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Investigation of Trace Elements Present in Local Milk-Based Sweetmeat: A Way to Verify the Food Adulteratio

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INVESTIGATION OF TRACE ELEMENTS PRESENT IN LOCAL MILK-BASED SWEETMEAT: A WAY TO VERIFY THE FOOD ADULTERATION

A thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy

BY

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This Thesis is Dedicated to My Beloved Parents

I declare that this thesis entitled **“INVESTIGATION OF TRACE ELEMENTS PRESENT IN LOCAL MILK-BASED SWEETMEAT: A WAY TO VERIFY THE FOOD ADULTERATION”** is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not currently submitted in candidature of any other degree

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Date :

“I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Doctor of Philosophy”

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Date :

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ABSTRACT

Dairy products are an important source of many nutrients and micronutrients in the diets of children and adults both. Considerably large amount of milk-based sweetmeats, produced in *Rajshahi* metropolitan city (area about 100 km^2) in Bangladesh, are being consumed regularly by the city dwellers. An attempt was made to investigate the physical quality, as well as the level of Ca and several trace elements e.g. Zn, Cu, Pb, Cd, Cr, Co, Mn, and As, for some common and popular indigenous local sweetmeats. Most of the analyses are done by Atomic absorption spectrophotometer, in which detection limits were obtained from fractional ppb (0.51 ppb for Arsenic) to as high as 71 ppb (for Lead). To justify the quality of products, the levels of the measured elements and other physical parameters were compared with the data from fresh milks and referred values for similar materials. Usually, levels of each mineral are expressed in terms of fresh weight of two selected milk-based sweet items considered here as a standard serving in a meal.

The levels of Ca and Cu were found more or less justifiable in relation with its level in liquid milk equivalent. However, amounts of Zn and Co, two important micronutrients, were found significantly lower in all sweetmeats compared to the certified reference values. On the contrary, Cr, Pb, Cd and As contents were found considerably higher, and interestingly the levels of As and Pb were well above of any other standard permissible limits.

A tentative conclusion was made that milk-based consumables are possibly adulterated with some inferior ingredients which would be the sources of those higher levels of toxic metals, and its consequences in public health are addressed in relation with scanty amount of per capita health expenditure of the country. Eventually, it is also assumed that chemists could put an important role in this direction by analyzing regularly more and more liquid milk and milk-based food items in their routine analysis by setting a well-equipped analytical laboratory, so that they can assure the standards of any consumables for the people of the country.

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LIST OF SYMBOLS

$	-	Dollar (USA)
µg	-	Microgram
µL	-	Micro Liter
μL^{-1}		Micro $Liter^{-1}$
µS	-	Micro Simen
μgd^{-1}		Microgram day^{-1}
μgg^{-1}		Microgram g^{-1}
µM		Micro Molar
AAS	-	Atomic Absorption Spectrophotometer
ADB	-	Asian Development Bank
AI	-	Adequate Intake
ALA	-	Alpha Linolenic Acid
As	-	Arsenic
ATP	-	Adenosine Tri Phosphate
Avg.	-	Average
B	-	Beliful
BBS	-	Bangladesh Bureau of Statistics
BC	-	Birth of Christ
BLITZ	-	Online news media
BP	-	Beliful Parasandesh
bp	-	Boiling point
BR	-	Beliful Rosogolla/Rosgolla
Ca	-	Calcium
CAB	-	Consumer association of Bangladesh
Cd	-	Cadmium
CE	-	Capillary electrophoresis
CHD		Coronary Heart Disease
cm	-	Centimeter
Co	-	Cobalt
Cr	-	Chromium

Cu	-	Copper
DDI		Daily Dietary Intake
DF	-	Degree of Freedom
DL	-	Detection Limit
EC	-	Electrical Conductivity
EDTA	-	Ethylene diamine tedra acetic acid
EPA		Environmental Protection Agency
FAO	-	Food and Agriculture Organization
g	-	gram
GC	-	Gas chromatography
GDP	-	Gross Domestic Product
h		hour
HEU		Health Economics Unit
HPLC	-	High performance liquid chromatography
HTST	-	High Temperature Short Time
K	-	Kelvin
Kg	-	Kilogram
L	-	Liter
LC	-	Liquid chromatography
LME	-	Liquid Milk Equivalent
LOD	-	Limit of Detection
LOL	-	Limit of Linearity
LOQ	-	Limit of Quantitation
M	-	Molar
MB	-	Mistibari/Misti Bari
MBP	-	Mistibari/Misti Bari Parasandesh
MBR	-	Mistibari/Misti Bari Rosogolla/Rosgolla
MBz	-	Mithaibazar/Mithai Bazar
MBzP	-	Mithaibazar/Mithai Bazar Parasandesh
MBzR	-	Mithaibazar/Mithai Bazar Rosogolla/Rosgolla
MC	-	Moisture content
MDI		Maximum Daily Intake
mg	-	milligram

Mg	-	Magnesium
mgd^{-1}		milligram day^{-1}
min	-	Minute
mL	-	Mili Liter
mM	-	Mili Molar
MMT	-	Million Metric Tone/Tones
Mn	-	Manganese
MOHFW		Ministry of Health and Family Welfare
mp	-	Melting point
MS	-	Milk Solid
MS	-	Mass spectrometry
mS	-	mili Simen
MT	-	Metric Tone/Tones
MV	-	Mistanno Vandar
MVP	-	Mistanno Vandar Parasandesh
MVR	-	Mistanno Vandar Rosogolla/Rosgolla
MW	-	Molecular weight
NAD	-	Nicotinamide Adenine Dinucleotide
NFMS	-	Non Fat Milk Solid
ng	-	nano gram
nL	-	Nano Liter
nm	-	Nano Meter
NRC	-	National Research Council
P	-	Parasandesh
Pkt	-	Packet
ppb	-	parts per billion
ppm	-	parts per million
R	-	Rosogolla
RDA	-	Recommended Daily Allowance
s	-	Second/Seconds
S	-	Simen
SD	-	Standard Deviation
SD		Standard Deviation

SE	-	Standard Error
SE		Standard Error
-SH	-	Thio group
SNF	-	Solid Not Fat
SS	-	Sum of Squares
Std	-	Standard
TFW	-	Total Fresh Weight
THE	-	Total Health Expenditure
TMS	-	Total Milk Solid
TS	-	Total Sugar
UHT	-	Ultra High Temperature
UK	-	United Kingdom
UNICEF	-	United Nations International Children's Emergency Fund
USA	-	United States of America
USDA		United States Development Authority
UV	-	Ultraviolet
VIS	-	Visible
WHO	-	World Health Organization
XRF	-	X Ray Fluorescence
y^{-1}	-	per year
Zn	-	Zinc

Chapter One
Introduction and Hypothesis

Part I: General background

1.1 Short history about milk

Milk is as ancient as mankind itself, as it is the substance created to feed the mammalian infant. All species of mammals, from man to whales, produce milk for this purpose. Many centuries ago, perhaps as early as 6000-8000 BC, ancient man learned to domesticate species of animals for the provision of milk to be consumed by them [1]. These included cows, buffaloes, sheep, goats, and camels, all of which are still used in various parts of the world for the production of milk for human consumption [1]. As a child we need it for growth and as a grown up we need it for replenishing our daily nutrition.

Technological advances have only come about very recently in the history of milk consumption, and our generations will be the ones credited for having turned milk processing from an art to a science [1]. The availability and distribution of milk and milk products today in the modern world is a blend of the centuries old knowledge of traditional milk products with the application of modern science and technology [1]. However, technologies (e.g., processing) have been developed to transform milk into its various consumer products, including beverages, fermented products, concentrated and dried products, butter and ice cream. Fermented products such as cheeses were discovered by accident, but their history has also been documented for many centuries, as has the production of concentrated milks, butter, and even ice cream [1].

1.2 Essentiality of milk as food

Milk was our very first food and played a major contribution to the human diet irrespective of any nationalities, creeds, or clans across the world. If we were fortunate it was our mother's milk, a loving link. If not mother's milk it was cow's milk or "formula". But the bulk materials of “formula” are obtaining from cattle’s milk, that recent melamine-related-horror is sufficient to understand that link [2].

Milk and dairy products have become a major part of the human diet in many countries. Milk has a wide range of positive nutritional benefits and supplies a variety of nutrients including protein for body building, vitamins, minerals, fat and carbohydrate for energy. The contribution of milk makes to individual diets will vary from country to country [3-4]. Variability also found between humans in their ability to absorb minerals present in milk.

There is also a great variety of complexity of foods which can affect the absorption of each nutrient called bioavailability. Tannins, for example, which are found in tea, and phytates from cereals can interfere with iron and other trace metals absorption. It is believed that casein from milk may inhibit the iron-chelating properties of tannins and phytates. Therefore the use of milk in tea and on cereals can improve bioavailability of trace metals not only originating in milk but also from other foodstuffs [5].

1.3 World milk production

Over the 280 million dairy cows in the world produce over 400 million tones of milk worth an estimated US$ 110 million y^{-1} [3]. Cow’s milk represents about 90.8% of the world milk production with buffalo, sheep and goats producing 6%, 1.7% and 1.5% respectively (see Table 1.1). Consumption statistics show that about 94% of the world milk supply is utilized as processed milk or milk products [6].

Table 1.1: World production of milk

Species	Million Tones	% of World Total
Cow's milk	427.9	91.06193
Buffalo's milk	27.2	5.788466
Sheep's milk	7.6	1.617365
Goat's milk	7.2	1.532241
Total	469.9	100

The following table (Table 1.2) shows the quantity of raw Cow's milk produced (in 2006) in leading countries of this kind around the world [2]. The production in the United States is tremendously high compared to any other countries in that list. Interestingly, being developing countries, production in India and China is considerably high among the rest.

Table 1.2: Cow's milk production (in million tones) in selected countries (2006)

Country	Milk Production (Million Tones)
United States	82.462
India	39.759
China	31.934
Germany	27.955
France	24.195
New Zealand	15.000
United Kingdom	14.359
Italy	11.186
Netherlands	10.995
Australia	9.550
Canada	7.854
Total (11 countries)	275.249 (64.32% of total world production)

Source: International Dairy Federation, Bulletin (2007)

To show the severity of the problem that would have been occurred through poor milk consumption, it is noteworthy to explain how milk is important for one's health throughout in his/her life. Therefore, detailing of milk composition is felt necessary and is putting in the following sections.

1.4 World milk consumption

The role of milk in the traditional diet has varied greatly in different regions of the world. The tropical countries have not been traditional milk consumers; therefore, they are not appeared in the Table. Whereas the more northern regions of the world, Europe (especially Scandinavia) and North America (e.g., United States of America, USA), have traditionally consumed far more milk and milk products in their diet. In tropical countries where high temperatures and lack of refrigeration has led to the inability to produce and store fresh milk, milk has traditionally been preserved through means other than refrigeration, including immediate consumption of warm milk after milking, by boiling milk, or by conversion into more stable products such as fermented milks [7-9].

Therefore, the total milk consumption (as fluid milk and processed products) per person varies widely from highs in Europe and North America to lows in Asia. However, even within regions such as Europe, the custom of milk consumption has varied greatly. Consider for example the high consumption of fluid milk in countries like Finland and Sweden compared to France where cheeses have tended to dominate milk consumption. Table 1.3 illustrates milk per capita consumption information from various countries of the world [2].

Table 1.3: Per capita consumption of milk and milk products in various countries (2005/2006) [2]

Country	Liquid Milk Drinks (L)	Cheeses (kg)	Butter (kg)	LME (Cheeses + Butter)	Total LME
Finland	183.9	19.1	5.3	101.26	285.16
Sweden	145.5	18.5	1.0	80.925	226.425
Ireland	129.8	10.5	2.9	55.61	185.41
Netherlands	122.9	20.4	3.3	98.355	221.255
Switzerland	112.5	22.2	5.6	115.37	227.87
United Kingdom (2005)	111.2	12.2	3.7	65.985	177.185
Australia (2005)	106.3	11.7	3.7	63.91	170.21
Canada (2005)	94.7	12.2	3.3	64.325	159.025
Germany	92.3	22.4	6.4	119.52	211.82
France	92.2	23.9	7.3	129.48	221.68
New Zealand (2005)	90.0	7.1	6.3	55.61	145.61
United States of America	83.9	16.0	2.1	75.115	159.015

LME, Liquid Milk Equivalent

1.5 Milk composition

As because of our main focus is to highlight the mineral portions of the milk and its beneficial role to our health in whole life time, therefore, a considerable amount of information about milk i.e., the bulk portions and its nature, particular amount of each ingredients, etc. are put here for the shake of conveniences of readers for their hassle-free reading. Together with a sizeable amount of references are cited for the further detail reading [10-28].

The composition of milk from different mammals varies enormously. In order to provide high energy to their offspring, whales and porpoises produce milk with a fat content in excess of 40% elephants produce milk of about 20% fat and reindeer, milk of over 15% fat. Cows, like humans, produce milk of a lower fat content which is close to 4%.

Milk composition varies depending on the species, breed, the animal's feed, stage of lactation and the health status. Herd management practices and environmental conditions also influence milk composition. Although there are minor variations in milk composition, the milk from different cows is stored together in bulk tanks and provides a relatively consistent composition of milk year round in the USA (see the Table) [11].

Table 1.4: Gross composition of milk of various breeds, g/100g [11]

Breeds	Body Wt. (kg)	Milk Yield (kg)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Total Solids (%)
Holstein	640	7360	3.54	3.29	4.68	0.72	12.16
Brown Swiss	640	6100	3.99	3.64	4.94	0.74	13.08
Ayrshire	520	5760	3.95	3.48	4.60	0.72	12.77
Guernsey	500	5270	4.72	3.75	4.71	0.76	14.04
Jersey	430	5060	5.13	3.98	4.83	0.77	14.42
Shorthorn	530	5370	4.00	3.32	4.89	0.73	12.9

Cows produce more milk than their offspring require and man has taken advantage of this since the dawn of time. Breeding programs have successfully increased the amount and quality of milk cows produce. In general, the gross composition of cow's milk is 87.7% water, 4.9% lactose (one kind of carbohydrate), 3.4% fat, 3.3% protein, and 0.7% minerals (referred to as ash) [11]. The average composition of cow's milk is shown in the flowing table (Table 1.5). Water is the main constituent of milk, therefore, much milk processing is designed to remove water from milk or reduce the moisture content of the product. However, it is also stated that cow's milk contains about 87.4% water and about 12.6% milk solids (total solids) the latter comprising about 3.9% fat, 3.2% protein, 4.6% lactose (anhydrous) and 0.9% 'other solids' i.e. minerals, vitamins, etc. Non-water constituents are present in different physical forms; dissolved (lactose), colloidally dispersed (protein) and emulsified in water (lipids or fats). These physical characteristics are used to facilitate the commercial and analytical separation of the major constituents of milk.

Table 1.5: Composition of cow's milk

Main constituent	Range (%)	Mean (%)
Water	85.5 - 89.5	87.0
Total solids	10.5 - 14.5	13.0
Fat	2.5 - 6.0	4.0
Proteins	2.9 - 5.0	3.4
Lactose	3.6 - 5.5	4.8
Minerals	0.6 - 0.9	0.8

Minerals are mainly chlorides, phosphates and citrates of sodium (Na), calcium (Ca) and magnesium (Mg). Although it comprises less than 1% of the milk they influence its rate of coagulation and other functional properties. Some minerals are present in true solution. The physical state of other mineral is not fully understood. Calcium,

Mg, phosphorous (P) and citrate are distributed between the soluble and colloidal phases (see Table 1.6). Their equilibria are altered by heating, cooling and by a change in pH.

Table 1.6: Distribution of milk salts between the soluble and colloidal phases

Minerals	Total	Dissolved	Colloidal
		(mg/100 mL of Milk)	
Calcium (Ca)	1320.1	51.8	80.3
Magnesium (Mg)	10.8	7.9	2.9
Phosphorus (P)	95.8	36.3	59.6
Citrate	156.6	141.6	15.0

1.5.1 Total solids in milk

The 'total solids' of milk are determined simply by evaporating the water and weighing the residue. For the reference method the conditions for the measurement are tightly controlled and rely on oven drying 5 g of milk at 102°C for 2 hours until constant mass is achieved. However, total milk solids are always considered to be a very crude measure of its quality. Total milk solids comprise fat (3.9%) and solids-not-fat (8.7%), sometimes referred to as non-fat-milk solids (NFMS).

1.5.2 Milk fats

1.5.2.1 Milk fat chemistry and physics

Milk contains approximately 3.4% total fat. Milk fat has the most complex fatty acid composition of the edible fats. Over 400 individual fatty acids have been identified in milk fat. However, approximately 15 to 20 fatty acids make up 90% of the milk fat. The major fatty acids in milk fat are straight chain fatty acids that are saturated, monounsaturated, and polyunsaturated fatty acids. Milk fat contains approximately

65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids. Saturated fatty acids are associated with high blood cholesterol and heart disease. However, short chain fatty acids (4 to 8 carbons) are metabolized differently than long chain fatty acids (16 to 18 carbons) and are not considered to be a factor in heart disease. Conjugated linoleic acid is a *trans* fatty acid in milk fat that is beneficial to humans in many ways.

Milk fat melts over a wide temperature range, from approximately -40°F to 104°F (40°C). This is best illustrated by the firmness of butter at refrigerator temperature versus room temperature. At refrigerator temperature butter is approximately 50% solid, but is only about 20% solid at room temperature, which is why it spreads more easily as the temperature increases. The melting properties of milk are a result of the melting points of the individual fatty acids.

The triglycerides of milk fat are in the form of globules. The globules are surrounded by a protein and phospholipid membrane that stabilizes the globules in the water phase (called serum) of milk (see Figure 1.1). The native globules range in size from less than 1μm to over 10μm. The uneven size distribution allows the larger globules to float in a process called creaming, thus resulting in a "cream line" at the top of the container. Milk is homogenized to reduce the size of the large globules to less than 1 μm, create a uniform distribution of globules throughout the water phase, and minimize creaming.

Under the microscope cream can be seen to consist of a large number of spheres of varying sizes floating in the milk (Figure 1.1). Each sphere is surrounded by a thin skin the fat globule membrane which acts as the emulsifying agent for the fat suspended in milk (Figure 1.1).The membrane protects the fat from enzymes and prevents the globules coalescing into butter grains. The fat is present as an oil-in-water emulsion: this emulsion can be broken by mechanical action such as shaking.

1.5.2.2 Deterioration of milk fat

Milk fat can be degraded by enzyme action, exposure to light, and oxidation. Each of these processes proceeds through different mechanisms. Enzymes that degrade fat are called lipases, and the process is called *lipolysis*. Milk *lipases* come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation, or somatic cells present in milk. Lipases remove fatty acids from the glycerol backbone of the triglyceride. Usually the action of *lipase* causes undesirable rancid flavors in milk.

Milk fat can also be degraded by a classical chemical oxidation mechanism, the attack on double bonds in the fatty acids by oxygen. Oxidation of the unsaturated phospholipids in milk produces off-flavors.

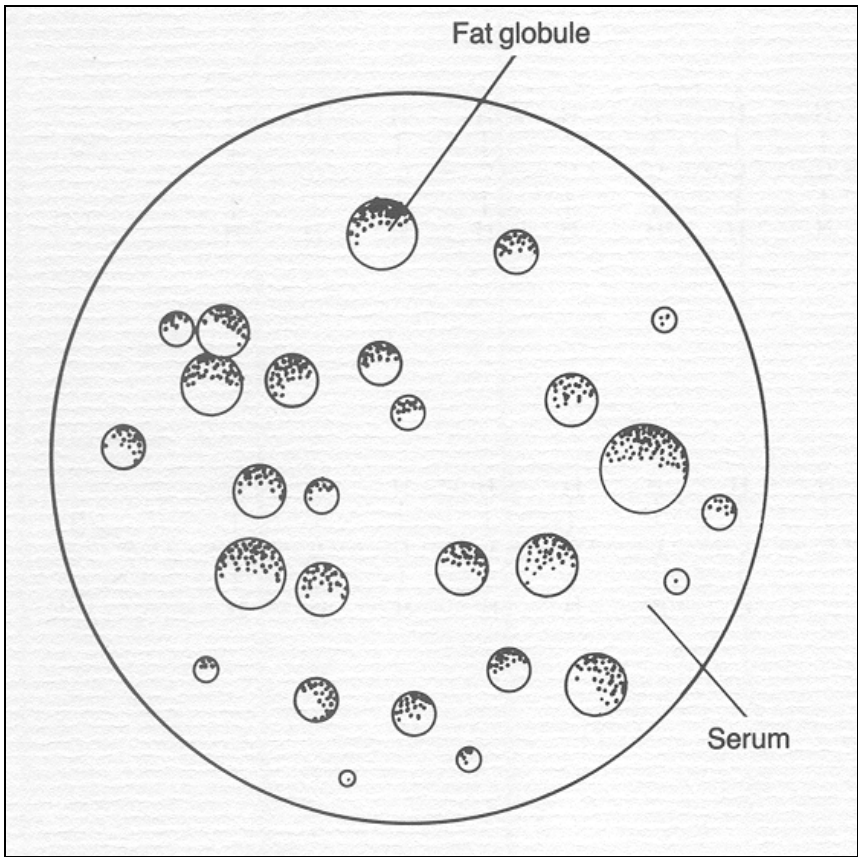

Figure 1.1: Fat globules in milk

1.5.2.3 Influence of heat treatments on milk fat

Typical high temperature short time (HTST) pasteurization conditions do not affect the functional and nutritional properties of milk fat. Higher heat treatments may stimulate oxidation reactions and cause fat deterioration and off-flavors. High heat treatments such as ultra high temperature (UHT) pasteurization can disrupt the milk fat globule membrane proteins and destabilize the globules, resulting in their coagulation.

1.5.3 Milk proteins

Proteins are the most valuable components of milk in terms of their importance in human nutrition and their influence on the properties of dairy products containing them. This, together with the availability of rapid instrumental methods of measurement, has led to increased use of protein as a quality parameter.

Proteins are large molecular weight complex organic compounds which contain carbon (C), hydrogen (H), oxygen (O) and nitrogen (N); sulphur (S) , P and other elements may also be present. Protein molecules are made up of amino acids, this link together via peptide bonds to form long chains. Milk protein and their fractions are shown in Figure 1.2.

Milk contains 3.3% total protein. Milk proteins contain all nine essential amino acids required by humans. Total milk protein content and amino acid composition varies with cow breeds and individual animal genetics.

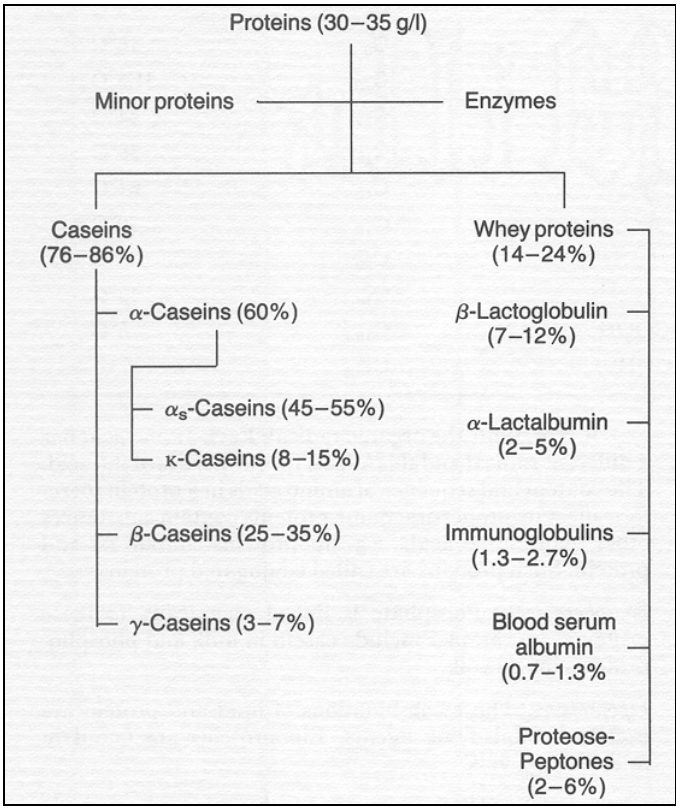

Figure 1.2: Milk protein with their variety of common fractions

Casein, the main fraction, is further made up of a number of fractions and is therefore heterogeneous. The whey proteins are also made up of a number of distinct proteins as shown in the Figure 1.2.

Milk provides easily digestible proteins of a high nutritional value and is a rich source of essential amino acids. Proteins are the body's building blocks' affecting our growth and immunity. Antibodies, enzymes and hormones all contain proteins. Thus the proteins we eat provide the amino acids needed to replace both these and essential ones. Body is able to synthesis some amino acids there are eight which it cannot make and these are the essential amino acids. Histidine is also considered to be essential for infants. The essential amino acids have to be supplied in our food proteins and all, unlike many other foods are found in milk. The acids conditions in the stomach untangle proteins laying them open to attack by enzymes called *proteases.* The broken

fragments are then used to provide the body's amino acid requirements. Proteins in excess of the body are used for energy. Essential amino acids, their daily requirement (g), source of milk proteins (in 100g milk protein), and total amount of liquid milk equivalent (LME) for daily requirement of essential amino acids (g) are given in Table 1.7.

Table 1.7: Essential amino acids present in milk proteins and their daily requirements

Amino Acids	Daily Requirement (g)	g/100g milk Protein	Milk (LME) for Daily Requirement (g)
Phenylalanine	1.1	5.5	747.6323
Methionine	1.1	2.8	1466.667
Leucine	1.1	12.1	414.8377
Valine	0.8	7.1	417.094
Lysine	0.8	7.4	570.7602
Isoleucine	0.7	6.7	423.8213
Tereonine	0.5	4.6	349.5702
Tryptophan	0.3	1.4	400
Histidine [a]	80	2.2	106666.67

[a]**mg/kg**

1.5.3.1. Deterioration of milk protein

Proteins can be degraded by enzyme action or by exposure to light. The predominant cause of protein degradation is through enzymes called *proteases*. Milk *proteases* come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation, or somatic cells present in milk. The action of *proteases* can be desirable, as in the case of yogurt and cheese manufacture, so, for these processes, bacteria with desirable properties are added to the milk. Undesirable degradation results in milk with off-flavors and poor quality. The most

important *protease* in milk for cheese manufacturing is *plasmin* because it causes *proteolysis* during ripening which leads to desirable flavors and texture in cheese. Two amino acids in milk, methionine and cystine are sensitive to light and may be degraded with exposure to light. This results in an off-flavor in the milk and loss of nutritional quality for these two amino acids.

1.5.3.2 Influence of heat treatment on milk proteins

The caseins are stable to heat treatment. Typical high temperature short time (HTST) pasteurization conditions will not affect the functional and nutritional properties of the casein proteins. High temperature treatments can cause interactions between casein and whey proteins that affect the functional but not the nutritional properties.

The whey proteins are more sensitive to heat than the caseins. HTST pasteurization will not affect the nutritional and functional properties of the whey proteins. Higher heat treatments may cause denaturation of *ß-lactoglobulin*, which is an advantage in the production of some foods (yogurt) and ingredients because of the ability of the proteins to bind more water. Denaturation causes a change in the physical structure of proteins, but generally does not affect the amino acids composition and thus the nutritional properties. Severe heat treatments such as ultra high pasteurization may cause some damage to heat sensitive amino acids and slightly decrease the nutritional content of the milk. The whey protein *α-lactalbumin*, however, is very heat stable.

1.5.3.3 Milk protein chemistry and physics

There are two major categories of milk protein that are broadly defined by their chemical composition and physical properties (see the Figure 1.3). The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein.

The casein family of protein consists of several types of caseins and each has its own amino acid composition, genetic variations, and functional properties. The caseins in milk form complexes called micelles that are dispersed in the water phase of milk (see Figure 1.3). Casein micelles are spherical and are 0.04 to 0.3µm in diameter, much smaller than fat globules which are approximately 1µm in homogenized milk. The casein micelles are porous structures that allow the water phase to move freely in and out of the micelle. Casein micelles are stable but dynamic structures that do not settle out of solution. They can be heated to boiling or cooled, and they can be dried and reconstituted without adverse effects. *ß*-casein, along with some calcium phosphate, will migrate in and out of the micelle with changes in temperature, but this does not affect the nutritional properties of the protein and minerals. The whey proteins exist as individual units dissolved in the water phase of milk. The casein micelles consist of subunits of the different caseins (e.g., α_{s1}, α_{s2} and *ß*) held together by Ca phosphate bridges on the inside, surrounded by a layer of six caseins which help to stabilize the micelle in solution. The caseins have a relatively random, open structure due to the amino acid composition. The high phosphate content of the casein family allows it to associate with Ca and form Ca phosphate salts. The abundance of phosphate allows milk to contain much more Ca than would be possible if all the Ca were dissolved in solution, thus casein proteins provide a good source of Ca for milk consumers.

The serum (whey) protein family consists of approximately 50% *ß-lactoglobulin*, 20% *α-lactalbumin*, blood serum albumin, *immunoglobulins*, *lactoferrin*, *transferrin*, and many minor proteins and enzymes. Like the other major milk components, each whey protein has its own characteristic composition and variations. Whey proteins do not contain phosphorus, by definition, but do contain a large amount of sulfur-containing amino acids. These form disulfide bonds within the protein causing the chain to form a compact spherical shape. The disulfide bonds can be broken, leading to loss of compact structure, a process called denaturing. Denaturation is an advantage in yogurt production because it increases the amount of water that the proteins can bind, which improves the texture of yogurt. The functions of many whey proteins are not clearly defined, and they may not have a specific function in milk but may be an artifact of milk synthesis. The function of *ß-lactoglobulin* is thought to be a carrier of vitamin A. It is interesting to note that *ß-lactoglobulin* is not present in human milk.

α-lactalbumin plays a critical role in the synthesis of lactose in the mammary gland. *Immunoglobulins* play a role in the animal's immune system, but it is unknown if these functions are transferred to humans. *Lactoferrin* and *transferrin* play an important role in iron absorption and there is interest in using bovine milk as a commercial source of *lactoferrin*.

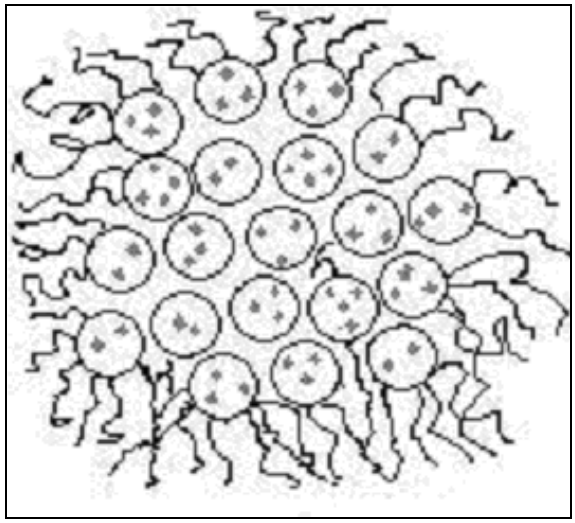

Figure 1.3: Pictorial view of Casein Micelle; ♦ Calcium Phosphate, O Sub-micelle

1.5.4 Milk carbohydrate chemistry

Milk contains approximately 4.9% carbohydrate that is predominately lactose with trace amounts of monosaccharide and oligosaccharides. Lactose is a disaccharide of glucose and galactose. The structure of lactose is depicted in Figure 1.4.

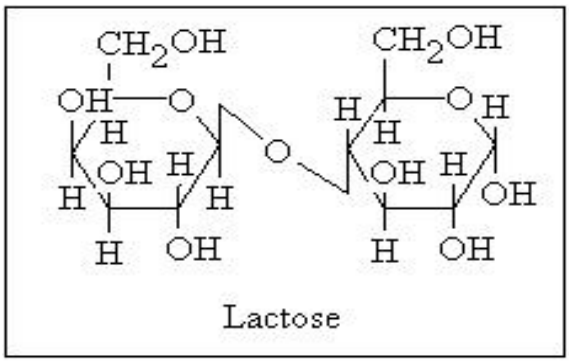

Figure 1.4: Structure of a lactose molecule present in almost all natural milk

1.5.4.1 Lactose

Lactose is at about 4.6% of the milk, the principal component of milk, yet it is the least important of the solids both nutritionally and commercially. Lactose is milk sugar that is the major carbohydrate in the milk of most mammals. Lactose consists of two molecules, D-glucose and D-galactose (Figure 1.4) and is digested or broken down into these constituent parts by the enzyme *lactase*.

Lactose plays a major role in the characteristics of condensed milk products. It also supplies the carbohydrate sources for bacterial action causing milk to sour in the presence of bacteria which grow at room temperatures (*mesophilia* bacteria) and is the carbohydrate source for beneficial cultures used in yogurts and cheese making during which process lactose is converted to lactic acid.

1.5.4.2 Lactose physical properties

Lactose is dissolved in the serum (whey) phase of fluid milk. Dissolved lactose in solution is found in two forms, called the α-anomer and ß-anomer that can convert back and forth between each other. The solubility of the two anomers is temperature dependent and therefore the equilibrium concentration of the two forms will be different at different temperatures. At room temperature (e.g., 20°C) the equilibrium ratio is approximately 37% α and 63% ß-lactose. At temperatures above 93.5°C the ß-anomer is less soluble so there is a higher ratio of α- to ß-lactose. The type of anomer present does not affect the nutritional properties of lactose.

Lactose crystallization occurs when the concentration of lactose exceeds its solubility. The physical properties of lactose crystals are dependent on the crystal type and can greatly influence their use in foods. Temperature affects the equilibrium ratio of the α- and ß-lactose anomers, as described above. Lactose crystals formed at temperatures below 20°C are mainly α-lactose crystals. The α-monohydrate lactose crystals are very hard in form, for example, when ice cream goes through numerous warming and freezing cycles. This results in an undesirable gritty, sandy texture in the ice cream.

Gums are often used in ice cream to inhibit lactose crystallization. The crystal form of ß-lactose is sweeter and more soluble than the α-monohydrate lactose and may be preferred in some bakery applications. When a lactose solution is rapidly dried it does not have time to crystallize and forms a type of glass.

1.5.4.3 Influence of heat treatments on lactose properties

The normal pasteurization conditions used for fluid milk have no significant effect on lactose. The higher temperatures used for UHT pasteurization of extended shelf life products can cause browning and isomerization reactions, which may affect product quality and nutritional properties. The browning reaction, called the Maillard reaction, occurs between the lactose and protein in milk and produces undesirable flavors and color, and decreases the available content of the amino acid lysine in milk protein.

1.6 Overall importance of milk as food across the global world

Milk is secreted by the mammary gland of mammals to feed their offspring. Cow's milk is commonly used as human food, but milk from sheep, goats, buffalo, yak, horses and camels is also used. Milk contains large amount of essential nutrients and has rightly been recognized as nature's single most complete food. For centuries man has taken advantage of this by taking millions of metric tons milk per year (y^{-1}) worldwide from cows, water buffaloes, goats and sheep and using it to make a significant contribution to his diet.

As a food, milk serves the following broad purposes: (a) growth, (b) reproduction, (c) supply of energy, (d) maintenance and repair and (e) appetite satisfaction. The requirements of these categories vary with the individual, and in some instances not all the stated functions of the food need to be served, e.g. adults no longer require food for growth whereas infants do. The functions of a food are served specifically through the various nutritionally important components, comprising proteins, carbohydrates, lipids, minerals, vitamins and water.

Nutritionally, milk has been defined as "the most nearly perfect food". It provides more essential nutrients in significant amounts than any other single food. Milk is an outstanding source of calcium and phosphorus for bones and teeth, and contains riboflavin, vitamins B_6, A and B_1 in significant amounts. It also contains B_{12}, the anti-pernicious anemia vitamin.

Milk fat or butterfat is the second largest component of milk and is of major commercial value. It serves nutritionally as an energy source and supplies essential fatty acids. Fat content is closely followed by milk proteins at about 3.4%. Milk proteins in turn are subdivided into casein, comprising approximately 76 to 80% of the total milk proteins, and the whey proteins, comprising roughly 20 to 24%. The whey proteins are of higher nutritional value than casein. Milk proteins are outstanding sources of essential amino acids.

Minerals have many roles in the body including enzyme functions, bone formation, water balance maintenance, and oxygen transport. In milk, Ca, Mg, and phosphate are the minerals bound within the casein micelle and the remainder are soluble in the serum phase. The fact that Ca and phosphate are associated as minerals bound with the protein does not affect the nutritional availability of either Ca or phosphate. Milk contains small amounts of Cu, iron (Fe), manganese (Mn), Na and many more elements, which are not considered major source of these minerals in the diet.

Magnesium in milk is essential for skeletal development, protein synthesis, muscle contraction and nerve function. One glass (ca. 200mL) of semi skimmed milk will provide a child of 6 years with 19% of their daily requirement for Mg and an adult (19 to 50 years) with 7.5%. The main sources of P in the diet come from milk and milk products. It is the second most abundant mineral in the body and plays a vital role in Ca and protein metabolism. Phosphorus is also essential for healthy bones and teeth as well as cell membrane structure, tissue growth and regulation of pH levels in the body. A glass of semi skimmed milk will provide a child of 6 years with 55% of their daily requirement for P and an adult with 36%.

Bovine milk is a poor source of dietary Fe; infants can develop anemia if not breast-fed with human milk (which contains a higher bioavailability of Fe compared to bovine milk) or if other dietary sources are not found.

Milk is a rich source of iodine in the diet. Iodine forms part of the hormones thyroxine and triiodothyronine. These hormones are produced in the thyroid, a gland in the neck and regulate the body's rate of metabolism (how quickly the body burns energy and the rate of growth). One glass of semi-skimmed milk will provide a child of 6 years with 96% of their daily requirement for iodine and an adult with 44%.

The nutritive value of milk products is based on the high nutritive value of milk as modified by processing. Over-processing and, in particular, severe heat treatment reduce the nutritional value of milk. Butter-making concentrates the fat-soluble nutrients, while cheese-making concentrates the milk fat and the major protein fractions.

It is not surprising, that the nutritional value of milk is high. Recently it has been known that the role of milk is not only to nourish but to provide immunological protection for the mammalian young [12]. All the essential amino acids and fatty acids present in the milk have that immunological protection capacity.

1.7 World health problems in relation with milk consumption

Dairy products are a good source of many minerals, particularly Ca where it furnishes up to 75% of the dietary need in the western world. The bioavailability of Ca from milk products is around 85%, compared to 20 to 75% from vegetable sources.

Calcium, P, Na and potassium (K) account of about 4% by weight of the fat-free human body, Ca therefore is a dietary essential [29]. Calcium is essential for the healthy growth and maintenance of teeth and bones and is a vital function in blood

clotting and muscle contraction. A glass of semi-skimmed milk can provide a 6 year old child with over half (55%) of his or her Ca requirement and can provide an adult (19-64 years) with over a third (35%) of his or her daily Ca requirement. The dairy council recommends the consumption of 3 portions of milk and dairy products per day (day^{-1}) in order to meet the full daily recommended intake for Ca. A portion includes 200mL milk, 30g or a matchbox sized piece of cheese and 150g or a pot of yogurt (appropriate portion sizes vary with age).

1.7.1 More about calcium

In many western countries milk and dairy products contribute between 50 to 60% of average Ca intake, and in a form which is readily utilized [16]. Vitamin D is essential in Ca absorption which is also enhanced in the presence of lactose [17]. Not all Ca eaten is absorbed (typically about 30%) and the presence of phytates, for example, from high-fiber diets further reduce Ca availability by locking it into a complex thereby preventing absorption and subsequent utilization [30]. Not only is milk a readily usable source of Ca, it is believed that it may also enhance the bioavailability of Ca form other food source [30].

Osteoporosis is a disorder of the skeleton which occurs during ageing and increases the risk of bone fractures. Osteoporosis is particularly prevalent amongst post-menopausal women although it may also occur in older men [31].

Nutritionists now believe that there is value in having a regular intake of Ca throughout one's lifetime in order to develop and maintain optimum bone health and that this creation of peak bone mass helps reduce the risk of osteoporosis occurring later in life. Bone mass peak is the 20 to 30 year age group after which it declines. Therefore it is of particular importance that teenagers do not forego milk and milk products as part of their diets. Low-fat milks, equally rich in Ca but lower in calories, are a valuable part of a healthy diet [30].

The following description about Ca and its essentiality, one text book titled 'Handbook of Dairy Foods and Nutrition' [32] is found worthwhile to cite here and considered the basis of the following discussions and most of the references were cited therein.

Low Ca dietary intake is generally recognized to contribute to osteoporosis and to predispose people to hypertension when consuming large amounts of salt. Bone mineralization requires a ratio of Ca to P of between 1.3 and 1.5 to 1, such as found in dairy products, while other nondairy sources of Ca have a much lower ratio.

The major mineral component in milk (and most dairy products) is Ca phosphate, an inorganic salt of low solubility in water. A high intake of Ca in the diet is believed to promote strong bone development, hence the recommendation of Ca in the diet of young children. The low solubility of Ca phosphate in water (and also in milk) would result in calcification in the mammary gland if it were not for the unique properties of the casein micelle in solubilizing this mineral.

Each of the four main casein molecules (α_{s1} casein, α_{s2} casein, β casein, and κ casein) contain at least one phosphate group that is capable of binding to the Ca phosphate mineral complex in milk. Some twenty-five thousand of these casein molecules, with bound Ca phosphate, aggregate to form the heavily hydrated casein micelle of molecular weight 108 to 109 Daltons (Da). Thus Ca phosphate is rendered soluble in milk and can be considered to be the binding agent that holds the micelle together. There is some controversy over the nature of the substructure of the casein micelle. The two main competing models are described elsewhere [14, 19, 27].

A small but absolutely essential amount of Ca is found in the blood and soft tissue. As a result of homeostasis, the body will take Ca from the bones if there is not enough circulating in the blood and extra-cellular fluid. The skeleton is constantly being absorbed and remodeled. Bone is made from Ca and phosphate combined into one crystal called hydroxyapatite. Osteoporosis results from many factors some of which

are inadequate Ca intake or absorption along with accompanying adequate vitamin D levels, hereditary factors, lack of bone stressors (e.g., exercise) throughout one's life, and hormone function.

Vitamin D is required for maximum Ca absorption. Calcium helps vitamin K function in blood clotting, functions in blood pressure regulation, and may be useful in lowering moderate hypertension, functions in many enzyme reactions inside and outside cells and is a cofactor for enzymes and proteins, functions in nerve impulse conduction, in neurotransmitter release, in hormone secretion, and in heart, smooth and skeletal muscle cell contraction. Calcium interacts with Zn, Mg, vitamin D and vitamin K. Calcium limits the absorption of lead (Pb) and exposure to Pb stored in the skeleton which can be mobilized by demineralization.

Calcium is available in many foods. Most people think of dairy when they think of Ca. Though cheese is a good source of Ca it is high in saturated fat. Eat a varied diet to get the best Ca absorption. It is estimated that only 30% of dietary Ca is absorbed. Factors which inhibit Ca absorption and may contribute to Ca loss are: aluminum, Al (foods cooked in Al cookware including the use of acidic foods with the cookware), Al foil, antacids containing Al and high levels of Mg. Zinc oxylates (a chemical that is found in sweet potatoes, dried beans, rhubarb and spinach), concentrated forms of phytic acid (such as found in wheat bran and dried beans) and dietary fiber inhibit Ca absorption. Alcohol, phosphates (in soft drinks and meats), sugar, and protein increase Ca excretion. High levels of Na may also be linked to Ca excretion. There is not enough research to state definitely how much effect caffeine has on Ca excretion but it may be very little. Athletes should focus on ingesting milk and other Ca healthy drinks rather than ingesting soft drinks and caffeinated beverages.

Increased levels of protein may also increase Ca excretion. An increase of 1.75mg of Ca per day may be needed to offset Ca loss set forth by increased protein intake of 1 g each over the RDA of 46g of protein per day for adult women and 56g of protein per day for adult men. Most people in the western society eat far more protein than the RDA.

During the peak bone development years, 9 to 17, it is reported that this age group drinks more soft drinks than milk, thereby limiting Ca intake and contributing to Ca excretion during their formative years. Pre-pubescent and adolescence are critical years in the formation of a strong skeleton. A strong skeleton can be developed through engaging in physical activity and a healthy diet which may prevent the development of or decrease the degree of osteoporosis in later years. Peak bone mass is achieved around the age of 30.

Following is the Examples of Adequate Intake (AI) of Ca and the dairy source:

AI (Adequate Intake)

Male and female adolescents 14 to 18 years of age = 1,300 mg day^{-1}

Male and female adults 19 to 50 years of age = 1,000 mg day^{-1}

UL (Upper Limit)

Everyone (except infants) = 2,500 mg day^{-1}

Source

Milk, 1 cup/8 oz. (tentatively 225mL milk) = 300 mg Ca

1.7.2 Low calcium intake and risk of chronic disease

Low Ca intake has been implicated in the etiology of several chronic diseases, such as osteoporosis (mentioned above) hypertension [30, 31] and colon cancer [32]. For the treatment of High Blood Pressure (BP) now recommends adequate intake of Ca, with K, Mg for the prevention of hypertension [32]. People from most of the African countries typically have a high incidence of hypertension, lactose maldigestion, and a low Ca intake. It has been suggested that low intake of Ca and K may be an important reason for increased incidence of hypertension among the African population compared to white western people [30-32]. Since many people with lactose

intolerance consume less milk and other dairy foods and thereby less Ca, appropriate management strategies must be taught to help insure adequacy of Ca and other nutrients in the diet.

1.7.3 Dairy foods and colon cancer

Colon cancer is the third leading cause of cancer morbidity and mortality is the USA. In 1993, an estimated 152,000 Americans developed this disease (109,000 colon cancer; 43,000 rectum cancer) and 57,000 died from it (50,000 colon cancer; 7,000 rectum cancer). An equal number of men and women are affected by colorectal cancer.

Both genetic and environmental factors contribute to cancer [33]. Among environmental factors, diet is estimated to be responsible for 30 to 60% of all cancers. Colorectal cancer is thought to be caused by an interaction between dietary factors and genetic predisposition. Although some dietary factors are suspected of contributing to specific cancers, others may be protective [33-37]. Recently it has begun to be appreciated that several components in dairy foods specifically Ca and vitamin D, bacterial cultures, and a class of fatty acids known as conjugated dienoic derivatives of linoleic acid may protect against colon cancer.

Based on the evidence to date, increasing intake of Ca and vitamin D, especially from dairy foods which provide the majority of these nutrients in our diets, appears to be a prudent measure to reduce risk of colon cancer [36-37] With respect to the amount of Ca necessary to reduce risk of colon cancer, Ca intakes of at least 1.5g day^{-1} for women and 1.8g day^{-1} for men and vitamin D intakes of 5μg day^{-1} are recommended by some researchers. Some findings presented on the relationship between Ca and various diseases are to recommend that individuals consume 1.5 to 2.0g dietary Ca to prevent colon cancer.

Amounts greater than the RDA (800mg day^{-1}) for most adults are recommended to protect against colon cancer, especially in individuals at risk of this disease. According to the 1989 NRC (national research council) report on "Diet and Health", recommend Ca intakes up to 2.5g day^{-1} are safe for healthy adults [35]. Although the report cautions against Ca intakes greater than 1.5g day^{-1} for individuals susceptible to kidney stones, a recent study reports that a high intake of dietary Ca reduces the risk of kidney stones.

There is suggestive evidence that intake of culture-containing dairy foods such as yogurt may protect against colon cancer, although more research is needed to confirm this finding. Dairy foods are an important source of Ca, vitamin D, and bacterial cultures, all of which have been suggested to protect against colon cancer. For this reason, all individuals, and especially those at risk of colon cancer, should consume the recommended number of servings from the milk and other food groups each day. Four servings a day of foods from the milk group can provide about 1.2g Ca. Additional Ca can be obtained by consuming other Ca containing foods.

1.7.4 Dairy foods and osteoporosis

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass with a consequent increase in bone fragility and susceptibility to fracture. Often called a "silent" disease, osteoporosis develops gradually over many years before the occurrence of clinical symptoms such as loss of height, curvature of the spine, and fractures, especially of the spine, hip, and wrist. For many individuals, a skeletal fracture is the first indication of osteoporosis. Osteoporosis is a major public health problem affecting more than 25 million people, mostly women. The causes of osteoporosis, similar to other chronic diseases, are multi factorial, involving, involving both genetic and environmental factors [31, 38-40]. Lifestyle factors have received considerable attention in recent years, especially since they can be manipulated, because more than 99% of the total Ca content of the body is found in the skeleton, it is not surprising that considerable interest lies in the role of Ca and vitamin D (which enhances Ca absorption in bone health).

Accumulating scientific evidence indicates that a sufficient intake of Ca throughout life protects against osteoporosis by achieving genetically program men peak bone mass reached at about 30 to 35 years of age or earlier and reducing postmenopausal and aging-associated bone losses.

Following is the RDA values suggested by Food and Nutrition Board, USA [30, 39]:

Recommended Dietary Allowances

(RDA) for Calcium

Infants

Birth-6 months	400 mg
6 months-1year	600 mg

Children and young adults

1-10 years	800 mg
11-24 years	1200 mg
Adults	800 mg
Pregnant and lactating women	1200 mg

If dairy products are excluded, the usual USA diet provides only about 300mg Ca a day, an amount far less than the current RDA of 800mg day^{-1} [31] for most adults, and the estimated amount of Ca (i.e., up to 1500mg day^{-1}) for individuals at risk of osteoporosis. A sequence of skeletal Ca retention during aging is given in the following chart 30, [39]:

Skeletal Ca retention during early life

Age(years)	Skeletal Ca (g)	Retention (mg/day)
0	25	440
10	400	200
13	800-1100	400
17	800-1100	80
35	900-1300	15

Bone mass increases during childhood and adolescence until linear growth has ended. It generally is accepted that following attainment of linear growth, consolidation of bone density continues until age 30 to 35 years (i.e. peak bone females indicate that peak bone mass at several skeletal sites may be ached much earlier (i.e., by late adolescence) [39, 40]. Calcium accretion in bone from about 25g at birth to 900 to 1300g at maturity parallels this change in bone density [31, 40]. After peak bone mass is reached, bone mass remains stable until menopause in most women. For aging in men, bone loss amounts to 3 to 5% a decade. However, in women, bone loss accelerates (i.e., average of 2 percent a year) at menopause and remains elevated for about five to ten years before slowing down to a rate similar to that of aging men. Women's relatively lower peak bone mass and high bone loss following menopause are a part of the reason why osteoporosis is more common in women than in men [40].

Age-related bone loss in both sexes results from the gradual thinning of both cortex and trabeculae. The accelerated loss of bone postmenopausally is thought to be mediated mainly by osteoclasts [31]. A gradual loss of cortical bone occurs with age and increases in women after menopause. The magnitude of peak bone mass and the rate and duration of postmenopausal and age-associated bone loss determine the likelihood of developing osteoporosis. When bone mass is low, less trauma in necessary to cause a fracture.

1.8 Milk as a good source of essential micronutrients

Milk and milk products play an important part in man's diet and contribute greatly to the diet of the young, therefore concern about the global pollution of the environment has led to many studies of the pathways of heavy metals into milk.

Several trace elements of nutritional importance found in milk, which is required for many enzyme and hormone metabolism [41-47], Zn for the function of some enzymes in the human body. Zinc is a vital mineral in milk and is a constituent of many enzymes in the body; its role is to fight infections, growth development, for sexual development, wound healing and for our sense of taste. One glass of semi skimmed milk will provide a child of 6 years with 12.3% of their daily requirement for Zn and an adult with 11%.

1.8.1 Trace elements in milk

The importance of trace elements in human nutrition is becoming more clearly understood and recent developments in trace analysis have meant that very low levels, from 1 part per billion (ppb) to a few parts per million (ppm), can now be measured with greater accuracy [44].

Trace elements tend to be classified as 'essential', 'non-essential' and 'toxic' [46]. There are 26 naturally occurring elements essential to animal life of which fifteen are accepted as being trace elements and many of these occur in milk. Certain elements such as arsenic (As) are essential at low levels but become toxic if consumed at high levels. Therefore, in many countries a minimum requirement for essential elements and a 'safe' dietary maximum level for the potentially dangerous toxic elements have been set and are discussed elsewhere [29, 41-47].

Trace elements in cow's milk largely originate from the feed. Milk, a naturally designed food for the young calf, is a good source of a broad-based cocktail of trace

elements in forms, unlike some mineral tablet supplements that can be readily assimilated by the body. This is why milk is referred to as an almost completed food.

Cow's milk contains traces of Al, As, barium (Ba), boron (B), bromine (Br), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), Fe, Pb, Mn, molybdenum (Mo), nickel (Ni), Na, selenium (Se), silicon (Si), silver (Ag), tin (Sn), vanadium (V) and Zn [4-6, 13-17, 46].

Here, only a common number of trace elements (should be used as synonymous of micro nutrients) will be discussed elaborately in relation with their importance to be related in testing the hypothesis of this research.

1.8.1.1 Zn in milk

Milk is a relatively important source of Zn, contributing for average milk drinkers about a quarter of the RDA of 10mg for children and 15mg for adults. Not only milk a good source of dietary Zn it is present in a highly bioavailable form [48-50]. Milk may also promote the absorption of Zn from other source such as cereals or vegetables which are rich in phytic acid, a Zn binding substance. The Zn concentration of human milk is appreciably lower than that of cow's milk, although individual variability is high. The possible significance of the fact that human milk contains less Zn and more Cu than cow's milk and therefore a much lower Zn:Cu ratio. The effect of subnormal and high dietary Zn intakes on the Zn level of human milk does not appear to have been specifically studied, but there is no doubt from work with other species that the level of Zn in milk reflects both low [51-54] and high [55-57] dietary Zn intakes.

Followings are examples of Zn present in dairy diets:

Milk, 8 oz. or 1 cup = 1.0mg;

Fruit yogurt, 1 cup = 1.8mg

1.8.1.2 Cu in milk

Subnormal Cu levels in the milk is the result of cows grazing Cu deficient pastures, with values as low as 0.01-0.02μg mL^{-1} (corresponding 10 to 20 ppb), have been reported [58]. Adding Cu to diets already adequate of this element has little effect on the Cu content of the milk of cows, goats, and human [59], However, substantial elevation of milk Cu were found for at least 4 weeks following subcutaneous injections of the cows with 300mg Cu as Cu glycinate. This was achieved without any increase in the incidence of spontaneous oxidized flavor in the milk, probably because of the small amount of Cu associated with the milk fat in early lactation. After 2 to 4 weeks of lactation only about 15% of the Cu in cow's milk is associated with the fat, whereas after 15 weeks the proportion so associated rises to some 35% [60].

1.8.1.3 Mn in milk

Investigations in several countries have shown normal cow's milk to contain 20 to 40 μg Mn L^{-1}, with concentrations of 130 to 160μg L^{-1} in colostrum [61]. The level in the milk responds rapidly to changes in dietary Mn intakes. The provision of high Mn feeds or feeding supplements providing only 3g Mn day^{-1} produced smaller increases [62]. The Mn level in human milk does not appear to be so well established, but there seems little doubt that this level is significantly lower than it is in the milk of cows, sheep, or goats [63]. A mean level of 15μg Mn L^{-1} (range 12 to 20μg) in breast milk, compared with 40 (32 to 52 μg) μg Mn L^{-1} in pasteurized cow's milk in New Zealand was reported [64].

1.8.1.4 Pb in milk

The levels of Pb in normal cow's milk is reported to be 20 to 40μg kg^{-1} (ca. 20 to 40 ppb) and that of ewe's milk in early lactation was found 110 to 150μg kg^{-1} [65]. Lead readily passes the mammary barrier so that dosing of the animals with Pb salts produces a marked increase in the level in the milk. The Pb content of market milk in USA cities ranged from 20 to 80μg kg^{-1}, with no significant differences between cities

and with a national weighted average close to 50μg kg^{-1} or about 50μg L^{-1} [(66]. A survey of bulk milk revealed a mean level of 40μg L^{-1} for 270 samples, with no samples greater than 200μg L^{-1} was found [67]. Human milk does not appear to have been so extensively studied.

1.9 Essential micronutrients and their role in good health

1.9.1 The nature of trace elements

Many mineral elements occur in living tissue in such small amounts that the early workers were unable to measure their precise concentrations with the analytical methods then were available. They were therefore frequently described as occurring in "traces" and the term trace elements arose to describe them [68-70]. This designation has remained in popular usage despite the fact that virtually all the trace elements can now be estimated in biological materials with great accuracy and precision. 15 elements generally accepted as trace elements. These Fe, Zn, Cu, Mn, Ni, Co, Mo, Se, Cr, iodine (I), F, Sn, Si, V, and As. A detailed classification of all essential elements is given in Table 1.8. The treatment of anemia with Fe and the association of I deficiency with goiter marked these as the only two trace element recognized as essential for animals well into the twentieth century. A more active 'modern' period 1957 to 2000; was based on the experimental induction of trace element deficiencies [68, 69]. These efforts have resulted in evidence supporting the essentiality of Cr, F, Ni, Si, V and most recent Li.

A gross classification of variety of trace elements is presented in table (Table 1.8). Essential elements are classified into the six bulk or structural elements, five macro minerals, three trace elements and sixteen ultra trace elements. The three prominent biologically active metals are Fe, Zn and Cu. All the remaining essential trace elements are considered ultra-trace since they are present less than 10mg in the adult human.

Table 1.8: Classification of the essential trace elements

Class	Class Name	Elements
a	Bulk Structural Elements	H, C, N, O, P, S
b	Macrominerals	Na, K, Mg, Ca, C,
c	Trace Elements	Fe Cu, Zn
d	Ultra trace Elements	Nonmetals: F, I, Se, Si, As, B Metals: Mn, Mo, Co, Cr, V, Ni, Cd, Sn, Pb

The importance of essential trace elements is manifold. In early period, the discovery of essential elements was hampered by relatively insensitive analytical methods. Fortunately, the recent developments of analytical techniques capable of determining ppb have opened new vistas for the discovery of new essential trace elements. Atomic absorption, atomic fluorescence, activation analysis and X-ray fluorescence (XRF), etc. have been employed for the determination of essential elements in biological systems. The past several decades have witnessed and explosive increase in our knowledge of the many elements that are essential for life and maintenance of plants and animals. This kind of research also encompasses the subject area which is frequently identified as bioinorganic chemistry.

1.9.2 Mode of action of trace elements

The only property that the essential trace elements have in common is that they normally occur and function in living tissues in low concentrations. These normal tissue concentrations vary greatly in magnitude and are characteristic for each element, they are usually expressed ppm or $\mu g\ g^{-1}$, and with some elements, such as I, Cr, Ni, and V, as ppb or $\mu g\ Kg^{-1}$. It should be noted that certain of the nonessential elements, such as Br occur in animal tissues in concentrations well above those of most of the essential trace elements.

1.9.3 Needs and tolerances

The minimum requirements of animals and man for the essential trace elements are commonly expressed in proportions or concentrations of the total dry diet consumed daily. The maximum intake of these and other elements that can be safely tolerated are usually expressed similarly. Since the availability of mineral elements is affected by the chemical form in which it is ingested, it is obvious that gross dietary intakes do not necessary reflect minimum requirements or maximum tolerances of wide or universal applicability.

Trace elements requirements and tolerances expressed as concentration such as ppb of the dry diet also carry the assumption that the whole diet is otherwise adequate and well balanced for the purpose of which it is fed, and that it is effectively free from other toxic factors capable of adversely affect the consumer health, appetite, or utilization of the element concerned. The question of appetite is especially important since the capacity of a particular dietary concentration of an element to supply the needs will clearly depend on the amount of the diet consumed daily or over a given period. Equally important is the level of other minerals or other nutrients which influence the availability or utilization of the element in question. A “true” or basic minimum requirement can thus be conceived as one in which all the dietary conditions affecting the element in this way are at an optimum. A series of minimum requirements therefore exist depending on the extent to which such interacting factors are present or absent from the whole diet.

Similarly, a series “ of safe” dietary levels of potentially toxic trace elements exist, depending on the extent to which other elements which affect their absorption and retention are present. These considerations apply to all the trace elements to varying degrees, but with some elements such as Cu they are so important that a particular level of intake of this element can lead to signs either of Cu deficiency or of Cu toxicity in the animal, depending on the relative intakes of Mo, S, or of Zn and Fe. The many mineral interactions of this type which exist are discussed in the following text sections.

Estimates of adequacy or safety also vary with the criteria employed. As the amount of essential trace elements available to the animal becomes insufficient for all the metabolic processes in which it participates, as a result of inadequate intake and depletion of body reserves, certain of these processes fail in the competition for the inadequate supply. The sensitivity of particular metabolic processes to lack of an essential elements and the priority of demand exerted by them vary in different species and, within species, with the age and sex of the animal and the rapidity with which the deficiency develops.

Ample evidence is available that the Mn requirements for growth are substantially lower than they are or satisfactory reproductive performance, recent evidence relating Zn intakes to rate of wound healing also raises important questions on the criteria for adequacy to be employed in assessing the Zn requirements for human.

1.9.4 The concept of essentiality

Biological trace elements arose from success in devising experimental methods to induce specific trace element deficiencies in laboratory animals. This involves the maintenance of animals on special formulated synthetic diets in controlled environmental chambers. The simplest definition of an essential element is that it is an element absolutely required for the maintenance of life; its absence results in severe malfunction of the organism or death. Experimentally, this rigorous condition cannot always be satisfied and this led to a broader definition of essentiality. The essential ultratrace elements are universally required for growth and survival of organisms. There is an impressive number of trace elements that have been shown to serve as required growth factors at extremely low concentrations (usually less than 1μM and as low as 10^{-10} M; e.g. 50μg day^{-1}).

1.9.5 Essential ultra trace metals

Amount essential ultlratrace metals (listed in Table 1.9), only Mn, Mo, Co, Ni have been clearly identified as forming metalloenzymes [66, 69, 70]. Mn is present in several important enzymes. Mn also appears to be rather directly involved in the

enzymic machinery of carbohydrate metabolism with possible links to lipid metabolism. In nitrogenase, the Mo acts as cofactor, a metal complex with a novel organic molecule called molybdopterin. Ni is known to function in several melattoenzymes e.g., urease, several hydrogenases and carbon monoxide dehydrogenase. A detailed description about the selective functions of cited ultra trace elements are given in Table 1.9.

Table 1.9: Properties of the essential ultratrace metals

Ultra Trace Metals	Deficiency Signs	Specific Functions
Mn	Growth depression bone deformities membrane abnormalities ; connective tissue defects	Carbohydrate metabolism *Superoxide dismutase* *pyruvate carboxylase*, etc.
Mo	Growth depression	*Oxidases*: aldehyde, sulfite, *xanthine molybdopterin*
Co	Anemia ; growth retardation	Constituent of vitamin B_{12}
Cr	Insulin resistance	Penetration of insulin action on carbohydrates and lipids; active as a bioorganic chromium complex
V	Growth depression	Control of sodium pump ; inhibition of a Tpase, P-transferases
Ni	Growth depression Reduced N utilization Reduced Fe metabolism	Constituent of *urease* ; Reduced hemopoiesis
Cd	Growth depression Reduced reproduction	Stimulates elongation factors in ribosomes
Sn	Growth depression	(Interactions with riboflavin)
Pb	Growth depression ; anemia	(Many enzyme effects)

1.9.6 Antagonism and synergism among essential trace elements

Synergistic effects have already been observed. For example, Zn absorption is impaired by Fe (II) [57]. High Zn intake induces a relative Cu deficiency, probably by interfering with Cu absorption. Mo and S also antagonize Cu by a temporary interaction involving the formation of Cu thiomolybdate, which also causes a reduced Cu uptake [71]. In general, toxic and heavy metal ions attack the active sites of enzymes, inhibiting essential enzyme function. Heavy metal ions in particular, Cd (II), Pb(II) or As(III) exert toxic action by attacking –SH groups of an enzyme, there by inhibiting enzyme action.

1.9.7 Detailed inspection of trace elements related to their presence in milk and milk products

1.9.7.1 Zn

Zinc is an essential constituent of over 40 of the body's enzymes and plays an important role in maintaining the appetite and in the promotion of wound healing dietary Zn deficiency can therefore lead to extensive skin lesions, a decline in the resistance to infection, depression, lethargy and a decline in appetite Zn deficiency has been associated with anorexia nervosa [53, 54]. Adequate dietary Zn is particularly important during periods of rapid growth and development and especially during recovery from infection or physical injury.

Zinc functions in cell/energy metabolism for growth and development, in cell signaling systems, in the immune system, in neurological development, and in reproduction. It is found in all body tissues and is particularly important in over 200 enzymes and hormone functions, and in vision, taste, smell, and in wound-healing processes. Its highest concentration is in muscles (65%), in red and white blood cells,

bone, skin, liver, kidneys, pancreas, eye retina, in the male prostate gland and sperm; it helps make cell membranes strong. Zinc absorption is decreased by drinking tea or coffee or eating vegetables or whole grains with phytic acid (found in fiber) with meals. With phytic acid Zn forms Zn-phytate which is not absorbed. High intakes of Ca, Fe and Cu may also limit Zn absorption. Some amino acids e.g., Cysteine and methionine improve Zn absorption in body.

1.9.7.1.1 Requirements

Minimum Zn requirement vary with the age and functional activities of the animal and with the composition of the diet, particularly the amounts and proportions of the many factors, organic and inorganic, which effect Zn absorption and utilization. Zinc requirements are also influenced by ambient Zn in the sweat, and by parasitic infestation with its attendant blood, and hence Zn, losses. The criteria of adequacy employed can also be important. For example, the requirements following trauma and disease are higher than "normal." RDA and UL are given in the following table (Table 1.10).

Table 1.10: RDA and UL for Zn in various age groups

Groups	**Age range, years**	**Requirements**
Male adolescents	14 - 18	11 mg day^{-1}
Female adolescents	14 - 18	8 mg day^{-1}
Male adults	19 years and older	11 mg day^{-1}
Female adults	19 years and older	8 mg day^{-1}
UL		
Adolescents	14 - 18	34 mg day^{-1}
Adults	19 years and older	40 mg day^{-1}

The minimum Zn requirements of humans compatible with satisfactory growth, health, and well-being vary with the type of diet consumed, climatic conditions, and the existence of stress imposed by trauma, parasitic infestations, and infections. However, the National Academy of Sciences, USA has recommended the following daily dietary allowances for Zn [45, 59] needed in various age groups (see Table 1.11), which can be assumed different from the reading given in the earlier Table.

Table 1.11: RDA for Zn in various age groups

Group/Species	Requirements/Limit, mg
infants (up to 1 year)	3-5
children (1 to 10 years)	10
Adult males (10 to 51+ years)	15
Pregnant women	20
Lactating women	25

These allowances apply to Western-style mixed diets and are not necessarily adequate for diets consisting predominantly of unrefined cereals high in phytate. In fact such diets consisting mainly of unleavened whole wheat or corn bread and beans, and supplying approximately the same amount of Zn (15mg day^{-1}) as a typical North American diet, are clearly inadequate in Zn for growth and sexual development in young meals in parts of Middle East countries [59].

Individuals at risk for Zn deficiency are strict vegetarians because Zn in plant food sources may be bio unavailable or are poorly absorbed, bowel inflammation diseases, long-term diarrhea, anorexic individuals, malabsorption syndromes, sickle cell anemia, infants and children, older adults, intravenously fed individuals, pregnant women and teens, lactating women and teens, HIV and AIDS infected individuals and those with liver disease. Zinc deficient individuals are more susceptible to infection and have an increased susceptibility to disease as the immune system is affected. Most individuals should be able to obtain enough Zn naturally in their diets.

1.9.7.1.2 Skeletal development

The long bones are shortened and thickened in proportion to the degree of Zn deficiency [52-56]. Changes and disproportions occur in other bones, giving rise to a disease histologically similar to that of Mn deficiency [61]. The mechanism of action of Zn in bone formation is still not fully understood. Decreased osteoblastic activity in the bony collar of the long bones occurs in Zn-deficient species.

1.9.7.1.3 Zinc and atherosclerosis

Indication has been obtained that Zn therapy can be beneficial in some cases of atherosclerosis. For Zn sulfate administrated orally to 13 patients with advanced vascular disease for 29 months, for twelve of these 13 showed marked clinical improvement, nine retuned to normal activity, and seven had a return of previously absent pulses [56, 57]. The mode of action of Zn in atherosclerosis is unknown. Hair and plasma Zn levels are usually subnormal in atherosclerosis and myocardial infarctions. Since atherosclerosis is thought to begin with some form of trauma it may be that, in part, an expression of inadequate arterial repair [53, 54].

1.9.7.1.4 Brain development and behavior

Zinc deficiency during the critical period for brain growth permanently affects brain function. When this deficiency is imposed throughout the latter third of pregnancy, brain size is decreased, there is a reduced total brain cell number, and the cytoplasmic nuclear ratio is increased, implying an impairment of cell division in this brain during the critical period of macroneuronal proliferation.

1.9.7.1.5 Distribution in tissues and fluids

Typical normal levels of Zn in the principal soft tissues of human are given in the following Table. Zinc occurs widely in relatively high concentrations through out the body [48-52]. The whole body of adult man is estimated to contain 1.4 to 2.3g of Zn, of which about 20% is present in the skin. The mean Zn concentrations of normal human epidermis and dermis have been reported to be 70.5 and 12.6μg g^{-1} dry weight, respectively [48, 49].

Table 1.12: Typical zinc concentrations of normal tissues[a] of human

Tissue	Human
Adrenal	12
Brain	14
Heart	33
Kidney	55
Liver	55
Lung	15
Muscle	54
Pancreas	29
Prostate	102
Spleen	21
Testis	17

[a]µg/g fresh tissue

1.9.7.1.6 In nails and hair

The level of Zn in the nails of 18 normal human subjects was reported to range from 93 to 292 ppm and to an average of 151 ppm [48, 50, 51]. The head hair of 46 subjects ranged similarly from 92 to 255 ppm and averaged 173 ppm Zn [48, 50]. This is similar to the mean of 167±5 ppm for healthy males and 172±9 ppm for females reported elsewhere [57].

1.9.7.1.7 Toxicity

The relatively low toxicity of Zn among the divalent cations, coupled with efficient homeostatic control mechanisms, make chronic Zn toxicity from dietary sources and unlikely hazard to man. Where Zn salts or compounds are given orally in large doses over prolonged periods, as in the treatment of chronic ulcers or the prophylaxis of cardiovascular disease, possibilities of toxic effects cannot be dismissed. Indeed dose

of 150mg Zn day^{-1}, which are equivalent to about 200 to 300 ppm of the total daily dry matter intake of an adult are ample and this is unlikely to be serious, but where they are low or marginal the Cu and Fe status of the individual could decline and Zn-induced manifestations of Cu and Fe deficiencies ultimately arise [56-58]. It is also found that no "biochemical evidence" of toxicity during 4 months of oral administration of Zn sulfate at the rate of 200mg three times a day to patients with venous leg ulcers, but Fe and Cu were not specifically investigated and the period is not long [56, 58]. On the other hand, Zn is also a metabolic antagonist of Cd, so that high Zn intakes would be expected to afford some protection against the potentially toxic effects of increasing Cd exposure from the environment [72].

Excessive long-term use of zinc (60mg day^{-1} or above of this value) may cause Cu deficiency. Therefore, drinking fluids or eating foods from galvanized metal containers should be avoided. Zinc absorption may be decreased if combined with certain medications such as antibiotics.

1.9.7.2 Cu

Copper absorption and retention is so strongly influenced by a number of other mineral elements and dietary components that series of minimum Cu requirements exist, depending on the extent to which these influencing factors are present or absent from the diet, and on the criteria of sufficiency employed.

1.9.7.2.1 Deficiency

Copper deficiency has been implicated in the etiology of three distinct clinical syndromes in the human infant. In the first of these, anemia, hypoproteinema and low serum Fe and Cu levels are present and combined Fe and Cu therapy is necessary to promote complete recovery [56, 58]. The hypocupremia results from and inability of the infants to obtain sufficient Cu from their Cu-low milk diets to prevent Cu depletion in the face of the increased loss of the plasma Cu protein into the bowel [73]. Even normal breast-fed infants are often unable to obtain sufficient Cu from the

milk to prevent some Cu depletion [74]. However, hypocupremia does not necessarily arise in infants fed exclusively on milk diets. For example, It has been detected that no differences in weight gains, hemoglobin values, serum protein, and plasma Cu levels in two groups of premature infants, one of which received a milk diet supplying only 14 µg Cu kg^{-1} body weight day^{-1} for 7 to10 weeks and other receiving up to six times of that amount of Cu, was found [74, 75].

1.9.7.2.2 Distribution in the body

The healthy adult body has been estimated to contain 80mg of total Cu [75]. Newborn and early young are normally richer in Cu per unit of body weight than adults of the human species [74, 75]. The levels in newborn are largely maintained throughout the suckling period, followed by a steady fall during growth to the time when adult values are reached.

The Cu concentrations in the inner and outer layers of human dental enamel have been reported as 11.3 and 9.5µg g^{-1}, respectively [73]. In a study of human dental enamel, the value found exceedingly wide range of 0.07-208µg Cu g^{-1}, with a median value of 0.7 and a mean of 6.8 ± 4.0µg g^{-1} [58, 74].

The normal range of concentration of Cu in the blood of healthy human adults can be given as 0.5-1.5µg mL^{-1}, with a high proportion of values lying between 0.8 and 1.2 µg mL^{-1} [58,73-75]. Copper concentration in the blood of human is given in the table (Table 1.13).

Table 1.13: Copper concentration in the blood of human

Age and condition	Mean copper concentration[a]	Ref.
Healthy adult male	1.10[c]	58,73-75
Healthy adult female	1.23[c]	do
Female at late pregnancy	1.92[c]	do
Pregnant female at delivery	2.69[c]	do

[a]measured as µg mL^{-1}; [c]in serum

1.9.7.2.3 Effect on disease

Abnormally high liver Cu levels are characteristic of a number of diseases in man. These include Mediterranean anemia, hemochromatosis, cirrhosis and yellow atrophy of the liver, tuberculosis, carcinoma, sever chronic diseases accompanied by anemia, and Wilson's disease (hepatolenticular degeneration) [73]. Extremely high liver Cu levels, as high as 4000ppm Cu (dry, fat-free basis), can occur in chronic Cu poisoning [73].

1.9.7.2.4 Bone disorders

The skeletal changes are a specific effect of the Cu deficiency unrelated to the concurrent anemia. The Histological changes in affected bones and in those of Cu-deficient animals [58, 74] were thinned cortices, broadened epiphyseal cartilage, and a low level of osteoblastic activity. The bones were normal in ash, Ca, P, and Mg contents of this ash were similarly found to be normal [58].

1.9.7.2.5 Toxicity

Chronic Cu poisoning may occur in animals (a) under natural grazing conditions, (b) as a consequence of excessive consumption of Cu-containing salt mixtures, common in cattle food, (c) from the unwise use of Cu-containing drenches, (d) from contamination of feeds with Cu compounds from agricultural or industrial sources, and (e) through animals given Cu supplements as growth stimulants if the basal diet is not suitably balanced with other minerals with which Cu interacts.

In all animals the continued ingestion of Cu in excess of requirements leads to some accumulation in the tissues, especially in the liver. The capacity for hepatic Cu storage varies greatly among species, and differences among species in tolerance to high-Cu intakes are also great. The liver Cu levels attained were much lower than those characteristic of chronic Cu poisoning occurred [58].

Copper interacts metabolically with so many other elements, such as Zn, Fe, Cd, and Mo, as considered elsewhere, that it is impossible to give maximum safe or minimum tolerable dietary Cu levels based on Cu alone. A series of such levels exist depending on the extent to which these interacting substances are present or absent from the diet [58, 74, 75].

1.9.7.2.6 The Zn:Cu Dietary ratio as a risk factor in coronary heart disease

An imbalance in Zn and Cu metabolism may contribute to the risk of coronary heart disease (CHD) [71]. This hypothesis was initially based on the results of experiments with rats consuming a cholesterol-free diet in which the intakes of Zn and Cu were varied by varying the ratio of salts of these elements in the drinking water. Water with a Zn:Cu ratio to other risk factors in CHD. For example, a relationship between the amount of fat and the ratio of Zn to Cu of foods was demonstrated [66, 69]. In some regular meals and diets in USA shown considerable variability to have Zn:Cu ratios in excess of those which produce hypercholesteremia [59, 61], and the mortality rate for CHD and the ratio of Zn:Cu in milk of 47 cities in the USA were found significant [76]. Attention was also drawn to the higher Zn:Cu ratio in cow's milk than in breast milk. It was suggested that one of the benefits that might be gained from a return to breast-feeding is a reduction in CHD. The above findings suggest that dietary Zn:Cu ratios must be given further critical consideration in future epidemiological and experimental studies of the etiology of CHD [58, 74, 76].

1.9.7.3 Mn

The body of a normal70 kg man is estimated to contain a total of 12 to 20mg Mn [63, 64]. This relatively small amount of Mn is distributed widely throughout the tissues and fluids, without notable concentration in any particular location and with comparatively little variation among organs or species, or with age [69]. However, Mn tends to be higher in tissues rich in mitochondria and is more concentrated in the mitochondria than in the cytoplasm or other organelles of the cell [69]. The pigmented portions of the retina are richer in this metal than most body tissues. The pigmented melanin-containing parts of the conjunctiva are higher in Mn than the non-pigmented parts [69, 70].

1.9.7.3.1 Requirements

The minimum dietary requirement of Mn vary with the species and genetic strain of animal, the chemical from in which the element is ingested, the composition of the rest of the diet, and the criteria of adequacy employed.

1.9.7.3.2 Sources and the amounts in human diets

The common foods in human dietaries are highly variable in Mn concentration. It is found that several major food groups contain Mn on the fresh basis [61, 69]. The concentrations ranged from 23ppm for the richest groups to as low as 0.2ppm for the poor groups.

1.9.7.3.3 Deficiency and functions

Manganese deficiency has been observed in man is association with a vitamin K deficiency [69, 77], The main manifestations of Mn deficiency, namely, impaired growth, skeletal abnormalities, disturbed or depressed reproductive function, and defects if lipid and carbohydrate metabolism are displayed in all species studied, but their actual expression varies with the degree and duration of the deficiency and its rate of development and with the age and stage of growth of the animal.

Hemoglobin levels do not appear to be significantly affected by lack of Mn [59, 66]. The growth inhibition of Mn deficiency results from both reduced food consumption and impaired efficiency of food use, but severe impaired appetence is not a conspicuous feature of Mn deficiency, as it is occurred in Zn and Co deficiencies [49].

1.9.7.3.4 Toxicity

Manganese toxicity in man arising from excessive intake in foods and beverages has never been reported and is difficult to visualize ever arising, except where industrial contamination occurs. Chronic Mn poisoning occurs among miners following prolonged working with Mn ores. Excess Mn enters the body mainly as oxide dust via

the lungs and also via the gastrointestinal tract from the contaminated environment [46, 66, 70]. The lungs apparently act as a depot from which the Mn is continuously absorbed. Manganese poisoning is characterized by a severe psychiatric disorder resembling schizophrenia, followed by a permanently crippling neurological disorder clinically similar to Parkinson's disease. Comparative studies of a population of "healthy" Mn miners and patients suffering from chronic Mn poisoning, revealed faster losses of injected ^{54}Mn from the whole body and from an area representing the liver and higher tissue Mn concentration in the former group than in those suffering from chronic Mn poisoning [70]. The presence of elevated tissue Mn levels is thus not necessary for the continuance of the neurological manifestations of the disease, and metal chelation therapy is unlikely to secure their remission.

1.9.7.4 Co

Cobalt is an essential trace element, being present in vitamin B_{12}. Although Co levels in milk are low, milk actually supplies about one-quarter of the average dietary contribution.

1.9.7.4.1 Requirements and relation with Vitamin B_{12}

Under grazing conditions, lambs are the most sensitive to Co deficiency, followed by mature sheep, calves, and mature cattle, in that order [78]. Field experience suggests that species differences among ruminants in Co requirements are small. Early evidence indicated that 0.07 or 0.08 ppm Co in the dry diet was just adequate for sheep and cattle [79]. This level of dietary Co, therefore, became accepted as the minimum requirement for these species. Later studies placed the minimum level of "pasture associated" Co required by growing lambs appreciably higher, namely, 0.11ppm on the dry basis. In a study of a marginally Co-deficient area it has been assessed that the mean values of 0.11ppm Co or more would probably exclude the likelihood of Co deficiency [79]. Mean values approaching 0.08ppm would suggest but not prove actual or potential existence of the disease." More precise estimates of minimum Co requirements applicable under all grazing conditions are difficult because of the influence of many variables such as seasonal changes in Co

concentrations, selective grazing habits, and soil contamination. It has been found that intake of Co to ensure optimum growth and hemoglobin production is 0.08 mg day^{-1} when supplementary Co is giver three times each week. Therefore, in growing lambs the requirement was stated to be higher, because for the diets with lower level of Co there is a significant reduction in vitamin B_{12} production [80]. .

1.9.7.4.2 Diagnosis of Co-vitamin B_{12} deficiency

The milder forms of Co deficiency in ruminants are impossible to diagnose with certainty on the basis of clinical and pathological observations alone. A secure diagnosis of Co-vitamin B_{12} deficiency can be achieved in these circumstances by measuring the response in temperament, appetite, and live weight that follows Co feeding or vitamin B_{12} injections. However, if the ration of grazing consistently contains less than 0.08ppm Co, cobalt-vitamin B_{12} deficiency can be predicted with confidence.

1.9.7.4.3 Requirements for human

Cobalt must be supplied in the diet of man entirely in its physiologically active form, vitamin B_{12}. Human tissues are unable to synthesize the vitamin from dietary Co, and their intestinal micro flora have an extremely limited capacity to effect this vital transformation at a point in the digestive tract where the vitamin can be absorbed. In these unique circumstances the Co status of human foods and dietaries is relatively unimportant; it is their vitamin B_{12} status that is crucial.

Reported data for the Co content of human foods and total diets are both meager and highly variable. Some of the variation undoubtedly from analytical errors or inadequacies, but some also reflects soil and climatic differences directly affecting the Co content of foods of plant origin and indirectly those of animal origin.

Among individual types of foods, the green leafy vegetables are the richest and most variable in Co content, while dairy products, refined cereals, and sugar are the poorest. Typical values for the former group are 0.2 to 0.6 ppm, and for the latter 10

to 30 ppb Co [70]. Plant products have been estimated to contribute up to 88% of the total Co of Japanese diets [70]. Normal cow's milk is very low in Co, with most values lying close to 0.05µg L^{-1} (ca. 0.05ppb). The organ meats, liver and kidney, commonly contain 0.15 to 0.025ppm, and the muscle meats about half of those levels. These foods contain much more Co than can be accounted for as vitamin B_{12}. Fruits, vegetables, and cereals contain none of their cobalt as vitamin B_{12}.

1.9.7.4.4 Distribution in tissues and fluids

The total Co content of the body of adult man has been reported to an average 1.1 mg, with about 43% of this total stored in the muscles, 14% in the bones, and the remainder distributed among other tissues [81]. Excessive accumulation does not occur in any particular organ or tissue, but the liver, kidneys, and bones usually carry the highest concentrations of this element. The Co concentrations reported for normal human tissues are found similar or appreciably higher to those of other species [81]. Such differences probably reflect analytical variations, although regional differences in Co intakes may also be a factor. On the basis of rather limited evidence it seems that Co does not accumulate in human tissues with age [81]. Representative values for Co levels in human and bovine tissues are given in the following Table 1.14.

