concentrations in milk from cows grazing on open fields in Kaduna were observed to be higher than the WHO-recommended allowed daily consumption (0.05 mg/kg body weight) (Lawal et al., 2006). The requirement to immobilize metals that have been released into the environment or mobilized by human technological operations and partially lost is what currently drives the highest demand for metal sequestration.

It is well known that dissolved metals, especially heavy metals, present a major health risk when they leak into the environment. Humans are at the top of the food chain, where they accumulate in living tissues, increasing the risk. Controlling environmental heavy metal emissions is therefore required. Environmental pollution became a serious issue as a result of the growth in global population and the development of industrial uses. Both liquid and solid garbage are produced by communities. The liquid wastewater is primarily made up of industrial effluent and water that residents discard after using it for a variety of purposes. Along with other contaminants, heavy metals have risen in recent years to harmful levels for the ecosystem in many areas. In recent years, there has been a lot of focus on the removal of hazardous and harmful heavy metals from water supplies, mine waters, and wastewater from industrial effluents.

Industrial wastewaters frequently contain a significant amount of heavy metals that, if discharged without sufficient treatment, would damage public health and the environment. Heavy metals are naturally occurring elements that are hazardous and are found in the earth's crust, including Cu (copper), Cd (cadmium), Ni (nickel), Pb (lead), Zn (zinc), Ag (silver), Cr (chromium), Hg (mercury), Fe (iron), Co (cobalt), and As (arsenic). They can't be destroyed or reduced to nothing. They sporadically get into our systems through food, water, and air. Some heavy metals, such as copper, selenium, and zinc, are necessary as trace elements to keep the human body's metabolism running smoothly. However, they can cause toxicity at higher amounts. For instance, drinking water contamination (such as from lead pipes), high ambient air concentrations close to emission sources, or ingestion through the food chain could all lead to heavy metal poisoning.

Table 2.2: The uses of some heavy metals and their effects on human health

Heavy metals	Uses	Health effects	Reference
Cadmium	fertilizers, mineral processing, battery manufacture, and electroplating	cancer as well as harm to the kidneys, liver, and lungs	Sharma, 1995
Chromium	mining, galvanometry, leather electroplating, metal plating, and dye manufacturing	harm to the liver, kidneys, and skin irritation	Kumer et al., 2006
Lead	Industries involved in metal plating, textiles, battery production, automobiles, and petroleum	spontaneous abortion, kidney, brain, and nervous system injury	Tunali et al., 2006
Nickel	Chemical manufacturing, metal finishing, and metallurgy industries	memory issues, tremors, an elevated heart rate, renal, and brain damage	Abia et al., 2005

2.7 Heavy metals and their effects

2.7.1 Lead

Lead (Pb) exposure accumulates over time. Lead poisoning can result in death or significant harm to the kidneys, brain, and central nervous system (Jennings et al., 1996). Common effects of Pb damage include memory and concentration issues, high blood pressure, migraines, slower growth, hearing issues, digestive issues, problems with both men's and women's reproductive systems, and discomfort in the muscles and joints. The effects of lead poisoning can last a lifetime, making lead the number one health risk to children. Lead poisoning not only retards a child's development, harms the neurological system, and results in learning difficulties, but it is also also thought to contribute to crime and antisocial behavior in kids (USGAO).

Due to the high calcium requirements for developing skeletal systems, children are particularly susceptible to lead (Esi saawa dadzie et. al., 2012). However, if large amounts of calcium (Ca) are consumed later, the lead in the bone may be replaced by Ca and become mobile. Lead that is deposited in bone is not dangerous. Lead may cause neurotoxicity, nephrotoxicity, and hypertension once it is released into the body (Salem et al., 2000). In their study, Salem et al. (2000) found a significant link between chronic disorders such renal failure, liver cirrhosis, hair loss, and chronic anemia and contaminated drinking water with heavy metals from parts of the Great Cairo Cities, Egypt.

These illnesses were reportedly linked to drinking water contamination from heavy metals as Pb, Ni, Cr, Cd, Mo, and Cu. Water contamination with copper and molybdenum caused liver cirrhosis, renal failure from lead and cadmium, hair loss from nickel and chromium, and chronic anemia from copper and cadmium. Numerous studies into these illnesses hypothesized that anomalous disease dispersion in particular regions was related to agricultural practices and industrial wastes that released harmful and hazardous substances into the groundwater, resulting in the contamination of drinking water in those locations.

2.7.2 Chromium

One of the heavy metals whose concentration in the environment is steadily rising as a result of industrial expansion, particularly the rise of the metals, chemical, and tanning sectors, is chromium (Cr) (Adeleken and Abegunde, 2011). Chromium VI and Chromium III are the two most prevalent types of the metal (Hilgenkamp et al., 2006). While chromium VI is carcinogenic, chromium III is an important component of a balanced diet for both people and animals. Chromium III shortage affects human glucose and lipid metabolism (Chernoff et al., 2005). Even though environmental chromium toxicity is uncommon, it nevertheless poses some concerns to human health since chromium can collect on the skin, dorsal spin, hair, nails, lungs, muscle fat, liver, and placenta, where it is linked to a number of medical disorders (Adeleken and Abegunde, 2011).

Lung cancer, chromium dermatitis, malignant neoplasia, and skin ulcers are some of the health consequences of exposure to chromium VI (Sarkar et al., 2005).

Bronchial asthma and nasal septal ulcers and perforations have also been documented. In their study, Sarkar et al. (2005) also showed that drinking water with chromium VI levels over the standard suggested range was linked to a fourfold rise in childhood leukemia. Adeleken and Abegunde (2011) claim that drinking water with chromium levels over 5 mg/l causes mucous membrane and skin ulcers, respiratory tract cancer, and gastrointestinal hemorrhage.

Among other things, dyes, textiles, paints, cement, leather, plastics, printing ink, cutting oils, photography materials, wood preservatives, detergents, and others are sources of chromium in the environment (Hilgenkamp et al., 2006). Rocks, liquid fuels, brown and hard coal, industrial waste, and water erosion of power plants are additional sources of chromium. According to Adeleken and Abegunde (2011), the non-biodegradability of chromium is what causes it to stay in the environment, and once combined with soil, it transforms into a variety of mobile forms before ending up in an environmental sink.

2.7.3 Cadmium

A particularly dangerous metal is cadmium. Cadmium is present in all rocks and soils, including coal and mineral fertilizers. Batteries, pigments, metal coatings, and polymers are just a few applications for cadmium. In electroplating, it is widely utilized. Effects on health Human carcinogens include cadmium and its compounds. Smokers are exposed to cadmium levels that are much greater than non-smokers. Breathing in high concentrations of cadmium can cause serious harm to the lungs.

- Extremely high consumption levels aggravate the stomach, which can result in vomiting and diarrhea.
- Long-term exposure to low levels causes a buildup in the kidneys, which may develop to renal disease. It also damages the lungs and weakens the bones. Regulated boundaries.
- According to the EPA, drinking water contains 5 parts per billion (ppb) or 0.005 parts per million (ppm) of cadmium.
- According to the Food and Drug Administration (FDA), the content in bottled water should not be higher than 0.005 ppm (5 ppb).
- For an 8-hour workday and a 40-hour work week, OSHA recommended an

average of 5 micrograms per cubic meter of working air.

2.7.4 Nickel

Human exposure to surroundings that are heavily nickel (Ni) contaminated results in a number of pathologic consequences. Agricola was the first person to notice the lung-damaging effects of nickel in the 16th century. Nickel carbonyl exposure was linked to a few fatal cases, and by the early 1930s, nickel was known to induce contact dermatitis. Workers exposed to nickel also experienced increased rates of lung and nasal cancer (Sunderman et. al., 1988; Seilkop and Oller, 2003). Nickel renounced the dishonorable title of "Allergen of the Year" in 2008. (Gillette et al., 2008). The doctor claims that nickel allergy is still on the rise and that it cannot be only attributed to trendy body jewelry and medical gadgets made of nickel (like endoprostheses and coronary stents).

