MICROWAVE ASSISTED DIGESTION OF MILK BASED DAIRY PRODUCT FOR DETERMINATION OF HEAVY METALS WITH ATOMIC ABSORPTION SPECTROPHOTOMETER

A
Thesis

Submitted to the
UNIVERSITY OF KOTA

in the Partial Fulfillment of the Requirements for the
Award of the Degree of
DOCTOR OF PHILOSOPHY

in
Chemistry
under the
Faculty of Science

Submitted by
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Under the Supervision of
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DECLARATION

I, **Aarti Bansal**, hereby certify that the research work presented in my Ph.D. thesis entitled **"Microwave Assisted Digestion of Milk Based Dairy Product for Determination of Heavy Metals with Atomic Absorption Spectrophotometer"** which is carried out by me under the supervision of **Professor Monika Dakshene** and submitted in the partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy of the University of Kota, represents my ideas in my own words and where others' ideas or words have been included in this thesis, I have adequately cited and referenced the original sources.

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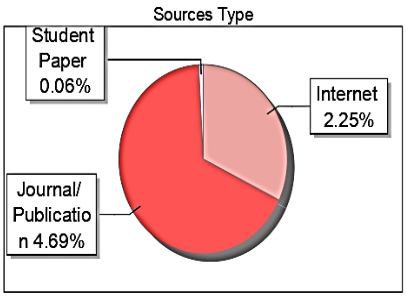

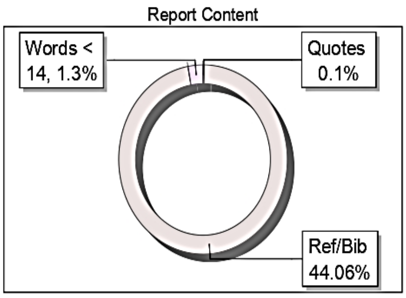

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A WORD OF GRATITUDE

Foremost, I would like to express my sincere gratitude to a brilliant mind, without whom this thesis would have only been a dream, Dr. Monika Dakshene, Professor & Head, Department of Chemistry, Government College, Kota.

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Regards,

Aarti Bansal
(Research Scholar)

Dedicated

To

My Father

Mr. Daulat Ram Bansal

&

My Mother

Mrs. Laxmi Bansal

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"Coming together is a beginning, staying together is progress, and working together is success"

-Henry Ford

These words are very true for the sincere efforts of people followed by adequate planning and dedication to achieve success in any field. My thesis is the result of arduous research and culmination of the process that included constant assistance, support & guidance by my guide.

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Some people are always there for you in happy or sad times, who refresh you, bring a smile to your face, and help you to carry on in life. This credit I give to my children **Shivy Mandhata** and **Tavish Mandhata** for patiently listening to my concerns and frustrations and for believing in me.

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ABSTRACT

Milk and milk based dairy products are an excellent source of proteins, lipids, carbohydrates, vitamins and minerals. Due to their nutritional benefits these dairy products especially yoghurt, butter and cheese considered as complete food throughout the world and consumed by humans. However, the presence of toxicants, particularly heavy metals, in dairy products can have detrimental effects on human health. Various anthropogenic pollutants including livestock husbandry, processing conditions, packaging and sanitation, may also have an influence on the concentration of heavy metals in food items. For this study, 240 samples of dairy products Yoghurt, Butter and Cheese (80 samples of each) were collected from the individual farms and local shops of different areas of kota division of Rajasthan to assess the heavy metal concentration in them. Assessment of six metals i.e. Pb, Cd, As, Al, Fe, and Zn in all dairy samples have been done by Atomic Absorption Spectrophotometer (Shimadzu-6300AA) after microwave digestion. Water, soil, fodder and milk samples were also collected from the same place to calculate the translocation factor and bioaccumulation factor. The result shows that concentration of heavy metal in dairy products collected from more polluted areas are found to be higher than in less polluted areas. From the result, it was also found that the Pb, Cd, Al, Fe and Zn concentrations in the dairy samples of local shops exceeded the permissible limit whereas the concentration of all metals were found to be within the limits from individual farms of less polluted areas. Translocation factor and biological accumulation factor results show that the value of BAF is less than 1, which shows that there is only absorption and no accumulation occurs in the plant. On observing the effect of processing and packaging with time, it was found that the metal concentration increases with time. Estimated daily intake (EDI) and health risk index (HRI), Metal pollution index (MPI) were calculated. For this study one way ANOVA is performed to determine the statistical evidence and significant difference among the cities taken, the pollution status and the types. Statistical analysis was carried out for each metal shows a significant difference for pollution status wise, while city wise and type wise the sum of means were significantly indifferent. To prove the null hypothesis of no difference and difference between the heavy metal concentration, means for all pairs of groups, a Tukey – Kramer test was also done.

Keywords: - Heavy Metals, Dairy products, permissible limits, public health, AAS.

PREFACE

The research presented in this thesis was conducted in the Department of Chemistry, Govt. College Kota under the supervision of Dr. Monika Dakshene, Professor & Head, Department of Chemistry, Govt. College Kota. This thesis is based on the risk associated with human health due to heavy metal contamination through Dairy Products.

The thesis comprises reports of research output in the form of six separate chapters.

I conceived and planned the study, performed the review of the literature, and wrote up the discussion of the literature and framework of our study in chapter I.

The second chapter comprises of research design, analytical procedure adopted, and materials used for the study.

Chapters III, IV, and V incorporate the research findings, data analysis, and interpretation of findings. These chapters consist of detailed heavy metal analysis in the yoghurt, butter and cheese samples of individual farms and local shops of more polluted and less polluted areas of the Kota division, Rajasthan. The effect of packaging and processing on dairy products has also been discussed.

The comparison between the findings of all types of dairy products and their deviation from RDA values is presented in chapter VI. This chapter also includes the methods to evaluate the health risk assessment by calculating EDI (estimated daily intake), MPI (metal pollution index), and HRI (health risk index).

In the course of my research, I learned that no task can be completed without discussions, interactions, and criticism. I owe much to those who supported and guided me with this expertise.

Aarti Bansal
(Research Scholar)

CONTENTS

ABBREVIATION

Pb	Lead
Cd	Cadmium
Al	Aluminium
As	Arsenic
Fe	Iron
Zn	Zinc
RSD	Relative Standard Deviation
BAF	Bio Accumulation Factor
EDI	Estimated Daily Intake
FAAS	Flame Atomic Absorption Spectrophotometer
AAS	Atomic Absorption Spectrophotometer
HRI	Health Risk Index
MPI	Metal Pollution Index
IF	Individual Farms
LS	Local Shops
KR	Kota Ranpur
KK	Kota Kaithoon
BC	Baran Chhabra
BM	Baran Mangrol
BnL	Bundi Lakheri
BnK	Bundi Kapren
JJ	Jhalawar Jhalarapatan
JA	Jhalawar Aklera
DD	Dry Digestion
WD	Wet Digestion
MD	Microwave Digestion
SD	Standard deviation
TF	Translocation Factor
BAF	Bio Accumulation Factor
MPI	Metal Pollution Index
EDI	Estimated Daily Intake
HRI	Health Risk Index
BIR	Beyond Instrumental Range

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CHAPTER - I
THE PERSPECTIVE

Chapter I includes a brief account of the subject and recent developments in the area, the origin of the research problem and the scope of the work.

1.1 Introduction

Milk and dairy products are fundamental components of many diets worldwide, offering a range of nutritional benefit and playing a significant role in various culinary traditions. They are bioactive substances that support the growth, development, nutritional, therapeutic and health benefits of mammals [1-3]. Thus, these products are regarded as almost complete food. So its consumption is increasing day by day [4-7]. Various importance of dairy products are shown in **Fig. 1.1**.

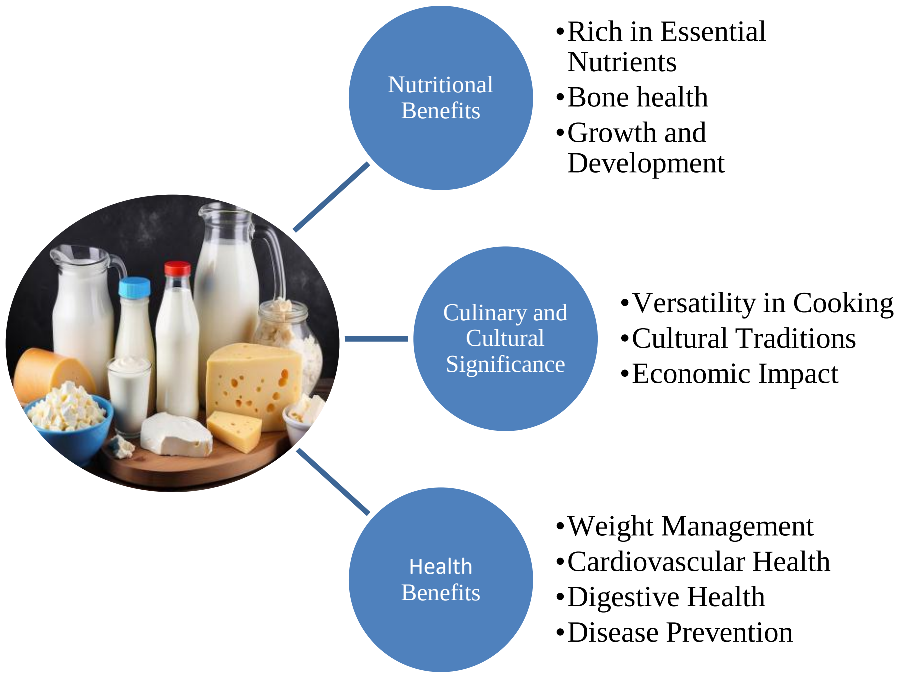

Figure 1.1: Importance of dairy product

Even though these products have numerous beneficial attributes, customers could be exposed to harmful effects on their health because they might include hazardous chemicals and toxic contaminants including heavy metals because of environmental pollution [8]. The continuous rising of the undesirable toxic substances due to urbanization, industrialization, irrigation of crops by the sewage water and industrial effluent, chemical fertilizers and pesticides used to protect the crop, which is the major concern for the health of animals and human beings [9-12].

Kota and its nearby districts like Bundi, Baran and Jhalawar have a wide range of industries like Kota Super Thermal Power Plant, Chambal Fertilizers and chemical limited (CFCL), Shriram Fertilizers, Shriram Rayons, Instrumentation Ltd. NTPC, Anta, Chhabra Termal Power Plant, Ruchi Soya Industries Ltd., ACC Ltd., Kali sindh Thermal Power Project Jhalawar. The neighborhood's primary industries are stone cutting and polishing, which produces a significant amount of slurry waste. The heavy metal concentration in the atmosphere is further increased by the extraction of CaO, MgO and SiO_2.

Kota Super Thermal Power Station (KSTPS) produces 3500 metric tons of fly ash per day, which is composed of various metal oxides, including SiO_2- 58%, Al_2O_3-19%, Fe_2O_3-8%, CaO-0.6%, MgO-0.6%, TiO_2- 1.3%, Na_2O-3.74%, K_2O_2-18%, PbO-0.008%, CuO-0.9%, ZnO-0.9%, and other residues of 3.0%. Due to various and dynamic industries in kota division, it is expected to have a higher level of heavy metal contamination [13]. Along with anthropogenic pollutants in addition to human activities, livestock husbandry [11], processing conditions, packaging [14-16] and sanitation, may also have an influence on the concentration of heavy metals in food items. As a by-product of mammary gland, milk may contain a variety of xenobiotics due to which heavy metals are present in milk products which can cause a serious risk to human health [17].

A number of researchers have reported the presence of potentially toxic metals (mainly Cd, Pb, As, Cr and Ni) in the animal milk, maternal mother milk and in the dairy products [18-21], but there is no such study is found in Rajasthan. So, the objectives of this study are to determine the concentrations of some heavy and trace elements in milk and dairy products and to evaluate their potential health risks to humans.

1.2 Production and Consumption of Milk and Dairy Product

India is the world's top producer of milk, contributing to 24.64% of the world's total production in the 2021–2022 period. The amount of milk produced in 1950 –1951 was only 17 million tons (MT). Before Operation Flood began, milk output was just 21.2 MT in 1968–69. By 1979–80, it had risen to 30.4 MT and by 1989–90, it had reached 51.4 MT. In 2020–21, it had reach 210 million tonnes.

Globally, milk production is currently increasing at an average of 2%, but in India, it is growing at a rate greater than 6%. Over the course of the last nine years or between 2014–15 and 2022–23, India's milk output has increased by 58%, exceeding 230.58 million tons in 2022–23.

Worldwide milk production shows in **Fig. 1.2** while **Fig. 1.3** shows milk production in India.

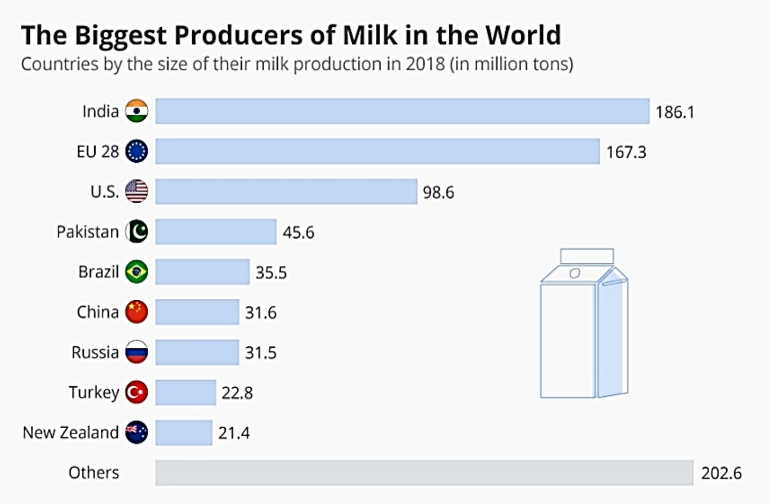

Figure 1.2: Milk production in World in million tons

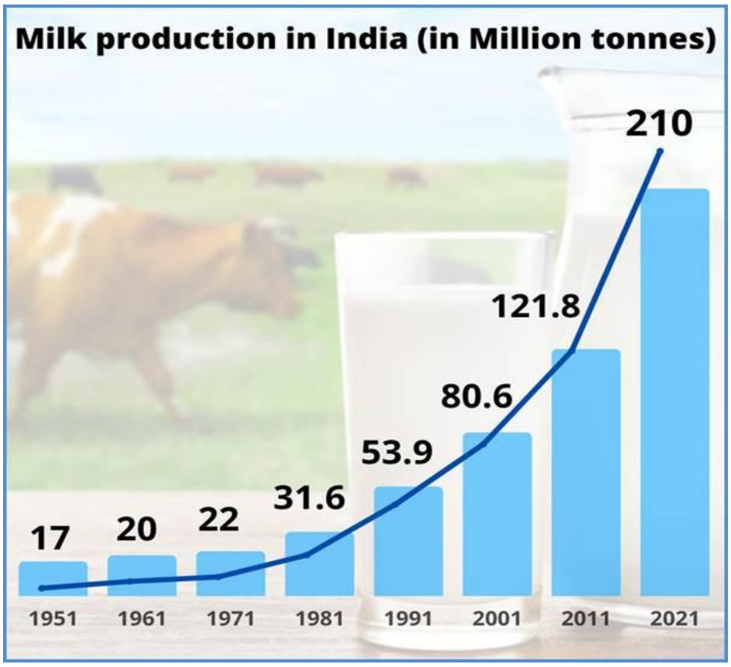

Figure 1.3: Milk production in India in million tonnes

The daily milk intake increased from 107 grams per person in 1970 to 427 grams per person in 2020–21 in India, compared to the global average of 322 grams per day in 2021, in just three decades (the 1980s, 1990s and 2000s). About 25% of all milk produced is considered to be commercially processed of that amount. 70% milk is sold as packaged milk while remaining 30% is utilized to make different dairy products.

Consumption of Dairy Products

Milk and dairy products are the most essential foods and are consumed by both adults and children worldwide [22]. **Fig. 1.4** and **Fig. 1.5** shows the Consumption of dairy products in the world as well as in India respectively.