Table 1.14: Co Concentration in human and bovine tissues[a]

Species	Liver	Spleen	Kidney	Heart	Pancreas
Normal human	0.18	0.09	0.23	0.10	0.06
Healthy sheep	0.15	0.19	0.25	0.06	0.11
Co-deficient sheep	0.02	0.03	0.05	0.01	0.02

[a]µg/g fresh tissue

The concentrations of Co in the tissues are below normal in Co deficiency [79-81], and can be increased above normal by Co injections or oral supplements. Levels in the liver have attracted special attention because of their possible value in diagnosing Co deficiency in ruminants in the field. It has been showed that Co, unlike Fe and Cu,

does not normally accumulate in the fetal liver. However, the Co (and vitamin B_{12}) content of the liver of the newborn lamb and calf is deduced below normal when the mother has been on a Co-deficient diet and can be raised to normal levels by prenatal Co administration [78, 81]. .

1.9.7.4.5 Toxicity

Cobalt has a low order to toxicity in all species studied, including man. Daily doses of 3mg Co kg^{-1} body weight, which approximate 150ppm Co in the dry diet or some 1000 times normal levels, can be tolerated by sheep for many weeks without visible toxic effects [78]. With a dose of 4 or 10mg Co kg^{-1} body weight, appetite and body weight are found severely depressed, the animals become anemic, and some deaths occur at the higher level. The anemia probably arises from a depression in Fe absorption by the very high intakes of Co. It has been estimated that a single dose of 300mg Co kg^{-1} body weight a soluble salt would usually be lethal, and that single doses as 40 to 60mg Co kg^{-1} body weight would occasionally be fatal [80-82].

1.9.7.5 Cd

Cadmium minerals are scarce, but as a result of its chemical similarity to Zn, Cd occurs by isomorphous replacement in almost all Zn ores. Growing plants require Zn and they also take up and concentrate Cd with the same biochemical process. The out break of Cd poisoning occurred in Japan in the form of “itai itai” disease. Many people suffered from this disease in which their bones became fragile. At high levels, Cd causes bone marrow disorders. Cadmium, like is a cumulative poison hence levels in food should be kept to a minimum. A significant part of man’s intake results form inhalation from air contaminated by Cd, and cigarette smokers increase their intake by 25-50%.

As with Pb, the only threat to milk comes from forage and fertilization of feedstuffs with sewage sludge containing Cd. Again, as with Pb, the cow acts as an effective biological filter and the proportion of ingested Cd finding access to milk is extremely small. Milk and milk products therefore contribute a negligible amount to the weekly acceptable intakes are recommended [69, 70, 83].

1.9.7.5.1 Source

Cadmium enters the biosphere through its increasing use in electroplating, in plastics as stabilizers, in paints as pigments, in Cd batteries, and as a contaminant in phosphate fertilizers and sewage sludges. In the absence of Cd releasing factories, the levels in the air approximate 0.001µg Cd m^{-3} which would lead to a maximum inhaled amount of 0.02µg per person day^{-1}. In large cities higher levels approaching 0.03µg m^{-3} may be found [59, 70]. The amount inhaled from the air in most circumstances is insignificant compared with that ingested with the food, with the exception of heavy smokers who could have an intake of 5µg Cd day^{-1} or more from this source alone [70]. Moreover such inhaled Cd is much better absorbed than ingested Cd. Most municipal waters contain less than 1-3µg Cd L^{-1} [70], even at that upper level the consumption of 2.5 µg Cd L^{-1} would provide only 25µg Cd day^{-1}. Food is thus normally the major source of Cd to animals and nonsmoking humans.

1.9.7.5.2 Distribution in animal tissues and fluids

Cadmium is virtually absent from the human body at birth and accumulates with age up to about 50 years. At this age the average person not exposed to abnormal amounts of Cd has a total body burden of 20 to 30mg Cd of which one-half to two-thirds occurs in the liver and kidneys [83]. The concentration of Cd in the liver and kidneys, particularly the kidney cortex, is apparent from many studies with several species. Normal human blood is low and variable in Cd content. The mean Cd concentration in human hair has been reported as 2.76 to 0.48µg g^{-1} in males and 1.77 to 0.24µg g^{-1} in females [83]. These levels are slightly higher than the 1 to 2ppm quoted for human hair [83, 84].

1.9.7.5.3 Toxicity

Cadmium is toxic to virtually every system in the animal body, whether ingested, injected, or inhaled. Histological changes have been observed in the kidneys, liver, gastrointestinal tract, heart, testes, pancreas, bones, and blood vessels [83], and hepatic protein-bound Cd has been associated with chronic pulmonary diseases in a

group of patients without unusual contact with Cd [83]. Anemia is a common manifestation of chronic Cd toxicity in all species, due at least in part to its metabolic antagonism to Cu and Fe. Zn deficiency can also be induced by Cd, acting similarly as a metabolic Zn antagonist. The most striking morphological change produced by the long-term ingestion of toxic Cd levels was hepatic malfunction and interstitial renal fibrosis.

1.9.7.6 Cr

A number of reports of the Cr content of human foods and dietaries have appeared but many of these are of little value for nutritional purposes because (a) analytical methodology and instrumentation in the past have been inadequate, and (b) little is known of the forms of Cr present and their relative absorbability and biological activity.

1.9.7.6.1 Requirements and sources

Dietary Cr intakes by man are clearly greatly influenced by the amounts and proportions of refined carbohydrates consumed. An institutional diet provided about 80 μg Cr person^{-1} day^{-1} [85], while in other studies with diabetics and old people in which some responses to Cr supplementation were obtained, the daily intake was estimated to be as low as 50 μg [86]. Chromium intakes in USA have been stated to "vary from 5 μg to over 100 μg day^{-1}" [86], higher levels than these were reported for well-balanced J diets [85, 87].

The minimum human Cr requirements compatible with satisfactory growth and long-term health and fertility cannot yet be given because of inadequate knowledge of the forms and availability of Cr in foods. It has been calculated that a daily intake varying from 20 to 500μg Cr, depending on the chemical nature of Cr in individual foods, would be needed to compensate for a urinary loss of 5μg Cr day^{-1} [85].

1.9.7.6.2 Deficiency and functions

Chromium deficiency is characterized by impaired growth and longevity in experimental animals and by disturbances in glucose, lipid, and protein metabolism. Cr supplementation prevents the appearance of the lesion on body tissues but does not cure the fully developed defect. The biochemical mechanism underlying this pathological change has not been understood and determined.

1.9.7.6.3 Distribution in animal tissues and fluids

Chromium is widely distributed throughout the human body in low concentrations without special concentration in any known tissue or organ, and that these levels decline with age, except in the lungs, Human stillborn and infant tissues carry higher Cr concentrations than those of adults, These levels decline rapidly in the first decade of life in the heart, lung, aorta, and spleen, while in the liver and kidney the neonatal concentrations are maintained until the second decade, when a decline occurs [85, 85]. Substantial variations in human liver and kidney Cr levels have been observed in different geographical regions [85], presumably as a reflection of regional differences in environmental Cr intakes. The reported levels of Cr in blood have declined markedly in recent years, but a reliable normal range for human blood can still not be given with complete confidence.

1.9.7.6.4 Toxicity

Hexavalent Cr is much more toxic than trivalent, In fact trivalent Cr has such a low order of toxicity that a wide margin of safety exists between the amounts ordinarily ingested and those likely to induce deleterious effects. Lifetime exposure to 5mg L^{-1} of Cr (III) in the drinking water induced no toxic effects, and similar exposure to mice, for three generations to Cr oxide at levels up to 20ppm of the diet had no measurable effect on mortality, morbidity, growth, or fertility (33). Chronic exposure to chromate dust has been correlated with increased incidence of lung cancer [85], and oral administration of 50ppm of chromate has been associated with growth depression and liver and kidney damage in experiments [86].

1.9.7.7 Pb

Lead (Pb) is a threat to man via food, drink or inhalation. Biological interest in Pb has centered principally on its properties as a highly toxic cumulative poison in man and animals. In recent years the problem of long term exposure to increased amounts of Pb in highly urbanized and motorized environments has engaged particular attention. The possibility that Pb in low concentrations performs some vital functions cannot be excluded, especially in the light of the suggestive evidence [88] that Pb is required for growth in animals. However, more clear-cut data are required before Pb can be included among the essential trace elements.

Chronic lead poisoning is caused by long exposure at levels of about 1mg day^{-1}. The major biochemical effect of Pb is its interference with heme synthesis, which leads to hematological damage. Lead inhibits several of the key enzymes involved in the overall process of heme synthesis whereby the metabolic intermediates accumulate. The overal effect is the disruption of the synthesis of hemoglobin as well as other related respiratory molecules such as cytochromes which require heme. Ultimately, lead does not permit utilization of O_2 and glucose for life sustaining energy production. This interference can be detected at a Pb level in the blood of about 0.3 ppm. At higher levels of Pb in the blood (> 0.5 ppm) cause kidney malfunction and finally leads to the damage of brain.

1.9.7.7.1 Sources

The “natural” level of lead in air, if there were no contribution from man-made pollution, has been estimated to be 0.005μg m^{-3} [89]. Actual atmospheric Pb levels found ranging from 0.4 to 7.6μg m^{-3} in different cities, at sites with varying motor traffic densities [70]. Most of this Pb comes from the exhaust fumes of cars burning petrol containing lead alky additives, so that intakes from this source will clearly be much greater in motorized urban communities than in rural areas. In such urban communities the amount of Pb absorbed via the lungs can be as much or greater than the amount retained from the diet, although most people ingest very much more from the diet than they inhale from the air [70, 88]. Atmospheric Pb contributes to total

intakes by dust fall as well as by inhalation. High concentrations of Pb have been found on roadside soil and grass with the Pb content declining with increasing distance from the road [70]. Some of this lead fallout from the atmosphere can be incorporated into vegetable crops and rainwater.

Surface waters used for domestic purposes vary greatly in lead content. Many domestic water supplies can exceed this limit where the water is soft and comes from lead-lined tanks and water pipes. The mean Pb contents of the cold tap water were found, in the order given, approximately 1000, 220 and 100μg L^{-1} [89, 90]. Consequently, the blood lead of the inhabitants showed a significant positive correlation and the erythrocyte – aminolevulinic dehydrase activity showed a significant negative correlation with water lead content.

1.9.7.7.2 Distribution in animal tissues and fluids

The total body burden of lead in "normal" adult man ranges from 90 to 400 mg [69, 70]. The affinity of bone for Pb and the much higher Pb concentrations in bone than in soft tissues are apparent in few studies [88-90]. Human soft tissues were reported to be a range from 0.13 to 0.50 ppm Pb in the brain and from 1.3 to 1.7ppm Pb (wet weight) in the liver. The levels in human fetuses of 7 to 8 months gestation ranged from 0.17ppm in the brain to 0.68ppm Pb in the liver [88]. Relatively high Pb concentrations in the tissues of stillborn infants were also demonstrated [88]. Lead was shown to accumulate in the tissues with age, up to 50 to 60 years particularly in the bones, kidney, liver, lung, and spleen [69, 70]. However, no comparable increase with age, except in aorta, was observed in tissues from Africa and the Middle East, and their median values were generally lower than those in the USA [69, 70]. Similarly, the mean concentrations of Pb in the tissues of English residents were also reported, where the pattern of distribution were with the highest levels in the bones and the lowest in the muscles, in similar in normal laboratory and farm animals to that in man, and concentrations are of the same order as those just cited for human tissues [69, 70, 88-90].

Lead concentrations increase at high Pb intakes in all tissues, except the muscles, and especially in the bones, liver, kidney, and hair. The hair of normal children has been reported to range from 2 to 95μg g^{-1}, compared with a range of 42-975μg g^{-1} for the hair of patients with chronic Pb exposure [88]. Lead does not accumulate significantly in the tissues at moderately high dietary Pb intakes because of corresponding increases in excretion, but at higher intakes, substantial tissue deposition occurs.

The Pb which is absorbed enters the blood and reaches the bones and soft tissues of the body, from which it is gradually excreted via the bile into the small intestine and thence eliminated in the feces. The increases in Pb concentrations that occur with age in human tissues indicate that excretion is not quite keeping in pace. However, there is little evidence that tissue Pb accumulations of those magnitudes are either harmful or harmless to human [88-90].

1.9.7.7.3 Toxicity

The symptoms and pathology of acute Pb poisoning lie outside the scope of this text and have been well documented [89]. Chronic Pb poisoning is characterized particularly by neurological defects, renal tubular dysfunction, and anemia [90]. Damage to the central nervous system causing Pb encephalopathy and neuropathy, which is a common feature, especially in children with their low Pb tolerance [90, 91]. In children, chronic Pb poisoning involves physical brain damage, including behavioral problems, intellectual impairment, and hyperactivity. The mechanism by which Pb affects the nervous system is largely obscure, although it is known to block both impulse transmission and acetylcholine release [90, 91].

The anemia that is a common feature of chronic Pb poisoning arises from effects on heme synthesis and red blood cells [90], and perhaps also from an effect of Pb on iron and copper metabolism. Heme synthesis is affected primarily by the inhibition of particular enzyme that regulated by the incorporation of Fe into the heme molecule. Lead also affects the fragility of red cells which have a shortened life span. These effects of Pb are the main cause of the anemia, but there are also reports that indicate the effects of Pb and Fe deficiency on impairment of hematopoiesis [90, 91].

1.9.7.7.4 Tolerance and criteria of safety

Tolerance to Pb varies with the age, the forms and sources of the Pb, and the composition of the diet being consumed. For most of the species studied, it was found obvious that the young animal is less tolerant than the adult counterpart. The higher absorption of dietary Pb occurred for the young also. Lower tolerance occurred to inhaled Pb than Pb to be ingested, particularly when the former is of low particle size, that relates to the substantially higher retention of inhaled than ingested Pb.

The exacerbation of Pb toxicity symptoms induced by dietary deficiencies of Ca and Fe, and the further interactions of Pb with P, Zn, and Cu, also discussed indicate clearly that tolerance to particular, potentially toxic, Pb intakes is greatly influenced by the dietary levels of these elements relative to that of Pb. However, results also pointed out that Ca and Fe intakes are most likely to be inadequate in children and pregnant women, the population groups most susceptible to Pb poisoning [56]. Therefore, the provision of adequate intakes of Ca and Fe appears to be highly desirable to minimize the potential hazards of chronic exposure of children and women to Pb.

Because tolerance to Pb is influenced by so many factors, a series of maximum, long term, “safe” tolerances exists, depending on the extent to which one or more of those factors continue to operate. Definition of the threshold or thresholds at which adverse affects arise is there for difficult and data and criteria on which such thresholds could be based are meager. It can be stated that about 100μg Pb must be assimilated daily by adult man for blood Pb concentrations to exceed 40μg $100g^{-1}$, the level at which most individuals reveal increased urinary alpha linolenic acid (ALA) excretion. On this basis, a blood lead of 40μg $100g^{-1}$ can tentatively be taken to indicate the threshold at which the body burden of Pb exceeds the homeostatic mechanisms and adverse effects could become evident. The WHO gives a provisional tolerable weekly intake of Pb by man as 3 mg per person or 0.05mg kg^{-1} body weight [92]. It is pointed out that these intake levels do not apply to infants and children. Efforts to decrease exposure to such readily assimilable forms of Pb as atmospheric Pb appear to be thoroughly justified by the available evidence.

1.9.7.8 As

Arsenic commonly occurs in insecticides and herbicides. Among its compounds, those of As (III) are the most toxic. Arsenic (III) exerts its toxic action by attacking-SH groups of an enzyme, thereby inhibiting enzyme action. The enzymes which generate cellular energy in the citric acid cycle are adversely affected. The inhibitory action is based on the inactivation of pyruvate dehydrogenase by complexation with As (III), whereby the generation of ATP is prevented.

1.9.7.8.1 Sources

Arsenic is widely distributed in the biosphere. It occurs in the air in areas where coal is burnt, particularly near smelters and refineries, in seawater to the extent of 2 to 5 ppb, and in public water supplies in concentrations which may exceed that of seawater [93-96]. Arsenic occurs in normal soils at levels ranging usually from 1 to 40ppm, although much higher levels can result from the continued use of arsenical sprays for insect control [95]. The amounts absorbed from these soils by the aerial parts of plants have been reported to be small, but recent evidence indicates that certain plants growing on As-enriched soils can accumulate extremely high As levels [96]. Concentrations as high as 2080 and 3470ppm As (dry basis) were obtained for the foliage of samples. Surface contamination of herbs, fruits, and vegetables with spray residues can also rise their As concentrations well above normal levels. Most human foods contain less than 0.5ppm As and rarely exceed 1 ppm on the fresh basis [97-99]. This applies to fruits, vegetables, cereals, meats, and dairy products.

The total amounts of As ingested daily are obviously greatly influenced by the amounts and proportions of sea origin foods included in the diet. As institutional diet containing no such foods was reported to supply 0.4mg As day^{-1} and an average diet to supply 0.9mg day^{-1}, these calculated daily intakes are substantially higher than the 0.07-0.17mg As day^{-1} reported for Japanese individuals [100].

1.9.7.8.2 Distribution in tissues and fluids

Arsenic is widely distributed throughout the tissues and fluids of the body in low and highly variable concentrations. In a study of healthy adult human tissues using neutron activation analysis, reported the mean As concentrations of most tissues to lie between 0.04 and 0.09ppm on the dry basis [101]. The variability was extremely high and the skin, nails, and hair contained substantially higher values which is consistently different from other tissues, with no evidence of marked accumulation in any internal organ or tissue. The levels of As reported for human blood vary widely among individuals and among investigations using different analytical methods.

Normal cow's milk contains 0.03-0.06ppm As [69, 70] with values up to 1.5 ppm in the milk of cows grazing As contaminated areas. High levels have also been found in the milk of women receiving As therapy for diseases.

1.9.7.8.3 Toxicity

The symptoms of acute As poisoning in man by the oral route-nausea, vomiting, diarrhea, burning of the mouth and throat, and severe abdominal pains-have frequently been described. Chronic exposure to smaller toxic doses results in weakness, and muscular aching with few gastrointestinal symptoms. Skin and mucosal changes usually develop, together with a peripheral neuropathy and linear pigmentations in the fingernails [102]. Headache, drowsiness, confusion, and convulsions are seen in both acute and chronic As intoxication. The biochemical basis for these disturbances is probably an inhibition by arsenite of a wide range of enzyme systems. Enzymes containing active thiol groups are effectively inhibited through combination of As with these groups [102].

Part II: Status of Bangladesh

1.10 Overview of Bangladesh

1.10.1 The Geo-Physical Environment of Bangladesh

Bangladesh is a country of about 144.862 square km including inland and estuarine water surfaces. It is located between 20° 34′ N and 26° 38′ N latitude and between 88° 10′ E and 92° 41′ E longitude. The country is bordered by India in the west, north and east except for a small portion in the south east by Myanmar. The entire south is occupied by the Bay of Bengal. Although the country is predominantly a plain surface, it is criss-crossed by a very high density of river systems. This gives the country a fluvial nature [103]. Bangladesh has a tropical monsoon climate. The seasonal distribution of climatic elements is broadly similar throughout the country but eastern parts are much wetter than western parts. With the exception of the relatively dry western region, where the annual rainfall is about 1600 mm, most parts of the country receive at least 2000 mm of rainfall per year [104-06]. Mean annual temperature everywhere is about 25°C. Mean monthly temperatures range between about 18°C in January to about 30°C in April-May. Extreme temperatures range between about 4°C & 43°C were also recorded.

1.10.2 Economical Status and Main Resource Bases

Bangladesh is primarily an agrarian economy. Agriculture is the single largest producing sector of economy since it comprises about 30% of the country's GDP and employing around 60% of the total labor force [105, 107]. The performance of this sector has an overwhelming impact on major macroeconomic objectives like employment generation, poverty alleviation, human resources development and food

security. In Bangladesh about 75% of the population depends directly or indirectly on agriculture for its livelihood. Meeting the nation's food requirements remains the key-objective of the government and in recent years there has been substantial increase in grain production. Rice being the staple food, its production is of major importance. Rice production stood at 28.3 million tons in 2009-2010 fiscal years. Crop diversification program, credit, extension and research, and input distribution policies pursued by the government are yielding positive results. The country is now on the threshold of attaining self-sufficiency in food grain production [105].

The Title of this research does not indicate of the areas where the research is took place; however, the issue would be described in the Methodology chapter (Chapter 2). Therefore, it is thought that basic information regarding the specific areas of interest ought to be presented prior to that description.

1.10.2.1. Food security situation: Bangladesh context

- Bangladesh has made substantial progress in increasing food grain production over the last two decades. The production of food grains in 2002 to 03 was 26.70 million metric tons, which was reached to 28.60 million tons in the year 2003 to 04. This has led to improve overall food security situation, where per capita availability of food grains for FY03 (fiscal year 2003) were 202 kg capita-^{1}year^{-1}.
- Poverty head count ratio remains at the level of 44.3% (5.5 million people lying under poverty line). The hard-core poverty head count ratio, though, declined over the year’s still counts more than 24.5 million people. Both rural and urban Poor have low incomes and thus low purchasing powers, which increase the chances of consuming food of poorer quality that may well be also unsafe.
- Nutrition and food utilization are increasingly recognized as key components of food security in Bangladesh, having one of the highest rates of malnutrition in the world. Economic analyses indicated that without improvements in the nutritional status of the population, 22.9 Billion US$ in productivity will be lost to the country between 2000 & 2010 (UNICEF & ADB, 1980) [105, 107].

- Urban population are gradually shifting from cereal-based diets and would likely generate a demand for fish, livestock, horticultural, forest produce as well as processed items, in turn necessitating safety load of associated transport, storage and marketing infrastructure.

1.10.2.2 Rajshahi: Main Western City Corporation of the Country

Rajshah is a city in western Bangladesh, and the divisional headquarters of Rajshahi Division as well as the administrative district that bears its name and is one of the seven metropolitan cities of Bangladesh [107]. Rajshahi is located in the north-west of the country and is situated on the northern banks of the river Padma (or Ganges which is one of the major rivers of the Indian subcontinent). Its total area is 96.69 km^2, and the estimated population is 853,000, which might show it's least density (in terms of population) compared to other metropolitan cities. Apart from the usual agricultural products of Bangladesh, such as rice, wheat, potatoes and lentils, this region also producing various crops such as mangoes, sugarcanes, lichies etc. Therefore, this region is considered as a production-surplus area of the county. In terms of dairy production, as an integral part of total agriculture, is not properly addressed in any authoritative publications (like BBS yearbooks [107]). In spite of being an important city and located on a riverbank, industrial development in Rajshahi has not taken place to any great extent.

1.11 Milk production in Bangladesh

Information about total milk production of the country is difficult to find in available documents even those are published by the related authority. However, data of milk consumption (appearing in the succeeding section) would be helpful to have a sense about the scale of production. However, to that effect, information about country's export and import of various milk commodities (see Table 1.15) need to be evaluated to account that provisional estimation.

Table 1.15: Country's Export and Import of milk powder and other dairy commodities for four fiscal years

Export				
Commodity	**Amounts (in ca. M. Tons)**			
	(2007-08)	(2008-09)	(2009-10)	(2010-11)
Milk powder	25	68.675	19.468	0.378
Import				
Commodity	**Amounts (in ca. M. Tons)**			
	(2007-08)	(2008-09)	(2009-10)	(2010-11)
Milk & cream	415.904	407.063	233.608	17132.721
Milk powder	47856.465	73907.389	47561.279	67580.066
Whey	5424.375	4003.137	3263.690	6849.866
Butter oil	174.518	52.887	115.757	87.156
Cheese and other	61.323	41.544	86.546	103.906
Ice-cream	About 50 tons in each fiscal year			

Information about the number of livestock (including commercial & subsistence) of the country (see Table 1.16) can be found in Year Handbook (e.g., BBS 2011 [108, 109]), but that also may not be helpful to the extent of that calculation of yearly milk production of the country. As because of there is no indication of what percentages of livestock are comprises as dairy cattle and the average amount of milk they produce.

Table 1.16: Some statistics of livestock of the country

Livestock	**Number in whole country, $X10^6$**	**Number in Rajshahi Division, $X10^6$**	**Number in Rajshahi District, $X10^6$**
Cattle & buffaloes	25.13	8.5 (33.83% of the whole country)	0.4 (4.7% of the division; and 1.6% of the country)

1.12 Milk consumption in Bangladesh

Bangladesh has lower levels of dairy product consumption than other countries in North America, Europe, South Asia and even lower than Sub-Saharan countries in Africa (see Table 1.18). This very poor milk consumption of the country could pose serious damage to the nation human resources and as well as country's economy.

1.13 Uses of milk for milk products

Bangladesh does not have a long history of a formal dairy industry, but indigenous milk production, processing and consumption have ancient roots. The proportion of milk that goes to sweets and other products is estimated at around 42% percent (Personal communication with authoritative personnel). However, BBS notion is 50% of total milk consumption is used as variety of milk products and milk-based sweetmeats [107-109].

An elaborate description about variety of milk products/derivatives are shown in through a pictorial diagram (see Figure in Appendix). However, most common milk derivatives are arranged and related themselves with their subcontinent names in the following table (Table 1.17), afterwards, a brief description of each derivative (both by their worldwide name and subcontinent names) are given below.

Table 1.17: Per Capita Availability of Milk and Milk Products for Domestic Consumption in Bangladesh for Several Years [107]

Year	LME Per Capita (Kg)	Year	LME Per Capita (Kg)
1991-92	8.6	1998-99	13.2
1992-93	8.3	1999-00	13.5
1993-94	8.5	2000-01	13.5
1994-95	9.5	2001-02	13.3
1995-96	10.7	2002-03	6.9
1996-97	13.3	2003-04	6.7
1997-98	13.1	2004-05	7.9

LME is Liquid Milk Equivalent

Table 1.18: Per capita annual dairy product consumption in Kenya, Ghana and Bangladesh in 1999 [2,9,107]

Year 1999	LME per capita (kg)
Bangladesh	19
Kenya	85
Avg., Sub-Saharan Africa	55
Avg., South Asia	72

LME is Liquid Milk Equivalent

Table 1.19: Common milk derivatives and their subcontinent analogous

International/worldwide name of the Derivative	Subcontinent name of the Derivative
Butter	*Makkhan*
Clarified butter	*Ghee*
Cheese	*Paneer (Chhana* or *Chana*)
Evaporated milk	***Khoa*** (*Moa* or *Maowa*)
Yoghurt (yoghourt or yogurt)	Curd plus Whey

1.13.1 Butter

Butter is a pale yellow colored dairy product made by churning fresh or fermented cream or milk. Butter consists of butterfat, milk proteins and water. Most frequently made from cows' milk, butter can also be manufactured from the milk of other mammals, including sheep, goats, buffalo etc [110]. Rendering butter produces clarified butter or *ghee*, which is almost entirely butterfat. Butter is a water-in-oil emulsion resulting from an inversion of the cream, an oil-in-water emulsion; the milk proteins are the emulsifiers. Butter remains a solid when refrigerated, but softens to a spread able consistency at room temperature, and melts to a thin liquid consistency at 32 to 35 °C. The density of butter is 911g L^{-1}.

1.13.2 Clarified butter

Clarified butter is milk fat rendered from butter to separate the milk solids and water from the butterfat. Typically, it is produced by melting butter and allowing the components to separate by density. The water evaporates, some solids float to the surface and are skimmed off, and the remainder of the milk solids sink to the bottom and are left behind when the butter fat is poured off.

1.13.3 Ghee

Ghee is a class of clarified butter that originated in South Asia and is commonly used in South Asian (Indian, Bangladeshi, Nepali, Sri Lankan, and Pakistani) cuisine and ritual. *Ghee* is a type of clarified butter that is prepared by boiling butter and removing the residue. *Ghee* has a long shelf-life and needs no refrigeration if kept in an airtight container to prevent oxidation. The texture, color and taste of ghee depend on the quality of the butter and the duration of the boiling.

1.13.4 Cheese

Cheese is a food derived from milk, produced in a wide range of flavors, textures, and forms. It consists of proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. It is produced by coagulation of the milk protein casein. Typically, the milk is acidified and addition of the enzyme *rennet* causes coagulation. The solids are separated and pressed into final form. Most cheeses melt at cooking temperature. Hundreds of types of cheese from various countries are produced. Their styles, textures and flavors depend on the origin of the milk, whether they have been pasteurized, the butterfat content, the bacteria and mold, the processing, and aging.

1.13.5 Paneer

Paneer is a fresh cheese common in South Asian cuisine. In eastern parts of Indian Subcontinent, it is generally called *Chhana* (or *Chana*). It is an un-aged, non-melting farmer-cheese or curd-cheese made through curdling heated milk with lemon juice, vinegar, or any other food acids. To prepare *paneer*, food acid (usually lemon juice, vinegar, citric acid or yogurt) is added to hot milk to separate the curds from the whey. The curds are drained in muslin or cheesecloth and the excess water is pressed out. The resulting *paneer* is dipped in chilled water for 2 to 3 hours to give it a good texture and appearance. From this point, the preparation of *paneer* diverges based on its use and regional variation.

1.13.6 Evaporated milk

Evaporated milk also known as dehydrated milk, is a shelf stable canned milk product with about 60% of the water removed from fresh milk. It differs from sweetened condensed milk, which contains added sugar. Sweetened condensed milk requires less processing since the added sugar inhibits bacterial growth. The product takes up half the space of its nutritional equivalent in fresh milk. When the liquid product is mixed with a proportionate amount of water, evaporated milk becomes the rough equivalent of fresh milk. This makes evaporated milk attractive for shipping purposes as it can have a shelf life of months or even years, depending upon the fat and sugar content. This made evaporated milk very popular before refrigeration as a safe and reliable substitute for perishable fresh milk, which could be shipped easily to locations lacking the means of safe milk production or storage.

1.13.7 Khoa

Khoa (or *Moa*, *Maowa*) is a yellow colored milk derivative widely used in Indian and Pakistani cuisine, made of either dried whole milk or milk thickened by heating in an open iron pan. It is similar to some kind of cheeses, but lower in moisture and made from whole milk instead of whey. A concentration of milk to $1/5^{th}$ volume is normal in the production of *khoa*. *Khoa* is used as the base for a wide variety of Indian sweets. About 600,000 metric tons *Khoa* are produced annually in India [110].

1.13.8 Yoghurt

Yogurt or yoghurt or yoghourt is a fermented milk product produced by bacterial fermentation of milk. The bacteria used to make yogurt are known as "yogurt cultures". Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and its characteristic tang. Worldwide, cow's milk, the protein of which mainly comprises casein, is most commonly used to make yogurt, but milk from water buffalo goats are also used in various parts of the world. In Western culture, the milk is first heated to about 80°C to kill any undesirable bacteria and to denature the milk proteins so that they set together rather than form curds. In some places, such as parts of India, curds are a desired component and milk is not pasteurized. The milk is then cooled to about 45°C. The bacterial culture is added, and the temperature is maintained for 4 to 7 hours to allow fermentation.

1.13.9 Curds

Curds are a dairy product obtained by *curdling* (coagulating) milk with *rennet* or an edible acidic substance such as lemon juice or vinegar, and then draining off the liquid portion. The increased acidity causes the milk proteins (especially casein) to tangle into solid masses, or curds. The remaining liquid, which contains only whey proteins, is the whey.

1.13.10 Whey

Whey or milk serum is the liquid remaining after milk has been curdled and strained. It is a by-product of the manufacture of cheese or casein and has several commercial uses. *Sweet whey* is manufactured during the making of *rennet* types of hard cheese like *cheddar* or Swiss cheese. *Acid whey* (also known as "sour whey") is a byproduct produced during the making of acid types of dairy products such as cottage cheese or strained yogurt.

Unlike the western trends of amount of milk-based products being produced each year from the raw milk they produce, a significant amount of liquid milk, here in Bangladesh, is regularly being used to produce variety of milk-based sweetmeats apart from common derivatives mentioned above.

1.14 Production of milk-based sweetmeat in Bangladesh

The sweetmeats are delicious, wholesome, nutritious and very fame item in Bangladesh. There is no such ceremony and festival, which goes without sweetmeats. Among this sweetmeat *Rossogolla* is the most popular on accounts of its high palatability and spongy texture. *Rossogolla* is one of the varieties of *Chhana* based sweetmeat. *Chhana* is highly recommended for diabetic patient on accounts of its high protein and low sugar content. A standard composition of *Chhana* is given in table in the following (Table 1.20). The various *Chhana* based sweetmeats are available in the markets. *Rossogolla* is being produced traditionally throughout the country. For this reason an attempt was taken to examine the product with a closer look.

As mentioned earlier that, *Chhana* consists of acid coagulated milk solids used for the preparation of many milk based sweets. It differs from *Paneer* in that no pressure is applied to remove the whey. *Chhana* is widely used in everywhere Bangladesh. Cow milk is preferred since it yields a soft bodied and smooth textured product. Both these characteristics are suitable for the production of high grade c*hhana*-based sweets like *Rossogolla*.

Buffalo milk produces a *chhana* with a slightly hard body, a greasy and coarse texture, and does not produce good quality *chhana*-based sweets.

Table 1.20: Composition of *Chhana*

Content (%)	Production from	
	Cow milk	Buffalo milk
Fat in dry matter	53.0	61.0
Protein	37.0	30.0
Lactose	4.6	4.8
Ash	4.4	4.1

Chhana has the same legal requirements as *paneer,* i.e. a maximum moisture content of 70% and a minimum content of milk fat in dry matter of 50%. *Chhana* from cow milk is light yellow in color, has a moist surface, soft body and smooth texture. On the contrary, *Chhana* derived from buffalo milk is whitish in color but yields a larger amount, but both have a pleasant sweetish, mildly acid taste.

1.14.1 Rasogolla: Chhana-based Sweets

This milk-based sweet is having been developed in nineteenth century (ca. in 1868) by a sweetmeat maker, *Nobin Chandra Das*, a Calcutta based enterprising [111]. It is prepared using fresh and soft-*chhanna*, in the form of balls, usually 30 mm in diameter, with a typical spongy body and smooth texture, often stored and served in dense sugar syrup [112-127]. The following descriptions are the condensed form of those articles cited here.

Freshly-made *chhana* is squeezed by hand in a muslin cloth to remove as much whey (or water) as possible. Possibly, 1 to 4 % of the wheat flour/semolina is mixed with the *chhana* in a container and kneaded thoroughly by hand to make dough. The dough is portioned and rolled into balls of about 15mm diameter having a smooth surface with no cracks, as 1 kg of *chhanna* presumably yields 90 to 100 *rasogollas*. The dough balls are cooked in specially prepared cooking medium with sugar for about 30 to 60 minutes. Once the cooking is complete, the balls are transferred to a container

with water at 30 to 35°C for texture stabilization and color improvement of the balls. After 5 to 10 min of texture stabilization in water, the texture stabilized balls are transferred to sugar syrup. The desired sugar syrup concentration in the final product is 45 to 50%. This is achieved by dipping the texture-stabilized balls first in 35 to 40% sugar syrup for 1 to 2 hours, followed by a second dipping in 58 to 60% sugar syrup. The product finally acquires the desired sugar concentration after equilibration between the sugar syrup inside and outside the balls is achieved.

The Bureau of Indian Standards has established the following specifications for *rasogolla* (see Table 1.21), however, such kind of standards are presumably absent in this country.

Table 1.21: specifications for *rasogolla* prepared by *chhana*

Constituents	**%**
Moisture	45 to 55.0
Milk fat	5.0
Sucrose	45.0
Protein	5.0

1.14.2 *Sandesh*: *Chhana & Khoa*-based Sweets

Sandesh is a sweet prepared from *Chhanna* and *khoa* with a somewhat firm body and a smoothy texture. The method of *sandesh* preparation varies from shop to shop or by the trader to trader [128-133]. The following descriptions are the condensed form of those articles cited here.

The art of sweet making is kept as a secret by the traders and they do not divulge the information at any cost to others. The traders prefer to use cow milk for *chhana* & *khoa* for *sandesh* preparation. *Chhanna* and *khoa* (more than 50%) and sugar are mixed and kneaded together and heated in a flat-bottomed vessel by continuous stirring with the help of flat edged wooden ladle after addition of color and flavor. Usually 600 to 950g of sugar is added to 2.5 kg of a mixture of *chhana* & *khoa* at a time, depending upon the variety of the sweet. Heating is continued at 75 to 85°C for 15–25 min, depending on the type of *sandesh*. The final temperature ranges from 60 to 70°C. The soft grade *sandesh* is usually moulded at room temperature. The heated mass is removed directly into moulds to give the desired shape. The sweets are now ready for eating. Alternatively, the processed mass is put into a tray, cooled and set. It can then be cut into desired shapes or moulded into required forms. There are several types of *sandesh* available in the market. One a drier variety made from old *Chhanna* and *khoa* is called *parasandesh*, often called only *para*. This is most preferable in quality *sandesh* and has a longer shelf-life than any other types which are softer and is having less shelf-life.

Varieties of *sandesh* available in the market may be broadly classified into three main groups: soft grade, hard grade (e.g., *para sandesh*) and high moisture grade. They are differentiated by their differing physical qualities and chemical composition. Hard grade *sandesh* contains smaller proportions of moisture, fat and protein, but higher amount of sucrose than soft grade *sandesh*. The sweet, besides being palatable, is also a rich source of milk proteins, fat, sucrose and fat soluble vitamins like A, D, E and K. Cow milk is usually preferred for *sandesh* making because of its soft body, smooth texture and small grains.

1.14.3 Composition of *Sandesh*

The final composition of the prepared *sandesh* is presented in the following table (Table 1.22). Significant differences in chemical composition from the earlier variety (*rosogolla*) can be noticed were probably due to the differences in the raw materials with probably different composition of milk and use of different protocol for preparation.

Table 1.22: Specifications for *sandesh* prepared by *chhanna* & *Khoa*

Constituents	%
Moisture	<20
Milk fat	20 to 30
Sucrose	<40.0
Protein	~20.0
Ash	~2.0
Acidity	<1.0

1.14.4 Textural parameters as affected by composition of *sandesh*

Use of milk with lower fat content i.e. decreasing fat from 6.0 to 1.5% significantly decreased the hardness of s*andesh*. However, relatively high moisture content in *sandesh* made from milk having lower fat content led to decrease in hardness. Fracturability was found negatively correlated with moisture content but positively correlated with fat content. The negative correlation between moisture content and fracturability of *sandesh* reveals that the samples having higher moisture content were softer and broke on application of even lower force. The cohesiveness, which reflects inter-particle binding force in any food product, did not seem to be influenced by compositional parameters. Correlation between cohesiveness and compositional parameters could not be established for *sandesh*. Decreasing the fat content in milk from 6% to 1.5% significantly decreased the gumminess of *sandesh*. Gumminess is a product of hardness and cohesiveness, hence followed similar pattern to that observed for hardness.

The overall textural parameters of *sandesh* were significantly influenced by the fat content of the milks. Most of the textural properties were influenced by moisture, fat and ash content. The cohesiveness of *sandesh* was not influenced by product composition.

1.15 Food adulteration in Bangladesh

Adulteration of food is the addition of substances to foods in order to increase the amount in bulk and reduces the cost, with an intention to defraud the purchaser. In the process of adulteration extraneous matters are directly added to food grains, therefore, common adulterants are water in milk, starch in spices, sands and crushed rock are also added to the food grains to increase its weight etc. Any food item may be considered as adulterated if its nature and quality are not up to the standard. Unscrupulous traders normally adulterate food for their economical benefit.

In Bangladesh, it is really hard to find any food items from fish to meat, vegetables to milk; or even from biscuits to juice that are not adulterated by any means. The hotels and restaurants are also serving these poisonous food items. Different reports show that adulterated foods are causing serious diseases including diarrhoea and other infected diseases round the year. Recently, the government and general public have been much worried about this issue. The government has set mobile courts to detect and punish dishonest people. And at least some steps are taken by the conscious people of the country. For example, they are trying to avoid some of these foods. But it is found not enough in this regard. Both the government and public have to work together in order to eradicate this problem for ever.

1.15.1 How people make commodities adulterated: Examples

Followings are the examples of adulteration in various commodities occurred in the country and appeared in mass media.

1.15.2 Grains

Mixing infested and damaged grains to good quality grains is a common practice. Sometimes grain polishing and husks are added to increase the weight. Now a days, plastic beads that have the shape of food grains are often mixed with cereal grains, e.g., colored beads are added to the pulses. Sometimes water is sprayed over the grain stock to increase the weight.

1.15.3 Edible Oils

Adulteration of fats and oils is easy and cannot be easily detected. *Ghee* is adulterated with hydrogenated oil and animal fats. Recently, because of the discovery of synthetic colors and flavors, any fat can be made to look like *ghee* and customers may easily be cheated. *Till* (sesame seeds oil) oil and coconut oil are often mixed with ground nut or cottonseed oil as the latter are cheaper. Adding allyl-isothio-cyanate to soybean oil or palm oil gives the characteristic pungent smell of mustard oil. Mixing of palm oil with soybean oil is a common practice among some traders for more profits.

1.15.4 Milk

The adulteration of milk is normally done with the addition of water and removal of fat. Sometimes extraneous substances like soybean and groundnut milk, wheat flour, etc are mixed. Selling diluted buffalo milk as cow milk is a common practice in rural areas. Addition of wheat flour, fine grained semolina, etc to milk powder is also common.

1.15.5 Tea

Tea leaves may be adulterated with the addition of used tea leaves, sawdust, and dried and ground leaves other than tea leaves.

1.15.6 Spices

Spices like chilies and turmeric powder are adulterated with the addition of lead pigment to impart brightness in color and good appearance. Chili powder is normally adulterated by adding brick powder.

1.15.7 Sweetmeat & soft drinks

Excessive use of wheat flour in place of milk protein (*chhana*) in the preparation of sweetmeat is an example of adulteration. Use of carboxy methyl cellulose in lieu of liquid glucose or sugar syrup in the preparation of soft drinks is an example of extortion. In the name of various fruit juices, imitation products are prepared by using artificial and prohibited ingredients instead of using original fruit juice. Recently, a special drink named mineral water is being prepared and marketed with little or no assurance of quality.

1.16 Milk and milk products adulteration in Bangladesh

When consumers buy milk they have a right to assume that it will be pure and unadulterated. Hence, there is an obligation on the dairy industry to provide adequate quality control systems. There are many potential adulterants listed below:

a) Extraneous water

b) Detergents/sterilants accidentally finding access to milk during production

c) Teat dips, udder salves, etc.

d) A Neutralisers used to mask developed acidity

e) Skim-milk powder used to elevate milk solids

f) Salt or sugar used to mask extraneous water or to elevate total solids

g) Preservatives such as formalin, hydrogen peroxide, hypochlorite, etc. used to mask poor hygienic quality

h) Foreign fats

Since milking plants and processing plants are wet-cleaned, therefore, the most common potential adulterant in milk is extraneous water.

The nature of adulteration of milk is multifarious as can be seen above. Neutralizers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate are also added. Formalin is poisonous though it can preserve milk for a long time. Sugar, poor quality glucose, starch, wheat flour, arrowroot, rice flour and urea is generally added in the milk or in the preparation of synthetic milk to increase the solids not fat (SNF) content of milk i.e. to increase the lactometer reading of milk, which was already diluted with water. The presence of sulphate (as ammonium sulphate) in milk increases the lactometer reading. Addition of salt in milk is mainly resorted to with the aim of increasing the corrected lactometer reading. The characteristic feature of milk is its fatty acid composition, which mainly consists of short chain fatty acids such as butyric, caproic, caprylic acid; whereas the vegetable fats consist mainly of long chain fatty acids and hence adulteration of vegetable fat in milk can be easily be found.

Addition of water and extraction of fat is very common and not harmful. But what when the milk one drink is not milk at all? Rather combination of urea, liquid detergent, a little sugar, vegetable oil and water - synthetic milk?

1.16.1 Extraneous Water: most common adulterant for milk

Milk is a variable biological fluid, and the fat, protein, lactose and even the natural water content all vary form cow to cow and from herd to herd. Compositional quality therefore cannot be used as a measure of the ‘purity’ of milk. Developing a method of controlling extraneous water in milk has therefore occupied the minds of food control authorities since the mid 1800s [134].

Although compositional quality has been used as an indication of adulteration, there is a need to survey milk quality in any specific country before one can seta standard; however a solids-not-fat (SNF) of 8.5% was at one time set as a presumptive legal standard in the UK [4-6]. Whilst this standard did not prove that milk below 8.5% SNF was adulterated nor that milk greater than 8.5% SNF was genuine it did act a crude indicator enabling the analyst to concentrate on suspect herds.

1.16.2 Nitrates as an indication of extraneous water

Cattles milk is virtually free from nitrates, even when cattle may have ingested them in food or drink. Most waters, on the other hand, contain traces of nitrates. The nitrate test can therefore be used as a qualitative test for extraneous water in milk. It must be emphasized, however, milk which doest not contain nitrates cannot be assumed to be free from extraneous water [4-5].

The Gerber method [2] may give an indication of the presence of nitrites or nitrates since an unusual golden-brown color is produced with milk containing nitrates when the butyrometer is shaken. The color is different from the gradual production of the purplish-brown coloration obtained with pure milk.

Unfortunately, no method for estimation of nitrate is established or currently available in the lab, therefore, attempt of nitrates estimation, a comparatively easy way to indicate of extraneous water of milk, was not incorporated in the objectives.

Recently, the quality of milk is now deteriorated due to adulteration either in collecting areas in different marketing channels. Adulteration of milk is usually done by adding inferior cheaper materials or elements like unsafe water, sugary materials and sometimes by powdered milk [134]. Milk is a very perishable product and its shelf life is only few hours. Health hazard chemicals are frequently used to the milk in different regions of Bangladesh as preservative for increasing its shelf life; however, nobody is using safer preservative for increasing the shelf life of milk in Bangladesh except some milk shed areas where market milk companies have been collecting milk [135, 136]. A number of evidences of milk adulteration (possibly as a whole of variety of foodstuff adulteration) are given in the following table (Table 1.23), as those are collected from popular print media.

Table 1.23: Few Evidences of adulterated of milk and milk products in Bangladesh are depicted here from various news sources

Date & Year	News Sources	Topic Title	Important Phrases appeared in the main text
August 17, 2006	BDNEWS24.COM (Online news sources) Dhaka, Bangladesh	Adulterated milk abounds in *Sherpur*, children falling ill	The children are allegedly suffering from different kinds of diseases including intestinal diseases after taking the **milk**;
July 31, 2009	The Financial Express, a daily newspaper of Bangladesh	Food adulteration by chemicals and diseases	Cutting oil has been used for making **milk**. When **milk** is made by cutting oil, there is no chance to grow any kind of virus/bacteria and so preservation is unnecessary;
March 4, 2012	The Financial Express, a daily newspaper of Bangladesh	Milk is not white	Dairy owners mix urea, detergents, caustic soda in small quantity of **milk** and add large volume of impure water to produce white liquid in bulk; This adulterated milk is also used in making sweetmeats;
July 16, 2012	BLITZ, Comprehensive Tabloid Weekly of Bangladesh	Palm waste and cow fat in condensed milk in Bangladesh	Palm oil extract waste are mixed with artificial sugar and milk thus producing condensed **milk**; Condensed milk is used in numerous dessert dishes in many countries;
July 18, 2012	Daily Sun a daily newspaper of Bangladesh	Food adulteration a 'silent killer' in Bangladesh	There is hardly any food substance - fish, **milk**, fruit or vegetable - that has not been poisoned by mixing toxic substances;
October 10, 2012	A blog which deals with public health problems and prospects of Bangladesh.	Adulterated food: a serious public health problem in Bangladesh	From raw vegetable and fruits to milk and **milk** products to fish, meat and processed food–every food item is contaminated;
February 19, 2013	Total News Bd.com, (Online news sources) Dhaka, Bangladesh	Over 50 percent food items in Bangladesh adulterated	Adulteration are found in all kinds of **milk** powder;

1.17 Situation of consumer rights in Bangladesh

The protection of consumer rights has become an essential part of socioeconomic policy even in developing countries [5, 134]. The growing interdependence of the world economy and the international character of many business practices had contributed to the development of a uniform and universal emphasis on the need for consumer rights protection.

In this country, the sense of the importance of consumer rights protection have increased significantly owing to the current role of the state by directing the judicial expeditions against adulterated foods together with other commodities produced locally. Although this movement was considered a milestone in the history of the consumer protection movement in this country and any forthcoming Act would provide better safeguards to the consumers against any kind of exploitation and/or deprivation. However, most of their (judicial mobile courts) findings were shocking and frightened the concerned consumer when they knew that many of the giant producers (in local perspective) are just using the fake levels of the ingredients and do not maintaining the minimum hygienic conditions where the foodstuffs are being processed.

1.17.1 Lacking authentic evidences of adulteration for supporting the consumer rights

The raids by the authority were undertaken following complaints of adulteration in milk and milk products that are sold in open market to unsuspecting customers, something that could have serious health implications (various reports on national news paper can be noticed in recent days). Here in this country, milk may be adulterated on purpose and mostly motivated by economic greed during milking or in

processing. The driving force behind most adulteration is to maximize revenues by using (partially) either a cheap ingredient as a substitute for a more expensive one, or (partially) removing the valued component in the hope that the altered product is undetected by the final user. Diluting milk with water or skimming off the cream are good examples of this long-standing practice.

Supplementation of a product with a cheap ingredient, also known as 'economic adulteration', does not usually carry a health hazard for consumers. This statement is not generally valid in respect of adulterated milk and milk products. Consumers who are allergic to some ingredients that may have been added in milk may suffer severely if they ingest. Consequently, it is necessary to set up a complicated legal framework to ensure proper consumer protection and encourage fair trade practices.

Detection of fraud is complicated by the fact that the quantities of certain indicators vary due to biological, climatic, agronomic and temporal factors. Moreover, processing can dramatically change the composition of minor constituents, therefore specifications that are too stringent cannot be set for food inspection as this will eventually increase the number of false-positive results. The Government alone may not competent to define the method of analysis including the sampling procedure. It is to ask the public analyst to analyze the collected samples on a priority basis and must submit a report within a considerable time frame. So far the Government has not prescribed any testing procedure for presence of any adulterant. As for the presence of deadly urea in the milk, a study conducted by the Health Ministry some time back revealed that the presence of urea in milk was mostly due to natural causes and not due to malpractices in the trade. Several studies have indicated that the high level of urea in milk was due to the presence of urea in the cattle feed.

Therefore, Essential and effective consumer protection laws are now essential, and before that a set of standards of analysis for every possible parameters are required. As such, consumer protection movement in India was very strong, and due to the government's support, there were over 5,000 consumer protection organizations and 3,000 Consumer Courts, which looked after consumer interests [132]. We assigned that current government-backed-anti-adulteration movement (also mentioned in earlier) is enjoying the mass support but peoples became shocked, very often, once

they found that locally made foods, including sweetmeats, are adulterated deliberately. Therefore, it is assumed that scientists, especially chemists, could put an important role to this movement by analyzing the suspected products and comparing the findings with what it should be in standard conditions. Whereby, to prepare all possible means to protect the consumers i.e., the human resources of nation will be easy. Therefore, urgency of legislation for consumer protection against food adulteration, or especially milk adulteration in Bangladesh is felt immensely.

1.18 Possible role of scientists in the country

Human population of many city areas is outnumbering of milk production in and around that particular city. Therefore, the major portion of milk usually comes from rural areas. Limited works have been done in different regions of Bangladesh regarding public health issues relating to adulteration of raw milk.

At this critical juncture, it is thought that the scientist community could come forward to put their logistic efforts by maintaining a regular surveillance in terms of providing information about the authenticity of the ingredients and the levels claimed by the producers on their products. In addition, the possible impact of several suspected ingredients on consumer (usually toddler) health, and the way the foods are being made could be assessed critically in relation with common reported health problems.

Therefore, present study (hypothesis is appeared in the succeeding section) is thought to be undertaken to determine the type of adulterants being added to the raw milk or in the milk products by the suppliers of the western city, Rajshahi.

Part III: Hypothesis and Objectives

1.19 Hypothesis

It would not be striking, as it should have been, if the level of consumption of milk and milk-based food items in our country is scanty. Therefore, it is hypothesized that the level of daily intake of Ca through dairy foods is significantly low and possibly not being mitigated through any other richer sources like animal proteins.

Secondly, although a sizeable amount of milk is produced each year in Bangladesh and a substantial part of it is being utilized to produce various items of milk-based sweetmeat in all over the country. A major portion of the city population of the country is consuming these varieties of sweetmeats and that is considered as an indirect consumption of milk which is important and essential for Ca supply for each and every people whatever their age groups are. When consumers buy milk or milk products they have the right to assume that it will be pure and unadulterated. But, unfortunately, this is not always the case, as just mentioned in preceding sections (Part I and Part II).

Claims of adulteration of liquid milk by adding simply water is not new, but together with milk based sweetmeats, adulteration can be committed through various ways. In general, adulteration of milk can be defined as offering a product to the market as milk but which, in fact, does not comply with its legal definition. Adulteration practices mostly involve the addition of water, whey, sugar, starch, plant fats, urea and many more for higher lactometer riding for SNF portion. Sometimes it is also claimed that chemicals like formalin, being an objectionable ingredients, is also added for long shelf life. And all of these adulterated milks are being used for all milk based products like various sweetmeats round the country. Claim for a second stage of adulteration for milk-based products is also prominent. Therefore, it is also hypothesized that measurements of key ingredients of milk or milk products are essential, and their levels are needed to compare with benchmark samples for the purpose of regretting or accepting those claim of adulterations.

The raids by the authority were undertaken following complaints of adulteration in milk and milk products that are sold in open market to unsuspecting customers, something that could have serious health implications. However, the Government alone may not competent to define the method of analysis including the sampling procedure.

Therefore, an attempt has been taken to verify the levels of ingredients present in locally made milk-based sweetmeats and to assess the probable detrimental effects of some selected ingredients on consumer health. The verification would be done periodically round the year assuming that the levels could be different if it is produced in different batch.

1.20 Objectives

The purpose of this study is manifold. First, to address the level of per capita consumption of milk and milk-based food items in the country compared to the other countries (which are economically affluent). To this effect, vulnerability of bone related problems especially in old ages will be focused; so that preventive measures can be taken by the respective authority well ahead.

To test the hypothesis, it is intended to analyze several liquid milk samples those were to be collected from the real markets either from the bulk portions or bought as packets, and as well as from the dairy farms. Milks those collected directly from the milking points of the firms were considered as standard as because of that milk was collected from the selected breed and with the presence of lab personnel.

Density, electrical conductivity (EC) and pH measurements of all the liquid milk samples were done in the fresh conditions. Amount of total milk solids (protein + fat + lactose + minerals) and their fractionation was also calculated.

Amounts of Ca in liquid milks were estimated in fresh condition as well as in different portions upon the fractionation of solids. Selected micronutrients e.g., Zn, Cu, Mn, Co, Cr, Cd, Pb and As of most of the liquid milk samples collected from were measured to verify their quality, and if possible, the trail of adulteration would have been reconstructed.

Addition to above, milk-based sweetmeats available in the Rajshai University Campus and in Rajshahi city area (details are appeared in the succeeding chapter) were selected and eventually collected to verify the status of the products and selected ingredients was also tested and comparison with liquid milk standards were also made.

It is assumed that the facilities available at the department e.g., pH and conductivity measurements, other quantitative analysis e.g. gravimetric and titrimetric analysis etc, and the services e.g., Atomic Absorption Spectrophotometry (AAS) provided by the University through the Central Science Research Laboratory would be sufficient to carry out this research. Afterwards, rigorous statistical analysis is to be applied, depending on the patterns of data obtained, and if any specific statistical treatment would be felt necessarily and justified would be adopted to any further extent.

Chapter Two

Materials and Methods

2.1 Study area: Highlights

Some basic information about the *Rajshahi* metropolitan city is given in the description of the preceding chapter. However, the region is an example of ancient inhabitation compared to the many important cities of the country. To that extent, it is also can be found (in the documents of locally published materials that can not be cited in the references) that local inhabitants had a history of making good quality of milk-based sweetmeats since they had m started to live here. As time passes, although it attracts more people to its exploitable and affordable resources, however, still the area is a less densely populated compared to any other old cities across the country (indicated earlier). Scientific studies regarding adulteration of local milk and milk products as a whole in the country are very scanty. However, virtually no study can be found where either adulteration of milk and/or milk-based sweetmeats is analyzed for this northwestern metropolitan city, *Rajshahi*. Physical boundary of the city, in which the city center and the University lie - from which most of the samples (milk-based sweetmeats are drawn for analysis) is presented in the following Figure.

2.2 Plan of studies

For a quick grab, tentative work plan with the time frame and duration is given in the following table (Table 2.1).

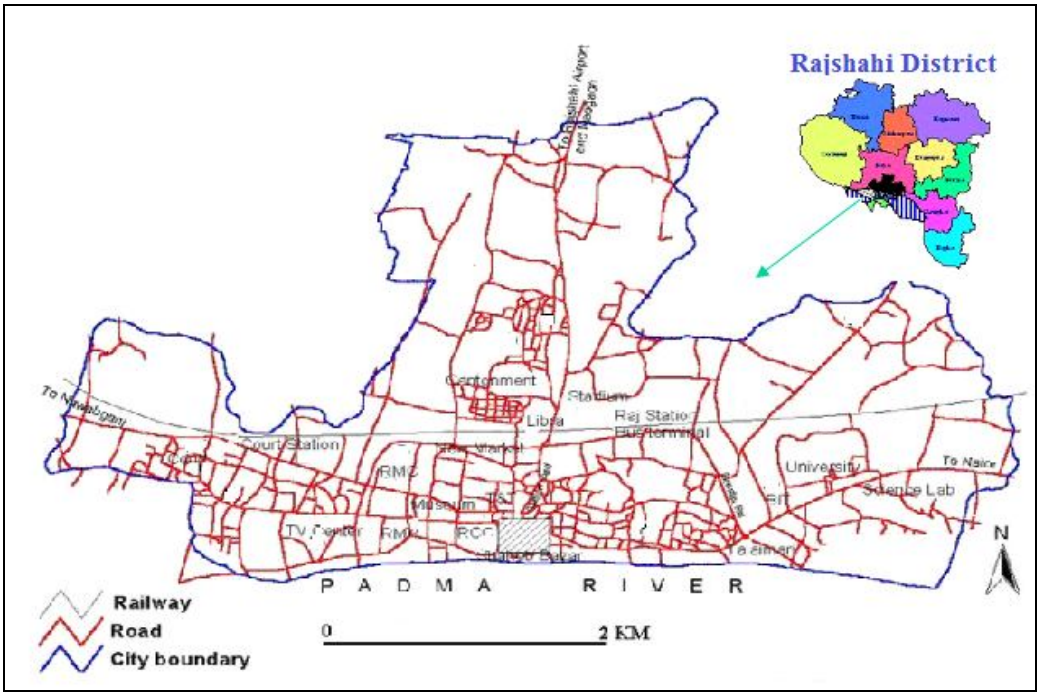

Figure 2.1: ***Rajshahi*** **City Corporation area: all the sample sources of the main study are present here**

Table 2.1: Tentative Time Frame of Plan of studies are presented in three steps

Steps	Nature of Work	Initiated on	Finished on	Duration
1	***Initial Study***	***January 2010***	***September 2010***	***9 months***
2	***Market Survey***	***October 2010***	***December 2010***	***3 months***
3	**Work Plan for Main Study**	----------		
4	***Main Study (First Batch)***	***March 2011***	***June 2011***	***4 months***
5	**Preparation and Delivery of First Oral Presentation**	----------		
6	***Main Study (Second Batch)***	***August 2011***	***December 2011***	***5 months***
7	***Main Study (Third Batch)***	***January 2012***	***April 2012***	***4 months***
8	**Preparation and Delivery of Second Oral Presentation**	----------		
9	***Main Study (Statistical Analysis)***	***July 2012***	***October 2012***	***4 months***
10	**All the remaining works need to be done afterwards**	----------		

2.3 Importance of initial study prior to the main studies

Prior to take a holistic program of analysis, a preliminary (here termed as Initial study) step is taken. In which, simpler parameters like pH, Electrical Conductivity, Density, two micronutrients (Cu and Zn), and two heavy metals Pb, Cd is incorporated for both liquid milks and milk products (sometimes referred as milk-based sweetmeats) from two selected sources. Results are to be evaluated and the plan (for choosing samples and sources as well as those parameters need to be considered for favorable in testing hypothesis) is to be set for main studies.

2.4 Materials for Initial Studies

2.4.1 Liquid Milk

Three categories of milk samples are used in this study. Their descriptions, identity (those are written in this discussion) and sources are given below in Table 2.2.

Table 2.2: Description, identity and description of milk samples those used in this study

Description	Identity	Sources	Characteristics
Packet Milk	Pkt-1	Bought from the grocer in the market, *Rajshahi*	½ Liter packet, UHT Milk
Packet Milk	Pkt-2	Bought from the grocer in the market, *Rajshahi*	½ Liter packet, UHT Milk
Standard Milk	Std-1	From the milk traders, at *Shirajgonj*	Collected in a clean and dry plastic bottle at the milking point
Standard Milk	Std-2	From the milk traders, at *Shirajgonj*	Collected in a clean and dry plastic bottle at the milking point
Standard Milk	Std-3	From the milk traders, at *Rajshahi*	Collected in a clean and dry plastic bottle at the milking point
Real Sample Milk	RS-1	Bought from a retail trader in the market, *Rajshahi*	Collected in a clean and dry plastic bottle
Real Sample Milk	RS-2	Bought from a retail trader in the market, *Rajshahi*	Collected in a clean and dry plastic bottle

Photograph of a packet is furnished in the Appendix-A, where all the necessary information can be found. Three milks were collected at the milking point and with own presence, therefore those three milks are treated as standard milk. Two milks were bought from the market, and it was bulk and open, therefore, these two milks are treated as real sample. Obtained information about three breeds where milks were collected as standards are given in Table 2.3.

Table 2.3: Information about three breeds where milks are collected as standards

Variety of Breeds	Reconstructed description from personal communication with herdsmen
Breed of Std-1 Milk	Herdsman claimed that it is a pure breed of *Jercy*, mainly feed on
Breed of Std-2 Milk	Herdsman's claim is that it is a cross breed of native variety and *Jercy*, feed on variety of fodder and very often grazed on green fields, drink water occasionally
Breed of Std-3 Milk	Herdsman claimed that it is a pure breed of *Fijian*,

2.4.2 Milk-based Products

Milk based product, a sweetmeat called *Rasogollah* (it is claimed that the product is always prepared from the common and popular milk derivative called *Chhana*) were collected from two different but popular shops. One of that located inside the Rajshahi University campus named *Silsila Restaurant* (in this discussion it was written as *Silsila* only).The other shop is located at the Rajshahi city center named *Jorkali Mistanno Bhandar* (in this discussion it was written as *Jorkali* only). Comparative information of two sources for the samples is given in the following table (Table 2.4).

Table 2.4: Comparative Information of Two Sources for Preliminary Study

Source	Tentative Establishment	Business Area	Business Nature	Number of Sweetmeat Items	Approximate Revenue day^{-1}
Silsila	1980	At the University Campus	Full meal, Snacks, sweetmeats	7 to 8	5000 to 7000 BDT
Jorkali	1960	City center	Only Variety of sweetmeats	About 20	About 5000 BDT

Commitment for carrying the business in future

2.4.3 Prior market survey of main study

Based on more than 50 questions, a detailed survey on production of sweetmeats and their raw materials (see Apendix-B) were done in *Rajshahi* Metropolitan City for 10 most popular sweet-meat producers (including the *Jorkali* is also there). Refined data are given in the Table 2.5.

Table 2.5: Required Raw Materials in Regular and in case of Overfull Demand

Name of Raw Materials →		**Milk L**	***Channa* Kg**	**Sugar Kg**	**Flour Kg**	***Khoa/ Maowa* Kg**
Brand/Outlet	**Demand**					
Rajshahi Mistanno Vandar	Regular	80	50	150	50	30
	Overfull	110	90	300	80	70
Rajshahi Mistee Bari	Regular	800	120	400	50	30
	Overfull	1200	200	1000	150	80
Shamim Sweets	Regular	30	65	150	30	25
	Overfull	55	85	190	45	38
Beliful	Regular	400	75	140	40	45
	Overfull	600	120	200	65	75
Sheebgonj Sweets	Regular	12	20	10	1.5	5
	Overfull	24	40	20	3	10
Jorakali Mistanno Vandar	Regular	20	25	30	10	2
	Overfull	35	40	35	10	4
Modhubon	Regular	15	20	50	3	5
	Overfull	30	40	100	6	10
Naborup	Regular	30	15	50	3	5
	Overfull	60	30	100	6	10
Mithai Bazar	Regular	350	90	120	40	40
	Overfull	500	150	160	65	70
Bindu Hotel & Restaurant	Regular	30	15	35	07	10
	Overfull	60	30	70	14	20

2.4.4 Selection of sources and materials from the survey

On the basis of the production amounts (which is corroborative to their use of raw materials in terms of amount of all milk derivatives, amounts of sugar and flour), four producers (here termed as Brand/outlet, but the 'sources' would be used in final results and discussions texts. Name of four sources and the abbreviated forms (would be used in the following texts) are given in Table 2.6.

As it is stated earlier (Chapter One) that in the subcontinents, milk-based sweetmeats those are prepared by either with *Chhana* (analogous to the Cheese) or with *Khoa* or *Maowa* (analogues to dry-matter of evaporated milk) are popular, available and being prepared in variety of ways. Therefore two items (called Rosogolla and Parasandesh) are to be selected for analysis in main study and are to be collected from all four sources. Therefore, both items are arranged with their sources in the Table 2.7 that is also stated their combined abbreviated from which are intended to use in the later discussions.

Table2.6: Name of four sources and their abbreviated forms (which would be used in the following texts)

Brand/Outlet	Abbreviations used for sources
Beliful	*B*
Rajshahi Misti Bari	*MB*
Rajshahi Mistanno Vandar	*MV*
Mithai Bazar	*MBz*

Table 2.7: Name of two items and their abbreviated forms on the basis of their sources (which would be used in the following texts)

Brand/Outlet	Items collected for Analysis	Abbreviations used only for items	Final abbreviations used in the texts
B	*Rosogolla*	*R*	*BR*
	Parasandesh	*P*	*BP*
MB	*Rosogolla*	*R*	*MBR*
	Parasandesh	*P*	*MBP*
MV	*Rosogolla*	*R*	*MVR*
	Parasandesh	*P*	*MVP*
MBz	*Rosogolla*	*R*	*MBzR*
	Parasandesh	*P*	*MBzP*

2.4.5 Selections of parameters need to be investigated

Following table (Table 2.8) is described the whole schedule of tasks that include initial study to main study, type of samples involved in each study, parameter chosen, and a hint methods used or adopted.

Table 2.8: Total schedule of the program with the methods used

Study	Sample Types	Sample Sources	Parameters Investigated	Methods Used/Adopted
Initial	Liquid Milk	Details appeared in above	Density, pH, Conductivity	Adopted (Cited in References)
			Ca	Titrimetric (Cited in References)
			Trace Elements: Zn, Cu, Pb, Cd	Flame AAS
	Sweetmeat: *Rosogolla*	*Silsila* (from University Campus) *Jorkali* (from City Center)	% of FW, MC, TS and TMS	Adopted (Cited in References)
			Trace Elements: Zn & Cd	Flame AAS
Main (1^{st}, 2^{nd} & 3^{rd} Batch)	Sweetmeat: *Rosogolla & Parasandesh*	*B, MB, MV, MtBz* (from City Center)	% of FW, LME, TMS, TS	Adopted (Cited in References)
			Ca	Titrimetric (Cited in References)
			Trace Elements: Zn, Mn, Cu, Pb, Cr, Cd, Co, As	Flame AAS (Hydride generation for As)

2.5 Methods, chemicals and instruments are to be used in all studies

Following two tables (Table 2.9 & 2.10) are depicted general chemicals (with their, formula, sources and minimum assay, if found available) and minor instruments (with make and source), respectively, used in all studies.

Table 2.9: Information about various chemicals is given in the following

Name	Formula	Source/Origin	Remarks
Buffer Solution	pH 10 (Borate)	Fisher scientific UK Limited, Bishop Meadow Road, Lough borough, Leicestershere LE115RG UK.	--------
Cadmium nitrate	$Cd(NO_3)_2.4H_2O$	Company: LOBA CHEMIE, LOBA CHEMIE PVT.LTD. BOX NO. 2042. Mumbai -400-002	Minimum assay - 99%
Eriochromic Black-T: (Indicator)		Matheson Coleman & Bell, Division of the Matheson Company. Int. Norwood (Cincinnati), Ohlo, East. Rutherford N.J.	--------
Ethylendiamine tedraacetic acid Tetrasodium Salt Tetrahtdrate	EDTA	Fluka Biochemika. Sigma- Aldrich Chemie GmbH, Riedstr 2, D-89555 Stein heim, Packet in Switzerland.	--------
Hydrogen peroxide	H_2O_2	Fisher scientific UK Limited, Bishop Meadow Road, Lough borough, Leicestershere LE115RG UK.	--------
Lead Nitrate	$Pb(NO_3)_2$	Matheson Coleman & Bell, Division of the Matheson Company. Int. Norwood (Cincinnati), Ohlo, East. Rutherford N.J.	--------

Led oxide	PbO	THOMAS BAKER (CHEMICALS) LIMITED. 62, MAKER CHAMBERS III, NARIMAN POINT, BOMBAY – 400021.	Minimum assay 98.0%.
Silver nitrate	$AgNO_3$	THOMAS BAKER (CHEMICALS) LIMITED, 62, MAKER CHAMBERS III, NARIMAN POINT, BOMBAY – 400021.	Minimum assay 99.9%
Sodium thiosuphatel (Pentahydrate)	$Na_2S_2O_3$, $5H_2O$	THOMAS BAKER (CHEMICALS) LIMITED, 62, MAKER CHAMBERS III, NARIMAN POINT, BOMBAY – 400021.	Minimum assay - (iodometri c) 99.0-100%
Universal indicator solution	pH 4-11 (Including color scale)	Merck Specialties, Private Limited. Shiv Sagar Estate 'A' Dr. Annie Besant Road. Worli Mumbai-400018.	--------

Table 2.10: Information about various minor instruments is given in the following

Name	Make	Source/Origin
Electric Balance	AB 204-5	METTLER TOLEDO, MADE IN SWITZERLAND
Multi - range Conductivity meter	HI 9033100	HANNA instruments, Microprocessor Conductivity Meter, UK.
GAS FERNACE		KARL LOLB, SCIENTIFIC TECHNICAL SUPPLIES, Buchschlag - Frandfurt. West Germany.
pH –METER		Wissenschaftlich- Techbiche WerkstaHan GmbH, D-82362 Weilheim, West Germany.

2.5.1 Methods

Following measurements and adopted techniques are described here.

2.5.1.1 Density measurement

All density measurements were done in 10.00 mL density bottle and a precision level was maintained to four decimal places.

2.5.1.2 pH Measurement

Following procedures were maintained in the measurements:

Electrodes with distilled water were washed and wiped them gently with tissue or filter paper. Temperature was set by the control. The pH meter was standardized against a buffer solution of known pH. Buffer solutions with pH as close as possible to that of the test solution were used. Before measuring the pH of the test sample, the electrodes were rinsed with distilled water and were dried. The electrodes were allowed to dip into the test solution and read the pH. Electrode tips were kept in distilled water between tests. pH of 1:1 dilution of each samples were also recorded.

2.5.1.3 Conductivity Measurement

Likewise in above, conductivity measurements were also done by the conductivity electrode, and similar to that conductivity of 1:1 dilution of each samples were also measured.

2.6 Determination of moisture content and total solids (TS) in milk and milk products

2.6.1 Apparatus

Pyrex beaker, about 3 cm in diameter, and not less than 2.5 cm deep; A glass stirring rod; A spoon or spatula and an oven.

2.6.2 Procedure

Weigh 10 g of milk or milk products were poured into a Pyrex beaker. Materials were heated over a temperature range about 383 K until it ceases foaming and a light-brown color appears. Upon heating the sample, the container would place on the asbestos-centre wire gauze on a tripod. This distributes the heat evenly across the bottom of the cup. After the moisture is driven from the milk or the products, samples were allowed to cool and reweighed.

2.6.3 Calculations

Percentage moisture content of the milk or milk products is calculated as:

Moisture % = (Original weight – final weight)/final weight × 100 (dry weight basis). The final weight is the solids present in the materials.

2.7 Determination of Ca: complexometric titration

Many metal ions form slightly dissociated complex ions. The formation of these can serve as the basis of accurate and convenient titrations for such metal ions. Such determinations are referred to as complexometric titrations. The accuracy of these titrations is high and they offer the possibility of determinations of metal ions at concentrations at the millimole level. Many cations will form complexes in solution with a variety of substances that have a pair of unshared electrons capable of satisfying the coordination number of the metal. The metal ion acts as a Lewis acid and the complexing agent is a Lewis base. The number of molecules of the complexing agent, called the ligand, will depend on the coordination number of the metal and on the number of complexing groups on the ligand molecule. Simple complexing agents such as ammonia are rarely used as titrating agents because a sharp end point corresponding to a stoichiometric complex is generally difficult to achieve. This is true, since the stepwise formation constants are frequently close together and not very large, and a single stoichiometric complex cannot be observed. Certain ligands that have two or more complexing groups on the molecule, however, do form well-defined complexes and can be used as titrating agents. One such reagent that is widely used is ethylenediaminetetraacetic acid (EDTA).

An organic agent which has two or more groups capable of complexing with a metal ion is called a chelating agent. The complex which is formed in this manner is called a chelate. Thus there are six complexing groups in EDTA. EDTA is usually represented by the symbol H_4Y, which recognizes the fact that it is a tetraprotic acid. The four hydrogens in the formula refer to the four acidic hydrogens on the four carboxyl groups. It is the unprotonated ligand Y^{4-} that is responsible for the formation of complexes with metal ions.

The present analysis is concerned with the determination of Ca by the use of a complexometric titration of the type that is described above [137]. The titration is performed by adding a standard solution of EDTA to the sample containing the Ca. The reaction that takes place is the following:

$$Ca^{2+} + Y^{4-} <===> CaY^{2-}$$

Before the equivalence point, the Ca^{2+} concentration is nearly equal to the amount of unchelated (unreacted) Ca since the dissociation of the chelate is slight. At the equivalence point and beyond, pCa is determined from the dissociation of the chelate at the given pH. The equivalence point is detected through the use of an indicator which is itself a chelating agent. The specific indicator used is Eriochrome Black T (EBT). It contains three ionizable protons and we will represent it by the formula H_3In. In neutral or somewhat basic solutions, it is a doubly dissociated ion, HIn^{2-}, which is blue in color. EBT cannot be used as an indicator for the titration of Ca with EDTA, since it forms too weak a complex with Ca to give a sharp end point. Therefore, a solution containing the magnesium (Mg) complex of EDTA, MgY^{2-}, is introduced into the titration mixture. Since Ca^{2+} forms a more stable complex with EDTA than Mg, the following reaction occurs:

$$MgY^{2-} + Ca^{2+} <===> CaY^{2-} + Mg^{2+}$$

The Mg that is released in this manner then reacts with the doubly ionized ion of the EBT. The complex that is formed between Mg and that ion is red; hence at the start of the Ca titration the solution is red. This reaction can be written as follows:

$$Mg^{2+} + HIn^{2-} <===> MgIn^{-} + H^{+}$$

(blue) <===> (red)

The solution is then titrated with a standard solution of EDTA. At the beginning of the titration, the EDTA reacts with the remaining Ca ion that has not been complexed. After all the Ca has reacted the next portion of EDTA reacts with the Mg complex which was formed earlier. The added EDTA competes favorably with the red magnesium-indicator complex ($MgIn^{-}$), to give MgY^{2-} and HIn^{2-} and thereby giving a blue color at the end point.

$$MgIn^{-} + H^{+} + Y^{4-} <===> MgY^{2-} + HIn^{2-}$$

(red) <===> (blue)

2.7.1 Materials required

0.0100 M EDTA standard solution, 0.01000 M Mg_2SO_4 solution, pH 10 buffer, either liquid milk or solution that was prepared from milk-based products, and Eriochrome black-T.

2.7.2 Equipment & glassware

Volumetric flask (250 mL, 100 mL, 50 mL and 25 mL), conical flask (400 mL and 250 mL), Burette (50 mL, graduated by 0.10 mL increment), Pipettes (transfer pipette 10 and 25 mL, graduated pipette 10 mL which is graduated by 0.10 mL increments), Was bottles.

2.7.3 Preparation of a 0.0100 M EDTA solution

About 2 g of EDTA dihydrate, $Na_2H_2Y_2$ $2H_2O$, were dried in a drying oven at 80°C for one hour. Then accurately weighed 0.95 g ± 0.l mg, and quantitatively transferred the EDTA into a 250 mL volumetric flask**,** distilled water were added and mixed thoroughly and made it up to the mark of the flask. The contents were mixed well by inverting and shaking the tightly stoppered flask. The solution was then labeled as "Standard EDTA".

2.7.4 Preparation of the Mg-EDTA complex indicator

Precisely 0.744 g of dried EDTA was mixed with 0.492 g of $MgSO_4$ in 100 mL of distilled water. The solution was divided into two 50 mL portions. To one portion, few drops of phenolphthalein were added, and sufficient of 0.1 M NaOH solution was added drop-wise by counting the drops to turn the solution faintly pink. Once the number of drops of NaOH has been determined, the solution was discarded. To the

second 50mL portion the same number of drops of 0.1 M NaOH solution was added as were added to the first portion, and then diluted to about 95 mL with distilled water. 2 mL of pH 10 buffer solution and few drops of EBT indicator solution were added. At this stage there are two possibilities, the solution is either red or blue. If the solution is red, Mg^{2+} is in excess. In that case, 0.0100 M EDTA solution was added drop-wise until the solution just turns blue. If the solution is originally blue then EDTA is in excess and in that case 0.01 M $MgSO_4$ solution was added drop-wise until the solution just turns red, then 0.100 M EDTA solution was added drop-wise to just turn the solution blue again.

2.7.5 Titration of liquid milk

Precisely 5 to 10 mL aliquot of the milk was pipette out into a 250 mL Erlenmeyer flask. 2 mL of pH 10 buffer, 10 mL of Mg-EDTA Indicator solution and 2/3 drops of EBT indicator was added. The mixture was titrated with the standard 0.0100 M EDTA solution to a color change from red to blue. At least triplicate milk samples were titrated using the tantamount of each reagents and indicator. Each time, blank titration was performed and volume of titer for blank was deducted from the sample titer volume.

2.7.6 Titration of the slurry prepared from milk products

As amounts of Ca in liquid milks are estimated in fresh condition but for solid samples similar method is used with reconstituting milk like slurry. Accurately weighed about few hundreds mg of milk ash prepared from TMS in an electrical furnace, and transferred into a 250 mL Erlenmeyer flask. It is to be noted that amount of ash actually corresponds to about 5 to 10 mL of fresh liquid milk. Approximately 100 mL of distilled water and few drops of concentrated nitric acid were added to that ash sample, and were stirred to dissolve. Let stand for a sufficient length of time, so that all bubbles were dispersed. If foams occurred it was suppressed by the addition of 1 or 2 drops of n-octanol. After that the slurry was titrated with standard 0.0100 M EDTA solution to a color change from red to blue. At least triplicate slurry samples

were titrated using the tantamount of each reagents and indicator. Each time, blank titration was also performed and volume of titer for blank was deducted from the sample titer volume.

2.8 Comparison of analytical techniques for trace analysis: a review

2.8.1 Introduction

Trace elemental analysis requires more than just knowledge of the analytical technique to be used. This requires knowledge of a whole range of disciplines that need to come together to create the final result. The disciplines required can be described as follows:

a. Sampling, sample storage and preservation, and sample preparation methodologies

b. Analytical technique

c. Data control, including calibration strategies and the use of certified reference materials for quality control

d. Data management, including reporting of results and their meaning

Therefore, the methodology for trace elemental analysis requires an understanding of a whole range of inter-related issues centered around the sample, sample preparation, analysis, data interpretation/presentation and quality assurance. The following section has highlighted some of the most important aspects. In addition, the main strategies for calibration are also discussed, including the preparation of a standard calibration curves.

For that purpose, modern instrumental techniques have been found are increasingly automated, making identification of mineral and analytical setup convenient, straightforward, and relatively rapid. However, the accessibility and familiarity of these techniques may also lead to problems. Therefore, it is tempting to apply those automated and reported methods to trace-element analysis if possible.

For trace elemental analysis, detection limits (DL) is matter of concern. However, it can be achieved only on state of art and latest version of equipment like atomic absorption spectrometer (AAS). Such high-tech equipments facilities and trained technical manpower to operate this equipment are lacking even in laboratories of many cities of the country.

This section gives a general overview of methods employed for the measurement of common micronutrients in milk and milk based sweetmeats from the perspective of an analytical chemist. Different methods are being in practice to analyze elements by using various equipments.

2.8.2 AS

Atomic spectroscopy (AS) is perhaps the most widely used method for trace metal determination [138-140]. AS based techniques require expensive and complex instrumentation, more sample preparation and calibration curved based task is needed. It is also involves the use of absorption characteristic of metals [139]. Alternatively, it can involve measuring the absorption or emission radiation and is named as Atomic Absorption Spectroscopy (AAS) or Atomic Emission Spectroscopy (AES), respectively. Various methods of AAS or AES are described below.

2.8.3 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

GFAAS is an approved method by EPA in USA for measuring traces metal in water samples [141]. Atoms absorb light at characteristic wavelengths. The amount of light absorbed by an element at a certain wavelength can be linearly correlated to the concentration of the element. It was described as well about the use of graphite tube in the GFAAS unit. It uses to supply linearly heat to a sample for atomization and vaporization. The DL for this method is usually 1 to 5 ppb. GFAAS requires electricity, but no refrigeration. This method can have other chemical interferences, which is partially remedied by the use of matrix modifiers. Safety issues associated with this method include exposure to intense magnetic fields and emissions.

2.8.4 Stabilized Temperature Platform Graphite Furnace Atomic Absorption Spectrometry (STP-GFAAS)

The same principles of STP-GFAAS as described above for GFAAS [141]. However, it uses a transversely heated graphite atomizer as a background corrector. The DL for the method is reported as 0.5 ppb. Chemical interactions associated with this method are similar to those for GFAAS, although use of a stabilized temperature platform and matrix modifiers help to eliminate these interferences. The safety issues associated with this method are also similar to GFAAS.

2.8.5 Gaseous Hydride Atomic Absorption (GHAA)

The principle of GHAA is to measure gaseous hydrides [142], under certain conditions, Arsenic forms a hydride that can be measured based on a characteristic wavelength. The DL for this method is to be 0.5 ppb. This method has similar chemical interactions and safety issues as observed in GFAAS. It oxidizes the hydride and can contaminate the hydride generator and can prevent recoveries under any conditions.