These investigations raised awareness of the effects of nickel on human health (Sivulka et al., 2005). Nickel can also attach to histidine and 2-macroglobulin (Glennon and Sarkar, 1982; Kasprzak et al., 2003), and in this form, it is disseminated throughout the tissues in addition to albumin, which is the primary transport protein of nickel in blood. Additionally, a significant number of nickel-binding proteins were described, including 1-antitrypsin, 1-lipoprotein, and prealbumin (Nielsen et al., 1994). The bone, lung, kidney, liver, endocrine glands, and brain often have the highest Ni amounts. Nickel can also be discovered in breast milk, saliva, hair, and nails. Rodents have been used to demonstrate nickel transplacental transfer. Nickel is eliminated in the urine, feces, bile, and perspiration rather than building up inside the body (Valko et al., 2005).

Nickel allergy manifested as contact dermatitis, cardiovascular and lung fibrosis, kidney disorders, and respiratory tract cancer is just a few of the harmful health impacts that nickel compounds can have on people (Oller et al., 1997; Mcgregor et al., 2000; Seilkop and Oller, 2003). Acute health impacts typically emerge from short-term exposure to high concentrations of pollutants, but chronic noncancerous health effects may occur from long-term exposure to relatively low concentrations of pollutants. Various clinical signs are how it shows up in people (nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness, and cough).

The most frequent reaction to nickel exposure at the point of contact is skin rash. Human allergic dermatitis can be brought on by skin contact with metallic or soluble nickel compounds. People at work and outside of it are affected by this health issue brought on by nickel exposure. According to studies using data from some authors, women are more likely to get dermatitis. This may be because they come into touch with nickel-containing objects more frequently, such as jewelry, buttons, watches, zippers, coins, some shampoos, pigments, and detergents (Vahter et al., 2002; SzczepaniaK and Prokop, 2004).

According to statistics, 2% of males and 10% of women in the population are extremely sensitive to nickel. Direct and persistent skin contact with objects that emit nickel ions typically results in metal sensitivity. Additionally, it was shown that the combination of the conventional carcinogen UV radiation (UVR) and nickel (II) chloride had a synergistic effect on the development of skin cancer in Skh1 hairless mice (Uddin et al., 2007). According to Uddin et al2007 .'s research, environmental metals like arsenic, chromium, and nickel may pose a co-carcinogenic risk to humans that may be more serious than the risk posed by the metals alone because humans are exposed to both UVR from sunlight and nickel through environmental exposure.

2.8 Cadmium, lead, nickel, and chromium levels in milk

According to Ahmad and Goni (2010), all milk samples tested positive for cadmium, lead, nickel, and chromium, with 100% prevalence in both India and

Bangladesh (lead: 0.05 mg/L, nickel: 0.01 mg/L, and copper: 0.005 mg/L). Another study revealed that there was 100% prevalence of nickel in milk in India, with 30% of the samples above the standard level (0.01 mg/L) (Reddy et al., 2012).

2.9 Risk factors for presence of cadmium, lead, nickel and chromium in milk

Cities, industrial areas, and agricultural operations (such as the use of heavy metals as fertilizers or medicines) have all been identified as risk factors for the occurrence of high concentrations of heavy metals in milk. Lead in milk may be brought on by transhumance along roads and/or motorways, polluted fodder, climatic elements such gusty winds and the usage of pesticide compounds, contaminated pasture with industrial effluents, and mining areas. According to Solano (2007), the biggest sources of heavy metals in the soil and water in Samburu County, Kenya, are cattle rustling, military trainings that resulted in heavy use and disposal of weapons, a growing population without adequate sewerage facilities, an increase in the number of aging automobiles, and fertilizers from the wheat and barley farms in the highlands. The second-largest and fastest-growing city in Bangladesh is Chattogram. Industrial activities, population expansion, agricultural practices, other manufacturing, industrial effluents, and oil and gas are discharged in the river known as the Karnaphuli due to the city's rapid growth, and in the early days, pollution was never even noticed in this healthy city. The majority of Bangladeshi companies along riverbanks lack efficient effluent treatment facilities, resulting in the discharge of toxic wastewater that hasn't been properly treated into rivers, which causes heavy metal contamination. Additionally, oil tankers capsized and sank at a jetty in the port of Chittagong, Bangladesh, the country's major port city, on the Karnaphuli River.

This is also largely to blame for the harmful heavy metal contamination of milk in the Cgattogram region. In Bangladesh, no previous attempts have been made to investigate factors related to the presence of heavy metals in milk. In addition, poor planning, particularly in Chittagong City, has led to overpopulation and a lack of a sufficient sewerage disposal system for waste, which could result in the contamination of milk and milk products with heavy metals. Therefore, it is appropriate to carry out the current investigation to identify the risk variables that affect the high prevalence of heavy metals in milk. 2018 (Rehman et al.)

2.10 Advantages of Atomic Absorption Spectrophotometer (AAS):

The main advantages of AAS are that it is relatively inexpensive and easy to use, while still offering high throughput, quantitative analysis of the metal content of solids or liquids. This makes it suitable for use in a wide range of applications. The specific advantages of AAS are given below:

- Simple & cheap instruments
- Low operating cost
- Very good precision
- Require less operator skill

Chapter 3 Materials and Methods

3.1 Study Area

Different dairy farms and super markets are belonging to Chittagong city area were selected for the current study. The selection of study area depends on the purpose of the research, convenient of collection and analyze of the sample Sampling locations are shown in Fig. 3.1 and the sampling conditions are illustrated in Table 3.1. Standard procedures were used to analyze the parameters of the milk sample. All laboratory works were performed at Chattogram Veterinary and Animal Sciences University and Bangladesh Council of Scientific and Industrial Research (BCSIR).

Figure 3.1: Location of the Study Area and the Sample Points

3.2 Study period

The study had conducted from September to December, 2021 and the study sites were purposively selected for collecting the milk and milk products samples.

3.3 Samples collection

Raw milk, pasteurized milk and powdered milk sample had collected from different dairy farms and super markets of Chattogram metropolitan area. 1500 mililiter polypropylene bottles were utilized for milk collection. All bottles had been cleaned with diluted acid, followed by distilled water, then oven-dried before being used for sample collection. Raw milk samples were placed in plastic bottles and transported to the lab at a freezing temperature.

Five different brands of pasteurized milk and full-cream powder milk samples were randomly selected from several super shops in the Chattogram metroplitan region. The sample vials had been carefully labeled with the date and the sampling location before being quickly chilled to 3° to 4°C. Additionally, yogurt and ghee (clarified butter) samples were gathered from several local markets, preserved in sterile glass bottles and analysis was begun as soon as possible following collection. Sample containers had been kept away from sources that might contain metals.

3.4. Sample preparation

3.4.1 Digestion of samples

Microwave Digestion of powder milk, yogurt and ghee sample

Two hundred milligram of the sample was placed in the digestion container together with seven milliliters of nitric acid and one milliliter of hydrogen peroxide. A clean PTFE or glass bar had been used to gently shake or swirl the mixture. Wait until the ship has closed for 20 minutes. After that, the mixture was warmed in the microwave using the program:

	Step	**1**	**2**	**3**
Temperature Program	**T[°C]**	145	170	190
	P[bar]	40	40	40
	Power[%]	80	80	80
	Ta[min]	2	5	2
	Time[min]	5	10	15

Wait until the vessels have cooled to room temperature to prevent foaming and splashing (about 20 min). Since a significant amount of gas will be produced throughout the digesting process, the digestion vessel had to be opened cautiously under a fume hood while wearing hand, eye, and body protection. The samples were allowed to cool to room temperature after digestion. The mixture had been filtered using Whatman filer paper No. 1 and transferred to a volumetric flask. The filtrate volume had then reached the desired level when combined with deionized water. (jorem et al.,2000)

Microwave Digestion of liquid (pasteurized and raw milk) milk sample

A set of digestion tubes had been filled with 0.5gm of each sample and 10 ml of each of the concentrated inorganic acids perchloric and nitric had been dispensed into the sample tubes. To speed up the chemical reactions, 1 ml of hydrogen peroxide was added. Carefully shake the mixture or whisk it with a glass or PTFE bar. Wait until the ship has closed for 20 minutes. Use the following program to heat in the microwave that is given in previous section.