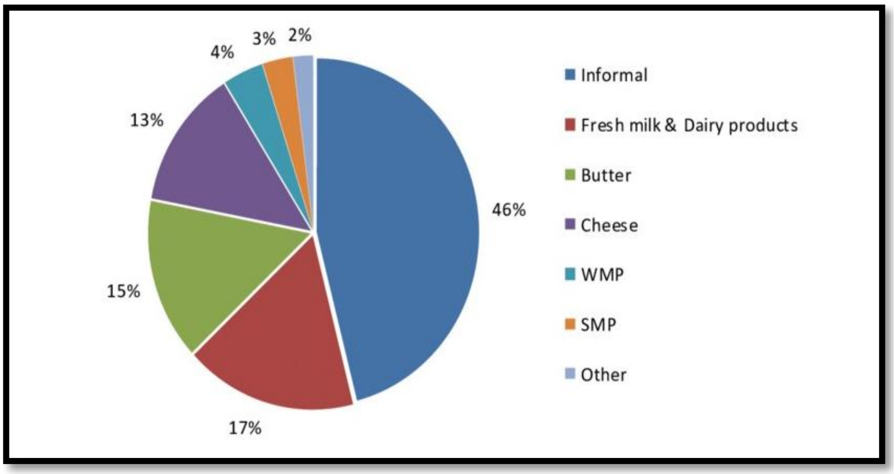

Figure 1.4: Worldwide consumption of dairy products

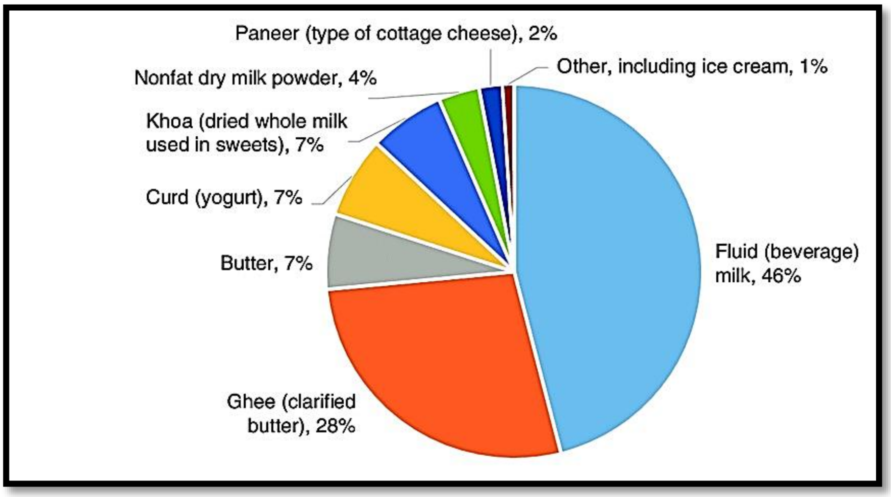

Figure 1.5: Consumption of dairy products in India

1.3 Composition of Dairy Products

Milk and dairy products contain all necessary macro and micronutrients such as minerals, fats, sugars and these are a good sources of calcium, riboflavin and phosphorus. It also contains protein, potassium, vitamin A, vitamin D, vitamin B-12 and niacin in good proportions. So dairy products are important sources of nutrition for human body. Therefore, the study of the composition of dairy products are necessary. The composition of dairy products (Yoghurt, Cheese and Butter) are shown in **Table 1.1**.

Table 1.1: Composition of Yoghurt, Cheese and Butter

Composition	Yoghurt	Cheese	Butter
Water	81 %	37 %	16-17.5
Protein	9 %	23 %	-
Fat	5 %	33 %	80-82
Carbohydrate	4 %	3.5 %	-
Sugar	4 %	-	-
Minerals and other compounds	-	3.5 %	1 %
Salt	-	-	1.5 %

Table 1.1 indicates that the maximum amount of water present in yoghurt i.e., 81% while in cheese 37% of water is present and in butter water content ranges from 16 - 17.5%. The amount of protein, which is the building blocks of living cells are 9% and 23% in yoghurt and cheese respectively. Fat content in yoghurt and cheese are 5% and 33%, while it ranges from 80-82% in butter. The level of carbohydrate and sugar in yoghurt is 4%. The remaining constituents like minerals, salts and other compounds are also found in less amount in dairy products and play a very important role in human's body [23,24].

1.4 Types of Contamination in Dairy Products

(I) Physical Contamination:

Physical Contaminants in dairy products include utensils that are not clean and other factors like dirt particles and hair are also responsible for the contamination. Physical contamination also results from washing milk equipment by uncleaned water. Chewing of tobacco by milkmen, dirty hands, dirty udders and udder infections can also lead to contamination [25]. The contamination can also occurs by milking equipment, storage containers and milk transportation. The dairy workers who handled the milking and processing are also accountable for any such contaminations [26]. Due to untreated water supply by natural resources, water accumulates lots of contaminants which directly or indirectly affects the quality of water and finally food and dairy items.

(II) Chemical Contamination :

The majority of chemical pollutants found in milk and dairy products come from veterinary drugs (sulphonamides and antibiotics), hormones, anthelminthic medicines and pesticides.

(A) Veterinary Drugs :

(i) Antibiotic : Most of the ailments of cattles can be treated by antibiotic drugs like penicillin, tetracycline, streptomycin and erythromycin etc.[27,28]. These drugs can be given orally and by injected directly in skin. Residues of these drugs are usually detectable in lactating cows [29].

(ii) Parasiticides Drugs : These drugs usually used for destroying internal parasites like – tapeworms and roundworms in cattles. A widely used parasiticides drug is albendazole. This drug is quickly absorbed in cattles gut and transformed in to metabolites [30,31]. These metabolites are present as residual in milk and dairy products and have toxicological significance. This drug and it's metabolites are considered as mutagenes [32].

(B) Hormone :

(i) Steroid Hormones: Another excellent source of steroid hormones is milk. The fat content of milk and dairy products affects the levels of lipophilic hormones. With increased fat content, not only progesterone but also estrone does rise.

It appears that food processing has no effect on the ratios and quantity of hormones. Cheese ripening appears to have an impact on the hormones in milk and dairy products, while food processing like heating or churning seems to have no effect at all. Testosterone was found in both fresh and mature cheese (0.1–0.5 mg kg^{-1}). During the fermentation process, testosterone is most likely formed not just by propionic acid bacteria but also by other fermenting bacteria or clotting enzymes [33].

(ii) Bovin Growth Hormone & Somatotropin Hormone :

Genetically designated hormone Bovine Somatotropin Hormone (BST) is identical to the natural pituitary product Bovine. It is used for lactating cattle to increase the milk production around 10-15%. [34-40]. There is a controversy raised regarding the use of BST hormone. United States and Europe has concluded that there is no adverse effect on humans whereas WTO (World Trade Organization) has partially accepted this [41].

(C) Pesticides and Insecticides :

Milk and dairy products are contaminated by pesticides and insecticides as they are used to protect the vegetation. The cattle when grazed the treated crops, these pesticides enter in their body and accumulate in their tissues and finally transfer to milk and dairy products. Some common pesticides like: DDT, dioxins, polychlorinated biphenyl (PCBs) are persist in the environment and through food chain they cause contamination [42]. Milk retains 20% of ingested chlorinated pesticides which adhere to butter and milk fat [43].

(D) Heavy Metals :

Metals like zinc (Zn), copper (Cu), iron (Fe), and selenium (Se) are necessary for the body in order to maintain its metabolism and are also necessary for chemical, biological and enzymatic interactions in both humans and animals. They are defined as heavy metals, which have atomic weight in between 63.5 to 200.6 g/mol and specific gravities more than 5 g/cm^3 [44-46]. Heavy metals can neither biodegrade nor undergo thermolysis and are found in all forms of nature. So they are harmful to humans even if they are present in trace amounts [47].

Heavy metals are a major contributor to the health risks associated with contaminated food. By removing the original metals from their native binding locations, these metals attach themselves to protein sites rather than their designated metals, which results in cellular malfunctioning and eventually poisoning [48].

Heavy metals are environmental pollutants whose toxicity poses a serious threat to nutritional, evolutionary, ecological and environmental balances [49,50]. They are highly poisonous and have negative effects on living beings because of their accumulation in ecosystem (water, soil, plant and animal) [51-53]. Both natural and man-made processes release the heavy metals into the environment.

1.5 Classification of Heavy Metals

According to a review of the literature, the following metals are classified as heavy metals: Arsenic, Aluminium, Mercury, Iron, Chromium, Lead, Copper, Cobalt, Zinc and Cadmium. Other names for heavy metals include "micronutrients," "trace inorganics," "toxic elements,". More than 60 elements in different parts of human body have been detected, but only 17 are available in living cells.

From a nutritional perspective, there are many elements found in milk and dairy products. These are classified into two categories i.e., essential and non-essential, which are given in **Table 1.2**.

Table 1.2 : Classification of metals as essential and non-essential metals

Essential Metals (Harmless)	Non Essential Metals (Toxic)
Zinc (Zn)	Zirconium (Zr)
Copper (Cu)	Lead (Pb)
Iron (Fe)	Arsenic (As)
Cobalt (Co)	Mercury(Hg)
Mangnese (Mn)	Cadmium (Cd)
Chromium (Cr)	Aluminium (Al)

Essential heavy metals exhibit lower toxicity at lower concentrations and act as coenzymes in biological processes. For example - Hemoglobin and myoglobin include iron and vitamin B12 contains cobalt. Heavy metals that are not essential have a strong harmful effect on living things, even at extremely low quantities.

These heavy metal categories have the potential to cause a range of health issues and have extremely dangerous harmful effects on consumers, when their concentrations exceeded the maximum permitted levels. Different elements have different levels of toxicity and the toxicity and necessity vary from element to element [54]. So on the basis of toxic level these elements also classified in to different categories, which is shown in **Table 1.3**.

Table 1.3: Classification of elements on the basis of toxicity

Low toxicity			Very toxic, relatively accessible			Toxic, low solubility		
H	C	F	Pb	As	Cu	Ti	Ga	W
Li	P	O	Co	Se	Ag	Zr	La	Os
Na	Mg	F	Te	Ni	Au	Hf	Nb	Rh
K	S	Sr	Pd	Be	Zn	Ir	Ta	Ru
Ca	Cl	Al	Bi	Sb	Sn	Re	Ba	
Rb	Br	Si	Cd	Hg	Pt			

The human body contains relatively few heavy metals, such as iron (Fe), manganese (Mn), selenium (Se) and molybdenum (Mo), which are considered as more significant vital micronutrients. Conversely V, Co, Ni, Cu, Zn, Cr and W are less significant and may be hazardous in excess, while heavy metals such as Pb, Cd, Mg, As and Sb are poisonous to living beings [55].

1.6 Physical and Chemical Properties of Heavy Metals

- **Lead (Pb) :** Lead poisoning is regarded as the most frequent environmental health hazard and is one of the most widely distributed environmental metal poisons [56-57].
- **Cadmium (Cd)**: The kidneys and liver are the primary organs where cadmium accumulates, as it is a highly harmful and unnecessary element for human health [58-59].

- **Aluminium (Al)**: Aluminium is a less hazardous and non-essential element for human health. Harmful activities of Al cause peptide denaturation or transformation, oxidative stress, immunologic changes, genotoxicity and a pro-inflammatory impact.
- **Arsenic (As):** Although arsenic is a metalloid, heavy metal toxicity is the term used to describe it because of its carcinogenic and poisonous properties. Exposure to arsenic affects the human health.
- **Iron (Fe)**: Small quantity of iron is required in the diet that is why it is a necessary metal but when present in excessive concentrations, it can be extremely detrimental to humans [60].
- **Zinc (Zn)**: One of the essential minerals for proper growth and development of the human body is zinc [61-62].

The **Table 1.4** displays the physical and chemical characteristics of a few chosen heavy metals for this study.

Table 1.4: Physical and chemical properties of some selected metals

Properties	Unit	Pb	Cd	Al	As	Fe	Zn
Atomic Number	-	82	48	13	33	26	**30**
Atomic Mass	g/mol	207.211	112.411	26.981	74.921	55.846	65.38
Electronic Configuration	-	$[Xe]4f^{14}5d^{10}6s^2 6p^2$	$[Kr]4d^{10}5s^2$	$[Ne]3s^2 3p^1$	$[Ar]3d^{10}4s^2 4p^3$	$[Ar]3d^6 4s^2$	$[Ar]3d^{10}4s^2$
Density (ρ)	g/cm^3	11.35	8.66	2.7	5.71	7.79	7.14
Melting Point	^0C	327.5	321.17	660.3	808.9	1535.5	419.5
Boiling Point	^0C	1739.8	764.8	2470	615.5	2751	907
Atomic Radius	A^0	1.54	1.61	1.18	1.14	1.56	1.42
Ionic radius	A^0	1.33	1.55	1.25	1.15	1.40	1.35
Vander Waals radius	A^0	2.03	1.56	1.84	1.86	1.27	1.39
Electronic Negativity	-	1.86	1.69	1.61	2.19	1.82	1.65
Energy of 1st ionization	kJ/mol	715.3	867	578	946	760	906.4
Energy of 2nd ionization	kJ/mol	1451	1621	1817	1798	1556.4	1733.3

1.7 Sources of Heavy Metals in Dairy Products

The earth crust and surface soils naturally contain heavy metals in varied amounts. The erosion of soil, the natural weathering of the earth's crust, industrial effluents, mining, urban runoff, sewage discharge, pesticides and disease control agents and fertilizers used on crops are few examples of the many natural and anthropogenic sources of heavy metals [63]. Nickel and Cadmium are the examples of heavy metals, which are widely distributed in the environment and typically found in industrial compounds such as phosphate fertilizers. Use of excessive fertilizer not only cause the soil infertility but also lowers soil microbial activity. These compounds are absorbed by the plants and transferred into animals and humans.

Soil has also contaminated by air and water, which is the primary cause of lead and mercury poisoning. Additionally, all plant parts like roots, stems, leaves and fruit, that are growing in contaminated soil can acquire metals [64]. Large concentrations of Pb and Cd can build up in plants such as rice, grass, some leguminous species which are used as cattle feed and also vegetables [65]. The majority of Cd builds up on grains, crops and grain-based goods [66]. Heavy metal contamination in different food sources are shown in **Table 1.5** .

Table 1.5: Food sources for heavy metal contamination

Contaminant	Food Sources
Lead	Carrots, lettuce, moonshine, beetroots, honey, smoked food, wine, beer, wheat, buckwheat, milk, breast milk, tamarind candy, mustard, some traditional medicines, raisins, almonds, calcium supplements, cocoa powder, rice, potatoes, paprika powder, and mussels
Cadmium	Wheat, corn oats, pig, duck liver paste, wine, beer, peanuts, soybeans, rice, medicinal herbs, culinary herbs, sunflower kernels, milk, cheese, egg, fish, mushroom, garlic, and spinach.
Mercury	Fish oil, seafood, mushrooms, eggs, cetacean products, and human milk
Arsenic	Fish, algal products, tomatoes, wheat, soy sauce, cooked spinach, chicken and bovine meat, carrots, green papaya, rice, sheep, wine, and milk

Heavy metals can be transfer from industrial sites to canals and rivers by direct discharge from contaminated locations. High concentrations of heavy metals may also be present in water storm runoff from city roadways. Commonly observed heavy metals in waste water include nickel, copper, zinc, chromium, lead and arsenic. Atmospheric deposition, waste disposal, vehicle exhausts and urban effluent are some of the major environmental sources of metal which is present in milk and dairy products [67].

The burning of fossil fuels (coal, petroleum oil and its by-products), along with industrial and municipal sewage, is the cause of zinc pollution in the environment. Zinc can migrate to ground waters from mine and industrial waste due to its high solubility[68].

Chemical pollutants that can be exposed in animal feed and found in milk residues after consumption include drugs, heavy metals, radionuclides, mycotoxins and pesticides [69].

The quantity of heavy metals in uncontaminated milk is obviously not very high but manufacturing, processing and packaging procedure have the potential to drastically modify and magnify their concentration [70].

All of these are hazardous to the ecosystem because they can easily accumulate in environment naturally or as a result of human activity, which makes it easier for them to enter in to the food chain and raises the risk to both animals and human's health [71].