2.8.6 Inductive Coupled Plasma Atomic Emission Spectroscopy (lCP-AES)

ICP-AES instrument utilizes optical spectrometry to measure the characteristic atomic emission spectra of the analyte. ICP-AES uses a modified version of AES [143]. The samples are aspirated into a region where they are vaporized and atomized by a flame, discharge or plasma. The excited atoms radiate at a characteristic wavelength, and the intensity is directly proportional to the concentration of the analyte. The DL for this method ranges from 5 to 8 ppb. ICP-AES can have several chemical interference's

such as Al, Sb, Cr, Co, Fe, Mo, Ni, and V when analyze for As. Sources of spectral line could overlaps, wings of intense spectral lines could be broadened at higher concentrations. Physical interferences affect sample nebulization and transport processes. Chemical interference's caused by molecular compound formation, ionization effects and thermo-chemical effects associated with sample vaporization and atomization in the plasma are documented [138-140]. Safety issues associated with this method are described as the toxicity of the reagents used is not fully understood and all mixing and acidification should be performed under a fume hood [30]. Secondly, radio frequency and UV radiation is emitted, 'when the instrument is in use. Finally, high voltages are present while the unit is in operation.

Many researchers now prefer to use ICP-AES as an analytical tool as it is less susceptible to interference and has lower DL than AAS; additionally, a number of elements can be analyzed at once. However, this method is not without problems; it is more expensive than AAS and the quality of results depends upon the experience of the operator. In simultaneous analysis there is also the problem of devising a sample decomposition which renders soluble a range of elements with diverse chemical properties. ICP-AES is marginally better for other elements, and it might have been interesting to pass the same sample through both machines to compare the magnitude of the difference. However, when Ca is present in sample in relatively high amounts (that is obvious for milk-based samples) it was thought impractical to use for this purpose.

2.8.7 Inductive Coupled Plasma Mass Spectroscopy (ICP-MS)

The theory of mass spectroscopy (MS) and ICP to determine analyte concentrations is explained [144]. Reports are available for MS technology to analyze different elements through utilization of differences about the charge and mass ratios [144]. Documented DL for this method is about fractional (<0.2) ppb. ICP-MS can have other analytical interferences, including high levels of Cl^{-1} that form complexes with the argon carrier gas and can be mistaken for As because of its proximity in mass.

2.8.8 Anodic Stripping Voltametry (ASV)

The uses and principle of ASV electrochemistry to separate metal ions in solution is described elsewhere [145]. A mercury electrode at a negative potential reduces metal ions in solution and the ions migrate to the electrode. The ions that have collected on the electrode are then re-oxidized using a ramped potential and a current signal is generated. This signal can then be used to calculate the concentration of the metal ions. The DL for this method is only 0.5 ppb if a deposition time of 80 seconds is used. ASV can have chemical interactions with Cu, Mg, Zn and bismuth. Safety issues associated with this method include the use of high voltages and toxic metals.

2.8.9 AAS for trace elemental analysis

In analytical chemistry, atomic absorption spectroscopy is a technique for determining the concentration of a particular metal element in a sample. Atomic absorption spectroscopy can be used to analyze the concentration of over 62 different metals in a solution. Although atomic absorption spectroscopy dates to the nineteenth century, the modern form (see Figure 3.1) was largely developed during the 1950s by a team of Australian chemists. They were led by Alan Walsh and worked at the CSIRO (Commonwealth Science and Industry Research Organization) Division of Chemical Physics in Melbourne, Australia.

FIGURE 2.2: Atomic Absorption Spectrophotometer (AAS) used in this study

2.8.10 Principle

The technique relies heavily on Beer-Lambert law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals for an instant by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured. In optics, the Beer-Lambert law, also known as Beer's law or the Lambert-Beer law or the Beer-Lambert-Bouguer law is an empirical relationship that relates the absorption of light to the properties of the material through which the light is travelling. Although several of the expressions often are used as Beer-Lambert law, the name should strictly speaking only by associated with the latter two. The reason is that historically, the Lambert law states that absorption is proportional to the light path length, whereas the Beer law states that absorption is proportional to the concentration of absorbing species in the material. The combined for the equation is as follows:

$$A = \varepsilon bc$$

Where, A is Absorbance, ε is molar extinction coefficient, b is the path length and c is the concentration.

2.8.11 Prerequisites

There are at least five conditions that need to be fulfilled in order for Beer's law to be valid. These are:

a. The absorbers must act independently of each other;

b. The absorbing medium must be homogeneously distributed in the interaction volume and must not scatter the radiation;

c. The incident radiation must consist of parallel rays, each traversing the same length in the absorbing medium;

d. The incident radiation should preferably be monochromatic, or have at least a width that is more narrow than the absorbing transition; and

e. The incident flux must not influence the atoms or molecules; it should only act as a non-invasive probe of the species under study. In particular, this implies that the light should not cause optical saturation or optical pumping, since such effects will deplete the lower level and possibly give rise to stimulated emission.

If any of these conditions is not fulfilled, there will be deviations from Beer's law.

2.8.12 Instrumentation

2.8.12.1 Types of Atomizer

In order to analyze a sample for its atomic constituents, it has to be atomized. The sample should then be illuminated by light. The light tranmsitted is finally measured by a detector. In order to reduce the effect of emission from the atomizer (e.g. the black body radiation) or the environment, a spectrometer is normally used between the atomizer and the detector. The technique typically makes use of a flame to atomize the sample, but other atomizers such as a graphite furnace or plasmas, primarily inductively coupled plasmas, are also used. When a flame is used, it arranged so that it is laterally long (usually 10 cm) and not deep. The height of the flame above the burner head can be controlled by adjusting the flow of the fuel mixture. A beam of light passes through this flame at its longest axis (the lateral axis) and hits a detector.

2.8.12.2 Light Sources

The light source is most often choosen so that it has a spectral width that is more narrow than that of the atomic transitions.

2.8.12.3 Hollow cathode lamps

In its conventional mode of operation, the light is produced by a hollow cathode lamp. Inside the lamp is a cylindrical metal cathode containing the metal for excitation, and an anode. When a high voltage is applied across the anode and cathode, the metal atoms in the cathode are excited into producing light with a certain emission spectrum. The type of hollow cathode tube depends on the metal being analyzed. For analyzing the concentration of Cu, a Cu cathode tube would be used, and likewise for any other metal being analyzed.

2.8.12.4 Diode lasers

AAS can also be performed by lasers, primarily diode lasers because of their good properties for laser absorption spectrometry. The techniqe is then either referred to as diode laser atomic absorption spectrometry (DLAAS or DLAS), or, since wavelength modulation most often is empoyed, wavelength modulation absorption spectrometry.

2.8.12.5 Background Correction methods

The narrow bandwidth of hollow cathode lamps make spectral overlap rare. That is, it is unlikely that an absorption line from one element will overlap with another. Molecular emission is much broader, so it is more likely that some molecular absorption band will overlap with an atomic line. This can result in artificially high absorption and an improperly high calculation for the concentration in the solution. Three methods are typically used to correct for this:

1. Zeeman correction - A magnetic field is used to split the atomic line into two sidebands (see Zeeman effect). These sidebands are close enough to the original wavelength to still overlap with molecular bands, but are far enough not to overlap with the atomic bands. The absorption in the presence and absence of a magnetic field can be compared, the difference being the atomic absorption of interest.

2. Smith-Hieftje correction (invented by Stanley B. Smith and Gary M. Hieftje) - The hollow cathode lamp is pulsed with high current, causing a larger atom population and self-absorption during the pulses. This self-absorption causes a broadening of the line and a reduction of the line intensity at the original wavelength.

3. Deuterium lamp correction - In this case, a separate source (a deuterium lamp) with broad emission is used to measure the background emission. The use of a separate lamp makes this method the least accurate, but its relative simplicity (and the fact that it is the oldest of the three) makes it the most commonly used method.

2.9 Choice of AAS as an analytical tool in this study

AAS was chosen as the method of analysis for various reasons, not least being its accessibility. The experimental work yielded large numbers of samples and it was thought important that these should be personally analyzed as and when required. This allowed control over preparation of samples and standards, and their actual passage through the machine, giving personal responsibility for accuracy, calibration and basic maintenance of parts, such as the burner head which unless perfectly clean can give incorrect readings. The Machine used was a Perkin-Elmer 5000 AA spectrophotometer Perkin-Elmer Corp., Connecticut, USA.

AAS is also a comparative method of analysis, comparing standards of known concentration with the test samples, resulting in related values rather than individual numbers which may mean little on their own. This means it is usually easier to spot when the machine is not functioning correctly; delays in noticing malfunctioning obviously result in loss of samples and wasted time.

The precision for each element measured does vary in AAS with Zn being the most precise due to the ease with which it can be nebulised into the flame. Of the other elements examined Mn is the next most precise followed by Cu, Cr, Cd, Co, and Pb. Only As was measured using GHAA technique, described above.

The preparation of standard solutions involved acid and matrix matching. These are important because matrix interference can cause suppression or enhancement of the analyte signal, and for accurate results sample and standard should be physically and chemically as similar as possible. Standards were made using a serial dilution technique from SpectrosoL standard solutions which are supplied at strength of 1000 mg L^{-1}. For analysis of samples prepared concentrations of the standards ranged from 10.0-0.05 mg L^{-1} for all the elements analyzed. All six elements were included in the same solution (for matrix matching), and the final molarities of each standard was adjusted to 0. 9M using SpectrosoL atomic absorption grade nitric acid to provide acid matching. All solutions were diluted to correct volume using Milli-Q ultra-pure water to lower the risk of contamination. To check the accuracy of the serial dilutions of the standard solutions for 5 and 0. 5 mg L^{-1} were then passed through the AAS machine and the resultant peaks on the chart compared with those produced by solutions at 5 and 0.5 mg L^{-1} prepared independently by another staff of the laboratory.

Micronutrients in the samples were in low concentrations and an impact bead was used in the nebuliser section of the burner head; this improves the sensitivity and DLs of the machine. For macronutrients in all samples, and higher concentrations of micronutrients in the samples, a flow spoiler was substituted as this minimizes the interference from matrix components. The burner angle was also altered as necessary to give the most accurate results; if high concentration solutions are nebulised into the flame the ions may be too dense for absorption to take place fully, and the reading is therefore too low. For analyses of Zn, Cu and Mn the burner was turned through 30° for higher concentrations and through a further 30° (to 60°) for concentrations for further higher concentration if it was required.

All the elements analysed required a flame burning air and acetylene. The Perkin-Elmer manual suggests varying gas mixtures for different elements but analytical staff has found that the machine gives more accurate results using a standard setting. The height of the burner head was adjusted for maximum efficiency at the beginning of each session, and individual lamps for each element were set to their optimum energy levels prior to usage.

The results for each element were printed out in the form of a peak height chart by a chart recorder to give a permanent record. For maximum accuracy standards were charted after every five replicates so that each separate set of samples was calibrated to a specific set of standards. Once the recording was finished the peak height of each standard and sample trace was measured and entered into a specifically designed programmed data sheet on "Microsoft Excel". By use of a regression equation this calculated the amount of element in each sample; mean values and standard error, standard deviation were also calculated. As noted previously to allow comparisons between sites the amounts of each element were converted into a concentration of the amount of element per g of sample dry weight.

2.9.1 Trace elements analysis of this study

The analysis of chromium, cadmium, lead and mercury was performed in a Varian AA200 atomic absorption spectrophotometer equipped with a graphite furnace (GFAAS). A microwave system was used for acid digestion of all the samples. All the samples (milk and dairy products) were dried at 70°C in a forced stove until dry weight. To a dry sample of 0.3 g, finely crushed, were added 6.0 ml of HNO_3 (65%) and 1.0 ml of H_2O_2 (30%). The solution was filtered, deionised water (50 ml) was added and the sample was then stored in plastic bottles. The accuracy of instruments and analytical procedures were checked with certified reference materials (Non fat milk powder, National Bureau of Standards, Gaithersburg, USA). Concentration range used for each element, the corresponding linear equation with square values of correlation coefficient, LOD values are given in the following Table.

Table 2.11: Equation of calibration curves, r^2, LODs (for S/N = 3) on the basis of the concentration ranges used for the elements investigated[#]

Element	Concentration Range ppm/ppb	Equation	r^2	LOD ppm/ppb	Typical Calibration Curve
[*]**Zn**	0.100 – 0.400 ppm	y = 0.63371x	0.999	<0.02 ppm	See Appendix C Page:
[*]**Cu**	0.500 – 2.000 ppm	y = 0.11956x	0.999	0.08 ppm	See Appendix C Page:
[*]**Mn**	1.000 – 5.000 ppm	y = 0.16668x	0.998	0.06 ppm	See Appendix C Page:
[*]**Pb**	2.500 – 10.000 ppm	y = 0.01413x	0.999	0.71 ppm	See Appendix C Page:
[**]**Cr**	0.500 – 2.000 ppm	y = 0.03285x	0.993	0.06 ppm	See Appendix C Page:
[*]**Cd**	0.100 – 0.400 ppm	y = 0.62028x	0.958	<0.02 ppm	See Appendix C Page:
[*]**Co**	1.000 – 5.000 ppm	y = 0.03710x	0.955	0.27 ppm	See Appendix C Page:
[*]**As**	5.000 – 20.000 ppb	y = 0.01973x	0.999	0.51 ppb	See Appendix C Page:

[#]y, absorbance; x, concentration; [*]LOD is calculated considering the lowest distinguishable absorbance as 0.01 for the system; [**]LOD is calculated considering the lowest distinguishable absorbance as 0.002 for the system;

2.10 Statistical analysis

Descriptive statistical analysis including mean comparison, correlation studies, simple linear regression, multivariate analysis (where necessary) are done using Microsoft (MS) Excel and SPSS through making a interface between them primarily for data transfer from Excel to SPSS.

Chapter Three

Results and General Discussions

3.1 Results from initial studies

3.1.1 Density, pH and conductivity

Density, pH and conductivity of all seven milk samples are given in Table 3.1. The quality of milk varies as the breed varies, together with that, for any selected breed, the quality could vary again as its foods and fodder varies. Therefore, it is understood there was no single standard value for the density that must be considered for all milk samples. But it is worth trying to measure that property and try to correlate the values with other parameters if that correlation provides any interesting feature to address.

Table 3.1: Density, pH and conductivity of all milk samples

Milk Identity	Density ($g\ cm^{-3}$)	*pH (fresh milk)	*pH (1:1 dilution)	*Conductivity ($mS\ cm^{-1}$)	
				(fresh milk)	(1:1 dilution)
Pkt 1	1.028	6.51	6.60	4.77	2.96
Pkt 2	1.015	6.51	6.50	4.98	2.89
Std 1	1.023	6.54	6.58	5.65	3.35
Std 2	1.025	6.57	6.63	5.20	3.01
Std 3	1.022	6.59	6.62	5.20	3.10
RS 1	1.023	6.52	6.56	4.36	2.60
RS 2	1.023	6.66	6.68	5.59	3.33

Mean of at least three replicates; Standard Error (SE) in a order of 0.00;

There were no labels of density for packet milks; however, it has been found on the packets those carrying identical levels but produced in different batches; they have quite different densities, ranging from 1.028 to 1.015, the later is significantly lower than the former. The variation in densities between packets could be the result of imperfection in quality control of the production, or it could be the uncertainties of the current measurements that were taking place in the laboratory. But the chances of uncertainties of the measurements are limited as both the measurements were found well précised when their standard deviations are very small. But more investigations are needed to blame the producer if their lack of seriousness for maintaining the consistency of quality of their products is the reason.

It was assumed that as because of three standard milks were collected upon surveillance, therefore, question of addition of extraneous water was impractical, therefore, comparatively lower densities can be found for the real milk samples those were bought from the bulk open markets. But irrespective of the sources, all five milk samples gave almost similar densities that were close to 1.02. This means that either all milks are appearing in the market without addition of any extraneous water (it is nice to think), or might be the traders, who are more clever and adding more chemical agents to bring back the densities as the products are genuine.

The pH of all milk samples were found similar to the reported values and comparable with each other except one occasion (the value is 6.6) which is a real sample (RS 2). It is difficult to make any speculation for this variation, but many adulterants are basic in nature, therefore if that is added a slight increase in pH could be the result. pH measurements for all milk samples were also done in 1:1 dilution (a solution prepared by1 part milk with 1 part distilled water). Always a trend of increase in pH was found in diluted sample except a minute single breech for milk of packet 2. It might be the inherent buffer characters and capacities of milk that actually acting against any pH changes occurred upon dilution. Therefore, it is assumed that variation in pH as a reflection of addition of extra water is difficult.

Conductivity varied significantly among three groups and even within a single group (see Table 3.1, column 5). Conductivity of milk in packet 2 is higher than the milk of packet 1, while the density of packet 1 was lower. This reveals that the dissolved mineral portions are higher in packet 2 while its total solids were less than the milk of packet 1. Therefore, a question of doubt about the different level of ingredients in those two packets can be raised again. Conductivity of the milks of three standards are always significantly higher, and in this group, conductivity of standard 1 is again considerably higher than the rest two. Variation in the conductivity within this group can be rationalized by considering their different breeds, ages and food habits.

However, variation in the conductivity between two real sample milks is enormous, one is the lowest among all milks (4.36mS cm^{-1}) and other is nearer to the highest value. These two milk samples (RS 1 and RS 2) showed different pH values and different conductivity but having identical density. Addition of extraneous water and any other materials, for compensating the density, in those two milk samples might have been handled differently; therefore suspicion of adulteration can be justified.

It can be summarized from the above findings that three standard milks showed little variations in their density, pH and conductivity, while both packet milks and both real milk samples showed a greater extent of variation in their properties. Therefore, a wider variation in the quality of available milks in the real markets can be found, and for the assurance of pure supply of milk in the market for the consumer, various instruments of the Government need to be functioned properly and efficiently.

3.1.2 Estimation of Ca in fresh milk samples

The method for Ca measurements which is described in preceding chapter (Chapter 2) is only for fresh milks. As both packet milks were UHT treated milk, therefore, these two milk samples were excluded for this particular estimation. But their Ca contents were measured in different ways that are descried where it is necessary. Amount of Ca in three standard milks and two sample milks are put in Table 3.2. Interestingly,

the Ca contents varied significantly in both milk groups (Standard & Real). Std 1 milk sample is containing least amount among all five milks, while the second standard (Std 2) contains highest Ca and that is almost 33% higher than Std 1, and Std 3 shows moderation between those two extreme values. Similarly, significant variation in Ca content is found in those two real sample milks, where Ca in RS 1 is exactly 22% higher than the RS 2, but the contents of Ca in both RS 1 & RS 2 milks are between the highest and lowest values of standard milks, and the average values of both groups (the last column of the Table 3.2) are nearly similar. Therefore, question of inferior in qualities in terms of poor Ca content to the real milk samples can not be put.

Table 3.2: Ca content of five fresh milk samples

Milk Identity	mg Ca in 100 mL fresh milk ± SD	Average mg of Ca in 100 mL fresh milk for each group
Std 1	128 ± 3	157
Std 2	191 ± 6	
Std 3	153 ± 1	
RS 1	164 ± 2	149
RS 2	134 ± 1	

It would be more interesting if the above results are compared with any other related works that published either in home or abroad. But unfortunately, no studies in our country of this kind are available. Therefore, data for Ca content of similar food items from USDA Nutrient Database are cited here (see Table 3.3).

Table 3.3: Data obtained from USDA nutrient database for standard reference *

SL†	Food Description	Weight, g (a measure)	Ca, mg per measure	Ca, mg per 100 mL fresh milk‡
1	Milk, sweetened, condensed, canned	306 (1 cup)	869	----
2	Milk, evaporated, canned	252 (1 cup)	658	----
3	Milk, evaporated, canned, nonfat	256 (1 cup)	742	----
4	Milk, fluid, 3.25% milk fat	244 (1 cup)	290	121
5	Cheese, cheddar	28.35 (1 oz)	204	86
6	Cheese, Swiss	28.35 (1 oz)	272	115

*** Release 15. ; † SL, Serial Number; ‡ considering the density is 1.02 and total solids are 12 %;**

The Ca content of 100 mL fresh milk equivalent for first three food items in Table 3.3 (SL 1, 2 and 3) can be assumed very high. But the reality is, Ca content of those food items can not be recalculated for its 100 mL fresh milk equivalent, as because of those items were either evaporated or condensed, therefore, as long as the moisture contents are unknown, their calculations would be irrational. Similarly, without knowing the real moisture content, Ca content of last two food items (SL 5 and 6) will also be impractical. But if it is attempted by considering them totally dry, the value can be obtained as low as 86 mg (for 5^{th} food item).

Only the calculation for fluid milk (4^{th} food item) is found justifiable, but that value 121 mg Ca in 100 mL fresh milk is quite lower than the value found here. It could be the breed responsible for this variation.

However, the five fresh milks in this study are quite rich in Ca and by any how Ca content of two real milk samples are still considerably higher than the values cited in Table 3.3. When clear evidences of any kind of adulteration are yet to establish and neither it is Ca depleted, therefore chances are poor to blame our real milk samples as an adulterated one. But on the other hand, it is not wise to rule out the possibility of adulteration that could occur even in the minor scale.

But the question of very little amount of milk that is being consumed (as per capita) in this country remained unanswered, and the consequences which can be the results for this nation's habit (or it is a question of economical capability but not the habit) is worth mentioning and need to be addressed with the scale of its severity.

3.1.3 Selected trace elements of standard and packet milks

Milk of two categories was analyzed for Zn, Cu, Pb and Cd. The amounts found are presented in Table 3.4. Typical calibration curves and LOD for all elements are presented in Appendix that mentioned earlier.

Zn contents in both milks are found almost same. And both values are significantly higher compared to the value cited in Table 3.5 for milks in abroad. However, Cu content is enormously higher in packet milks and that is more than 100 % than the standard milks. The amount of Cu present in standard milks is also tremendously higher than value cited in Table 3.6 for milks in abroad. The values of any parameters could vary in country to country as lots of variables are associated here. But a concern can be raised upon the variation found in this study for both milk samples.

Table 3.4: Contents of Zn, Cu, Pb and Cd in two milk samples

Milk Identity	Zn (mg in 100 mL fresh Milk) ± SD	Cu (µg in 100 mL fresh Milk) ± SD	Pb (µg in 100 mL fresh Milk) ± SD	Cd (µg in 100 mL fresh Milk) ± SD
Pkt 2	0.6 ± 0.1	78 ± 13	82 ± 29	1.4 ± 0.3
Std 3	0.57 ± 0.09	29 ± 4	0.0 ± 0.0	0.9 ± 0.1

Table 3.5: Data obtained from USDA nutrient database for standard reference *

SL†	Food Description	Weight, g (a measure)	Zn, mg per measure	Zn, mg per 100 mL fresh milk‡
1	Milk, sweetened, condensed, canned	306 (1 cup)	2.88	----
2	Milk, evaporated, canned	252 (1 cup)	1.94	----
3	Milk, evaporated, canned, nonfat	256 (1 cup)	2.30	----
4	Milk, fluid, 3.25% milk fat	244 (1 cup)	0.93	0.39
5	Cheese, cheddar	28.35 (1 oz)	0.88	0.37
6	Cheese, Swiss	28.35 (1 oz)	1.11	0.47

* Release 15. ; † SL, Serial Number; ‡ considering the density is 1.02 and total solids are 12 %;

Table 3.6: Data obtained from USDA nutrient database for standard reference *

SL†	Food Description	Weight, g (a measure)	Cu, µg per measure	Cu, µg per 100 mL fresh milk‡
1	Milk, sweetened, condensed, canned	306 (1 cup)	46	----
2	Milk, evaporated, canned	252 (1 cup)	----	----
3	Milk, evaporated, canned, nonfat	256 (1 cup)	41	----
4	Milk, fluid, 3.25% milk fat	244 (1 cup)	24	10
5	Cheese, cheddar	28.35 (1 oz)	9	4
6	Cheese, Swiss	28.35 (1 oz)	9	4

* Release 15. ; † SL, Serial Number; ‡ considering the density is 1.02 and total solids are 12 %;

The amount of Pb present in packet milks certainly overtook any standard permissible values. A plausible explanation for the presence of Pb in milk is the roadside grasses if the cattle feed on. The reason could be the leaded fuels which are still considering cheaper compared to the other fuels those having different anti knocking agents [26]. Being a similar heavy metal, the presence of Cd can be justified as for their fate at the same points but the source of their flux is still unknown.

In conclusion, it is obviously that milk consumption in our country is alarmingly low compared to the amount that one should drink minimum in daily basis. It is obvious that Ca intake is directly related to the consumption of milk and milk based products, if the shortfalls are not corrected by other rich sources which are unusual. Low Ca intake would aggravate other illness some of them can be life threatening e.g., hypertension, kidney stones.

The above findings suggested that whatever the source of the milk, i.e., available in the markets either in packets or in bulks, or even it is directly collected from milking points, they still comply with standard values, and most importantly, beneficial parameters like, Ca, Zn and Cu are also present in appreciable amounts. However, it is also assumed that manipulation can happen and possibilities are there for false results. Therefore, it is necessary to set standards for selecting parameters and the methods that would be précised and sensitive. So that the authentic and reliable analysis can be followed and their documentations would be a fundamental means for legislative advancements for the authority about milk adulteration and by which consumer's right will be protected.

3.1.4 Status of two sweetmeats

Fresh weight, water content, sugar content and the amount of milk solids of two sweetmeats of both shops are given in Table 3.7.

The products of *Jorkali* are clearly better than its counterpart (*Silsila*) in terms of precisions those found here for selected parameters (see Table 3.7). Standard deviations (SD) which found significantly lower for fresh weight, water content, sugar content and *milk solid*s for *Jorkali*. Not only that, if both fresh weights are compared, it is clearly seen that in fresh conditions *Jorkali* products are at least couple of grams heavier. This means, possibly customers are getting more return at *Jorkali* than *Silsila*. However, it is difficult to say, without having customers' real feelings, that in terms of tests and enjoyments, they are really become more satisfied at *Jorkali*.

Milk solids, a desirable substance, are nearly double in *Jorkali's* item than its counterpart. On the contrary, water and sugar contents are comparatively lower in *Jorkali* than *Silsila*, again which would be a considerable issue in customers' choice. "Less sugar content" simply means that items are bathing in less sugary syrup, which may unveil their freshness but in unusual way. Because it is assumed that, once the items are prepared for consumption it would have been in comparatively dilute syrup,

but in following days, items bathed in syrup was continuously heated and boiled for their longer shelf-life, therefore, gradually the syrup became more dense, so the content of sugar in a fresh product became higher.

Table 3.7: Fresh weight, water content, sugar content, amount of milk solids of two sweetmeats

Item	†Fresh Wight (g) ± SD	†Water content (%) ± SD	†Sugar Content (g) ± SD	†Milk Solid (g) ± SD
Silsila	28 ± 1	69 ± 8	11 ± 2	5.4 ± 0.7
Jorkhali	29.7 ± 0.4	62 ± 2	8.7 ± 0.1	9.65 ± 0.04

† All figures given here are the average of at least three replications

As because of our intention is to find any contrasting properties in sweetmeat samples, therefore, we were found interested in trace elements rather than bulk elements Ca. Therefore, possible trace elements (in this case Zn, Cu, Pb and Cd) were selected and tested. Zn and Cd contents of both sweetmeats are given in Table 3.8.

Interestingly in both items, Zn contents found quite similar while the amounts of milk solids in *Jorkali* was significantly higher. An immediate explanation can be made that a certain portion of that milk solid (of *Jorkali*) might come from cheap alternative which have lower inherent Zn content, as any local cereal/vegetable origins could meet that demands. But to establish that assumption, partitions followed by analysis of milk solids of each product are essential, that is recommended here.

Cd is virtually absent in that products of *Silsila*. But for the *Jorkali*, although it is negligible and below the WHO permissible limit, Cd does present, and the doubt of the possibility of mixture of non-dairy materials with milk solids heightened further.

Table 3.8: Zn and Cd contents of both sweetmeats

Item	†Zn content (mg) in each piece (fresh weight basis)	†Cd content (µg) in each piece (fresh weight basis)
Silsila	0.18 ± 0.05	0.0 ± 0.0
Jorkhali	0.18 ± 0.07	3.2 ± 0.1

† All figures given here are the average of at least three replications

Unfortunately, due to lack of shortage of fuel gas (Acetylene) for AAS, estimation of Cu and Pb in milk products was not possible even in that due course of time. Therefore, these tasks initially planned but remained undone.

Therefore, on the basis of all of those above findings and speculations suggestions can be made for the valuable customers and consumers that products of *Jorkali* can be better than the *Silsila* in some respects. However, to do that, quality comparisons of both products from both sources should be done few times more, which is a real limitation (as time is a constraint here), and, therefore, further studies are obviously required.

To meet that demand, a more holistic approach is taken to analyze popular sweetmeats, produced locally in reputed outlets, in terms of their basic quality parameters and most importantly their Ca contents, levels of micro nutrients and to verify the potential of presence of common heavy toxic metals they might contain. This approach is therefore termed as 'main study', and it is assumed that results of this elaborate attempt would produce sizeable amount of information regarding those aforementioned parameters, and might be worthwhile to test the hypothesis about the possibility of adulteration and any kind of deprivation that might have been occurred for the consumers in terms of inferior nutritional status of the locally made consumable milk products.

3.2 Results from main studies

As mentioned earlier that the main studies were done in three batches, but each time replicated samples of both items from sources were collected and analyzed accordingly. The arrangements of results presented in the following figures on the basis of follows criteria:

1. All results are given as mean with its standard deviations (SD),

2. Four physical parameters, e.g., Fresh weight (FW), LME, Total milk solids (TMS) and Total sugar (TS) appeared first and with that sequence, afterwards, results of minerals e.g., Ca, Zn, Mn, Cu, Pb, Cr, Cd, Co and finally As are given with the stated sequence.

3. For each parameter (physical and minerals) results are given for *Rosogolla* (R) first of four sources followed by the *Parasandesh* (P). Afterwards, results for two time intervals are compared by their values with similar sequences e.g., results of R followed by results of P.

4. Mean values of all minerals are actually the values obtained from a single item (R or P) in their fresh weight. As it is assumed that, whatever the measure of servings for milk products that vary in country to country, therefore, a single item (a *Rosogolla* or *Parasandesh*) is considered here as a serving in a standard diet or meal.

3.2.1 Results of FW

Mean FW of both items of four sources and their comparisons in two time intervals are given in Figure 3.1 to Figure 3.4.

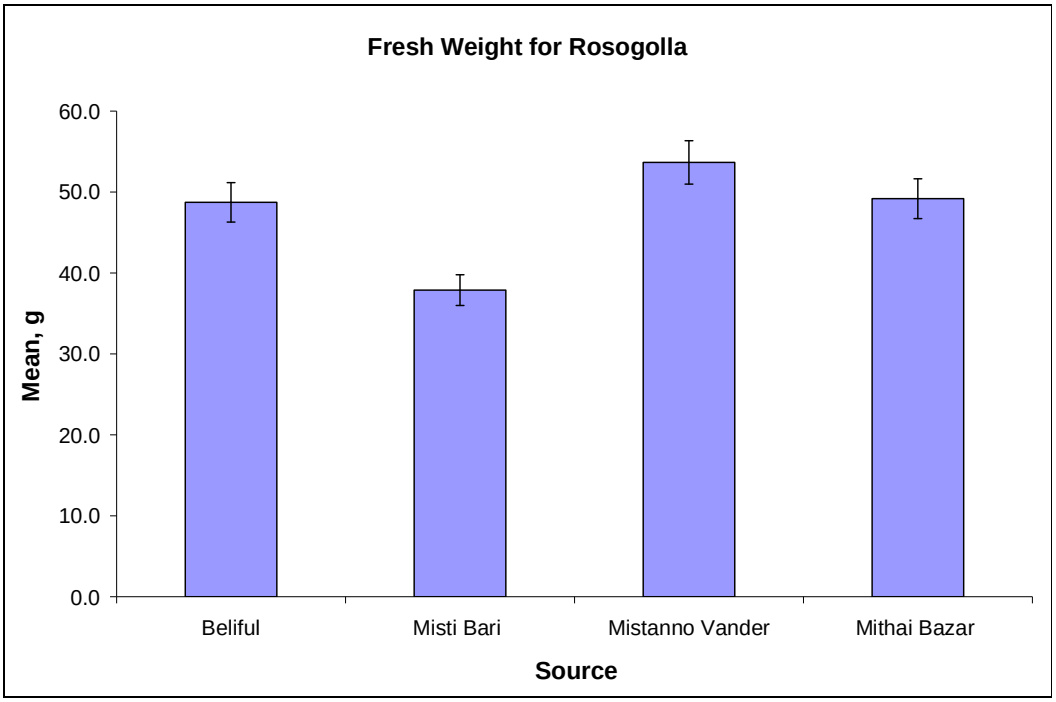

Figure 3.1: Mean Fresh Weight (FW) with SD of Rosogolla from four sources

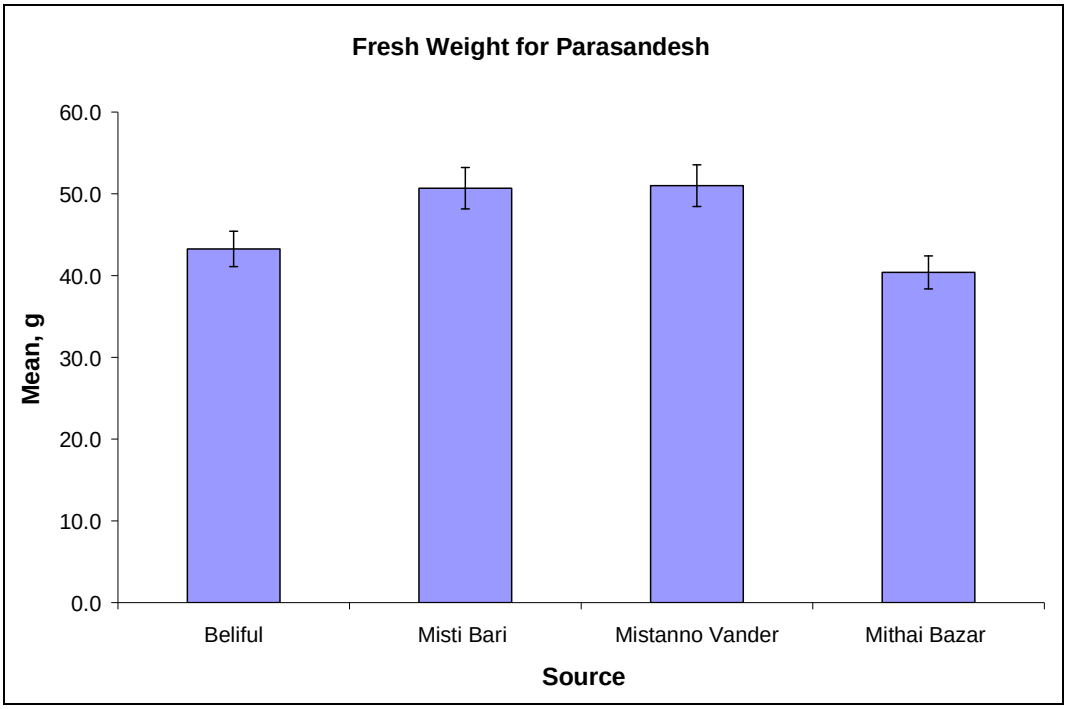

Figure 3.2: Mean Fresh Weight (FW) with SD of Parasandesh from four sources

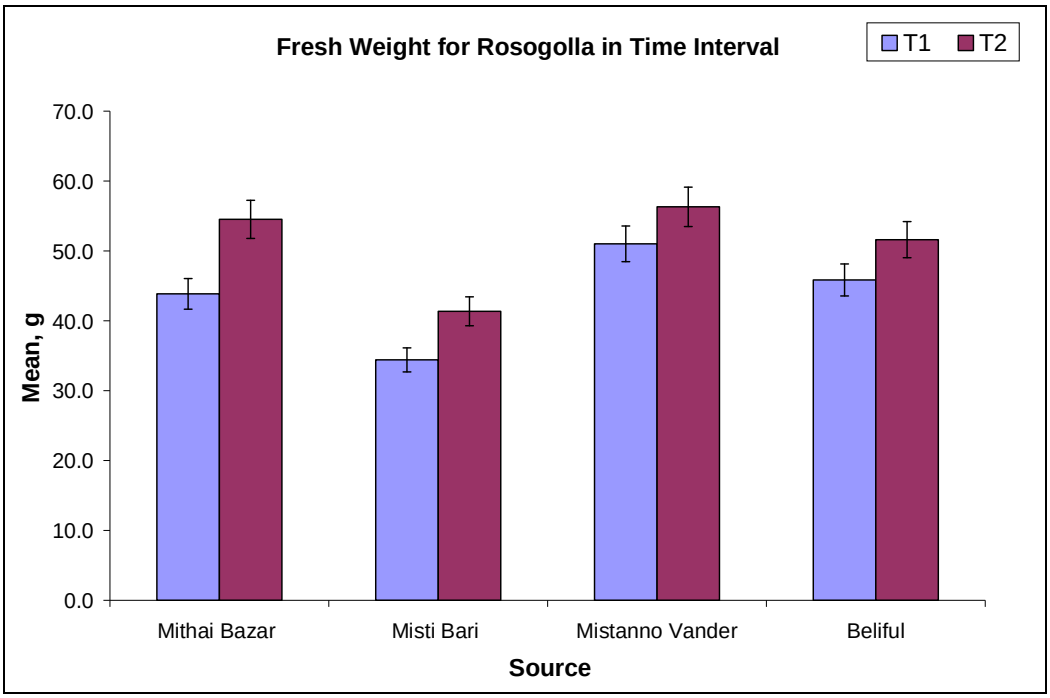

Figure 3.3: Mean Fresh Weight (FW) with SD of Rosogolla from four sources in time interval

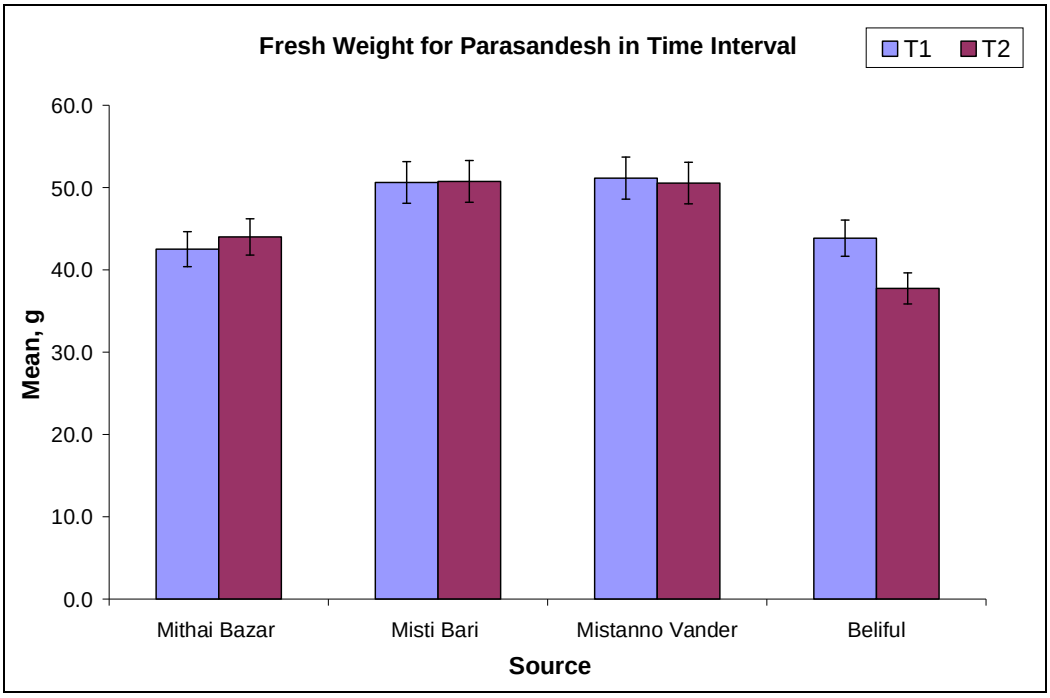

Figure 3.4: Mean Fresh Weight (FW) with SD of Parasandesh from four sources in time interval

Fresh weights vary moderately among four sources being the highest values found in MV samples and the lowest values found in MB. So the range is 50 to a little less 40g with a difference of about 10g is for that extreme case. Replicated values for all sources produced minor variability (that can be speculated as small values of SD) in their FW. However, the mean weight of P samples from MB and MV are almost similar and that is more or less 50g, on contrary, the other two sources, B and MBz offered their product of 40 to 45g of weight.

Interestingly, all four sources produced their R products significantly heavier in the time when second batch was analyzed. However, for P products in second batch, three sources maintained the weight of their P products except for the B source.

Therefore, tentatively, it can be said that all four sources are producing either of their product (R or P) of 40 to 50g as their FW.

3.2.2 Results of LME

Mean value of LME of both items of four sources and their comparisons in two time intervals are given in Figure 3.5 to Figure 3.8.

Here both items are the solid products and prepared from milk with containing other materials like sugar, water etc. Therefore, LME is considered as an important parameter and expressed the product in terms of equivalent amount of real milk.

LME for all R products showed a gradual decrease from the source B to MBz (see Figure 3.5). The highest value in B is almost 90mL that dropped for MBz is close to 70mL only that corresponded more than 20% variation occurred between those two sources. On the other hand, LME for P products showed different trend. MB and MV products have LME of nearly 250mL. The LME of this product for the source B is close to 200mL, while the LME of the product of MBz is only as close as only 150

mL. So, the difference the quality, in terms of LME, is found between highest and lowest values are more than 40% that is really a significant aspect to be considered.

LME values for all sources except the source B, produced in time intervals showed minor variability in their LME. Therefore, it can be assumed that two sources (Mb and MV) are producing better quality products compared to other two sources.

3.2.3 Results of TMS

Total milk solids are the measure of actual portion of milk present in that particular products, apart from water, sugar etc. Mean value of TMS of both items of four sources and their comparisons in two time intervals are given in Figure 3.9 to Figure 3.12. TMS is, in true sense, is strongly related to the LME parameter, therefore, the nature and trends found in those figures (Figure 3.9 to 3.12) can be found similar to the figures for LME, but only distinction is their units (that is in g).

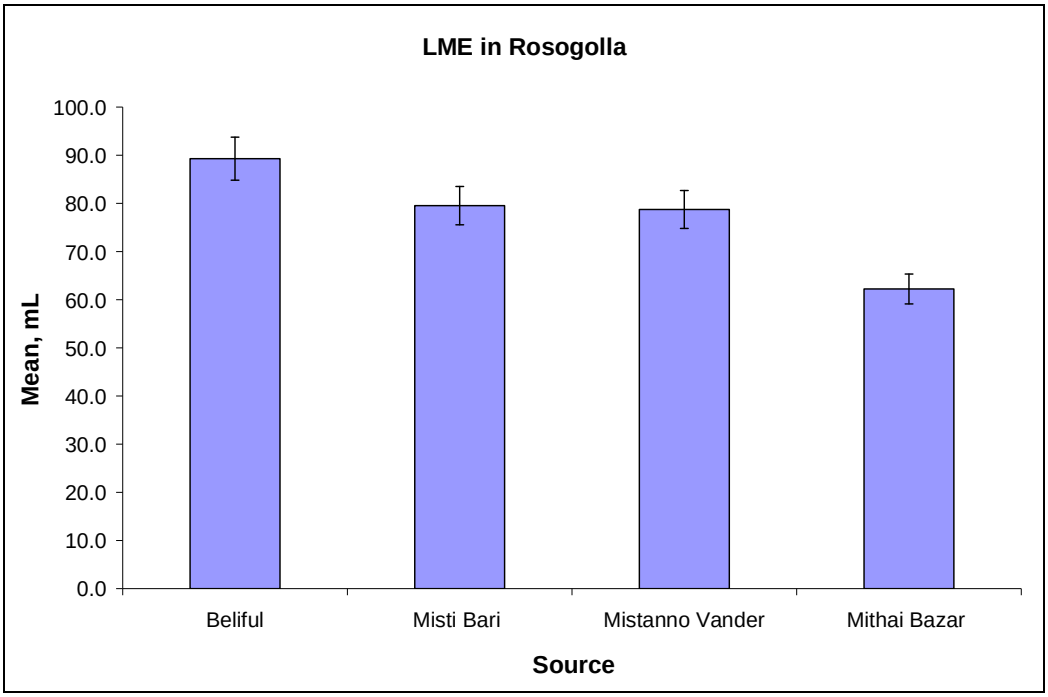

Figure 3.5: Mean LME with SD of Rosogolla from four sources

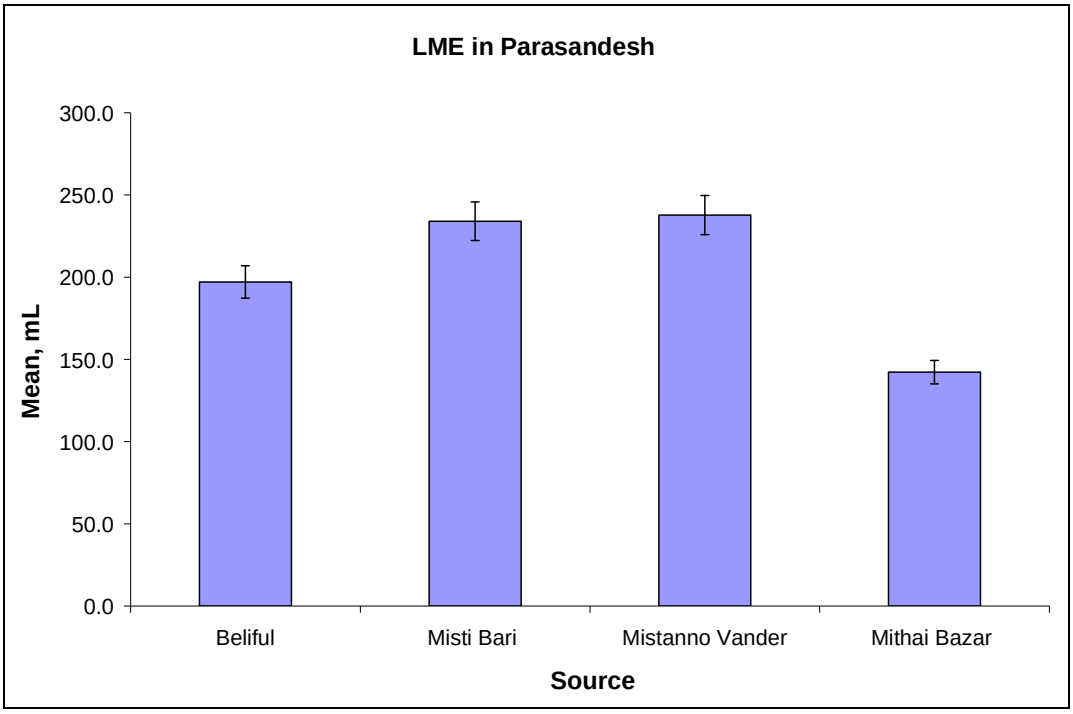

Figure 3.6: Mean Fresh Weight (FW) with SD of Parasandesh from four sources

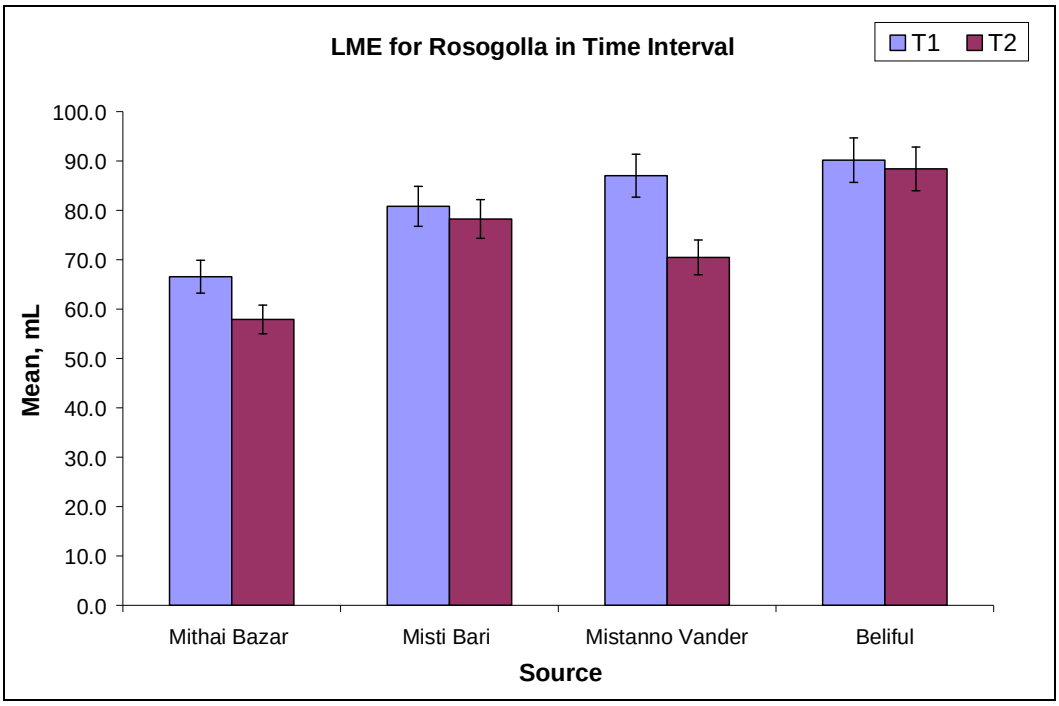

Figure 3.7: Mean LME with SD of Rosogolla from four sources in time interval

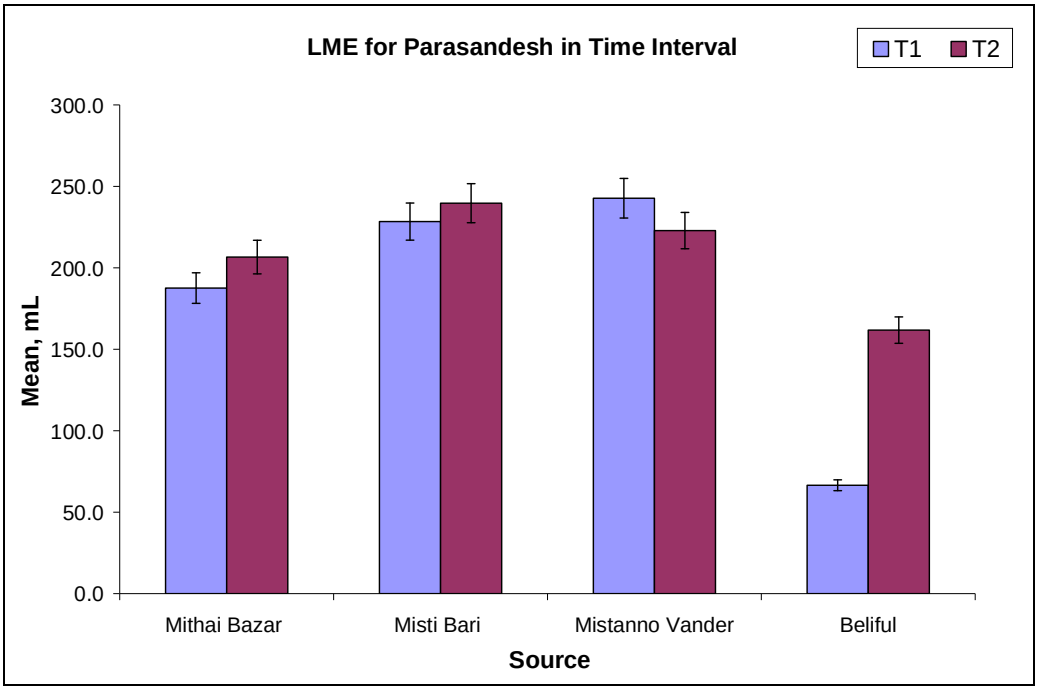

Figure 3.8: Mean Fresh LME with SD of Parasandesh from four sources in time interval

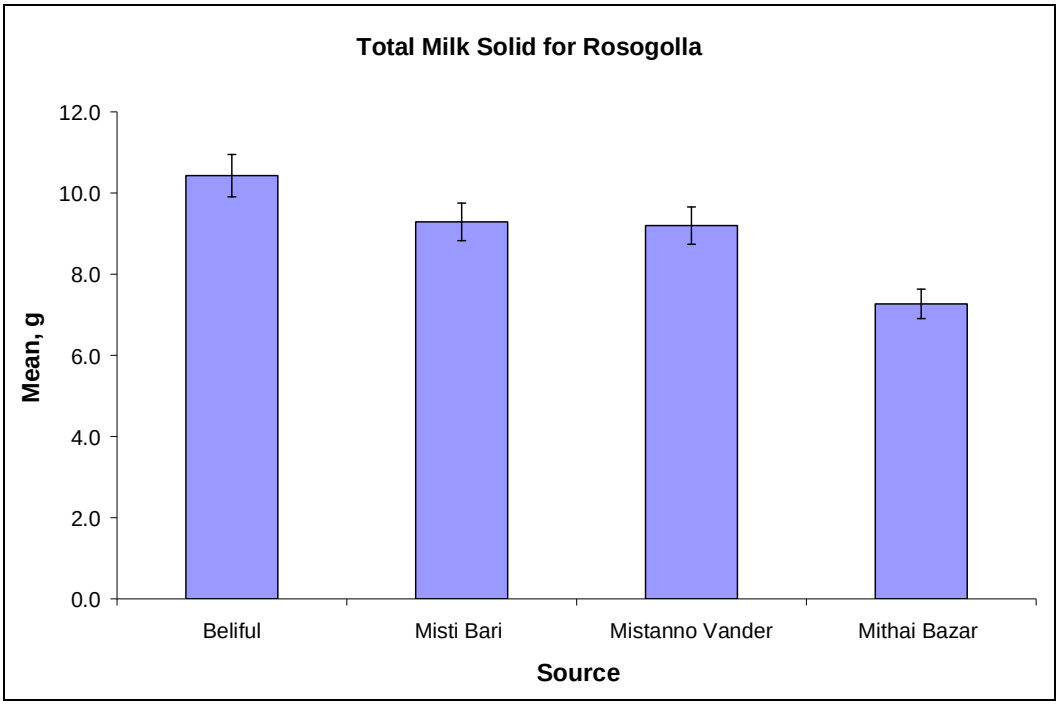

Figure 3.9: Mean TMS with SD of Rosogolla from four sources

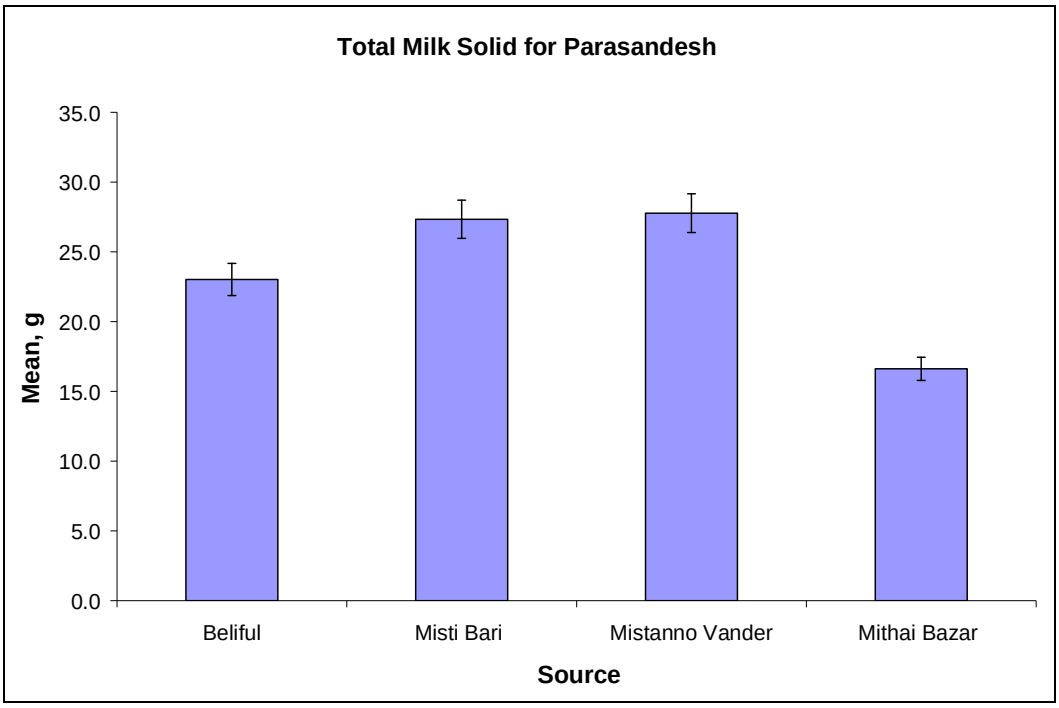

Figure 3.10: Mean TMS with SD of Parasandesh from four sources

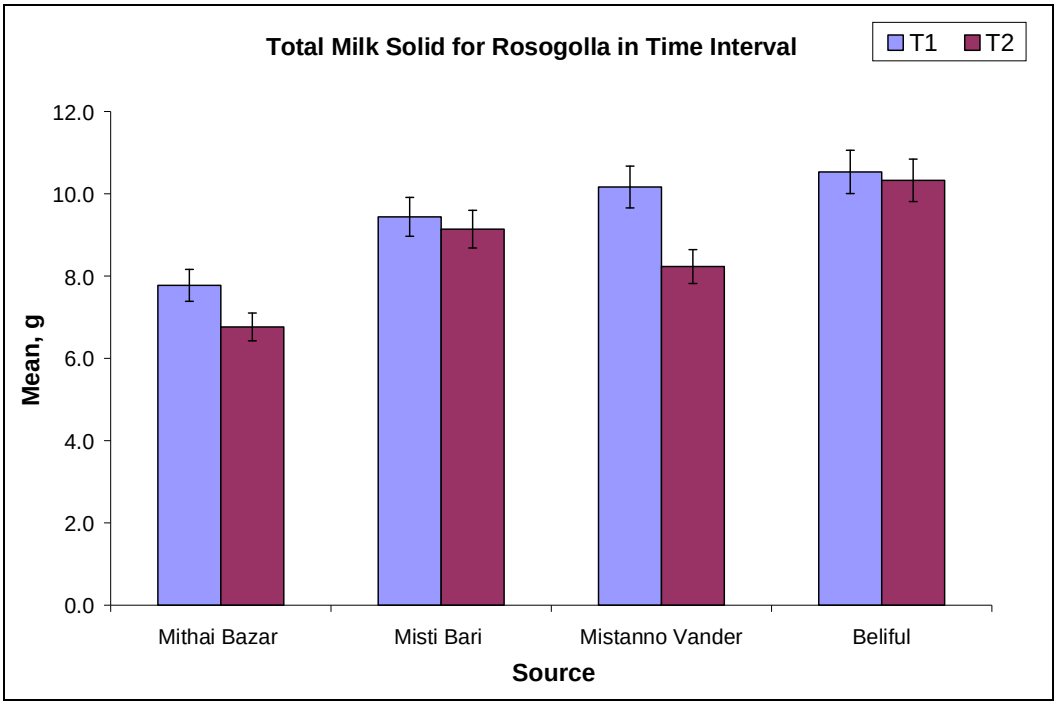

Figure 3.11: Mean TMS with SD of Rosogolla from four sources in time interval

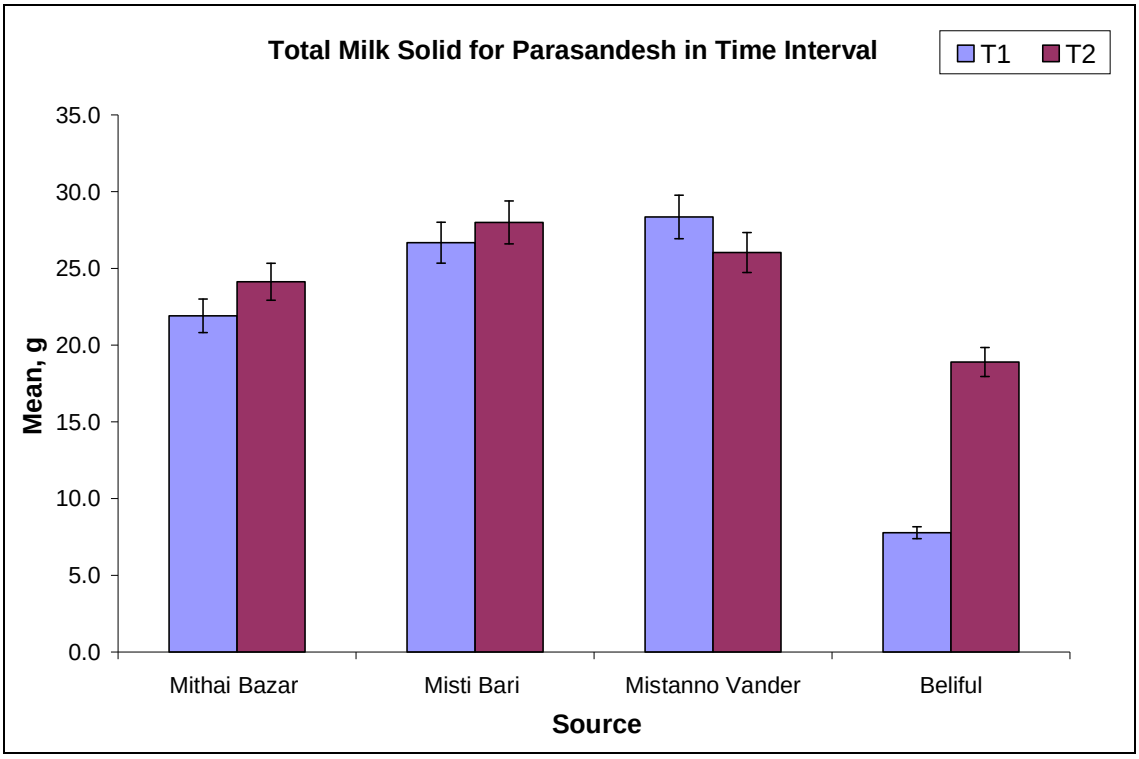

Figure 3.12: Mean TMS with SD of Parasandesh from four sources in time interval

3.2.4 Results of TS

Mean value of TS of both items of four sources and their comparisons in two time intervals are given in Figure 3.13 to Figure 3.16.

It is somewhat interesting to see that the products R, whose FW is roughly about 50g, but contains 20 to nearly 30g sugar. Only the source MB is using less sugar than other three counterparts. The TS values of P products in all sources are comparatively lower than their R products. The amount of TS in all P products is between 15 to 20g.

In time intervals, TS values for R products of MBz varied greatly, while the TS values for P products of the source B showed significant variation. In both time intervals, variability among replicates are not found significant.

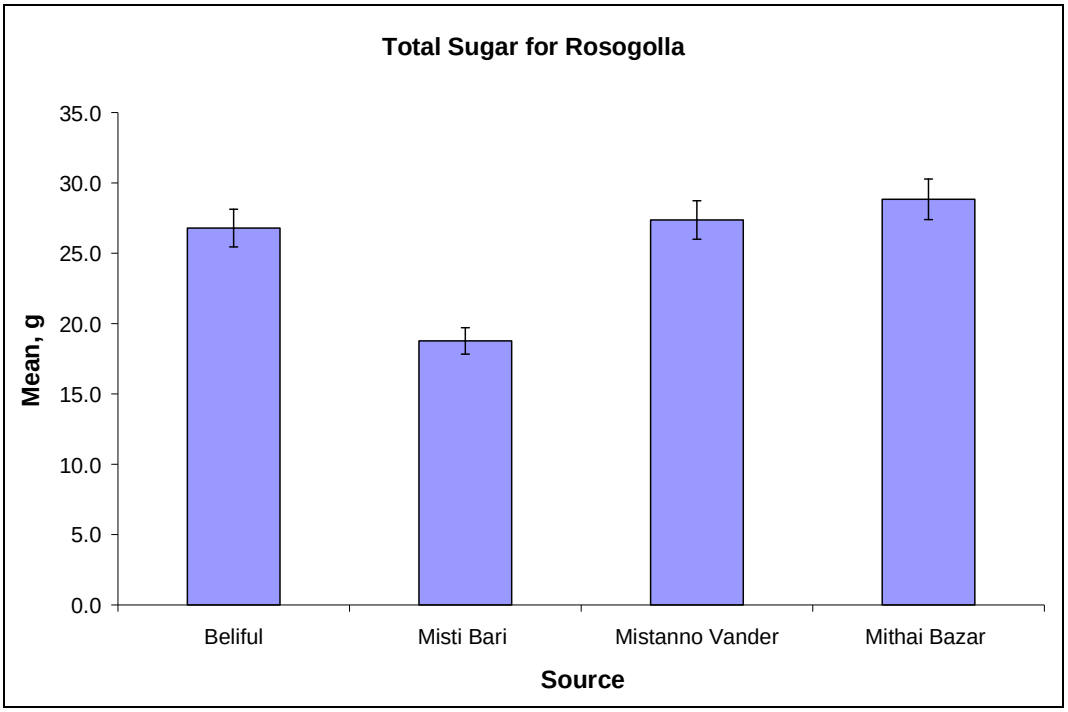

Figure 3.13: Mean TS with SD of Rosogolla from four sources

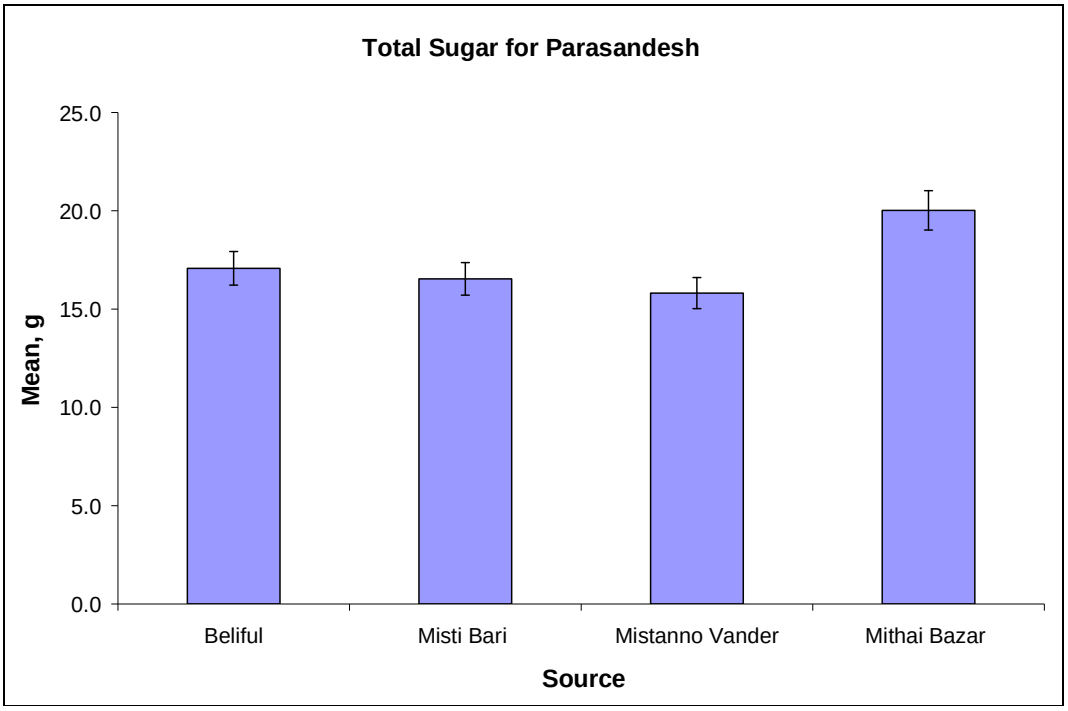

Figure 3.14: Mean TS with SD of Parasandesh from four sources

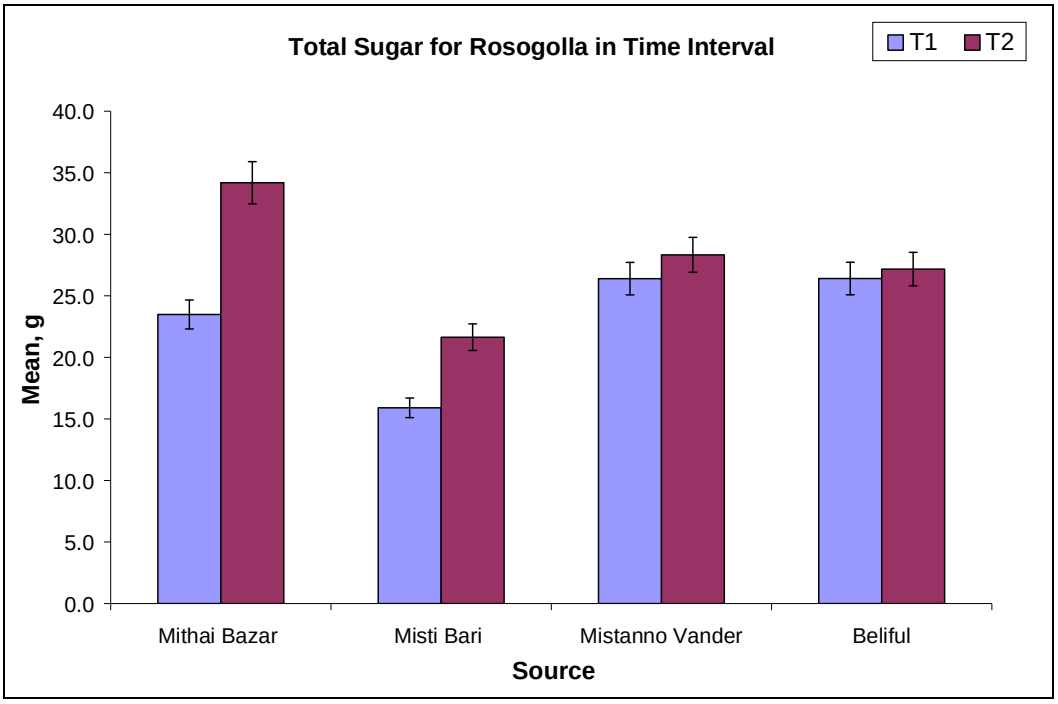

Figure 3.15: Mean TS with SD of Rosogolla from four sources in time interval

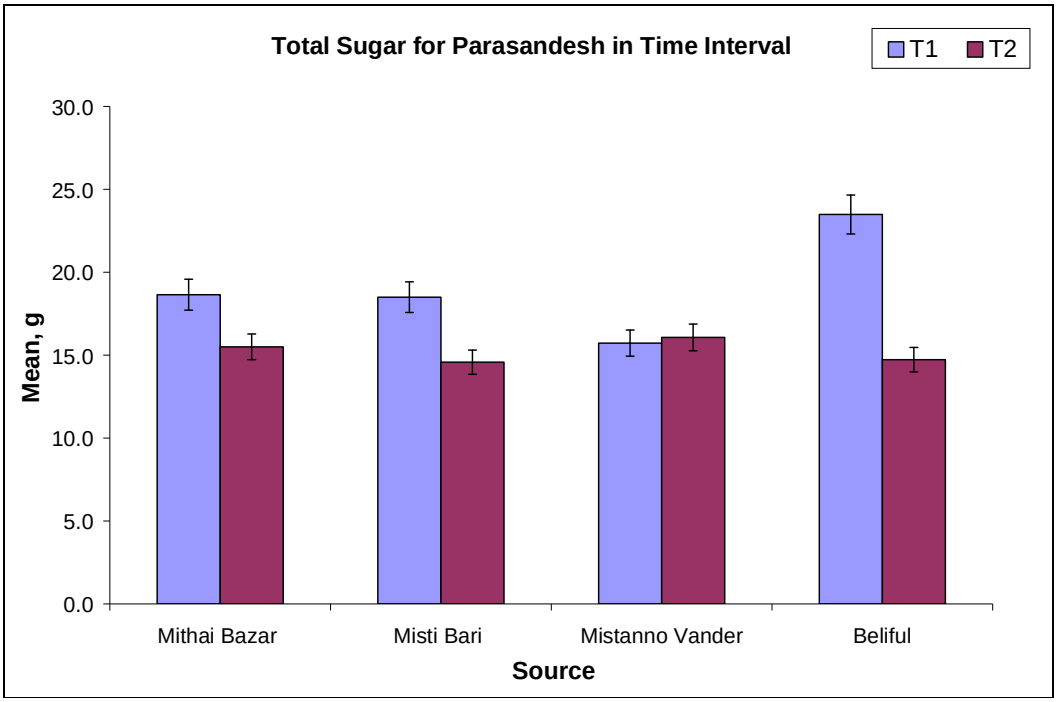

Figure 3.16: Mean TS with SD of Parasandesh from four sources in time interval

3.2.5 Results of Ca

Mean value of Ca of both items of four sources and their comparisons in two time intervals are given in Figure 3.17 to Figure 3.20.

Calcium contents in R products varied between a little over 50mg to almost 90mg, the lowest value found for the source MB, and the highest value is for the source B. So it is surprising that this crucial nutrient is varied up to 80% among the sources.

The scenario is different for P products, the highest values (over 250mg) found for MB and MV, and the rest two (B and MBz) producing the products contain nearly similar amount of Ca that is about 200mg. In terms of %, the variation is about 20 to 25%. But it is obvious that all P products contain nearly double (or more) the amount of Ca than its R counterparts.

It can be said that the level of Ca of the both products (R and P) of source B varied greatly in time intervals, therefore it is assumed that they may not be so particular to maintain their products overtime. However, the variability or error levels in the analysis of both batches are quite appreciable.

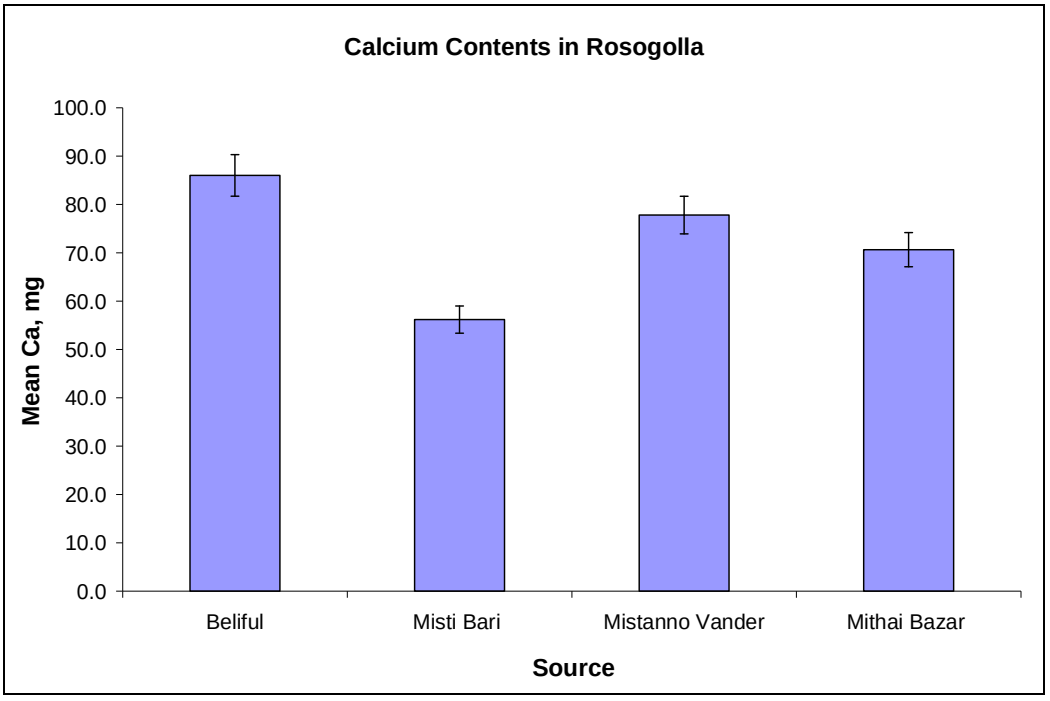

Figure 3.17: Mean Calcium contents with SD of Rosogolla from four sources

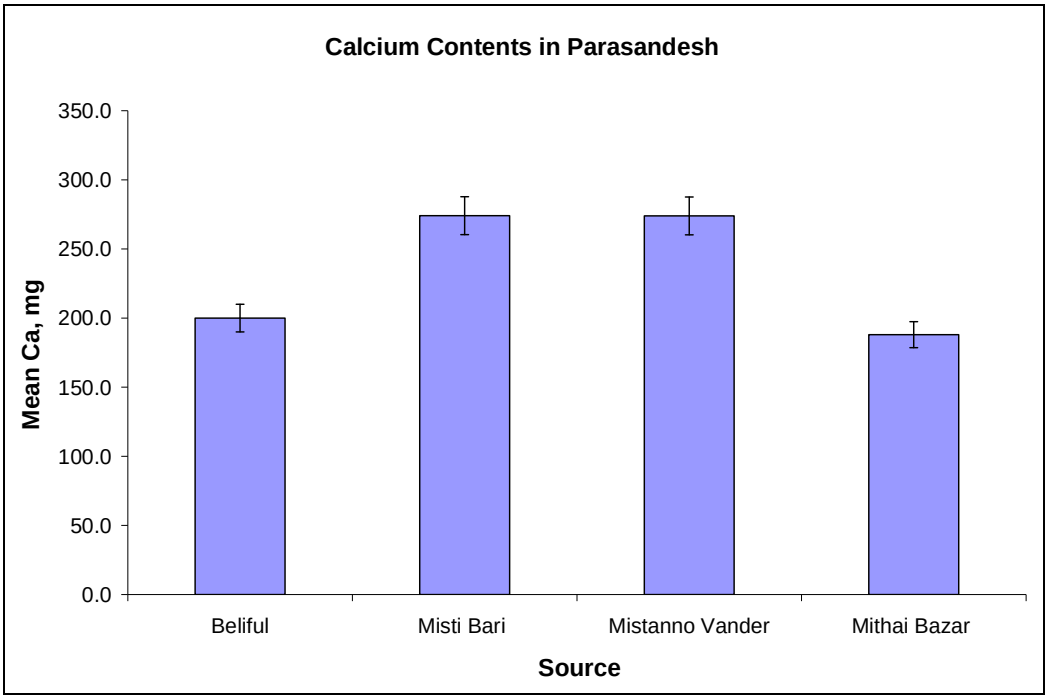

Figure 3.18: Mean Calcium contents with SD of Parasandesh from four sources

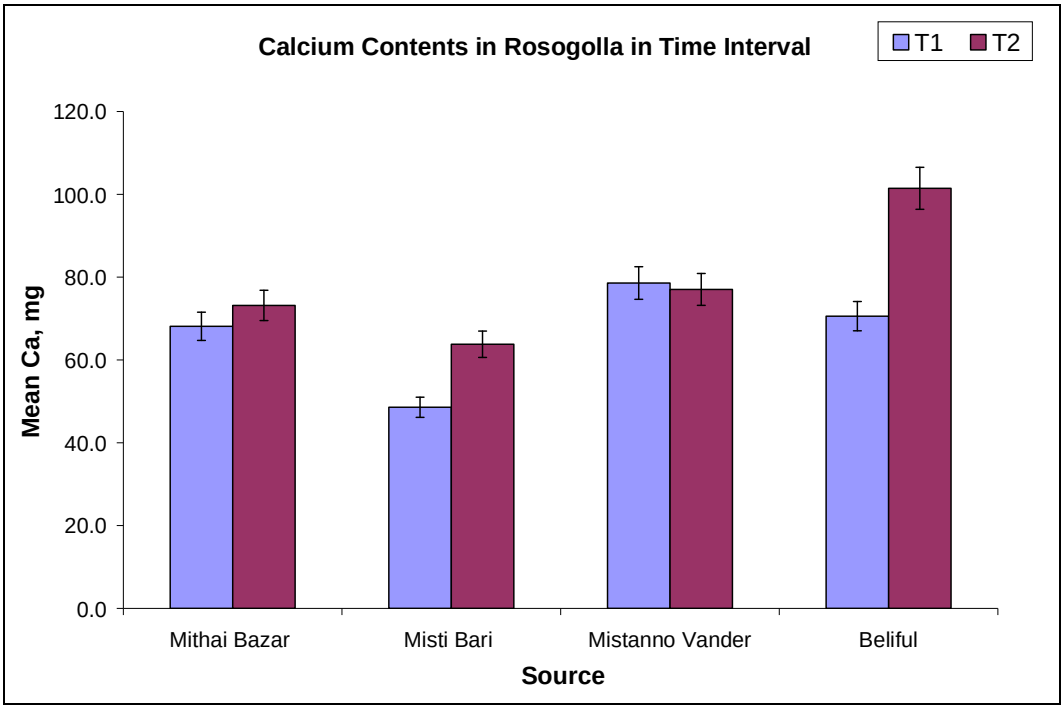

Figure 3.19: Mean Calcium contents with SD of Rosogolla from four sources in time interval

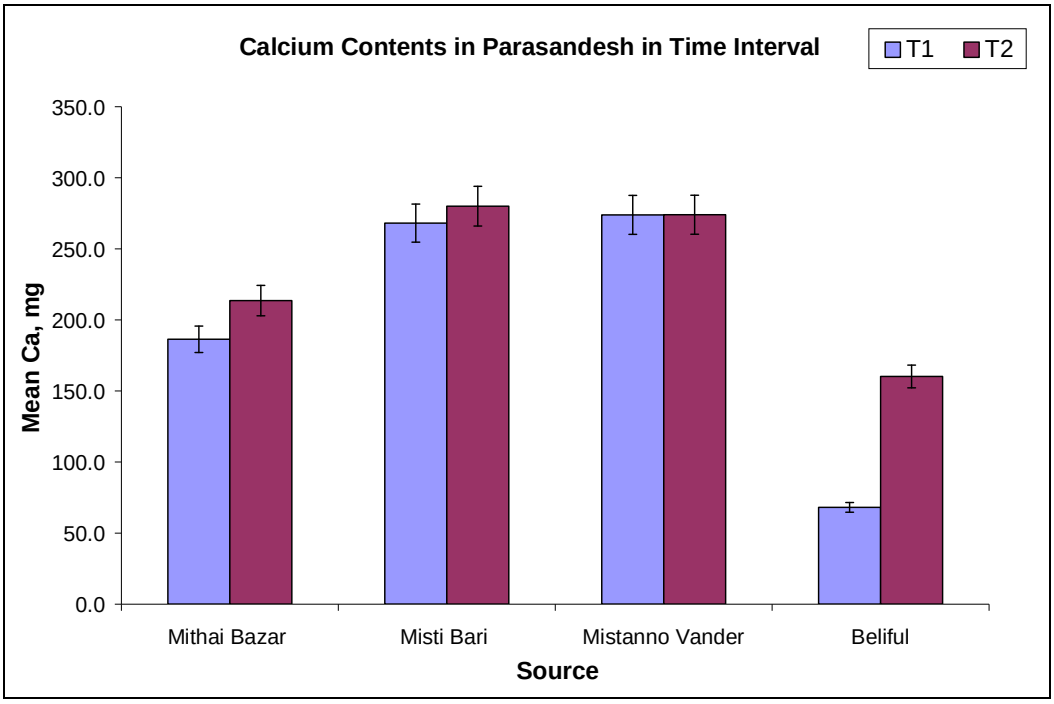

Figure 3.20: Mean Calcium contents with SD of Parasandesh from four sources in time interval

3.2.6 Results of Zn

Mean value of Zn of both items of four sources and their comparisons in two time intervals are given in Figure 3.21 to Figure 3.24.

Zinc contents in R products varied between a little over 0.08mg (80µg) to about 0.13mg (130µg), the lowest value found for the source MB, and the highest value is for the source MV. Likewise Ca, this element too is varied between 40 to 60% among the sources, which is significant.

Again for P products (similar to Ca), the highest values (about 250µg) are found for MB and MV, and the rest two (B and MBz) producing the products contain Zn that is well above 150µg and little below of 200µg, respectively. Significant variation in Zn contents is obvious for two products; the highest values are always for the P products.