To avoid foaming and splashing wait until the vessels have cooled to room temperature (about 20 min). Carefully open the digestion vessel in a fume hood wearing hand, eye and body protection since a large amount of gas will be produced during the digestion process. At the end of digestion, the samples were allowed to cool to room temperature. Transfer the solution to a volumetric flask and filtered through Whatman filer paper No 1.Then the volume of the filtrate was made up to mark with deionized water.

3.4.2 Solid Sample preparation

Solid samples were prepared Dry ashing method. Dry ashing or oxidation is usually performed by placing the sample in an open vessel and destroying the combustible (organic) portion of the sample by thermal decomposition, normally in the presence of an ashing aid, using a muffle furnace. Typical ashing temperatures were 450 to 550°C at atmospheric pressure, and the ash residues were dissolved in 5-10% nitric acid. (Hoenig et al., 2003)

3.4.3 Techniques for Analysis

The materials were examined using an air acetylene flame and an atomic absorption spectrophotometer (Type: AA- 6200, Shimadzu, Japan). All of the spectroscopic analyses of the sample solutions and standard metal solutions were performed at the wavelengths where the highest absorptions λ max.

3.4.3.1 Atomic Absorption Spectrometry (AAS)

If a sample containing metal element is heated on high temperature, vapor is produced which contains the atoms of the same element. The quantity of energy that is absorbed by a gaseous metal atom in the form of radiation with a specific wavelength is proportional to the concentration of the metal atom in the solution. The foundation of AAS is the estimation of the radiation transmitted in such a transition (using Beer Lambert's-equation). Beer Lambert's-law states that:

$$\mathrm{Log}T^{-1} = a\ b\ c$$

Here

a = the absorptivity in grams per litre-centimetre

b = the atom width in centimeters

c = the concentration of atoms

According to the metal concentration, the AAS entails measuring the change in light intensity from beginning radiation (I_o) to final radiation (I). Logarithmic values are automatically converted to absorbance by modern instruments (Nollet et al., 2011). The AAS instrumentation is shown in Figure 3.1 below.During sample analysis all the samples were evaluated by Atomic Absorption Spectrophotometer as per protocol described by (Ahmad et al., 2010**).** Approximately 12 ml of processed samples were taken in a cup suitable to fit into the sample tray of the Atomic Absorption Spectrophotometer (AA-6200, Shimadzu, Japan). On starting the machine around 2-3 ml processed sample were automatically drawn for a single metal detection (lead/nickel/chromium).

The sample solution was drawn into an aerosol using a pneumatic analytical nebulizer, which was then used to condition the aerosol such that only the smallest droplets (less than 10 m) could penetrate the flame. The sample was then combined with the flame gases in the spray chamber. Only approximately 5% of the aspirated sample solution made it to the flame due to this conditioning process. A burner head that produced a flame that was thin and only a few millimeters deep were located on top of the spray chamber. This flame was traversed by the radiation beam along its longest axis, and the gas flow rates were altered to produce the highest concentration of liberated atoms.

In order to achieve the best sensitivity, the burner height was also adjusted so that the radiation beam went through the area of the flame with the highest density of atom clouds. The calibration curves for different heavy metals are given below:

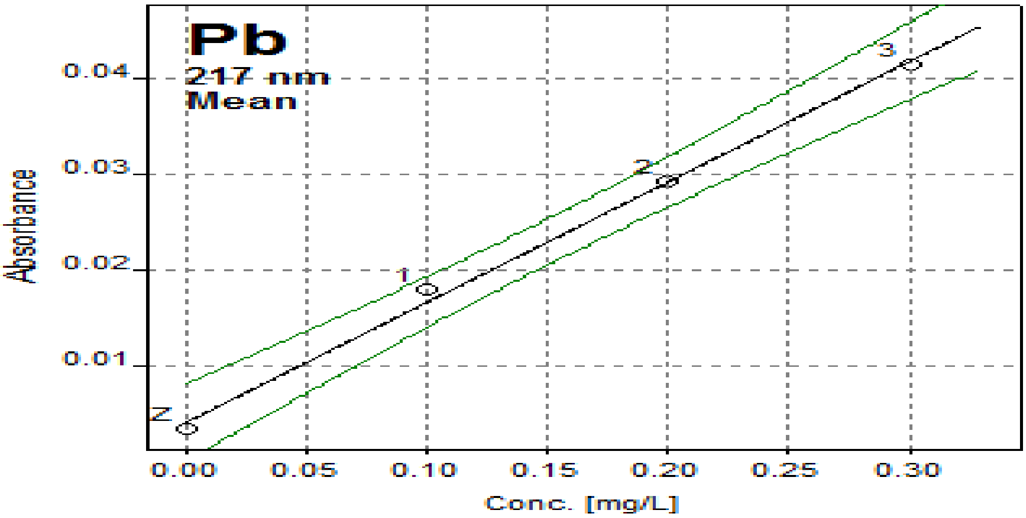

Figure 3.2: Calibration curve of Lead (Pb)

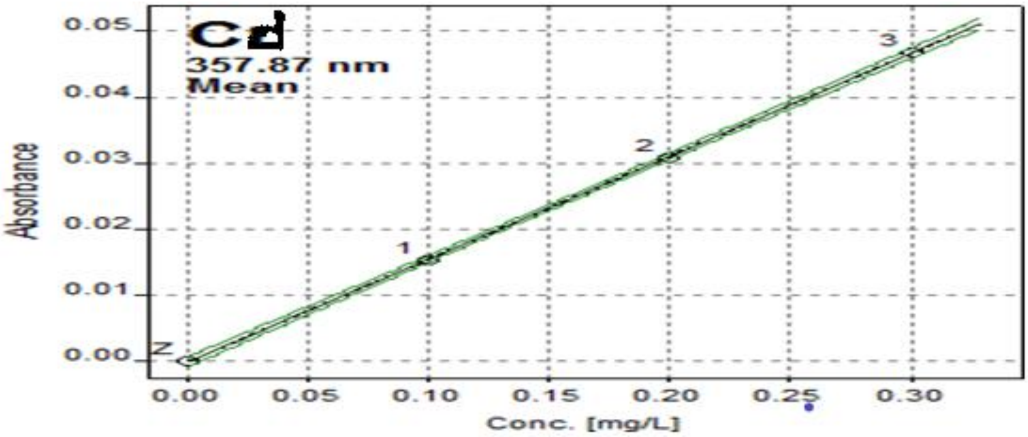

Figure 3.3: Calibration curve of Cadmium (Cd)