1.8 Incorporation of Heavy Metals in to the Food Chain

It has been extensively documented worldwide that human populations consume harmful heavy metals via food chains [82]. Heavy metal concentrations in water, air and soil have increased as a result of industrial and agricultural activity. Even when waste water is treated at sewage, toxic metals are typically left behind. Heavy metal concentrations in water, air and soil have increased as a result of industrial and agricultural activity. This raises the possibility of soil contamination by heavy metals. After that, they are absorbed by plants and crops. When cattle graze this contaminated crop and drink polluted water on grassland, these heavy metals and other pollutants can accumulate in the tissues of animals such as cows, sheep and buffalos, posing a health risk to them. As a result, milk and dairy products get contaminated by heavy metals [73-76].

There are several other ways that heavy metals can enter the food chain. **Fig. 1.6** shows the multiple routes of heavy metals entering into food chain.

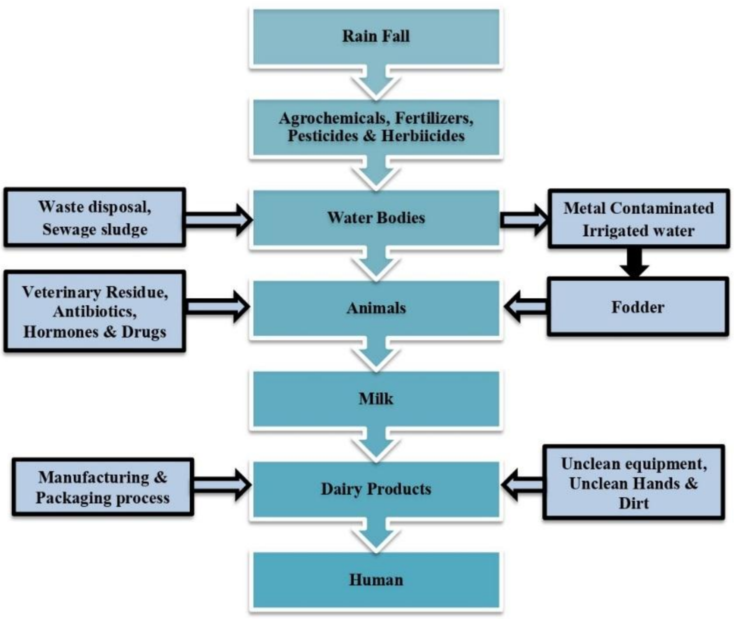

Figure 1.6 : Factors responsible for milk & Dairy Product contamination

There are multiple routes in the food chain where humans could come into contact with trace metals. These metals represent a serious risk to the health of people and animals because they are incorporated into raw milk and dairy products through the food chain [77].

1.8.1 Incorporation of Heavy Metals in to Dairy Products due to Processing & Packaging

Dairy products may get contaminated during the manufacturing and packaging processes [78]. Packaging is an essential factor of processing, so it affects the quality of dairy products by various contaminants. Plastic containers, PET bottles, polycarbonate bottles, LDPE pouches, paperboard laminate cartons and tetra packs are a few examples of packaging materials. Packaging can raise the risk of cancer by 12% [79]. Too much added sugar is blame for stomach disorders, high blood pressure and diabetes. Added artificial substances such as colour, texture, odour and additives are also responsible [80–82]. Human health may be at risk due to the migration of contaminants from packaging materials into the milk and dairy products [83]. Canned dairy products are considered as a source of heavy metal toxicity due to migration of metals from can to product, during long storage period [84].

Dairy product packed in plastic containers have different element levels than samples packed in tin containers [85].

1.8.2 Chemistry involved in heavy metal pollution

$$2\ FeS_2 + 2\ H_2O + 7\ O_2 \longrightarrow 2\ FeSO_4 + 2\ H_2SO_4$$

$$2\ FeSO_4 + 2\ H_2SO_4 \longrightarrow Fe_2(SO_4)_3 + SO_2 + 2\ H_2O$$

$$Fe_2(SO_4)_3 + 2\ FeAsS + 9/2\ O_2 + 3\ H_2O \longrightarrow 2\ H_3ASO_4 + 4\ FeSO_4 + S$$

Presence of water and bacteria can lead to the formation of monomethyl mercury and dimethyl cadmium.

$$M + \text{organic matter} \text{ ---}H_2O\text{, Bacteria} \longrightarrow CH_3M + (CH_3)_2M$$

These organic forms are said to be extremely dangerous and can contaminate subsurface water by leaching.

1.9 Hazardous Effects of Heavy Metals

Human need trace levels of iron, cobalt, copper, manganese, molybdenum and zinc but in greater amounts, all metals are hazardous [86]. Other heavy metals such as lead, mercury, arsenic and cadmium are poisonous and have no known vital effects on organisms. It is reported that over the time, these metals accumulates in the bodies of animals and cause serious illness. These are extremely dangerous because of their too long biological half lifetime and inability to biodegrade [87]. Chronic exposure to heavy metals can have detrimental effects on the circulatory, central and peripheral neurological systems.

Heavy metal contamination of milk poses a serious risk to human health because even small amounts of the metals can accumulate to significant concentrations in the body. Such as lead and mercury persist in the body and exert their toxic effects by combining with one or more reactive groups which are necessary for normal physiological functions. This can result in cellular disruptions [88,89].
Extremely hazardous substances such as lead, mercury, cadmium and their impact on health are demonstrated in **Table 1.6** [90-93].

Table 1.6 : Metals and their toxic effects on humans health

Metal	Toxic effect
Lead (Pb)	Development of different cancers, damage to the liver, heart, blood vessels and reproductive systems, anaemia, weakening of the immune system, and problems with the central and peripheral nerve systems also include kidney failure. Furthermore induce hepatitis and encephalitis.
Cadmium (Cd)	Chronic cumulative poisoning damages the kidneys, bones, lungs, liver, heart and blood vessels.
Mercury (Hg)	The fetus is harmed in addition to the central and peripheral nervous systems by long-term brain and liver damage.
Chromium (Cr)	Lung cancer and DNA damage.
Nickel (Ni)	Causes local infections as well as a range of malignancies of the brain, bone and blood.
Arsenic (As)	Cause cancer of the lungs, liver and urinary bladder.
Zinc (Zn)	Cause cramping in the abdomen, nausea, vomiting, diarrhea, bleeding in the stomach in rare situations.
Copper (Cu)	Menke's disease (severe mental retardation, unusual hair, neurological damage) and Wilson's disease (excessive buildup of Cu in the brain and cornea).
Manganese (Mn)	Neurotoxic condition that can impact behaviour regulation and dopamine balance.

Heavy metal toxicity can lower energy levels and harm the liver, kidney, brain, blood composition, lungs and other important organs. Persistent exposure to some metals can cause degenerative processes that mimic multiple sclerosis, Alzheimer's, Parkinson's and muscular dystrophy as well as worsening neurological, muscular and physical symptoms. Certain metals and their compounds can even cause cancer if exposed to them repeatedly over an extended period of time.

Heavy metals can have cumulative harmful effects that lead to chronic degenerative changes, particularly in the brain system, liver and kidneys [94].

In certain situations, they can also have teratogenic and carcinogenic effects due to which heavy metals are considered hazardous [95].

Therefore, it is necessary to investigate the concentrations of heavy metals in dairy products made from milk and assess the potential health concerns.

1.10 Sources, Toxicity Mechanism and Hazardous Effects of Heavy Metals selected for Study

1.10.1 Lead (Pb):

- **Source: -** Pb is present in the environment due to various anthropogenic activities like mining, smelting, industrialization, fossil fuels burning, gasoline, plumbing pipes, house paint etc. as well as naturally occurring sources [96-98]. In batteries, cosmetics lead is most commonly used [99]. Approximately two metric tons of lead is being released by vehicles which affects the soil, plants and water bodies [100]. The percentage of lead in the environment cause global pollution and can pollute air, water, soil and the food chain [101]. Pb exposure in humans is primarily caused by drinking contaminated water and eating contaminated food.
- **Toxicity Mechanism:** Lead metal toxicity in healthy cells is mainly caused by an ionic mechanism which involves an imbalance among the synthesis of highly reactive intermediates and antioxidants to deactivate them. The primary reason of the ionic mechanism for lead toxicity, which disrupts the biological metabolism of the cell, is the ability of lead metal ions to substitute monovalent and bivalent cations [102]. Lead toxicity affects a number of biological processes, including protein folding, maturation, ionic transportation enzyme control, cell adhesion and apoptosis and significant changes have also been observed [103].
- **Hazardous Effect: -** It has been documented that excessive Pb levels in the human body cause edema, behavioral and mental impairments and seizures [104–106]. The Environmental Protection Agency classifies lead (Pb) as a carcinogen [107]. Chronic lead poisoning damage brain and kidney [108]. Blood lead level (BLL) is the outcome of lead accumulation in the human body.

  Both inorganic and organic forms of lead are present in nature, but the form which initially emerged in the environment was an inorganic form which is unfavorable to human for neurodevelopment and the nervous system. The central nervous system is the main organ affected by Pb poisoning [96]. Lead poisoning damages the nervous system and lowers the activity of several biosynthetic enzymes and Pb toxicity has also linked to neurobehavioral problems [109,110], which includes mental impairment and delayed development of the nervous system and other body organs [111,112]. The lead toxicity in human causes lung cancer, intestinal cancer and central nervous system.

  The types of carcinogenic effects of lead toxicity were explained in **Fig. 1.7**.

Figure 1.7 : Carcinogenic effect of Lead

Institute for Health Metrics and Evaluation studies of 2017 reported that lead exposure was responsible for 24.4 million disability-adjusted life years (DALYs) and 1.06 million fatalities [113].

Table 1.7 : Sources of contamination of Lead and their effects

Heavy Metal	Sources of contamination	Effect on human health	Reference
Pb	Ammunition, vehicle exhaust, batteries, corrosive container lids, contaminated soil, cosmetics, fertilizers, foods (if grown in contaminated soil), hair coloring products, insecticides, pesticides, paints that contained lead, lead-glazed pottery, solder, tobacco smoke and water (if transported through lead pipes), among other items.	The neurological system, kidneys, bones, heart and blood are the main organs that are impacted by poisoning with lead, and pregnant women and infants are especially significant risks. It may also result in attention deficit disorder (ADD), trouble with learning, behavior abnormalities and other developmental difficulties. It can also have a deadly effect on development and impede growth.	[96,103,104, 114]

Major symptoms of lead poisoning encompass inflammatory stomatitis, irregularities in sperm count, reduced libido, infertility and blue gingival tissue. Additionally, menstrual disorders including spontaneous miscarriage and irregular ovarian cycles may be experienced by women who exposed to lead [115].

1.10.2 Cadmium (Cd):

- **Source: -** Cadmium is widely utilized in the battery, PVC stabilizer, alloy and pigment/coating industries [116]. Cadmium is used extensively in some industrial processes, such as the manufacturing of fertilizers, iron and steel, cement, and fossil fuels [117]. Excess amount of Cadmium is produced by smelting of zinc from its ore i.e., sphalerite (zinc sulphide) as sphalerite can include up to 3% of cadmium sulphide [118]. Cadmium is mostly utilized in plastic stabilizers, coatings and plating [119].
- **Toxicity Mechanism: -** It has been reported that cadmium easily connect with ligands such as cysteine, histidine, aspartate, etc. that cause iron deficiency [120]. Although the exact mechanism of cadmium toxicity is not known but its effects on cells are well known [121]. Organic matter in soils has a considerable adsorptive capacity for cadmium. Food absorbs more Cd when it is present in the soil, which is quite harmful [122].

  In acidified soils more Cd is absorbed by plant, as a result uptake of Cd through food will rises [123]. Due to chemical similarities of cadmium and zinc, they have certain toxicological characteristics [124]. Cadmium is bio-persistent element which stays in living organisms for many years [112].
- **Hazardous Effect**: Cadmium is regarded as one of the metals that causes the greatest risk to human health [125]. After it's prolonged exposure, a normal cell changes into a cancerous cell [126]. Increased cadmium levels cause infertility by lowering sperm count [127]. Cardiovascular disease is also caused by cadmium exposure [128]. The metabolic pathways for vitamin D are affected by cadmium. Kidney injury is caused by elevated blood cadmium levels. Urinary cadmium has numerous detrimental effects on various tissues, including the mammary glands, the lungs, periodontal tissues, excessive blood pressure and diabetes [122,129-131]. Even low concentrations of cadmium exposure to humans can have negative health effects [132]. Cadmium poisoning is the cause of "Itai-itai" sickness, which is characterized by severe pain in the spine and joints [133]. The types of carcinogenic effects of cadmium toxicity has given in **Fig. 1.8.**

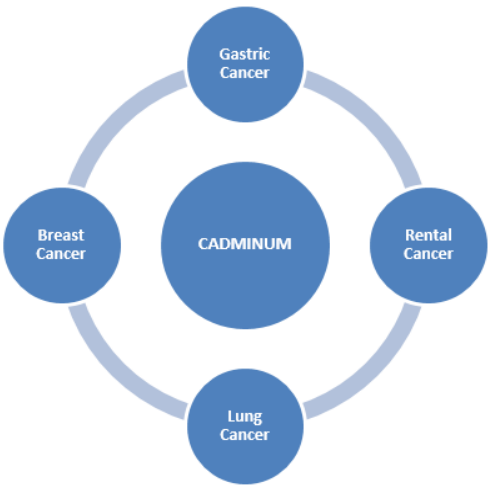

Figure 1.8: Carcinogenic effect of Cadmium

Cadmium shows its toxic effects on the gastric system and leads to gastric cancer, lung cancer, breast cancer and it also effects the excretory system and leads to renal cancer.

Table 1.8: Sources of contamination of Cadmium and their effects

Heavy Metal	Sources of contamination	Effect on human health	Reference
Cd	Polluted air, batteries, ceramic glazes and enamels, tap and well water, food (if cultivated in soil contaminated with cadmium), and second hand and first hand cigarette smoke.	When cadmium products are used, manufactured, or consumed, one may be exposed to cadmium by inhalation or ingestion. The main cause of cadmium toxicity, which mainly affects the kidneys, bones, lungs and immune system, is cigarette smoke. In addition to causing yellow teeth and anaemia, it may cause lung cancer, prostate cancer and heart disease. It appears that cadmium also has an impact to autoimmune thyroid conditions.	[116,117, 119, 121]

1.10.3 Aluminium (Al) :

- **Source: -** Aluminium with 8.13%, is the third highest available element in the earth's crust. It can be found naturally in food, water, minerals, rocks and soil. Numerous human and natural sources allow aluminium to enter the food chain. Cereal, sweets, drinks and dairy products are the primary dietary sources of aluminium [134]. Aluminium is widely present in human diets. Main sources of Al exposure to humans are water, airborne dust and pharmaceuticals [135]. Food contamination during preparation, cooking and storage may cause people to consume large amounts of aluminium.

  A number of different sources allow aluminium to get into milk and milk products. Before milking, the feed and fodder given to the dairy cows contaminates the milk. Furthermore, aluminium may inadvertently find its way into milk and milk products through manufacturing processes or contamination from metal machinery [136]. Leaching of aluminium from various utensils is affected by the quality of the containers. The use of aluminium containers for milk processing and storage may increase the level of this metal in milk and milk products significantly [137,138].
- **Toxicity Mechanism: -** Pesticides such as aluminium phosphide (AlP) is used to protect the crops, but research has shown that it is also extremely hazardous to living organisms [139]. Aluminium phosphide exposure displayed altered sensorium, nausea, vomiting and acute respiratory distress syndrome. AlP causes

toxicity when aluminium phosphide reacts with acids or water to produce phosphine. It has been shown that phosphine-induced cell damage results from repeated interactions with the respiratory chain, an enzymatic cascade system located in the mitochondrial membrane. The inhibition of cytochrome oxidase may accurately represent the primary target of phosphine [140].

$AlP + 3\ H_2O \rightarrow Al(OH)_3 + PH_3$

$AlP + 3\ H^+ \rightarrow Al^{3+} + PH_3$

Furthermore, a process that depends on aluminium toxicity could result in cell death.