In this case, little variations are found in the results for both products in time intervals for all four sources.

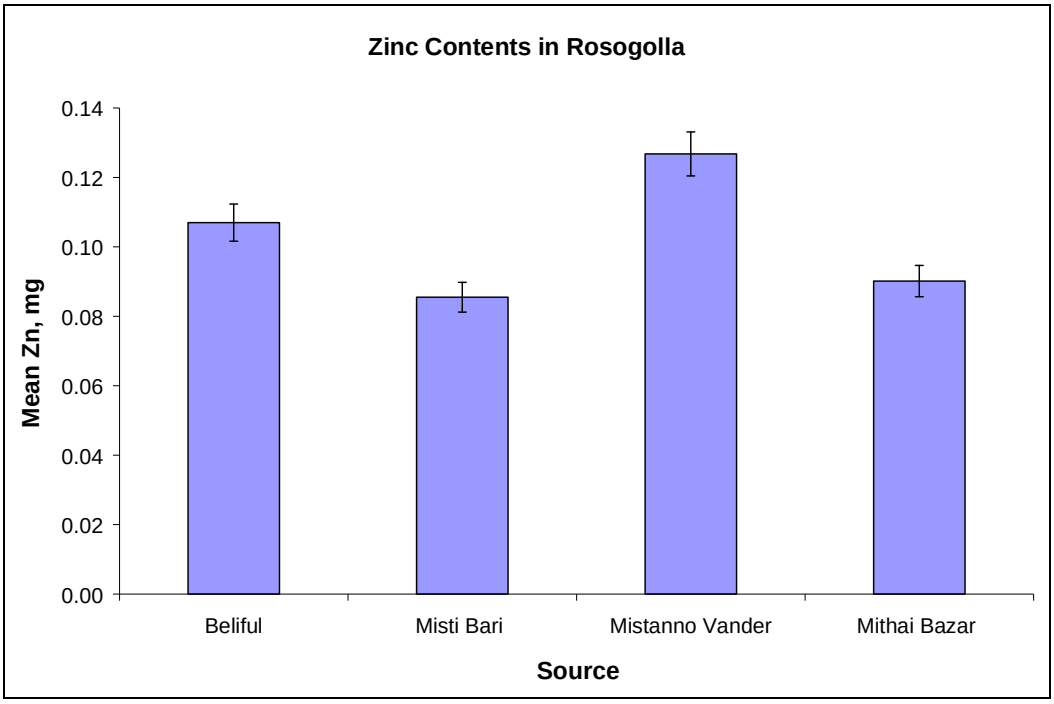

Figure 3.21: Mean Zinc contents with SD of Rosogolla from four sources

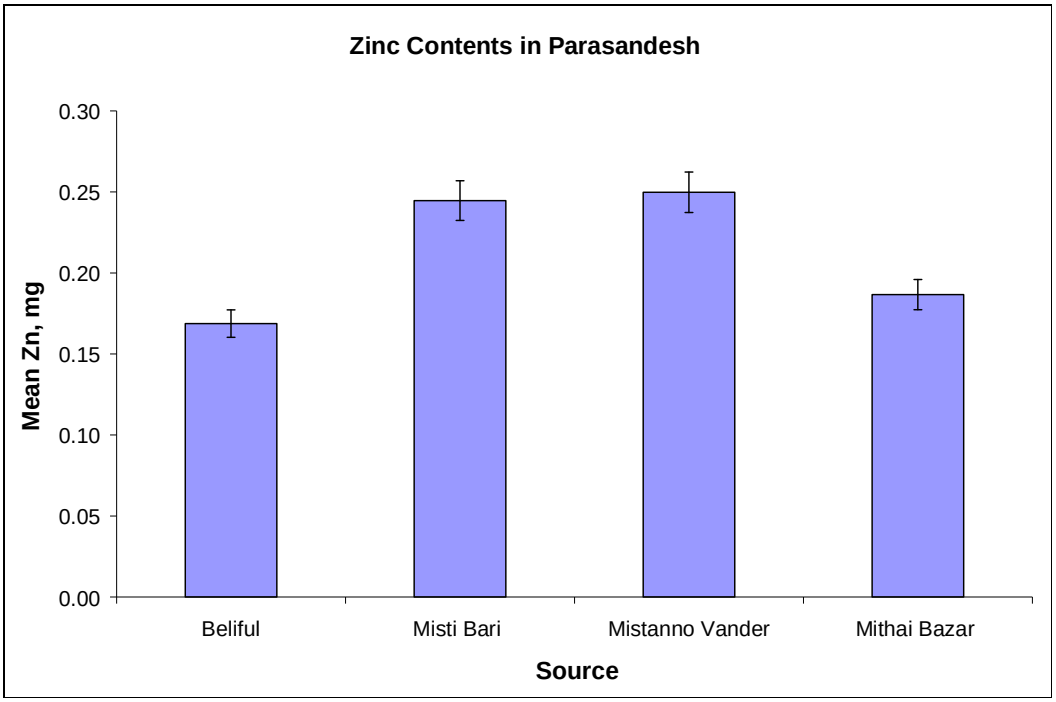

Figure 3.22: Mean Zinc contents with SD of Parasandesh from four sources

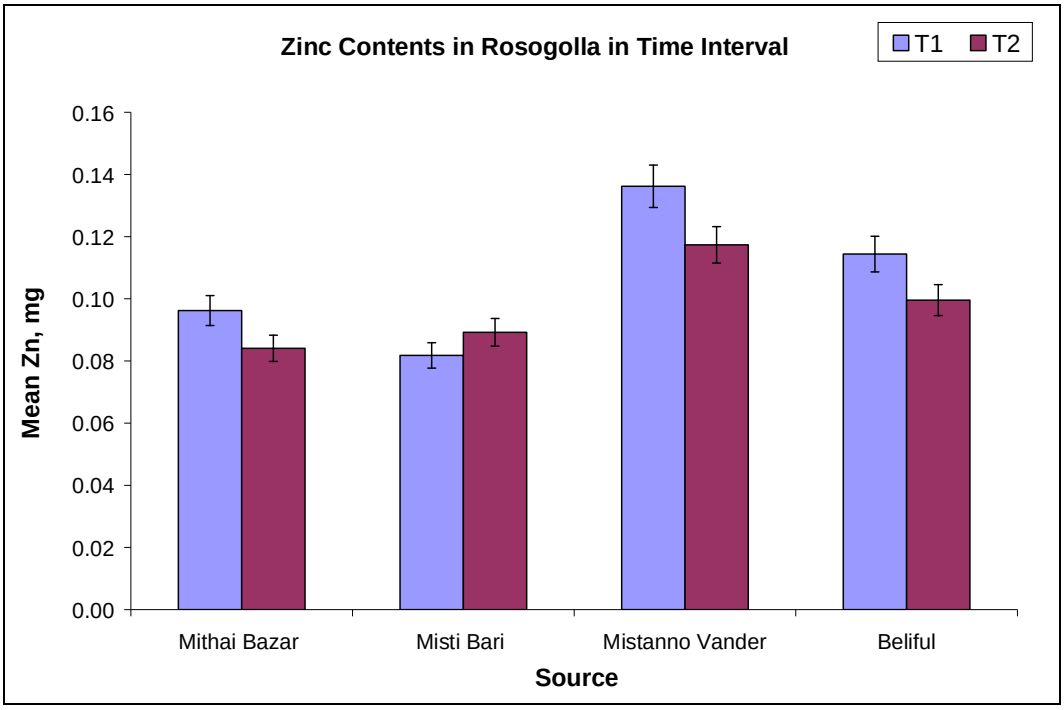

Figure 3.23: Mean Zinc contents with SD of Rosogolla from four sources in time interval

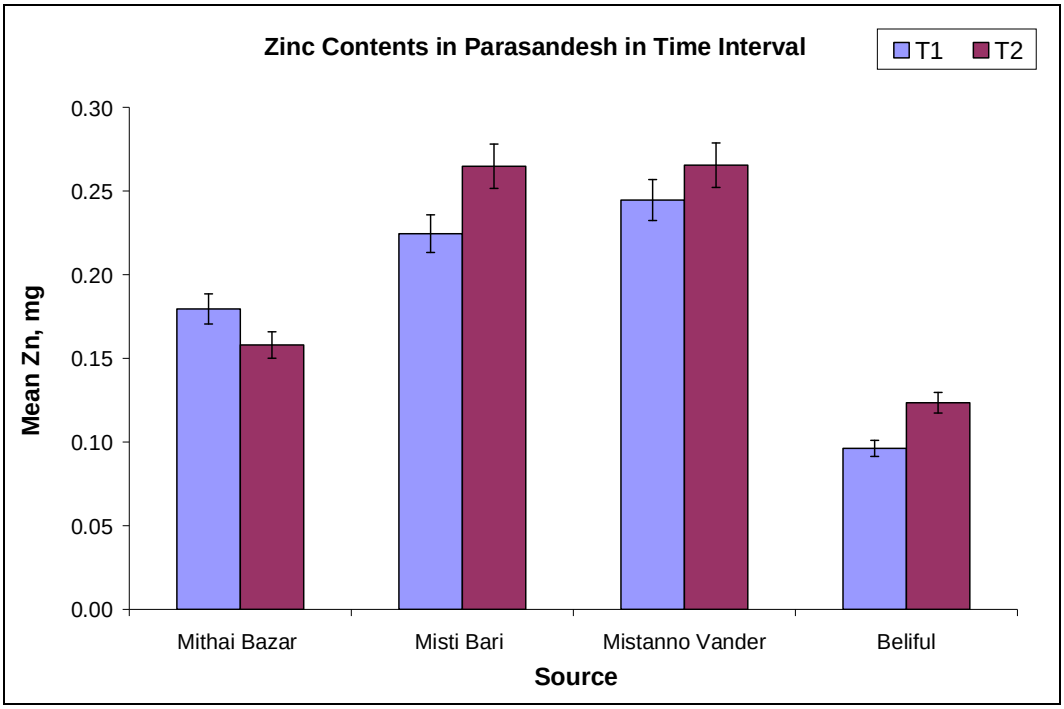

Figure 3.24: Mean Zinc contents with SD of Parasandesh from four sources in time interval

3.2.7 Results of Mn

Mean value of Mn of both items of four sources and their comparisons in two time intervals are given in Figure 3.25 to Figure 3.28.

Manganese contents in R products varied between a little below of 0.04mg (40µg) to about 0.12mg (120µg), again the lowest value found for the source MB, and the highest value is for the source MV. The variation can be calculated as up to 200% among the sources, which is really a significant issue.

However, for P products, the highest values (about 150µg) are found only for MBz and the rest three (B, MB and MV) producing the products contain Mn that is only about 60µg. Significant variation in Mn contents is found again obvious for two products; the highest values are found usually for the P products.

It is to be noticed that for both products the Mn contents are significantly higher in the samples, of all sources, used in second batch. This huge variations in Mn contents indicate that the quality of products is being not properly managed the producers. Variations in error levels are found arguably minimal.

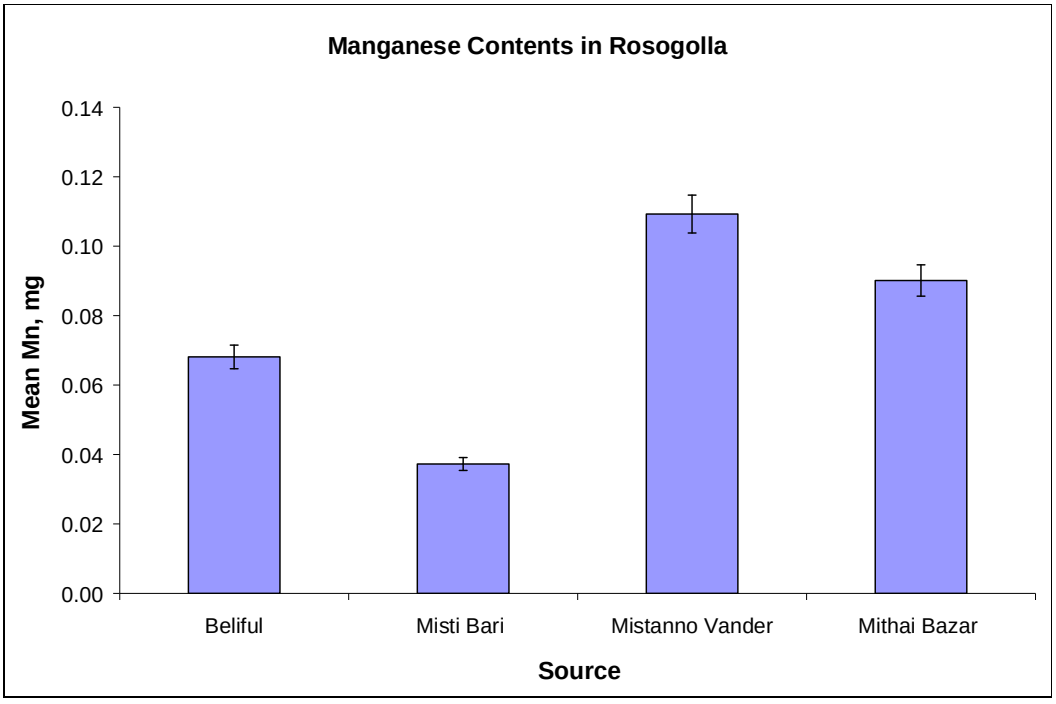

Figure 3.25: Mean Manganese contents with SD of Rosogolla from four sources

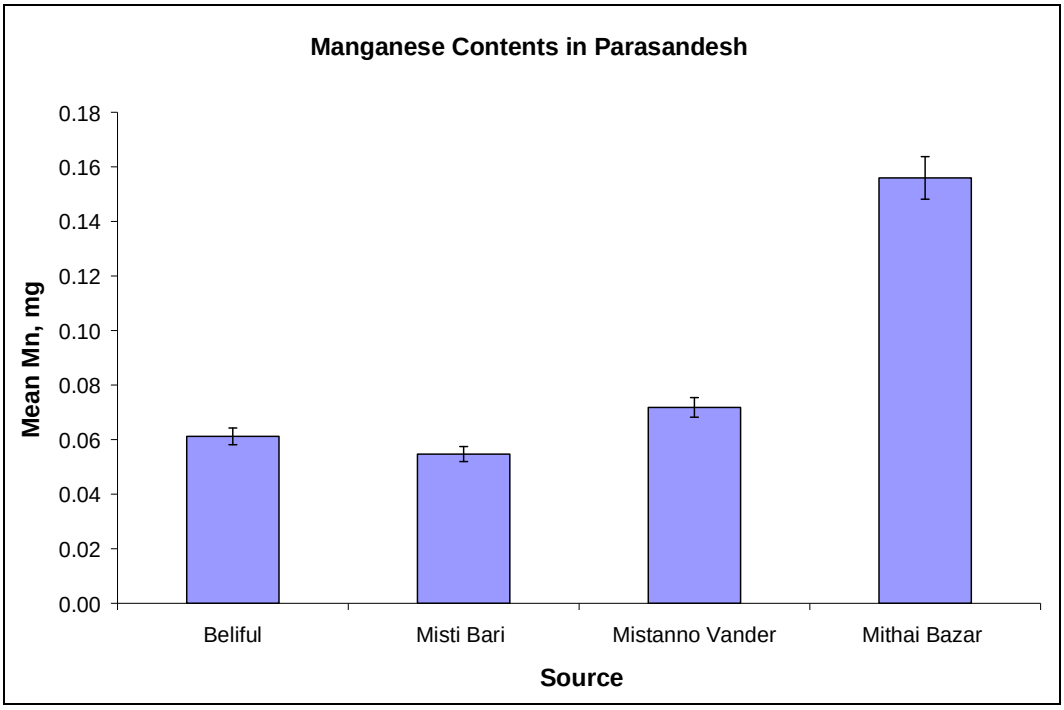

Figure 3.26: Mean Manganese contents with SD of Parasandesh from four sources

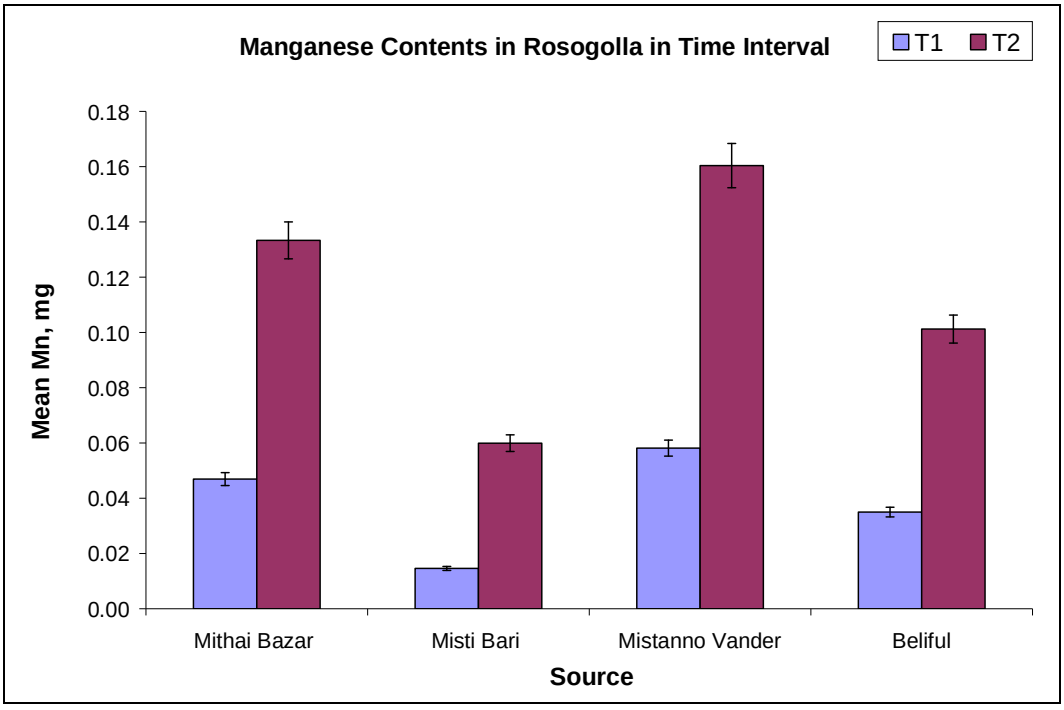

Figure 3.27: Mean Manganese contents with SD of Rosogolla from four sources in time interval

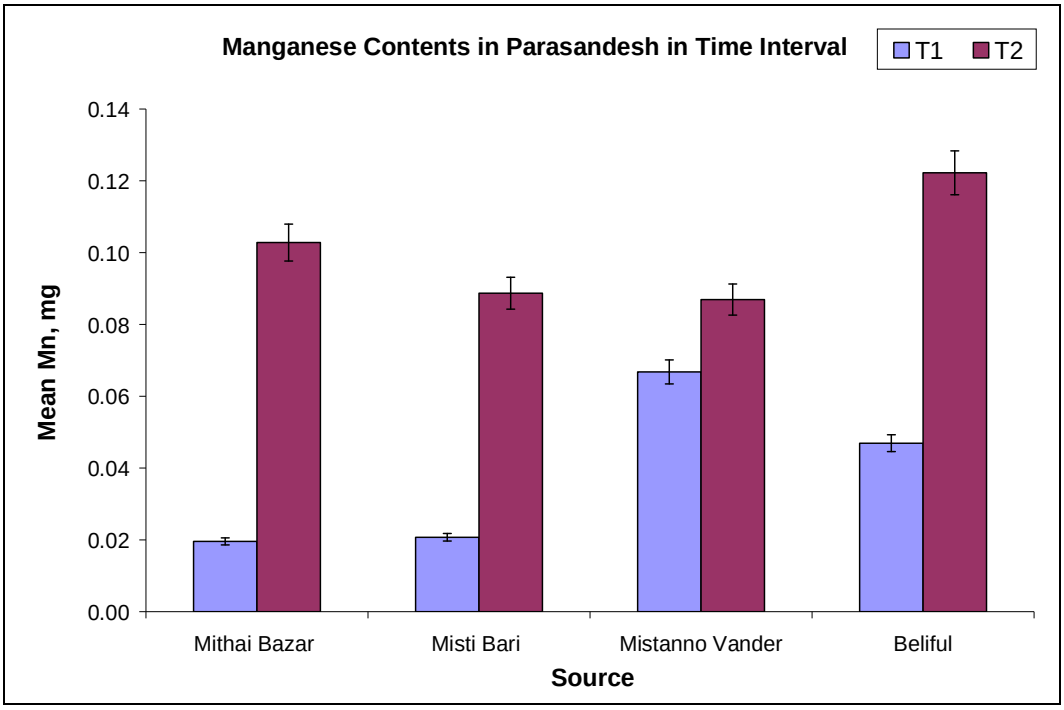

Figure 3.28: Mean Manganese contents with SD of Parasandesh from four sources in time interval

3.2.8 Results of Cu

Mean value of Cu of both items of four sources and their comparisons in two time intervals are given in Figure 3.29 to Figure 3.32.

Copper contents in R products varied between a little below of 0.004mg (4µg) to about 0.012mg (12µg), the lowest value found for the source MB, and the highest value is for the source MV. The variation also can be calculated as up to 200% among the sources, which is off course a significant figure, and this trend is similar to Mn found just earlier.

For P products, the highest values (about 16µg) are found only for MBz and the rest three (B, MB and MV) producing the products contain Cu that is only about 6 to 8µg. Significant variation in Cu contents can not be appreciably attributable for two products.

It is again to be noticed that for both products the Cu contents are significantly higher in all the samples from all sources but those used in second batch only. That attributes that huge variations in Cu contents are result of mismanagement being occurring in the production levels. Variations in error levels are again found arguably minimal.

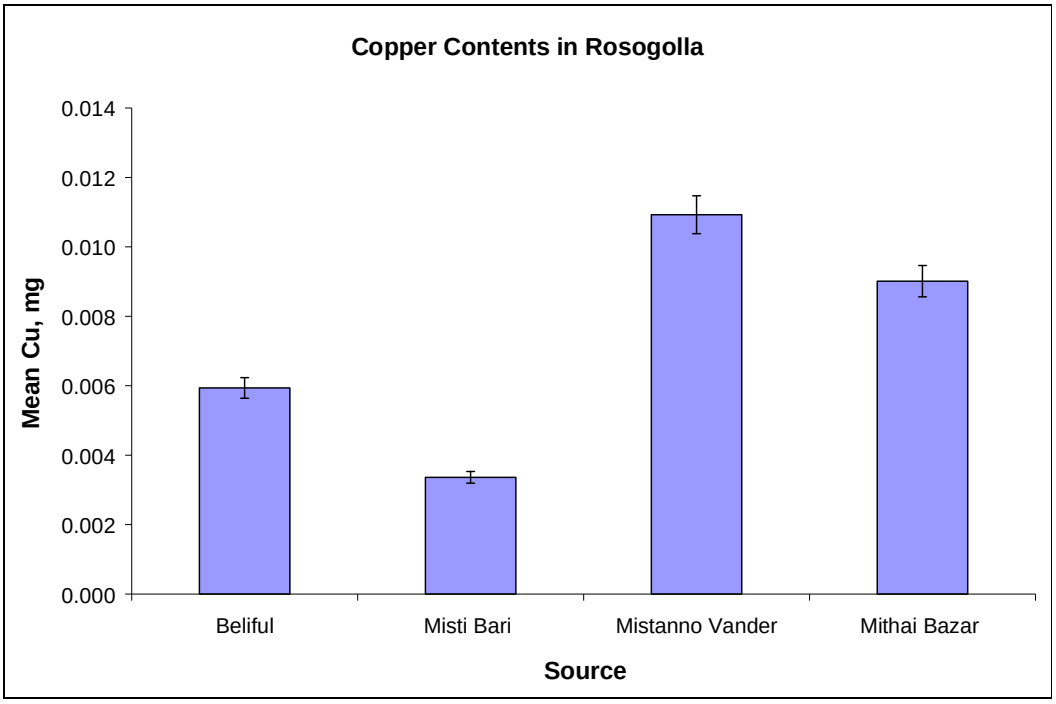

Figure 3.29: Mean Copper contents with SD of Rosogolla from four sources

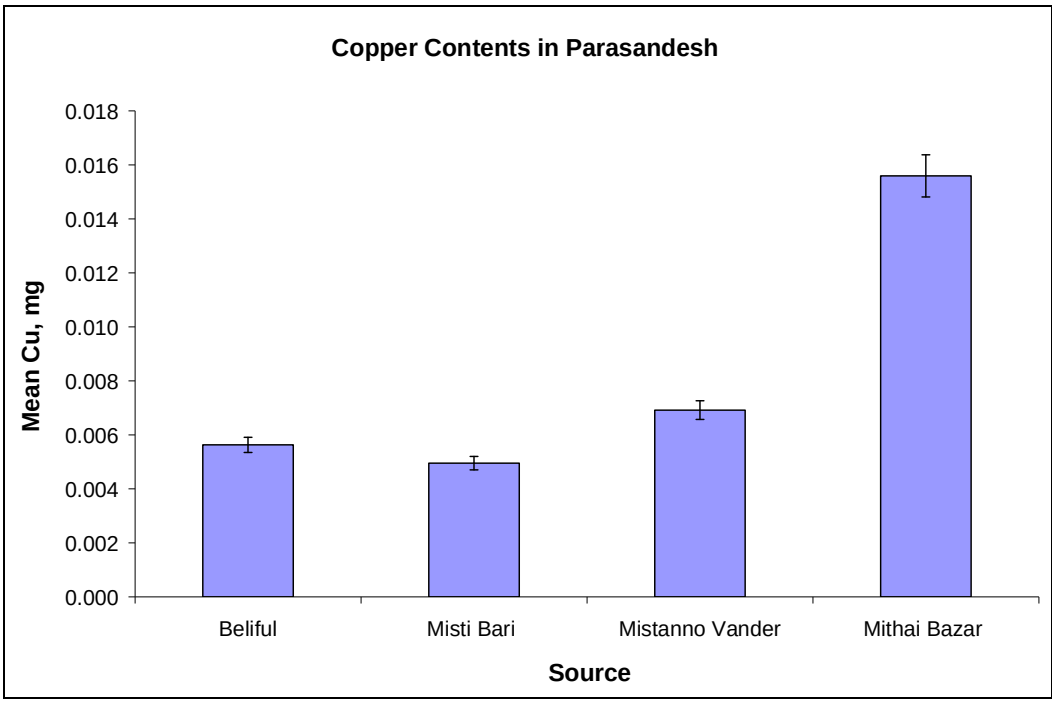

Figure 3.30: Mean Copper contents with SD of Parasandesh from four sources

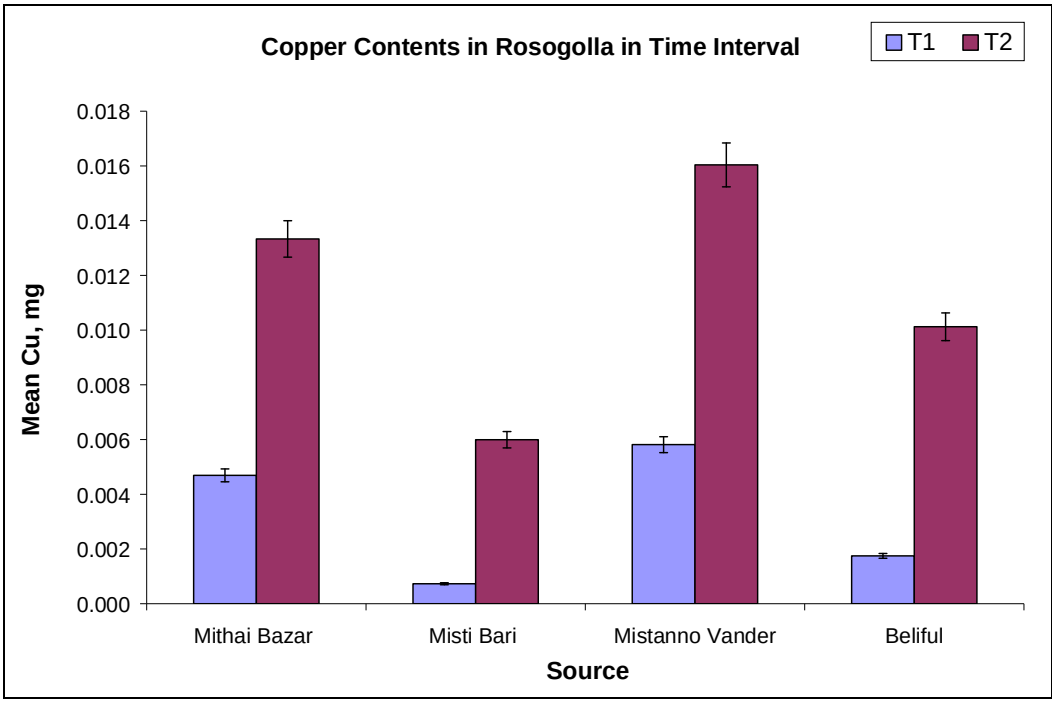

Figure 3.31: Mean Copper contents with SD of Rosogolla from four sources in time interval

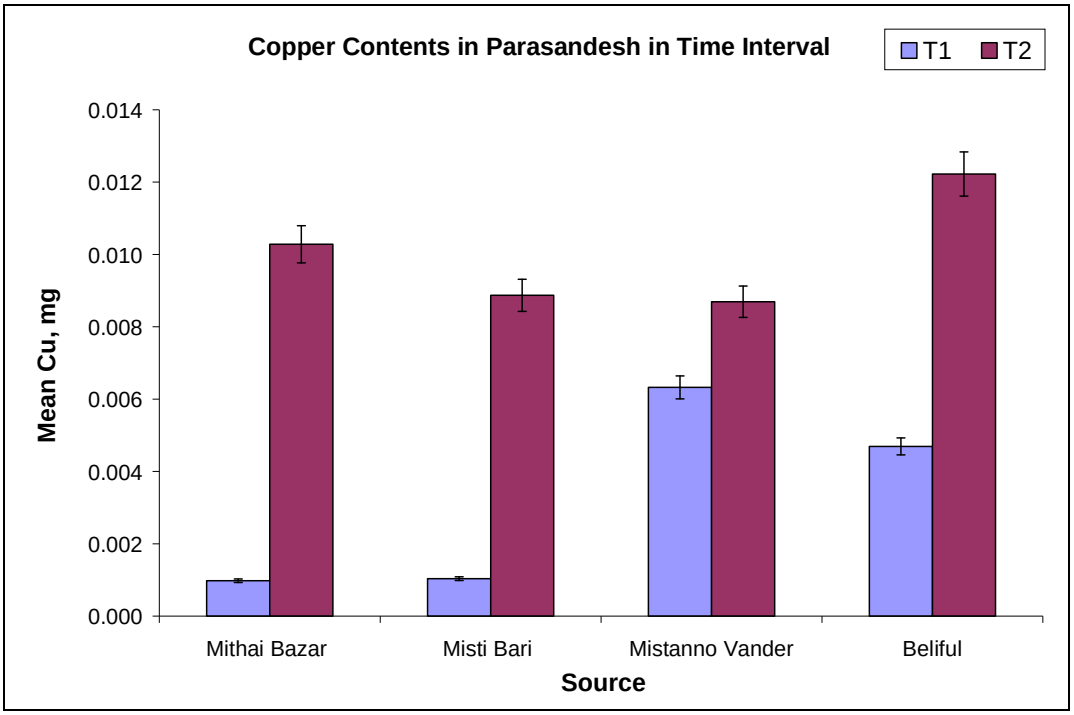

Figure 3.32: Mean Copper contents with SD of Parasandesh from four sources in time interval

3.2.9 Results of Pb

Mean value of Pb of both items of four sources and their comparisons in two time intervals are given in Figure 3.33 to Figure 3.36.

Those previous minerals e.g., Ca, Zn, Mn and Cu are considered as essential elements (some of them are micro nutrients), therefore their presence and the level in milk products are considered beneficial, while the presence and the level of metal ion Pb is to be considered differently. As its presence, with certain degrees, is a matter of concern and usually termed as contaminated or toxicity occurred in the edible foods.

Lead contents in R varied negligibly, between a little below of 0.05mg (50µg) to about 0.07mg (70µg), the lowest value found for the source MV, and the highest value is for the source MBz. Therefore, the variation is about 40% among the sources of highest and lowest values. Obviously, this level of Pb present in any regular consumables is really matter of concern.

For P, the values hiked further, about 40µg is found in products of MBz, but for MB it is well above 100µg. Significant variation in Pb contents for two products is hard to notice.

For both products, the Pb contents are significantly lower (unlike to Cu) in all the samples from all sources but those used in analysis as second batch. This huge variation in time interval (either positively or negatively) upholds the earlier statement of poor quality control of production line for all producers examined here. Variations in error levels are fully justifiable.

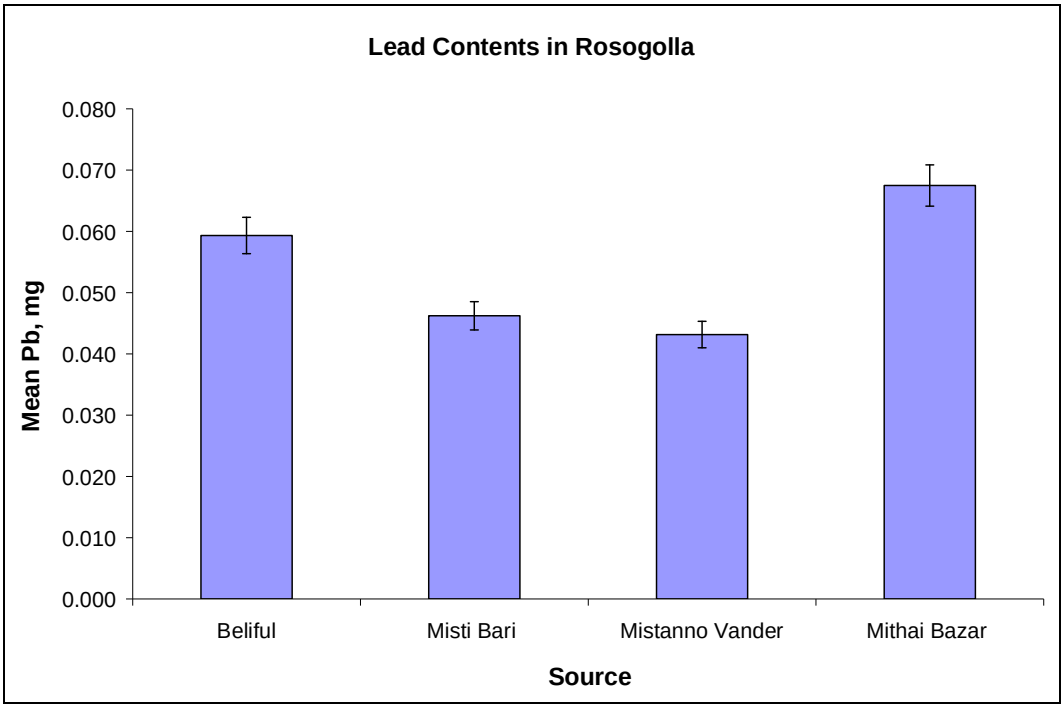

Figure 3.33: Mean Lead contents with SD of Rosogolla from four sources

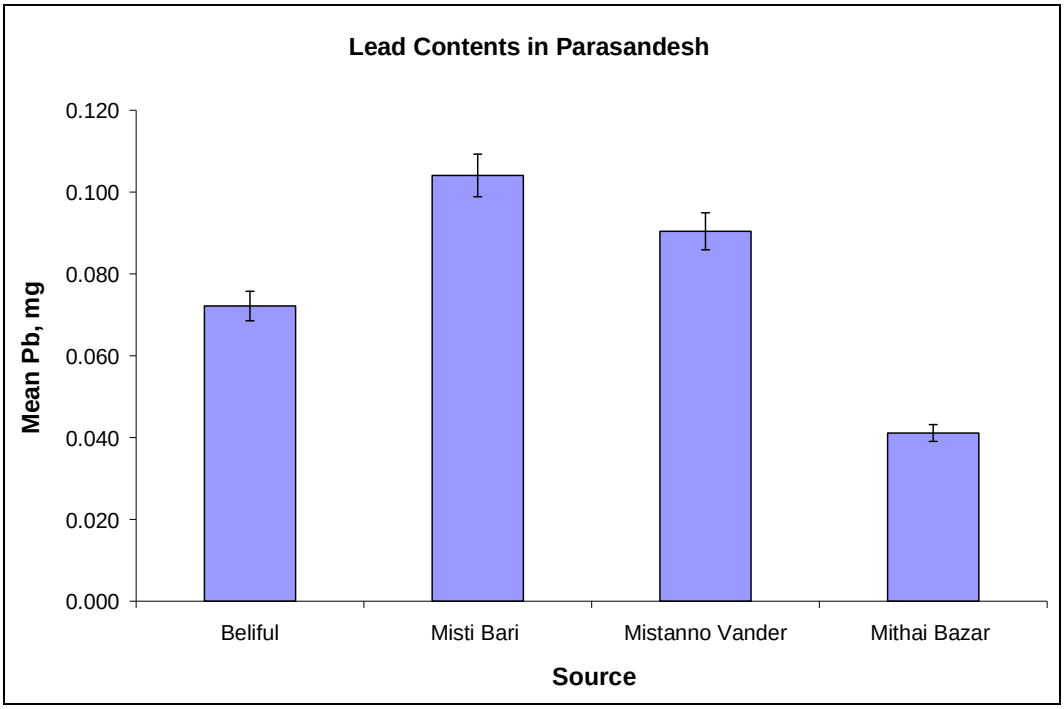

Figure 3.34: Mean Lead contents with SD of Parasandesh from four sources

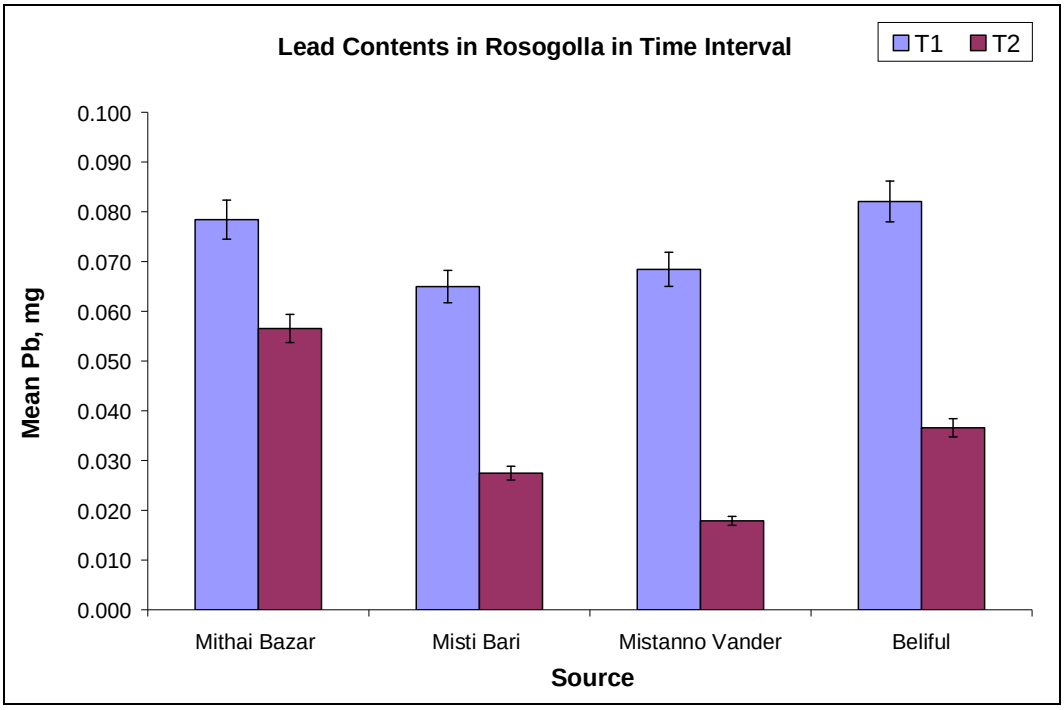

Figure 3.35: Mean Lead contents with SD of Rosogolla from four sources in time interval

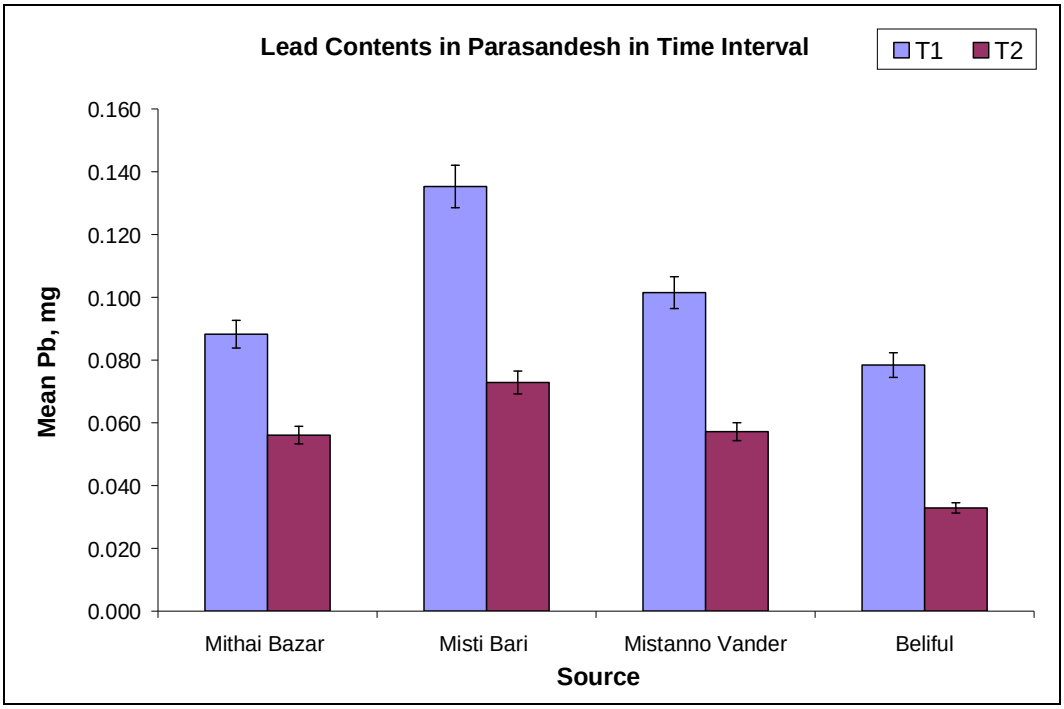

Figure 3.36: Mean Lead contents with SD of Parasandesh from four sources in time interval

3.2.10 Results of Cr

Mean value of Cr of both items of four sources and their comparisons in two time intervals are given in Figure 3.37 to Figure 3.40.

Chromium is an essential element for human nutrition, but its toxicity level is not far above of its appreciable levels and that is depends of its oxidation states. Therefore, sometimes its presence in food is also a matter of concern.

On the basis of chromium contents in R, all four sources can be classified in two groups. The source B and MB producing products with less amount of Cr which is only about 5µg, while for the other two (MV and MBz), the level is found to be 25 µg, which is exactly five times higher of the former two. Therefore, the variation is up to 400% among two groups of four sources, similar kind of trend is not found in earlier cases.

Chromium contents for P showed a linear trend, in which products from source B contain only 10 µg which is gradually increased to be 40µg for the products of MBz, and the rest two is in between. Variation in Cr contents for two products can not be mentioned as significant.

For both products, the trends of increasing nature in Cr contents for second batch sample are easy to notice. However data revealed that products R from the source B and MB were not incorporated in first batch, possibly because of a short breakdown of AAS machine. Similarly, products P from the source MB and MBz were not incorporated in that first batch of analysis. As usual, variations in error levels are considerably justifiable.

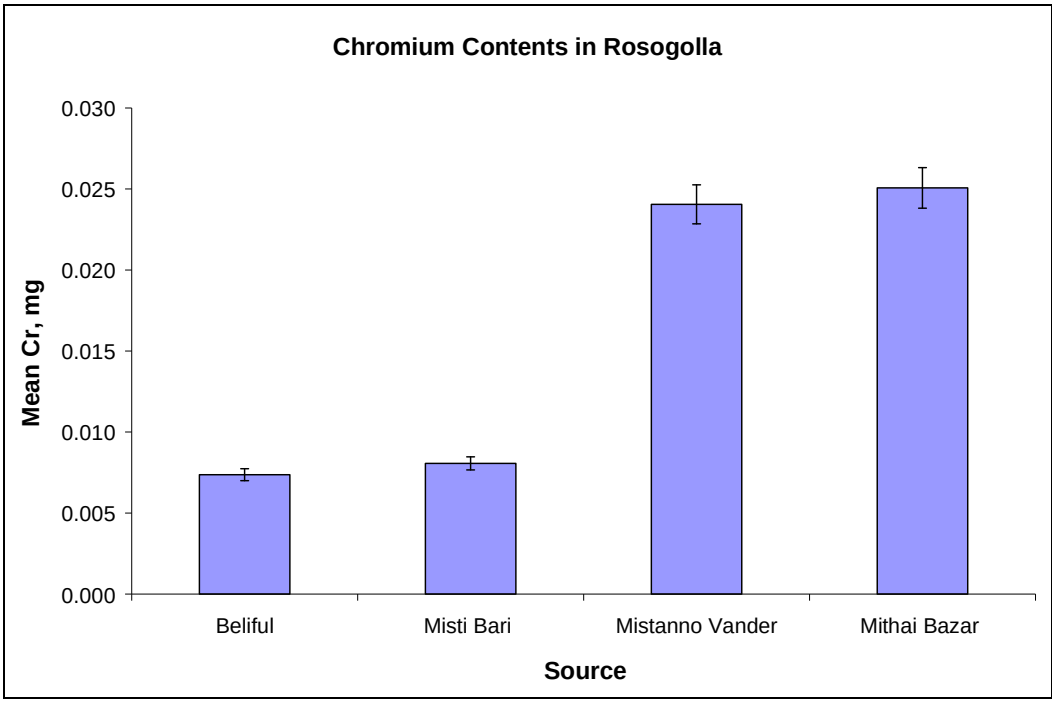

Figure 3.37: Mean Chromium contents with SD of Rosogolla from four sources

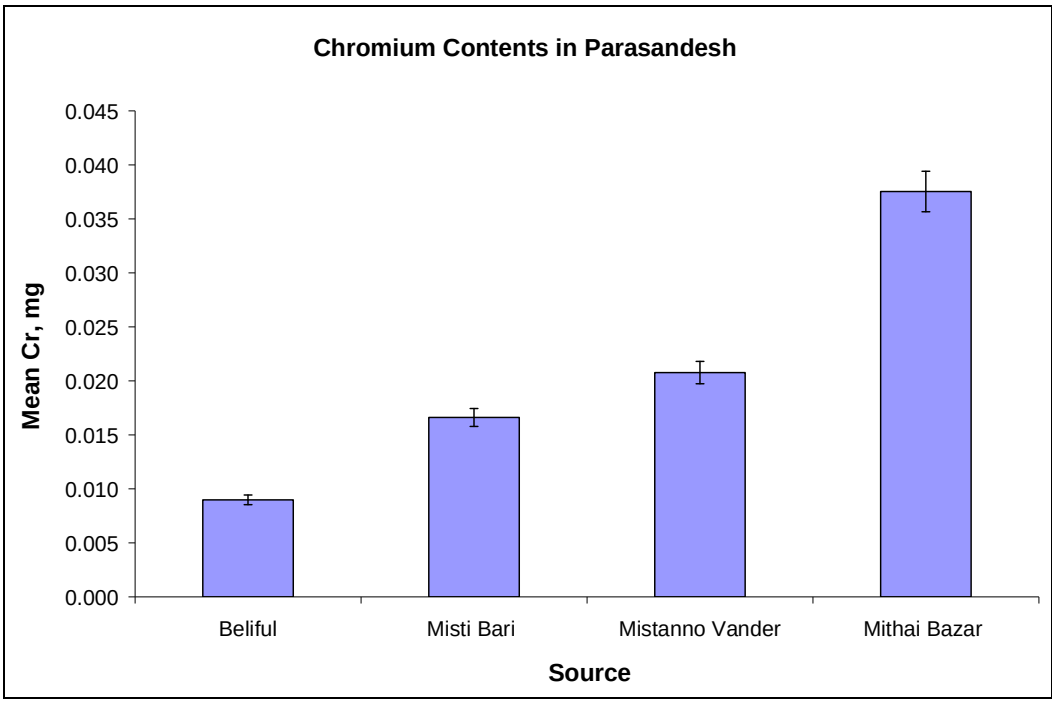

Figure 3.38: Mean Chromium contents with SD of Rosogolla from four sources

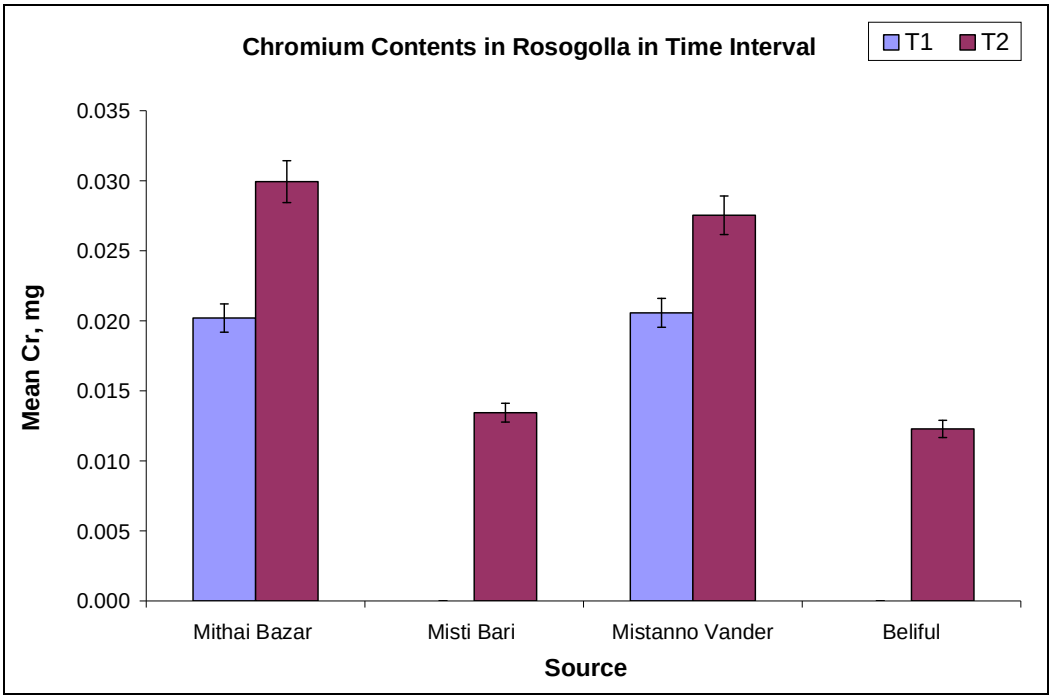

Figure 3.39: Mean Chromium contents with SD of Rosogolla from four sources in time interval

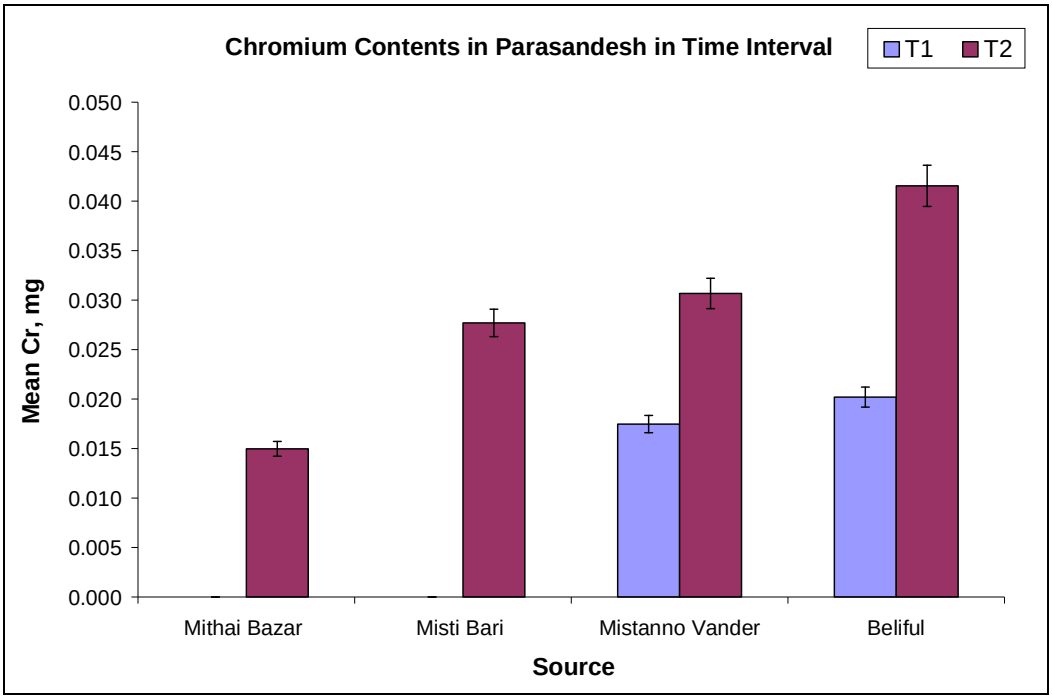

Figure 3.40: Mean Chromium contents with SD of Parasandesh from four sources in time interval

3.2.11 Results of Cd

Mean value of Cd of both items of four sources and their comparisons in two time intervals are given in Figure 3.41 to Figure 3.44.

Cadmium is an element, controversial about its essentiality for human nutrition, but it is always treated and compared with the level of Pb as both are heavy metals.

Similar to Cr, by the cadmium contents in R, four sources can be also classified in two groups. The source B and MB producing products with far less amount of Cd which is only about 1µg, while for the other two (MV and MBz), the level is found over 4µg, which is exactly four times higher of the former two. Therefore, the variation is up to 300% among two groups of four sources, similar kind of trend is only found in earlier cases of Cr.

Cadmium contents for P of three sources (B, MB and MV) are only 1 to 1.5µg, but products from MBz alone is showed a hike that is to be well above of 4µg. Therefore, variation in Cd contents for two products can not be mentioned as significant.

For both products, the trends of increasing nature in Cd contents for second batch sample can be noticed. However data revealed that products R from the source B and MB were not incorporated in first batch, possibly because of a short breakdown of AAS machine. Similarly, products P from the source MB and MBz were not incorporated in that first batch of analysis. As usual, variations in error levels are considerably justifiable.

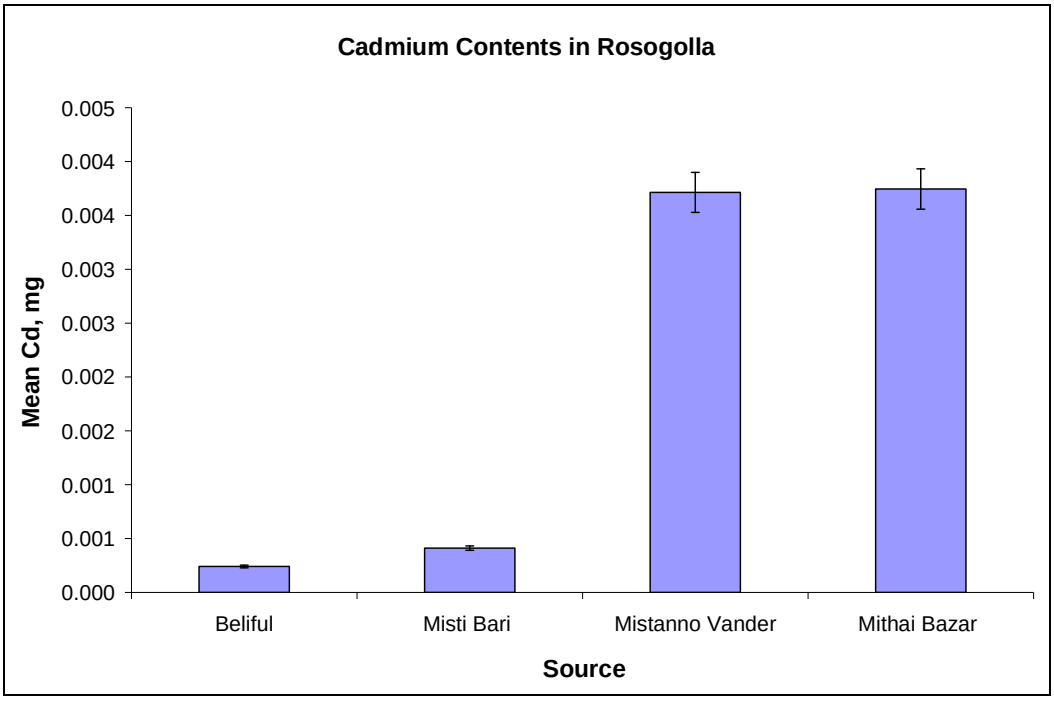

Figure 3.41: Mean Cadmium contents with SD of Rosogolla from four sources

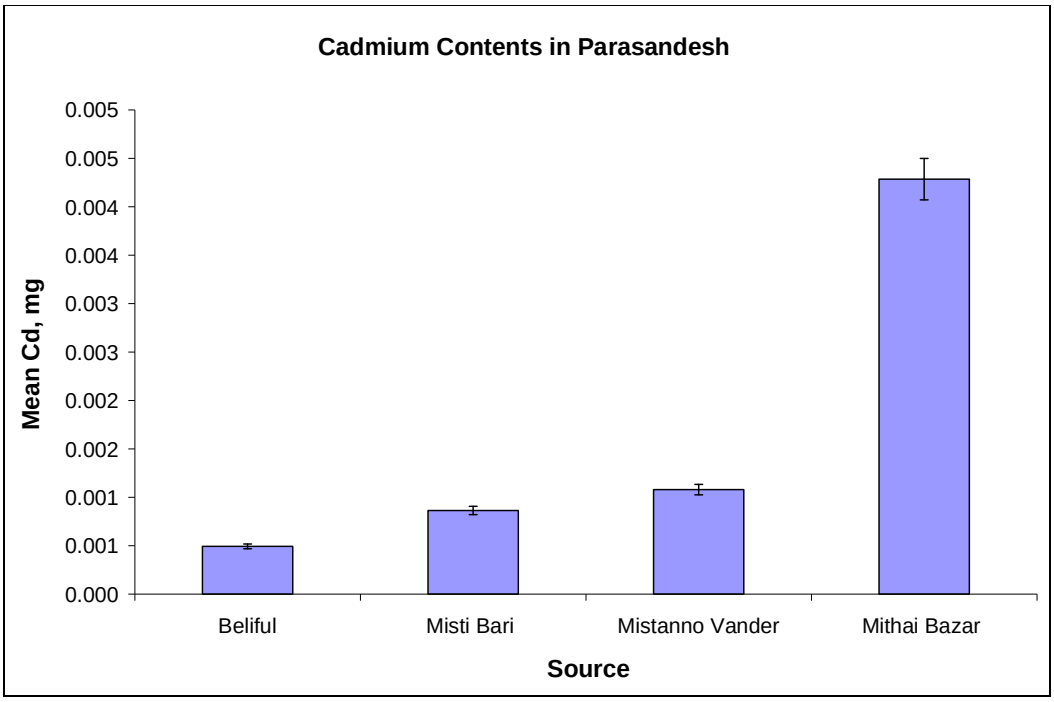

Figure 3.42: Mean Cadmium contents with SD of Parasandesh from four sources

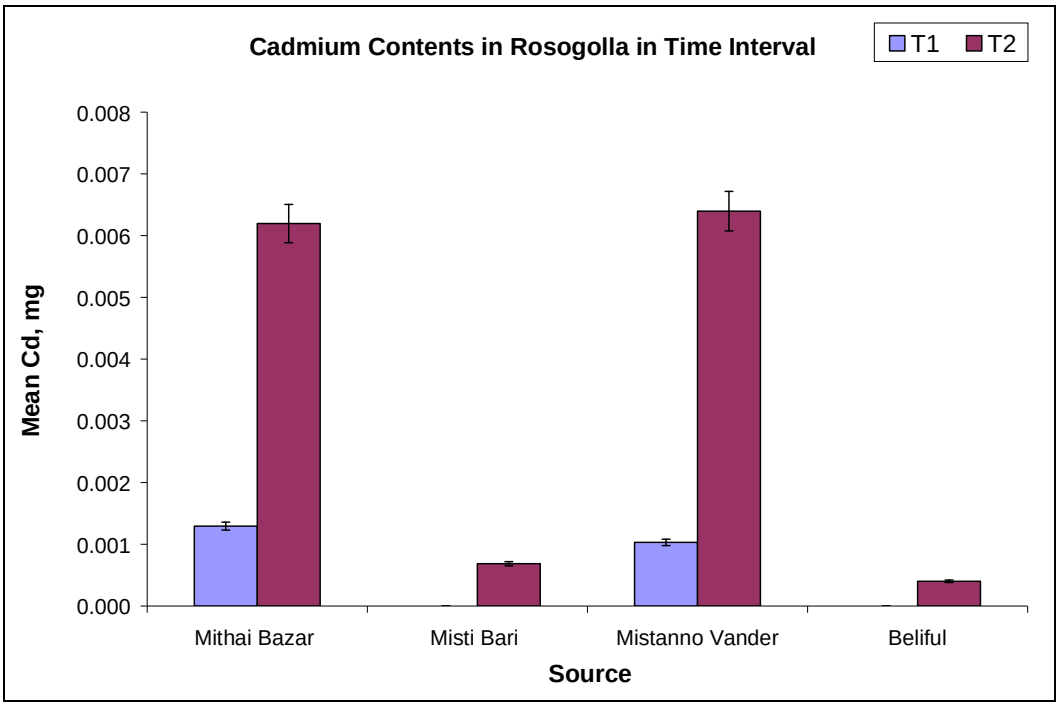

Figure 3.43: Mean Cadmium contents with SD of Rosogolla from four sources in time interval

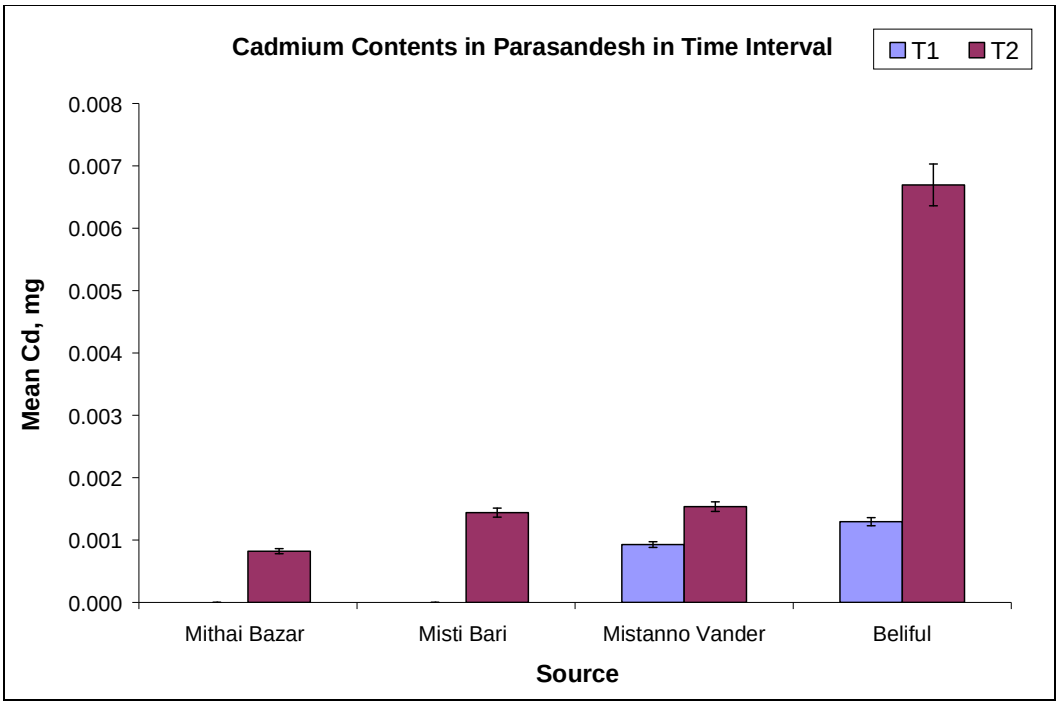

Figure 3.44: Mean Cadmium contents with SD of Parasandesh from four sources in time interval

3.2.12 Results of Co

Mean value of Co of both items of four sources and their comparisons in two time intervals are given in Figure 3.45 to Figure 3.48.

Cobalt is an essential element for human nutrition, which is a precursor of vitamin B_{12}. Therefore, its presence in milk or milk products is desirable, although milk or milk product is not a good source of Co unless the herd of cattle is fed by Co fortified food.

Cobalt contents in products R are found leas than 5µg for the source B and MB, while it well above 10µg for the sample of MV, however, the values went up to nearly 30µg for the products of MBz. So the level varied widely among the sources.

Cobalt contents for P showed a gradual increase from the source B to MBz with values nearly 6µg to 12µ, respectively. Unlike many other minerals, discussed above, the variation in Co contents for two products is eminent, and which is higher in the items R rather than items P that containing more milk solids.

For both products, the trends of increasing nature in Co contents for second batch sample appeared narrowly. However data revealed that products R from the source B and MB were not incorporated in first batch, possibly because of a short breakdown of AAS machine. Similarly, products P from the source MB and MBz were not incorporated in that first batch of analysis. As usual, variations in error levels are considerably justifiable.

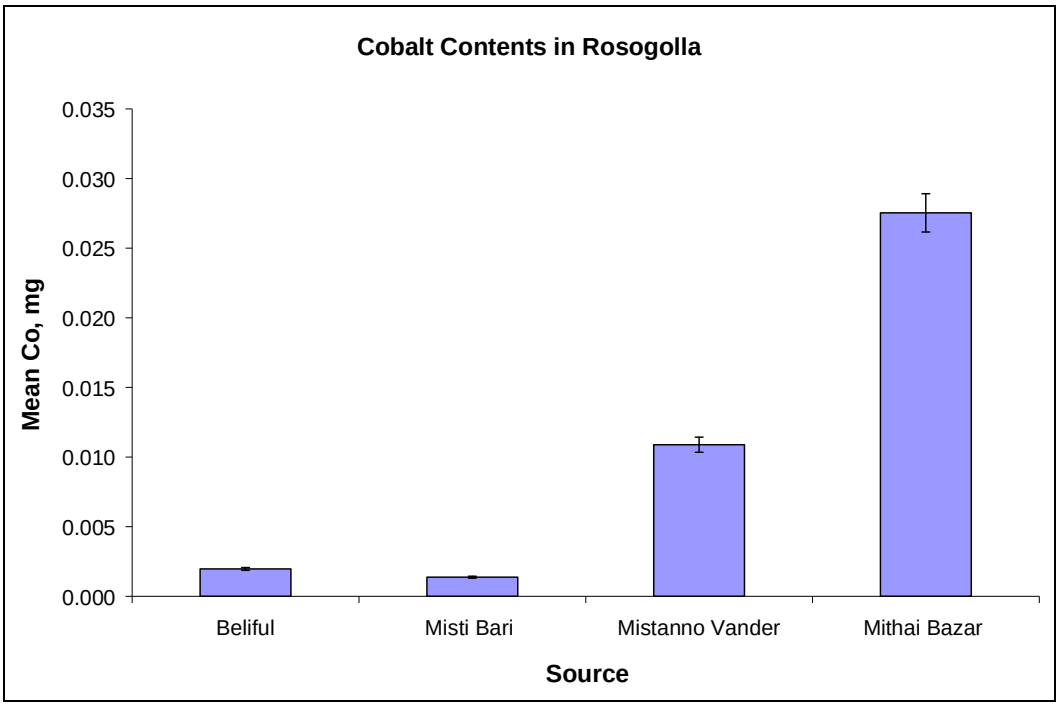

Figure 3.45: Mean Cobalt contents with SD of Rosogolla from four sources

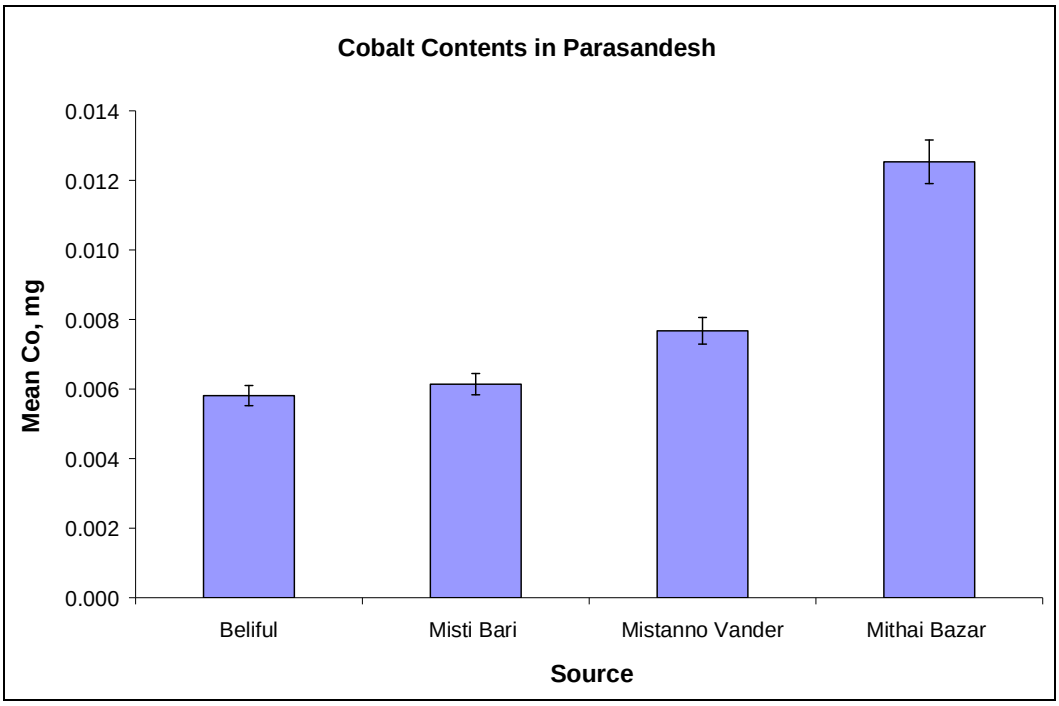

Figure 3.46: Mean Cobalt contents with SD of Rosogolla from four sources

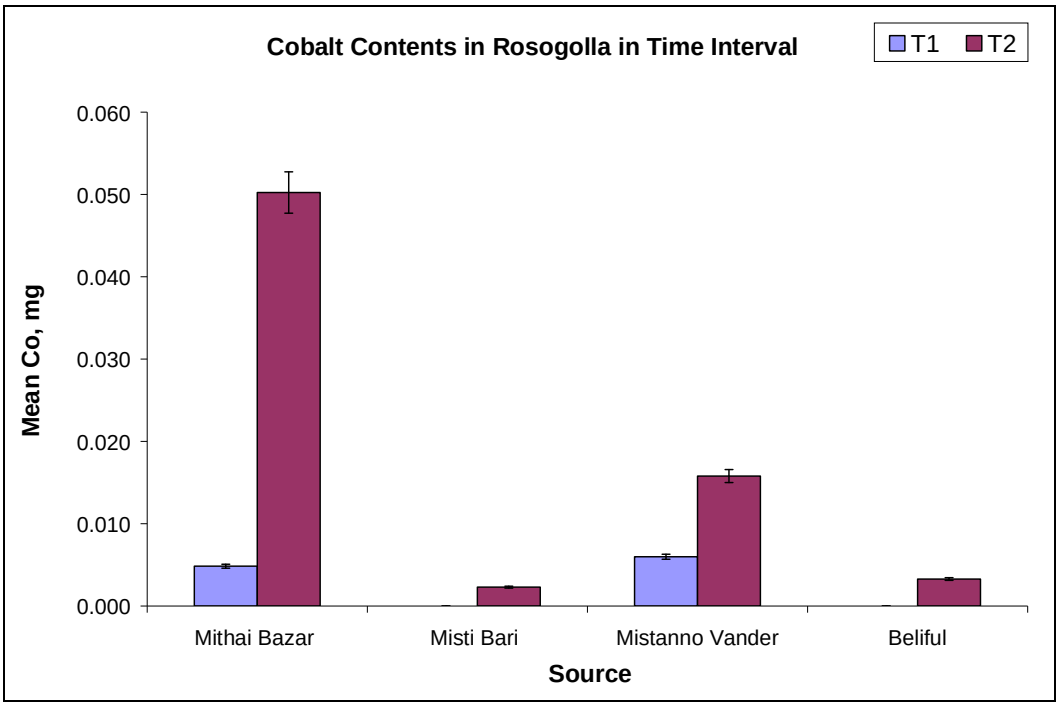

Figure 3.47: Mean Cobalt contents with SD of Rosogolla from four sources in time interval

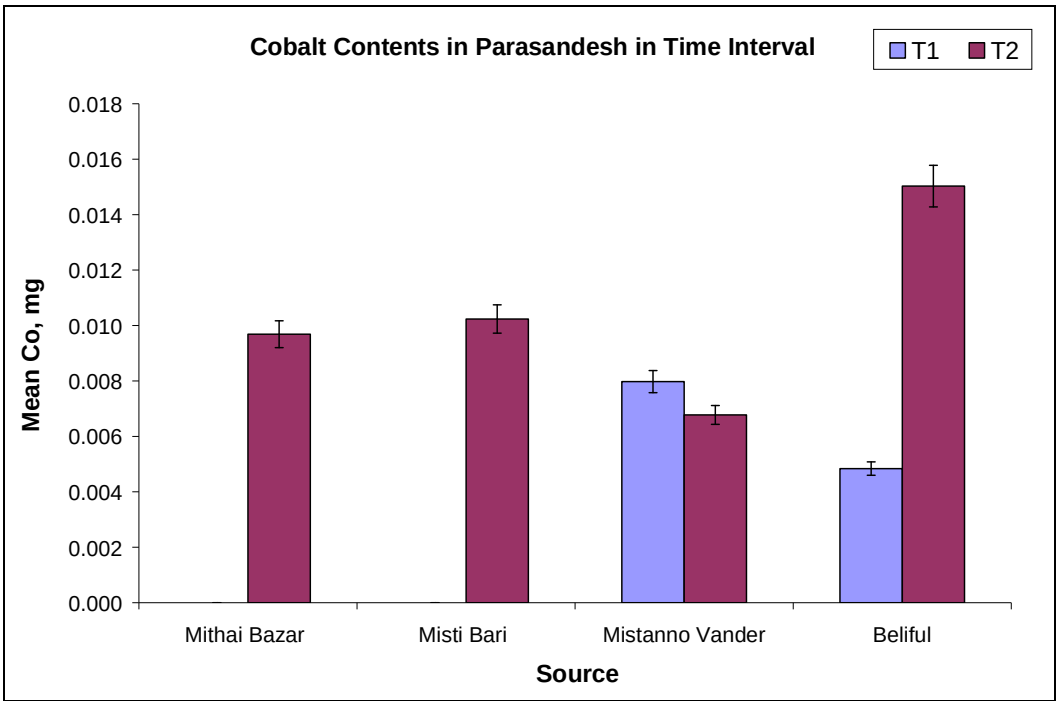

Figure 3.48: Mean Cobalt contents with SD of Parasandesh from four sources in time interval

3.2.13 Results of As

Mean value of As of both items of four sources and their comparisons in two time intervals are given in Figure 3.49 to Figure 3.52.

As is an essential element for plant kingdom but a debate is existed for its essentiality in human nutrition. However, its presence in milk or milk products is may not be desirable, and ideally milk or milk product are not a good source of As unless the herd of cattle is fed by As contaminated foods and drinks.

Arsenic contents in products R are found huge which is about 300µg for the products of all four sources. However, arsenic contents for P showed further increments to the values that appeared as 400 to 800µg. gradual increase from the source B to MBz with values nearly 6µg to 12µ, respectively. The variation in As contents for two products is not significantly prominent, but higher values are found in the items P compared to its R counterparts.

Data revealed that products R were analyzed singly, as samples from two sources were taken and analyzed separately, and there is no option remained for comparison for time intervals. Similarly, products P were also analyzed singly except in one occasion for the source MV, therefore comparison of time intervals is also found unjustifiable. As usual, variations in error levels are totally agreeable with all previous findings.

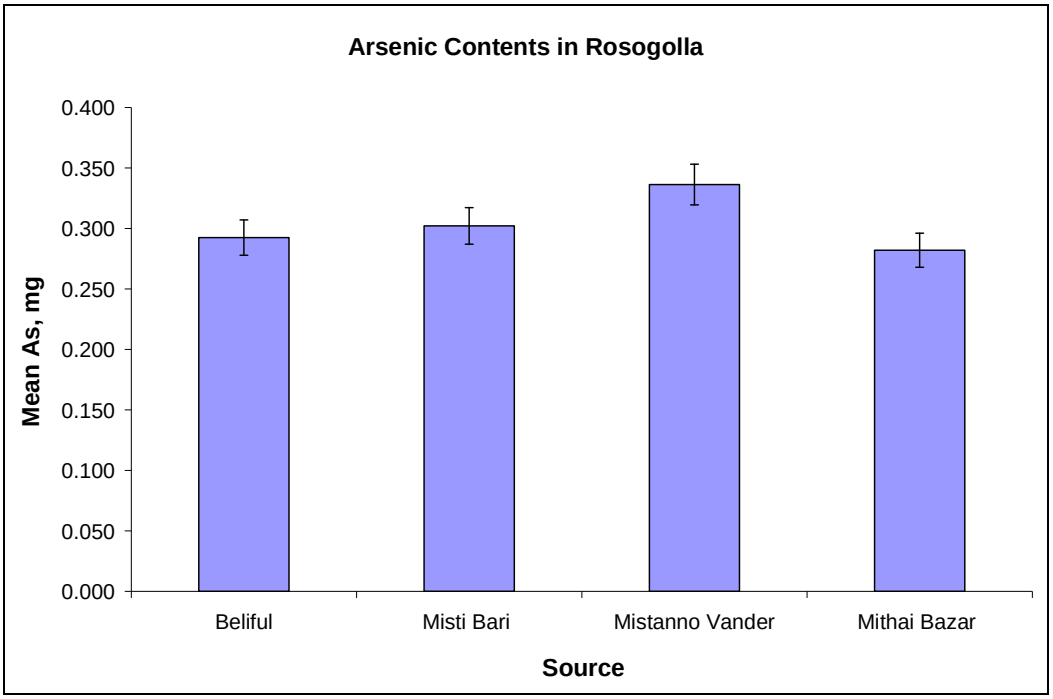

Figure 3.49: Mean Arsenic contents with SD of Rosogolla from four sources

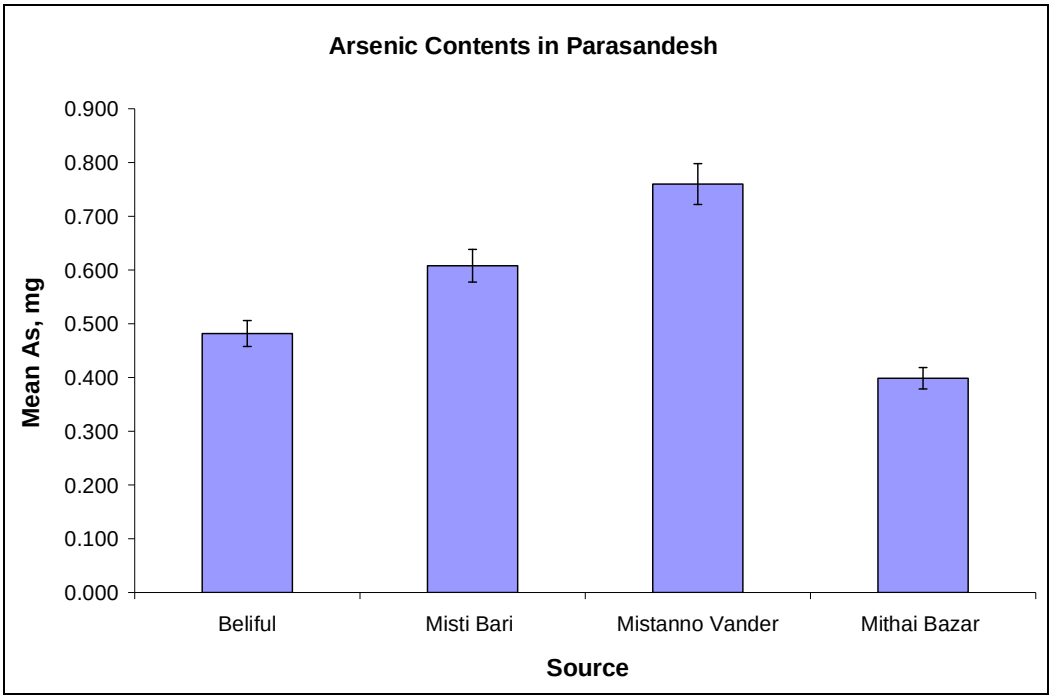

Figure 3.50: Mean Arsenic contents with SD of Rosogolla from four sources

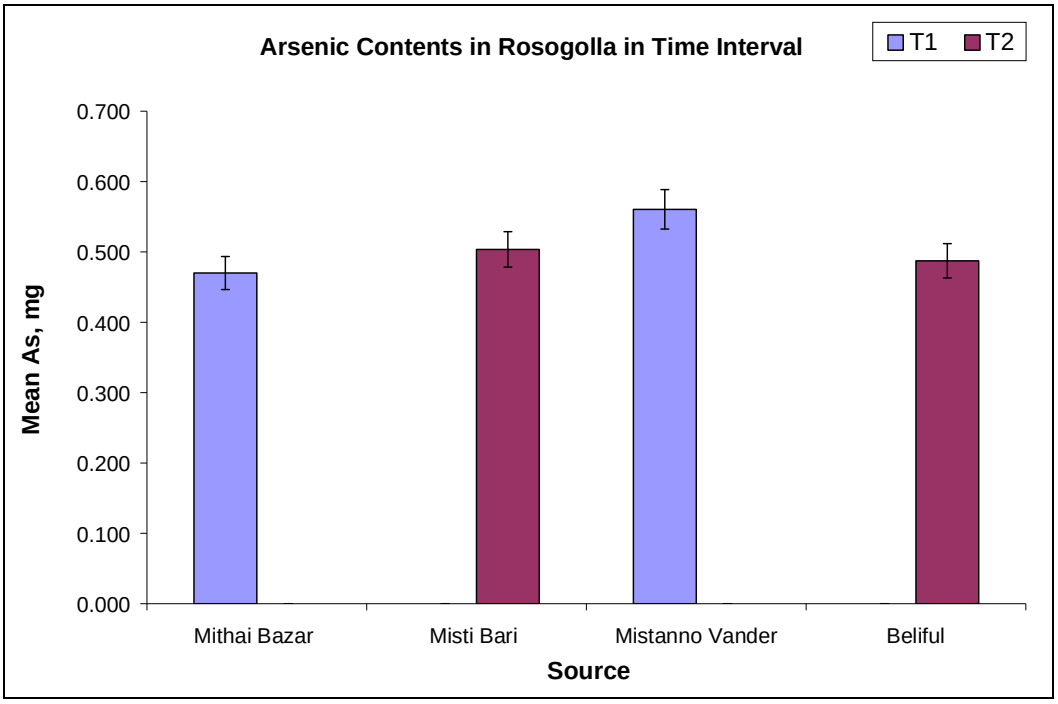

Figure 3.51: Mean Arsenic contents with SD of Rosogolla from four sources in time interval

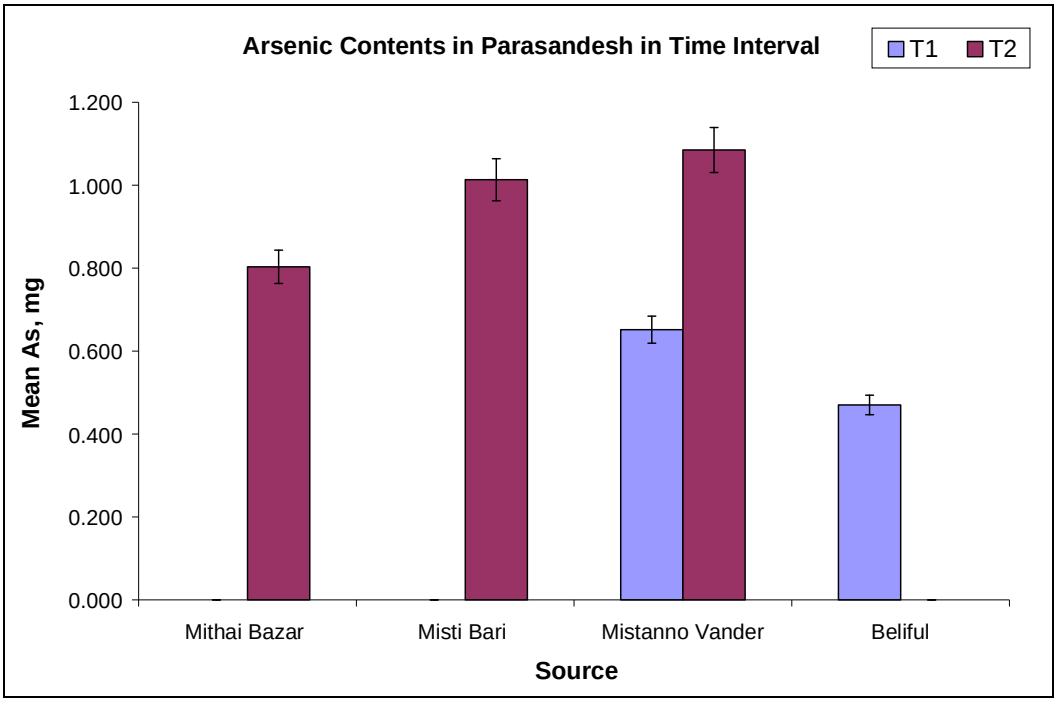

Figure 3.52: Mean Arsenic contents with SD of Parasandesh from four sources in time interval

3.3 Statistical Analysis

3.3.1 Mean comparisons

It is to be noted that in the text above, or forthcoming, the term 'item' should be read synonymously as 'products', and similarly the term 'source' or 'sources' should be read synonymously as 'producer' or 'producers'.