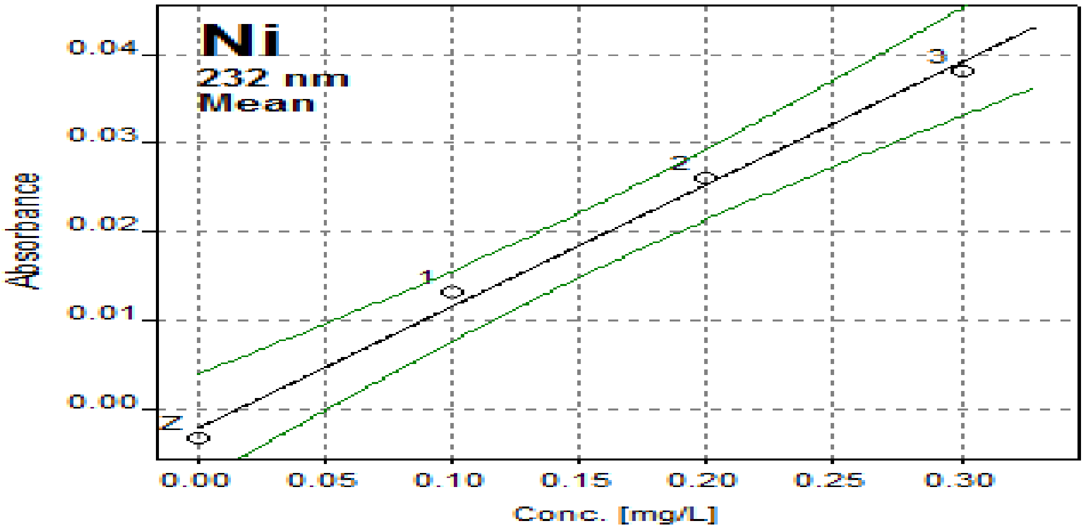

Figure 3.4: Calibration curve of Nickel (Ni)

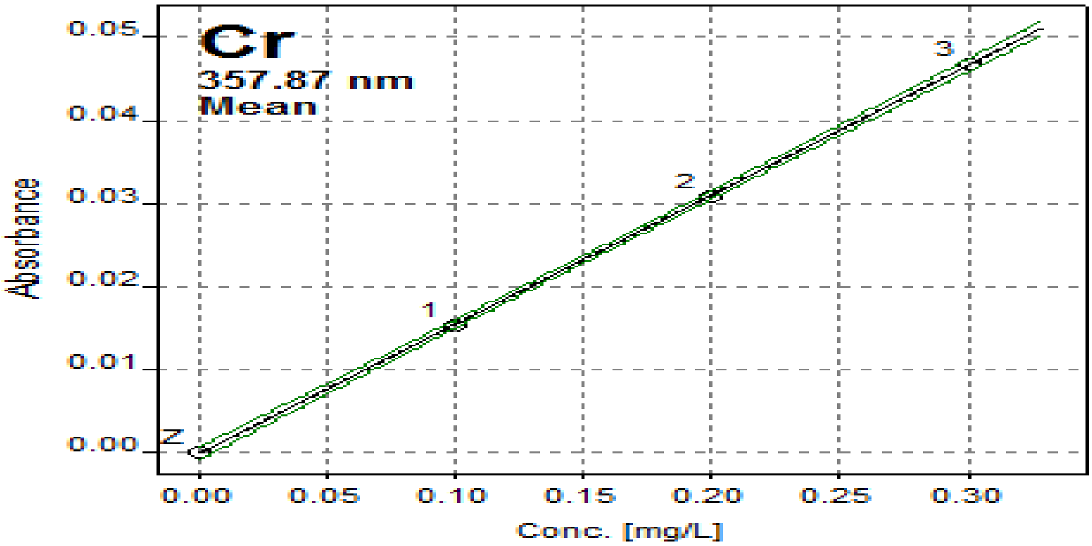

Figure 3.5: Calibration curve of Chromium (Cr)

There are the following stages in a flame's processes:

1. Desolation (drying): The solvent evaporated, leaving behind the dried sample's

nano-particles.

2. Vaporization: The gaseous molecules were created from the solid particles.

3. Atomization: The molecules separated into their individual free atoms.

4. Ionization: Atoms were partially transformed to gaseous ions depending on the ionization potential of the analytic atoms and the energy present in a particular flame. In flame atomic absorption spectrometry, a steady-state signal was produced while the sample was being aspirated, and the results were displayed on the device's screen with the metal concentrations expressed in average mg/L.

3.5 Statistical analysis

For statistical analysis, data were exported from Microsoft Excel 2007 and stored there before being entered into STATATM 11.0 (Stata Corporation, College Station, TX, USA). Utilizing percentages, means, and standard deviation for various variables, descriptive analysis was carried out. The level of heavy metal content in milk and milk products from various sites in Chattogram was lastly compared using a one-way ANOVA.The level of consequence was set $P \leq 0.05$.

Chapter 4 Results

4.1. Concentration of Pb, Ni, Cd and Cr in raw milk

In the raw milk sample, lead (Pb) and nickel (Ni) were likewise determined to be at concentrations of 0.159 mg/L and 0.126 mg/L respectively. Chromium (Cr) was at a greater concentration (0.193 mg/L) than Cadmium (Cd), which was at a lower quantity (0.022 mg/L) (Table 4.1).

Table 4.1: Concentration of Ni, Pb, Cr and Cd in Raw milk

Heavy metals	Number of sample	Concentration (mg/L) Mean ± SD	Min – Max (mg/L)	Maximum permissible limit
Pb	05	0.025±0.002	0 – 0.029	0.21
Ni	05	0.159±0.018	0 – 0.18	0.13
Cr	05	0.193±0.058	0 – 0.27	0.2
Cd	05	0.022±0.005	0 –0.025	0.046

4.2. Concentration of Ni, Pb, Cr and Cd in fresh milk of different dairy farms

When compared to raw milk from dairy farm B (0.022 mg/L), which had a lower concentration of Pb, raw milk from dairy farm E had a greater concentration of Pb (0.029 mg/L) (Table 4.2) in unpasteurized milk At a greater level (0.18 mg/L), Ni was present in dairy farm D. A lower concentration (0.136 mg/L) was found in the raw milk of dairy farm E. While Cr concentration in raw milk from dairy farm B was lower (0.136 mg/L), it was greater (0.27 mg/L) in that from dairy farm E. Dairy farm E had a greater concentration of cadmium (Cd) (0.030 mg/l), but dairy farm A's raw milk had a lower value (0.018 mg/l) (Table 4.2). The concentration of heavy metals (Pb, Ni, Cr, and Cd) in raw milk was lower than the acceptable limit, according to a comparison with the reference value (Fig. 4.1)

Table 4.2: Concentration of Ni, Pb, Cr and Cd in fresh milk of different dairy

farms

Heavy metals	Farms	Number of sample	Concentration(mg/l) Mean± SD	Permissible Value (PV)
Pb	A	03	0.025±0.009	0.21 (JECFA, 2003)
	B	03	0.022±0.005	
	C	03	0.023±0.004	
	D	03	0.027±0.004	
	E	03	0.029±0.003	
Ni	A	03	0.146±0.037	0.13 (JECFA, 2003)
	B	03	0.173±0.041	
	C	03	0.16±0.055	
	D	03	0.18±0.036	
	E	03	0.136±0.015	
Cr	A	03	0.136±0.015	0.2 (Oliver, 1997)
	B	03	0.156±0.025	
	C	03	0.163±0.025	
	D	03	0.24±0.045	
	E	03	0.27±0.052	
Cd	A	03	0.018±0.006	0.046 (JECFA, 2003)
	B	03	0.020±0.001	
	C	03	0.025±0.002	
	D	03	0.019±0.007	
	E	03	0.030±0.004	