- **Hazardous Effect: -** Due to comparatively low bioavailability of Al and highly efficient elimination in urine, it was believed for a long time that it is safe for human health [141]. Research has shown that aluminium may have a role in the emergence of dangerous brain conditions including Alzheimer's disease, dialysis, dementia, microcytic anaemia without iron deficiency, osteomalacia [142-146].
  Some primary indications of aluminium toxicity are:
  Reduced mental capacity, forgetfulness, difficulty focusing, impairment in speech and language, personality changes, mood swings, depression, auditory or visual hallucinations.

Table 1.9 : Sources of contamination of Aluminium and their effects

Heavy Metal	Sources of contamination	Effect on human	Reference
Al	Earth, rocks, minerals, and even food, such as cereal, drinks, desserts and dairy products.	It has recently been linked to osteomalacia, anemia and a neurological condition known as dialysis encephalopathy, which is more common in those with chronic renal failure. weariness and weakness primarily associated with microcytic anemia.	[147,148]

1.10.4 Arsenic (As) :

- **Source**: - Through various kinds of natural processes including environmental reactions, volcanic emissions and human activity, arsenic accumulates in the environment. Arsenic levels in the earth, rocks, soils and natural waterways are naturally elevated. Exposure of As occur by soil and groundwater that contains naturally occurring arsenic as well as in vegetation that is grown or irrigated by contaminated water [149,150]. The majority of environmental arsenic issues arise from mobilization in natural settings, mining operations, fossil fuel burning and the use of arsenical herbicides [103,151].
- **Toxicity Mechanism: -** When arsenic is bio transformed, toxic inorganic arsenic is methylated by fungus, humans and algae, producing mono and dimethyl arsenic acid (MMA and DMA) [103].

As (V) → As (IV) → MMA (V) → MMA (III) → DMA (V).

During the detoxification process, MMA (III) persists inside the cell as an intermediate product and turns out to be extremely poisonous and carcinogenic when converted into MMA(V) and DMA(V) and are eliminated by the urine. Compared to inorganic As, IARC is regarded as a type-I hazardous chemical [133]. In plants and animals when arsenic reacts with hydrogen and carbon, it produces organic compounds, while it forms inorganic arsenic when it reacts with sulfur, oxygen and chlorine.

- **Hazardous Effect**

  Arsenic has been identified as a human carcinogen its long-term exposure causes skin cancer, while lung cancer is caused by its inhalation [152]. Health issues have been brought on by the populace's exposure to arsenic from mining and smelting operations in a number of nations. Burning arsenic-rich coal in houses has been linked to major health effects [153]. Consuming water with high As concentrations is a serious concern. Drinking water contaminated with arsenic causes skin ulcers in those who are exposed, and skin cancer incidence is also higher in these people [154–157]. **Fig. 1.9** Described the various carcinogenic effects of arsenic poisoning.

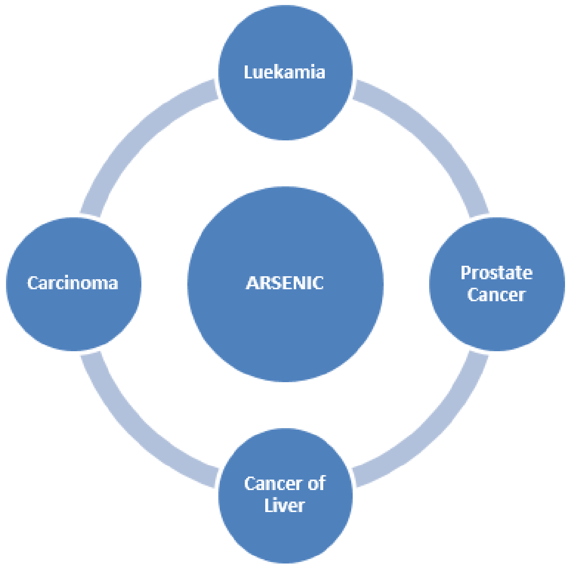

Figure 1.9: Carcinogenic effect of arsenic

Table 1.10 provides an overview of the sources and health implications of Arsenic mentioned above. Due to its toxic and carcinogenic effects on prostatic glands, it can induce prostate cancer, leukemia and lesions in the hepatic areas, causes liver cancer.

Table 1.10 : Sources of contamination of Arsenic and their effects

Heavy metal	**Sources of contamination**	**Effect on human**	**Reference**
As	Cigarette smoke, drinking water, meats and seafood, metal foundries, ore smelting facilities, soil, pesticides, fungicides, herbicides, insecticides, weed killers, wood preservatives, metal alloys and so on are examples of pollutants found in the environment.	Arsenic is a colorless, odorless, extremely deadly substance that can enter the body through the skin, lungs and mouth. In addition to malignancies of the skin, liver, bladder and lungs, arsenic poisoning appears to mostly impact the skin, lungs and gastrointestinal tract. It can also result in neurological abnormalities, impaired motor coordination, respiratory conditions and kidney impairment.	[114,155, 157]

1.10.5 Iron (Fe) :

- **Source: -** Anthropogenic mining activities are the source of the iron in the surface water. Groundwater has a far higher quantity of dissolved iron than freshwater does. It is one of the essential elements of living things as well as the proteins that carry oxygen [158].
- **Toxicity Mechanism: -** Iron is the most abundant metal in the world. All living things depend on iron for their growth and survival. Numerous dangerous free radicals are produced when this iron is unable to bind with proteins. The gastrointestinal system and biological fluid are corroded by the circulated unbound iron. The brain, heart and liver are penetrated by these free irons, which also interfere with oxidative phosphorylation, which changes ferrous ion into ferric ion and raises metabolic acidity. Lipid peroxidation, which is brought on by free iron, has also been shown to cause severe damage to cell organelles [159,160]. Iron-mediated harm to tissue resulted from iron poisoning. Additionally, it generates free radicals that attack DNA directly, causing cellular damage, mutation and cancerous transformation [161]. As a component of haemoglobin, cytochromes and other proteins, iron (Fe) is a necessary trace element that catalyzes a number of metabolic events and is important for the movement, storage and use of oxygen. It functions as a cofactor for a number of enzymes and deficiencies cause anaemia and other diseases [162]. However, too much iron can lead to tissue damage, organ failure and an increased risk of cancer because it can produce reactive oxygen species (ROS) [163,164]. Due to its catalytic impact on the oxidation of lipids with the formation of a disagreeable odour, bounding mainly proteins and membrane lipoproteins of milk

fat globules, a high Fe concentration in milk and dairy products might pose an issue in processing technology [165].

Table 1.11 : Sources of contamination of Iron and their effects

Heavy Metal	Sources of contamination	Effect on human	Reference
Fe	Soil, burning fuel, groundwater, processing unit and cutlery.	Organ failure and tissue damage nausea, vomiting, diarrhea, constipation, nausea, abdominal pain and cancer risk.	[158,159, 161,162, 164]

1.10.6 Zinc (Zn) :

- **Source: -** The air, water and soil contain zinc due to human activity as well as natural processes. The majority of zinc that ends up in the environment comes from mining, processing, and burning waste materials, as well as from the manufacturing of steel, coal and cadmium ores. Zinc levels in the atmosphere may rise as a result of these actions. Zinc can enter waterways through waste streams from the industries that manufacture zinc and other metals as well as from home waste water and runoff from zinc-containing soil. The primary causes of the rise in soil zinc levels are the dumping of coal ash from power plants and waste zinc from the metal manufacturing industry. Increased zinc levels in the soil are also a result of fertilizer and sludge.
  Animals that consume zinc-containing soil or water may absorb zinc. Furthermore, contamination from metal processing equipment or industrial processes can introduce zinc into milk and milk products [136, 166].
- **Toxicity Mechanism: -** The expression of thionine is induced by the plasma membrane and the metal-regulatory transcription factor (MTF)-1. Reactive oxygen species (ROS) or nitrogen species (RNS) oxidize thiols to generate oxidized protein thionine (Tox).
- **Health effect: -** Increased zinc concentrations may hinder development, reproduction, cause cramping in the abdomen, nausea, vomiting, diarrhea and in rare situations, bleeding in the stomach. Consuming excessive amounts of zinc for a few months may harm the pancreas, lower levels of high-density lipoprotein (HDL) cholesterol and induce anaemia. Diarrhea is a typical indicator in babies and kids. Alopecia, slowed growth and recurrent infections are more common in older kids. Zinc deficiency can retard the growth in new-borns and kids as well as cause appetite loss and reproductive issues in adults [167-170]. A zinc shortage may cause problems for the taste and smell sensations. Many different tissues and organs are impacted by zinc deficiency [171].

Table : 1.12 Sources of contamination of Zinc and their effect

Heavy metal	Sources of contamination	Effect on human	Reference
Zn	Earth's crust, industrial processes including steel production, fertilizer and pesticide production, mining, and the burning of coal and garbage	The pancreas, kidney, skin, lung, prostate, liver, gastrointestinal tract, brain, and heart Skin, bones, the reproductive, digestive, central nervous, and immune systems are just a few of the bodily systems that can be impacted by zinc deficiency . Excessive consumption of zinc may result in headaches, nausea, vomiting, upset stomach, and appetite loss.	[167-171]

1.11 Recommended Permissible Limit of Heavy Metal

Since milk is consumed by a variety of age groups, including youngsters and the elderly people, who are the most susceptible to the harmful effects of heavy metals. Because of their extreme toxicity, several regulatory bodies have set acceptable limits for the presence of heavy metals in milk. The government has created rules and regulations that are subject to legal enforcement in order to safeguard public health. The United States Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA) are just a few of the organizations that have created regulations about potentially hazardous substances.

The National Institute for Occupational Safety and Health (NIOSH), the Bureau of Indian Standards (BIS), and the Agency for toxic Substances and Disease Registry (ATSDR) (ATSDR 1994a, ATSDR 1994b) are among the federal agencies that create guidelines or recommendations for hazardous substances.

- ❖ The European Union Commission (EC) no. 1881 European Union (2006) and the Codex Alimentarius Commission (2015) have proposed a maximum allowable level of lead (Pb) in milk at 0.02 mg/ml.
- ❖ The highest amount of lead that can be found in milk, as advised by the FAO/WHO (Codex Alimentarius Commission, 1999), is 0.01 mg/ml. The highest levels of lead that are allowed in milk, as per Indian rules (FSSAI, 2011), are 0.02 and 0.1 mg/ml.
- ❖ The European Union Commission (European Union, 2006) set a maximum allowable limit of 0.1 mg/ml for milk.

The heavy metal acceptable values are listed below in **Table 1.13** :-

Table 1.13 : Permissible limits for extremely hazardous substances according to internationally recognized agencies

Metal/ Permissible Limit	Lead (mg/Kg)	Cadmium (mg/Kg)	Aluminium (mg/Kg)	Arsenic (mg/Kg)	Iron (mg/Kg)	Zinc (mg/Kg)	References
Codex Alimentarius Commission (2014)	0.02	0.0026	0.020	0.01	0.37	0.328	[172-176]
IDF Standard (2014)	0.02	0.0026	0.020	0.01	0.37	0.328	
IDF Standard (1979)	0.02	-	-	0.01	-	0.328	
IDF Standard (1977)	-	-	-	-	-	0.328	
European Commission (2014)	0.02	0.0026	0.020	0.01	0.37	-	

1.12 Previous Studies on Heavy Metal Contamination in Milk and Dairy Products

Table 1.14 : Previous Studies on heavy metal concentration in milk and dairy products

Studied Samples	Elements	Country	Result	Reference
Milk	Cd, Co, Pb, Cu, Ni	Pakistan	The mean concentrations per kg were 0.028, 0.061, 0.014, 0.738, and 0.001 mg. Results from the study demonstrated that levels of Pb and Cu in milk from the studied regions may be harmful to consumers and beyond the standard codex.	[177]
Cow's milk and cheese produced	Ni, Cr, Cu, Zn, Pb and As	Mexico	The mean value of Pb and As were 0.03 and 0.12 mg kg^{-1}, respectively, above the value set by the Codex Commission standards. Ranchero cheese and Oaxaca had higher levels of Pb, at 0.17 and 0.16 mg kg^{-1} respectively.	[178]
Milk, Yoghurt, cheese	Pb, Cd, Cu, Se, Zn	Iran	Mean values of Pb, Cd, Cu, Zn, and Se was 14.0, 1.11, 427, 571, and 2.19 µg kg^{-1} in raw milk, 9.59, 1.0, 378, 447, and 1.78 µg kg^{-1} in pasteurized milk, 14.5, 1.25, 428, 586, 1.68 µg kg^{-1} in cheese, and 7.54, 0.99, 399, 431, and 1.23 µg kg^{-1} in yoghurt, respectively.	[179]

Cow milk, goat milk, butterfat, soft cheese, Yoghurt	Pb, Cd	Nigeria	The mean of Pb ranged from 0.0025 to 0.0061 (ppm), with dairy products having the highest concentration at 0.0125 to 0.0175 ppm. In soft cheese and goat milk, Cd levels exceeded the maximum residue limits.	[180]
Milk, dairy products	Pb, Cd, Zn, Cu, Fe	Egypt	The concentrations of Pb, Cd, Zn, Cu, Fe, and 0.008-0.179, 0.888-18.316, 0.002-1.692, and 1.3208-45.6198 ppm were found in milk and dairy products, respectively.	[181]
Raw cow and ewe milk	Pb, Cd, Hg	Iran	The mean concentrations of pb, cd, and Hg in samples of cow milk were 12.9 ± 6.0, 0.3 ± 0.3, and 3.1 ± 0.3 ng g-1. Mean values in ewe milk were 14.9 ± 7.8, 1.6 ± 1.2, and 3.1 ± 0.3 ng g-1.	[182]
Pasteurized milk, Yoghurt, Yoghurt drinks, cheese	Cd, Pb, Hg, Se, As, Al	Iran	The concentration of Cd, Pb and Hg in dairy products were found 168.25± 92.2 (30.6 - 356.5), 3.2 ± 1.95 (0.4 - 8.1), 5.9 ± 4 (1.1 - 16), 4.55 ± 2.6 (0.6 - 10.6), 15.4 ± 8.53 (3.1- 40.2) and 23.15 ± 10.4 (6.8 - 50.2) μg/kg	[183]
Camel milk, sheep milks	Pb, Ni, Co, Zn, Mn, Fe, Cd	Saudi Arabia	The average Pb concentration (ppm) in camel milk samples from Riyadh and Qassim was 0.54 and 0.59, while the average nickel concentration in camel milk protein was 1.51 and 2.1 in Riyadh and Qassim, respectively, and the average concentration in sheep milk samples was 0.80 and 2.21.	[184]
Yoghurt and milk	Cd, Pb, Cr, Se, Cu, Mn, Zn	Korea	Cd and Mn were high in fruit mixed Yoghurts, while selenium was high in milk samples. The level of toxic trace elements, including As, Cd and Pb, was very low.	[185]
Cheese, cream and butter	Li, Al, Cr,Co, Mn, Mo, Sr	Croatia	The amount of Li 0.008-0.056, Al 0.01-3.93, Cr 0.005-1.66, Co < 0.005, Mn 0.068-5.37, Mo 0.003-0.225and Sr 0.085-3.49.	[186]
Cheese samples packaged in plastic, tin containers	Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Zn	Turkey	The samples of cheese packed in Chinese containers and plastic containers differ significantly from one another, indicate the effect of cheeses and packing materials.	[187]
Milk and Yoghurt	Pb, Ni	Turkey	Limits of detection were found to be 0.15 ng $mL^{-1}$for Pb and 0.75 ng $mL^{-1}$for for Ni. The lead concentrations were in the range of 15-61 ng mL^{-1} and 21-42 ng mL^{-1} for yoghurt.	[188]