Mean comparisons (t-tests) among all parameters of two items of four sources in time intervals are given in the following tables (Table 3.9 to Table 3.12). It can be seen from those tables that most of the time compared means are significantly different (p values are 0.05 or less than that), so this statistical results do uphold the above findings of variations found in parameters those presented in earlier figures. As the compared means are found statistically different in very often, therefore, detailed descriptions about each comparison is not intended rather important remarks are made which have followed the respective tables.

Table 3.9: Mean comparison of two items with time interval for the source B

Factors	FW	LME	TS	Ca	Zn	Mn	Cu	Pb
R in Intervals	NS	NS	NS	5.6**	NS	>17**	>27**	7.1**
P in Intervals	NS	2.78*	NS	4.2*	NS	>53**	>63**	6.2**
R & P in T_1	NS	>11**	4.47*	>15**	3.6*	6.0*	6.0**	NS
R & P in T_2	4.7**	>29**	6.8**	>27**	3.0*	NS	NS	4.2*

NS, Not Significant; *, Significant in 95% Confidence Levels; **, Significant in 99% Confidence Levels

The item R is preciously produced during the interval of two samplings that can be reflected by their milk and sugar levels but this trend is not corroborative to their mineral constituents. The other item P is not maintained as neatly as R (in terms of milk portion), which is partly reflected by the differences in other mineral constituents. It is almost clear that both items are inherently different, and these differences can be described by their level of significances for most of the parameters in both intervals.

Table 3.10: Mean comparison of two items with time interval for the source MB

Factors	FW	LME	TS	Ca	Zn	Mn	Cu	Pb
R in Intervals	NS	NS	4.3*	3.9*	NS	>21**	>32**	4.9**
P in Intervals	NS	NS	3.3*	NS	NS	>16**	>20**	5.4**
R & P in T_1	6.2**	>24**	NS	>10**	>10**	NS	NS	>12**
R & P in T_2	4.6**	>14**	6.6**	>36**	>24**	7.1**	7.1**	3.6*

NS, Not Significant; *, Significant in 95% Confidence Levels; **, Significant in 99% Confidence Levels

Both items (R and P) can be termed as precised products over those intervals except their sugar contents (TS) and for their mineral portions in many occasions. It can be easily attributed that both items are inherently different in terms of their mineral constituents, and these differences are clearly attributable in the second batch analysis too.

Table 3.11: Mean comparison of two items with time interval for the source MV

Factors	FW	LME	TS	Ca	Zn	Mn	Pb
R in Intervals	NS	3.3*	NS	NS	NS	>16**	NS
P in Intervals	NS	>13**	7.5**	5.5**	>17**	>16**	NS
R & P in T_1	7.2**	NS	4.0*	>60**	7.6**	4.7**	NS
R & P in T_2	8.6**	>19**	8.4**	>30**	NS	4.9**	NS

NS, Not Significant; *, Significant in 95% Confidence Levels; **, Significant in 99% Confidence Levels

In this case (Table 3.11) products R are not preciously produced in terms of its milk portion which can be justified by variations in several mineral constituents. The other item, P, showed remarkable variations in its physical and chemical constituents. Qualitative and quantitative differences in two items are clearly demonstrated in analysis of second-batch samples.

Table 3.12: Mean comparison of two items with time interval for the source MBz

Factors	FW	LME	TS	Ca	Zn	Mn	Pb
R in Intervals	NS	NS	4.28*	NS	NS	4.1*	NS
P in Intervals	5.4**	NS	NS	3.0*	>20**	>17**	NS
R & P in T_1	NS	NS	NS	>16**	>16**	7.4**	3.6*
R & P in T_2	6.2**	>13**	>14**	4.2*	7.3**	NS	NS

NS, Not Significant; *, Significant in 95% Confidence Levels; **, Significant in 99% Confidence Levels

Here (Table 3.12) the products R are preciously produced but TS, and this trend can be a mismatch with the variation in several mineral constituents. The items P are found also precised, which is barely reflected by the differences in other mineral constituents. It is to be noted that the contrasting properties of two items are visible by mineral portions in first-batch analysis, and by physical constituents in second-batch analysis.

3.3.2 Concluding remarks on comparisons

In terms of LME, TMS and the level of other essential nutrients milk products (both R and P) produced by the source B might be superior followed by MV. The quality of MBz is could be the most inferior in that respect. Calcium is significantly low in most of the sources, the level of Zn is found an abrupt depletion from any referred values; while, the level of Mn, Cu and especially for Pb and As showed a huge hike that must be uncommon in all the references cited.

The items R from all four sources were not significantly different for Zn, Mn, Cu, Pb except one or two cases (for MB and MV). But if the FW, LME, & TMS parameters were found highly significant then Ca, Zn, Mn were also found significant in 99% - 99.9% confidence levels (CL). Therefore it can be promptly ascertained that the quality of the item vary if the changes of LME & TMS are occurred.

The products P from all sources showed highly significant (99% - 99.9% CL) for FW, LME & TMS. Therefore, it can be ascertained that the mineral (and the micronutrients) are highly significant among the sources except one or two cases. As the product P are generally prepared from the whole milks, so they are actually rich with milk & mineral portions. This product (P) in all of the sources contain higher amount of Ca, Zn, Mn & Cu that signify its quality over its R counterparts.

3.3.3 Correlation studies

An advantage of the correlation method is that one can make predictions about things when he/she knows about correlations. If two variables are correlated, one can predict one based on the other. Therefore, a correlation tells us that the two variables are related, but we cannot say anything about whether one caused the other. This method does not allow us to come to any conclusions about cause and effect; however correlation studies do have merits and therefore variables of both items from each were correlated. Chart of total correlation studies are presented in Appendix (Appendix D).

Only those combinations which are significantly correlated (either positively correlated or negatively correlated) are tabulated here (see Table 3.13 to 3.20). Out of four sources, the order of considerations is maintained as B is followed by MB then MV and in the last MBz came. For items, all the products R came first.

As mentioned earlier too, the calculations of LME was virtually dependent on TMS, therefore, in the following tables of correlation matrices either LME or TMS were excluded. In some cases, Mn and Cu showed heavily positive correlation, therefore, if it happened either Mn or Cu were excluded too.

Table 3.13: Correlations matrix for BR (n = at least 18)

	BRTS	BRCa	BRMn	BRCu	BRPb
BRTFW	.799*	.881*	.740*		
BRCa			.939**	.934**	-.873*
BRMn				.999**	-.942**
BRCu					-.952**

***significant at the 0.05 level; **significant at the 0.01 level**

For BR, only nine combinations are found significantly correlated, most of them are positively correlated but only Pb is negatively correlated with Ca, Mn and Cu. And Ca and Cu is highly positively correlated with Mn, and interestingly TFW (TFW is actually the same parameter which is earlier stated as FW only) positively correlated with TS, Ca and Mn.

Table 3.14: Correlations matrix for BP (n = at least 18)

	BPTS	BPCa	BPZn	BPMn	BPCu	BPPb
BPTFW		.837*				
BPLME	-.849*	.939**		.823*	.819*	-.873*
BPTS			.751*	-.777*	-.774*	.860*
BPCa				.904**	.902**	-.874*
BPZn				-.783*	-.782*	.850*
BPMn					1.000**	-.962**
BPCu						-.960**

***significant at the 0.05 level; **significant at the 0.01 level**

For BP, nineteen combinations are found significantly correlated; almost half of them are negatively correlated. Here also Pb is negatively correlated with Ca, Mn and Cu and with LME also, positively correlated with TS and Zn. The correlation between Cu and Mn is highly positive (p value is almost 0). Interesting feature is that the TS are negatively correlated with LME, Mn and Cu. Except with Pb, Ca is always positively correlated with TFW, LME, Mn and Cu. Positive correlation between LME and Ca is fully justified. Here Zn is negatively correlated with Mn and Cu but positive with TS. The natures of correlations (both positive and negative) among variables are difficult to justify except in few occasions.

Table 3.15: Correlations matrix for MBR (n = at least 18)

	MBRTS	**MBRCa**	**MBRZn**	**MBRMn**	**MBRCu**	**MBRPb**
MBRTFW	.936**	.912**	.825*	.794*	.773*	
MBRTS		.970**	.781*	.940**	.929**	
MBRCa				.918**	.907**	
MBRMn					.999**	-.894**
MBRCu						-.904**

***significant at the 0.05 level; **significant at the 0.01 level**

For MBR, fourteen combinations are found significantly correlated; most of them are positively correlated. Pb is negatively correlated with Mn and Cu only. TFW is always positively correlated with TS, Ca, Zn, Mn and Cu. TS too showed positive correlations with Ca, Zn, Mn and Cu. As usual, Mn and Cu is highly correlated, and Ca is also positively correlated with Mn and Cu.

Table 3.16: Correlations Matrix for MBP (n = at least 18)

	MBPCa	MBPZn	MBPMn	MBPCu	MBPPb
MBPTFW	-.765*				
MBPTS		-.901**	-.842*	-.854*	.752*
MBPZn			.808*	.819*	-.756*
MBPMn				.999**	-.899**
MBPCu					-.907**

***significant at the 0.05 level; **significant at the 0.01 level**

For MBP, only eleven combinations are found significantly correlated; most of them are negative correlations. As usual, Pb is negatively correlated with Zn, Mn and Cu but showed positive correlation with TS only. Here, TFW is only negatively correlated with Ca only. TS showed negative correlations with Zn, Mn and Cu. Zinc, Mn and Cu are positively correlated with each other. The natures of correlations among variables are again found difficult to justify.

Table 3.17: Correlations matrix for MVR (n = at least 18)

	MVRTS	MVRZn	MVRCu	MVRPb	MVRCr	MVRCd	MVRCo	MVRAs
MVRTFW	.926**		.735*		.912**			
MVRLME		.889**	-.813*	.930**		-.862*	-.755*	
MVRTS					.867*			
MVRZn			-.745*	.819*		-.731*		
MVRMn			1.000**		.809*	.967**	.893**	
MVRPb						-.730*		
MVRCr						.796*	.755*	
MVRCd							.918**	
MVRCo								.999*

***significant at the 0.05 level; **significant at the 0.01 level**

For MVR, as many as twenty one combinations are found significantly correlated; most of them are positive correlations. Pb is found negatively correlated with Cd, but positive with LME and Zn.

Here, TFW is showed positive correlation with TS, Cu and Cr. Apart from Zn and Pb, LME is negatively correlated with Cu, Cd and Co. In one occasion, TS showed a positive correlation with Cr. Among minerals, Zn in negatively correlated with Cu and Cd, while it is positively correlated with Pb. Mn and Cu showed identical trends in correlation here, both of them are positively correlated with Cr, Cd and Co. Here Cd is positively correlated with Cr. Cobalt is positively correlated with Cr and Cd. In only one occasion, As is highly positively correlated with Co. The complex nature of correlations among variables, especially among minerals, is again found which are actually difficult to justify those trends obtained here.

Table 3.18: Correlations matrix for MVP (n = at least 18)

	MVPTS	**MVPCa**	**MVPZn**	**MVPCu**	**MVPCr**	**MVPCd**	**MVPCo**
MVPLME	-.987**	.961**	-.983**	.973**		.914**	.855*
MVPTS		-.939**	.968**	-.945**		-.849*	-.801*
MVPCa			-.948**	.939**		.860*	.955**
MVPZn				-.996**	-.740*	-.934**	-.863*
MVPMn				1.000**	.750*	.958**	.872*
MVPCr						.790*	
MVPCd							.825*

***significant at the 0.05 level; **significant at the 0.01 level**

For MVP, as many as twenty five combinations are found significantly correlated; the number is the highest compared to any other table of matrix presented here. Nearly half of the combinations are negatively correlated.

LME is negatively correlated with TS and Zn, but apart from Ca, rests are positively correlated as with Cu, Cd and Co. TS has negative correlations with Ca, Cu, Cd and Co, but found only positive with Zn.

Among minerals, Ca has positive correlations with Cu, Cd and Co but possesses negative relationship with Zn. Zinc also showed negative correlations with Cu, Cr, Cd and Co. Both Mn and Cu showed positive correlations with Cr, Cd and Co, and Cr is also positively correlated with Cd and Co. Again this complex nature of correlations among variables put difficulties to justify cause of relationships among them.

Table 3.19: Correlations matrix for MBzR (n = at least 18)

	MBzRTS	**MBzRZn**	**MBzRCu**	**MBzRPb**	**MBzRCr**	**MBzRCd**	**MBzRCo**
MBzRTFW	.960**		.870*		.894**	.742*	.793*
MBzRLME		.757*					
MBzRTS			.907**		.895**	.886**	.812*
MBzRCa				-.783*			
MBzRMn			1.000**		.972**	.893**	.826*
MBzRCr						.823*	.796*

***significant at the 0.05 level; **significant at the 0.01 level**

For MBzR, seventeen combinations are found significantly correlated; except in one occasion, all combinations are positively correlated. TFW is positively correlated with

TS, Cu, Cr, Cd and Co, while LME is only correlated with Zn. Sugar content (TS) on the other hand is positively correlated with Cu, Cr, Cd and Co.

Among minerals, Ca has only negative correlations with Pb. As before, Mn and Cu showed positive correlations with Cr, Cd and Co, and Cr is also positively correlated with Cd and Co. To make any generalized comments on the trends on correlation patterns is found hard again.

Table 3.20: Correlations matrix for MBzP (n = at least 18)

	MBzPTS	**MBzPCa**	**MBzPZn**	**MBzPCu**	**MBzPCd**	**MBzPAs**
MBzPTFW	.732*	.938**	.959**	.944**	-.952**	
MBzPLME	-.928**					
MBzPTS		.821*	.741*	.738*		
MBzPCa			.856*	.851*	-.897**	
MBzPZn				.987**	-.967**	
MBzPMn				1.000**	-.982**	-.994*
MBzPCo						-1.000**

***significant at the 0.05 level; **significant at the 0.01 level**

For MBzP, here eighteen combinations are found significantly correlated; most of them are positive correlations. As before, TFW is positively correlated with TS, Ca, Zn, Cu, and Cd, while LME is only negatively correlated with TS, but TS is found positively correlated with Ca, Zn and Cu.

Among minerals, Ca is found negative correlations with Cd, but positively related with Zn and Cu. Apart from that Zn is positive with Cu but negative with Cd. As before, Mn and Cu showed similar trends, and those have negative correlations with

Cd and As. Interestingly, As showed totally negatively correlated with Co. Further to say that to make any generalized comments on the trends on correlation patterns is not plausible.

3.3.4 Regressions: simple linear and polynomial

3.3.4.1 Background

Unlike correlation, linear regression is an approach to modeling the relationship between a scalar dependent variable and one or more explanatory variables denoted. The case of one explanatory variable is called simple linear regression.

In linear regression, data are modeled using linear predictor functions, and unknown model parameters are estimated from the data. Such models are called linear models. Like all forms of regression analysis, linear regression focuses on the conditional probability distribution of y given x, rather than on the joint probability distribution of y and x, which is the domain of multivariate analysis.

Linear regression was the first type of regression analysis to be studied rigorously, and to be used extensively in practical applications. This is because models which depend linearly on their unknown parameters are easier to fit than models which are non-linearly related to their parameters and because the statistical properties of the resulting estimators are easier to determine.

Linear regression models are often fitted using the least squares approach, but they may also be fitted in other ways, such as by minimizing the "lack of fit" in some other norm (as with least absolute deviations regression), or by minimizing a penalized version of the least squares loss function as in ridge regression. Conversely, the least squares approach can be used to fit models that are not linear models. Thus, although the terms "least squares" and "linear model" are closely linked, they are not synonymous.

Polynomial regression is a form of linear regression in which the relationship between the independent variable and the dependent variable which is modeled as an nth order polynomial. Polynomial regression fits a nonlinear relationship between the value of x and the corresponding conditional mean of y, and has been used to describe nonlinear phenomena. Although polynomial regression fits a nonlinear model to the data, as a statistical estimation problem it is linear, in the sense that the regression function is linear in the unknown parameters that are estimated from the data. For this reason, polynomial regression is considered to be a special case of multiple linear regressions. Although polynomial regression is technically a special case of multiple linear regressions, the interpretation of a fitted polynomial regression model requires a somewhat different perspective. It is often difficult to interpret the individual coefficients in a polynomial regression fit, since the underlying monomials can be highly correlated. Although the correlation can be reduced by using orthogonal polynomials, it is generally more informative to consider the fitted regression function as a whole. Point-wise or simultaneous confidence bands can then be used to provide a sense of the uncertainty in the estimate of the regression function.

3.3.4.2 Analysis adopted here

To find out clear relationship between variables, only those combinations in correlation studies (presented earlier) showed the CL levels 99% or higher (p value 0.01 or less) are regressed for simple linear model. However, if the model did not produce the agreements 80% or above (corresponding r^2 values is 0.8), then second order polynomial regressions were tried, and interestingly these attempts in most of the cases produced better agreements and its corresponding linear option. All linear regressions (with polynomial fits if attempted) with equations and r^2 values are presented in Appendix (Appendix.). The arrangement pattern can be found identical to correlation matrices presented in earlier tables.

3.3.4.3 Special treatments in regressions

Those combinations were regressed (either for linear fits and/or polynomial fits) are tabulated again (See Table 3.21.) to point out which combinations appeared at least

four times out of eight occasions. Those are highlighted here (see the bold faced rows of the Table 3.21.), for them simple linear regressions further tried taking all data series are in the considerations, and termed here as 'total regression' (applicable only for that particular combination). In these regression models, error levels for both coordinates are given.

Table 3.21: All possible combination considered in the regression analysis

Variables → **Combination ↓**	**BR**	**BP**	**MBR**	**MBP**	**MVR**	**MVP**	**MBzR**	**MBzP**
Ca-Mn	×	×	×			×		
Ca-Cu	×	×	×					
Mn-Pb	×	×	×	×				
Cu-Pb	×	×	×	×				
LME-Ca		×				×		
TWA-TS			×		×		×	
TFW-Ca			×					×
TS-Ca			×			×		
TS-Mn			×			×	×	
TS-Cu			×					
TS-Zn				×		×		
TFW-Cr					×		×	
LME-Zn					×	×		
LME-Pb					×			
Mn-Cd					×	×	×	×
Mn-Co					×			

Table 3.21: Continued

Cu-Cd	×			
Cu-Co	×			
Cd-Co	×			
Co-As	×			×
LME-TS		×		×
LME-Mn		×		
LME-Cd		×		
Ca-Zn		×		
Ca-Co		×		
Zn-Mn		×		×
Zn-Cd		×		×
TS-Cr			×	
TS-Cd			×	
Mn-Cr			×	
TFW-Zn				×
TFW-Mn				×
TFW-Cd				×
Ca-Cd				×

× is indicating that any kind or regression is found with good agreements (r^2 values is 0.8 or above)

3.3.5 Total regressions

Total regressions of those selected combinations are given in the Figures 3.53 to 3.56.

Total regression of Ca-Mn is presented in the following figure (Figure 3.53). Simple linear regression is significant but r^2 values is only found less than 0.5 which is improved enormously to 1 by the second order of polynomial fits. Here all error bars are given as SE (standard error) and found appreciably good. By and large, it can be noted that Mn might have a relationship with Ca contents of either products investigated here irrespective of any sources studied .

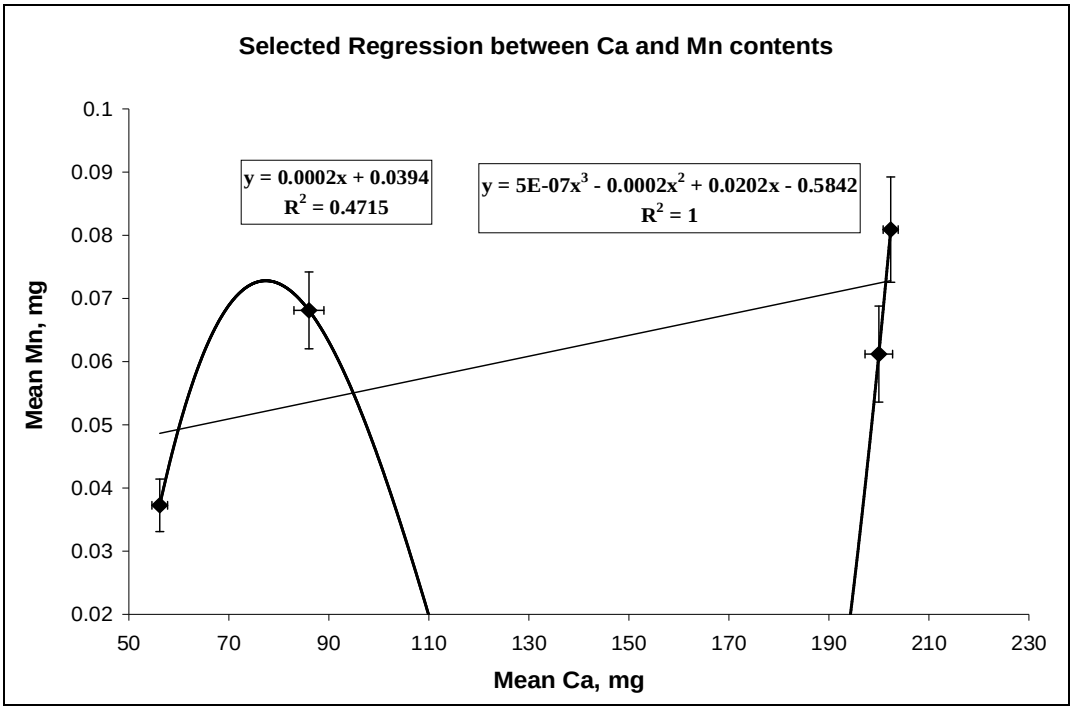

Figure 3.53: Total regression between Ca and Mn for both items of four sources (error bars are in SE)

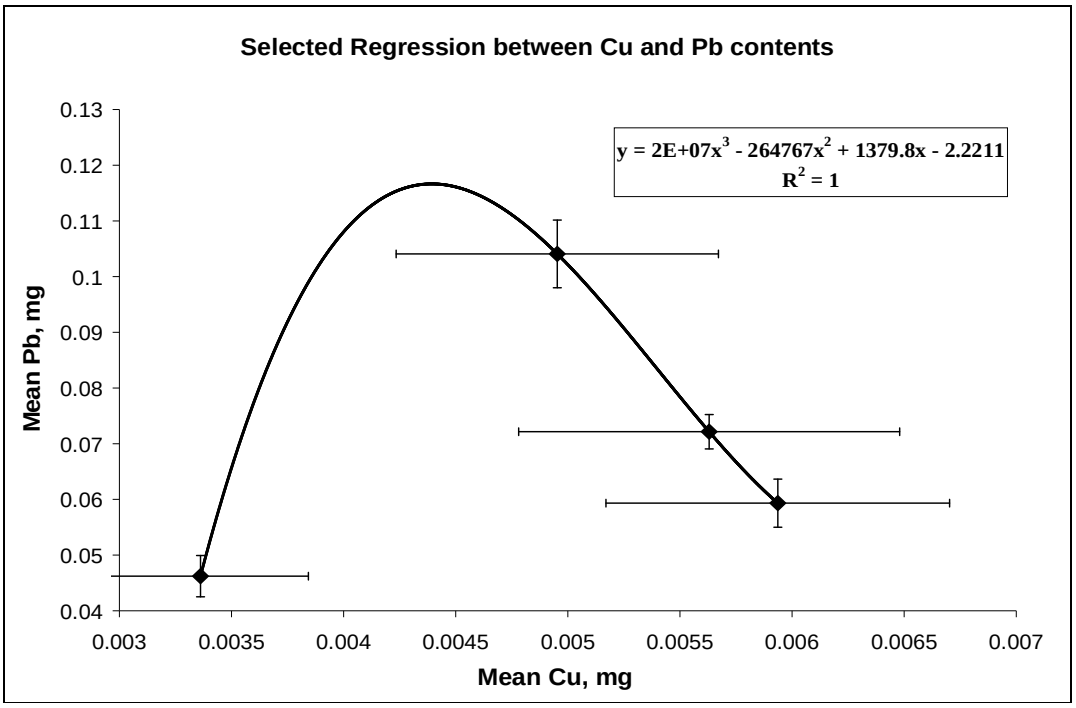

Figure 3.54: Total regression between Cu and Pb for both items of four sources (error bars are in SE)

Total regression between Cu and Pb is presented in the Figure 3.54. Simple linear regression is found not significant in terms of r^2 values which are improved to again 1 by the second order of polynomial fits. All error bars are given as SE, although the error levels for x-coordinates are quite large. However, Cu might have a relationship with Pb contents of either product investigated here irrespective of any sources , even though the relationship is more complex rather than any simpler form. The relationship between Mn and Pb might have produce similar fits as Cu-Pb does, as both minerals would show similar correlations with other minerals, which ascertained several times in above.

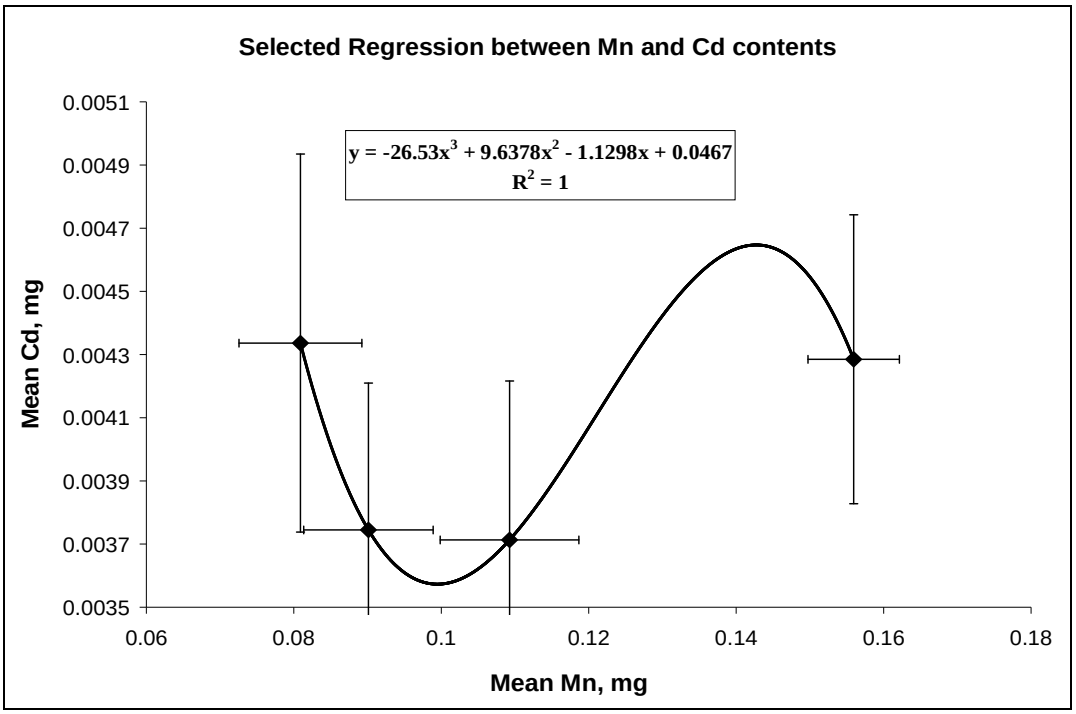

Figure 3.55: Total regression between Mn and Cd for both items of four sources (error bars are in SE)

Total regression between Mn and Cd is presented in the Figure 3.55. As before in this case too, simple linear regression is found not significant in terms of r^2 values which are improved to again 1 by the second order of polynomial fits. All error bars are given as SE, although the error levels for both coordinates are quite large in size. However, it is plausible that Mn might have a relationship with Cd contents of either product investigated irrespective of any sources studied, even though the relationship is more complex rather than any simpler form. The relationship between Cu and Cd might have produce similar fits as Mn does, the resons is ascertained in earlier.

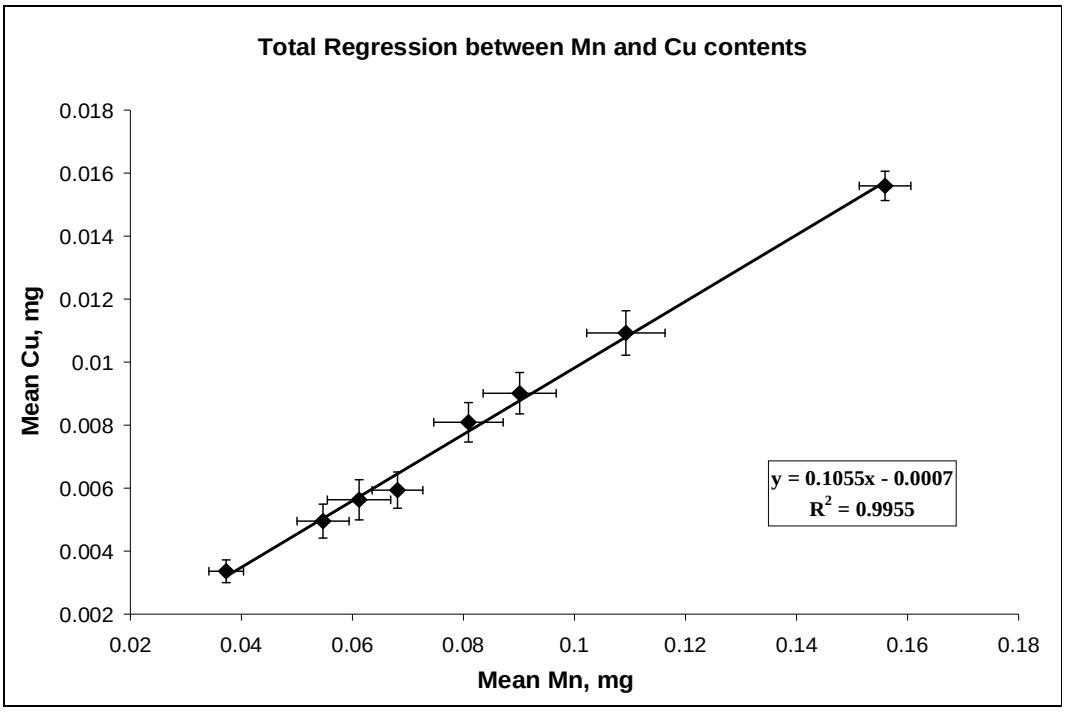

Figure 3.56: Total regression between Mn and Cu for both items of four sources (error bars are in SE)

Total regression between Mn and Cu is presented in the Figure 3.56. Simple linear regression is found absolutely significant in terms of r^2 value which is very close to one. Second order of polynomial fit is not necessary here. All error bars are given as SE, all the error levels for both coordinates are quite small in size. Therefore, it can be said that Mn has a simple linear relationship with Cu contents of both products investigated irrespective of any sources studied here.

3.3.6 Recovery of minerals

In the following tables (Table 3.22 to Table 24), the percentages of recovery (mean with SD) of each elements, analyzed in all three batches are given accordingly. Here, 100% recovery means the amount of that particular element obtained from the fresh weight of each item in terms of its TMS, is the amount that should obtain in relation with cited values in majority of early works referred here. For example, 400% recovery means the amount found is four times higher than the expected values. Therefore, less than 100% recovery means the amount found is less than expected values.

Table 3.22: Percentage recovery of minerals in first batch

Sample Identity	Ca Mean ± SD	Zn Mean ± SD	Mn Mean ± SD	Cu Mean ± SD	Pb Mean ± SD
BR	30 ± 7	68 ± 9	1261 ± 211	422 ± 92	8993 ± 400
BP	12 ± 3	76 ± 1	264 ± 15	3023 ± 40	4620 ± 495
MBR	46 ± 5	74 ± 2	528 ± 69	383 ± 121	8006 ± 1278
MBP	3 ± 11	75 ± 2	217 ± 55	229 ± 298	5824 ± 262

Recoveries for Ca and Zn, in first batch, found are less than expected values, that reveals that the original milk from which the products are prepared (even with other materials involved, e.g., flour, sugar etc.) is some how inherently depleted of Ca and Zn. Values for Mn and Cu varied widely, which is 2 times to 30 times higher than expected levels.

However, the presence of Pb is abruptly high, where the range is showed 40 times to even 80 times higher than the expected levels. Although its error levels (in terms of SD) is also high but those are not in the degree which could ultimately offset the severity of its levels.

Except in very few occasions, recoveries for Ca, Zn, Cu and Cd in second batch, found are usually less than the expected values, only Co showed almost 100% recovery for all sources. So again the results found corroborative to the results obtained from the previous batch. Values for Mn, Pb, Cr and As varied enormously, which are sometimes several 100 times higher than any expected values. But if the error levels for Pb and As is considered then their effective levels could be lower than their mean values, but this is not true for Mn or Cr.

Table 3.23: Percentage recovery of minerals in second batch

Sample Identity	Ca, Mean ± SD	Zn Mean ± SD	Mn Mean ± SD	Cu Mean ± SD	Pb Mean ± SD
BR	2 ± 6	71 ± 10	3904 ± 384	13 ± 7	4054 ± 538
BP	8 ± 4	81 ± 1	1634 ± 26	51 ± 1	2620 ± 391
MBR	27 ± 5	71 ± 4	2593 ± 274	29 ± 17	3329 ± 978
MBP	4 ± 10	72 ± 2	1190 ± 76	58 ± 3	2914 ± 563
MVR	20 ± 8	61 ± 2	2229 ± 30	16 ± 11	7545 ± 3534
MVP	92 ± 13	46 ± 3	1287 ± 2823	12 ± 6	5152 ± 1761
MBzR	8 ± 11	64 ± 5	2218 ± 1276	53 ± 24	11741 ± 784
MBzP	110 ± 136	34 ± 44	6986 ± 4957	7 ± 75	5635 ± 4935
Sample Identity	**Cr Mean ± SD**	**Cd Mean ± SD**	**Co Mean ± SD**	**As Mean ± SD**	
BR	1640 ± 235	55 ± 42	97 ± 3	108629 ± 40078	
BP	806 ± 81	60 ± 7	96 ± 2	77292 ± 25291	
MBR	2042 ± 100	15 ± 49	98 ± 1	127474 ± 41316	
MBP	1354 ± 231	39 ± 8	97 ± 1	85116 ± 14768	
MVR	2853 ± 198	2780 ± 18	95 ± 3	129315 ±25354	
MVP	3345 ± 329	35 ± 76	97 ± 2	154339 ±42241	
MBzR	3645 ± 454	89 ± 46	95 ± 3	144219 ±43048	
MBzP	4596 ± 3682	110 ± 164	93 ± 5	145378 ±105457	

In third batch, Zn and Co were recovered as less than 100%. The remainders, Mn, Cu (this is the first time, as not the case in first two batches), Pb, Cr and Cd were recovered always more than 100%. For those four elements, the range was found 3 to 60 times higher than the expected values. The results found again the corroborative to the results obtained from the both batches that level of some elements are considerably higher; therefore, the causes and its implications need to be addressed properly. Here the error levels for Pb is found again higher, so it's effective levels could be lower than their mean values.

Table 3.24: Percentage recovery of minerals in third batch

Sample Identity	**Zn** **Mean ± SD**	**Mn** **Mean ± SD**	**Cu** **Mean ± SD**	**Pb** **Mean ± SD**
MVR	58.46±3.4	7836 ± 506	520 ± 100	2322 ± 4196
MVP	82.47±0.88	2754 ± 262	253 ± 50	2704 ± 812
MBzR	63±4.4	8030 ± 750	499 ± 37	10870 ± 8415
MBzP	81±1.5	2534 ± 136	217 ± 92	1966±1531
Sample Identity	**Cr** **Mean ± SD**	**Cd** **Mean ± SD**	**Co** **Mean ± SD**	
MVR	4797 ± 944	813 ± 193	85.00468	
MVP	2250 ± 294	386 ± 120	88.09±5.	
MBzR	6508 ± 1033	1011 ± 345	42.8±29.3	
MBzP	3102 ± 587	314 ± 72	93.79±1	

To understand more clearly, results of all recoveries for all of the above parameters (for four sources) are rearranged in the following tables (Table 3.25 to Table 3.26). The first table (Table 3.25) is for the items R and the second one (Table 3.26) for the items P. The digits (4, 3, 2 and 1) used there representing as follows: 4, highest values; 3, next to highest values; 2 next to second highest values; and 1 the lowest values; The superscript letters representing the recoveries status: *a* for 0 to 100% recovery; *b* for 101 to 1000 % recovery; *c* for 1001 to 10000% recovery; and *d* for 10001 to 100000 % recovery.

Table 3.25: Figure of merits for recoveries of R

Source	Parameters								
	FW	**LME**	**TMS**	**TS**	**Ca**	**Zn**	**Mn**	**Cu**	**Pb**
B	2	4	3	2	4^a	3^a	2^c	2^b	3^c
MB	1	3	2	1	1^a	1^a	1^c	1^b	2^c
MV	3	2	4	3	3^a	4^a	4^c	4^b	1^c
MBz	4	1	1	4	2^a	2^a	3^c	3^b	4^d

For the item R, the highest levels of milk & milk solids are present in the source B, followed by MV; the quality of MBz is the most inferior in that respect; Ca is significantly low in most of the sources, the level of Zn is an abrupt depletion from the referred values; while, the level of Mn, Cu and especially for Pb showed a huge hike that must be uncommon in all the references cited. As Cr, Cd, Co and As were not included in all three batches, therefore, the gross assumptions about them are not made here.

Table 3.26: Figure of merits for recoveries of P

Source	Parameters								
	FW	**LME**	**TMS**	**TS**	**Ca**	**Zn**	**Mn**	**Cu**	**Pb**
B	3	3	2	2	2^{a}	2^{a}	2^{b}	2^{b}	3^{c}
MB	4	4	3	1	4^{a}	4^{a}	1^{b}	1^{a}	4^{c}
MV	1	2	4	3	3^{a}	1^{a}	3^{c}	3^{b}	2^{c}
MBz	2	1	1	4	1^{a}	3^{a}	4^{c}	4^{b}	1^{c}

For the item P, products from the source MB are clearly rich in milk & milk solids, and again MBz is lowest in that grade in which milk solids are being compensated by the highest level of sugar. The amount of Ca is little over than the expected values with one occasion produced in less recoveries. Zinc is showed always huge poor recoveries. And As before, Mn, Cu & Pb are remarkably high in all occasions. As Cr, Cd, Co and As were not included in all three batches, therefore, the gross assumptions about them are not made here.

Chapter Four

Consumption of Ca by Local Inhabitants and its Consequences

4.1 Role of Ca in human health

There are a great variety of Ca dependent biochemical and physiological processes, and the element plays an important role in growth and development having both extra-cellular and intracellular functions. It is required for cell elongation and division, being involved in microtubule and cell plate formation. However, in contrast with other cation species, Ca plays a comparatively minor role in enzyme activation. It is thought that it may inhibit the activating effect of Mg by displacing that element from functional sites, whilst the protein calmodulin (which incorporates Ca) regulates the enzyme nicotinamide adenine dinucleotide (NAD) kinase. The enzyme glutamate dehydrogenase, found mainly in mitochondria, is also dependent upon the presence of Ca ions for maximal activity. A more detailed description about its essentiality and consequences can be found in chapter 1.

4.2 Status occurring in the study area

As mentioned in the hypothesis and expressed the concern that a substantial part of liquid milk is being utilized to produce various items of milk-based sweetmeat in all over the country. Consequently, a major portion of the city population is consuming those varieties of sweetmeats and that is considered as an indirect consumption of

milk which is important and essential for Ca supply for each and every people of the area of interest.

Analysis of milk products are fully based on the city area (city center of Rajashahi City Corporation and in some extent the university area), therefore, results are need to be, and ought to be, evaluated in terms of its significance on health of local population.

As a reminder, the contents of Table 4.1 are readdressing the issue of Ca contents in any usual milk samples, and the amounts needed to meet the RDA for an adult. In relation with this figure, results (usually the average value) obtained in this research are put in the following table (Table 4.2). At least four glasses of milk is necessary to have 1g Ca in a day, which is a bit unrealistic. However, other main protein sources, like meat, fish also contain substantial amount of Ca, therefore referred that at least half of the RDA of Ca should meet through milk and milk-products. According to that assumption, two glasses of milk is now needed to meet the daily need, which is sensible and can be consumed after two major meals in a day.

As indicated earlier, a single item of R or P is considered here as a single (or ideal) serving in local cuisine. A single product of R may contain only 75mg of Ca, therefore, 13 pieces of this item are need to meet the RDA, but still need more than 7 pieces for half of the RDA for an adult, that sounds still ridiculous. On the other hand, the other product, P could be a good provider of Ca that contains 250mg Ca in a single item; therefore, 2 pieces are need for the half of RDA of Ca. So the logical allocation would be in the meals is to be one piece in a meal. However, both products (R and P, see the last row of Table 4.2) in combined are not sufficient as the source to provide 500mg Ca in a day.

Table 4.1: Ca contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
mg	276	226	about 25	at least 4 glasses (if RDA is 1L milk/1g Ca

(Compiled from the USDA Nutrient Database); [#] 1 serving = 1 cup (8 oz; 244 g); [*] 1 glass = 200 mL;

Table 4.2: Ca contents in items investigated (recovered from chapter 3)

Item	Average LME, mL	Ca, mg	% of RDA (by 1 serving)	Amounts needed to meet RDA
R	85	75	7.5	more than 13 pieces
P	225	250	25	at least 4 pieces
R + P	310	325	32.5	need to have three times in a day

At this point, it is necessary to see, if possible, the tentative amounts of products (e.g., in terms of LME) is being consumed per capita (per year) taking place in the locality. To that extent, earlier information (see Table 4.3) is reintroduced with some modified additions (those rows in the table are in bold faced).

Table 4.3: Required raw materials in regular and in case of overfull demand

Name of Raw Materials →		**Milk**	***Channa***	**Sugar**	**Flour**	***Khoa/***
Brand /Outlet	**Demand**	**L**	**Kg**	**Kg**	**Kg**	***Maowa*** **Kg**
MV	Regular	80	50	150	50	30
	Overfull	110	90	300	80	70
MB	Regular	800	120	400	50	30
	Overfull	1200	200	1000	150	80
B	Regular	400	75	140	40	45
	Overfull	600	120	200	65	75
MBz	Regular	350	90	120	40	40
	Overfull	500	150	160	65	70
Total	**Regular**	**1630**	**335**	**810**	**180**	**145**
	Overfull	**2410**	**560**	**1660**	**360**	**295**
Average		**2020**	**448**	**1235**	**270**	**220**
Increase in % for overfull		**150**	**170**	**210**	**200**	**200**

On the basis of above information in Table 4.3, following assumptions are adopted about the use of raw materials by four producers (outlet, here mentioned as sources) are:

1. Average production could be the logical amount of each raw materials being used in any typical day, because, if one producer got their overfull demand, possibly that day the other producer is running an ordinary day. However, in festive time, possibly most of the producers are busy with their overfull demand. On contrary, in public holydays or in the midst of political unrest, all producers might run in blank or business could be very close to zero earning. Therefore, the consideration of average amount of each raw material is justified.

2. Liquid milks could be used for whey and/or variety of cruddy products, therefore that amount is producing nearly half of the final products. So, 2020 L liquid milk is producing 1010 kg final products.

3. The rest all are mostly dry, therefore it is thought, those four raw materials might produce higher amount of final products. For example, if the average moisture contents of 40% are considered, then 1 kg raw materials actually produce 1.4 kg final products. According to that assumption, 2173 kg raw materials are producing 3042 kg (considering 40% MC) final products.

4. So in total, 4052 kg final products are being produced by those four producers.

Further extrapolation of assumptions about the total productions (milk products) of the city area is:

1. Four producers are producing probably one half of the total being produced in the city areas that means the rest of all producers are producing the rest half of the total sales of the city and the around. So the total productions now stand at 8104 kg across the city in a typical day.

2. It is difficult to assume who is the buyer; rather it can be considered that each one is a potential buyer in many occasions round the year.

Now, considering 360 business days in a year, the calculated total productions would be 2917440 kg. The total population of the city can be found cited in BBS documents, which are just little above of 500, 000. So the per capita milk product consumption round the year is to be nearly 6kg (compared to 6L). BBS always reported that tantamount of either liquid milk or milk products are consumed per capita, therefore, a person can highest consumes 12kg (or 12L) liquid milk or equivalent to that in a year. This assumption can support the national average which is reported in the part 2 of chapter one. The eventual figure can be calculated, and that is only 33mL LME is being allocated for each consumer in a day which is only less than 10% of LME accounted from two P products or just 10% if R and P are considered. Therefore, the obvious look out (see Table 4.4) is that people, being the city dweller, is still heavily being deprived from the standard requirements of Ca through milk-based consumables, and facing consequences in terms of many complicated physical impairments that addressed in the following table (Table 4.4).

Table 4.4: Figure of merits for Ca and related aspects

Level found in the study	**Typical contents in milk (reported earlier)**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
325 mg (R+P)	up to 1.33g L^{-1}	1.0 to 2.5g	Possibility of deficiency	Osteoporosis, hypertension, colon cáncer etc.

4.3 Scenario of national health services: a snap shot

The Bangladesh National Health Accounts, 1999-2001, termed as "NHA-2", represents the second endeavor of the Health Economics Unit (HEU) of the Ministry of Health and Family Welfare (MOHFW) of the Government of the People's Republic of Bangladesh to compile and update estimates of the health expenditures of the country [146]. Following information (presented either in tables or in figures) is obtained from that source, and considered here as necessary to evaluate the issues of health problem facing its population, irrespective of size under investigation.

Table 4.5 presenting the trend of national health expenditure, termed here as total health expenditure (THE) from the year of 1996 to 2007, which is more clearly depicted in Figure 4. 1. The rend is a gradual and steady increase and this similar increment in increase can be found its per capita expenditure (Figure 4.2) and the growth rate of national GDP (Figure 4.3).

Table 4.5: Total health expenditures, 1996-97 to 2001-02 and 2002-03 to 2006-07

Year → Expenditure↓	**1996-97**	**1997-98**	**1998-99**	**1999-00**	**2000-01**	**2001-02**
THE in million Taka	55,763	62,022	68,281	74,785	80,966	88,313
Year → Expenditure↓	**2002-03**	**2003-04**	**2004-05**	**2005-06**	**2006-07**	
THE in million Taka	89,709	102,229	17,085	138,955	160,899	

THE, Total Health Expenditure;

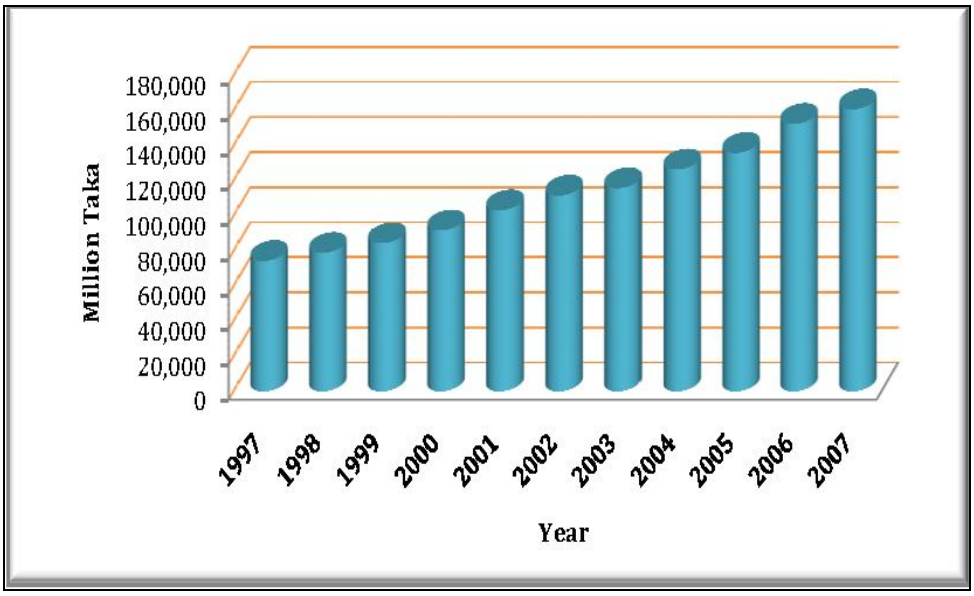

Figure 4.1: Total health expenditures, 2002-03 to 2006-07

However, together with the meager amount of THE (if total population is considered), an insignificant amount of that THE is only going to curative purpose (see the fourth column of Figure 4.4). Out of that curative expenditure, about 30% of that allocation is only for outpatient curative services (see Figure 4.5), and it is assumed that all the consumers facing a shortfall of Ca intake would be classified as those outpatients category. Therefore, the long term imbalance of diets, in terms of Ca deficiency, and its plausible consequences are not properly mitigated by the national expenditure.

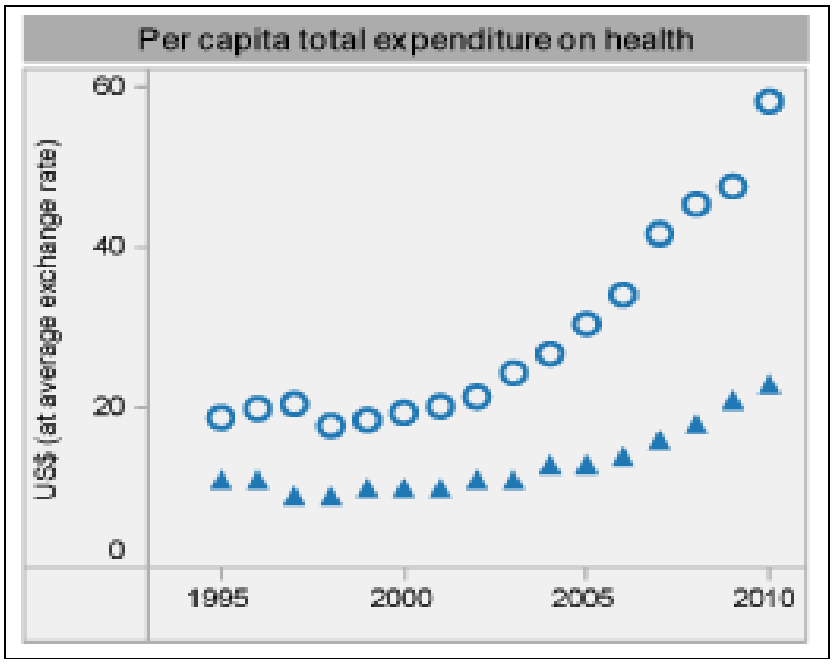

Figure 4.2: Per capita THE in Bangladesh from 1995 to 2010

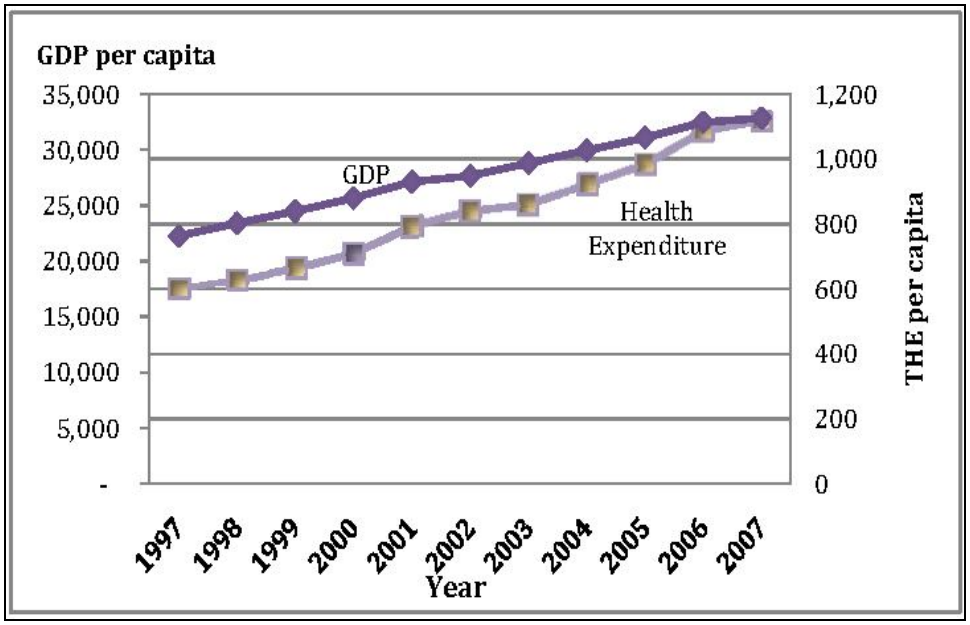

Figure 4.3: Per capita health expenditure and per capita GDP (Taka), 1997 – 2007

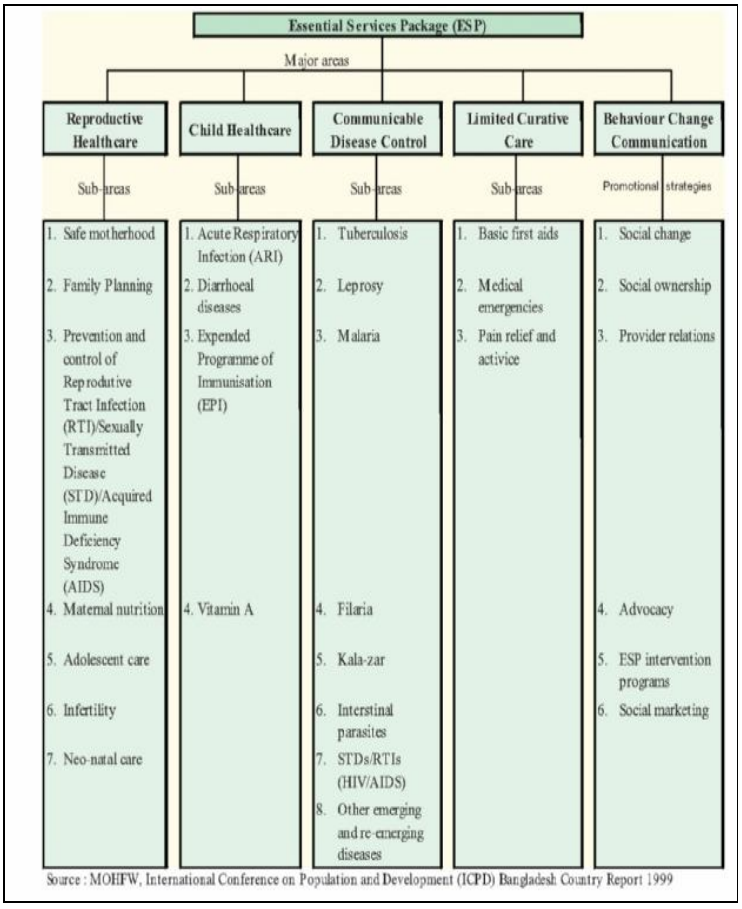

Figure 4.4: Structure of essential services package (from NHA-2)

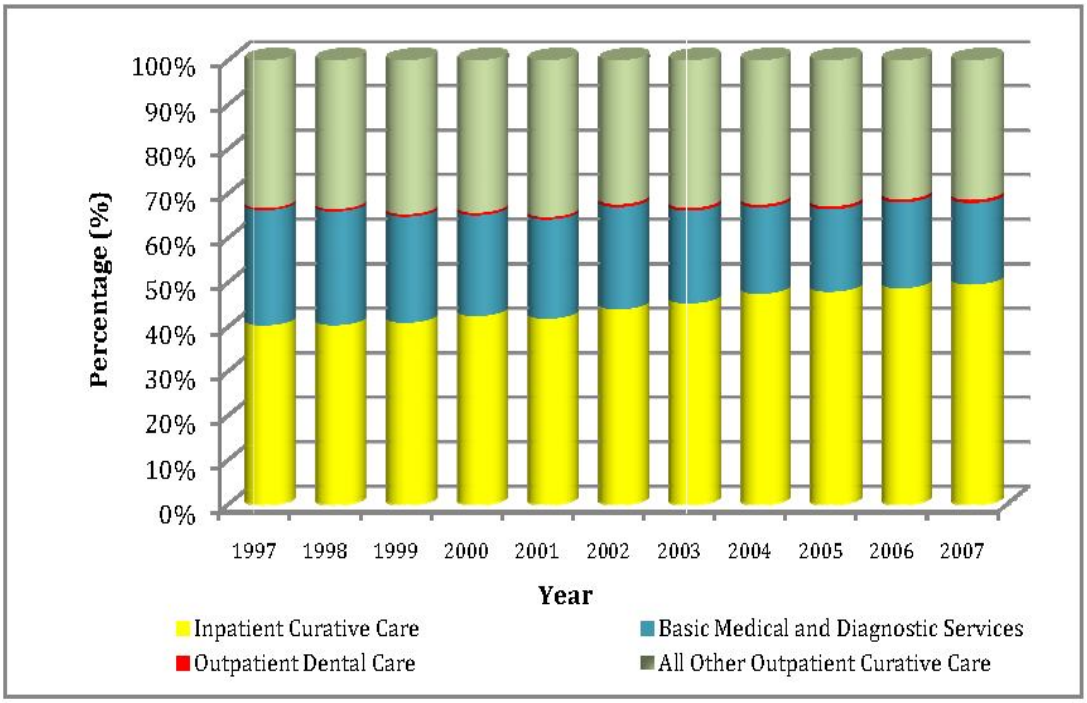

Figure 4.5: Services of curative care, 1997 - 2007

To address the issue of health risk factor, one example is ascertained here (see Figure 4.6) that adult blood pressure (hypertension) as a risk factor in national scale is a matter of concern. As hypertension is a common illness associated with low intake of Ca, therefore, inabilities in this regards (as people are really having less amount, and may prevailing its consequences through poor per capita THE of the country) is to be addressed properly, and at the same time the way of it's remedy is also to be set for the sake of national benefits and interests in long run.

To ascertain the issue of regional variations in health expenditure, following figure (Figure 4.7) and table (Table 4. 6) is inserted here from that same source, to see the actual comparison of regions of the country in terms of its health expenditure. It is assumed that larger percentages are due to the larger number of population of the region (see the case for Dhaka in Figure 4. 7), therefore, per capita expenditure remained almost similar in figure, which is deliberately a scanty amount to what the actual needs.

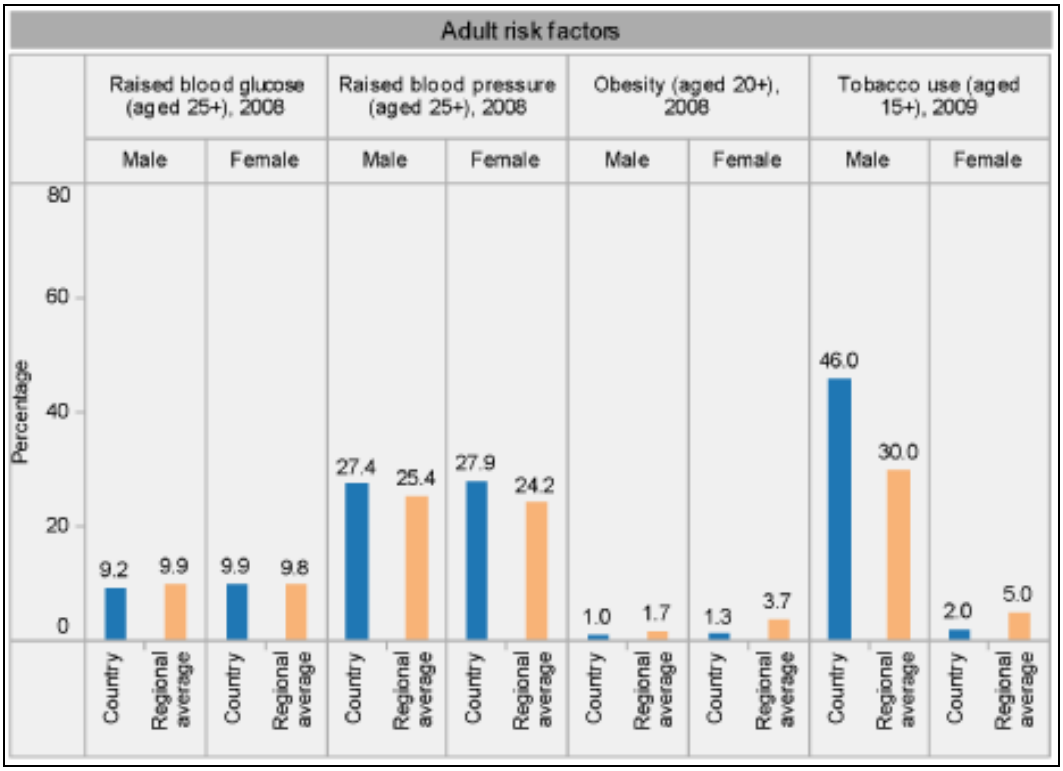

Figure 4.6: Adult risk factor of year 2006/2007 (from NHA-2)

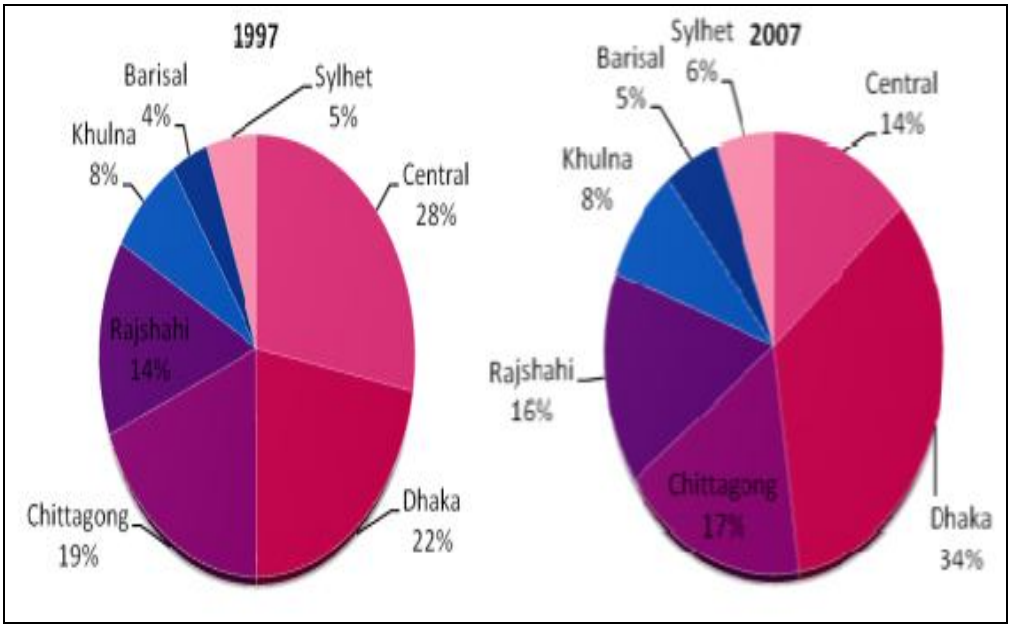

Figure 4.7: Percentage share of health expenditure by division

Table 4.6: Per capita spending on health by geographic region, 2007

Region	Population (2007) (in millions)	Expenditure (Million Taka)	Per capita spending (Taka)
Dhaka	40.67	4,970	122
Chittagong	26.53	3,929	148
Rajshahi	33.1	5,297	160
Khulna	16.06	2,619	163
Barisal	18.87	1,654	88
Sylhet	8.69	1,426	164

4.3.1 Remarks on national capacity and the outlook

The reality is people all over the country (possibly a very thin section of population is beyond the reach of any ominous touch of national shortcomings) is depriving from their daily dietary requirements of Ca minerals (justified in above), and all sorts of possible consequences on the basis of that poor intake of Ca is not supplemented through any kind of national initiatives and/or expenditure. Therefore, people are compensating this deprivation in a gradual deterioration of their own physical and ultimately in mental heaths.

However, sustainable economical development is the target of each developing country, as this country is thriving for. However, that achievement is difficult to attain leaving a vast majority of people under poor health condition which obviously a prerequisite of any kind economical development of the country.

4.4 Possible consequences of the prevalent status

It is clear that milk consumption in our country is so meager compared to any developed countries cited in the Chapter one and reassured in earlier sections of this chapter. When that poor per capita milk consumption is the reality [107-109] then

there is a serious possibility of risk factor impacting negatively on health for the entire population. And ultimately government has to spend a substantial amount of public money for tackle that situation.

For instance, studies suggested that poor Ca intake would actually enhance the risk of hypertension [147] (see Figure 4.8). While for only one g of Ca intake in a day the risk is reduced by 20 %. As myriad times it is advised that milk is a rich sources of Ca, and a drink of milk in regular basis would benefit in various ways by maintaining a good health even in older ages too [147, 148].

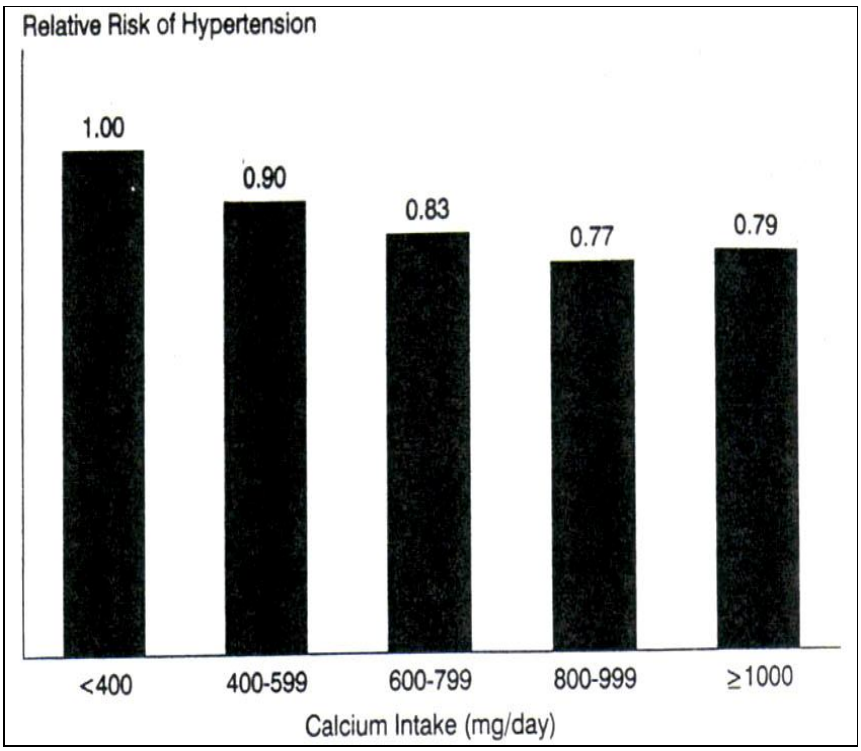

Figure 4.8: Relative risk of hypertension by level of energy-adjusted daily intake of Ca by US female nurses. (Adopted from Witteman, J. C. M., et a., *Circulation*, 80, 1320, 1989)

Similarly, the risk of kidneys malfunction through stone formation can be reduced up to 50 % if that prescribed amount of Ca (about 1 g, but slight more than 1 g is better) is taken regularly [149] (see Figure 4.9). And it is assumed that only drink of milk is the easiest way to maintain that prescription. Study also raveled that there is always a positive correlation existed between bone density and the amount of Ca intake in daily basis [150] (Figure 4.10).

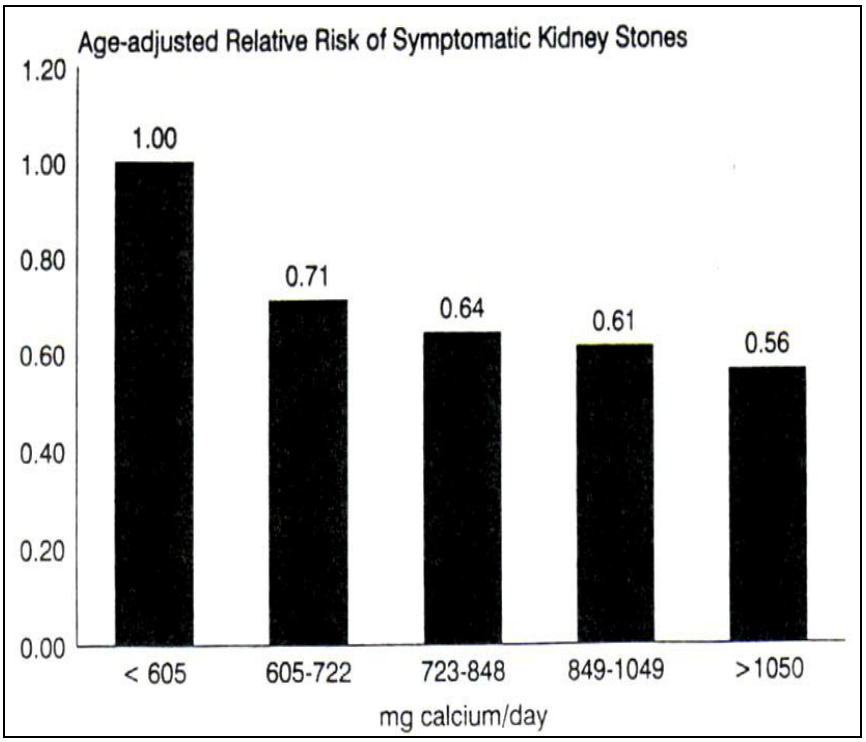

Figure 4.9: Age-adjusted relative risk of symptomatic kidney stones in adult male health professionals, according to dietary Ca intake. (Adopted from Curhan, G.C., et al., *N. Engl. J. Med.*, 328, 833, 1993)

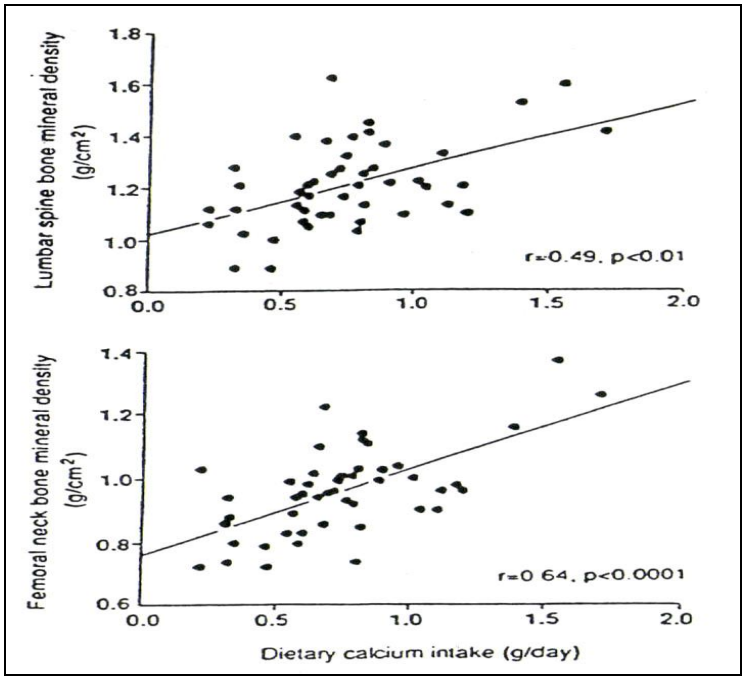

Figure 4.10: Relation between dietary Ca and bone mineral density at lumbar spine and femoral neck in normal men. (From Kelly, P. J., et al., *Br. Med. J.*, 300, 1361, 1990)

Chapter Five

Consumption of Trace Elements and its Consequences

5.1 Role of micronutrients in human health

Eight trace elements are analyzed in main study. Out of eight five (Zn, Cu, Mn, Co and Cr) is essential to human nutrition, the rest (Pb, Cd and As) is defined as heavy metals often pose threat for environment and eventually to the human health. A detailed description about essentiality and of each element and the consequences occurred due to their insufficient intakes or by overdose can be found in chapter 1.

5.2 Status of essential micronutrients

5.2.1 Zn

Status of Zn for human nutrition, it's sources, distribution in physiological sites, RDA and many more aspects about Zn is elaborately described in part 2 of Chapter 1. Like as Ca, following is the information presented in tables are to evaluate the status of Zn, it's common level in dairy sources, it's RDA values and possible (and prominent) consequences for it's deficiencies if occurred.

According to Table 5.1, nearly 100 glass of milk or its equivalent is needed to meet the RDA of Zn if no other source is considered, which would be an unrealistic approach if it is suggested to do. However, it is quite obvious that dairy milk or milk products is not a rich source for Zn, rather other protein sources like, all sorts of meats, sea foods, leafy vegetables and seasonal fruits are the richer sources of Zn. Apart from that, milk is still considered as fair source of Zn to be available for human nutrition.

Table 5.1: Zn contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
mg	1.0	0.80	<1,	100 glasses (if RDA is considered at average 25mg)

(Compiled from the USDA Nutrient Database); [#] 1 serving = 1 cup (8 oz; 244 g); [*] 1 glass = 200 mL;

Table 5.2 is presenting the average amount of Zn can be found in a single combination of two products (R + P), and evaluated this level with it's RDA and readdresses the consequences in a short term.

Table 5.2: Figure of merits for Zn and related aspects

Level of this study	**Typical contents in milk**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
(R+P) 0.34mg (LME 310 mL)	up to 4mg L^{-1}	10 to 40mg	Possibility of deficiency may be manifested	Skeletal development, Atherosclerosis, Brain development,

LME 310 mL is corresponding the serving by a combination of R & P, should contain more than 1mg Zn, but actual amount found is about 0.34 mg which is only a small portion of expected values (less than 30%). Therefore, possibility of Zn deficiencies in local inhabitants may not be uncommon, or even it could be prevalent with identifiable manifestations. As a result, human nutrition, for all age groups, would not properly being maintained, and again national health expenditure would be in stress further if the deficiency of Zn is to take in account.

5.2.2 Cu

Status of Cu for human nutrition, it's sources, distribution in physiological sites, RDA etc. is discussed in a length in part 2 of Chapter 1. Following tables (Table 5.3 and 5.4) are to evaluate the status of Cu, it's common level in dairy sources, RDA values and plausible consequences for it's deficiencies if found occurred.

According to Table 5.3, nearly 100 glass of milk or its equivalent is needed to meet the RDA of Cu if no other source is considered. Obviously dairy milk or milk products is not a rich source for Cu, rather other protein and cereal sources like, all sorts of meats, sea foods, leafy vegetables, whole meal food grains and seasonal fruits are the richer sources of Cu. However, milk is sometimes considered as a source of Cu for human nutrition. Table 5.4 is presenting the average amount of Cu can be found in a single combination of two products (R + P), and evaluated this level with it's RDA and readdresses the consequences in a single term.

Table 5.3: Cu contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
µg	27	22	about 1	nearly 100 glasses (if RDA is considered at average 2mg)

(Compiled from the USDA Nutrient Database); [#] 1 serving = 1 cup (8 oz; 244 g); [*] 1 glass = 200 mL;

LME 310 mL is corresponding the serving by a combination of R & P, should contain more than 6µg Cu, but actual amount found is about 16µg which is far more than expected values. Therefore, possibility of Cu deficiencies in local inhabitants may be uncommon, or not be prevalent with identifiable manifestations.

Table 5.4: Figure of merits for Cu and related aspects

Level of this study	Typical contents in milk	Average RDA/UL cited earlier in texts	Perception of deficiency /overdose	Consequences at a glance
(R+P) 16 µg (LME 310 mL)	up to 20µg L^{-1}	up to 2mg	Possibility of deficiency is virtually absent	----------

5.2.3 Mn

Status of Mn for human nutrition, it's sources, distribution in physiological sites, RDA etc. is discussed in a length in part 2 of Chapter 1. Following tables (Table 5.5 and 5.6) are to evaluate the status of Mu, it's common level in dairy sources, RDA values and plausible consequences for it's deficiencies if found occurring.

According to Table 5.5, huge number of glasses of milk or its equivalent is needed to meet the RDA of Mn if no other source is considered. Dairy milk or milk products is not a rich source for Mn, rather other sources like, all sorts of meats, sea foods, leafy vegetables, whole meal food grains, variety of seeds and nuts and seasonal fruits are the richer sources of Mn. Table 5.6 is presenting the average amount of Mn that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA and readdresses it's consequences.

Table 5.5: Mn contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	% of RDA (by 1 glass)	Amounts needed to meet RDA
µg	7	6	<1	figure could be unrealistically high

(Compiled from the USDA Nutrient Database); [#] 1 serving = 1 cup (8 oz; 244 g); [*] 1 glass = 200 mL;

LME 310 mL is corresponding the serving by a combination of R & P, should contain more than 6µg Mn, but actual amount found is about 200µg which is far far above than the expected values. Therefore, possibility of Mn deficiencies may be uncommon, or not be prevalent with identifiable manifestations, rather deficiencies from other sources could be compensated by the serving (R+P) considered here.

Table 5.6: Figure of merits for Mn and related aspects

Level of this study	**Typical contents in milk**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
(R+P) about 200µg (LME 310 mL)	up to 20µg L^{-1}	up to 5mg	Possibility of deficiency is virtually absent	----------

5.2.4 Co

Cobalt is a component of vitamin B_{12}, and this is the only known function of Co. Status of Co for human nutrition, it's sources, RDA and few more issues are discussed in part 2 of Chapter 1. Following tables (Table 5.7 and 5.8) are to evaluate the status of Co, it's common level in dairy sources, RDA values and plausible consequences for it's deficiencies if found occurring.

Dairy milk or milk products are not a good source for Co, while other sources like, all sorts of meats are good source for Co intake. The maximum dietary tolerable level of Co for common drinks (e.g. liquid milk) is cited as 10 ppm, as because of it's toxicity or deficiency has never been clearly demonstrated. Considering that 10 ppm, calculated information is presented in the following table (Table 5.7). However, Table 5.8 is presenting the average amount of Co that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA and readdresses it's consequences.

Table 5.7: Co contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
mg	2.4mg	2.0mg	not set	-------

LME 310 mL is corresponding the serving by a combination of R & P, should contain up to 5mg Co, but actual amount found is about 20µg which is far above below the expected values. Therefore, possibility of Co deficiencies might be common, and it's deficiency could be prevalent with identifiable manifestations.

Table 5.8: Figure of merits for Co and related aspects

Level of this study	**Typical contents in milk**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
(R+P) about 20µg (LME 310 mL)	up to 10mg L^{-1}	not set	Possibility of deficiency may be manifested	

5.2.5 Cr

Status of Cr for human nutrition, it's sources, RDA and few more issues are tried to discuss in part 2 of Chapter 1. Following tables (Table 5.9 and 5.10) are to evaluate the status of Cr, it's common level in dairy sources (if found), RDA values and plausible consequences for it's deficiencies/toxicity if assumed to occur.

The Cr (VI) (Cr^{+6}) form is toxic to human, although its occurrence is not common in the environment. Cr^{+6} is 100 times more toxic to human than trivalent Cr (Cr^{+3}). The solubility of both forms is significantly affected by pH, the lowest solubility occurs

between pH 5.5 and 8.0. It's toxic intake is considered as 200mg. Absorption of Cr is influenced by the presence of chelating agents and other metals, particularly Zn and Fe, and it is excreted primarily through urine. Chromium is required for normal carbohydrate and lipid metabolism in the body, and its deficiency affects many biological functions. Chromium supplementation improves glucose tolerance and blood lipids. The daily requirements of Cr for human adult are 50 to 200µg. Dairy milk or milk products certainly are not a good source for Cr. The maximum dietary tolerable level of Cr for common drinks (e.g. liquid milk) is cited as not above than 50 ppb, as because of it's toxicity has never been demonstrated occasionally. Considering that 200µg, calculated information is presented in the following table (Table 5.9). However, Table 5.10 is presenting the average amount of Cr that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA and readdresses it's consequences.

Table 5.9: Cr contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
µg	about 12	10	100 x 20	figure could be unrealistically high

LME 310 mL is corresponding the serving by a combination of R & P, should contain up to 17µg Cr, but actual amount found is about 40µg which is nearly double than the expected values. Therefore, possibility of Cr overdose might be realistic, and it's consequences could be prevalent with identifiable manifestations.

Table 5.10: Figure of merits for Cr and related aspects

Level of this study	**Typical contents in milk**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
(R+P) 40µg (LME 310 mL)	ca. up to 20µg L^{-1}	200µg	Possibility of deficiency is virtually absent	

5.3 Status of heavy metals

5.3.1 Pb

Status of Pb in human consumables, it's main sources, maximum daily intake (MDI) and few more issues are tried to discuss in part 2 of Chapter 1. Following tables (Table 5.11 and 5.12) are to evaluate the status of Pb, it's common level in dairy sources (if it is found in the common citations), RDA (if reported) values and plausible consequences for it's deficiencies/toxicity if assumed to occur.