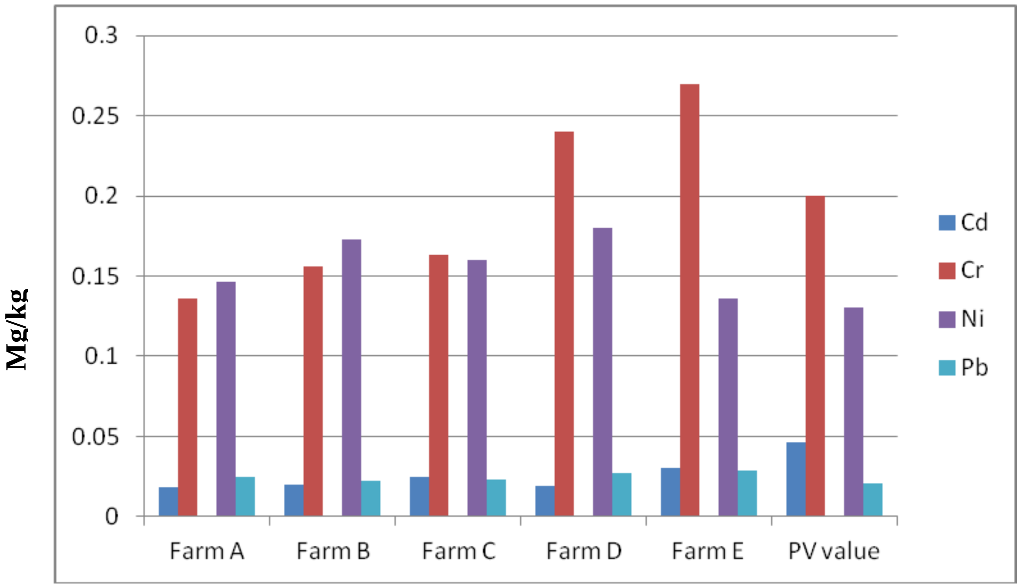

Fig 4.1.Comparison of metal concentration of raw milk with permissible (PV) value

4.3. Concentration of Ni, Pb, Cr and Cd in pasteurized milk

Cadmium (Cd) and nickel (Ni) were the two heavy metals that were identified in the highest and lowest concentrations respectively of the four. In brand-name pasteurized milk, chromium (Cr) and lead (Pb) concentrations were likewise found to be 0.177 mg/L and (0.223 mg/L) respectively. (Table 4.3)

Table 4.3: Concentration of Ni, Pb, Cr and Cd in pasteurized milk

Heavy metals	Number of sample	Concentration (mg/L) Mean ± SD	Min – Max (mg/L)	Maximum permissible limit
Pb	05	0.223 ± 0.013	0 – 0.243	0.21
Ni	05	0.291 ± 0.026	0 – 0.32	0.13
Cr	05	0.177 ± 0.009	0 – 0.19	0.2
Cd	05	0.035 ± 0.013	0 – 0.059	0.046

4.4. Concentration of Ni, Pb, Cr and Cd in different brand pasteurized milk

In comparison to other pasteurized milk samples, the brand R pasteurized milk sample had the highest lead (Pb) levels (0.243 mg/L), whereas the brand S pasteurized milk sample had the lowest (0.21 mg/L) (Table 4.4). Brand S pasteurized milk sample had the highest concentration of nickel (Ni) (0.32 mg/L), whereas brand Q and R pasteurized milk sample had the lowest concentration (0.263 mg/L). In the instance of Cr, the brand S pasteurized milk sample had a greater concentration (0.19 mg/L), but the brand T pasteurized milk sample had a lower value (0.166 mg/L). Brand P pasteurized milk sample had the highest content of cadmium (Cd) among pasteurized milk samples (0.059 mg/L), whereas brand T pasteurized milk sample had the lowest concentration (0.025 mg/L) (Table 4.4). The concentration of heavy metals (Pb, Ni, Cr, and Cd) in pasteurized milk was lower than the acceptable limit, according to a comparison with the reference value (Fig. 4.3)

Table 4.4: Concentration of Ni, Pb, Cr and Cd in different brand pasteurized

milk

Heavy metals	Brands	Number of sample	Concentration(mg/l) mean± SD	Permissible(PV) Value
Pb	P	03	0.22±0.138	0.21 (JECFA, 2003)
	Q	03	0.213±0.040	
	R	03	0.243±0.050	
	S	03	0.21±0.05	
	T	03	0.233±0.035	
Ni	P	03	0.303±0.110	0.13 (JECFA, 2003)
	Q	03	0.263±0.061	
	R	03	0.263±0.050	
	S	03	0.32±0.07	
	T	03	0.31±0.026	
Cr	P	03	0.173±0.061	0.2 (Oliver, 1997)
	Q	03	0.183±0.041	
	R	03	0.176±0.040	
	S	03	0.19±0.065	
	T	03	0.166±0.040	
Cd	P	03	0.059±0.038	0.046 (JECFA, 2003)
	Q	03	0.034±0.011	
	R	03	0.031±0.007	
	S	03	0.026±0.003	
	T	03	0.025±0.007	

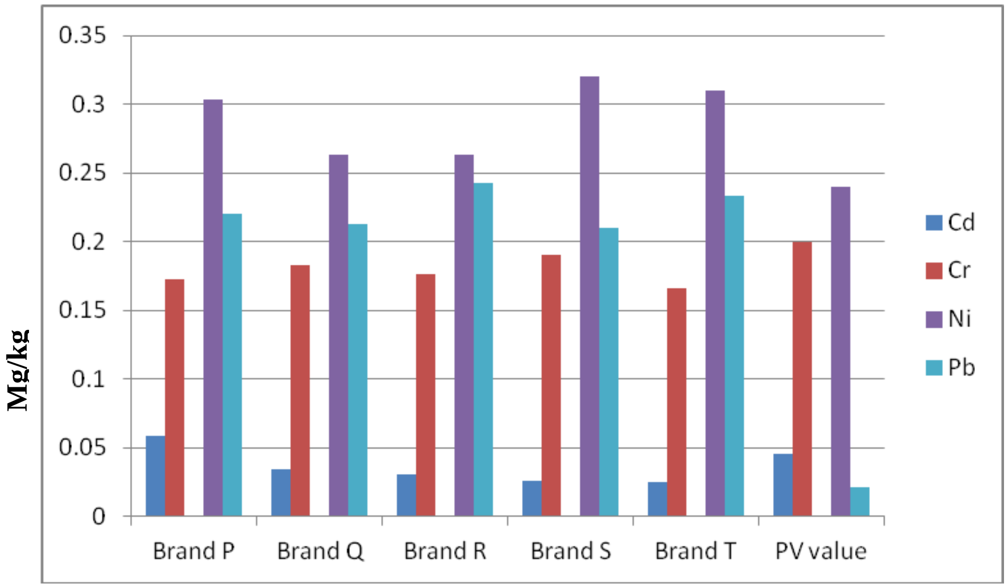

Fig. 4.2: Comparison of metal concentration of pasteurized milk with permissible (PV) value

4.5. Concentration of Ni, Pb, Cd and Cr in Powder milk

Lead (Pb) was the heavy metal with the highest concentration (0.189 mg/kg), whereas cadmium (Cd) had the lowest concentration (0.040 mg/kg). Additionally, 0.154 mg/kg and 0.280 mg/kg of chromium (Cr) and nickel (Ni) were found in brand-name powder milk, respectively. (Table 4.5)

Table 4.5: Concentration of Cr, Pb, Ni and Cd in Powder milk

Heavy metals	Number of sample	Concentration(mg/kg) Mean ± SD	Min – Max (mg/kg)	Maximum permissible limit
Pb	05	0.189 ± 0.008	0 – 0.193	0.21
Ni	05	0.280± 0.014	0 – 0.303	0.13
Cr	05	0.154 ± 0.010	0 – 0.166	0.2
Cd	05	0.040 ± 0.004	0 – 0.047	0.046

4.6. Concentration of Cd, Pb, Cr and Ni in different Powder milk

Pb levels were 0.189, 0.19, 0.176, and 0.186 mg/kg in brand-name powdered milk samples V, W, X, Y, and Z respectively. Since brand Y powder milk samples had a lower content of nickel (Ni) (0.264 mg/kg), brand Z powdered milk samples had a greater concentration (0.303 mg/kg). Nickel (Ni) levels were 0.276, 0.273, 0.286, 0.266, and 0.303 (mg/kg) in brand-name powdered milk samples V, W, X, Y, and Z, respectively (Table 4.6) In comparison to other brand powder milk samples, brand Z had the highest concentration of chromium (Cr) (0.166 mg/kg), while brand V had the lowest concentration (0.143 mg/kg). Chromium (Cr) levels were 0.163, 0.146, and 0.156 (mg/kg) in brand powder milk samples W, X, and Y, respectively. In comparison to other brand powder milk samples, brand V had the highest concentration of cadmium (Cd) (0.047 mg/kg), while brand Z had the lowest concentration (0.037 mg/kg) (Table 4.6). The concentration of heavy metals (Pb, Ni, Cr, and Cd) in powdered milk was lower than the acceptable level, according to a comparison with reference values (Fig. 4.5)