Milk	Pb	Iran	Lead levels ranged from 1 to 46 ng/ml, with a standard deviation of 8.8 and an average of 7.9 ng/ml.	[189]
Camel milk, Cattle, Buffalo, Goat	Cd, Ni, Cr, Mn, Zn, Fe	Pakistan	The camel had significant quantities of Mn (0.004 ± 0.094 mg/kg), Fe (0.530 ± 1.580 mg/kg), and Zn (mg/kg 0.021 ± 5.150 mg/kg), according to the data. significant levels of toxic metals were found in buffalo milk (0.010 ± 0.223 mg/kg) and Cd (0.186 ± 0.186 mg/kg), whereas significant levels of nickel (0.045 mg/kg 15.15) and chromium (0.045 mm/kg 1.152) were found in goat milk.	[190]
Camel milk	As, Pb	Kenya	The samples' arsenic concentrations ranged from 0.007 ppm to 0.099 ppm. As and Pb levels in the samples were higher than those in the codex.	[191]
Forage, Camel Milk, Fermented Camel Milk	Cu, Fe, Mn, Zn, As, Pb	Kazakhstan	Camel milk concentrations for Cu, Fe, Mn, Zn, As, and Pb were 0.07 ± 0.04, 1.48 ± 0.53, 0.08 ± 0.03, 5.16 ± 2.17, less than 0.1, and 0.025 ± 0.02 ppm, respectively. The mean content of shubat, or fermented milk, was 1.57 ± 0.46, 0.088 ± 0.02, 7.217 ± 2.55, and 0.007 parts per million.	[192]
Kassr cheese	Pb, Cd, Fe, Cu, Zn	Turkey	Nearly all of the analyzed elements have decreased throughout the milk conversion to fresh cheese because whey juice dissolves these metals into whey.	[193]
Camel Milk	Cu, Fe, Mn, Zn	Saudi Arabia	The average concentrations of Zn, Mn, Cu, Fe, Ca, Na, and K in milligrams kg-1 (dry matter) of cow's milk were 0.28 ± 2.0, 431.21 ± 2.43, 1.80 ± 1.10, 4.214 ± 1.78, 66.91 ± 41.95, 91.4 ± 3.45, and 7.84 ± 5.84 for camels. For goats: 10.13.11, 93.93 ± 94.9, 78.7 ± 90.68, 72.77 ± 72.7, 0.77 ± 0.07, and 12.12 ± 0.99, in that order. For sheep: 113.36 ± 822.5, 47.54 ± 95.4, 0.91 ± 3.09, 1.144 ± 0.05, 0.22 ± 0.62, 3.24 ± 5.101, and 127.11 ± 11.11.	[194]
Raw cows' milk and assessment of transfer to Comté cheese	Pb, Cd, Cu, Zn	France	While the metal concentrations (dry weight) in the corresponding cheese were significantly higher (Cd: 0.68–11.37 ng/g; Pb: 0.020–0.925 lg/g; Cu: 5.35–21.34 lg/g; Zn: 33.66–63.41 lg/g), the concentrations in the raw milk were very low (Cd: 0.34–1.01 ng/g; Pb: 0.009–0.126 lg/g; Cu: 0.28–1.71 lg/g; Zn: 20.62–30.96 lg/g).	[195]
Infant Formula Milk	Pb, Cd Ni, Mn	Pakistan	The range of concentrations for Fe, Zn, and Ni was 45.40-97.10, 29.72-113.50, and less than 0.001-50.90 µg/kg, respectively.	[196]

Infant Formulas Milk	As, Hg, Pb	Philippines	Lead and arsenic levels in three different types of tests were negative; however, a mercury sample tested positive and exceeded the weekly permissible limit.	[197]
Infant Formula, powdered and fluid (fresh & processed) cow milk	Ca, Mg, Cu, Zn, Fe, Mn, Pb, Cd, Cr, and Ni	Pakistan	The amounts of the hazardous metals were within permissible bounds and did not manifest in a way that would be harmful.	[198]
White and fruit parts of Yoghurts	Cu, Cd, Pb, Mn, Cr, Co, Ni, Zn, Hg	Slovak Republic	The fruit portions of the Yoghurt samples contained high quantities of hazardous elements (Cd and Pb), sometimes tolerably beyond the limit. There were no harmful elements present in the Yoghurt's white portion.	[199]

1.13 Scope of the Work/ Futuristic Approach

Considering present scenario of Rajasthan, ever-increasing industrialization and the likelihood that heavy metal pollution will continue to grow in the future, the current study project has been undertaken to assess the levels of heavy metals in milk-based dairy products as they move up the food chain or as a result of other factors such as the type of container used for processing or packaging. Despite the fact that various scientists worldwide have studied the identification of these metals, there is currently no information available regarding the degree of heavy metal contamination in dairy products from Rajasthan, India. The goal of the current plan is to measure and track the amounts of hazardous heavy metals in dairy products (Yoghurt, cheese and butter) from various less and more polluted areas of Rajasthan.

1.14 Objectives of Our Study

1. To assess the hazardous concentration of heavy metal in soil, water, and fodder samples in order to examine the transfer of metals from these sources to dairy products.
2. To investigate the concentration of heavy metals in various of samples of yoghurt, butter and cheese, collected from the different areas of Kota division, Rajasthan.
3. To investigates how manufacturing procedures and packaging material affect the heavy metal concentration in dairy products.
4. To compare the recommended dietary allowance (RDA) values established by several national and international organizations with the metal ion concentration found in dairy products.
5. To analyze the data using statistical analysis, correlation analysis.
6. To evaluate the health risk by calculating, estimated daily intake (EDI), metal pollution index (MPI) and health risk index (HRI).

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CHAPTER – II
MATERIAL AND METHOD

This chapter illustrates the detailed information about the study area and describes the methodologies adopted during the entire research work, mainly pertaining to field sampling and sample preparations in the laboratory. Translocation factor is also given in this chapter.

In this chapter the instruments, sources of chemicals used and the methods adopted for the work will briefly be outlined.

2.1. Equipments

2.1.1 Atomic Absorption Spectrophotometer (Model Shimadzu 6300 AA)

The heavy metals lead (Pb), cadmium (Cd), aluminium (Al), arsenic (As), iron (Fe), and zinc (Zn) were analysed in this investigation using the Atomic Absorption Spectrophotometer model (SHIMADZU 6300AA, Japan) depicted in **Fig. 2.1**. Using the Direct Air – Acetylene Flame method, the concentrations of all six metals (Pb, Cd, Al, As, Fe, and Zn) are determined. The standard solution for each element was prepared using four distinct concentrations of 0.01, 0.1, 1.0, and 5.0 ppm from Merck KGaA, Darmstadt, Germany.

Figure 2.1: Atomic Absorption Spectrophotometer (Model-Shimadzu-6300 AA)

Table 2.1: Wavelengths optimized for the analysed metals in AAS technique

Elements	Symbol	Wavelength
Lead	Pb	283.3
Cadmium	Cd	228.8
Aluminium	Al	309.3
Arsenic	As	189.0
Iron	Fe	248.3
Zinc	Zn	285.2

Wavelengths of 283.3 nm for lead, 228.8 nm for cadmium, 309.3 nm for aluminum, 189.0 nm for arsenic, 248.3 nm for iron, and 285.2 nm for zinc were set for the instrument.

The analytical instrument was warmed up to thirty minutes before the sample analysis began. A hollow cathode lamp was utilized as the light source, and the light path was modified to achieve optimal sensitivity. To guarantee quality control, we employed certified reference materials (CRM) for metal analysis. The calibration curves of the six components were constructed using MERCK Certipur® grade standards. One common laboratory analysis instrument for metals is a Flame Atomic Absorption Spectrometer (FAAS).

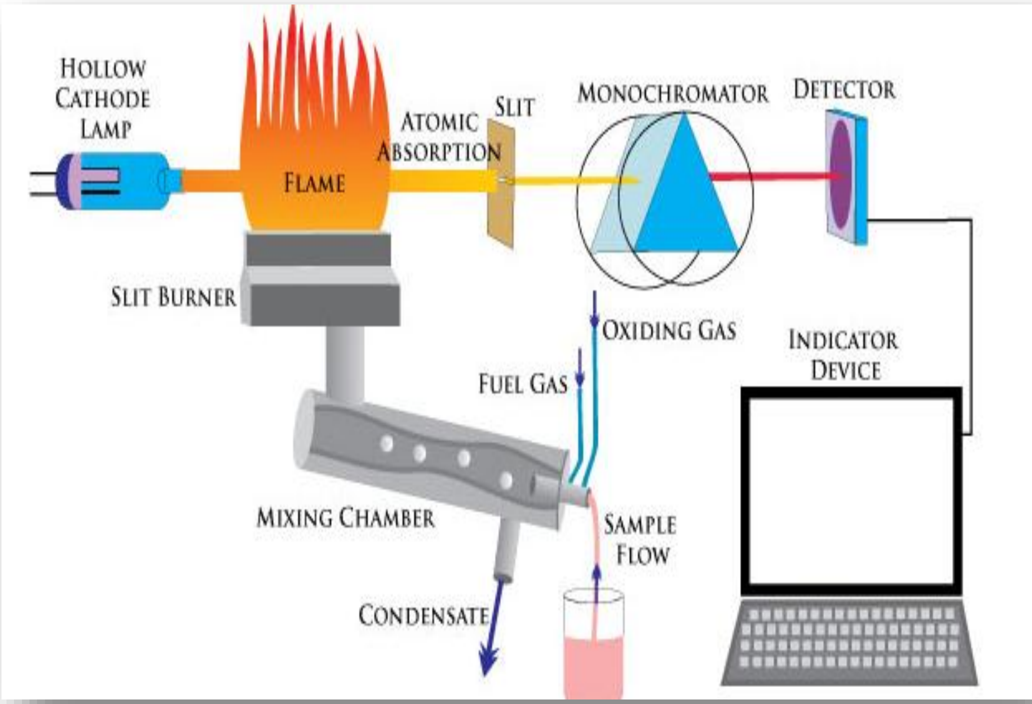

Figure 2.2: Schematic representation of FAAS

2.1.1.1 Instrument Calibration

The instrument was calibrated using the normal addition process prior to sample analysis. By measuring standard solution signals and plotting a graph allowed one to determine the elemental content of an unknown solution. To eliminate errors that could be systematic or random, a blank test was conducted.

2.1.1.2 Control and Assurance of Data Quality

The detection limit was examined to make sure the data was of high quality. Prior to statistical analysis, the detection limit for accuracy measures should be less than the lowest or minimum value of our data, the error should be below 10% and the standard deviation should be below 10% for good precision.

2.1.1.3 Detection Limits

The detection limits are found by measuring the blank's intensity. Equation (2.1) has been used to calculate DL3s or the 3s detection limit. The measured detection limits are given in **Table 2.2** Such data, whose elements concentrations are below the detection limit, are not employed in statistical analysis [1].

$$\boldsymbol{DL3s} = \mathbf{3}\,\frac{s}{m} \qquad \ldots(2.1)$$

Where- $DL3s$ = $3s$detection limit, s = Standard deviation   = Slope of the calibration curve

Table 2.2: Detection Limits for the instruments used in the analysis

Elements	Lead (Pb)	Cadmium (Cd)	Aluminium (Al)	Arsenic (As)	Iron (Fe)	Zinc (Zn)
Detection Limit (mg/L)	0.0005	0.0002	0.001	0.0001	0.002	0.003

2.1.1.4 Check the Accuracy

The accuracy of a measured value is its proximity to the standard. To assess an instrument's accuracy, standard reference materials of MERCK Certipur® grade have been used.

2.1.1.5 Precision and Relative Standard Deviation

Standard deviation can be used to quantify precision. The degree to which different groupings are near to one another might be characterized as precision. Standard deviation, which is measured as a function of relative standard deviation (RSD), is a crucial precision instrument [2]. It can be expressed as equation (2.2) below.

$$\%\ \mathrm{RSD} = \frac{s}{m} \times 100 \qquad \ldots.(2.2)$$

Where s = standard deviation,
m = arithmetic mean.

Table 2.3 represents the percentage relative standard (% RSD) of our work, which indicates that all of the results are less than 10, indicating that the analytical approach was sufficiently exact.

Table 2.3: Relative standard deviation

Elements	RSD (%)
Lead	4.6
Cadmium	4.7
Aluminium	5.6
Arsenic	1.2
Iron	6.5
Zinc	6.2

2.1.2 Microwave Oven

For this work, the CEM Phoenix microwave oven, shown in **Fig. 2.3**, was used to heat materials to high temperatures and carry out full ashing.

Figure 2.3 : Microwave Oven - CEM Phoenix

2.1.3 Muffle Furnace

As seen in **Fig. 2.4**, a muffle furnace is used for ashing of soil and fodder samples for elemental analysis. Temperature of muffle furnace reaches up to 1000 ^{o}C to 1200 ^{o}C.

Figure 2.4: Muffle Furnace

2.1.4 Deep freezer

Every collected sample was preserved at -20^{O}C in the LLOYD Deep freezer that is shown in **Fig 2.5**.

Figure 2.5: Deep freezer (-20^{O}C) LLYOD

2.1.5 Ion meter EC-pH / Ion Meter

Soil and water samples were tested for pH using a modal Eutech-pH/Ion meter (EC- pH 6500 42S Model). Prior to conducting the experiment, a pH meter calibration was carried out using standard buffer solutions with pH values of 4.00, 7.00 and 10.00.

2.1.6 Millipore water purification system

Millipore A.S., 67120 Molsheim Elix UV-3 water purification system: - The solutions were prepared by using the double-distilled water of Millipore water purification systems, as shown in **Fig. 2.6**.

Figure 2.6 : Ultra-Pure Water Purification System (Millipore Elix UV3)

2.1.7 Mechanical /Electrical shaker

A laboratory stirrer (Remi 2 LH magnetic stirrer) was utilized to digest the material at the required temperature and at a fixed and optimum rpm (revolutions per minute).

2.1.8 Microbalance

The most important instrument in every chemical laboratory is the analytical balance, that is used to weigh both the sample and the analytical reagents. Devices for calculating percentages, piece counting, and formulation memory are all included in modern instruments. For weighing purposes, we utilised a Citizen C x 200 electronic balance. The balance with the lowest count is 0.0001 mg.

2.1.9 Drying / Heating Oven

The oven model Labpro 101, shown in **Fig. 2.7**, was used to heat the samples and dry the glassware. A temperature range of 25°C to 150°C or higher is feasible. It is thermostatistically regulated and has an air-circulating fan.

Figure 2.7: Heating Oven

2.2. Chemicals

In this experiment, a number of analytic grade chemicals were used to clean glassware and to assist various dairy product, milk, soil, water, and fodder sample digestion processes. To create standards, samples, and blanks for the examination of metal concentration in collected samples, ultra-pure and deionized water was used.

Experimental Chemicals and reagents

- ❖ Nitric Acid (65%), Merck, Germany
- ❖ Hydrogen Peroxide (30%), Merck, Germany
- ❖ Hydrochloric acid (35%), Merck, Germany
- ❖ Sulphuric acid, Merck, Germany

Table 2.4 : Physical properties of common acids and oxidizing agents used for digestion

Compound	Formula	Molecular weight	Concentration		Boiling Point (ºC)	Density (Kg L^{-1})
			W/W	Molarity (%)		
Nitric acid	HNO_3	63.01	68	16	122	1.42
Hydrogen peroxide	H_2O_2	34.01	30	10	106	1.12
Perchloric acid	$HClO_4$	100.46	70	12	203	1.67
Hydrochloric acid	HCl	36.45	36	12	110	1.19
Sulfuric Acid	H_2SO_4	98.08	98	18	338	1.84

2.3. Sample Types

Selection of milk based dairy products and criteria for their selection

Not only milk but dairy products are the essential source of the diet in India, they are also regularly consumed by people all over the world. Three dairy products i.e., Yoghurt, Butter and Cheese have been selected for the study because of their wide range of applications in various forms and significant nutritional value.

2.3.1 Yoghurt

Yoghurt, a dairy product that has undergone fermentation, grows similarly to Streptococcus thermophilus and Lactobacillus bulgaricus. Other species like Streptococcus lactis and Lactobacillus acidophilus, are also commonly found in Yoghurt. Yoghurt is a great way to get vital minerals that are essential for a human's diet. The amount of calcium and magnesium required each day to maintain physiological processes may be significantly impacted by this. Yoghurt is a great source of phosphorus and calcium, which is the very essential for bones [3]. Since during manufacturing and processing different heavy metals may also enter in yoghurt [4,5]. So to measure the metal concentration in yoghurt we use spectrometric approach.

2.3.2 Butter

Butter is a solid emulsion of fat globules, water and inorganic salts that is produced by churning the cream from cow's milk. Its color varies from yellow to white. Butter has traditionally been used as a spread and as a cooking fat. The physical changes that take place during creating butter are more complex, despite the fact that butter is a simple product made of only a few elements.