Lead is one of the well-known toxic heavy metals and is a major pollutant. It is primarily introduced into the atmosphere by the use of lead-containing gasoline and then introduced into the food chain by deposition on crop plants and soil dust inhalation. Lead is the least mobile of the heavy metals in the environment due to its poor aqueous solubility. It is not readily soluble in water and is found in relatively low concentrations. The maximum permissible level in any drinking fluid (e.g., milk) is 50 ppb, as milk should be devoid of any traces of Pb.

Daily dietary intake (DDI) is 60 to 500µg while its toxic intake is cited as 1mg. Lead is both carcinogenic and teratogenic. It adversely affects young human, primarily impacting their intellectual development.

Considering the middle value of the range of DDI (mentioned above), and the concentration of liquid milk as 50 ppb, calculated information is presented in the following table (Table 5.11). The Table 5.12 is presenting the average amount of Pb that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA (here as DDI) and readdresses it's consequences.

Table 5.11: Pb contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
µg	about 12	10	100 x 28	figure could be unrealistically high

LME 310 mL is corresponding the serving by a combination of R & P, should contain up to 16µg Pb, but actual amount found is about 140µg which is nearly seven times higher than the expected values. Therefore, possibility of Pb toxicity may not be unrealistic, and it's consequences could be prevalent with identifiable manifestations.

Table 5.12: Figure of merits for Pb and related aspects

Level of this study	Typical contents in milk	Average RDA/UL cited earlier in texts	Perception of deficiency /overdose	Consequences at a glance
(R+P) 140µg (LME 310 mL)	ca. up to 50µg L^{-1}	280µg	Possibility of deficiency is virtually absent	

5.3.2 Cd

Status of Cd in human consumables, it's main sources, maximum daily intake (MDI) and few more issues are tried to discuss in part 2 of Chapter 1. Following tables (Table 5.13 and 5.14) are to evaluate the status of Cd, it's common level in dairy sources (if it is found in the common citations), RDA (if reported) values and plausible consequences for it's deficiencies/toxicity if assumed to occur.

Cadmium is becoming an element of concern due to its presence in waste products, primarily sewage sledges. Cadmium contents in the environmental matrices are strongly influenced by human's activity. DDI is cited the range of 7µg to 3.0mg, quite a long range, and its toxic intake is defined as 30mg. However, the mean daily intake of Cd is estimated to be about 40µg, and its UL is described as 200µg. The safe limit of daily intake of Cd is set by the WHO, FAO and US, EPA are 57, 71 and 70µg respectively (see the citations in earlier). The major dietary source of Cd is cereal grains.

Considering the common value of DDI (mentioned above), and the concentration of liquid milk as 50 ppb, calculated information is presented in the following table (Table 5.13). And the Table 5.14 is presenting the average amount of Cd that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA (here as DDI) and readdresses it's consequences.

Table 5.13: Cd contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
µg	about 12	10	100 x 7	figure could be unrealistically high

LME 310 mL is corresponding the serving by a combination of R & P, should contain up to 16µg Cd, but actual amount found is about 5µg which is nearly one third of the expected values. Therefore, possibility of Cd toxicity may not be realistic, and it's consequences could not be prevalent with any kind of identifiable manifestations.

Table 5.14: Figure of merits for Cd and related aspects

Level of this study	**Typical contents in milk**	**Average RDA/UL cited earlier in texts**	**Perception of deficiency /overdose**	**Consequences at a glance**
(R+P) 5µg (LME 310 mL)	ca. up to 50µg L^{-1}	70µg		

5.3.3 As

Following is the information presented in tables (Table 5.15 and Table 5.16) is to evaluate the status of As, it's common level in dairy sources (if found), it's RDA/UL values and possible consequences for it's deficiencies if occurred.

Arsenic is not highly mobile and therefore would not be expected to accumulate from movement within the food chain. The major source of As would be from physical deposition or from consuming products high in As, such as seafood. The bioaccumulation index for As is moderate to high.

DDI is to be 40µg to 1.4mg; however, daily required intake for adult human is 12 to 25µg, whose toxic intake is about 5mg in a day. Arsenic is believed to be carcinogenic but the level of intake could be high enough which is not very common in normal supply level which is usually 0.5ppb.

Considering the highest value of daily required intake for adult human as 25µg, and the concentration of liquid milk as 50 ppb (the national standard), calculated information is presented in the following table (Table 5.14). And the Table 5.14 is presenting the average amount of As that can be found in a single combination of two products (R + P), and is evaluated this level with it's RDA (here as average of DDI) and readdresses it's consequences.

Table 5.15: As contents of cow's milk (whole)

Unit	**In one** serving[#]	**In one glass**[*]	**% of RDA (by 1 glass)**	**Amounts needed to meet RDA**
µg	about 12	10	100 x 2.5	----------

LME 310 mL is corresponding the serving by a combination of R & P, should contain up to 16µg As, but actual amount found is about 940µg which is a huge hike than the expected values. However, if the middle value of DDI (cited above) is considered, then the amount of 940µg present in the sample can be considered as a moderately high. However, if that level of 940µg is to be consumed in regular basis, the

continuous exposure would be a real threat for the consumer. Therefore, the possibility of As toxicity could be obvious, and it's consequences might be prevalent with noticeable manifestations.

Table 5.16: Figure of merits for As and related aspects

Level of this study	Typical contents in milk	Average RDA/UL cited earlier in texts	Perception of deficiency /overdose	Consequences at a glance
(R+P) 940µg (LME 310 mL)	up to 50µg L^{-1}	720µg	Possibility of overdose	Toxicity may eminent in various organs

5.4 Concluding remarks

Except Zn, milk or milk products are not the good sources of the other elements, however their clear presences are reported and their findings in the products analyzed here uphold that claims. However, some of their presence, or even for the higher levels of expected elements, might be the basis to claim that milk products are might be the results of deliberate adulteration and/or contamination by various means. Even though, the way of the processes of adulteration and to find out the point, from milking point to be opened for sale, where it is taking place is difficult to identify with certainty.

From the perspective of nutritional interests, it is unfortunate that the level of most beneficial micronutrients (like, Zn and Cu) found here are quite below the level of required values. On the other hand, undesired elements like Pb and As contents are in the level for which one might express his/her concern in relation with their role in human health and the damages that might be eminent for a long time exposure if persisted due to regular consumption of that two products if recommended.

Chapter Six

Summary and Recommendations for Further Studies

6.1 Summary of the study

Chapter 1 (Introduction & Hypothesis) is subdivided into three parts, in which the part I and II offered the details about milk, its world-wide production compared to our own production level (in Bangladesh), per capita milk consumption in developed countries compared to our own per capita consumption with a reference to what actually dieticians are suggesting to have in daily basis. Secondly, it is also addressed if there are any shortages that possesses for the lack of nutrients intake those should have been acquiring through drinking milk or milk products in daily basis. Even though a scanty amount of milk is consumed each person in the country, the level of the quality of that milk is also a matter of concern, therefore, the importance of the analysis of milk which are available in the market is also addressed here.

In relation with that, sizeable amount of information about common mineral nutrients, e.g., Ca, and micronutrients e.g., Zn, Cu, Mn, Co and Cr, and common suspects of heavy metals, e.g., Pb, Cd and As are given methodically. Afterwards, uses of milk, usually in subcontinents, in preparing variety of sweetmeats with verity of milk derivatives (their English analogous name is also given) are described considerably. The situation of food adulteration that might have been occurring in the country is tried to address by referring several news sources, as these kind of information are virtually absent in the scientific documents. For all kind of information, references are usually cited from related published works. At the last, in part III, statement of the

current attempt which is taken through mentioning the hypothesis and the way it would have been tested are presented in objectives.

Chapter 2 (Materials and Methods) provided information about the sample those were tested in two different studies (termed here as initial and main study) with its probable time schedule; information about the chemicals, glass-ware and instruments those were used in the analysis. Several liquid milk samples, collected from markets and milking points together with several milk-based sweetmeats were considered to be analyzed in both stages of analysis. In main study, the basis of selecting items and their sources is defined in relation with market survey by using important questioners answered by the several sweetmeat producers in the city area. Possibility of verity of instrumental techniques for trace elemental analysis is also given in consolidated form. However, an elaborate description about AAS that is finally used in this study for trace elemental analysis presented in this chapter is noteworthy to mention, where numerous references are also cited.

In Chapter 3 (Results and General Discussions), obtained results were either put in the tables, figures or mentioned directly in the discussions. In figures, results are given in mean with standard deviations. However, in writings, efforts were made to use the results, if possible, to prove the hypothesis, if not, then it was tried to use it for the null hypothesis. But at the same time it is to be mentioned that the developments in the discussions were systematic and logical and notion was kept to maintain it till it finishes. Mean comparisons of all parameters (physical and elemental), their correlation studies, and on the basis of that correlation studies, regression analysis were one to find out the actual relationship existed among parameters. On the basis of all important findings, concise concluding remarks are generally given at the end of the chapter.

Chapter 4 is a special discussion of reconstructive work about the status of Ca intake by the local inhabitants through the milk products available within their reach. Here, possibility of low Ca intake is readdressed, and as because of its ill consequences, in terms of many common manifestations, is discussed at the later part of the chapter, therefore, insufficiency of national expenditure towards all kind of health services provided are as meager compared to what need is tried to bring into light.

Chapter 5 is also a kind of reconstructive work but for the micronutrients and suspected heavy metals. It is to be noted that, the level of these nutrients and heavy metals are always expressed in terms of fresh weight of two selected items, *Rosogolla* and *Perasandesh*, considering those items as a standard serving in local cuisine. Unfortunately, the level of essential nutrients like, Zn, Cu, Mn, Co and some extent Cr are found, most of the time, less than the required levels, on the other hand, heavy metals like, Pb and As are contained in a level that is obviously unexpected, however, this findings actually corroborative to the hypothesis that milk-based sweetmeats could be contaminated with available chemical compounds for various reasons, one of them could be to increase its shelf life.

6.2 Weaknesses of the study

It is fair to confess the shortcoming and irregularities of research if occurred. Inadequacies of technical shortcomings were hindering the work schedule in some extent. For instance, the analytical laboratory used in the study lacking some special accessories for which few parameters of analysis e.g., the important nutrients like Fe, Mg can not be done due to the shortage of special kind of lamps needed for that analysis. Another drawback is that, certified reference materials were not available in the laboratory (because of its highly expensive values), those reference materials are usually used in each batch of analysis for the sake of reliability of methods as to ensure that recoveries of elements are neatly done that can be a problematic feature for many sample preparation procedures used in analysis.

Financial capabilities are another constrains, as analysis of more liquid milk samples and as well as more milk products from many sources around the city area would have been better, that could lift the work further by adding another dimension to it.

6.3 Recommendations for further study

A part of this study was to highlight of per capita consumption of milk and milk products in the country by showing the data that found in the government references.

The reason of this highlight is to show the severity of the effect on consumers if it is found that milk and milk related consumables are really adulterated with objectionable ingredients or by inferior ingredients for unjustified profits.

Consumers are the largest stakeholders in any country. They should be treated with due respect and must be protected by strong and effective consumer protection laws. But unfortunately, in developing countries, the situation is quite the opposite. Due to the lack of effective consumer laws and consumer courts in the country, consumers are being cheated by misleading advertisements and are being poisoned by substandard, adulterated and counterfeit products. Therefore, it is assumed that scientists, especially chemists, could put an important role to this movement by analyzing the suspected products and comparing the findings with what it should be in standard conditions.

So far to the best of our knowledge, this is surely a pioneering work, of its kind, in our country; therefore, it really holds a special credit. Ultimately, the results of this research may contribute to the overall knowledge of mechanisms governing the healthy aging related to intake of minerals and micronutrients throughout the human lifespan and that may help by improving understanding and development of therapeutic approaches to dealing with increasingly prevalent any degenerative disorders.

To elucidate the role of Ca minerals and the dietary intake of micronutrients for the consumers, additional works is necessary; this may be done by determining a list of experiments for those identified consumers those have nutrient-deficient impairments. Newer type of experiment could also help to clarify the issue prevailing in the locality.

The challenge for food law enforcement agencies is to be one step ahead and to constantly develop new methods to gain a better insight into the complex chemical structure representing food, in order to identify a set of possible marker components for authentication purposes. We assume that respective authority is active in limiting adulteration and other forms of fraud because these practices can seriously undermine consumer confidence and the overall development of the industry. Fraudulent

practices create unfair competition, leading to market distortions which, in turn, may impact the country's economy. Therefore, the authentication of milk and milk products is of primary importance to both consumers and manufacturers, and at all stages of the production chain. Although actions were taken so far by the respective authority to limit adulteration and, in this regard, we the scientist community wants to work closely with the relevant government departments.

The results of current researches could be improved in several ways. For instance, continuous monitoring of all kind of products, either liquid milks selling in bulk or in groceries under special treatments (e.g., UHT), and/or all milk products selling for direct consumptions are needed and for that purpose, a common well-equipped laboratory for ultra-trace chemical and biological analysis (could be run by the respective national authority) is an urgency to be set within convenient time. To that extent, potential resource persons are also in need to develop for maintaining any kind of routine analysis that should be done in regular intervals.

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Appendix-A

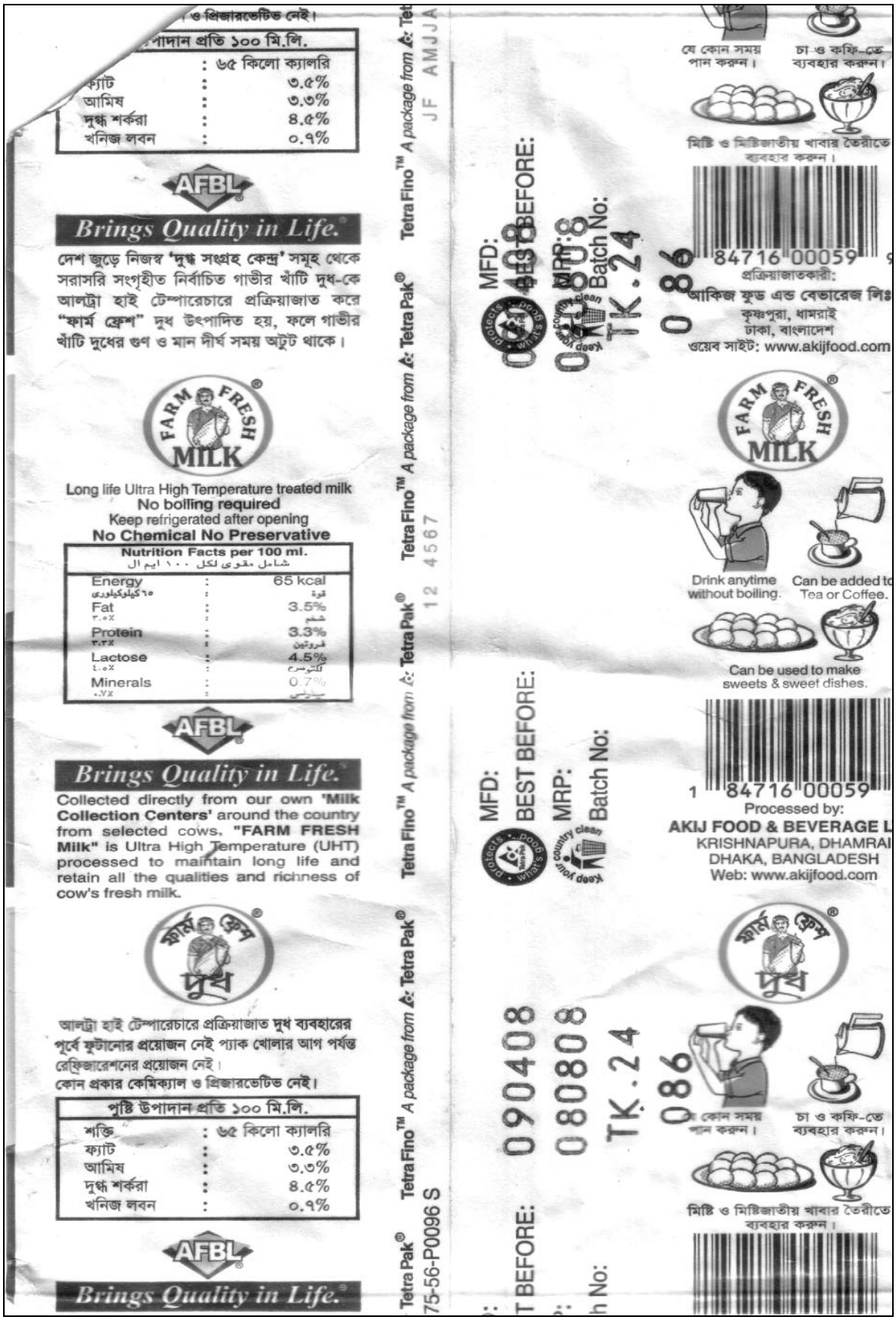


Figure A-1: Scanned photograph of the backside of the packet of packet milks used in this study

Appendix-B

Production of Sweetmeats: A Survey on *Rajshahi* Metropolitan City

Name of the Outlet/Brand: **Address:**

1. What is the year of establishment of your business?

   .. ..

2. How many numbers of employees in your organization at present?

   .. .. ..

3. Where do you produce your items?

   a. Own factory

   b. Others factory

   c. Others (please explain).. .. ..

4. How do you collect the capital?

   a. Own capital b. Bank Loan c. Others

5. What are the main objectives of your business?

   a. profit b. Brand Image c. Loyal customer

6. What materials do you essentially need for your business?

a. Milk b. *Channa* c. Sugar d. Flour e. Color

7. From where do you collect these materials?

8. Please mention the required amount (per day/week) of the above raw materials.

a. Milk b. *Channa* c. Sugar d. Flour e. Color

9. Please mention the amount (in kg) of raw materials needed in the due time of different occasions.

a. Milk b. Chana c. Sugar d. Flour e. Color

10. How do you forecast your need for the materials?

.. ..

11. Do you get the required raw materials smoothly from the suppliers, all over the year?

.. ..

12. Do you get raw materials of a good standard round the year?

a. yes b. No

13. Do you get additional raw materials when you face overfull demand?

a. Yes b. No c. Sometimes

14. Are you confident enough that you can get the raw materials at the time and of the quantity you need ?

a. Yes b. No

15. If no, then please stratify the causes

.. ..

16. Dou you have to face high price of raw materials for different occasions (Eid, Puja, New Year etc)?

17. Up to which limit of quantity you are capable enough to receive and deliver/serve argent orders ?

18. What is your preferred delivering system for collecting materials?

a. you collect them from the location of supplier

b. supplier delivers those at your convenient location.

19. How much time (lead time) do you keep as safety period before placing the order to supplier?

a. mMinimum, because these are readily available anytime

b. week, because the supplier need some preparation time

20. What factor/factors are restricted you to fix the capability limit?

.. ..

21. Do you used to stock materials in advance?

a. Yes

b. No

c. Sometimes

d. For specific items, please specify .. ..

22. Do you have any additional facility for storing raw materials?

.. ..

23. What payment system do you follow to pay the bill of suppliers?

a. Advance b. Cash c. Check d. Debt

24. Please mention the amount (in kg) of sells per day

.. ..

25. Is there any particular time in a day when the amount of sells increase?

a. No b. If yes then please mention the time duration .. .. ..

26. Is there any particular season in a year when the amount of sells increase?

a. No

b. If yes, then please mention the name of season .. ..

27. Please mention the amount (in kg) of sells in the due time of different occasion.

28. Please mention the amount (in kg) of sells for last seven days.

a. Day 01- b. Day 02- c. Day 03-

d. Day -4- e. Day 05- f. Day 06-

g. Day 07-

29. Do you segment the market? If yes, then which variable/variables do you consider for segmenting market ?

a. Geographic b. Demographic c. Psychographic d. Behavioral

30. How many product lines do you have?

.. ..

31. Please mention the number & name of items of your product-line length

a.	b.	c.	d.	e.
f.	g.	h.	i.	j.
k.	l.	m.	n.	o.
p.	q.	r.	s.	t.
u.	v.	w.	x.	y.

32. Do you engage in diversify or simplify the product line, which ?

a. Diversification (not depending on single or a few product items rather offering a big variety)

b. Simplification (reducing less profitable variety & concentrating on the profitable item).

33. Do you engage in product differentiation for achieving competitive advantages ?

a. Yes b. No

34. If yes, on which basis do you differentiate?

a. Form b. Style c. Design d. Featuring low price

e. The latest or most trendy product f. Efficient and rapid service

g. Coverage h. Communication i. If others please specify .. .. ..

35. Do you think that your customer prefer your product for your name and goodwill?

36. Do you think that the packaging of your products is attractive, good to preserve the quality, easy to carry and containing the necessary information?

a. Yes b. No

37. If no, then do you have any thought for its improvement?

a. Yes b. No

38. Do you have any plan to introduce any new product item?

a. Yes b. No c. We are thinking about this

39. What factors would you consider to determine the price of your product?

.. ..

40. What method/ methods do you follow to set up pricing?

a. Cost-based pricing b. Break- even pricing

c. Value-based pricing d. Competition-based pricing

41. Do you have any price discount or allowance?

a. Yes b. No

42. If yes, then on which factor/factors it's depended?

a. Large volume purchase b. Loyal customer c. Seasonal offer

d. If pay the bill in advance e. Others (please stratify) .. .. ..

43. How do you charge your customer when you offer a new product item?

a. Skimming (high price to ensure quick profit)

b. Penetration (low price to get market acceptability)

c. Going rate pricing (following the competitors)

44. What type/types of distribution channel are you using?

.. ..

45. How many outlets do you have?

a. One b. Two c. Three d. Four e. Five f. More than five

46. Do you think that by the number of this / these outlet you can meet up the demand of your target customer ?

.. ..

47. How do you motivate your channel members for working efficiency?

.. ..

48. Is there any facility for trained up your workers for quality control & improvement of service ?

a. Yes b. No

49. Do you feel any difficulty in storing your finished products?

a. Yes b. No c. Sometimes

50. Please disclose the transportation and warehouse facilities of your organization.

.. ..

51. What are your current promotional tool/tools?

a. Advertising b. Sales promotion c. Public relation

d. Personal selling e. Direct marketing

52. Which tools are you emphasizing more and why?

.. ..

53. Do you offer for a trial to the customer?

.. ..

54. What is your current slogan?

.. ..

55. How much do you spend for your yearly promotion?

56. Do you have any new promotional strategy?

.. ..

57. What are the dimensions of challenges you usually face from the activities of your competitors ?

a. Bargaining power of supplier

b. Bargaining power of customers

c. Impact on pricing

d. Investing on decoration

e. Bargaining power of the stuff

f. Quality competition

g. Others

58. Do you have any complaint system for the customers?

a. Yes b. No

Thank you for your kind cooperation

Appendix C

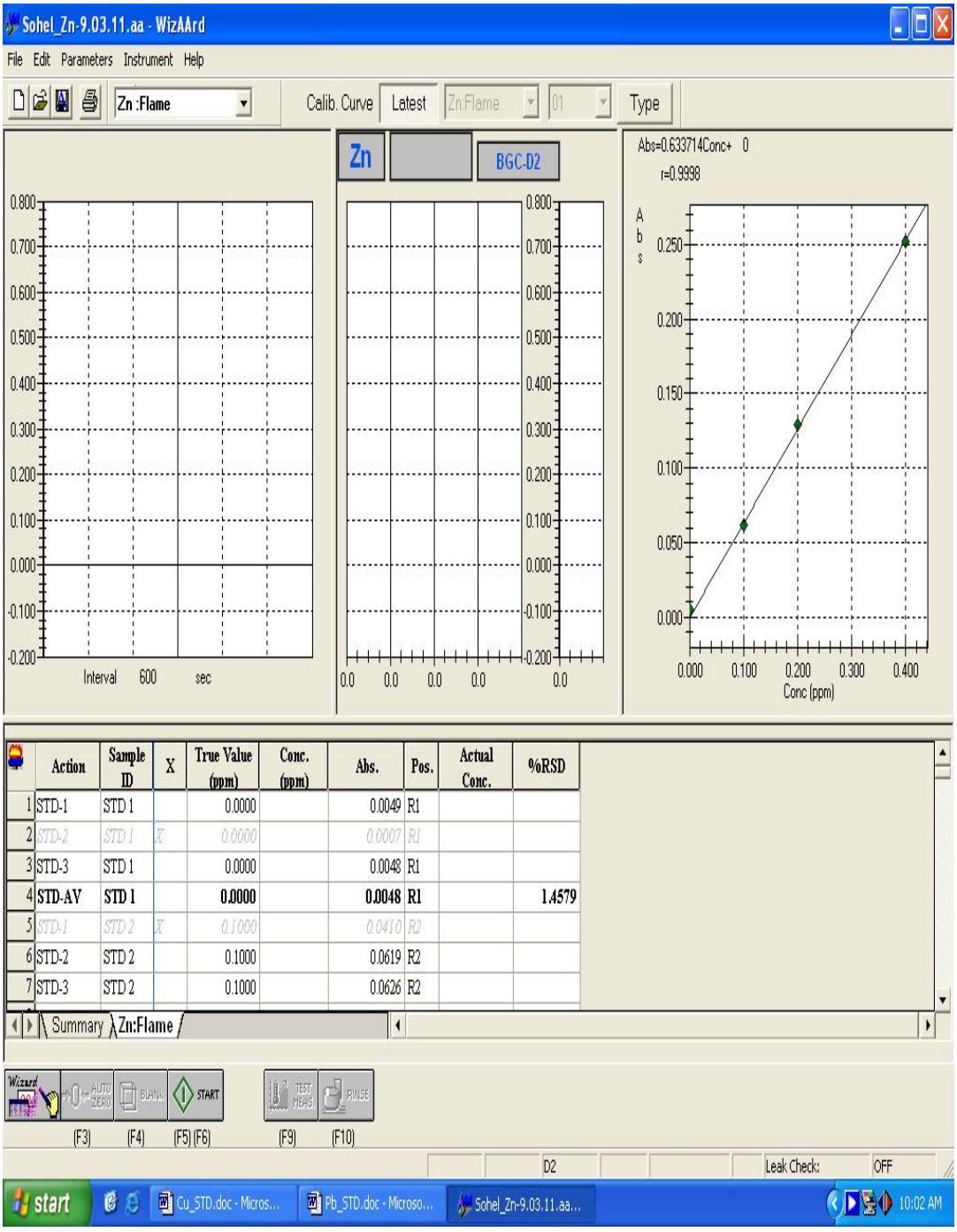


Figure C-1: Calibration of Zn in AAS with its background noise level (transported from operating software)

Appendix C

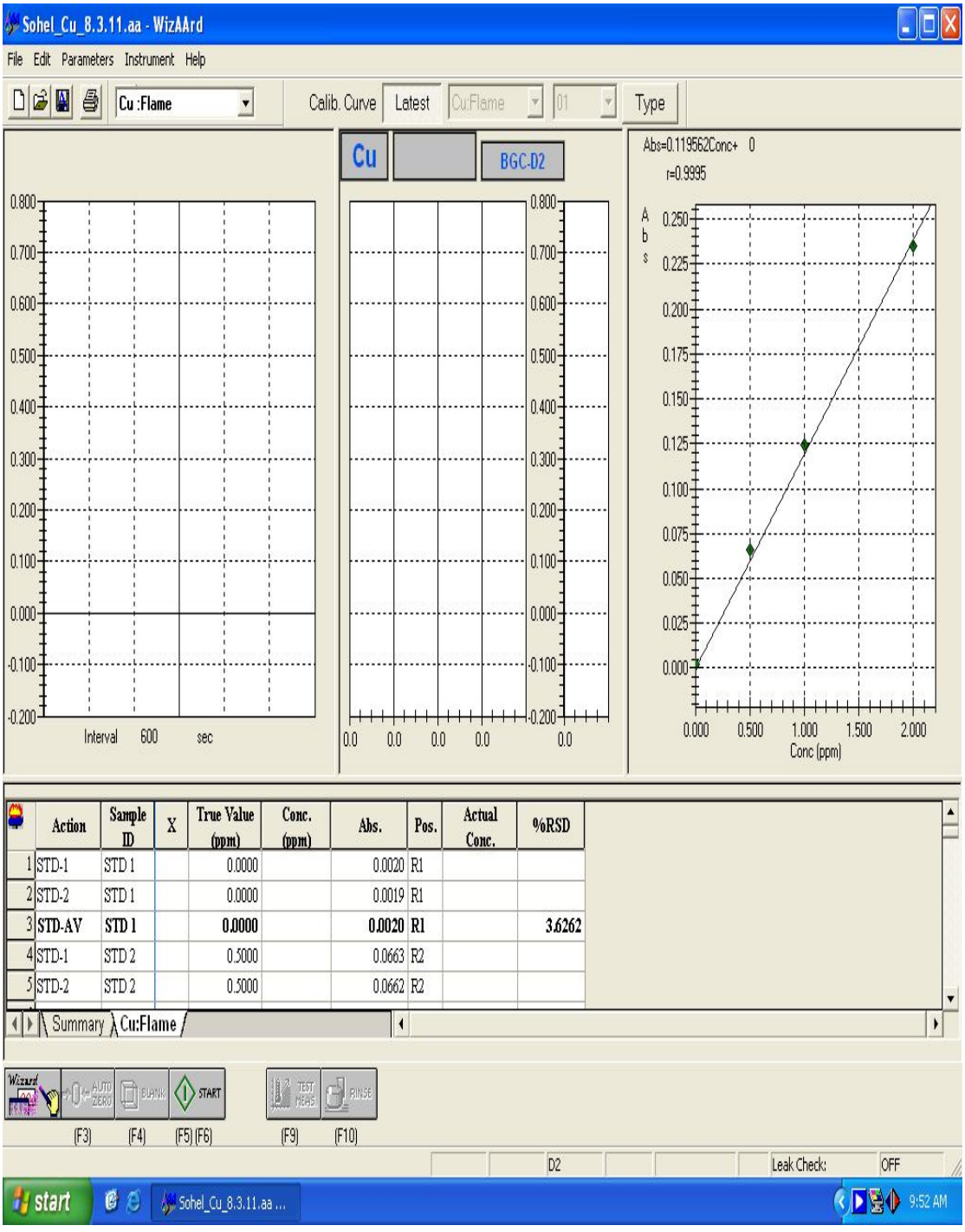


Figure C-2: Calibration of Cu in AAS with its background noise level (transported from operating software)

Appendix C

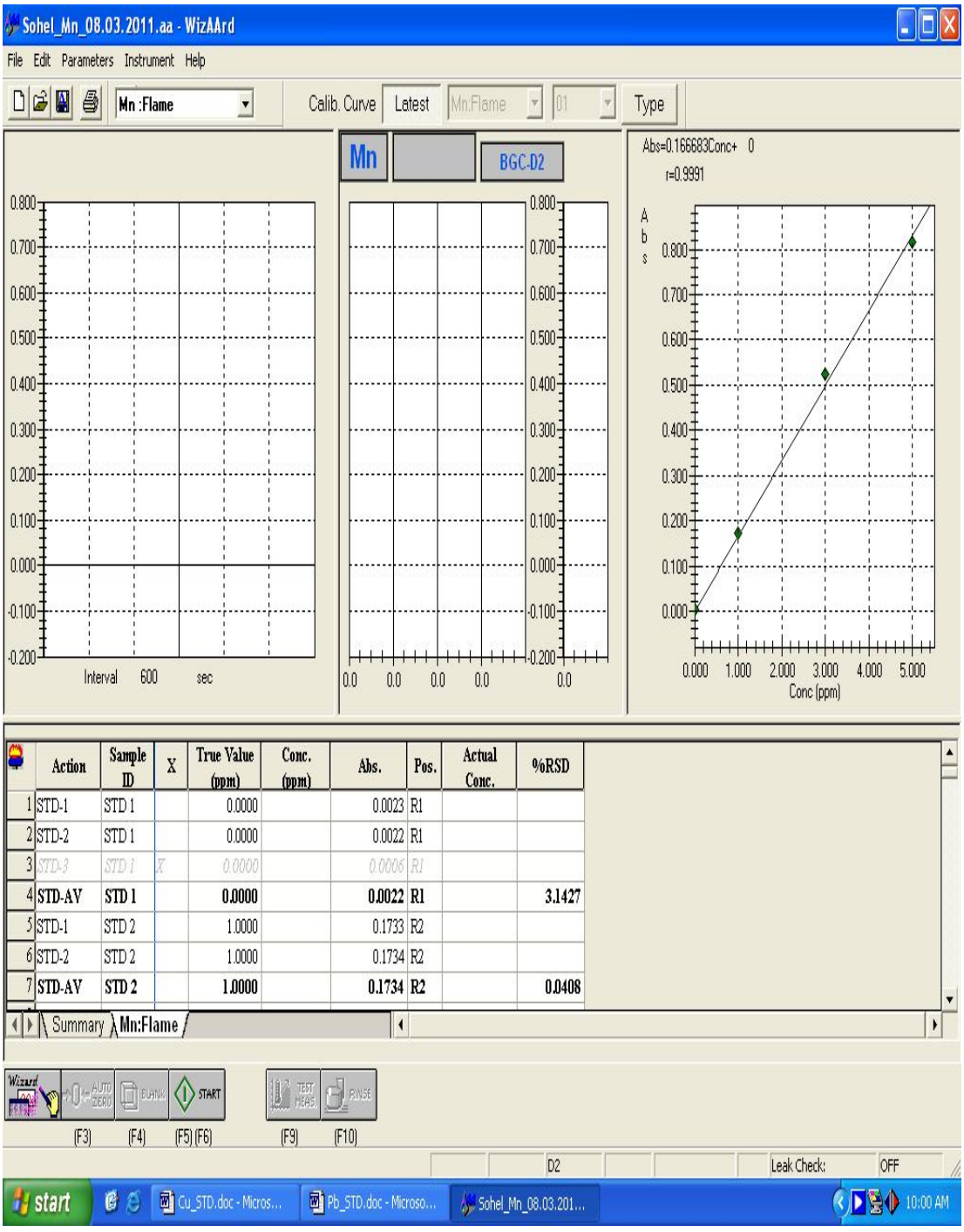


Figure C-3: Calibration of Mn in AAS with its background noise level (transported from operating software)

Appendix C

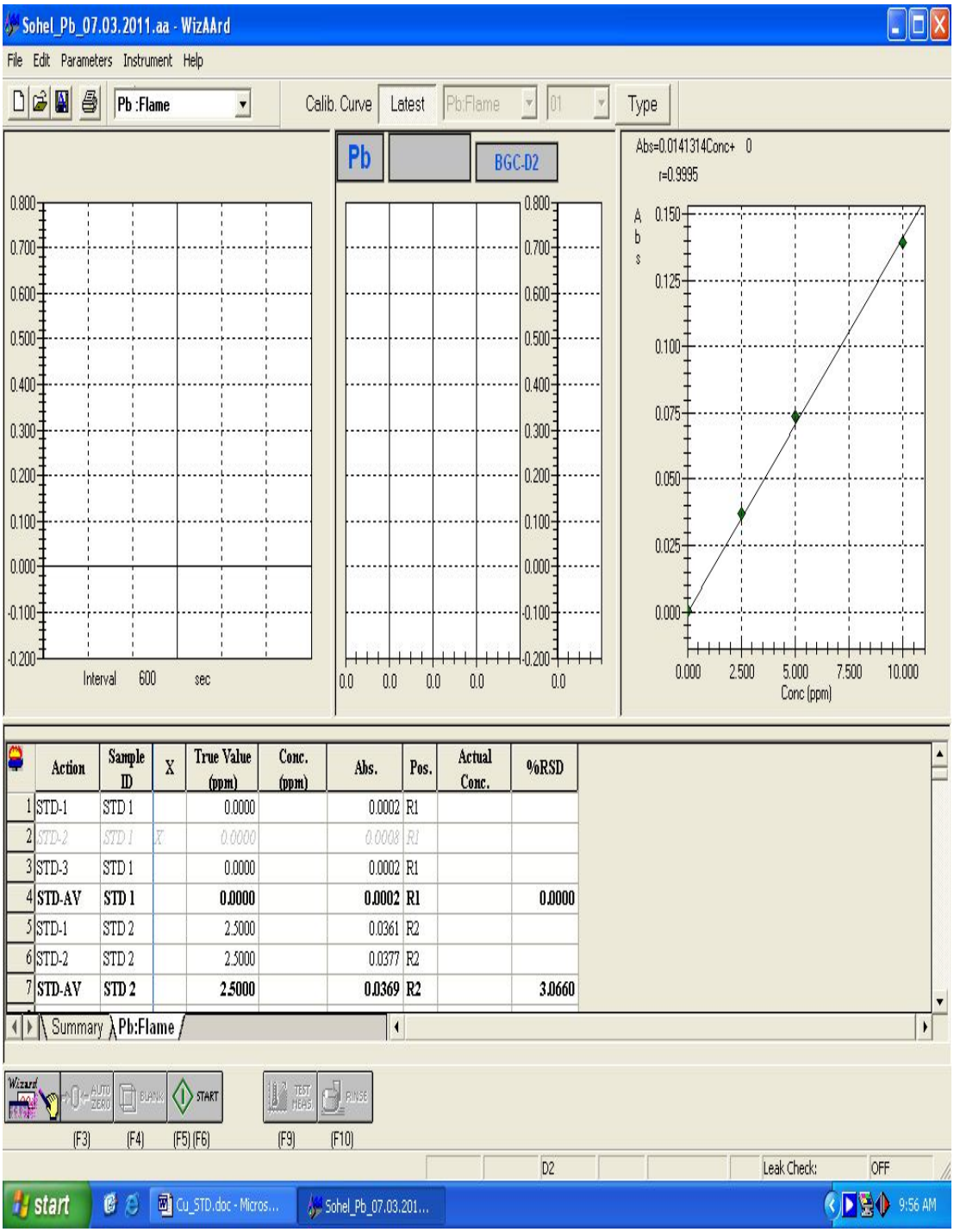


Figure C-4: Calibration of Pb in AAS with its background noise level (transported from operating software)

Appendix C

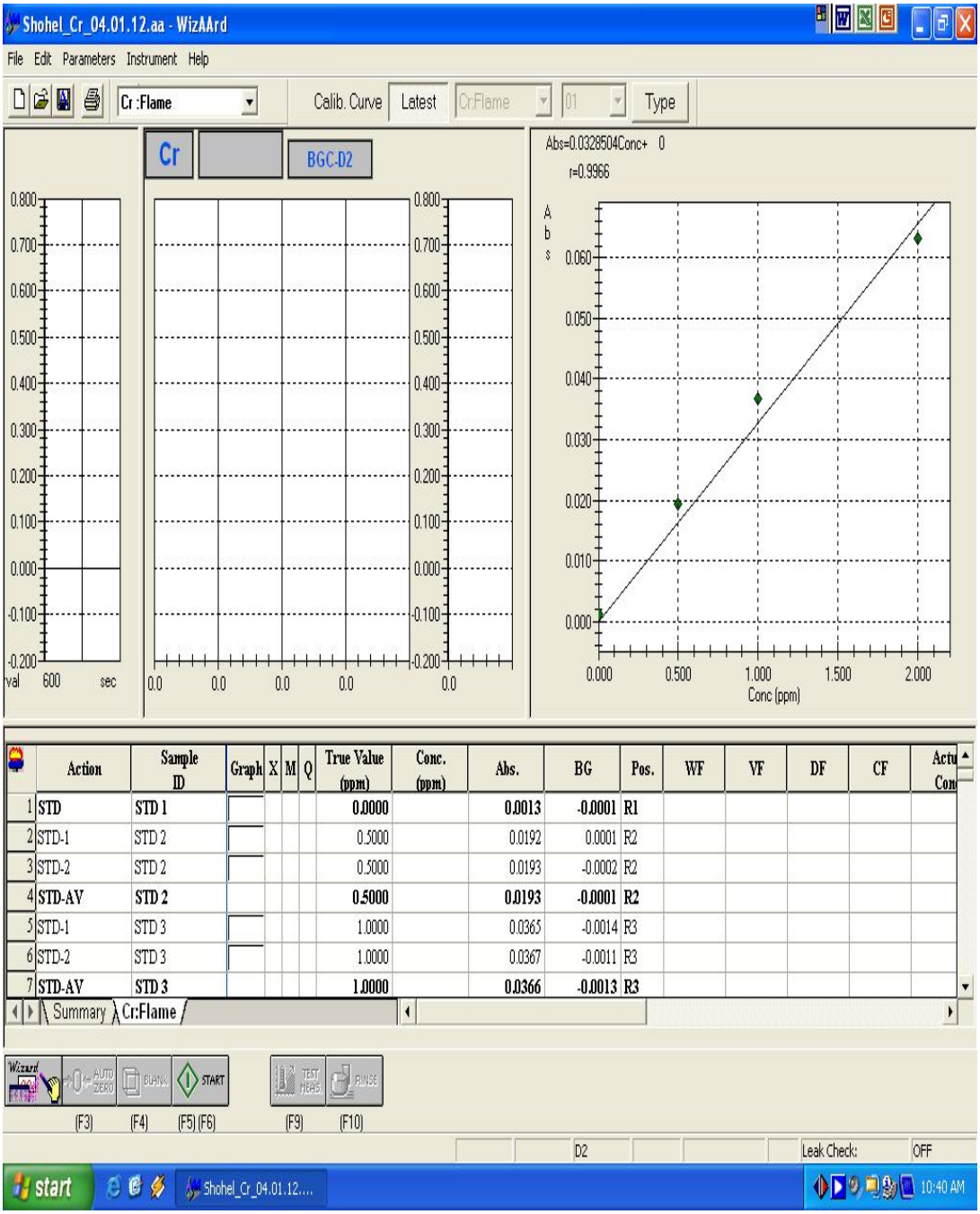
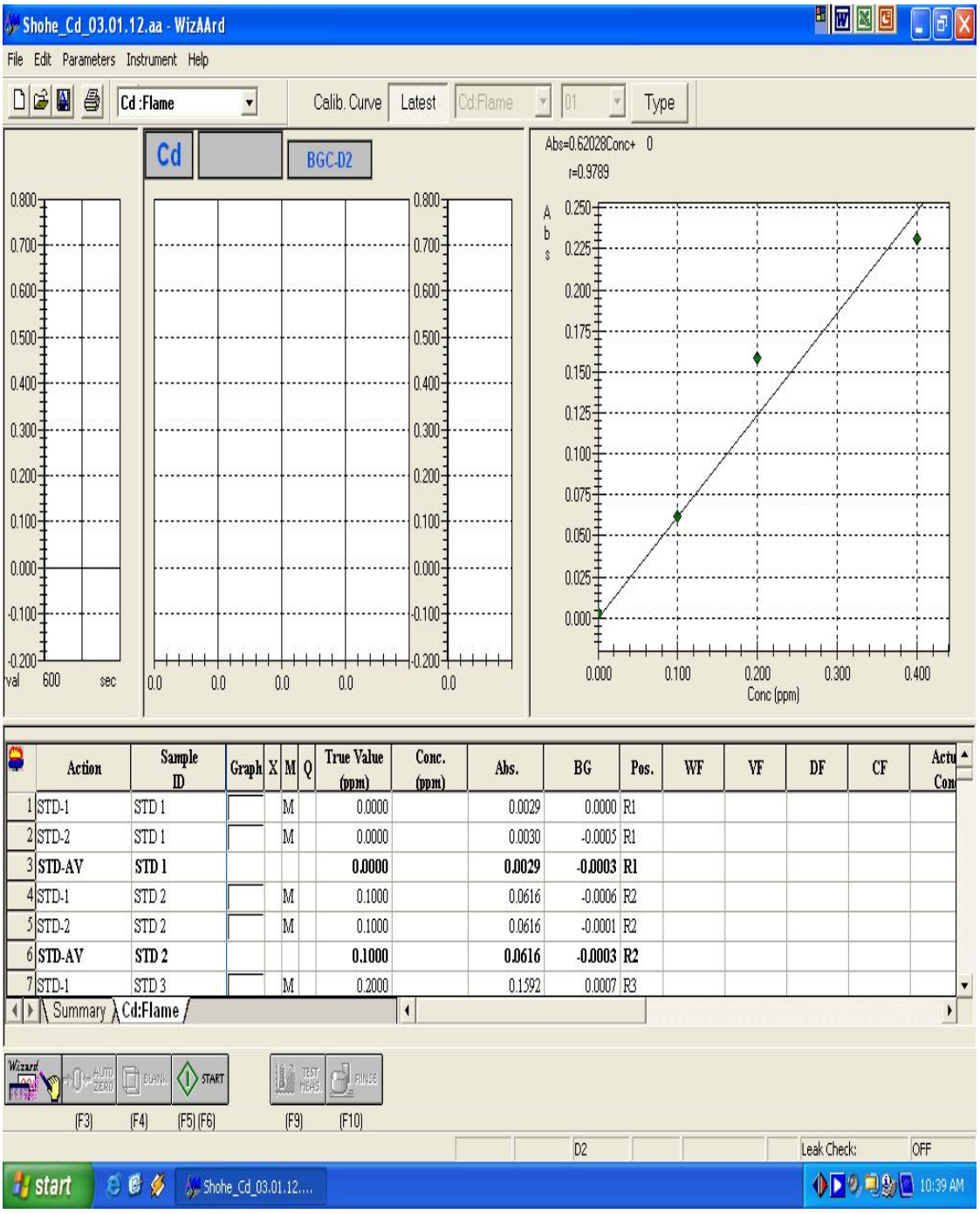


Figure C-5: Calibration of Cr in AAS with its background noise level (transported from operating software)

Appendix C



	Action	Sample ID	Graph	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Pos.	WF	VF	DF	CF	Actu Con
1	STD-1	STD 1			M		0.0000		0.0029	0.0000	R1					
2	STD-2	STD 1			M		0.0000		0.0030	-0.0005	R1					
3	**STD-AV**	**STD 1**					**0.0000**		**0.0029**	**-0.0003**	**R1**					
4	STD-1	STD 2			M		0.1000		0.0616	-0.0006	R2					
5	STD-2	STD 2			M		0.1000		0.0616	-0.0001	R2					
6	**STD-AV**	**STD 2**					**0.1000**		**0.0616**	**-0.0003**	**R2**					
7	STD-1	STD 3			M		0.2000		0.1592	0.0007	R3					

Figure C-6: Calibration of Cd in AAS with its background noise level (transported from operating software)

Appendix C

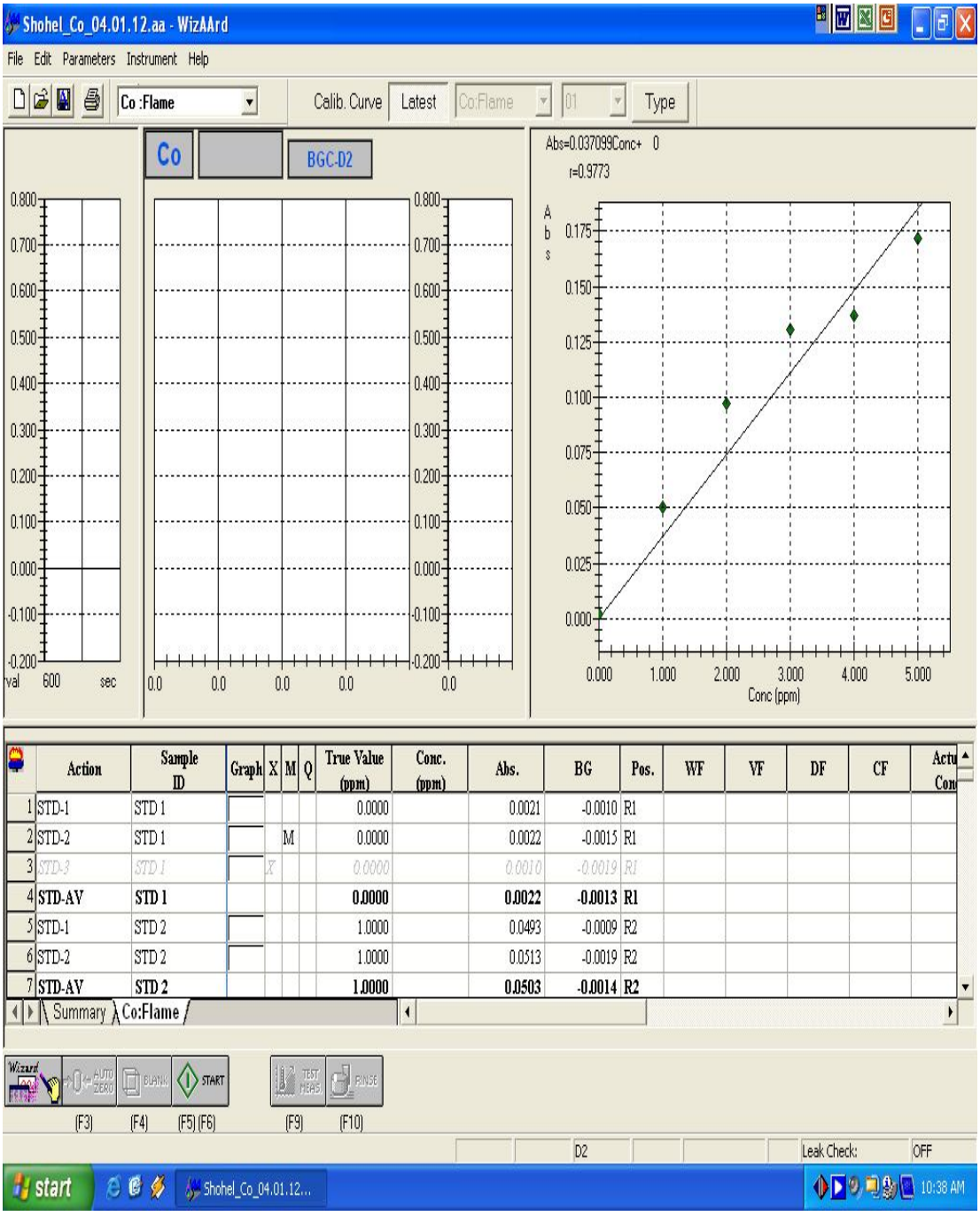
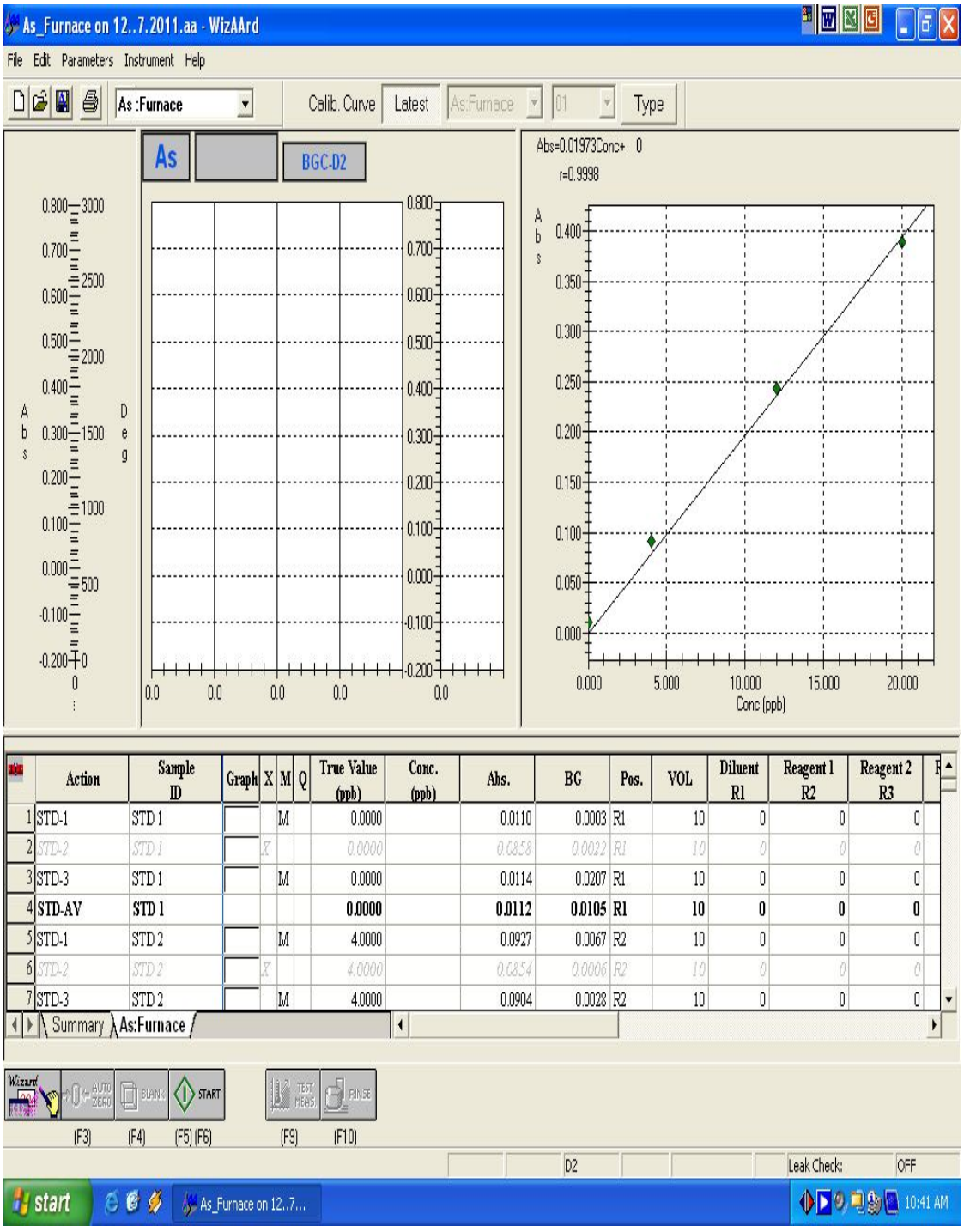


Figure C-7: Calibration of Co in AAS with its background noise level (transported from operating software

Appendix C



	Action	Sample ID	Graph	X	M	Q	True Value (ppb)	Conc. (ppb)	Abs.	BG	Pos.	VOL	Diluent R1	Reagent 1 R2	Reagent 2 R3
1	STD-1	STD 1			M		0.0000		0.0110	0.0003	R1	10	0	0	0
2	STD-2	STD 1		X			0.0000		0.0858	0.0022	R1	10	0	0	0
3	STD-3	STD 1			M		0.0000		0.0114	0.0207	R1	10	0	0	0
4	**STD-AV**	**STD 1**					**0.0000**		**0.0112**	**0.0105**	**R1**	**10**	**0**	**0**	**0**
5	STD-1	STD 2			M		4.0000		0.0927	0.0067	R2	10	0	0	0
6	STD-2	STD 2		X			4.0000		0.0854	0.0006	R2	10	0	0	0
7	STD-3	STD 2			M		4.0000		0.0904	0.0028	R2	10	0	0	0

Figure C-8: Calibration of As in AAS with its background noise level (transported from operating software

Appendix-D

Table D-1: Correlations matrix for BR (n = at least 18)

	BRTFW	BRLME	BRTMS	BRTS	BRCa	BRZn	BRMn	BRCu	BRPb
BRTFW	1	.152	.152	.799*	.881*	-.061	.740*	.722	-.655
BRLME		1	1.000**	.189	.029	-.148	-.152	-.151	.329
BRTMS			1	.189	.029	-.148	-.152	-.151	.329
BRTS				1	.432	.169	.209	.181	-.151
BRCa					1	-.244	.939**	.934**	-.873*
BRZn						1	-.178	-.220	.392
BRMn							1	.999**	-.942**
BRCu								1	-.952**
BRPb									1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-2: Correlations matrix for BP (n = at least 18)

	BPTFW	BPLME	BPTMS	BPTS	BPCa	BPZn	BPMn	BPCu	BPPb
BPTFW	1	.698	.698	-.316	.837*	-.166	.698	.697	-.615
BPLME		1	1.000**	-.849*	.939**	-.532	.823*	.819*	-.873*
BPTMS			1	-.849*	.939**	-.532	.823*	.819*	-.873*
BPTS				1	-.711	.751*	-.777*	-.774*	.860*
BPCa					1	-.516	.904**	.902**	-.874*
BPZn						1	-.783*	-.782*	.850*
BPMn							1	1.000**	-.962**
BPCu								1	-.960**
BPPb									1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-3: Correlations matrix for MBR (n = at least 18)

	MBRTFW	MBRLME	MBRTMS	MBRTS	MBRCa	MBRZn	MBRMn	MBRCu	MBRPb
MBRTFW	1	.424	.424	.936**	.912**	.825*	.794*	.773*	-.490
MBRLME		1	1.000**	.124	.242	.117	-.083	-.107	.387
MBRTMS			1	.124	.242	.117	-.083	-.107	.387
MBRTS				1	.970**	.781*	.940**	.929**	-.708
MBRCa					1	.668	.918**	.907**	-.667
MBRZn						1	.624	.598	-.446
MBRMn							1	.999**	-.894**
MBRCu								1	-.904**
MBRPb									1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-4: Correlations Matrix for MBP (n = at least 18)

	MBPTFW	MBPLME	MBPTMS	MBPTS	MBPCa	MBPZn	MBPMn	MBPCu	MBPPb
MBPTFW	1	.314	.314	.218	-.765*	-.259	.161	.133	.161
MBPLME		1	1.000**	-.698	.269	.497	.540	.539	-.182
MBPTMS			1	-.698	.269	.497	.540	.539	-.182
MBPTS				1	-.631	-.901**	-.842*	-.854*	.752*
MBPCa					1	.589	.205	.237	-.325
MBPZn						1	.808*	.819*	-.756*
MBPMn							1	.999**	-.899**
MBPCu								1	-.907**
MBPPb									1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-5: Correlations matrix for MVR (n = at least 18)

	MVRTFW	MVRLME	MVRTMS	MVRTS	MVRCa	MVRZn	MVRMn	MVRCu	MVRPb	MVRCr	MVRCd	MVRCo	MVRAs
MVRTFW	1	-.312	-.312	.926**	-.076	-.254	.735*	.735*	.001	.912**	.629	.568	-.442
MVRLME		1	1.000**	-.294	.507	.889**	-.813*	-.813*	.930**	-.535	-.862*	-.755*	.164
MVRTMS			1	-.294	.507	.889**	-.813*	-.813*	.930**	-.535	-.862*	-.755*	.164
MVRTS				1	-.266	-.195	.577	.577	.018	.867*	.479	.402	-.856
MVRCa					1	.130	-.203	-.203	.563	-.443	-.379	-.373	.232
MVRZn						1	-.745*	-.745*	.819*	-.327	-.731*	-.521	.575
MVRMn							1	1.000**	-.607	.809*	.967**	.893**	.108
MVRCu								1	-.607	.809*	.967**	.893**	.108
MVRPb									1	-.293	-.730*	-.607	.064
MVRCr										1	.796*	.755*	-.417
MVRCd											1	.918**	.950
MVRCo												1	.999*
MVRAs													1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-6: Correlations matrix for MVP (n = at least 18)

	MVPTFW	MVPLME	MVPTMS	MVPTS	MVPCa	MVPZn	MVPMn	MVPCu	MVPPb	MVPCr	MVPCd	MVPCo	MVPAs
MVPTFW	1	.156	.156	-.002	.181	-.205	.285	.285	.599	.228	.514	.365	-.720
MVPLME		1	1.000**	-.987**	.961**	-.983**	.973**	.973**	-.187	.709	.914**	.855*	-.910
MVPTMS			1	-.987**	.961**	-.983**	.973**	.973**	-.187	.709	.914**	.855*	-.910
MVPTS				1	-.939**	.968**	-.945**	-.945**	.292	-.699	-.849*	-.801*	.149
MVPCa					1	-.948**	.939**	.939**	-.243	.506	.860*	.955**	-.607
MVPZn						1	-.996**	-.996**	.254	-.740*	-.934**	-.863*	-.501
MVPMn							1	1.000**	-.212	.750*	.958**	.872*	.633
MVPCu								1	-.212	.750*	.958**	.872*	.633
MVPPb									1	-.026	.032	-.190	-.962
MVPCr										1	.790*	.350	.662
MVPCd											1	.825*	-.547
MVPCo												1	-.617
MVPAs													1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-7: Correlations matrix for MBzR (n = at least 18)

	MBzRTFW	MBzRLME	MBzRTMS	MBzRTS	MBzRCa	MBzRZn	MBzRMn	MBzRCu	MBzRPb	MBzRCr	MBzRCd	MBzRCo	MBzRAs
MBzRTFW	1	.152	.152	.960**	.626	.096	.870*	.870*	-.533	.894**	.742*	.793*	.548
MBzRLME		1	1.000**	-.116	.646	.757*	-.008	-.008	-.317	.082	-.391	-.091	-.227
MBzRTMS			1	-.116	.646	.757*	-.008	-.008	-.317	.082	-.391	-.091	-.227
MBzRTS				1	.432	-.152	.907**	.907**	-.468	.895**	.886**	.812*	.670
MBzRCa					1	.440	.385	.385	-.783*	.397	.051	.572	.641
MBzRZn						1	-.297	-.297	.045	-.126	-.521	-.292	.622
MBzRMn							1	1.000**	-.458	.972**	.893**	.826*	-.418
MBzRCu								1	-.458	.972**	.893**	.826*	-.418
MBzRPb									1	-.326	-.312	-.530	.184
MBzRCr										1	.823*	.796*	-.148
MBzRCd											1	.671	-.317
MBzRCo												1	.326
MBzRAs													1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Table D-8: Correlations matrix for MBzP (n = at least 18)

	MBzPTFW	MBzPLME	MBzPTMS	MBzPTS	MBzPCa	MBzPZn	MBzPMn	MBzPCu	MBzPPb	MBzPCr	MBzPCd	MBzPCo	MBzPAs
MBzPTFW	1	-.436	-.436	.732*	.938**	.959**	.944**	.944**	.621	-.585	-.952**	-.327	.762
MBzPLME		1	1.000**	-.928**	-.604	-.439	-.438	-.438	-.572	.029	.399	.469	-.384
MBzPTMS			1	-.928**	-.604	-.439	-.438	-.438	-.572	.029	.399	.469	-.384
MBzPTS				1	.821*	.741*	.738*	.738*	.640	-.256	-.705	-.505	.401
MBzPCa					1	.856*	.851*	.851*	.590	-.320	-.897**	-.272	.645
MBzPZn						1	.987**	.987**	.504	-.576	-.967**	-.447	.745
MBzPMn							1	1.000**	.442	-.550	-.982**	-.329	-.994*
MBzPCu								1	.442	-.550	-.982**	-.329	-.994*
MBzPPb									1	-.681	-.377	-.404	.624
MBzPCr										1	.441	.186	.791
MBzPCd											1	.258	.842
MBzPCo												1	-1.000**
MBzPAs													1

***Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level**

Appendix – E1

Regressions for BR

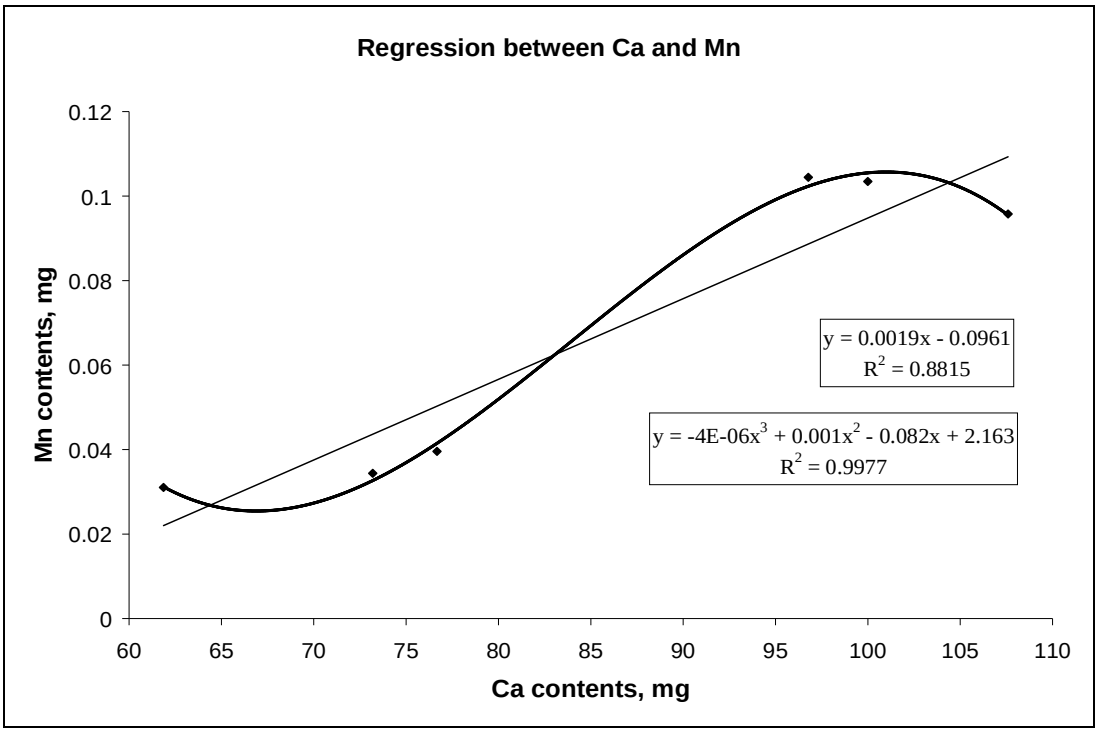


Figure E-1.1: Regression between Ca and Mn contents for R from the source B

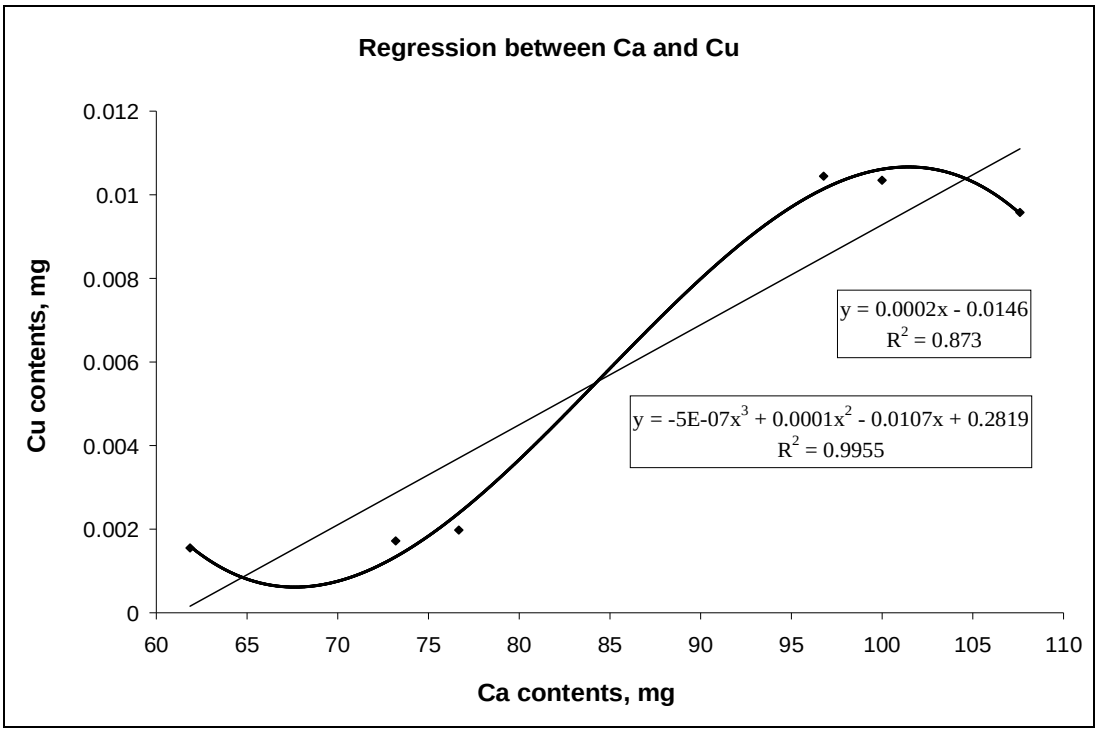


Figure E-1.2: Regression between Ca and Cu contents for R from the source B

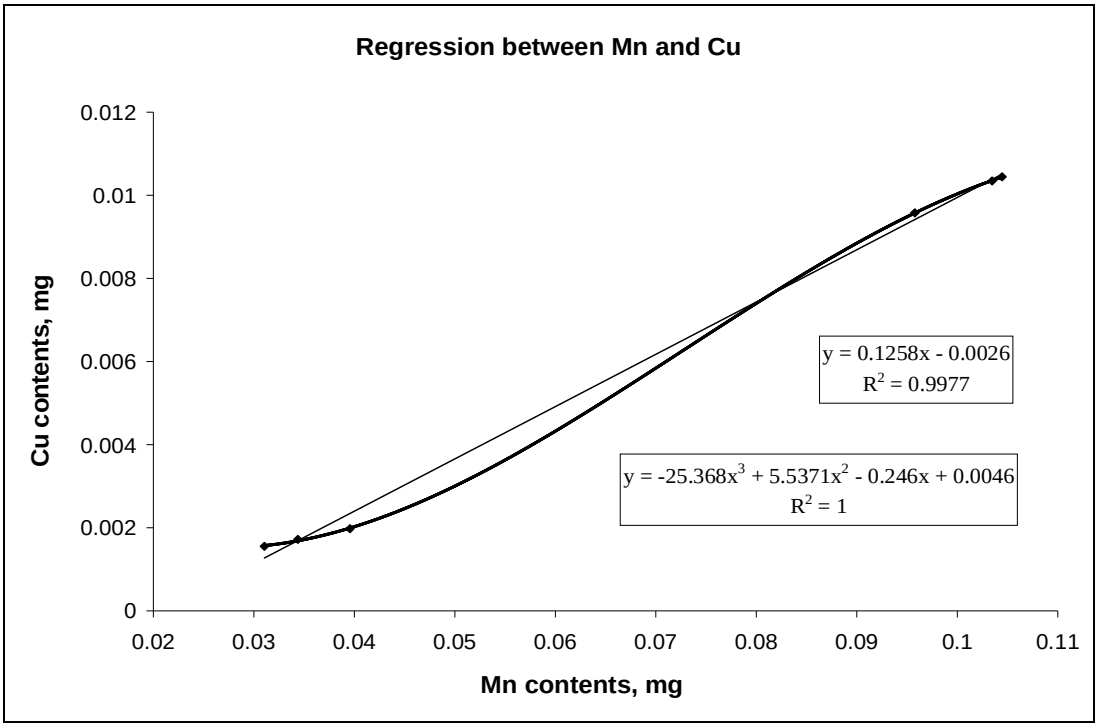


Figure E-1.3: Regression between Mn and Cu contents for R from the B source

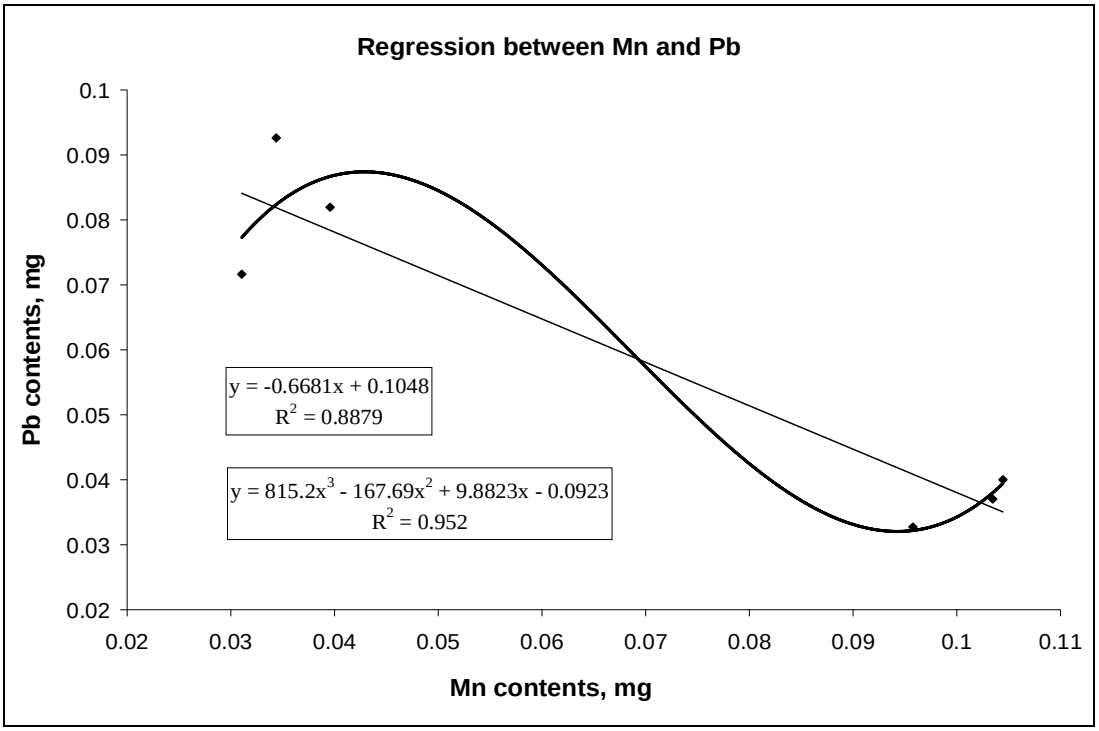


Figure E-1.4: Regression between Mn and Pb contents for R from the B source

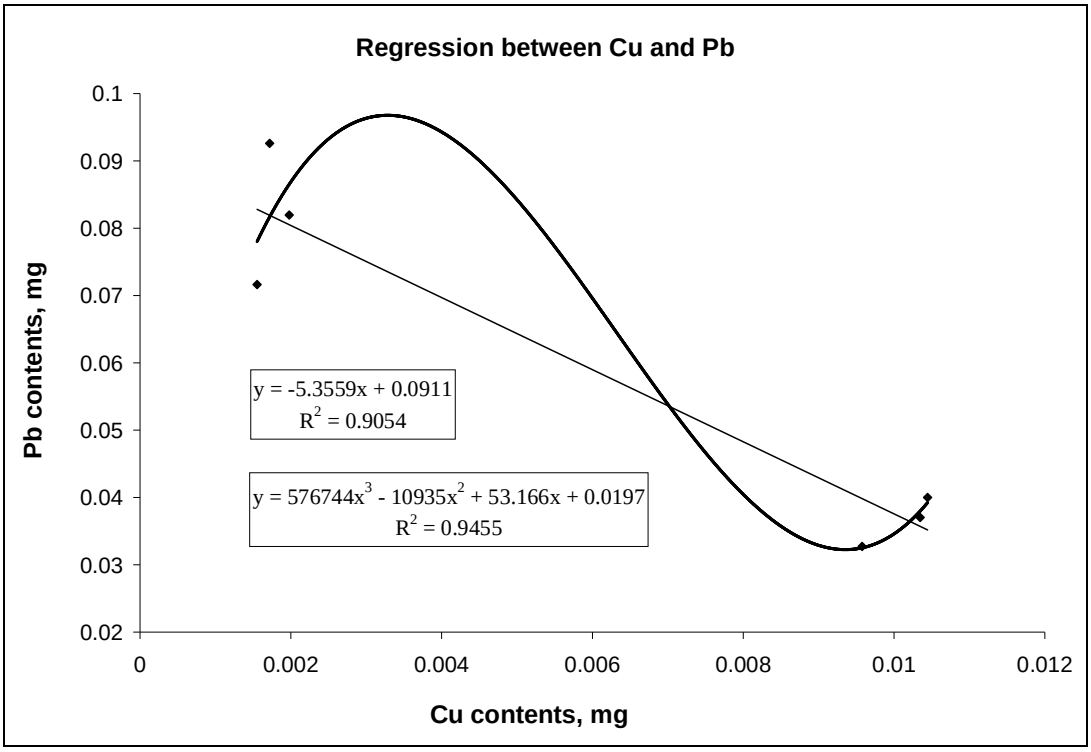


Figure E-1.5: Regression between Cu and Pb contents for R from the B source

Regressions for BP

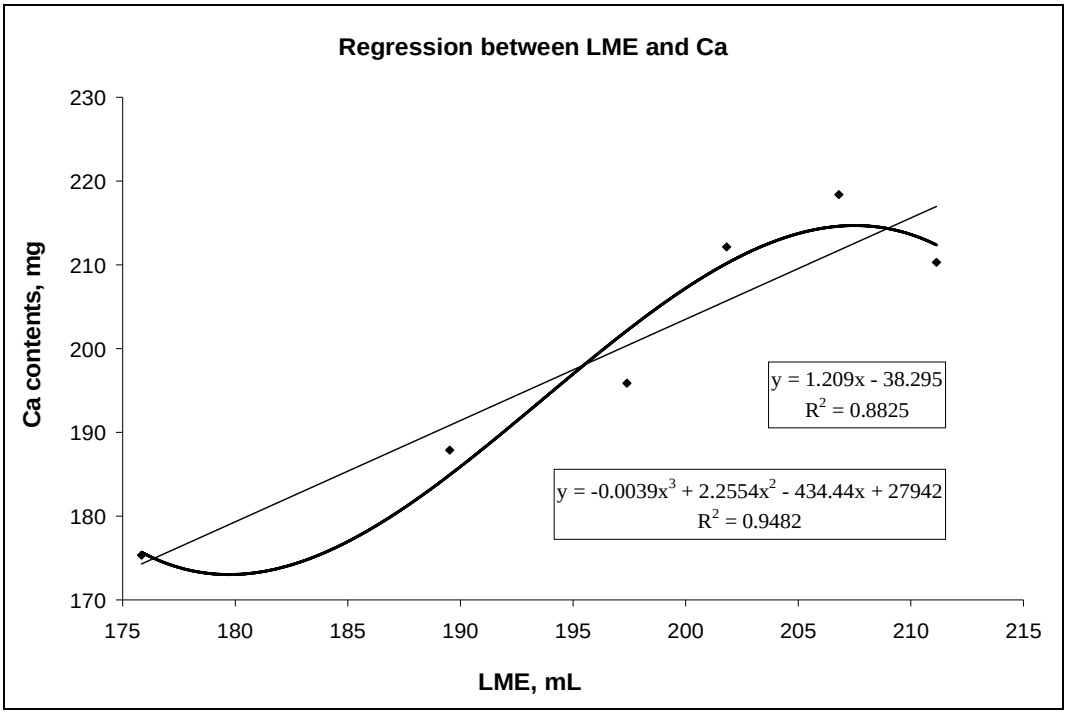


Figure E-2.1: Regression between LME and Ca contents for P from the B source

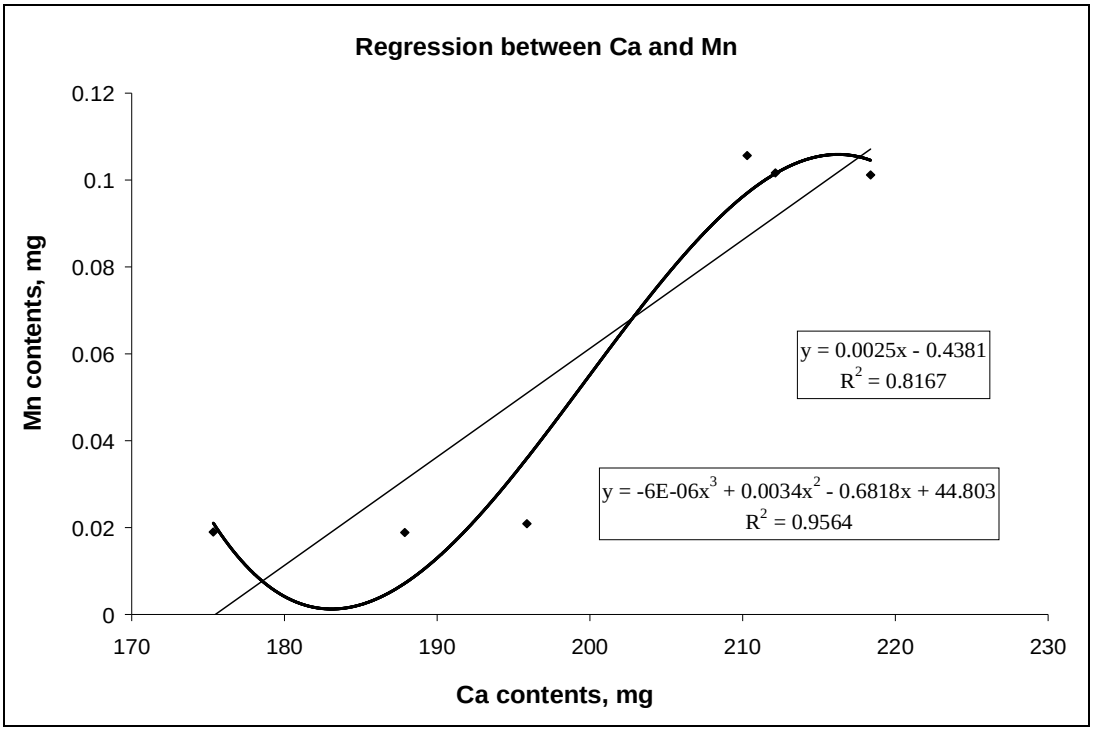


Figure E-2.2: Regression between Ca and Mn contents for P from the B source

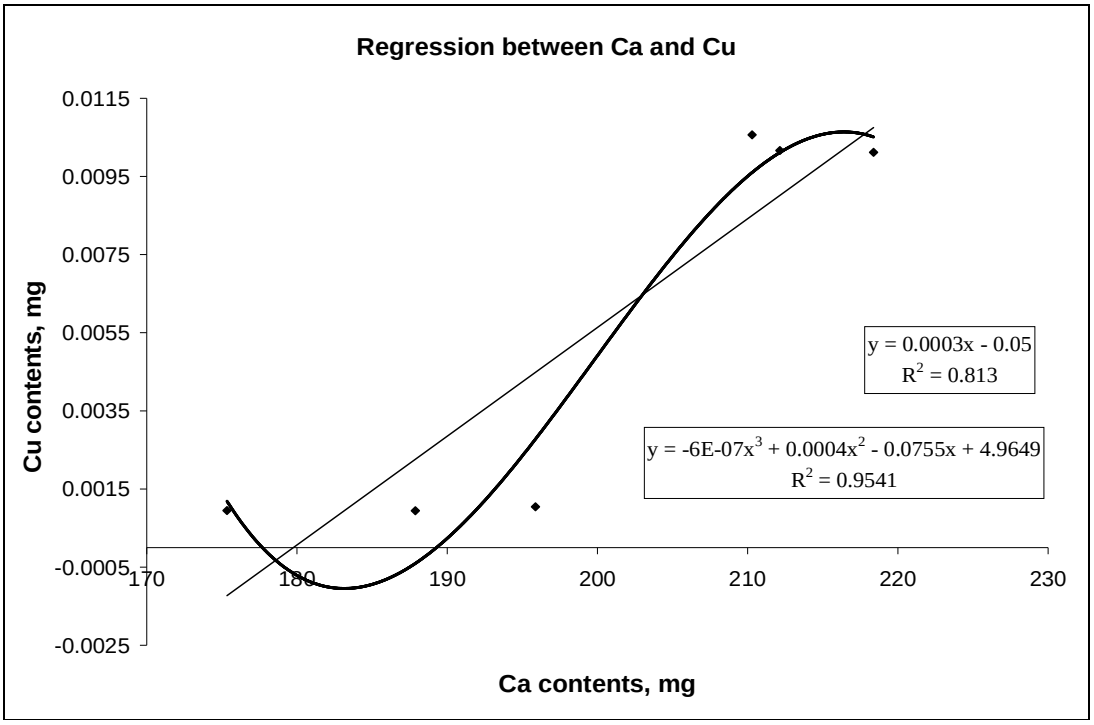


Figure E-2.3: Regression between Ca and Cu contents for P from the B source

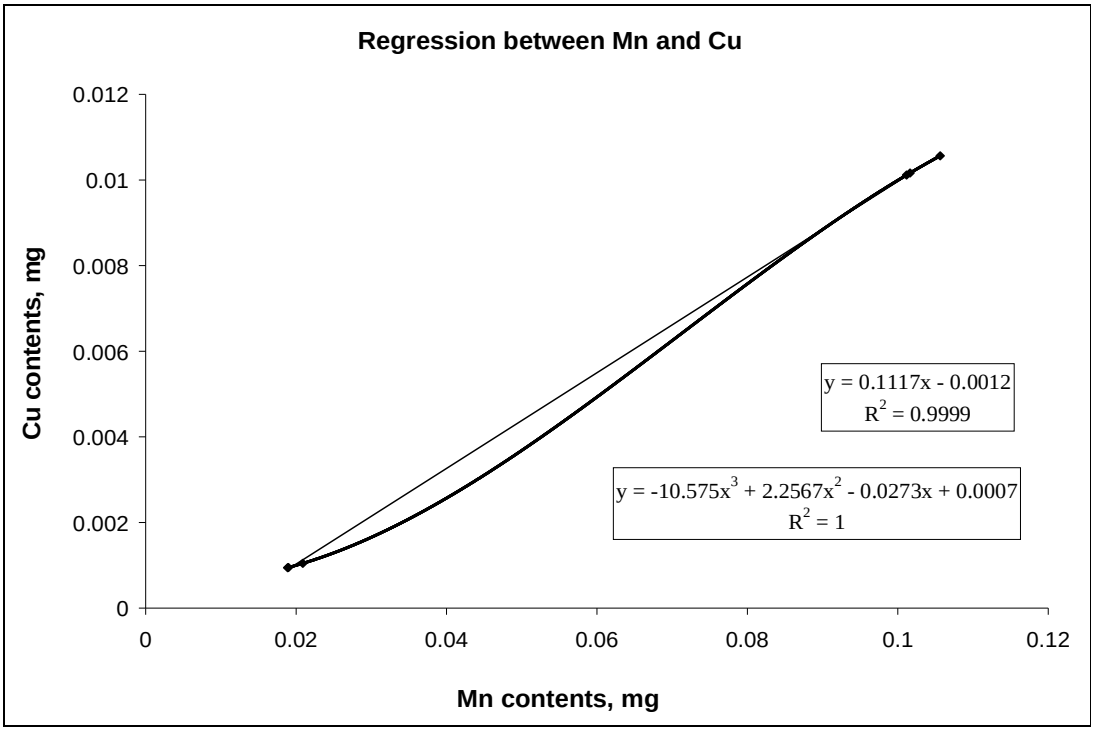


Figure E-2.4: Regression between Mn and Cu contents for P from the B source

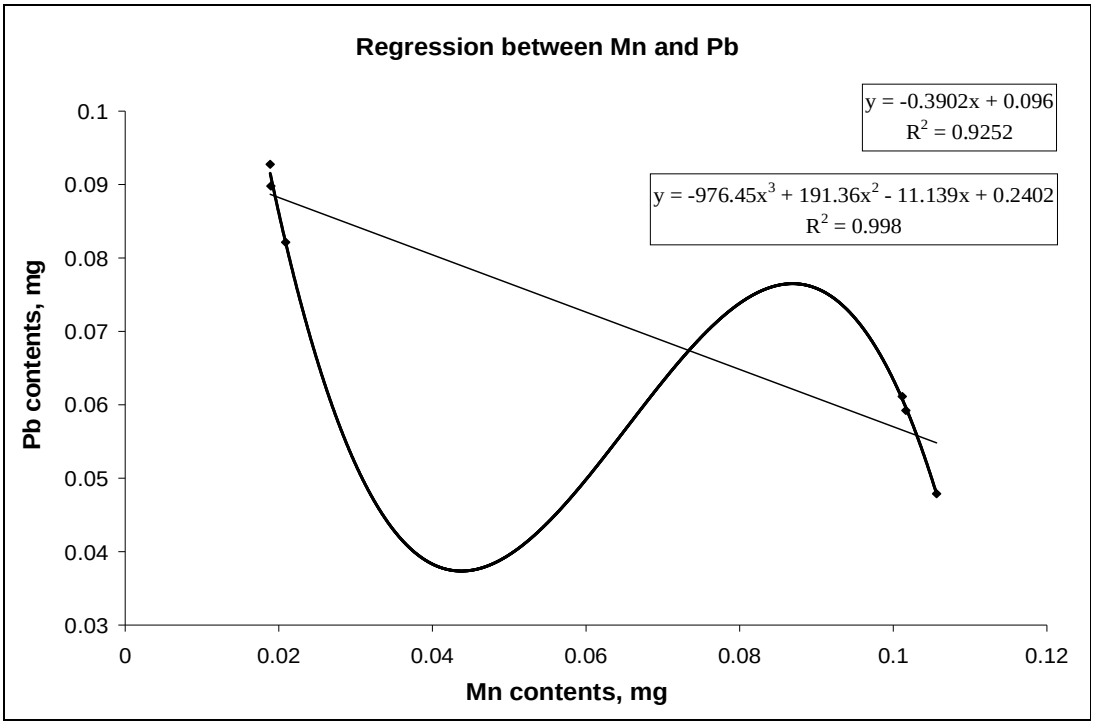


Figure E-2.5: Regression between Mn and Pb contents for P from the B source

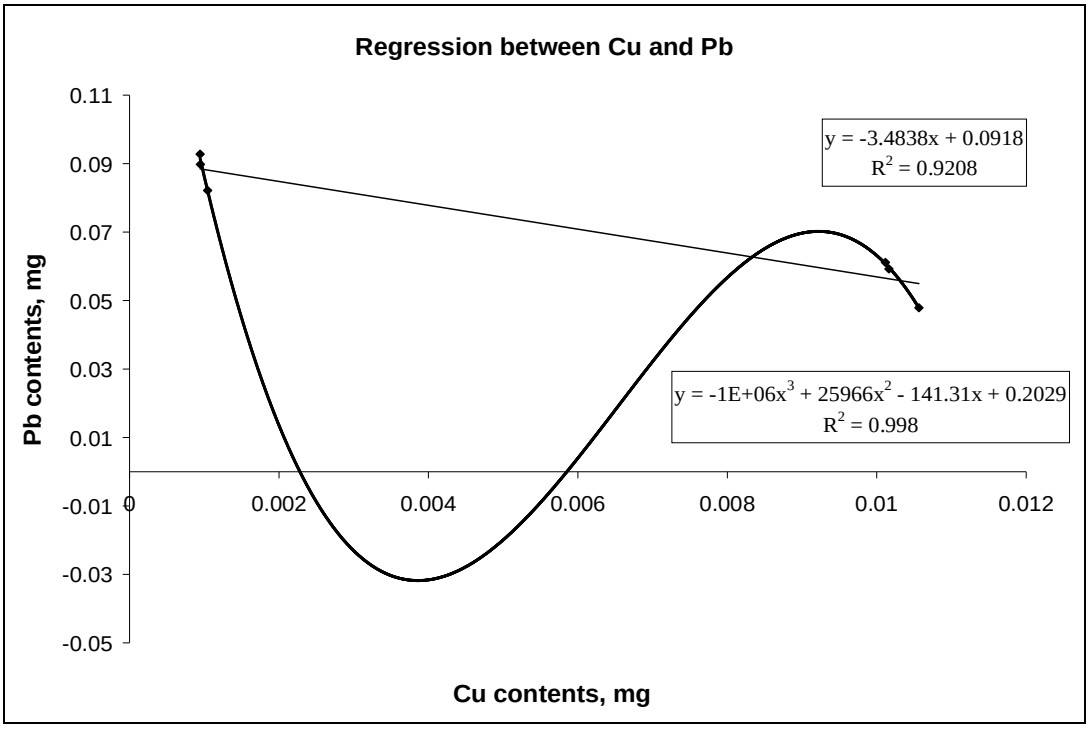


Figure E-2.6: Regression between Cu and Pb contents for P from the B source

Regressions for MBR

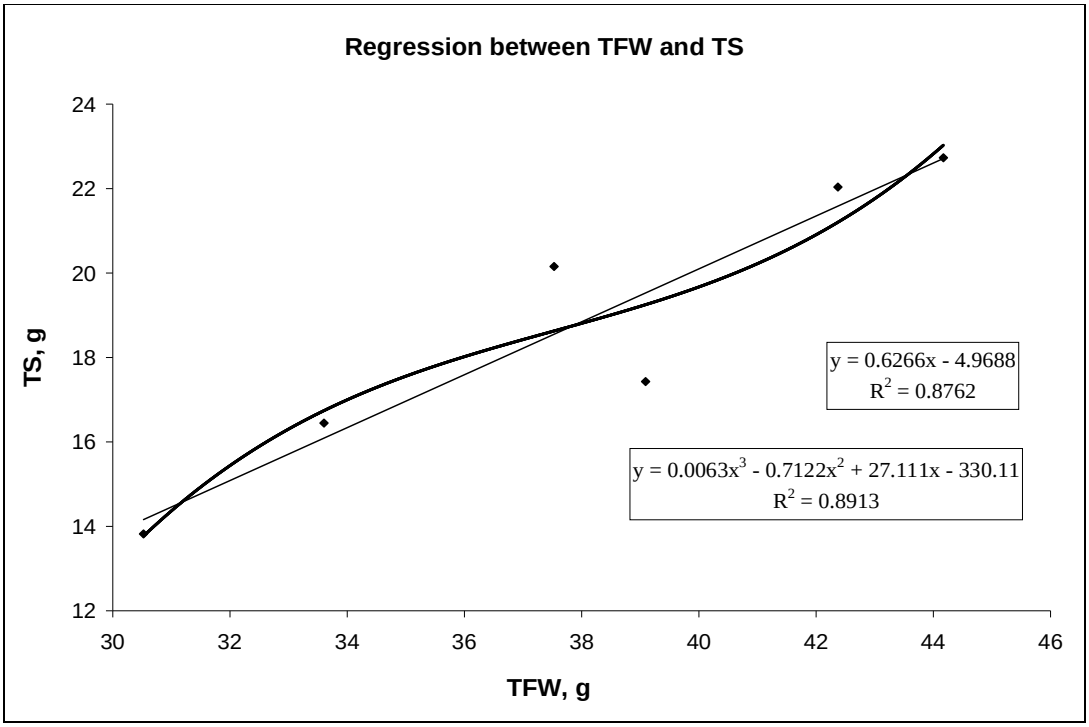


Figure E-3.1: Regression between TFW and TS contents for R from the MB source

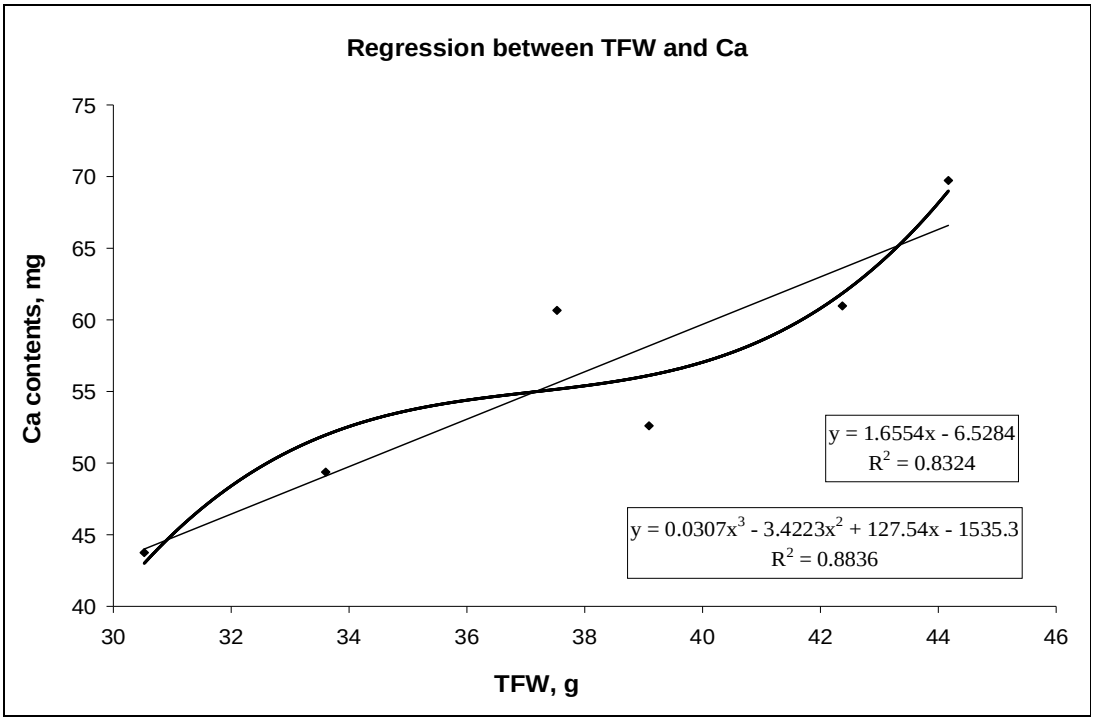


Figure E-3.2: Regression between TFW and Ca contents for R from the MB source

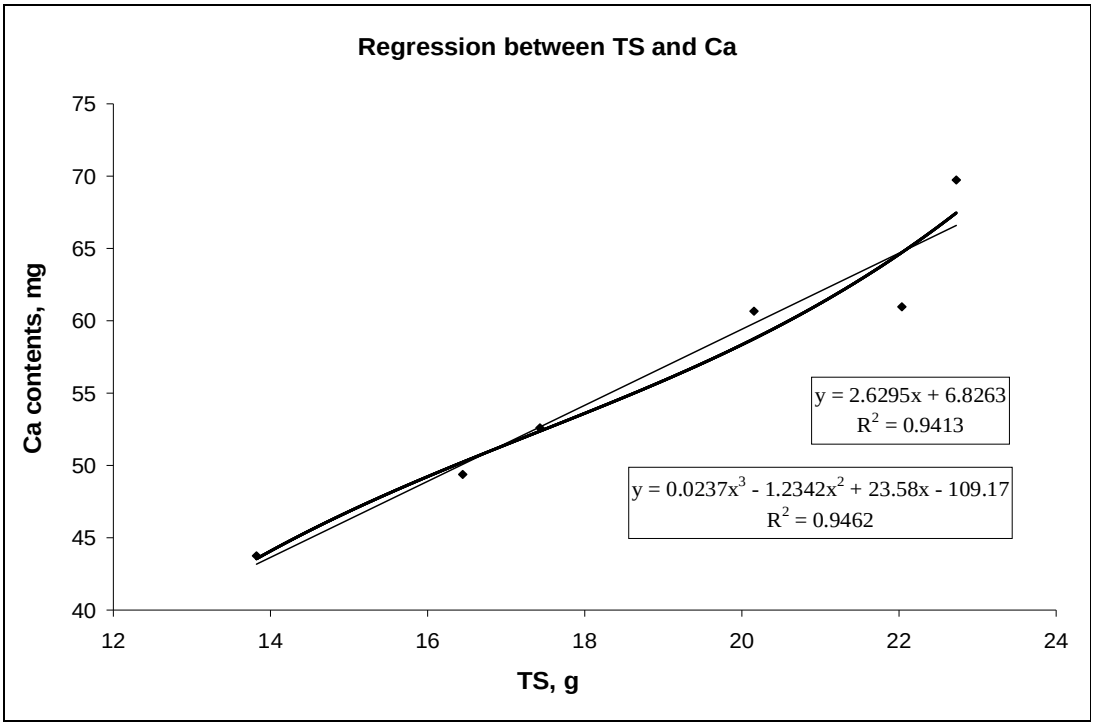


Figure E-3.3: Regression between TS and Ca contents for R from the MB source

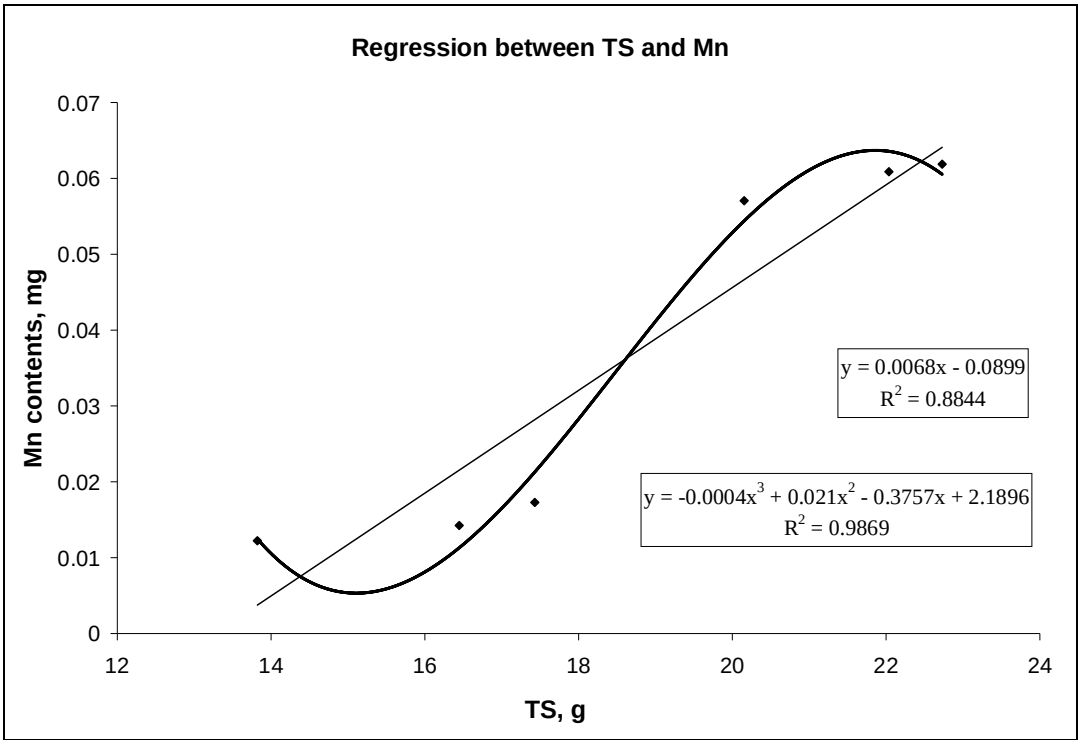


Figure E-3.4: Regression between TS and M contents for R from the MB source

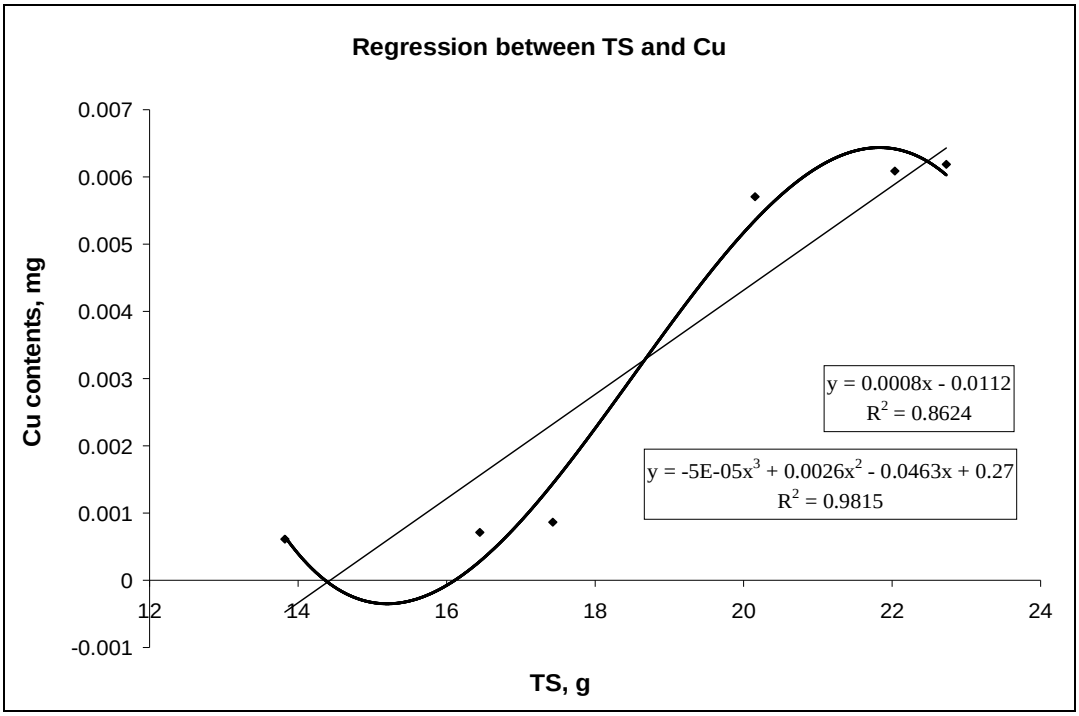


Figure E-3.5: Regression between TS and Cu contents for R from the MB source

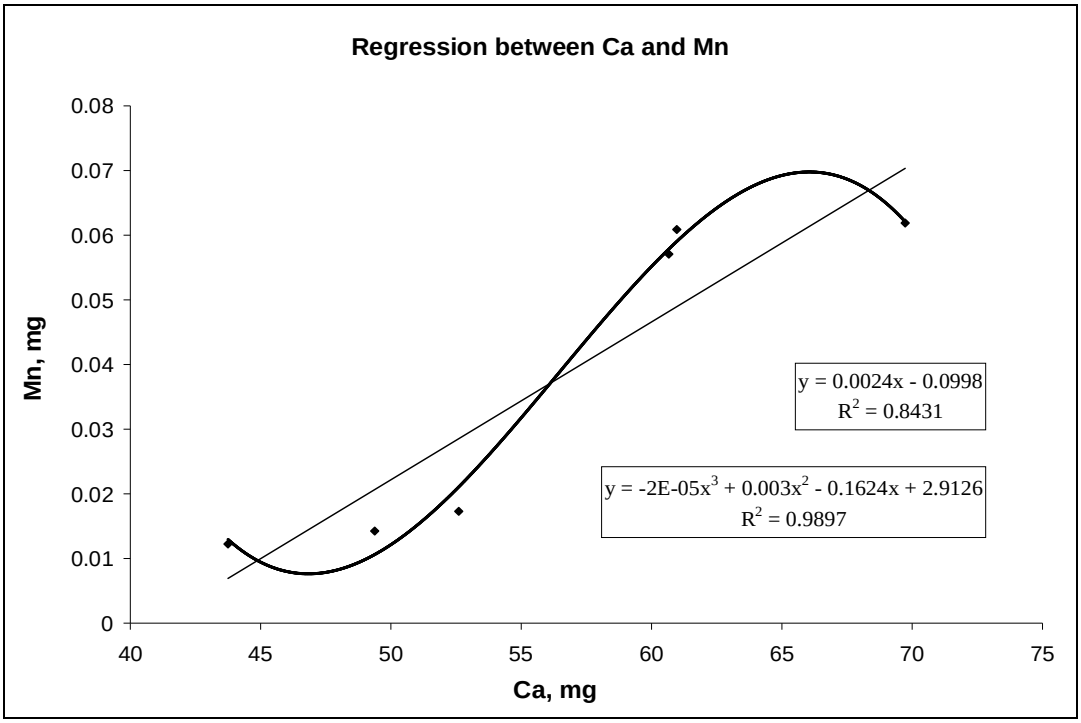


Figure E-3.6: Regression between Ca and Mn contents for R from the MB source

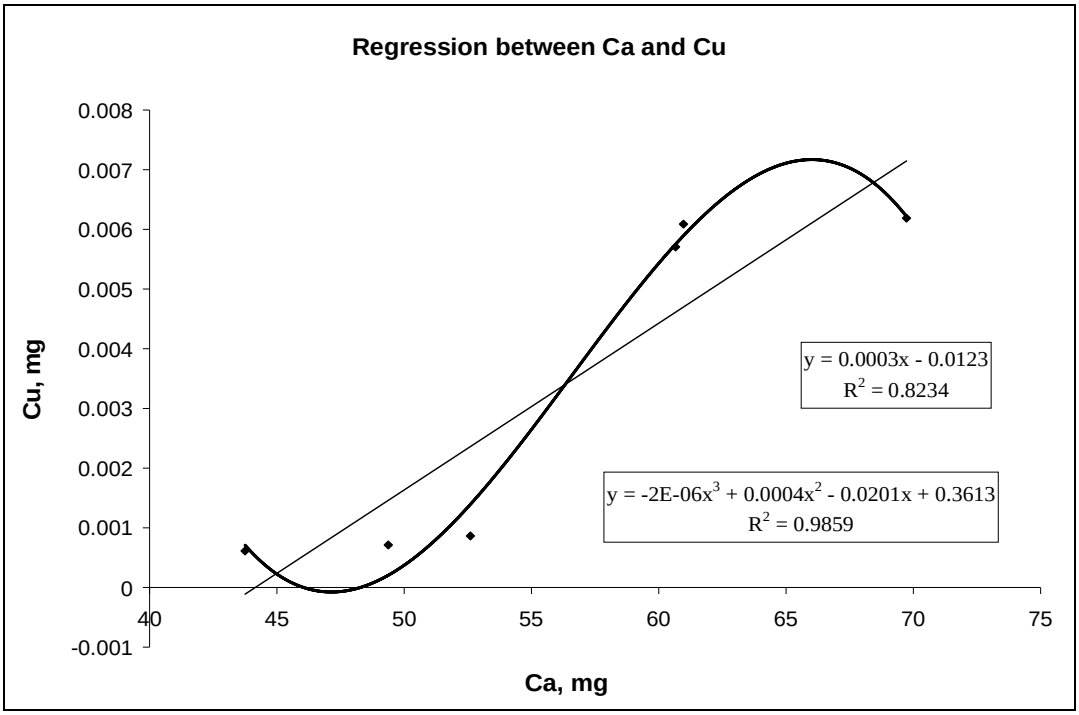


Figure E-3.7: Regression between Ca and Cu contents for R from the MB source

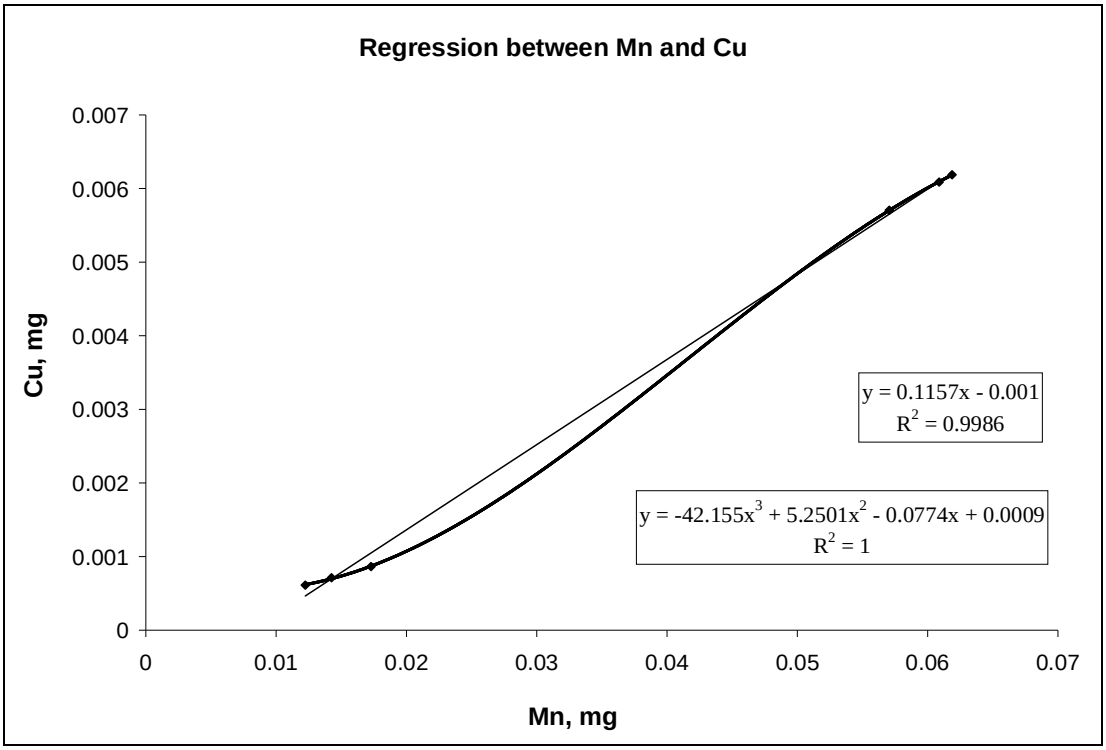


Figure E-3.8: Regression between Mn and Cu contents for R from the MB source

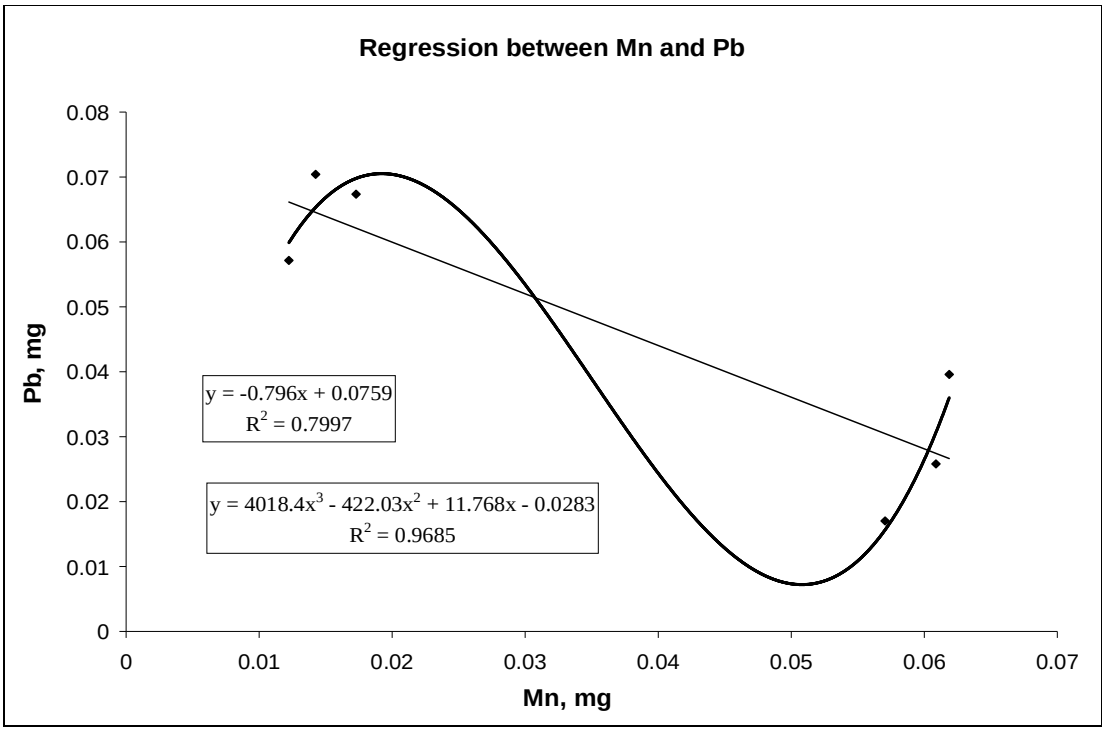


Figure E-3.9: Regression between Mn and Pb contents for R from MB source

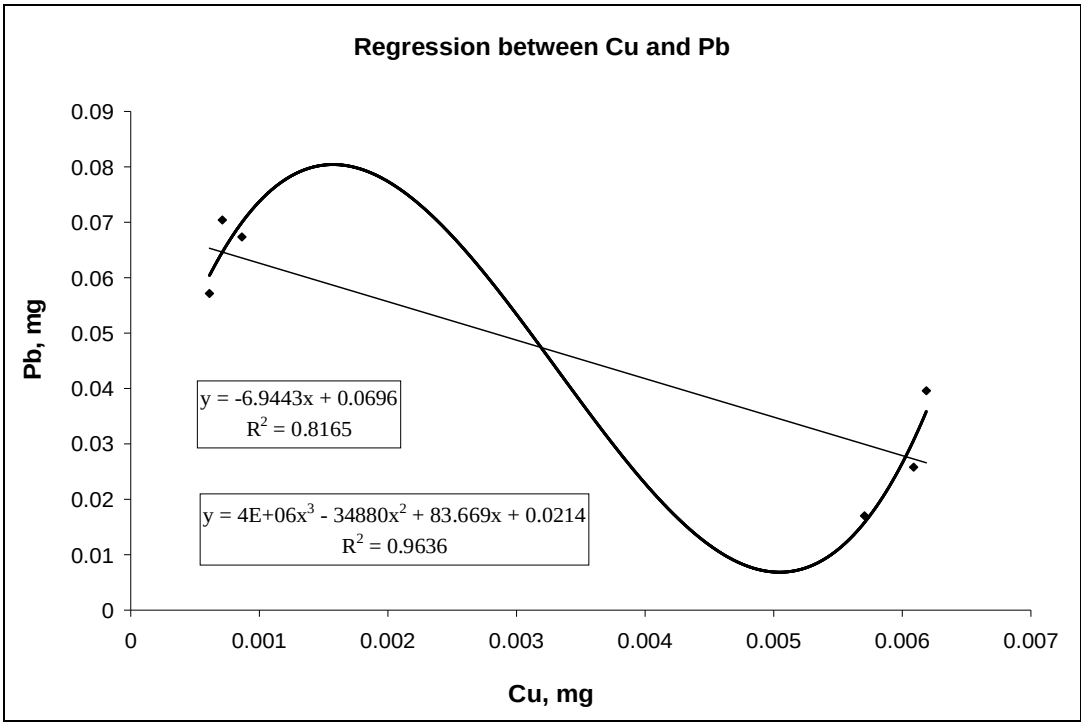


Figure E-3.10: Regression between Cu and Pb contents for R from the MB source

Regressions for MBP

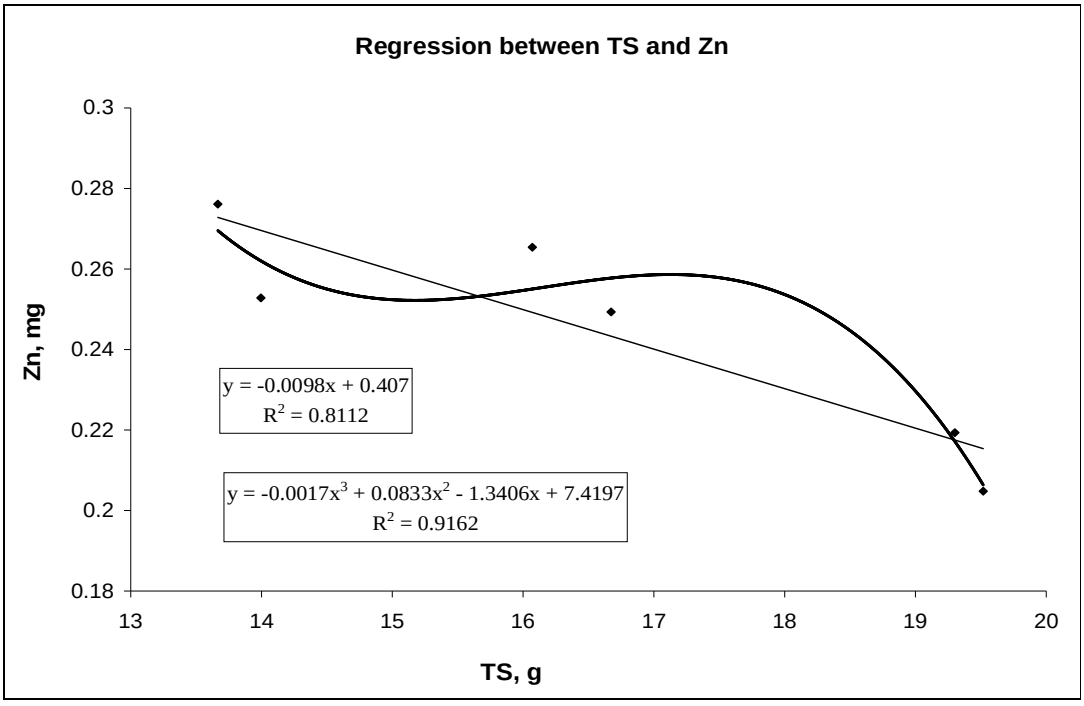


Figure E-4.1: Regression between TS and Zn contents for P from the MB source

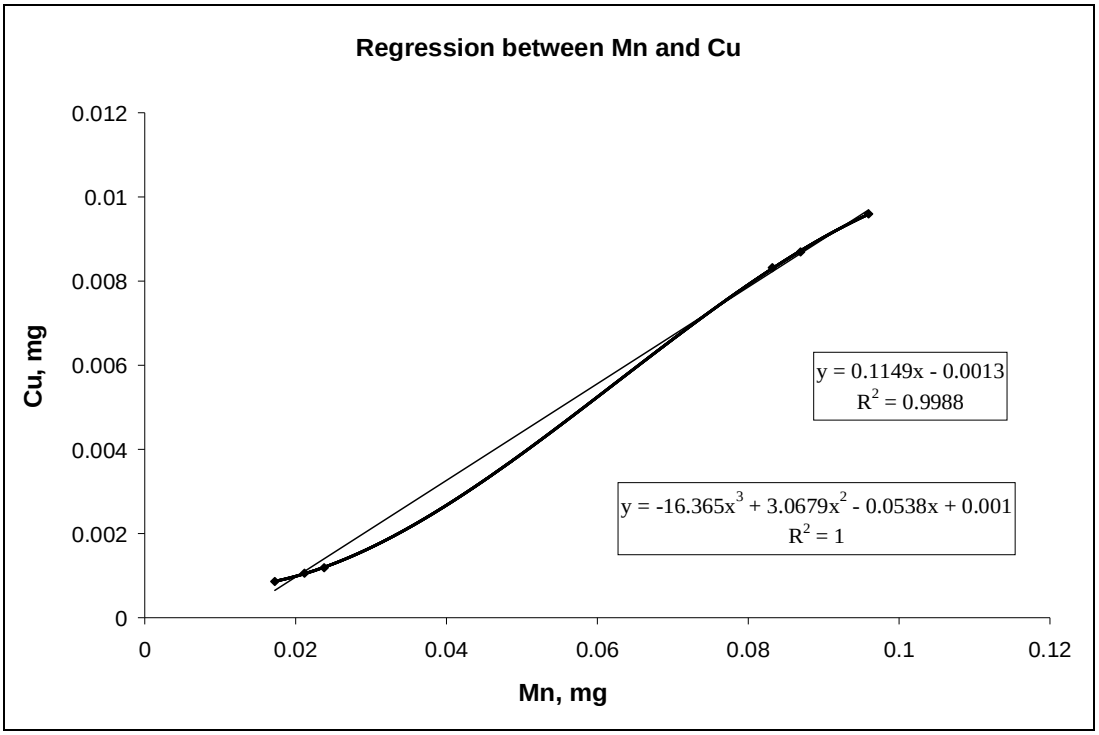


Figure E-4.2: Regression between Mn and Cu contents for P from the MB source

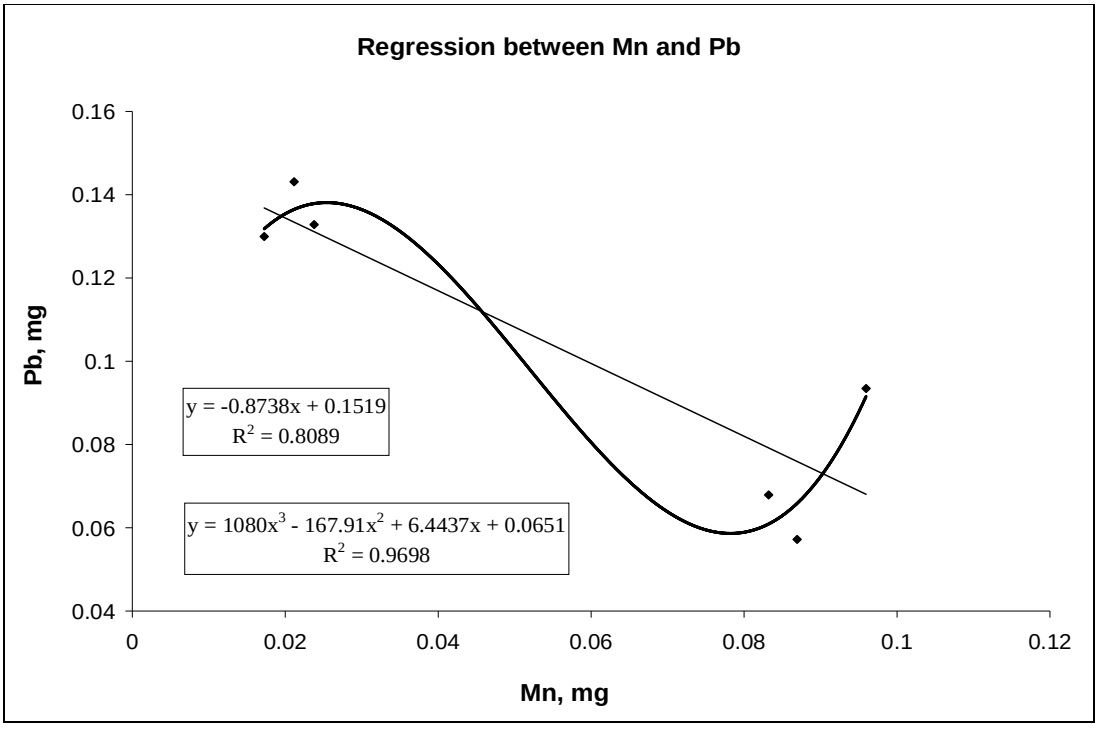


Figure E-4.3: Regression between Mn and Pb contents for P from the MB source

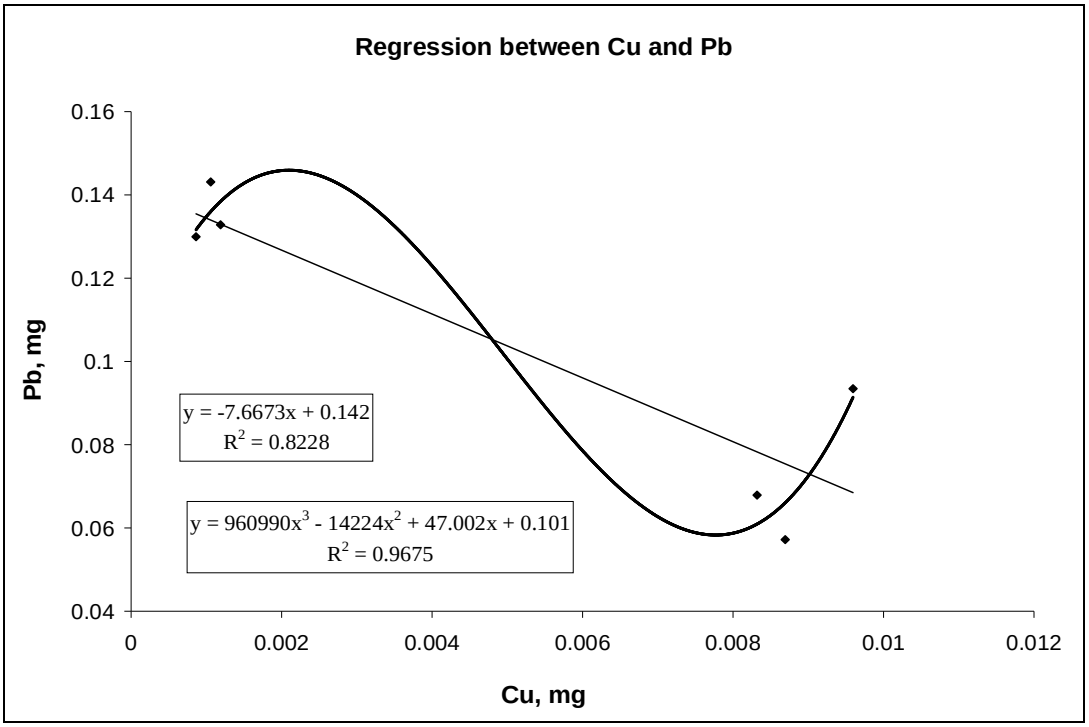


Figure E-4.4: Regression between Cu and Pb contents for P from the MB source

Regressions for MVR

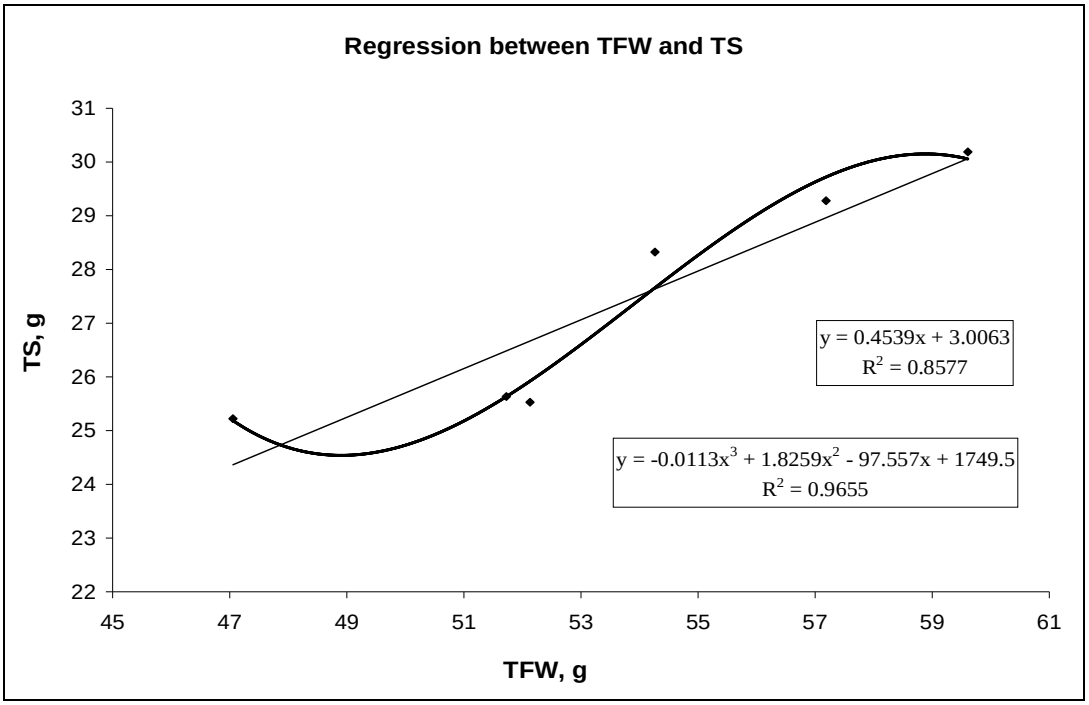


Figure E-5.1: Regression between TFW and TS contents for R from the MV source

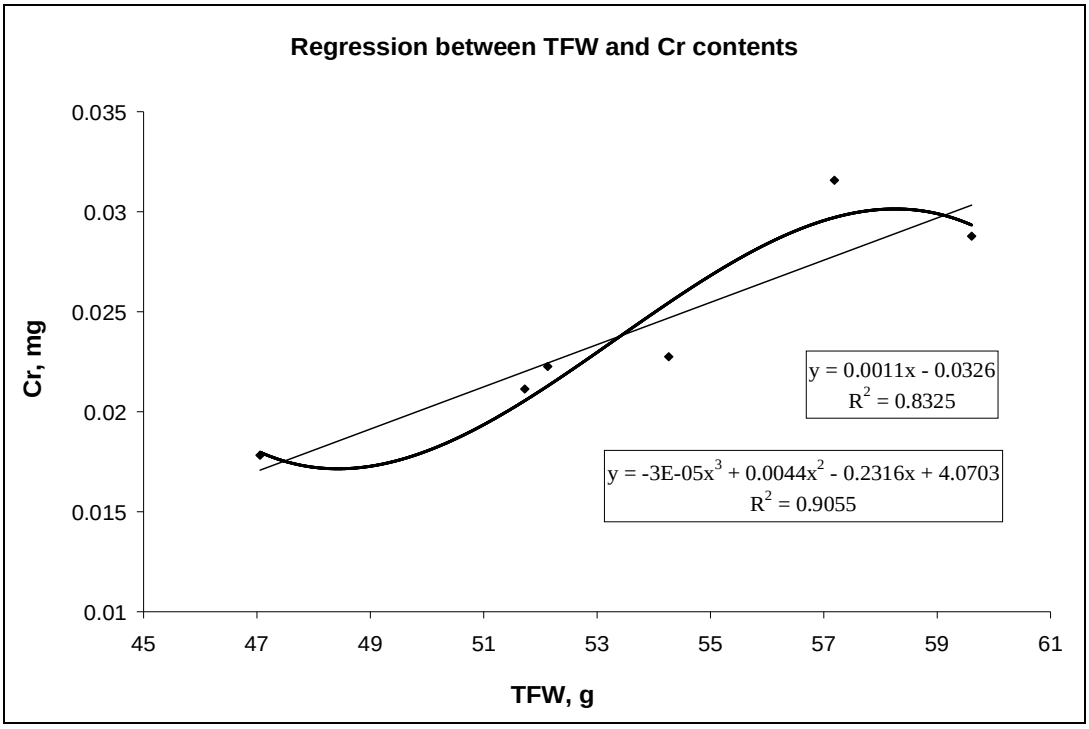


Figure E-5.2: Regression between TFW and Cr contents for R from the MV source

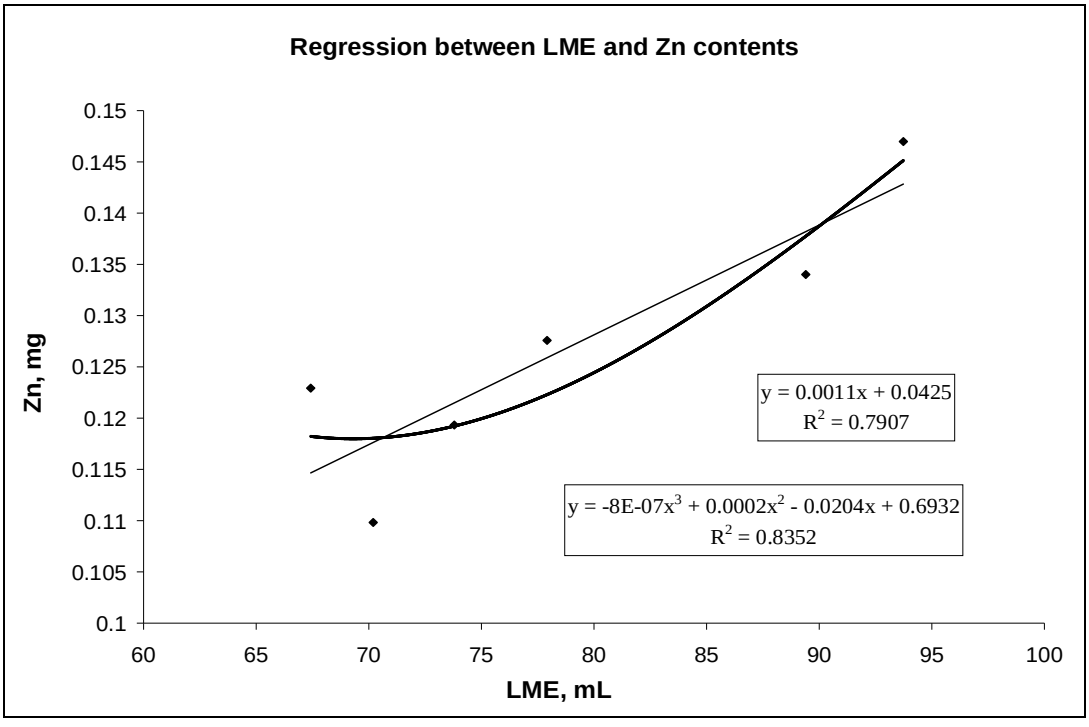


Figure E-5.3: Regression between LME and Zn contents for R from the MV source

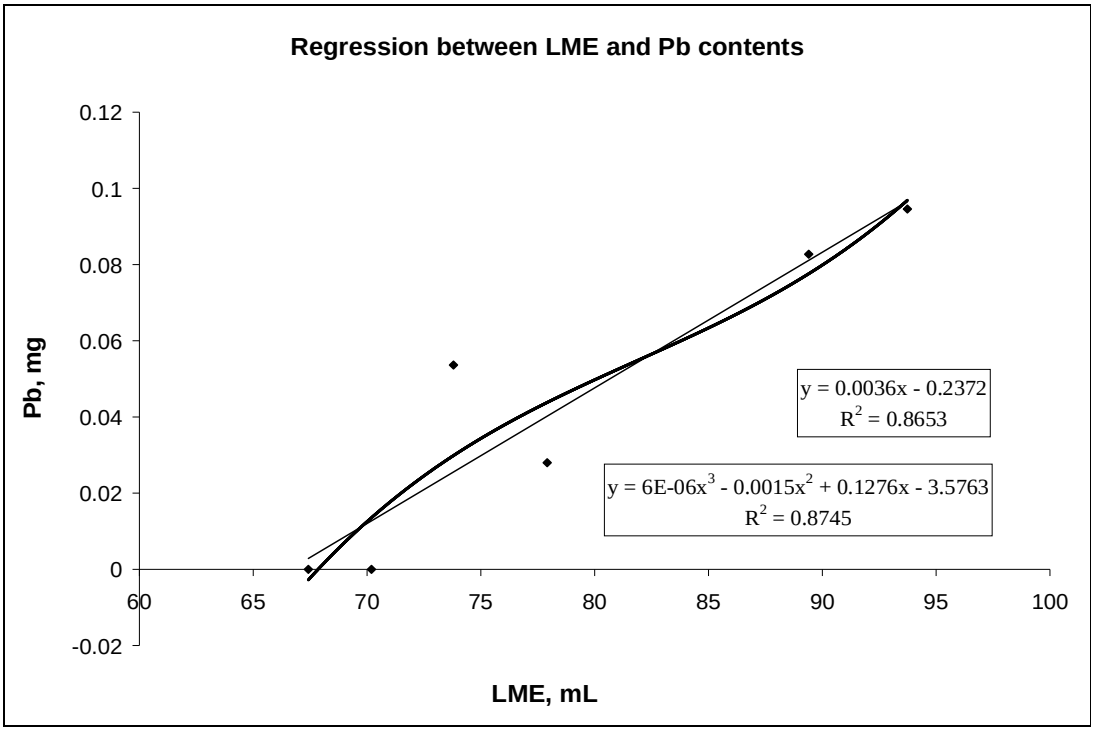


Figure E-5.4: Regression between LME and Pb contents for R from the MV source

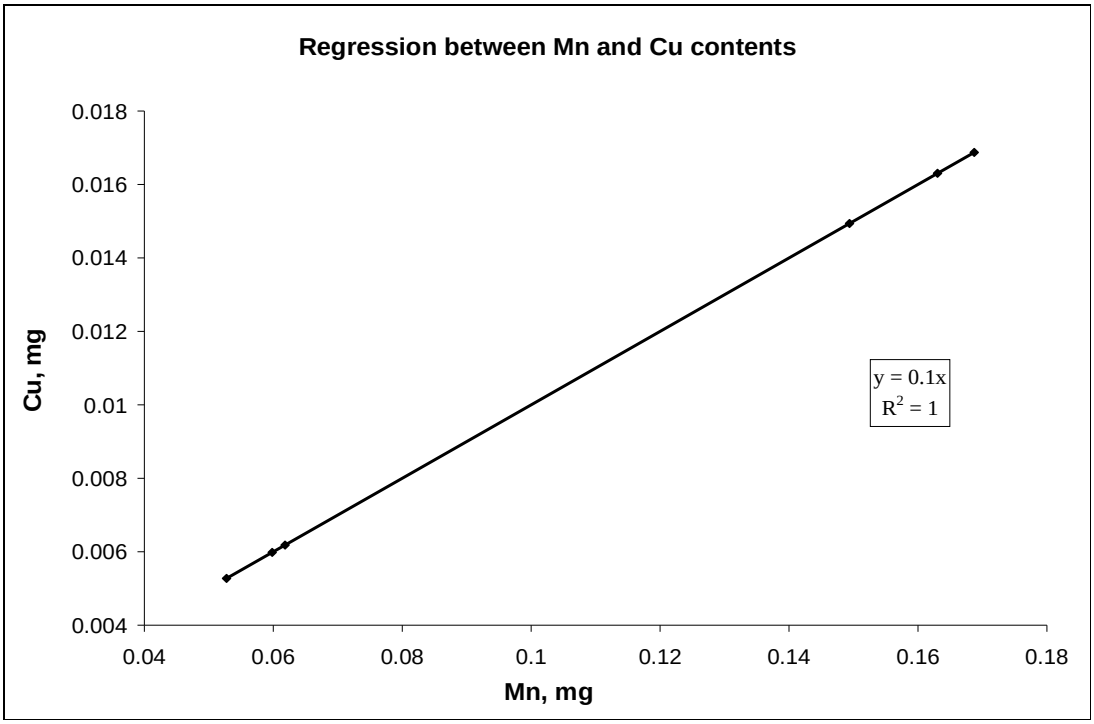


Figure E-5.5: Regression between Mn and Cu contents for R from the MV source

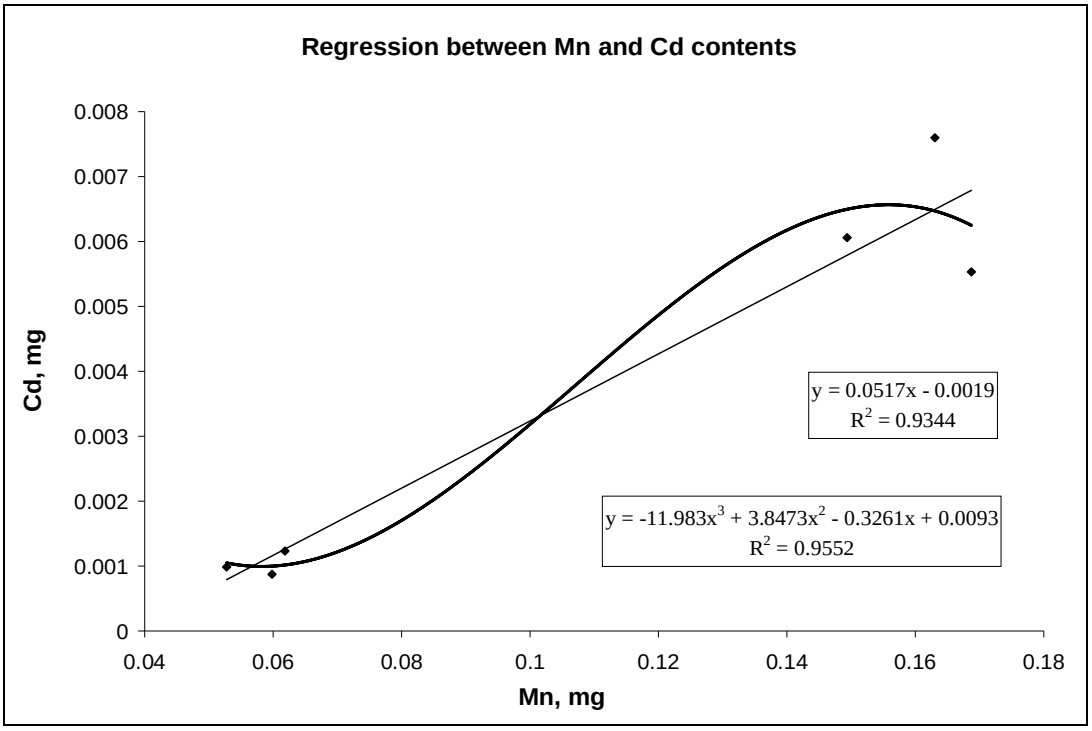


Figure E-5.6: Regression between Mn and Cd contents for R from the MV source

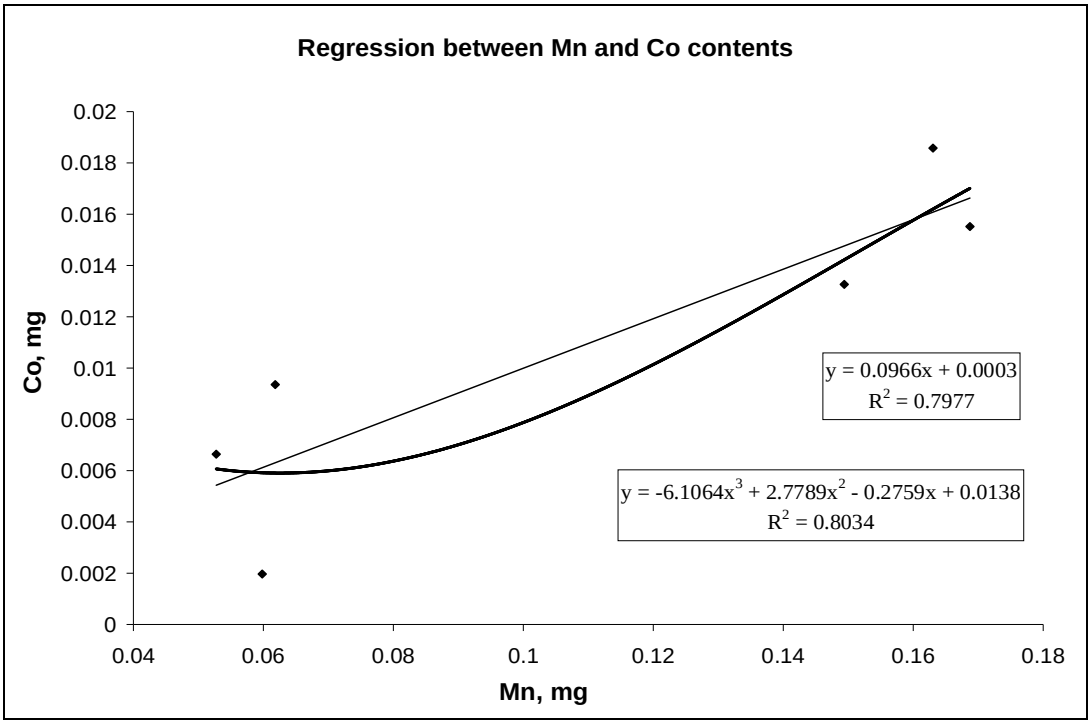


Figure E-5.7: Regression between Mn and Co contents for R from the MV source

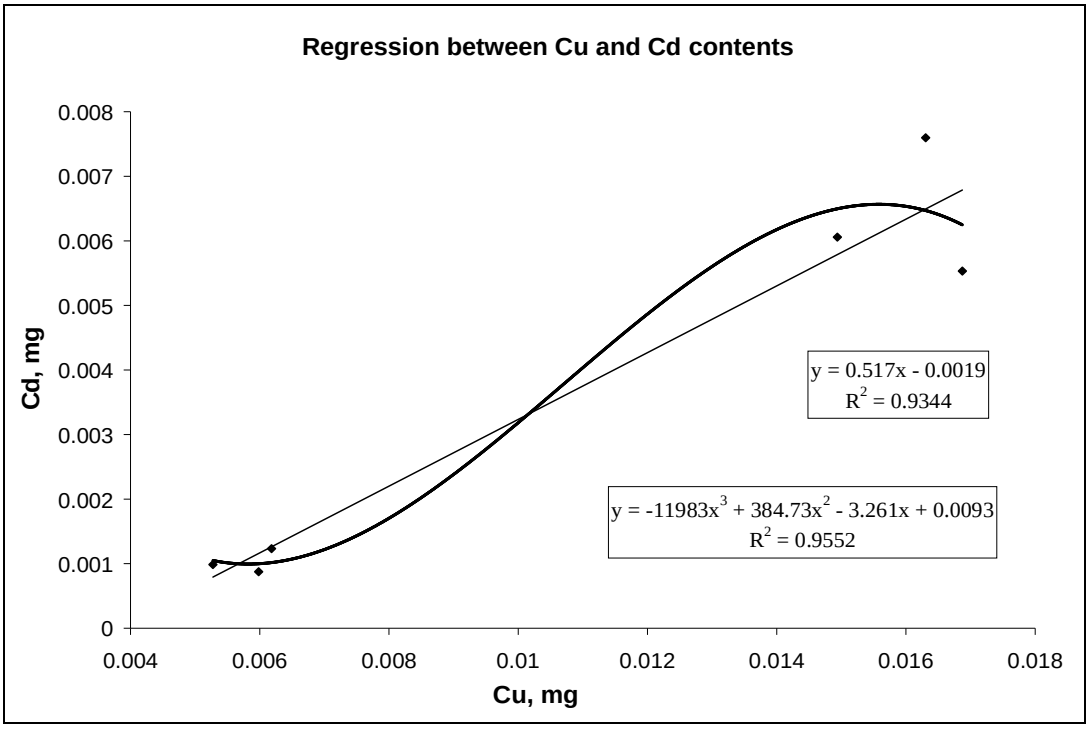


Figure E-5.8: Regression between Cu and Cd for R from the MV source

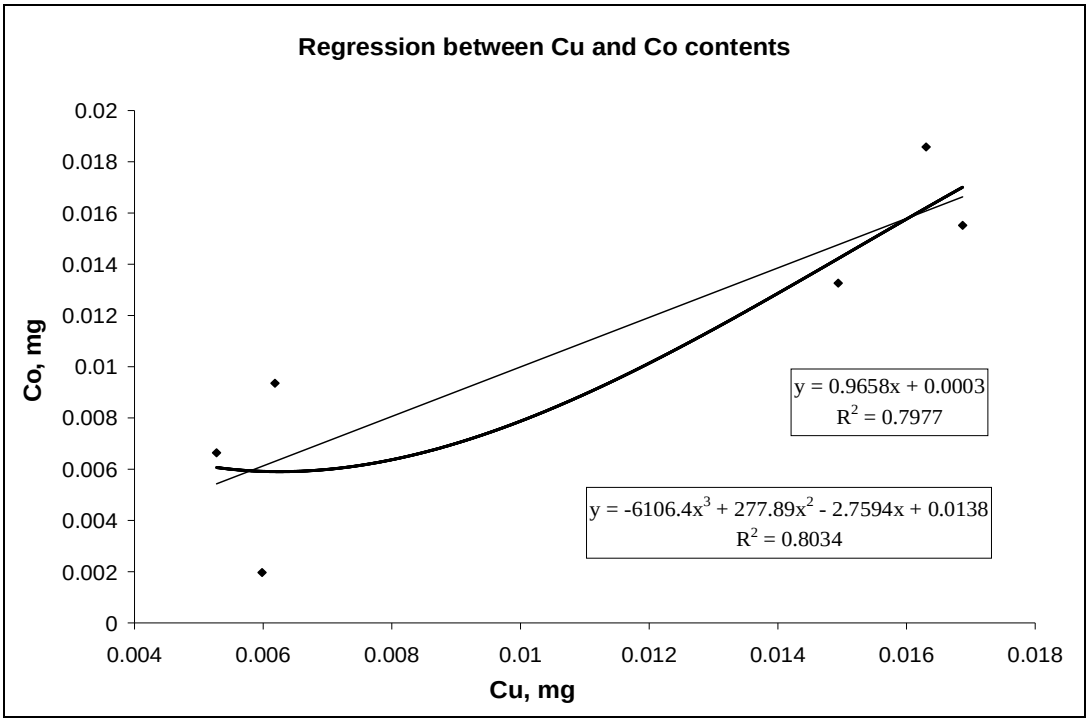


Figure E-5.9: Regression between Cu and Co contents for R from the MV source

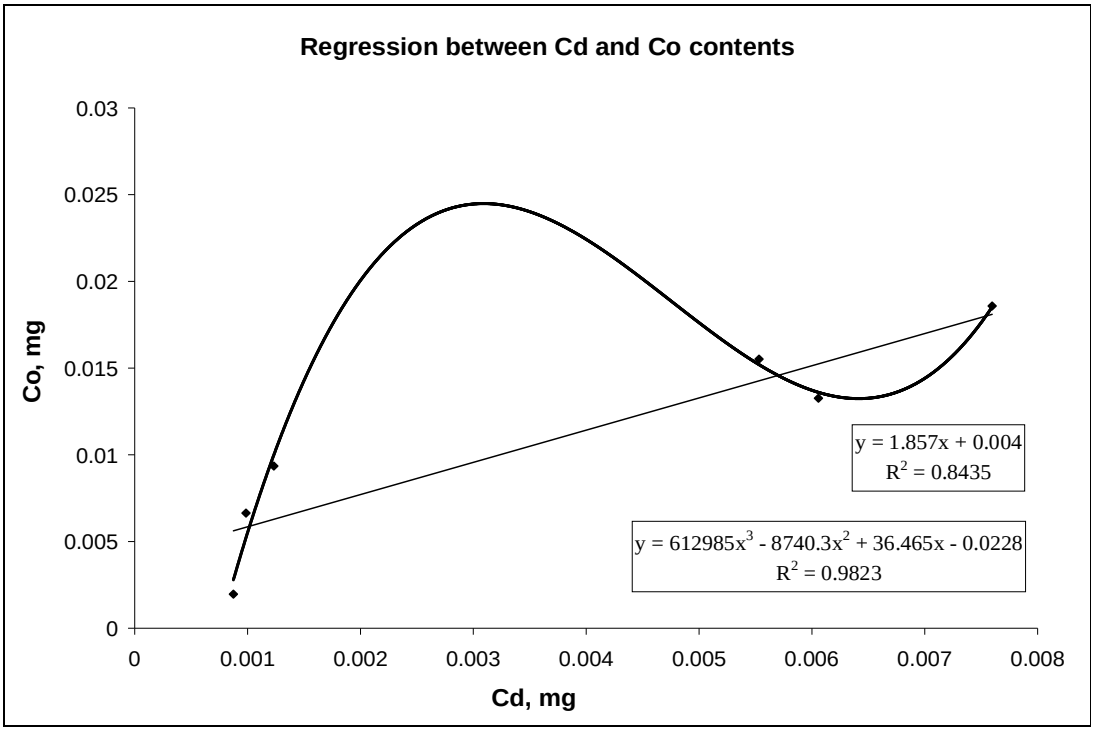


Figure E-5.10: Regression between Cd and Co contents for R from the MV source

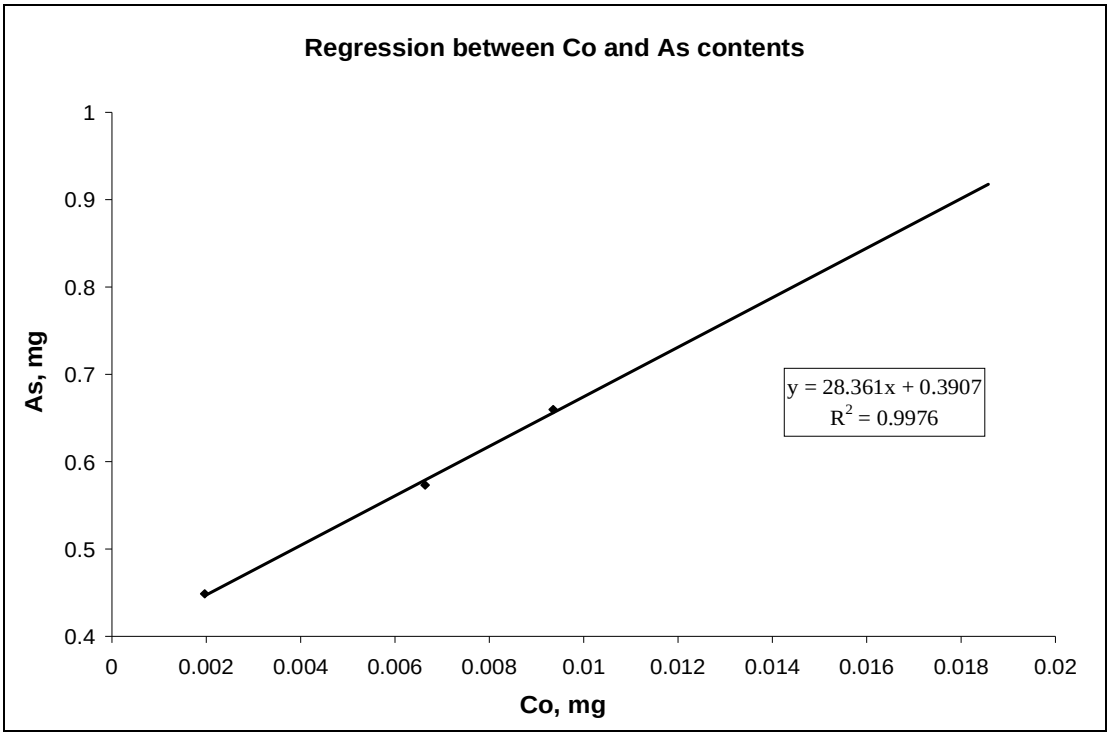


Figure E-5.11: Regression between Co and As contents for R from the MV source

Regressions for MVP