Table 4.6: Concentration of Cr, Pd, Ni and Cd in different Powder milk

Heavy metals	Brands	Number of sample	Concentration (mg/l) mean± SD	Permissible (PV) Value
Pb	V	03	0.193±0.025	0.21 (JECFA, 2003)
	W	03	0.2±0.036	
	X	03	0.19±0.055	
	Y	03	0.176±0.055	
	Z	03	0.186±0.081	
Ni	V	03	0.276±0.136	0.13 (JECFA, 2003)
	W	03	0.273±0.130	
	X	03	0.286±0.065	
	Y	03	0.266±0.075	
	Z	03	0.303±0.040	
Cr	V	03	0.143±0.032	0.2 (Oliver, 1997)
	W	03	0.163±0.025	
	X	03	0.146±0.025	
	Y	03	0.156±0.015	
	Z	03	0.166±0.032	
Cd	V	03	0.047±0.019	0.046 (JECFA, 2003)
	W	03	0.043±0.007	
	X	03	0.039±0.006	
	Y	03	0.038±0.007	
	Z	03	0.037±0.007	

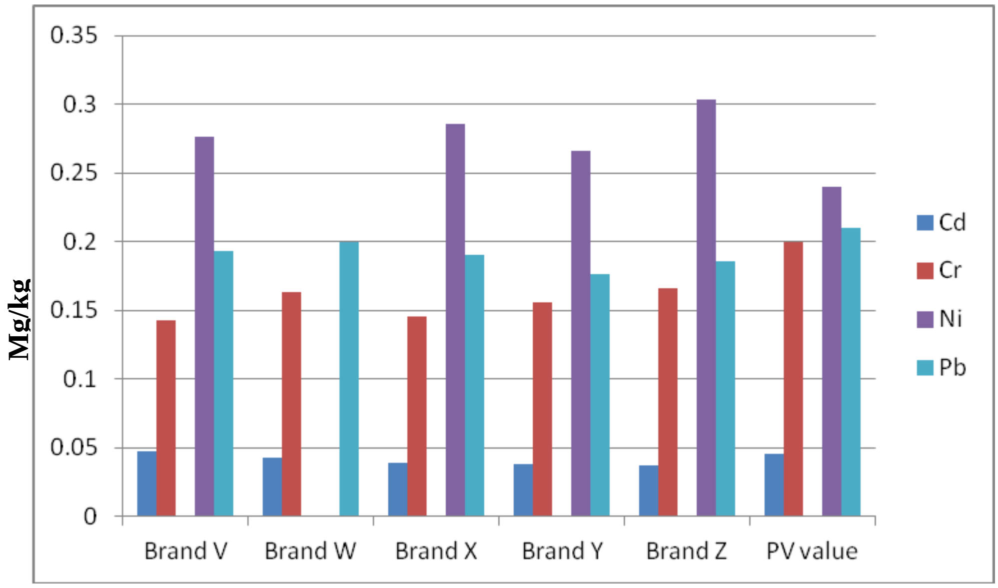

Fig. 4.3: Comparison of metal concentration of powder milk with permissible (PV) value

4.7. Concentration of Cr, Cd, Ni and Pb in Yogurt

Lead (Cr) was discovered to have the highest concentration of the four heavy metals (0.385 mg/kg), whereas lead (Pb) had the lowest value (0.010 mg/kg). Nickel (Ni) and cadmium (Cd) levels in yogurt were also determined to be (0.027 mg/kg) and (0.029 mg/kg) respectively. (Table 4.7)

Table 4.7: Concentration of Cd, Cr, Pb and Ni in Yogurt

Heavy metals	Number of sample	Concentration(mg/kg) Mean ± SD	Min – Max (mg/kg)	Maximum permissible limit
Pb	03	0.010 ± 0.005	0 – 0.011	0.21
Ni	03	0.029± 0.023	0 – 0.035	0.13
Cr	03	0.385 ± 0.235	0 – 0.46	0.2
Cd	03	0.027 ± 0.006	0 – 0.031	0.046

4.8. Concentration of Ni, Pb, Cr and Cd in different Yogurt

Branded yogurt samples C, D, and E had Pb concentrations of 0.011, 0.01, and 0.011 (mg/kg) respectively. Nickel (Ni) was present in higher concentrations in brand D yogurt samples (0.035 mg/kg) compared to brand E yogurt samples (0.025 mg/kg). Nickel (Ni) was present in brand yogurt samples C, D, and E at concentrations of 0.029, 0.035, and 0.025 respectively (Table 4.6) Brand D yogurt sample had the highest concentration of chromium (Cr) (0.46 mg/kg), whereas brand E yogurt sample had the lowest concentration (0.251 mg/kg). In brand yogurt samples C, D, and E, the amounts of chromium (Cr) were 0.445, 0.46, and 0.251 (mg/kg), respectively. When compared to other brand yogurt samples, brand C had the highest concentration of cadmium (Cd) (0.031 mg/kg), while brand E had the lowest (0.02 mg/kg) (Table 4.8). The concentration of heavy metals (Pb, Ni, Cr, and Cd) in pasteurized milk was lower than the acceptable limit, according to a comparison with the reference value (Fig. 4.7)

Table 4.8: Concentration of Cd, Ni, Pb and Cr in different Yogurt

Heavy metals	Brands	Number of sample	Concentration (mg/kg) mean± SD	Permissible (PV) Value
Pb	C	03	0.011 ± 0.006	0.21 (JECFA, 2003)
	D	03	0.01 ± 0.005	
	E	03	0.011 ± 0.005	
Ni	C	03	0.029 ± 0.024	0.13 (JECFA, 2003)
	D	03	0.035 ± 0.026	
	E	03	0.025 ± 0.021	
Cr	C	03	0.445± 0.267	0.2 (Oliver, 1997)
	D	03	0.46 ± 0.265	
	E	03	0.251 ± 0.174	
Cd	C	03	0.031 ± 0.005	0.046 (JECFA, 2003)
	D	03	0.03± 0.007	
	E	03	0.02 ± 0.005	

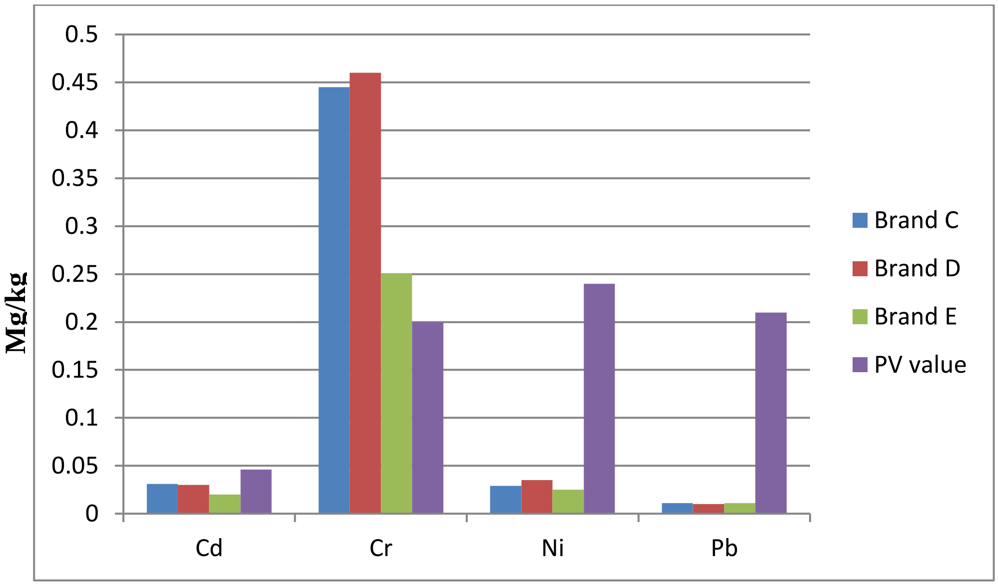

Fig. 4.4: Comparison of metal concentration of Yogurt with permissible (PV) value