2.3.3 Cheese

Milk, a coagulant and bacterial cultures are the three basic ingredients used to make cheese. The primary ingredient in cheese is milk, which can come from buffalo, sheep, goats, cows or a combination of these. An ingredient added to milk to aid in the formation of solids from the liquid portion is called a coagulant. So during manufacturing process we use different equipment, by which heavy metals might enter in dairy product and amount of metals depends on the process [6].

2.4. Sample Sites

Keeping in mind the overall objective of the study, the sampling sites were chosen so as to cover locations where there is a high probability of contamination in milk-based dairy products in different cities of Rajasthan .The carefully selected dairy products derived from milk that are easily accessible in these areas and are consumed by the local population.

The following factors were considered for selecting the sample locations -

(i) Areas where specific industries are assumed to be more likely to be contaminated.
(ii) The area around highways, accounting for exhausts, gas emissions, and traffic volume.
(iii) The areas used for cultivation.
(iv) Locations where there is a greater chance of contamination because of the irrigation technique and sources .
(v) Locations where elevated contamination may be brought by sewage treatment facilities.
(vi) Locations near dumpsites and landfills owned by municipal corporations.
(vii) Areas with higher anthropogenic activity.

Table 2.5 : Location, characteristics, and identification code of various sampling sites

S. No.	Study Area	Sub Area	Code	Characteristic
1	**Kota District**	Ranpur	KR	Highly polluted area (Industrial Area)
		Kaithoon	KK	Less polluted area
2	**Baran District**	Chhabra	BC	Highly polluted area
		Mangrol	BM	Less polluted area
3	**Bundi District**	Lakheri	BnL	Highly polluted area
		Kapren	BnK	Less polluted area
4	**Jhalawar District**	Jhalarapatan	JJ	Highly polluted area
		Aklera	JA	Less polluted area

A selection of commercial, industrial and residential locations were used in the random sampling process to choose the study sites, all of them could be different sources of heavy metal contamination. These areas are separated into those where human activity is more and less prevalent.

2.4.1 Kota District

2.4.1.1 Ranpur

It is located at a short distance from Kota city. In this industrial area there are various food processing plants, several chemical industries, and manufacturing industries are located. Improper disposal of sewage along with variety of agricultural practices, industrial effluents were observed.

2.4.1.2 Kaithoon

Kaithoon is well-known for producing Kota Doriah sarees situated 20 Km away from Kota city. So the soil surface may have been impacted by textile and printing effluents (dyes) and agricultural activities also.

2.4.2 Baran District

2.4.2.1 Chhabra

Chhabra is a town in Baran district where one of the coal-fired power stations of Rajasthan situated, which is a major source of pollution of this area.

Figure 2.8: Chhabra Thermal Power Plant

2.4.2.2 Mangrol

Mangrol is located in the Baran district. Where anthropogenity is seems to be less as compared to chhabra.

2.4.3 Bundi District

2.4.3.1 Lakheri

The coordinates of Lakheri are 25.67°N 76.17°E, located in the southeast of Rajasthan. A cement manufacturing unit ACC is situated in this area, which is asia’s longest running cement plant.

Figure 2.9: ACC Lakheri Cement Plant

2.4.3.2 Kapren

Kapren is located in the Bundi district. Local steppe climate is the term used to describe the predominant climate of Kapren. The entire year is dry with little rainfall. In Kapren, the yearly average temperature is 26.6°C.

2.4.4 Jhalawar District

2.4.4.1 Jhalarapatan

Jhalarapatan is a town of Jhalawar district, in which Kalisindh Thermal Power Plant is located. The distance between Jhalawar town and Kalisindh Thermal Power Plant is 12 km. It is emitted lots of gases, which are harmful for environment. The discharge of waste water from the power plant affect the soil and water quality and of course vegetation.

Figure 2.10 Kalisindh Thermal Power Plant Jhalawar

2.4.4.2 Aklera

Aklera is located at 24.42°N 76.57°E . It has moderate pollution level as compare to Jhalarapatan.

A set of sites were chosen and classified into two groups in order to ascertain the metal ion concentration in various dairy products. These regions are divided into those with more and less anthropogenic activity.

These two locations are-

(i) Individual aFrms

(ii) Local Shops

Table 2.6 : Identification code of the sample sites of various individual farms and local shops of Rajasthan

S.No.	Area	Highly Polluted		Less Polluted	
		Individual farms		Local shops	
1.	KOTA	Ranpur		Kaithoon	
		KRIF 1	KRLS 1	KKIF 1	KKLS 1
		KRIF 2	KRLS 2	KKIF 2	KKLS 2
		KRIF 3	KRLS 3	KKIF 3	KKLS 3
		KRIF 4	KRLS 4	KKIF 4	KKLS 4
		KRIF 5	KRLS 5	KKIF 5	KKLS 5
2.	BARAN	Chhabra		Mangrol	
		BCIF 1	BCLS 1	BMIF 1	BMLS 1
		BCIF 2	BCLS 2	BMIF 2	BMLS 2
		BCIF 3	BCLS 3	BMIF 3	BMLS 3
		BCIF 4	BCLS 4	BMIF 4	BMLS 4
		BCIF 5	BCLS 5	BMIF 5	BMLS 5
3.	BUNDI	Lakheri		Kapren	
		BnLIF 1	BnLLS 1	BnKIF 1	BnKLS 1
		BnLIF 2	BnLLS 2	BnKIF 2	BnKLS 2
		BnLIF 3	BnLLS 3	BnKIF 3	BnKLS 3
		BnLIF 4	BnLLS 4	BnKIF 4	BnKLS 4
		BnLIF 5	BnLLS 5	BnKIF 5	BnKLS 5
4.	JHALAWAR	Jhalarapatan		Aklera	
		JJIF 1	JJLS 1	JAIF 1	JALS 1
		JJIF 2	JJLS 2	JA IF 2	JALS 2
		JJIF 3	JJLS 3	JA IF 3	JALS 3
		JJIF 4	JJLS 4	JA IF 4	JALS 4
		JJIF 5	JJLS 5	JA IF 5	JALS 5

2.5. Sample Collection

Samples were taken from various local shops and individual farms from various parts of Rajasthan in order to evaluate and compare the uptake of heavy metals by particular milk-based dairy products.

2.5.1 Sampling Sites

A total of 16 locations within the Rajasthan state were chosen for study and 5 samples of each dairy product (yoghurt, butter, cheese) were collected from one location.

2.5.2 Sampling containers

Sterile screw-topped, high-quality 100 ml PTFE bottles (polyethylene containers with zippers) were used for sampling that had been previously cleaned with high-grade nitric acid.

2.5.3 Sample Quantity

10 gram of each sample was taken in order to assess the amount of heavy metals in the dairy products. Three main dairy products i.e., yoghurt, butter and cheese were taken for the study. So samples of each dairy products were collected from the areas given in above **Table 2.6** .

2.5.4 Sample labelling

Each sample was appropriately labelled and identified using a permanent marker. To prevent any mistakes or misunderstandings, all the details about the sampling sites, source, collection date, and assigned codes were noted in the observation register.

2.5.5 Sample preservation

Within two to three hours after sampling, all dairy products were taken to the laboratory from the locations. The samples were then kept in a deep freezer at -20°C.

2.6. Sample Digestion

The methods utilized for the digestion of samples of soil, water, fodder, milk, and other dairy products are listed below.

2.6.1 Digestion of Soil

To ascertain the amounts of heavy metals, a wet digestion technique was applied to the dried samples [7]. Each air-dried and sieved sample, weighed 1.0 g, ashen for three hours at 450^{o}C in a muffle furnace. The obtained ash was digested using 20 ml of Aqua-Regia (3 parts concentrate HCl + 1 part concentrate HNO_3) for a total of nine hours at different temperatures as shows below **Table 2.7**.

Table 2.7 : Digestion parameters for Soil

Time (Hours)	Temperature	Hold Time (Minute)
2	25^0C	10
2	70^0C	10
2	90^0C	10
3	120^0C	10

After digestion, the residue was allowed to cool, which was taken filtered and placed into a 25 ml volumetric flask. Double-distilled water was used to make the solution up to the mark.

2.6.2 Digestion of Water

10 ml of concentrated nitric acid (HNO_3) was added to 50 ml of water sample, and the mixture was heated to 85^{o}C until it turned clear [8]. Then the obtained mixture was filtered with the help of Whatman No. 42 filter paper, and deionized distilled water was added to make up the 50 ml of volume.

2.6.3 Digestion of Fodder

Fodder sample collection was done from the different locations. The samples were first cleaned with 1% HCl, then three or four times with water to remove any foreign substances. Finally, they were spread out on clean paper to dry properly. Dry samples were dehumidified once again by heating them to 65 ^{o}C to 75 ^{o}C in an oven. Later, these samples were digested in an acidic solution of HNO_3 and $HClO_4$ [9].

2.6.4 Digestion of Milk

An electric hot plate set at 90^{o}C was used to digest 5 ml of milk sample that had been treated with 5 ml of 65% nitric acid (HNO_3) and 2 ml of 30% hydrogen peroxide (H_2O_2). Then, the temperature was increased to 115^{o}C to 120 ^{o}C until brown fumes disappeared. After that, the clear solution was cooled, filtered and diluted with DI water in a 25 ml volumetric flask [10, 11].

2.6.5 Digestion of dairy products (Yoghurt, Butter, Cheese)

Dry, wet, and microwave digestions were the three different techniques used to analyze the dairy product samples. The perfect conditions for digestion are listed below.

2.6.5.1 Digestion Techniques:

(a) Dry Digestion :

One gram of sample was placed in a porcelain crucible and dried in a furnace at 100^{o}C. Then temperature was gradually increase from 100-500˚C. Then obtained sample was ashed for approximately seven or eight hours, and a white or gray ash residue was found. 5 ml of 25% v/v HNO_3 was used to dissolve the residue. After dissolution, the mixture was poured into a volumetric flask of 10 ml and brought to the volume. The solvent was used alone to conduct a blank control in the same manner.

(b) Wet Digestion:

One gram of sample was treated with 5 ml of nitric acid (65% HNO_3) and 2 ml of hydrogen peroxide (30% H_2O_2), and it was digested on an electric hot plate at a temperature of 90ºC. The temperature then raised up to 120ºC until the brown vapors vanished, signifying that the oxidation of the organic matrix was complete. After the organic matrix was broken down its elements were left in a clear solution. After cooling the samples filtration was done by filtering the clear solution into a volumetric flask of 25 ml capacity and made up to the mark by adding DI water.

(c) Microwave Digestion:

One gram of sample was digested with 4 ml of HNO_3 (65%) and 2 ml of H_2O_2 (30%) in microwave digestion system. The digestions of samples were carried out at different conditions summarized in **Table 2.8**.

Table 2.8 : Digestion conditions

Step	Time (min)	Power (W)
1	2	200
2	2	200
3	4	400
4	6	400
5	8	600
6	8	VENT

Resulting solution was transferred in to 10 ml volumetric flask and diluted with deionized water. In the same way a blank digestion was carried out. All sample solutions were clear.

2.6.5.2 Validation of the optimized method

In order to determine the best digestion method among the three methods (dry, wet, and microwave), we run a recovery test in which the material was treated with a known quantity (spiked concentration). In order to make sure that there would be no discernible variation from the quantity typically present in the main sample, the spike's numbers were limited to 5-8% [12]. Following AAS analysis of these samples, the recovery percentages were computed using the following formula:

$$\%\, \boldsymbol{recovery} = \frac{\text{Conc.in spike sample} - \text{Conc.in sample}}{\text{Amount spiked in sample}} \times 100 \qquad ..(2.3)$$

The results are shown in **Table 2.9** .

Table 2.9 : Results of optimized method validation

S. No.	Metal	Method ID	Amount Spiked (mg/L)	Concentration in Sample (mg / L)	Concentration in Spiked Sample (mg/L)	Recovery %
1	**Pb**	DD	0.0149	0.0984	0.1121	91.95
		WD	0.0149	0.0984	0.1125	94.63
		MW	**0.0149**	**0.0984**	**0.1129**	**97.32**
2	**Cd**	DD	0.0069	0.0211	0.0268	82.61
		WD	0.0069	0.0211	0.0272	88.41
		MW	0.0069	0.0211	0.0278	**97.10**
3	**Al**	DD	0.0373	0.0543	0.0886	91.96
		WD	0.0373	0.0543	0.0891	93.30
		MW	0.0373	0.0543	0.0898	**95.17**
4	**As**	DD	0.0048	0.0241	0.0283	87.50
		WD	0.0048	0.0241	0.0284	89.58
		MW	0.0048	0.0241	0.0286	**93.75**
5	**Fe**	DD	0.561	0.6781	1.1901	91.27
		WD	0.561	0.6781	1.2008	93.17
		MW	0.561	0.6781	1.2189	**96.40**
6	**Zn**	DD	0.287	0.5682	0.8242	89.20
		WD	0.287	0.5682	0.8269	90.14
		MW	0.287	0.5682	0.8427	**95.64**

DD: Dry Digestion, WD : Wet Digestion , MW : Microwave Digestion

Based on precision, accuracy and recovery (**Table 2.9**), we determined that the microwave digestion method is the most effective way for breaking down dairy products.

2.6.5.3 Advantages of the Proposed Method

By adopting this proposed microwave method, we may analyse the concentration of heavy metals in different dairy products in laboratories, assuring that they follow food safety standards and protecting public health. The advantages of the recommended strategy are shown in **Fig. 2.11** below.

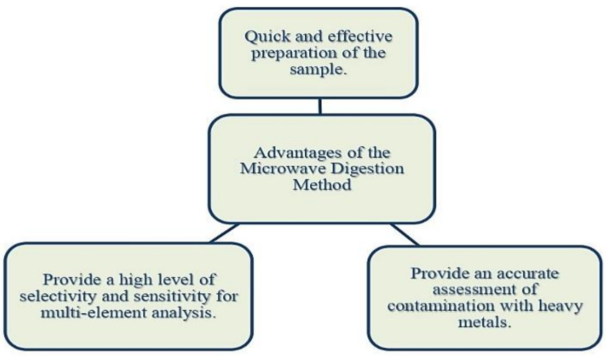

Figure 2.11 : Advantages of proposed method

2.7. Statistical Analysis

For calculation of data processing, correlation matrices and descriptive statistics (minimum, maximum, arithmetic mean, geometric mean and standard deviation) have all been carried out using MS-Excel 2016. JMP software were utilized for the static analysis.

2.7.1 Mean

The term "mean" refers to an average, which is determined by dividing the total number of data by the total number of data points.

If there are n samples, $n1, n2, n3 \dots \dots nN$

$$Mean = \frac{\text{n1} + \text{n2} + \text{n3} \cdots \text{nN}}{n} \qquad \dots\dots\dots\dots(2.4)$$

2.7.2 Standard Deviation and Variance

Variance (σ^2 or Var (X)) measures the average squared deviation of each data point from the mean of the dataset.

$$\textbf{Var (X)} = \frac{1}{n} \sum_{i=1}^{n} (x_i - \mu)^2 \qquad \dots\dots\dots(2.5)$$

Where :
X_i are the individual data points,
μ is the mean of the dataset,
n is the number of data points.

Standard deviation (σ or SD) is the square root of the variance, which indicates that how much on average, each data point differs from the mean values. The formula for standard deviation is:

$$\sigma = \sqrt{Var(X)} \qquad \dots\dots\dots\dots(2.6)$$

Standard deviation and variance are two essential concepts in statistics that describe the dispersion of a dataset around its mean (average).

2.7.3 One way ANOVA

If there is both categorical and quantitative data, the concentration of heavy metals in dairy products can be found using a one-way ANOVA test.
A t-test is a statistical test. This t-test is commonly used when there are two groups (samples) and wants to test whether their means are significantly different from each other.

t - test is used for comparing the means of two main groups. Two sample t test or unpaired t-test is used to examine whether the difference of means of two independent or unrelated group are statistically significant or not.

Tuckey Kramer HSD Tukey – The Tukey-Kramer Honestly Significant Difference (HSD) test, often referred as the Tukey test or Tukey's HSD, which is used after an ANOVA, indicates that there are significant differences between group means. It helps to pinpoint which specific group means are significantly different from each other.