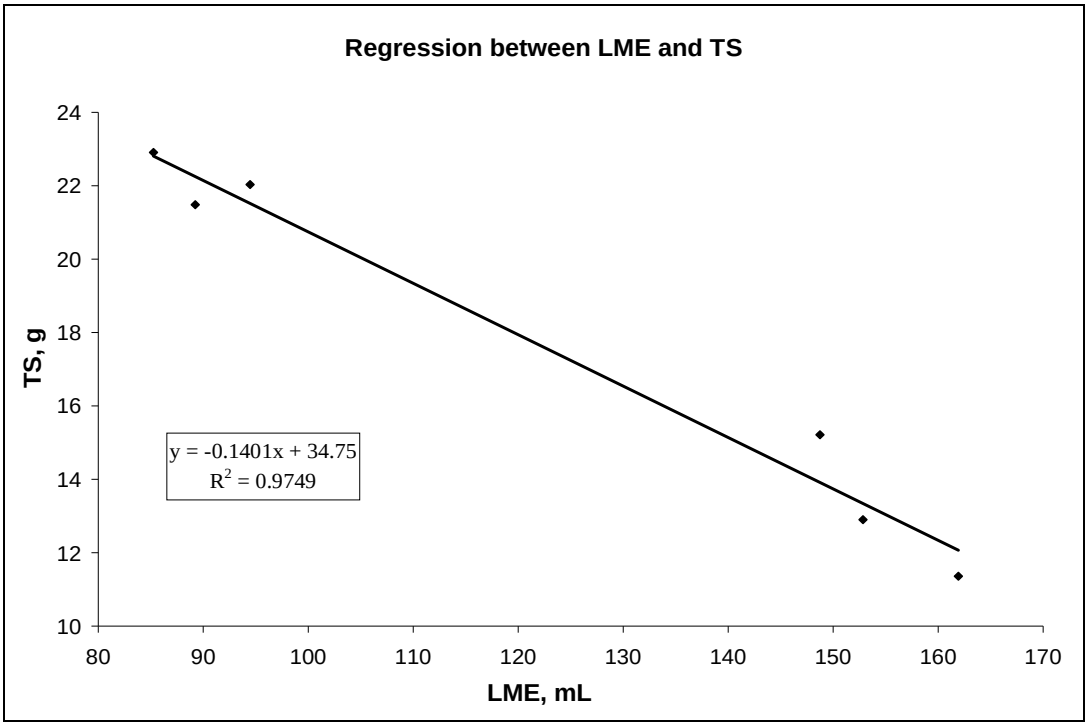


Figure E-6.1: Regression between LME and TS contents for P from the MV source

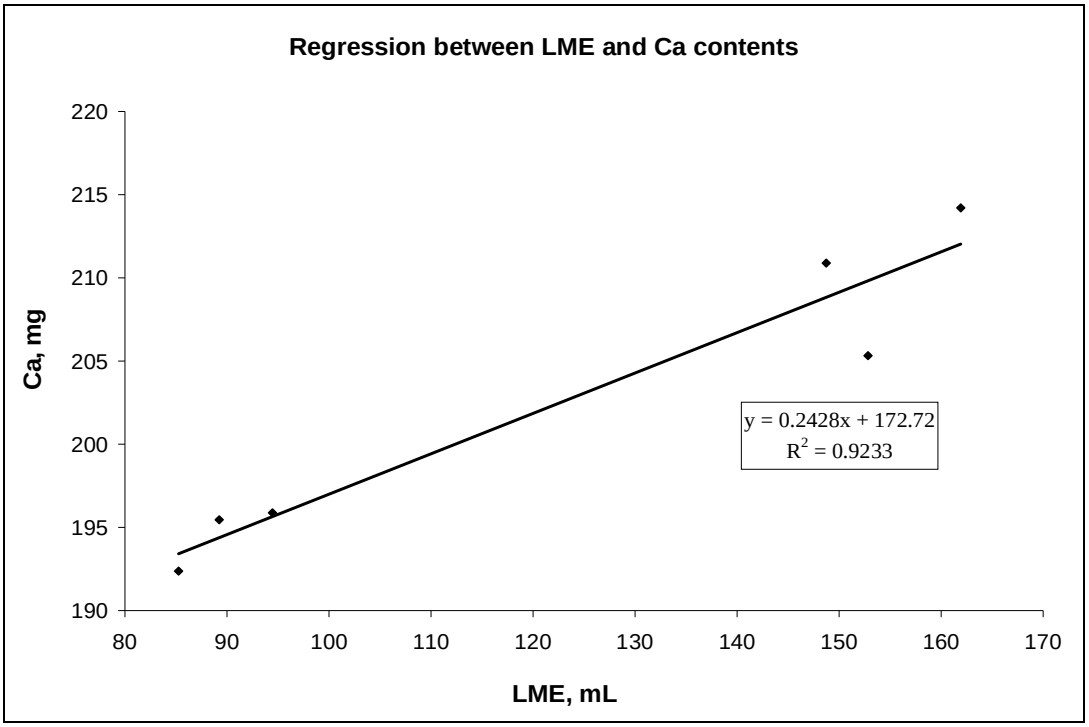


Figure E-6.2: Regression between LME and Ca contents for P from the MV source

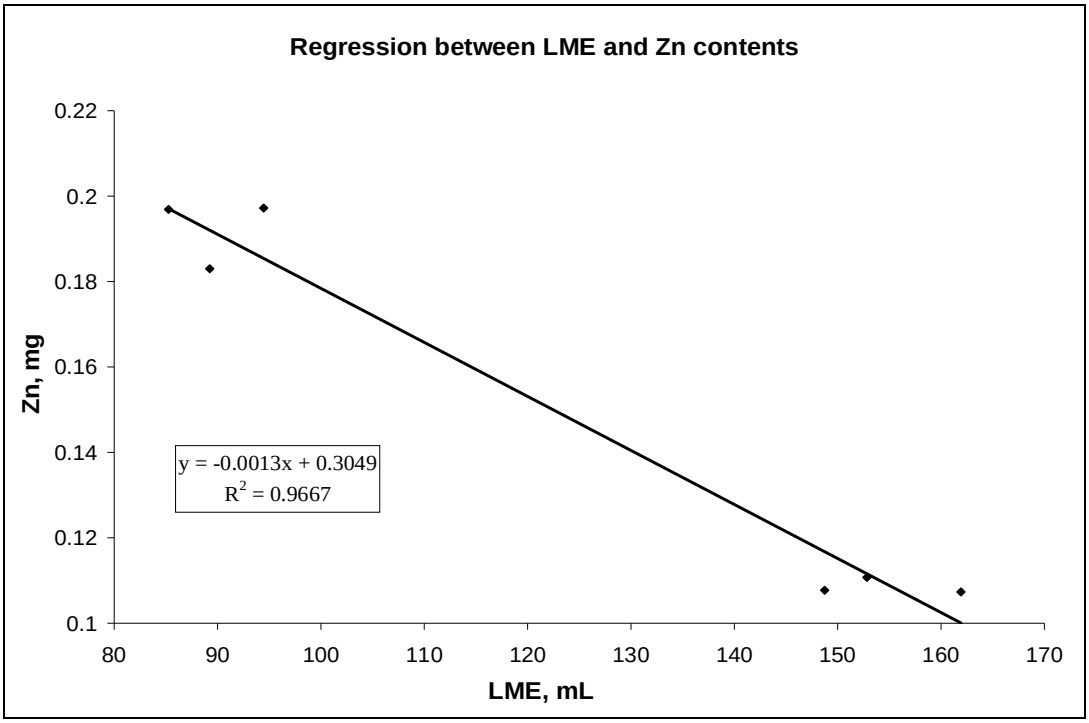


Figure E-6.3: Regression between LME and Zn contents for P from the MV source

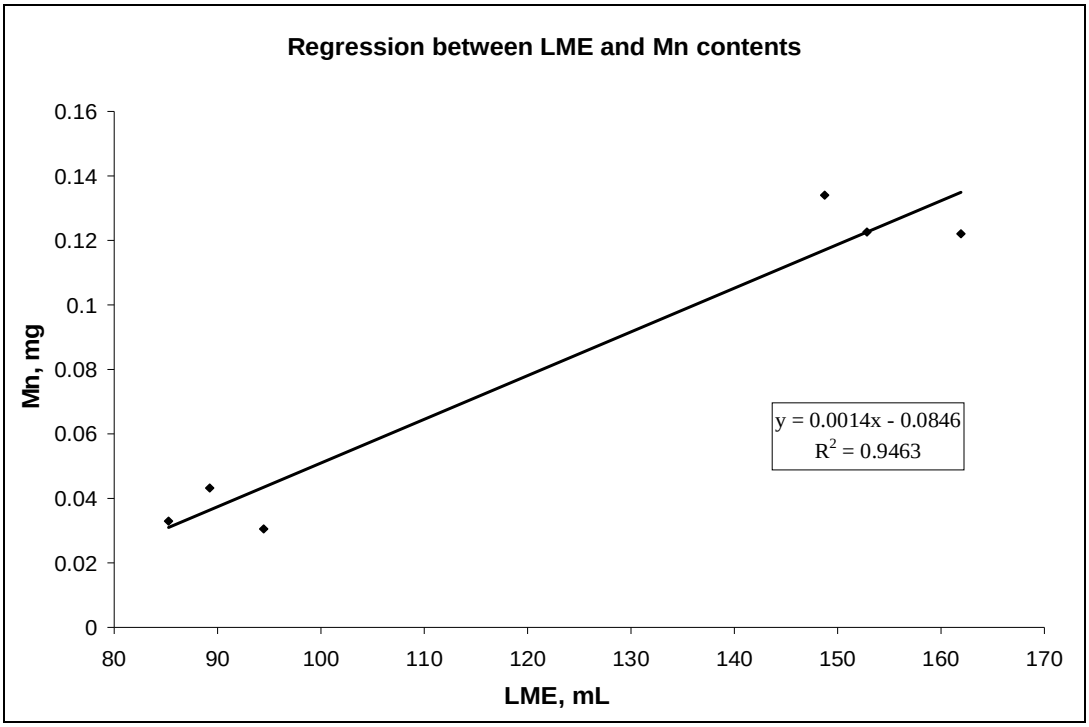


Figure E-6.4: Regression between LME and Mn contents for P from the MV source

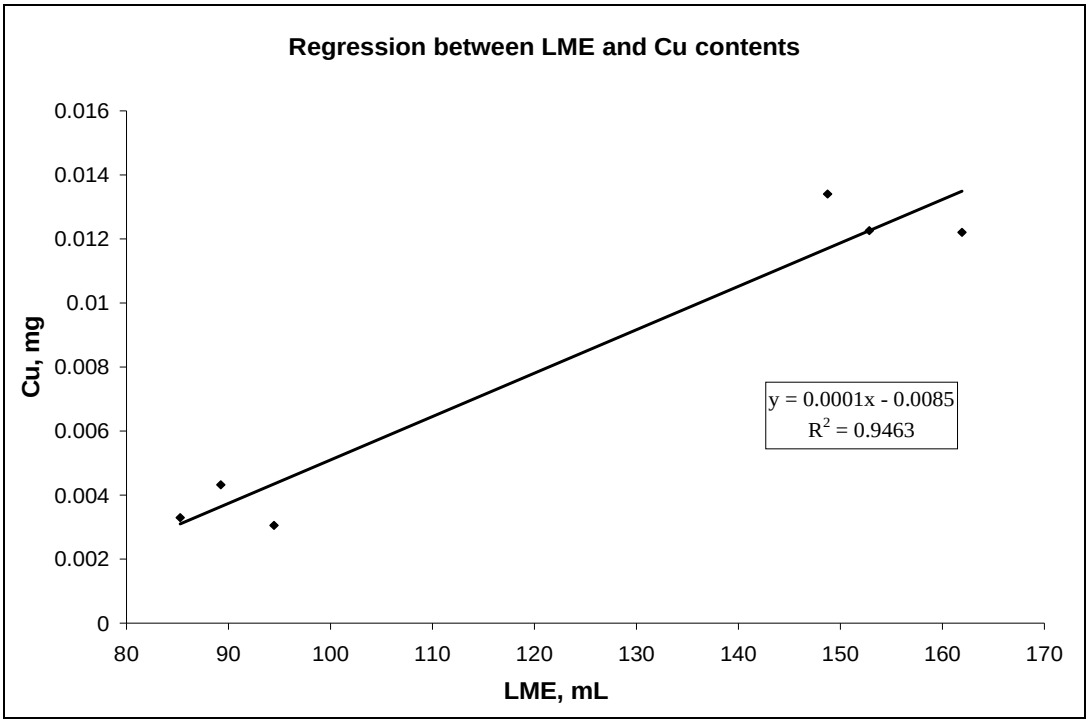


Figure E-6.5: Regression between LME and Cu contents for P from the MV source

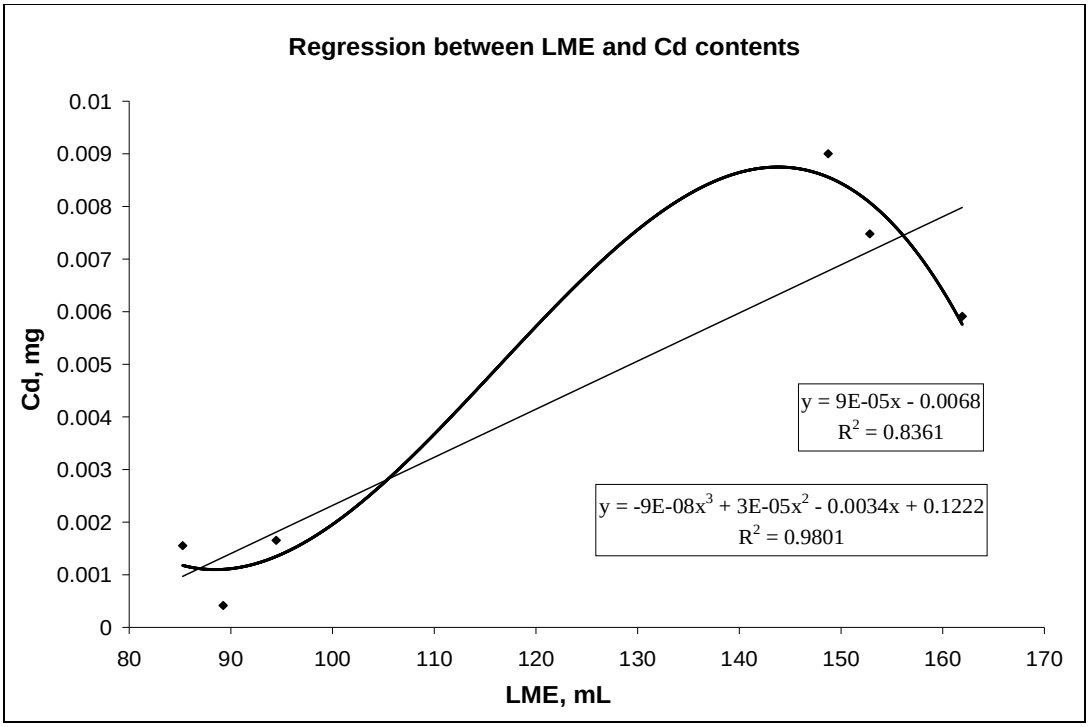


Figure E-6.6: Regression between LME and Cd contents for P from the MV source

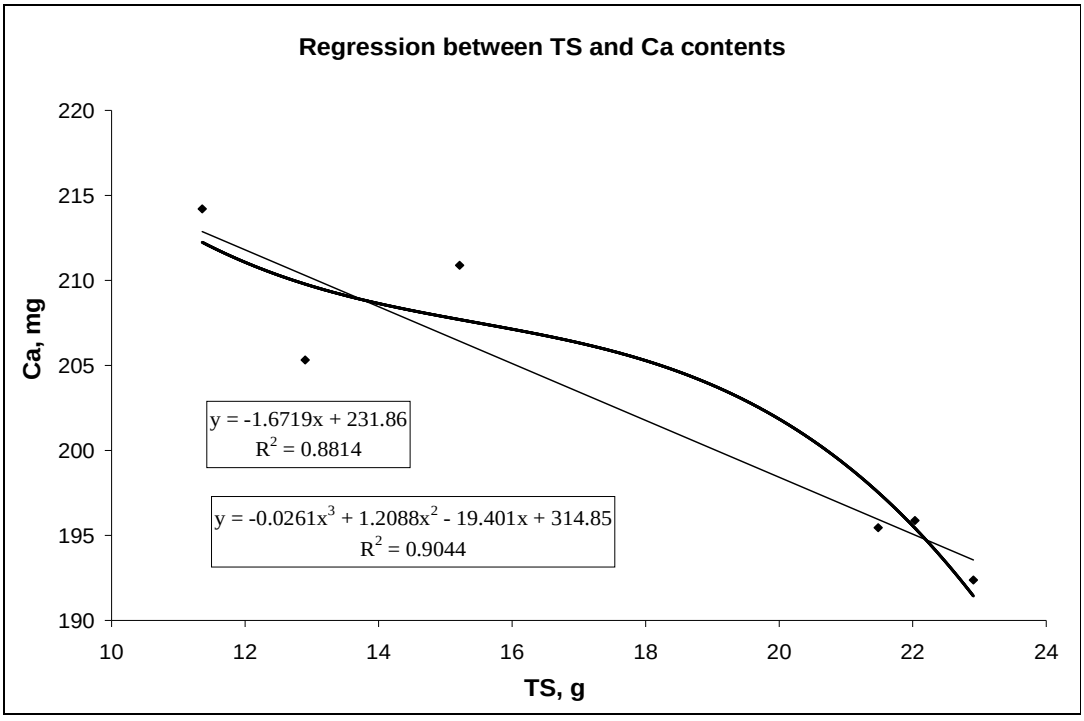


Figure E-6.7: Regression between TS and Ca contents for P from the MV source

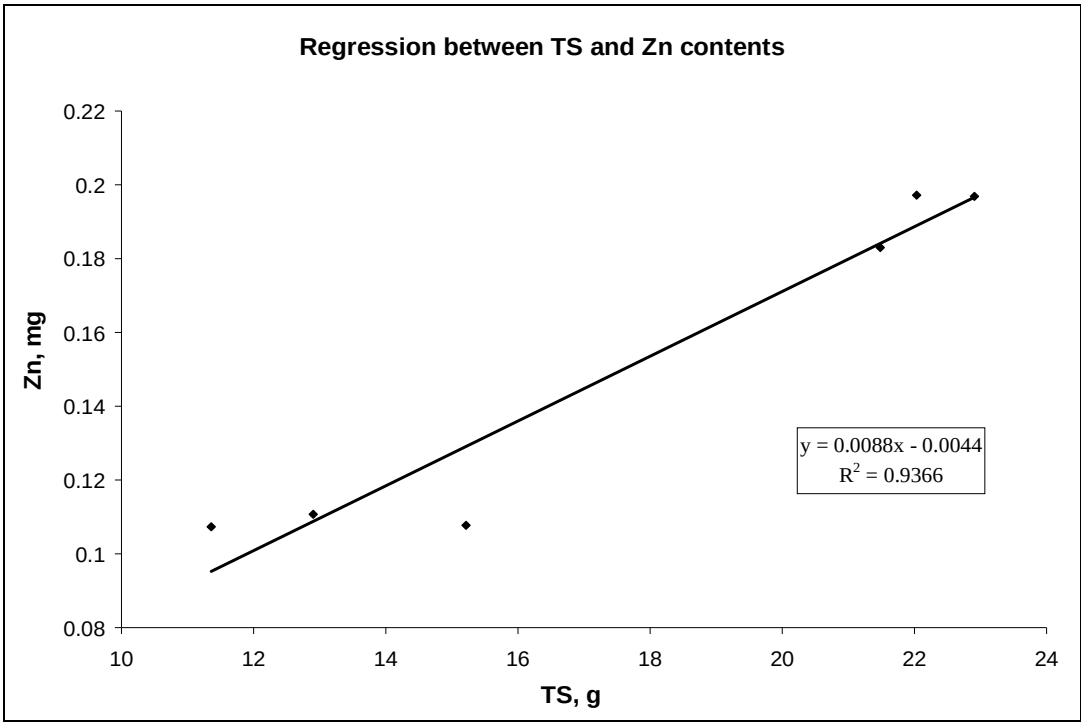


Figure E-6.8: Regression between TS and Zn contents for P from the MV source

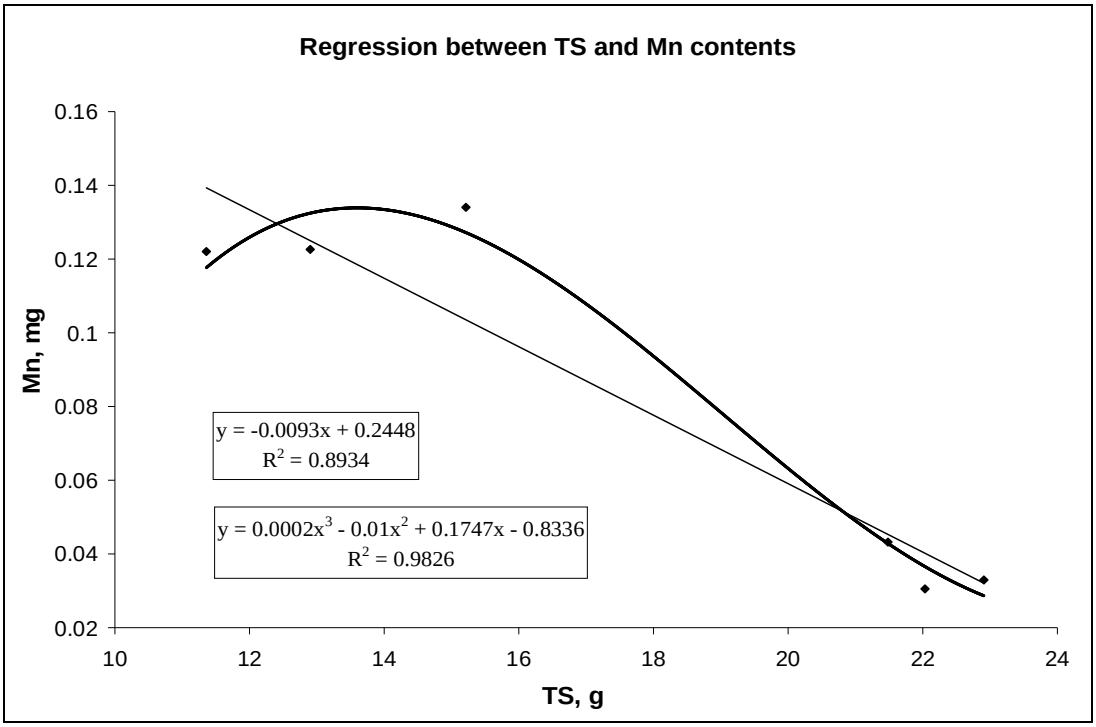


Figure E-6.9: Regression between TS and Mn contents for P from the MV source

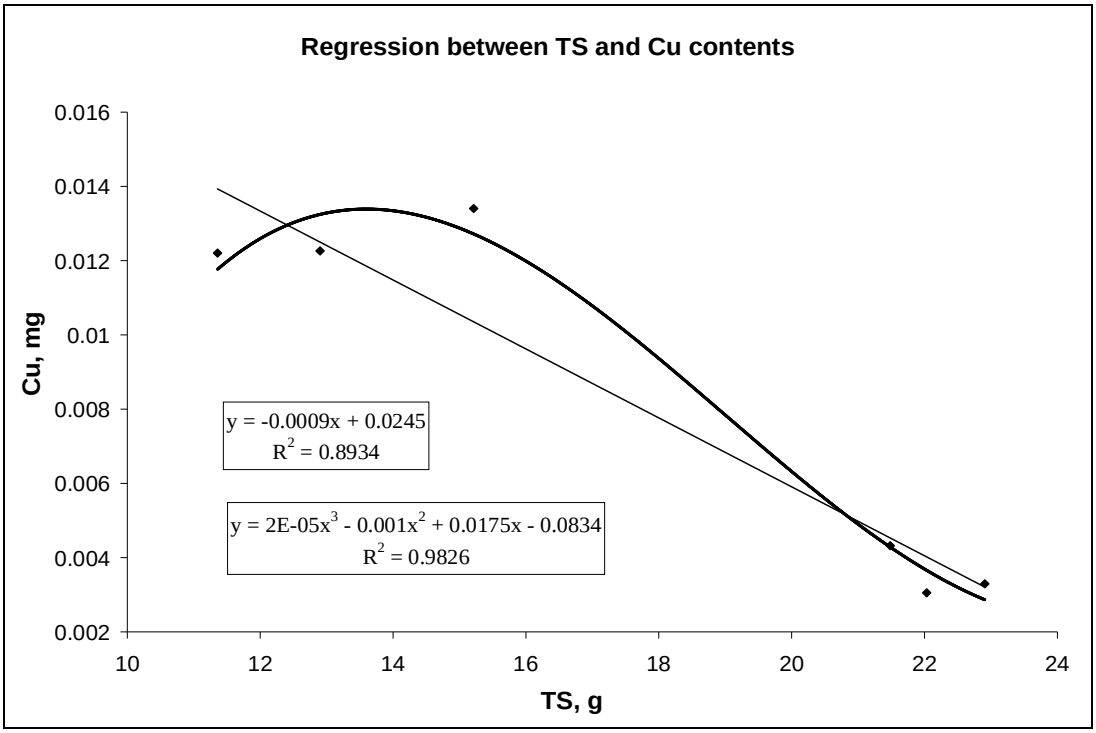


Figure E-6.10: Regression between TS and Cu contents for P from the MV source

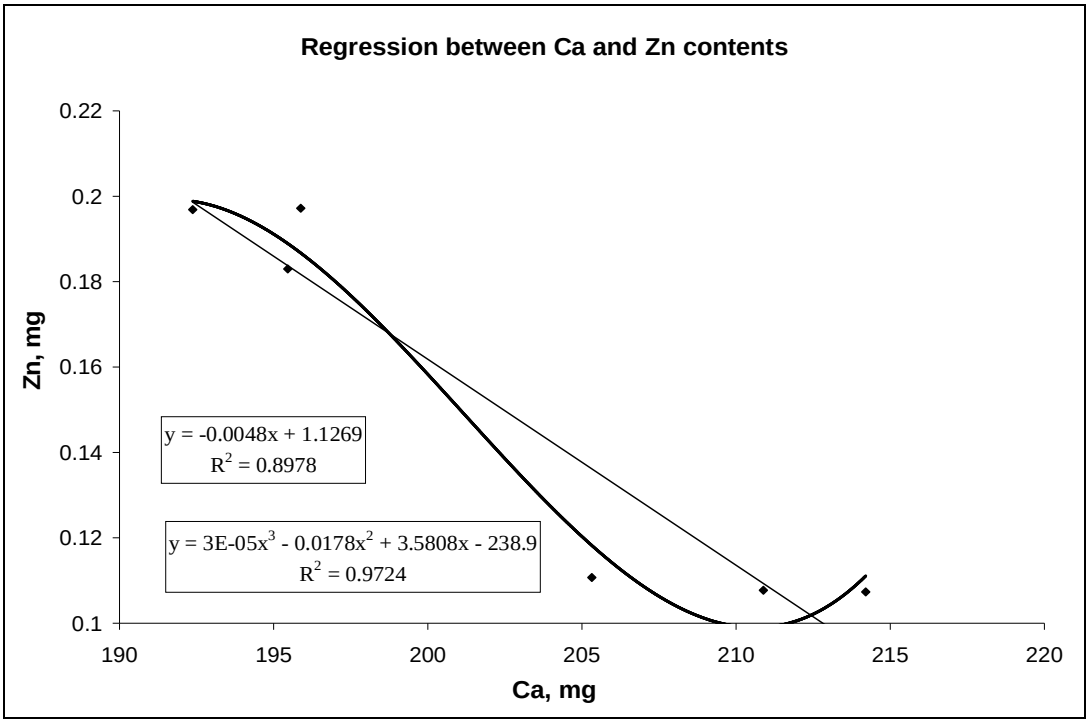


Figure E-6.11: Regression between Ca and Zn contents for P from the MV source

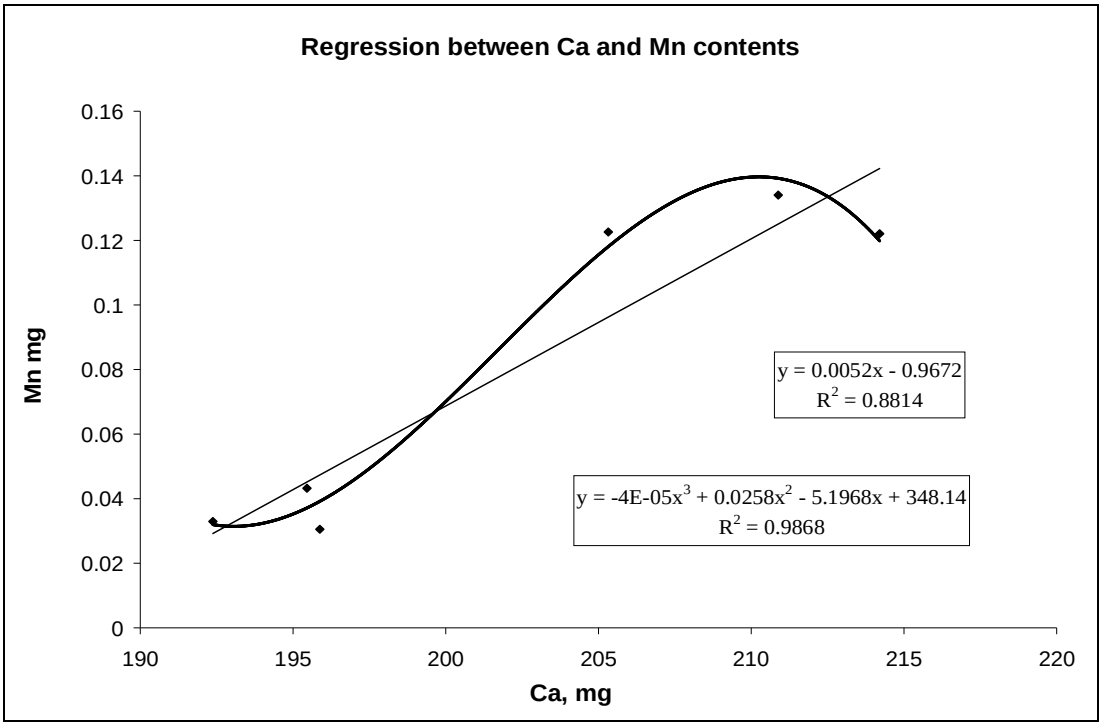


Figure E-6.12: Regression between Ca and Mn contents for P from the MV source

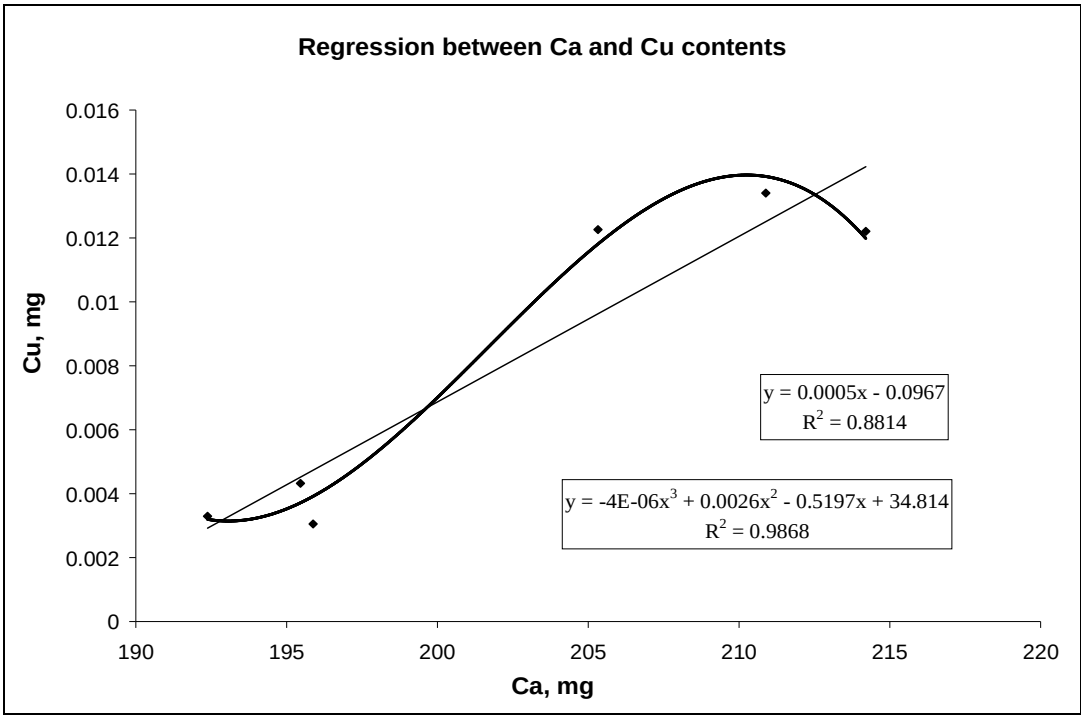


Figure E-6.13: Regression between Ca and Cu contents for P from the MV source

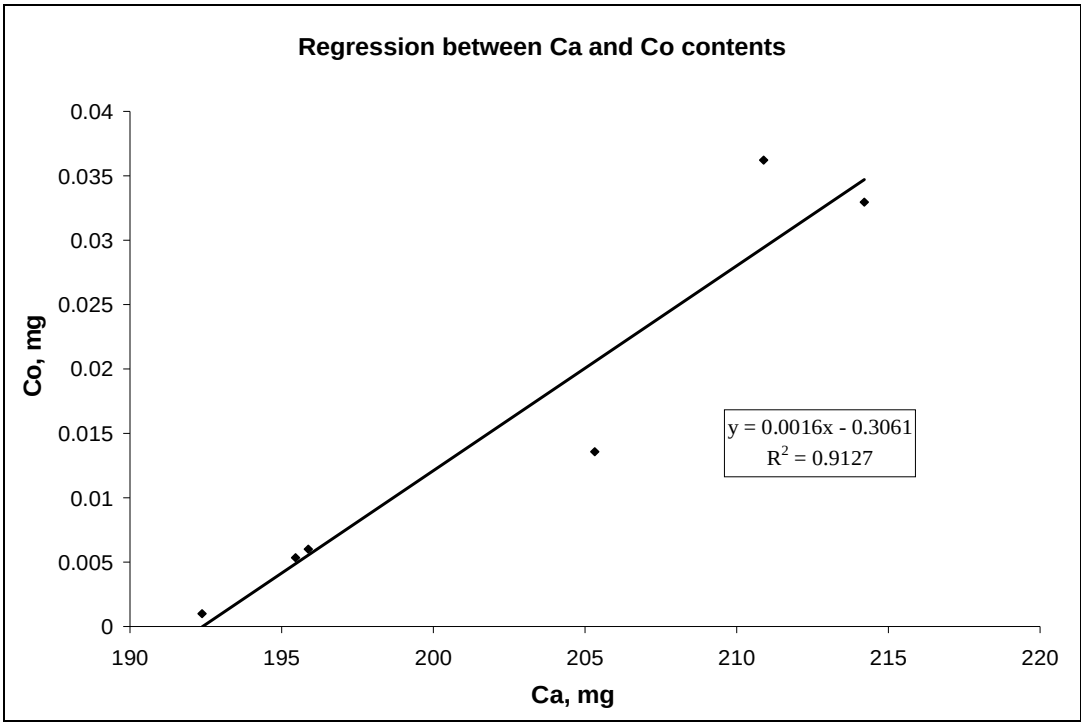


Figure E-6.14: Regression between Ca and Co contents for P from the MV source

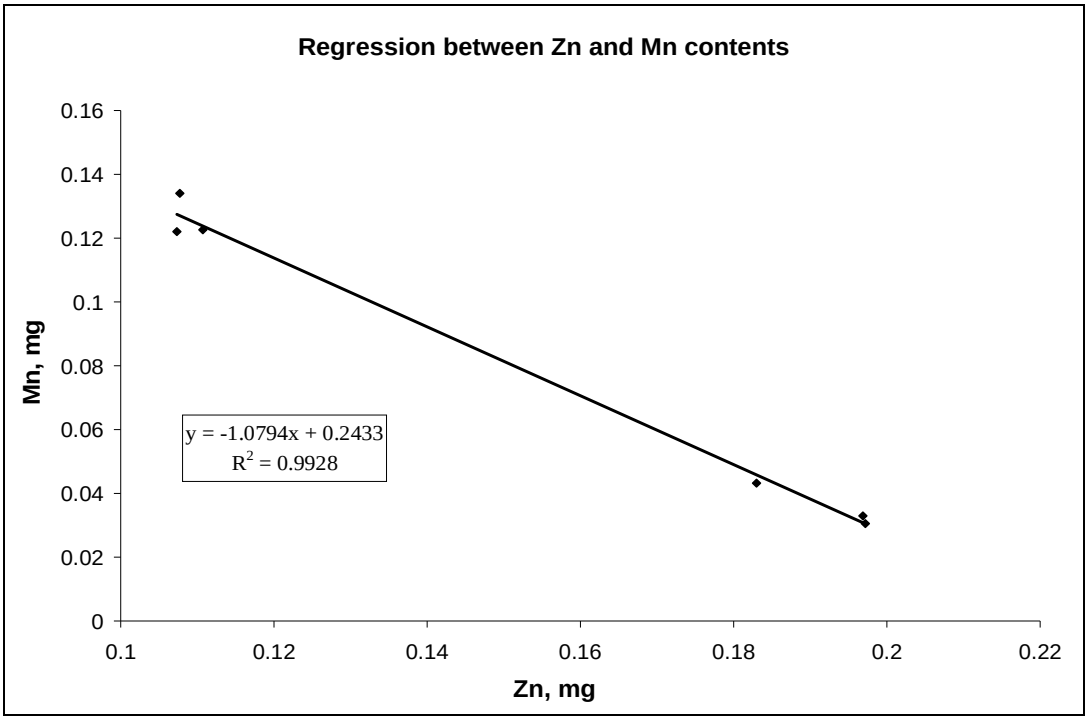


Figure E-6.15: Regression between Zn and Mn contents for P from the MV source

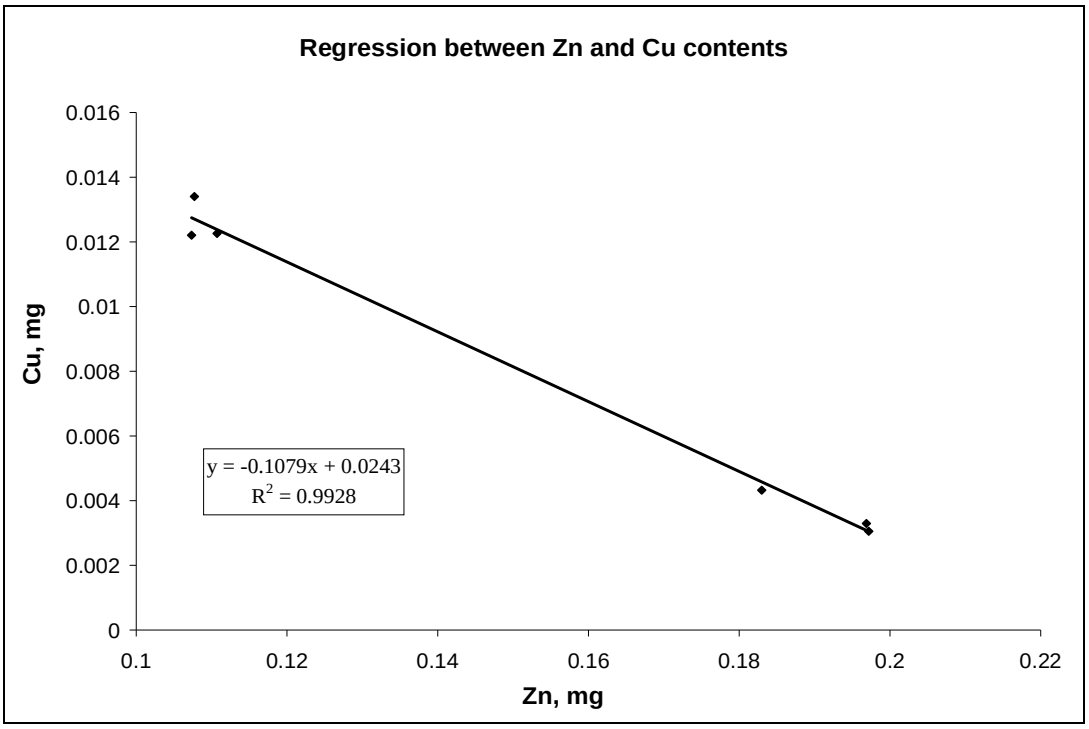


Figure E-6.16: Regression between Zn and Cu contents for P from the MV source

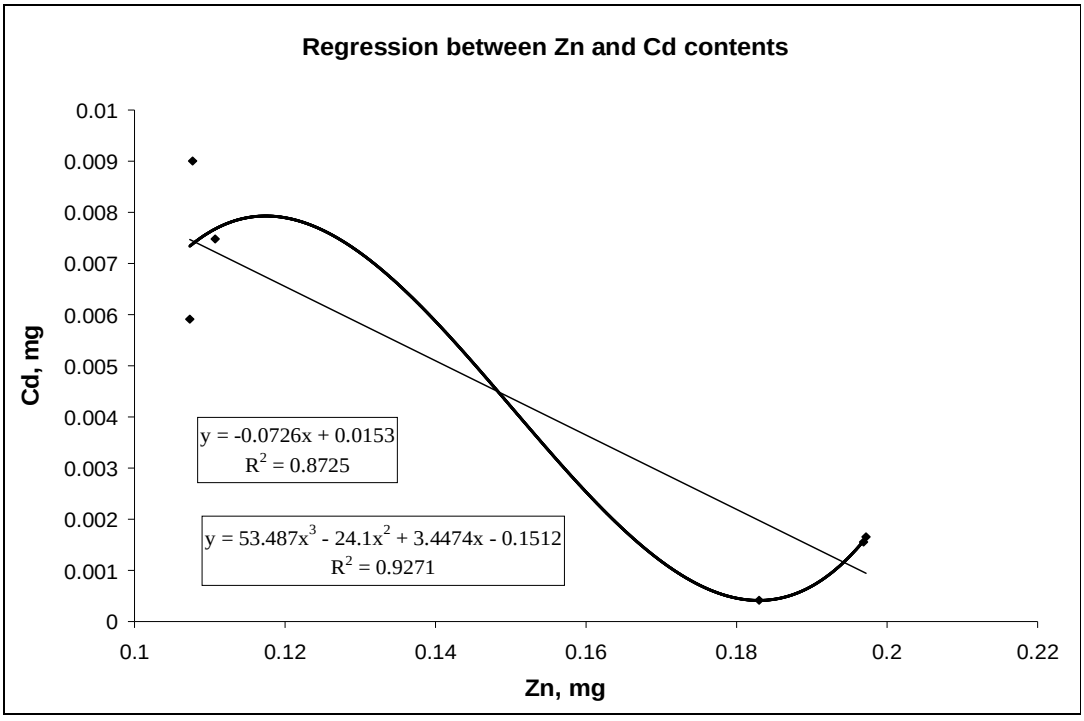


Figure E-6.17: Regression between Zn and Cd contents for P from the MV source

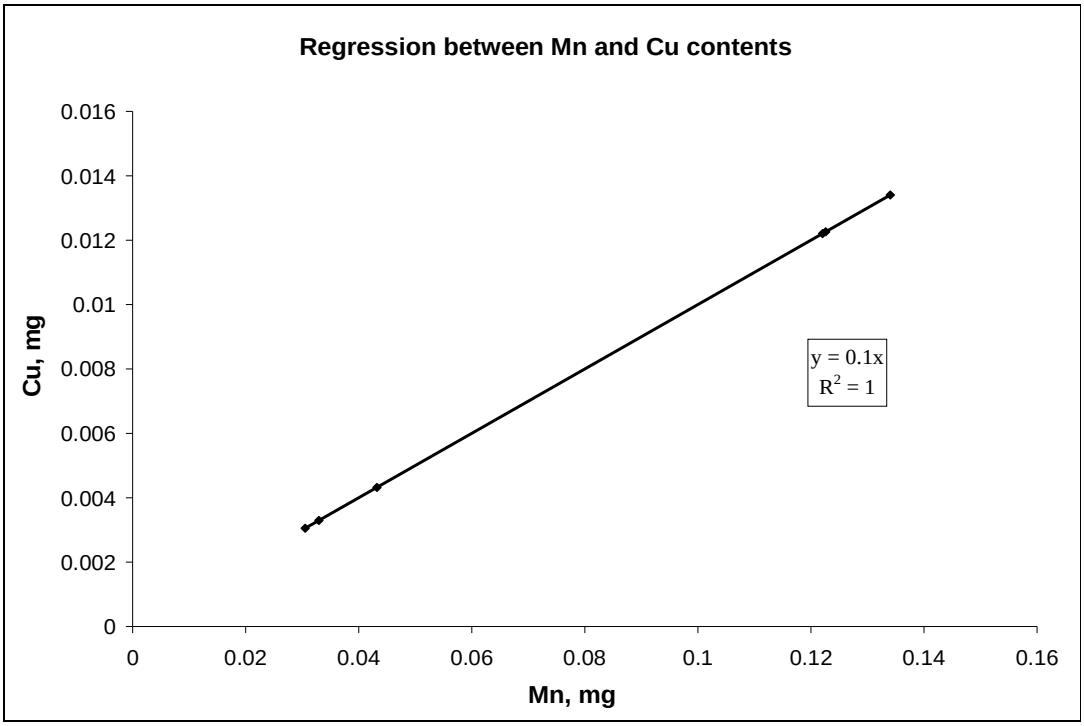


Figure E-6.18: Regression between Mn and Cu contents for P from the MV source

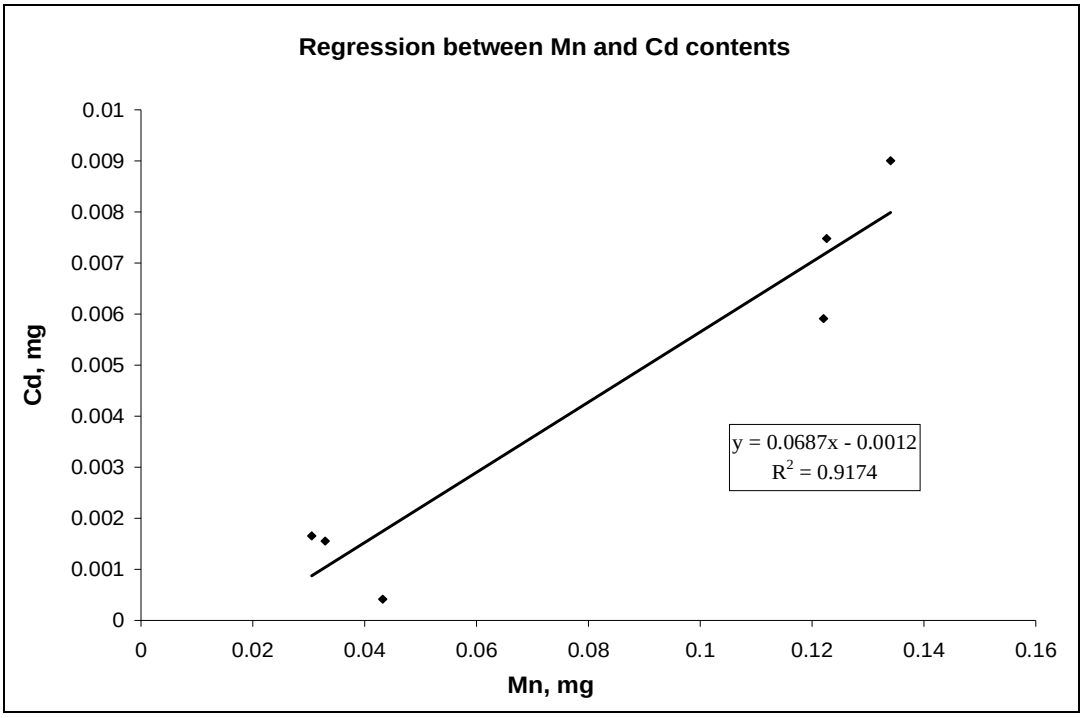


Figure E-6.19: Regression between Mn and Ca contents for P from the MV source

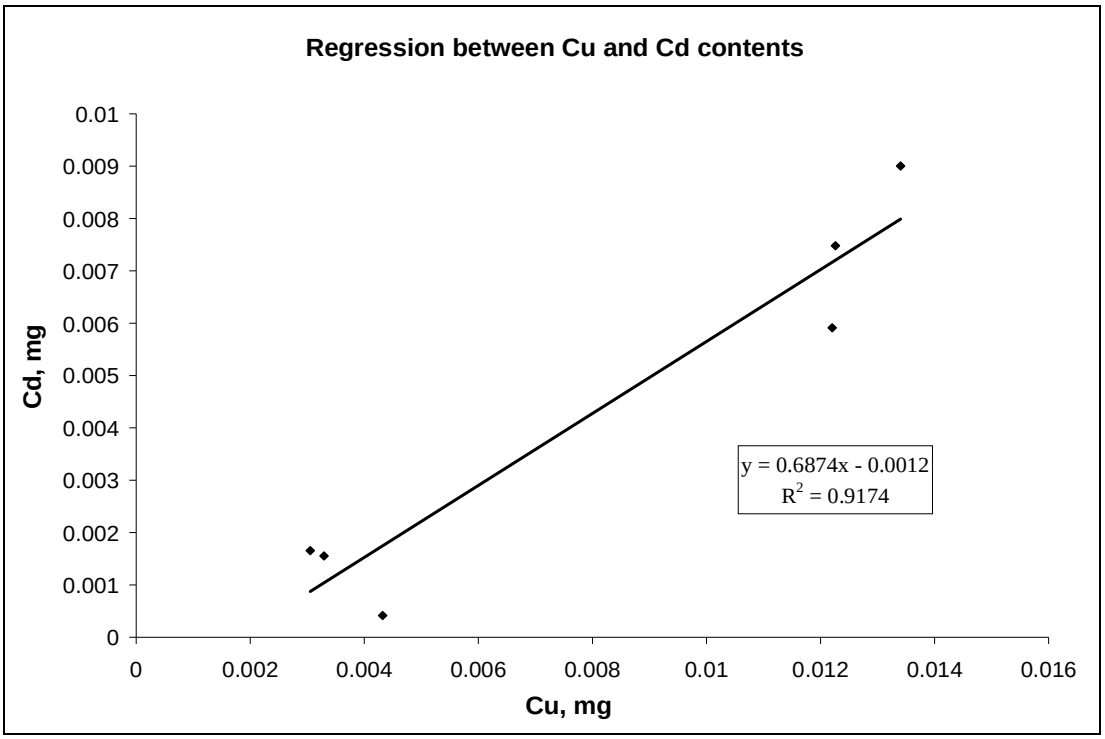


Figure E-6.20: Regression between Cu and Cd contents for P from the MV source

Regressions for MBzR

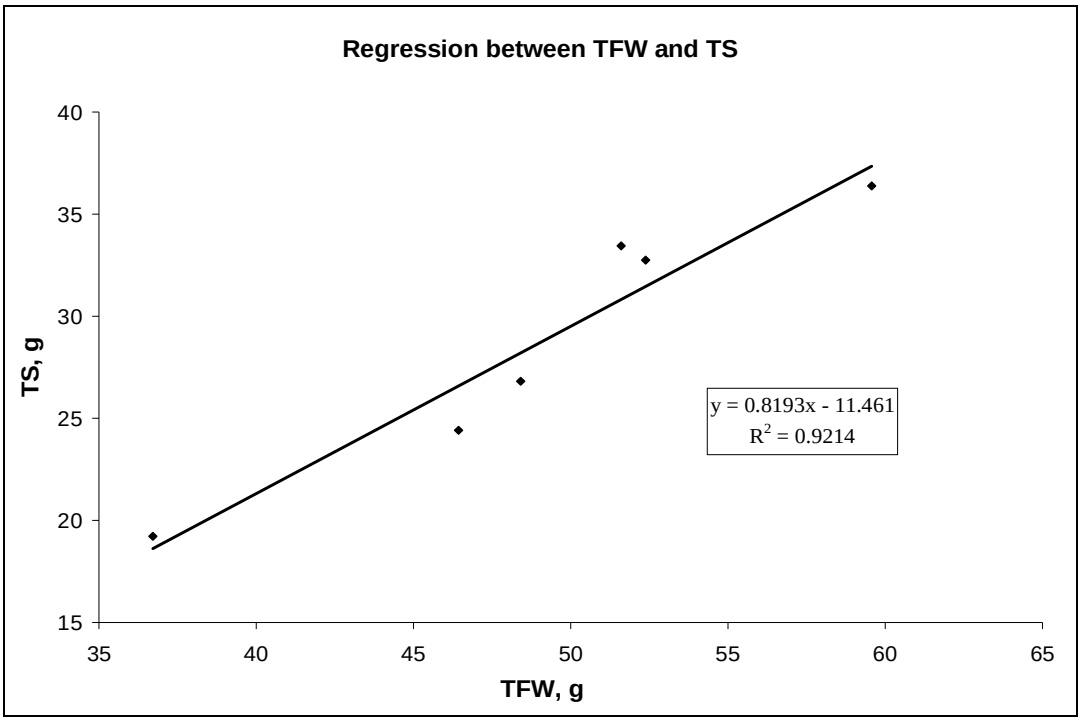


Figure E-7.1: Regression between TFW and TS contents for R from the MBz source

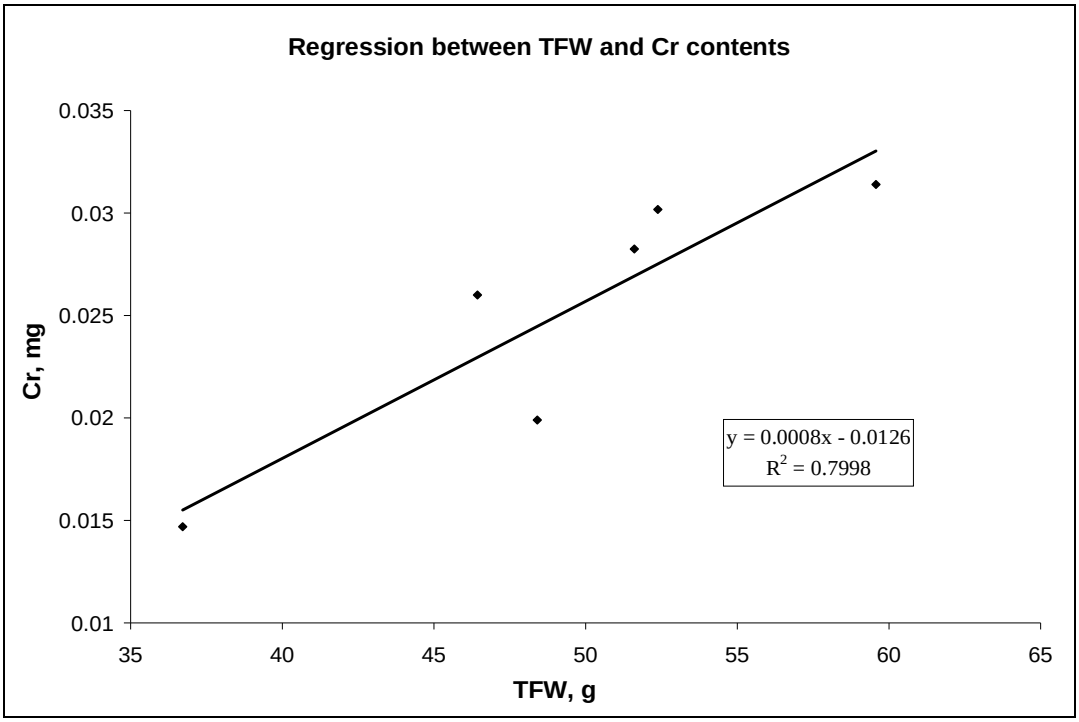


Figure E-7.2: Regression between TFW and Cr contents for R from the MBz source

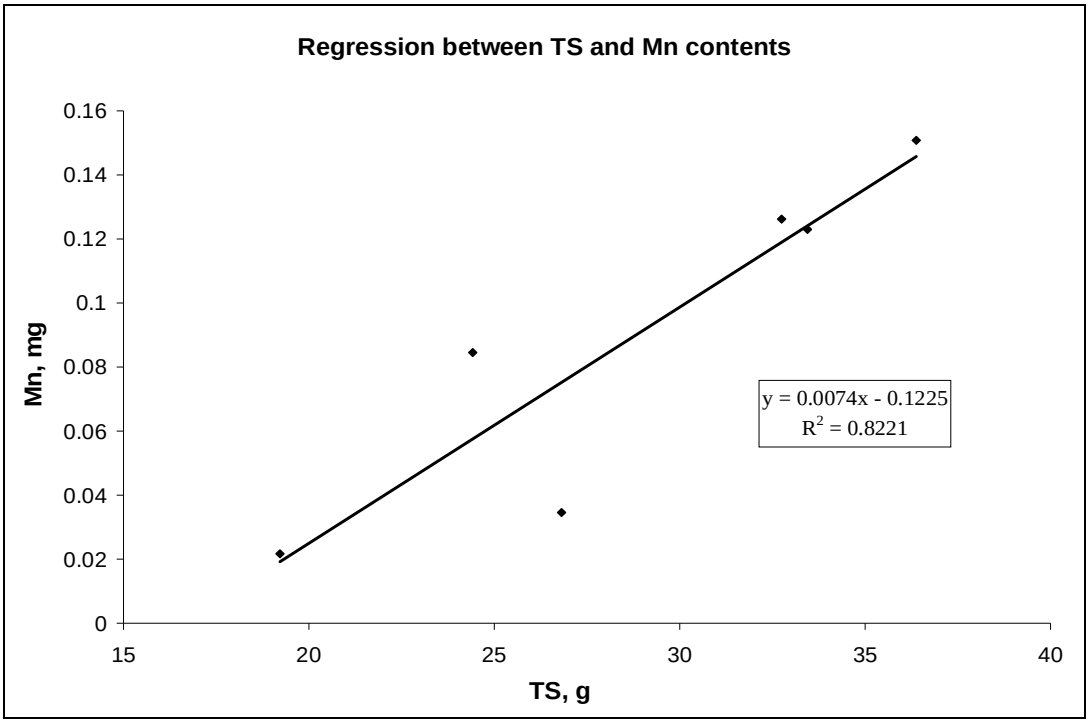


Figure E-7.3: Regression between TS and Mn contents for R from the MBz source

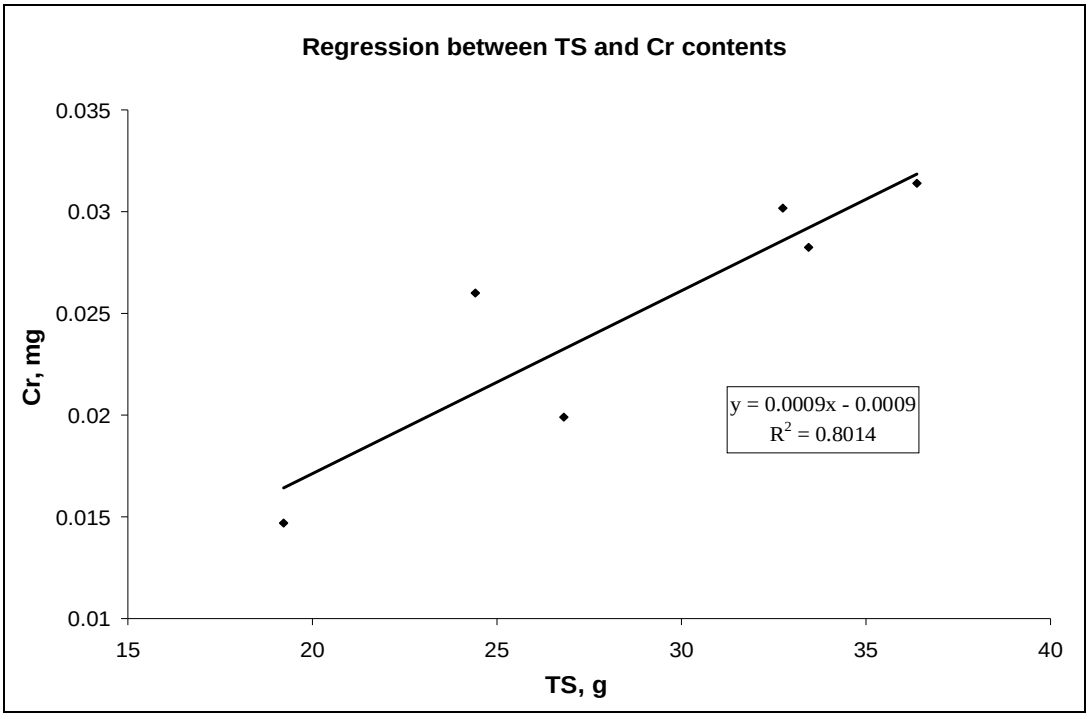


Figure E-7.4: Regression between TS and Cr contents for R from the MBz source

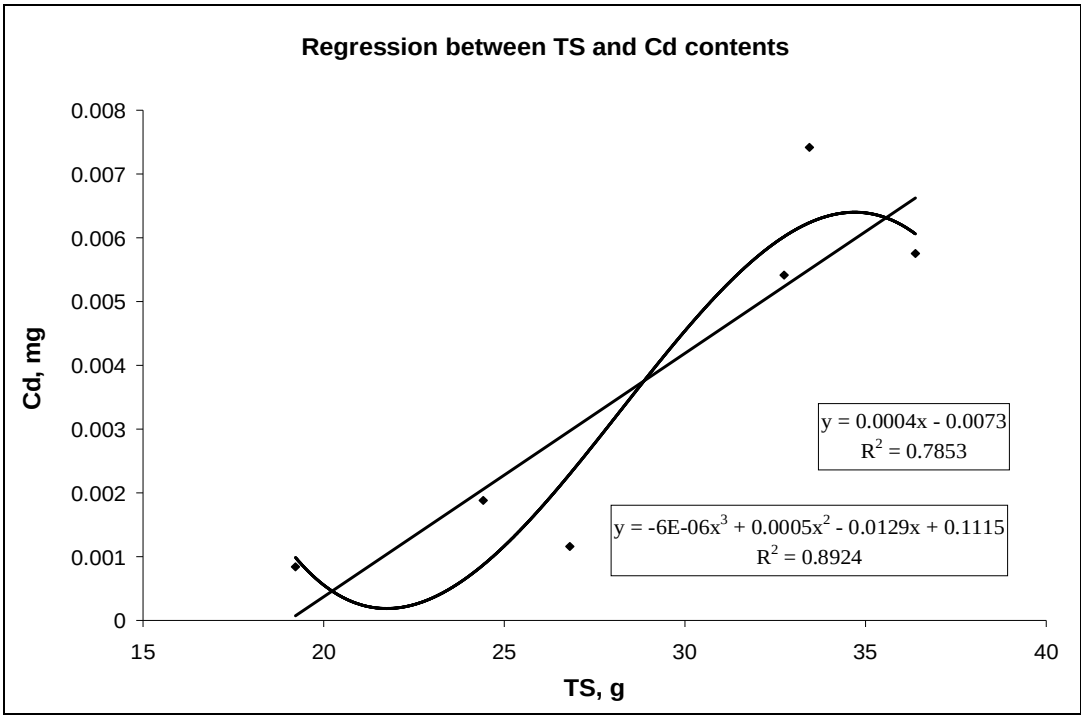


Figure E-7.5: Regression between TS and Cd contents for R from the MBz source

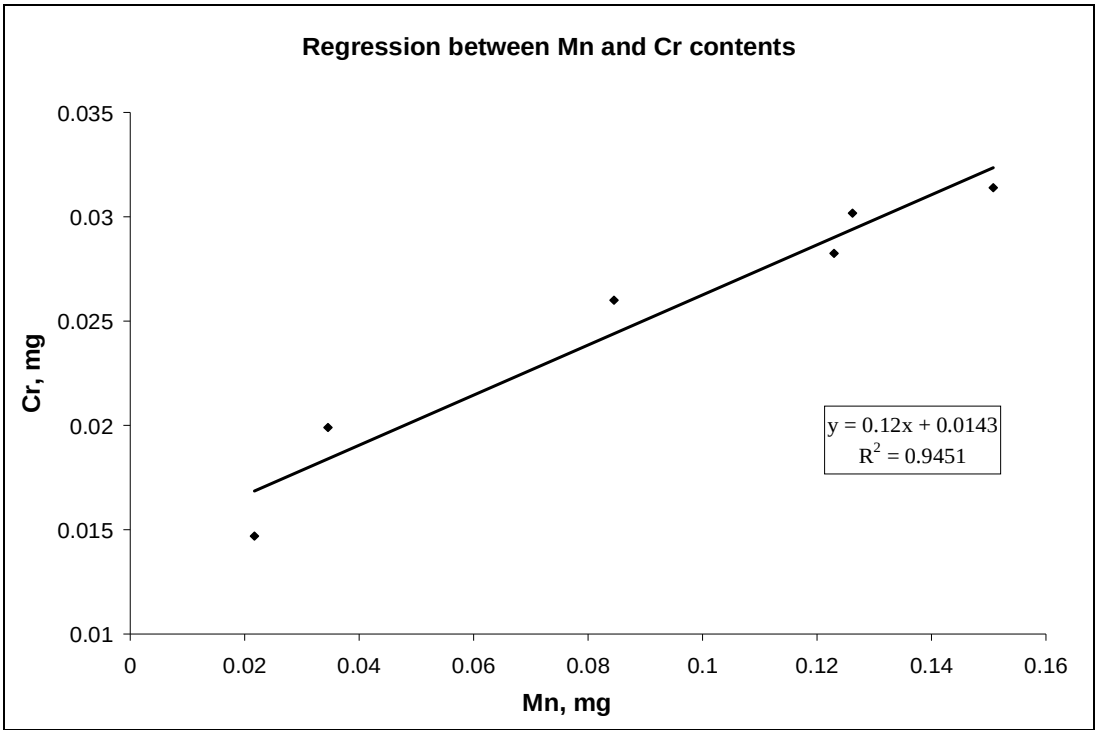


Figure E-7.6: Regression between Mn and Cr contents for R from the MBz source

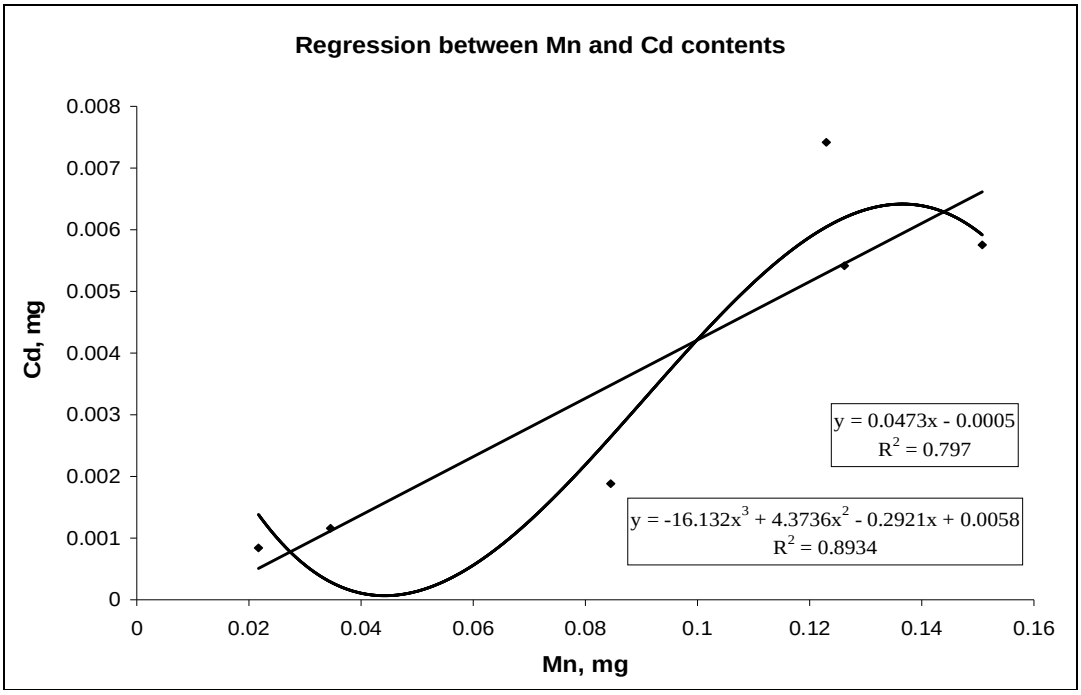


Figure E-7.7: Regression between Mn and Cd contents for R from the MBz source

Regressions for MBzP

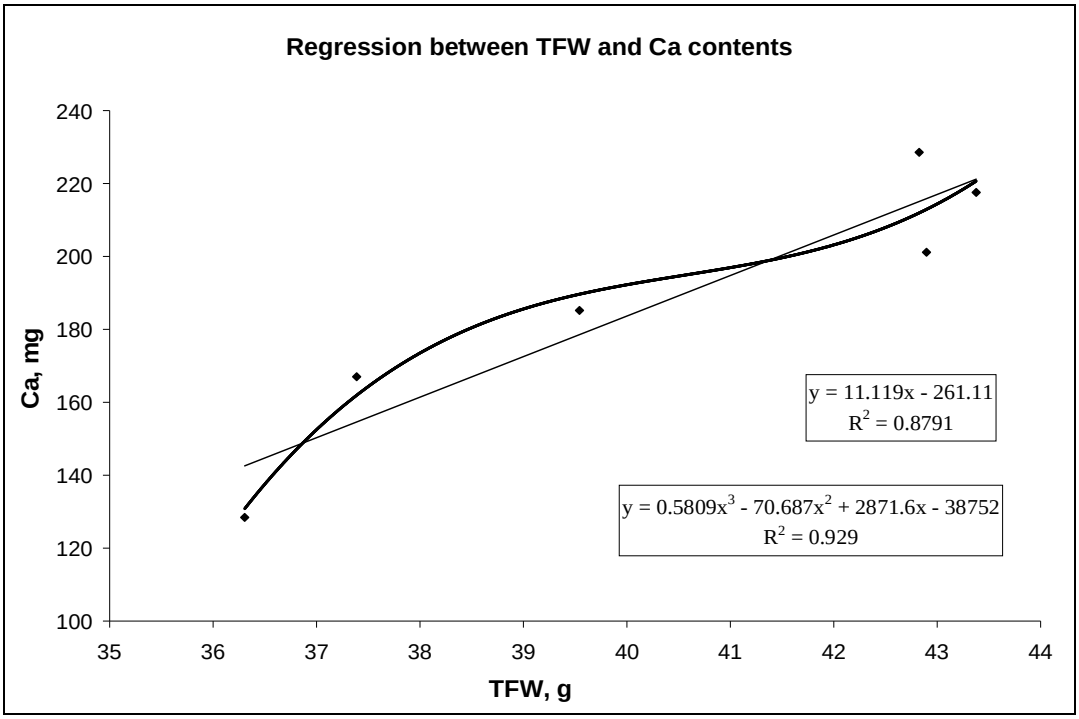


Figure E-8.1: Regression between TFW and Ca contents for P from the MBz source

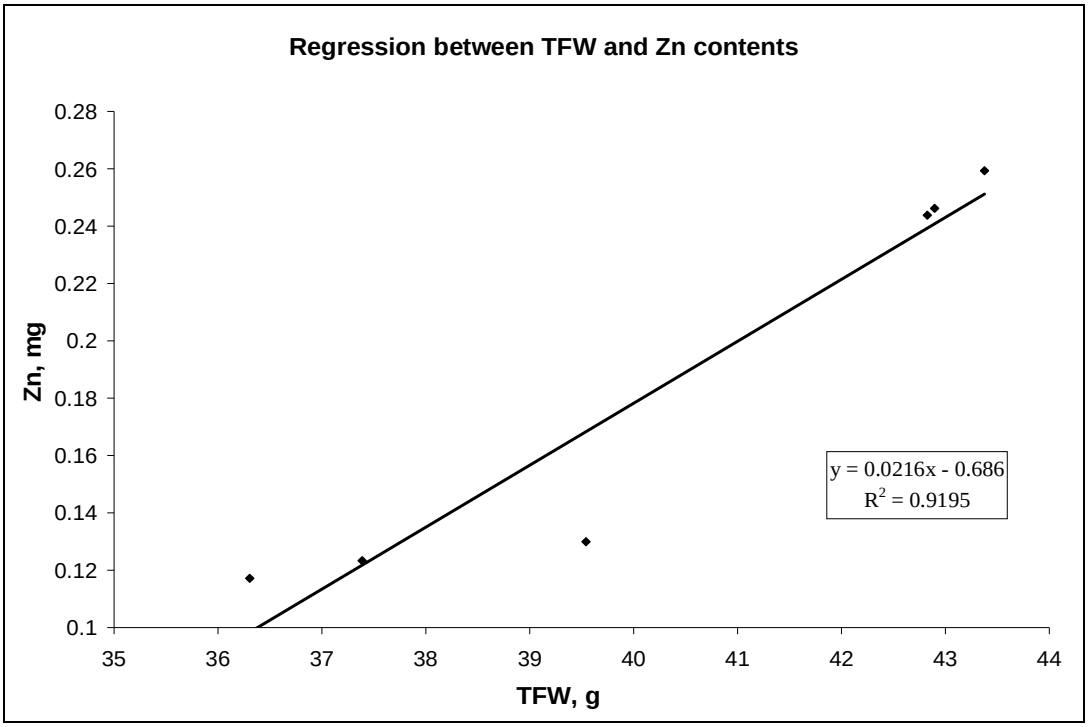


Figure E-8.2: Regression between TFW and Zn contents for P from the MBz source

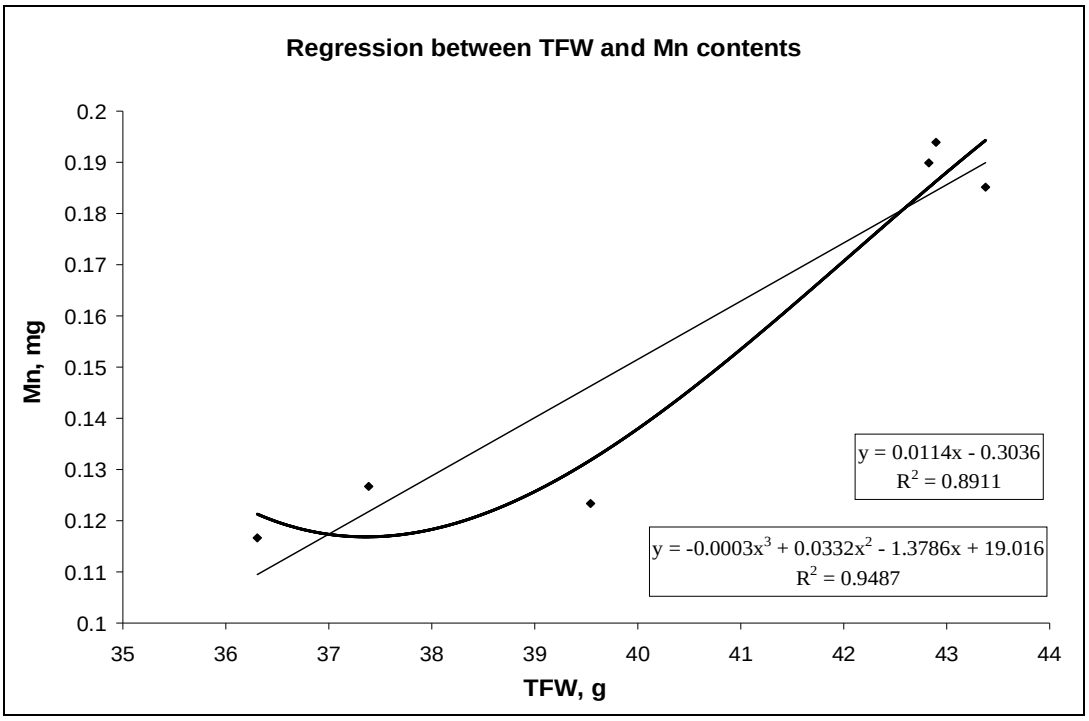


Figure E-8.3: Regression between TFW and Mn contents for P from MBz source

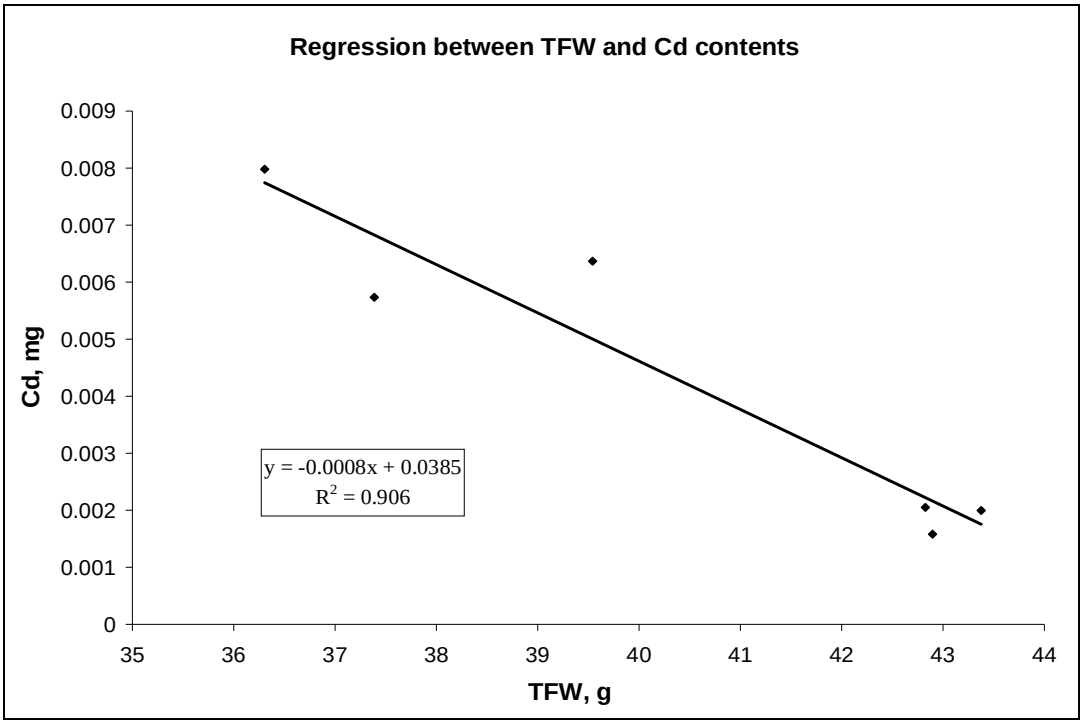


Figure E-8.4: Regression between TFW and Cd contents for P from the MBz source

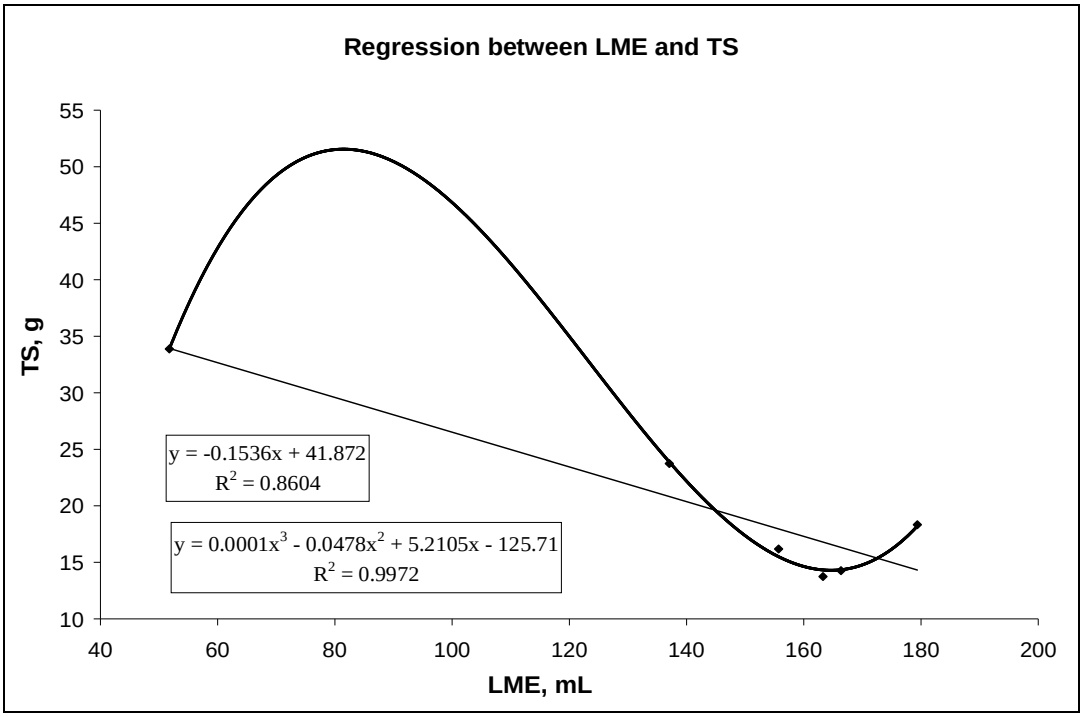


Figure E-8.5: Regression between LME and TS contents for P from the MBz source

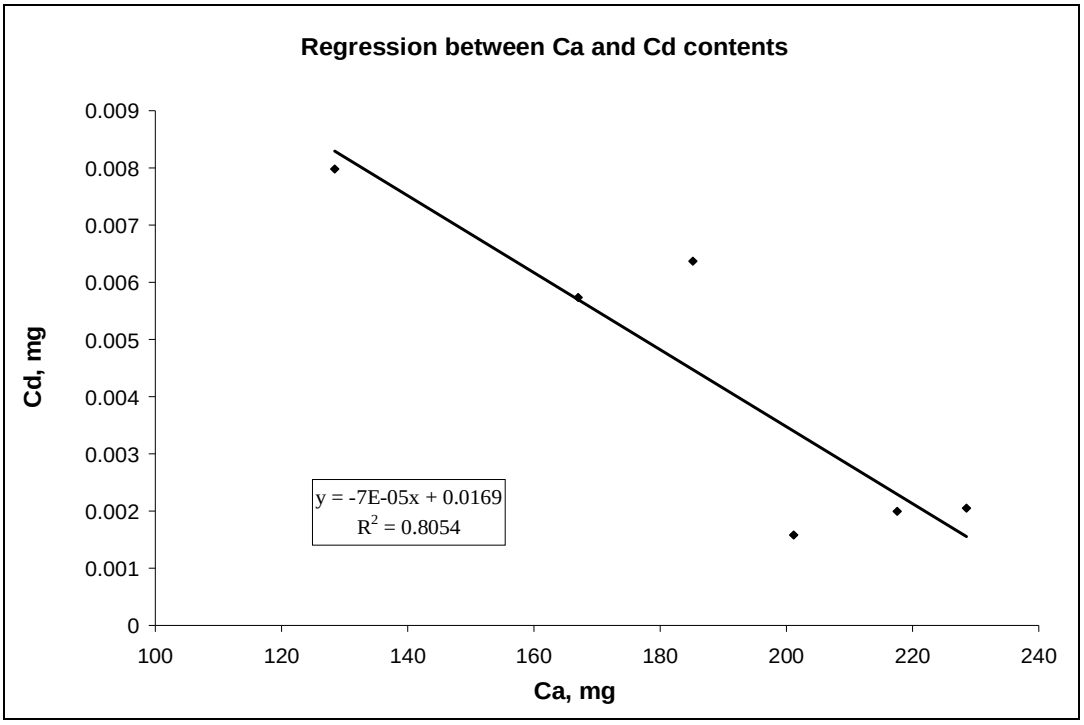


Figure E-8.6: Regression between Ca and Cd contents for P from the MBz source

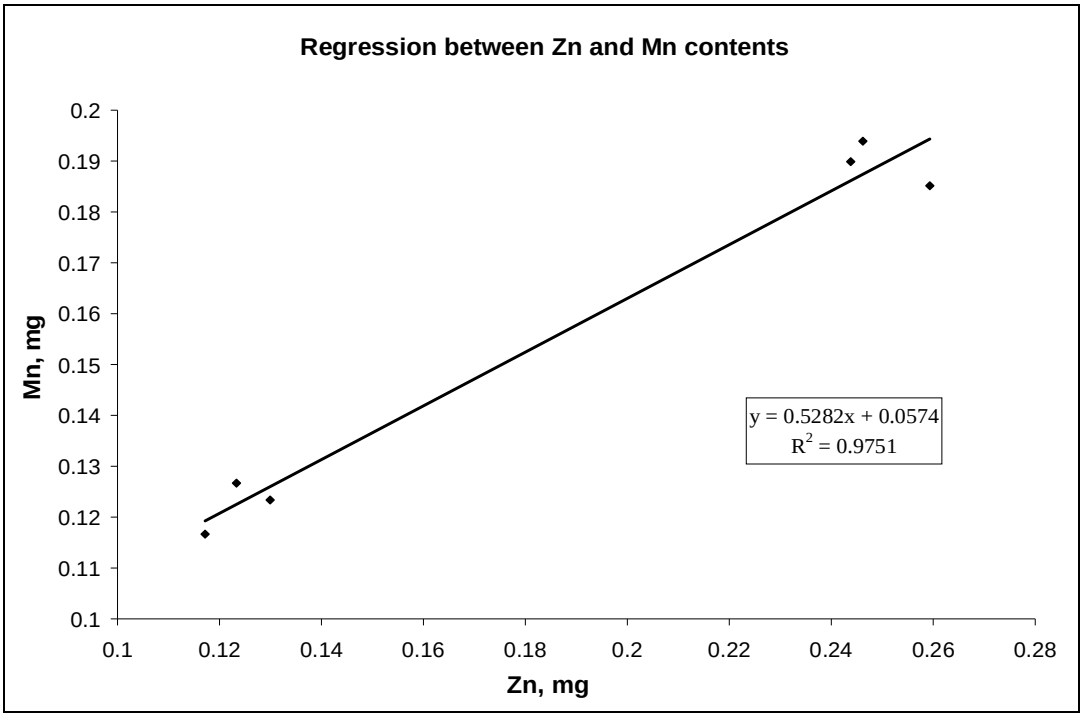


Figure E-8.7: Regression between Zn and Mn contents for P from the MBz source

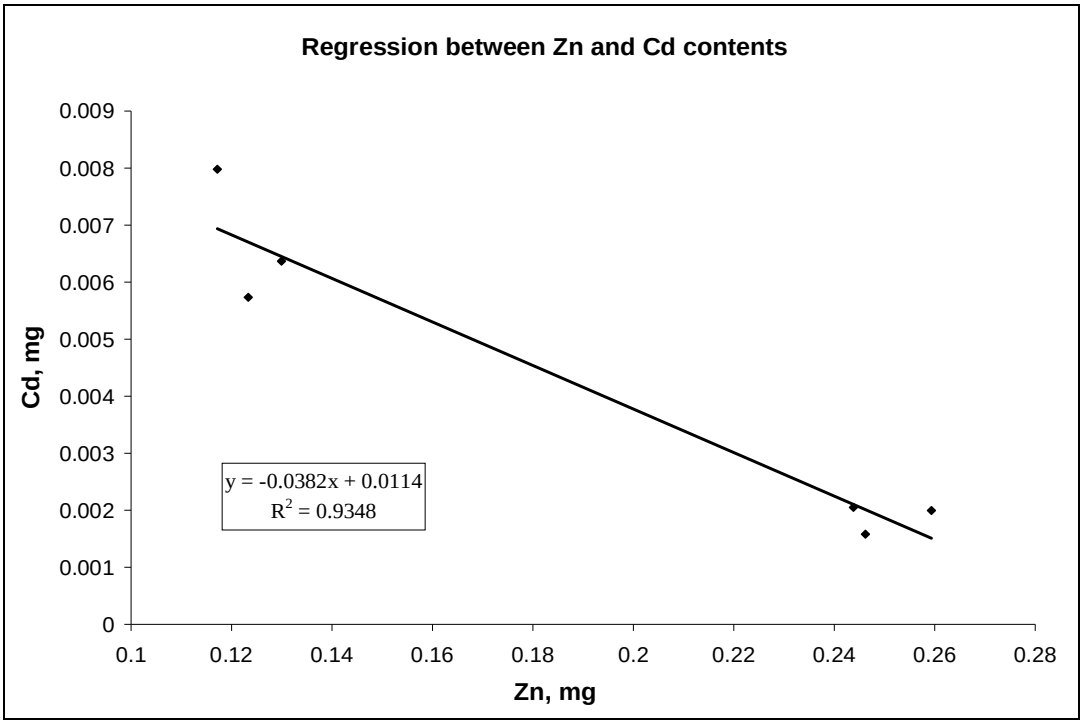


Figure E-8.8: Regression between Zn and Cd contents for P from the MBz source

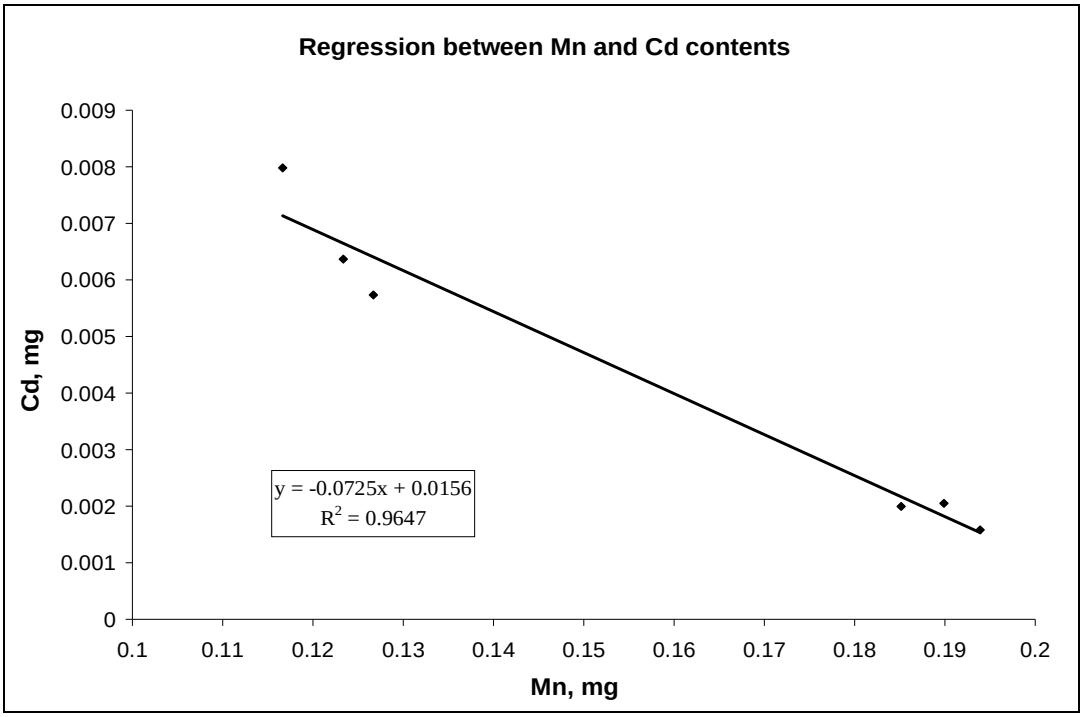


Figure E-8.9: Regression between Mn and Cd contents for P from the MBz source

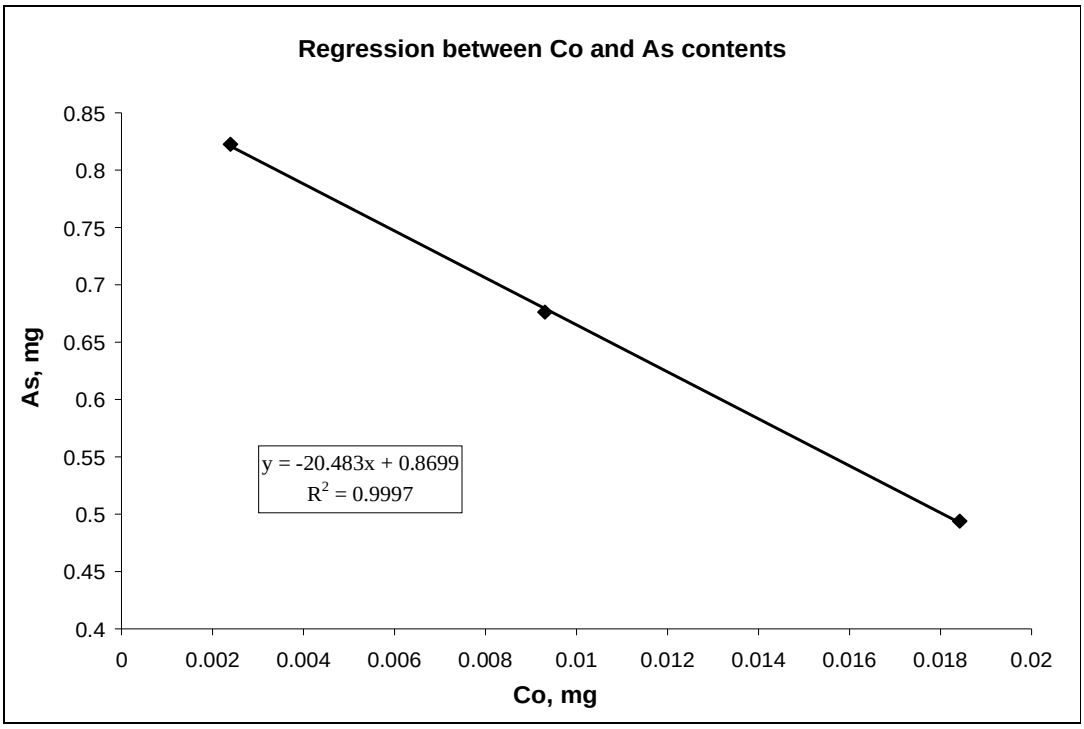


Figure E-8.10: Regression between Co and As contents for P from the MBz source