4.9. Concentration of Ni, Cr, Pb and Cd in Ghee

Lead (Pb) was the heavy metal with the highest concentration (0.237 mg/kg), whereas cadmium (Cd) had the lowest concentration (0.043 mg/kg). Nickel (Ni) and chromium (Cr) concentrations in brand ghee were similarly found to be 0.130 mg/kg and 0.167 mg/kg respectively. (Table 4.9)

Table 4.9: Concentration of Cd, Ni,Pb and Cr in Ghee

Heavy metals	Number of sample	Concentration(mg/kg) Mean ± SD	Min – Max (mg/kg)	Maximum permissible limit
Pb	05	0.237 ± 0.005	0 – 0.270	0.21
Ni	05	0.130± 0.004	0 – 0.150	0.13
Cr	05	0.167 ± 0.005	0 – 0.220	0.2
Cd	05	0.043 ± 0.006	0 – 0.050	0.046

4.10. Concentration of Cr, Pb, Cd and Ni in different Ghee

Brand ghee samples L, M, and N have Pb concentrations of 0.250, 0.190, and 0.270 (mg/kg) respectively. Brand L ghee sample had a greater content of nickel (Ni) (0.150 mg/kg), whereas brand N ghee sample had a lower value (0.110 mg/kg). Nickel (Ni) levels in brand ghee samples L, M, and N were 0.150, 0.13, and 0.110 (mg/kg), respectively (Table 4.6) Chromium (Cr) concentration in the brand M ghee sample was greater (0.220 mg/kg) than in the brand N sample (0.11 mg/kg), which had a lower value. In brand ghee samples L, M, and N, the amounts of chromium (Cr) were 0.170, 0.220, and 0.110 (mg/kg) respectively. Brand M ghee sample had the highest content of cadmium (Cd) among brand ghee samples (0.050 mg/kg), whereas brand L sample had the lowest concentration (0.035 mg/kg) (Table 4.10). Heavy metal concentrations (Pb, Ni, Cr, and Cd) in ghee were lower than the acceptable level, according to a comparison with reference values (Fig. 4.9)

Table 4.10: Concentration of Cd, Ni, Pb and Cr in different Ghee

Heavy metals	Brands	Number of sample	Concentration (mg/l) mean± SD	Permissible (PV) Value
Pb	L	03	0.250 ± 0.007	0.21 (JECFA, 2003)
	M	03	0.190 ± 0.004	
	N	03	0.270 ± 0.005	
Ni	L	03	0.150 ± 0.004	0.13 (JECFA, 2003)
	M	03	0.13 ± 0.003	
	N	03	0.110 ± 0.004	
Cr	L	03	0.170 ± 0.005	0.2 (Oliver, 1997)
	M	03	0.220 ± 0.006	
	N	03	0.110 ± 0.005	
Cd	L	03	0.035 ± 0.008	0.046 (JECFA, 2003)
	M	03	0.050 ± 0.003	
	N	03	0.044 ± 0.006	

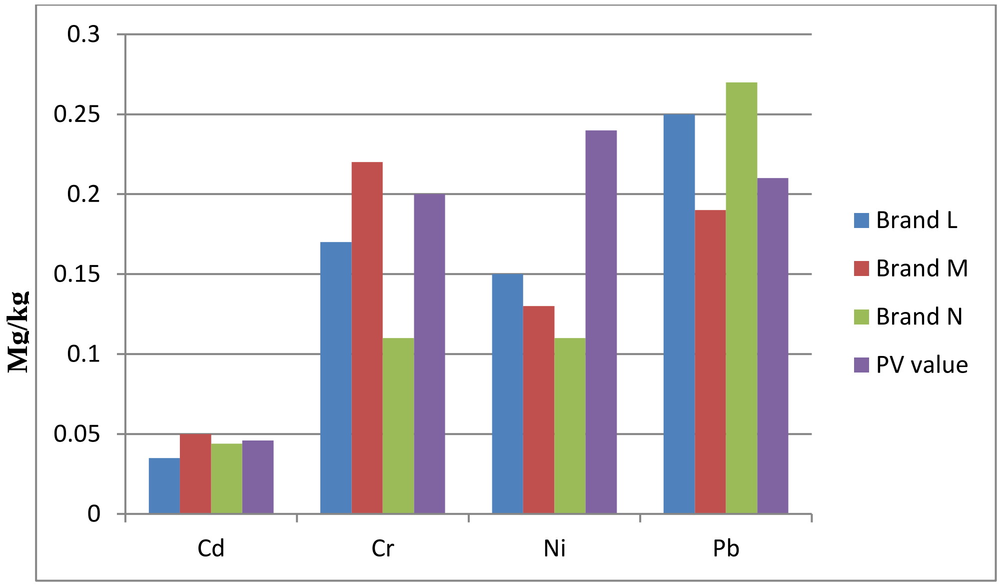

Fig. 4.5: Comparison of metal concentration of Ghee with permissible (PV) value

Chapter 5

Discussion

5.1 General

The present study was done in Chattogram and evaluates the accumulation behavior of heavy metals among different milk and milk products. The recent chemical profiles of milk and milk products in several studies indicate that the milk quality beyond the acceptable limit which becomes the serious threats to humans. In this section all important findings in the current study with its limitation, conclusions and recommendations have been discussed.

5.2 Pervasiveness of Ni in different samples

The present study demonstrated that Ni was found below the WHO standard's allowable limit of milk and milk products (WHO, 1993, 2004 and 2011) in all stations during the analysis. The findings of the study exhibits that Ni concentration varied from one farms to another has changed on the presence of industrial zones. The peak concentration of Ni was found in sample collected from brand S pasteurized milk sample (0.32±0.07 mg/L) and the relatively low in brand E yogurt sample (0.025±0.021 mg/L). This trend indicates that the areas with heavy industrialized zones having higher Ni contamination than that of low industrialized zones. In general, the sources of nickel come from urea fertilizer industry as a catalyst during the breakdown on methane gas. So urea fertilizer and their waste contain nickel at a certain level. By the use of urea fertilizer and disposal of waste from fertilizer industry pollute the environment. This statement is helped by many other studies (Klan et al., 2011; Pirsaheb et al., 2013).

5.3 Pervasiveness of Cd in different samples

The overall prevalence of Cd was found the result in every sample. The peak concentration of Cd was found in sample collected from brand E yogurt sample (0.02±0.005 mg/kg) and the relatively low in brand M Ghee sample (0.050±0.003 mg/kg). This high concentration of Cd is due to the presence of industrial wastes, Leaded fuel and other human-made sources in Chattogram area.

The same finding is stated by (Beavington et al., 2004; Fernandes et al., 2000; Khillare et al., 2004; Al-Masri et al., 2006). The findings of the study exhibits that Cd concentration varied from one farms to another has changed on the presence of industrial zones.

5.4 Pervasiveness of Pb in different samples

The highest permitted amount of lead in diet is 1–5 mg/kg, and the recommended daily allowance for lead is 0.3 mg (IAEA, 1980). Lead exposure is known to have negative effects on children's cognitive development and intellectual ability as well as adults' blood pressure and risk of cardiovascular disease (Anonymous, 2005). According to the study's findings, milk contains 0.228–0.290 mg/kg of lead. Lead bioaccumulation over a lengthy period of time is indicated by the muscle's high lead content. The amount of lead in this study was higher than the FAO/WHO limit of 0.5 mg kg^{-1}.