2.7.4 Correlation Coefficient

The Pearson correlation coefficient is a measurement of the linear relationship between two variables. It is denoted by r and ranges from (-1) to (+1) [13].

If

r = +1 represents a perfect positive linear relationship,

r = - 1 represents a perfect negative linear relationship,

r = 0 represents no linear relationship.

To calculate the Pearson correlation coefficient, following formula is used

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n \sum x2 - (\sum x) 2][n \sum \quad y2 - (\sum \quad y)2]}} \qquad \text{………(2.7)}$$

Where, n = Number of pairs of scores;

$\sum x$ = Sum of x scores;

$\sum x\ 2$ = Sum of squared x scores;

$\sum y$ = Sum of y scores;

$\sum y\ 2$ = Sum of squared y scores;

$\sum xy$ = Sum of the products of paired scores

It quantifies the degree to which a pair of variables is linearly related, with values closer to 1 or -1 indicating stronger linear relationships [14].

2.8. Health Risk Assessment

The following parameters can be used to analyse the toxicity level on human health.

Translocation Factor (TF) :

The translocation of heavy metals from soil to fodder and subsequently to milk involves the uptake of metals by plants from contaminated soil. These metals can accumulate in plants tissues, transferring to fodder. When animals consume this fodder, metals can then be transferred to milk and through milk these heavy metals can transfer in milk based dairy products (like yoghurt, butter and cheese etc.), posing potential risks to human health through the food chain.

The formula used to calculate the translocation factor is : [15,16].

$$TF_1 = \frac{C_{fodder}}{C_{soil}} \qquad \text{………(2.8)}$$

$$TF_2 = \frac{C_{milk}}{C_{fodder}} \quad \text{.........(2.9)}$$

Where

C_{fodder} = Concentration of metal in fodder

C_{Soil} = Concentration of metal in soil

C_{milk} = Concentration of metal in milk

Bioaccumulation Factor (BAF) :

The Bioaccumulation factor (BAF) of a heavy metal is a measure that quantifies the extent to which a substance such as a heavy metal, accumulate in a cattle relative to its concentration in the surrounding environment such as water or soil.

The bioaccumulation Factor (BAF) [17] can be calculated using the following formula :

$$\text{BAF} = \frac{C_{milk}}{C_{soil}} \quad \text{.........(2.10)}$$

Where

C_{Soil} = Concentration of metal in soil

C_{milk} = Concentration of metal in milk

A higher BAF indicates that the cattle accumulate the metal to a greater extent compared to its environment, which can have implications for the food chain and ecosystem health.

Metal Pollution Index (MPI) :

The Metal Pollution Index (MPI) is a quantitative measure used to assess the level of metal contamination in dairy samples. It provides a single value that represents the combined concentration of multiple metals, helping to evaluate the overall metal pollution status of an area. The MPI is particularly useful in environmental monitoring and risk assessment.

The formula to calculate the metal pollution index can vary but a common approach is [18] :

$$\text{MPI}_{(\mu g\ g^{-1})} = (\text{Cf}_1 \times \text{Cf}_2 \times \ldots \times \text{Cf}_n)^{\frac{1}{n}} \quad \text{.........(2.11)}$$

Where

Cf_n = concentration of metal n in the sample.

By calculating the geometric mean of the metal concentrations, the MPI provides a composite measure of metal pollution, allowing for easier comparison between different sites or over time. A higher MPI indicates greater metal pollution.

Estimated Daily Intake (EDI) :

The Estimated Daily Intake (EDI) of dairy products refers to the average amount of dairy products consumed by an individual on a daily basis. This measurement is used to assess dietary patterns, nutritional intake and potential exposure to contaminants or nutrients present in dairy products [19].

$$EDI = \frac{(C_{metal} \times W_{DP})}{BW} \qquad \text{.......(2.12)}$$

Where

C_{metal} = Mean concentration of metal in milk (in mg/kg)

W_{DP} = The average consumption of dairy products per day, which is 125 g/day for Yoghurt, 28 g/day for cheese and 15 g/day for butter in India [20]

Bw = Average body weight of an Indian adult (in kg) which is used as 60 kg for the study [21,22].

Health Risk Index (HRI) :

The Health Risk Index (HRI) is a quantitative measure used to evaluate the potential health risks posed by exposure to hazardous substances, such as chemicals, pollutants or heavy metals. The HRI helps in assessing the likelihood and severity of adverse health effects resulting from exposure to these substances. [22,23]

$$HRI = \frac{EDI}{R_f D} \times 10^{-3} \qquad \text{............(2.13)}$$

Where,

EDI = Estimated daily intake of metal (mg day^{-1})

$R_f D$ = Oral Reference Dose (mg kg^{-1}day^{-1})

$R_f D$ for Pb, Cd, Al, As, Fe and Zn is 0.0035, 0.001, 1.0, 0.004, 0.7, and 0.3 mg/Kg per day respectively [24].

HRI < 1 : Indicates that the exposure is below the reference dose, suggesting a low risk of adverse health effects.

HRI = 1 : Indicates that the exposure is equal to the reference dose, suggesting a threshold level where adverse effects might start to occur.

HRI >1 : Indicates that the exposure exceeds the reference dose, suggesting a higher risk of adverse health effects and the need for risk management or mitigation measures.

2.9. Instrumental Analysis

2.9.1 Soil

Collection, Digestion & Analysis

Soil samples were collected using soil auger from various locations within the sites from the similar depth i.e., 0-15 cm and placed in a clean container and labelled appropriately to track their origin. All sample were dried to remove excess moisture, sieved to get homogenised sample and kept for 3 hrs in muffle furnace at 450ºC. The resulting ash was further digested with 25 ml of aqua regia and finally analysed with the help of AAS maintaining the conditions.

Table 2.10: Heavy metal concentrations in mg/L in soil samples collected from different sites

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	1.6231	0.1492	1.9842	0.0083	24.146	26.4361
	KKIF	0.4342	0.0754	1.5893	0.0332	4.0932	8.0834
Baran	BCIF	1.4377	0.1463	2.2356	0.0986	13.452	16.9372
	BMIF	0.5432	0.0652	1.4842	0.0222	3.7841	5.9312
Bundi	BnLIF	1.3878	0.1603	2.2237	0.0743	10.452	14.7842
	BnKIF	0.3429	0.0653	0.9832	0.0278	2.9841	3.9741
Jhalawar	JJIF	1.5329	0.0986	1.3826	0.0223	12.8432	10.4632
	JAIF	0.3985	0.0342	0.08832	0.0198	1.6973	2.4632

Table 2.10 shows the mean concentration of metals in soil samples, which clearly indicates that among all metals concentration of iron is found in abundance whereas arsenic concentration is found to be the lowest. It is clear from the table that in all 4 zones overall metal concentration is found maximum in the sample collected from the proximity of industrial facilities.

2.9.2 Water

Collection, Digestion & Analysis

Water samples (n =16) were collected using clean container from the similar sites from where soil samples were collected and proper labeling was done to track their origin. Digestion of water samples involved acid digestion method using concentrated HNO_3. Resulting samples were analyzed by AAS.

Table 2.11: Heavy metal concentrations in mg/L in water samples collected from different sites

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	0.1231	0.0092	0.1842	0.0083	3.2361	5.2156
	KKIF	0.0525	0.0044	0.0763	0.0000	0.9321	0.8634
Baran	BCIF	0.0677	0.0083	0.1562	0.0068	0.6451	1.4521
	BMIF	0.0432	0.0072	0.0442	0.0000	0.8853	0.6741
Bundi	BnLIF	0.0692	0.0082	0.0844	0.0073	0.7242	0.5825
	BnKIF	0.0327	0.0059	0.0367	0.0046	0.8561	0.4741
Jhalawar	JJIF	0.0653	0.0053	0.0502	0.0053	0.6272	0.6323
	JAIF	0.0485	0.0042	0.0332	0.0048	0.4974	0.4632

Table 2.11 shows the results of water sample analysis. From the results it is interpreted that the mean concentration of arsenic and cadmium varies from 0.000 - 0.0083 and 0.0042 – 0.0092 respectively, which is very very low. The mean concentration of zinc is highest among all samples.

2.9.3 Fodder

Collection, Digestion & Analysis

Collecting, digesting and analyzing fodder samples (n=16) involved gathering samples from various sources, breaking them down with a diacid mixture of HNO_3 and $HClO_4$ and analyzed by AAS.

Table 2.12: Heavy metal concentrations in mg/L in fodder samples collected from different sites

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	0.1134	0.0089	0.0823	0.0068	6.4521	8.3452
	KKIF	0.0354	0.0037	0.0545	0.0000	2.5343	4.4472
Baran	BCIF	0.0778	0.0093	0.0664	0.0066	4.1321	5.3175
	BMIF	0.0392	0.0071	0.0385	0.0000	1.6942	1.9536
Bundi	BnLIF	0.0667	0.0081	0.0784	0.0069	2.4322	2.3721
	BnKIF	0.0318	0.0049	0.0323	0.0039	0.7565	0.9742
Jhalawar	JJIF	0.0609	0.0049	0.0492	0.0048	1.7982	0.8948
	JAIF	0.0389	0.0024	0.0302	0.0000	0.5935	0.5653

Table 2.12 shows almost similar trend as **Table 2.11** .

On comparing the concentrations of all six metals in soil, water and fodder samples, the concentration of metals in soil is higher as compared to water samples. The mean concentrations of Fe and Zn are found to be higher in fodder samples. Based on the aforementioned result, it may be inferred that the plant absorbs more Fe and Zn than the other metals. That could have been caused by a number of previously documented observations like synthesis of chelating agents, the redox reaction and other phenomenone including permissibility, selectivity and absorption capacity [25].

2.9.4 Milk

Our study is focused mainly on Yoghurt, Butter, Cheese. They all are the products based on the milk. Therefore it is necessary to analyse the base product milk first before analysing the above given three dairy products.

Collection, Digestion & Analysis

For the evaluation of milk samples (N=16) gathered from various locations, they were collected in 100 ml PTFE bottles and kept at -20ºC. To digest milk sample 5 ml of its quantity was taken with 5 ml of nitric acid (65%) and 2 ml of H_2O_2 (30%) and heated initially at 90ºC. After that, the temperature was progressively raised to 120ºC until the brown fume vanished. During digestion, the organic matrix is broken down, leaving the constituent parts in a transparent solution. This clear solution was cooled and then filtered into a 25 ml volumetric flask. Prepared samples were then analysed with help of AAS.

Table 2.13: Heavy metal concentrations in mg/L in milk samples collected from different sites

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	0.1095	0.0088	0.0323	0.0058	0.6341	0.4452
	KKIF	0.0243	0.0037	0.0545	BIR	0.3442	0.3434
Baran	BCIF	0.0677	0.0098	0.0568	0.0058	0.4323	0.3875
	BMIF	0.0094	0.0039	0.0234	BIR	0.3532	0.3436
Bundi	BnLIF	0.0379	0.0044	0.0384	0.0053	0.4322	0.4721
	BnKIF	0.0281	0.0032	0.0193	0.0021	0.3565	0.3742
Jhalawar	JJIF	0.0409	0.0037	0.0411	0.0031	0.4182	0.4148
	JAIF	0.0288	0.0013	0.0152	BIR	0.3936	0.2653

BIR : Beyond Instrumental Range

The results of milk analysis are shown in **Table 2.13** from which it can be seen that the samples of all four areas contain all six metals either in lesser or higher quantity.

2.10. Translocation Factor

Table 2.14: Translocation factor (TF_1) (soil to fodder)

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	TF_1KRIF	0.06986631	0.0597	0.0415	0.0343	0.2672	0.3157
	TF_1KKIF	0.08152925	0.0557	0.0371	0.0000	0.6191	0.5502
Baran	TF_1BCIF	0.05411421	0.0636	0.0297	0.0669	0.3072	0.314
	TF_1BMIF	0.07216495	0.1089	0.0259	0.0000	0.4477	0.3294
Bundi	TF_1BnLIF	0.04806168	0.0505	0.0353	0.0929	0.2327	0.1604
	TF_1BnKIF	0.09273841	0.075	0.0329	0.1403	0.2535	0.2451
Jhalawar	TF_1JJIF	0.03972862	0.0497	0.0356	0.0469	0.1400	0.0855
	TF_1JAIF	0.09761606	0.0702	0.3419	0.0000	0.3497	0.2295

Table 2.15: Translocation factor (TF_2) (fodder to milk)

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	0.965608	0.988764	0.392467	0.852941	0.098278	0.053348
	KKIF	0.686441	0.880952	0.923729	0.000000	0.135817	0.077217
Baran	BCIF	0.870180	1.053763	0.855422	0.878788	0.104620	0.072873
	BMIF	0.239796	0.549296	0.607792	0.000000	0.208476	0.175880
Bundi	BnLIF	0.568216	0.543210	0.489796	0.768116	0.177699	0.199022
	BnKIF	0.883648	0.653061	0.597523	0.538462	0.471249	0.384110
Jhalawar	JJIF	0.671593	0.755102	0.835366	0.645833	0.232566	0.463567
	JAIF	0.740360	0.541667	0.503311	0.000000	0.663184	0.469308

Bio Accumulation factor (BAF) (MILK, SOIL)

Table 2.16 : Bio Accumulation Factor

Areas	Sites	Pb	Cd	Al	As	Fe	Zn
Kota	KRIF	0.067463	0.058981	0.016279	0.029249	0.026261	0.016841
	KKIF	0.055965	0.049072	0.034292	0.000000	0.084091	0.042482
Baran	BCIF	0.047089	0.066986	0.025407	0.058824	0.032136	0.022879
	BMIF	0.017305	0.059816	0.015766	0.000000	0.093338	0.057931
Bundi	BnLIF	0.027309	0.027449	0.017269	0.071332	0.041351	0.031933
	BnKIF	0.081948	0.049005	0.019630	0.075540	0.119467	0.094160
Jhalawar	JJIF	0.026681	0.037525	0.029727	0.030303	0.032562	0.039644
	JAIF	0.072271	0.038012	0.172101	0.000000	0.231898	0.107705

Table 2.14 & **2.15** shows the results of translocation of metals from soil to fodder and fodder to milk, suggesting that plants actively absorb the metals from soil which is then transferred to the cattle milk.

Table 2.16 represents the bioaccumulation factor results. According to the results no significant accumulation of heavy metals occurs as all BAF values are less than one. BAF values below one indicate a lower risk of biomagnification.

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CHAPTER - III

ASSESSMENT OF HEAVY METALS IN YOGHURT : INSTRUMENTAL AND STATISTICAL ANALYSIS

This Chapter is divided into four major sections i.e Introduction, Instrumental analysis, Statistical analysis and Conclusion. Instrumental analysis has been done by AAS, JMP is used for statistical analysis.

3.1 Introduction

Yoghurt is an excellent source of nutrients like protein, vitamin, minerals, fat etc. Thus among different dairy products yoghurt is a great choice as a nutritional food. Therefore yoghurt is included in the human diet and consumed by peoples of all age groups, especially women and children [1,2] and promote a healthy metabolism and balanced energy levels [3]. On the basis of fat contents, yoghurt can be found as regular, low fat and non-fat yoghurt [4]. Yoghurt is a coagulated milk which is prepared from fermentation of lactic acid by lactobacillus delbrueckus spp. Lactobacillus bulgaricus (Lb. bulgaricus) and Streptococcus thermophiles from milk.

3.2 Manufacturing Process of Yoghurt

Yoghurt is prepared through several steps including blending, pasteurization, inoculation and fermentation and cooling.

The fermentation process takes 4-8 hours, during this process bacteria continue to consume lactose from milk and produce lactic acid and further acidifying and thickening it. For better fermentation process, the temperature is controlled very carefully and the yoghurt is cooled to stop fermentation for stabilizing it. During this step fruit, honey or granola can be added as sweeteners, flavourings agents or as other ingredients.