The main causes of the high Pb levels in milk products are contaminated diets and water sources. Lead in milk was found at mean levels of 3.15 mg/kg^{-1}, according to Mariam et al. (2004). These numbers were substantially higher than the levels discovered in this investigation.The permissible limit of lead in milk is 0.21mg/kg (Zmudzki and Szkoda, 1996).The result showed that lead concentration of raw milk and pasteurized milk were 0.193-0.20 mg/kg and 0.243-0.21mg/kg. It was revealed that from this study more milk samples contain higher amount of lead but minimum sample contain lower amount. Meluzzi et al, (1996) reported 0.315 ppm Pb in raw milk which is lower than our analyzed results.

The majority of milk contaminants, including heavy metals, are known to be more soluble and addictive in a milieu rich in lipids and rapidly oxidable, favoring the chelation of highly reactive metallic ions inside the organic catena of phospholipids (Bargellini et al.,2008). Therefore, values published in the literature for raw milk were lower than our findings, including 0.12ppm Pb (Bargellini et al., 2008), 0.036ppm Pb (Polonis and Dmoch, 2007), and 0.397ppm Pb (Meluzzi et al., 1996). It could be because the greater ionic radii of Ca^{+2} have prevented Pb from migrating across raw milk.

An overview of the latest research on the transfer of harmful metals from feed to milks by Kan and Meijer (2007) is provided. In trace amounts, chromium contributes significantly to how the body works (insulin's metabolic co-factor), but when levels are exceeded, it becomes poisonous.

5.5 Pervasiveness of Cr in different samples

According to estimates, adults need between 0.02 and 0.5 mg of chromium each day. The mineral chromium (Cr) is necessary for the body's utilization of sugar, protein, and fat while also having carcinogenic effects (Institute of Medicine, 2002). Chromic acid in its trivalent form is thought to be necessary for healthy lipid and carbohydrate metabolism (NRC, 1980). In nature, Cr (III) is found in abundance in the soil, water, air, and biological components. The type of chromium that causes the most worry, Cr (VI), is carcinogenic to humans. Dermatitis, allergic and eczematous skin reactions, skin and mucous ulcerations, perforation of the nasal septum, allergic asthmatic reactions, bronchial carcinomas, gastro-enteritis, hepatocellular deficiency, and renal oligo-anuric deficiency are among the most significant toxic effects following contact, inhalation, or ingestion of hexavalent chromium compounds (Baruthio et. al., 1992).

The maximum amount of chromium that can be found in raw milk and milk products is 0.2 mg/kg. The investigation therefore revealed that the concentration of chromium is higher in some samples and lower in some milk samples. In comparison to Hui's measurements, ours for Cr content in milks were higher. According to Iwegbue et al. (2006), the permitted limit was exceeded by the Cr concentration in raw milk, which ranged between 0.10 and 0.20 $mgkg^{-1}$. The results of our investigation agree with those of Akan et al. (2010), who discovered increased content in raw milk (0.29 ppm).

Chapter 6

Conclusion

The assessment of the heavy metal content of milk and milk products from Bangladesh is part of the current study. The general public's health is at danger from heavy metals like Cr, Pb, Cd, and Ni. Chromium (Cr) concentration in raw milk samples in the current investigation was greater than nickel (Ni) concentration in brand Z powder milk (0.303 mg/kg). According to the findings, brand R pasteurized milk (0.243mg/L) had the highest Pb levels among the brand-name pasteurized milk samples. Through tainted milk and powdered milk, they enter the human food chain. To establish the standard allowable limits for heavy metals in all forms of consumable milk and dairy products, more research is required. This demands that every effort be made, including raising awareness and conducting efficient surveillance as well as checking for the presence of heavy metals in products generated from animals. Additionally, the present findings may be helpful to dairy scientists, veterinarians, and farmers. Therefore, it can be said that the current results will greatly aid in raising public awareness of health issues. Last but not least, the current study will help us understand the amount of heavy metals in milk and milk products.

This study measured the levels of heavy metals in milk and milk product samples from various Chattogram locations. Cu was found in the highest concentration of the measured heavy metals, and Pb was found in the lowest concentration. The results suggested that consuming these samples of milk and milk products posed no possible non-carcinogenic or carcinogenic health hazards. The results of this study will assist consumers in understanding any potential health hazards associated with consuming milk and milk products. The presence of certain heavy metals in the milk samples may be the cause of their presence in the samples of milk products. To lessen the contamination from heavy metals, proper rules should be upheld. Cattles should be fed clean food and fresh water. The grasses that grow in the contaminated areas shouldn't be fed with catstles. Industrial wastewater needs to be handled adequately. Additionally, it is important to routinely check samples of milk and yogurt used for human consumption to ensure the absence of any hazardous metals and the availability of vital nutrients.

Chapter 7 Recommendations and Future perspectives

On the basis of the presence of heavy metals in milk and milk products of various selected sites in Chattogram district, Bangladesh, this chapter offers the following recommendations and future perspectives:

- The study was conducted on one occasion only in the winter season. So, further investigations to be carried out at different seasonal periods to check the seasonal variation of heavy metals concentration in milk.
- It's important to limit the sources of heavy metals in milk products, such as cattle meals and utensils used in processing. The sources of several heavy metals, including lead, zinc, and cadmium, which have been found in significant concentrations in milk and milk products, are known to be cattle diets and equipment.
- This investigation found higher concentration of metals in the industrial sites. So, further studies should continue for monitoring of the milk quality around these sites.
- This present research reveals that the areas with higher industrial zones show relatively higher concentration of heavy metals than that of lower industrial sites. Therefore, further investigations should be carried out with higher industrial zones to monitor the chemical profile of milk.
- To determine their contamination, a further investigation should be conducted that includes metals such as copper, arsenic, mercury, etc. This is because the equipment used to prepare milk products may contain certain metals.
- Similar researches to this present one are carried out with other important

industrial sites in Bangladesh to establish correlation of heavy metal contamination between the Chattogram district and other industrial sites of Bangladesh.

- Further investigations on the bioaccumulations and effects of heavy metals, and other persistent organic and inorganic pollutants on animals to understand the effects of pollutants on zoological environment.
- In order to do this, it is necessary to keep researching and monitoring the raw milk at industrial sites.
- People need to be aware of the dangers posed by the contamination of milk and milk products with heavy metals.

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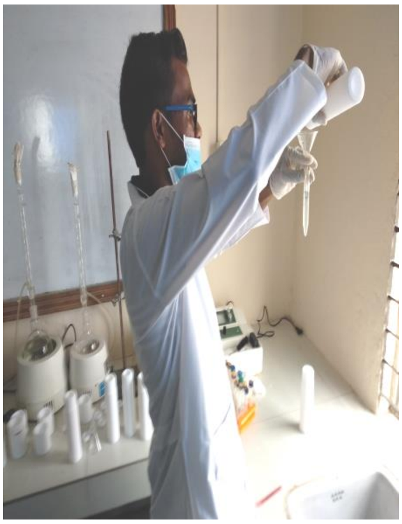
APPENDIX

Picture Gallery

Weighing sample

Addition of nitric acid



Prepared sample

AAS reading

BRIEF BIOGRAPHY

Md. Laysur Rahaman passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. Md. Laysur Rahaman obtained his B.Sc. (Hons.) in Food Science & Technology in 2017 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Department of Applied Chemistry and Chemical Technology in Food Chemistry and Quality Assurance under Food Science & Technology Faculty, CVASU. He has immense interest to work in food safety issues including food chemistry, quality assurance, food quality control, environmental chemistry, product development and processing, malnutrition, reduction of nutritional changes in food processing etc.