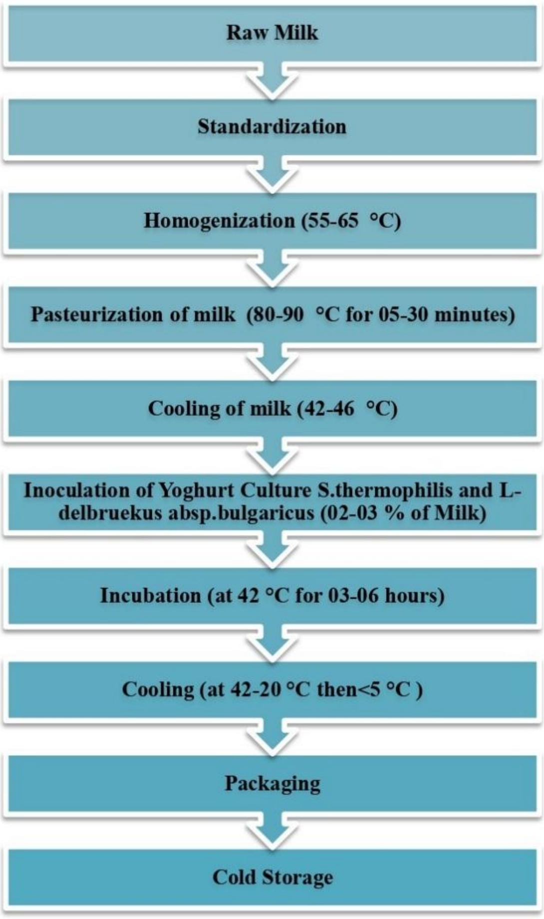


Figure 3.1: Flow Chart of Manufacturing of Yoghurt

Types of Yoghurt

3.3.1 Regular Yoghurt

One of the most beneficial foods is regular yoghurt. This yoghurt contains many useful ingredients like vitamins, minerals, etc. Also it is a great solution of milk substitute and has a high nutritional value like milk. It contains beneficial bacteria and elements like protein, calcium, vitamin-A, vitamin-6, vitamin-B12, phosphorus etc. It improves immune system of our body and helps to strengthen bones and teeth.

3.3.2 Kefir

A less thick dairy kefir is made from fermented milk, like yoghurt. It is more tangy and contains more probiotics than any yoghurt.

3.3.3 Greek Yoghurt

A Greek yoghurt is thicker, heavier and creamier in textures as it involves extra step during its manufacturing process. Greek yoghurt contains slightly less sugar and more protein.

3.3.4 Icelandic Yoghurt

Skyr is thick, creamy, fermented skim-milk cheese which is similar to yoghurt in taste and looks. It also has a higher protein content. It’s made up of heirloom Icelandic bacterial cultures. To make it thicker and creamier four times of milk is used than the milk used to make yoghurt.

3.3.5 French Yoghurt

French yoghurt is first fermented and then pot-set, like regular yoghurt. Regular yoghurt is usually poured into pots after fermentation process.

3.3.6 Australian Yoghurt

To make australian yoghurt whole milk is used. Straining is not done for this type of yoghurt. Unlike regular yoghurt slow and long fermentation process is used. To make it more thicker extra cream is used.

3.3.7 Lactose-free Yoghurt

This type of yoghurt is used by the lactose intolerant people. It is not same as dairy free yoghurt. For making this kind of yoghurt lactase enzymes is used to break lactose into simple sugars which is easier to digest.

3.3.8 Dairy-free Yoghurt

Dairy-free yoghurts are prepared from various plant-based milk, such as almond, coconut, soy, oat or cashew milk.

In yoghurt there are many micro (Fe, Zn, Cu, Mn, se) and macro elements (Ca, K, P) are present which are beneficial to human health and play an very important role in various physiological functions of the human body whereas other elements like As, Cr, Cd, and Pb, are hazardous and could have a negative impact to human health [5].

Different environmental factors, agriculture practices, such as spraying pesticides, irrigation of crops by contaminated water may be directly or indirectly responsible for the presence of heavy metals in yoghurt [6].

Yoghurt can also be contaminated by various other factors like containers and equipment used for manufacturing procedure and by packaging processes etc. [7-11].

Our main study is focused on the Regular yoghurt as it is highly consumed and the presence of heavy metals in yoghurt can cause a serious risk to human health [12]. Effect of extra added flavours and processing/ packaging have also been studied in this chapter.

The investigation of heavy metals in yoghurt in various regions of Kota division of Rajasthan is covered in this chapter. Various samples were collected from different areas of Kota division, Rajasthan. For this, the areas have been split up into four zone Kota, Baran, Bundi and Jhalawar. Within each of these zones, there are then two subzones: Less polluted and More polluted (industrial area). The goal of the current study was to evaluate six specific metals like Pb, Cd, Al, As, Fe and Zn in 80 yoghurt samples that were collected from various local shops and individual farms.

3.4 Collection, Digestion & Analysis of Yoghurt

Yoghurt samples were collected from more and less polluted areas of four districts. All areas are subdivided into two subareas. Five samples of yoghurt (50 gm) were collected from each subarea in PTFE bottles and stored at -20ºC before digestion. Digestion technique MW was finalised after performing recovery test. Percentage recovery for all six metals Pb, Cd, Al, As, Fe and Zn were found 97.32, 97.1, 95.17, 93.75, 96.4 and 95.64 % respectively (as presented in **Table 2.4** in chapter 2).
So the MW method was used to digest all yoghurt samples. In this method 1 gm of sample was digested with 4 ml of 65% HNO_3 and 2 ml of 36% H_2O_2 in microwave oven using the condition as per given in **Table 2.1** in chapter 2. Resulting solution was transferred into 10 ml volumetric flask and diluted with deionised water. After preparing the samples, elemental analysis has been done by AAS.

The detailed instrumental analysis results are given below:

3.4.1 KOTA DISTRICT

3.4.1.1 More Polluted Area (Ranpur)

(a) Individual Farms

Table 3.1: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of KRIF

Samples/Heavy metals	Pb	Cd	Al	As	Fe	Zn
KRIF - Y1	0.0487	0.0062	0.0329	0.0031	0.4979	0.3136
KRIF - Y2	0.0594	0.0087	0.0298	BIR	0.3872	0.2685
KRIF - Y3	0.0351	0.0282	0.1056	0.0028	0.6991	0.2612
KRIF - Y4	0.0541	0.0063	0.0294	0.0042	0.5411	0.2863
KRIF - Y5	0.0256	0.0174	0.0829	0.0038	0.4931	0.4983
Minimum	0.0256	0.0062	0.0294	BIR	0.3872	0.2612
Maximum	0.0594	0.0282	0.1056	0.0042	0.6991	0.4983
Mean	**0.0446**	**0.0134**	**0.0561**	**0.0028**	**0.5237**	**0.3256**
SD	0.0125	0.0085	0.0320	0.0015	0.1013	0.0882
Variance	0.0002	0.0001	0.0010	2E-06	0.0103	0.0078

KRIF : Kota Ranpur Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 3.1 presented the data of more polluted area Ranpur of kota district. Heavy anthropogenic activities are carried out their as it is surrounded by so many industries. Heavy metal concentration of yoghurt samples of Ranpur area given in **Table 3.1**. From the table it can be seen that Fe and Zn are found in abundance as compared to other metals. It is also evident from the table that the order of mean concentration are Fe > Zn > Al > Pb > Cd > As. As is found to be below detection limit in sample Y2 .

(b) Local Shops

Table 3.2: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of KRLS

Samples/Heavy metals	Pb	Cd	Al	As	Fe	Zn
KRLS - Y1	0.0798	0.0182	0.0529	BIR	0.4947	0.3136
KRLS - Y2	0.0374	0.0197	0.1018	0.0089	0.8872	0.2985
KRLS - Y3	0.0519	0.0082	0.0952	0.0037	0.7991	0.5612
KRLS - Y4	0.0396	0.0194	0.1694	0.0121	0.8931	0.5531
KRLS - Y5	0.0447	0.0097	0.0484	0.0107	0.6732	0.4883
Minimum	0.0374	0.0082	0.0484	BIR	0.4947	0.2985
Maximum	0.0798	0.0197	0.1694	0.0121	0.8931	0.5612
Mean	**0.0507**	**0.0150**	**0.0935**	**0.0071**	**0.7495**	**0.4429**
SD	0.0154	0.0050	0.0436	0.0045	0.1502	0.1147
Variance	0.0002	-	0.0019	2E-05	0.0226	0.0132

KRLS : Kota Ranpur Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

For local shop samples similar trends in concentrations can be seen. The minimum and maximum concentration of Pb, Cd, Al, As, Fe and Zn are found to be 0.0374, 0.0082, 0.0484, BIR, 0.4947, 0.2985 and 0.0798, 0.0197, 0.1694, 0.0121, 0.8931, 0.5612 mg/L respectively. For KRLS also, Arsenic is absent in samples Y1.

3.4.1.2 Less Polluted Area (Kaithoon)

(a) Individual Farms

Table 3.3: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of KKIF

Samples	Pb	Cd	Al	As	Fe	Zn
KKIF - Y1	0.0122	0.0022	0.0253	BIR	0.4291	0.3812
KKIF - Y2	0.0148	0.0024	0.0114	BIR	0.3411	0.2855
KKIF - Y3	0.0122	0.0021	0.0231	BIR	0.1491	0.2628
KKIF - Y4	0.0224	BIR	0.0241	BIR	0.3101	0.2541
KKIF - Y5	0.0145	BIR	0.0106	BIR	0.4733	0.3223
Minimum	0.0122	BIR	0.0106	BIR	0.1491	0.2541
Maximum	0.0224	0.0024	0.0253	BIR	0.4733	0.3812
Mean	**0.0152**	**0.0013**	**0.0189**	BIR	**0.3405**	**0.3012**
SD	0.0038	0.0011	0.0065	BIR	0.1123	0.0464
Variance	-	-	-	-	0.0126	0.0022

KKIF : Kota Kaithoon Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 3.4: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of KKLS

Samples	Pb	Cd	Al	As	Fe	Zn
KKLS - Y1	0.0122	BIR	0.0142	0.002	0.1614	0.3222
KKLS - Y2	0.0181	BIR	0.0124	0.0014	0.4221	0.2255
KKLS - Y3	0.0121	0.0032	0.0221	0.0006	0.4558	0.3871
KKLS - Y4	0.0274	0.0021	0.0282	0.0034	0.4325	0.2415
KKLS - Y5	0.0145	0.0034	0.0206	0.0017	0.3241	0.4172
Minimum	0.0121	BIR	0.0124	0.0006	0.1614	0.2255
Maximum	0.0274	0.0034	0.0282	0.0034	0.4558	0.4172
Mean	**0.0169**	**0.0017**	**0.0195**	**0.0018**	**0.3592**	**0.3187**
SD	0.0057	0.0015	0.0057	0.0009	0.1086	0.0762
Variance	-	-	-	8E-07	0.0118	0.0058

KKLS : Kota Kaithoon Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 3.3 and **3.4** shows the results of less polluted area of Kota that is Kaithoon. Similar results were found for both subareas. It is evident from both the tables that the hazardous metal As is totally absent in KKIF yoghurt samples whereas minimum amount of As is found in KKLS samples. Minimum concentration of Cd has also found to be below the detection limit.

3.4.2 BARAN DISTRICT

3.4.2.1 More Polluted Area (Chhabra Motipura)

(a) Individual Farms

Table 3.5: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BCIF

Samples	Pb	Cd	Al	As	Fe	Zn
BCIF - Y1	0.0335	0.0076	0.0398	0.0019	0.3809	0.3226
BCIF - Y2	0.0464	0.0057	0.0638	BIR	0.4372	0.4085
BCIF - Y3	0.0287	0.0095	0.0291	0.0056	0.2981	0.3102
BCIF - Y4	0.0321	0.0047	0.0564	0.0032	0.5121	0.2541
BCIF - Y5	0.0423	0.0112	0.0631	0.0028	0.4658	0.4322
Minimum	0.0287	0.0047	0.0291	BIR	0.2981	0.2541
Maximum	0.0464	0.0112	0.0638	0.0056	0.5121	0.4322
Mean	**0.0366**	**0.0077**	**0.0504**	**0.0027**	**0.4188**	**0.3455**
SD	0.0066	0.0024	0.0137	0.0018	0.0738	0.0657
Variance	-	-	0.0002	3E-06	0.0055	0.0043

BCIF : Baran Chhabra Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 3.5 represents the comparison of heavy metal concentration in yoghurt sample of various individual farms of Chhabra Motipura of Baran district. Each row displays the concentration of all six metals in one sample. The minimum concentration of Pb, Cd, Al, As, Fe, and Zn are 0.0287, 0.0047, 0.0291, BIR, 0.2981, 0.2541 mg/L respectively and maximum concentrations are 0.0464, 0.0112, 0.0638, 0.0056, 0.5121, 0.4322 mg/L. The mean concentration order for all metals are 0.0366, 0.0077, 0.0504, 0.0027, 0.4188 and 0.3455 mg/L respectively.

(b) Local Shops

Table 3.6: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BCLS

Samples	Pb	Cd	Al	As	Fe	Zn
BCLS - Y1	0.0537	0.0075	0.0601	0.0023	0.3811	0.3225
BCLS - Y2	0.0366	0.0089	0.0441	0.0051	0.4371	0.4087
BCLS - Y3	0.0289	0.0046	0.0792	0.0057	0.6982	0.3107
BCLS - Y4	0.0324	0.0151	0.0683	0.0037	0.6124	0.4543
BCLS - Y5	0.0426	0.0114	0.0532	0.0073	0.4661	0.4325
Minimum	0.0289	0.0046	0.0441	0.0023	0.3811	0.3107
Maximum	0.0537	0.0151	0.0792	0.0073	0.6982	0.4543
Mean	**0.0388**	**0.0095**	**0.0610**	**0.0048**	**0.5190**	**0.3857**
SD	0.0087	0.0036	0.0121	0.0017	0.1178	0.0584
Variance	0.0001	-	0.0001	3E-06	0.0139	0.0034

BCLS : Baran Chhabra Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Samples collected from local shops of more polluted area of Baran are presented in **Table 3.6** and similar trends are observed. On comparing the heavy metal concentration in IF and LS , the value of Pb and Zn are almost similar in both the places whereas Cd, Al, As and Fe are little higher in samples collected from local shops.

3.4.2.2 Less Polluted Area (Mangrol)

(a) Individual Farms

Table 3.7: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BMIF

Samples	Pb	Cd	Al	As	Fe	Zn
BMIF - Y1	0.0098	0.0066	0.0145	BIR	0.3999	0.2226
BMIF - Y2	0.0149	0.0013	0.0238	BIR	0.4234	0.3275
BMIF - Y3	0.0244	0.0012	0.0124	BIR	0.2411	0.3148
BMIF - Y4	0.0121	0.0009	0.0234	BIR	0.2301	0.2973
BMIF - Y5	0.0293	BIR	0.0216	BIR	0.3348	0.4992
Minimum	0.0098	BIR	0.0124	BIR	0.2301	0.2226
Maximum	0.0293	0.0066	0.0238	BIR	0.4234	0.4992
Mean	**0.0181**	**0.0020**	**0.0191**	**BIR**	**0.3259**	**0.3323**
SD	0.0075	0.0023	0.0048	BIR	0.0793	0.0910
Variance	0.0001	-	-	-	0.0063	0.0083

BMIF : Baran Mangrol Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 3.7 highlights the heavy metal concentration in yoghurt samples of various individual farms of Mangrol of Baran district which is less polluted area as there is no anthropogenic activities are going on. It can be seen from the table that As is totally absent and Cd concentration is also found very low in IF.

(b) Local Shops

Table 3.8: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BMLS

Samples	Pb	Cd	Al	As	Fe	Zn
BMLS - Y1	0.0114	0.0024	0.0109	0.0011	0.3561	0.4287
BMLS - Y2	0.0167	0.0032	0.0231	0.0018	0.4336	0.2719
BMLS - Y3	0.0147	0.0023	0.0242	0.0007	0.3192	0.2981
BMLS - Y4	0.0294	0.0029	0.0233	0.0031	0.2134	0.3294
BMLS - Y5	0.0166	BIR	0.0225	0.0032	0.3506	0.3171
Minimum	0.0114	BIR	0.0109	0.0007	0.2134	0.2719
Maximum	0.0294	0.0032	0.0242	0.0032	0.4336	0.4287
Mean	**0.0178**	**0.0022**	**0.0208**	**0.0020**	**0.3346**	**0.3290**
SD	0.0061	0.0011	0.0050	0.0010	0.0713	0.0535
Variance	-	-	-	1E-06	0.0051	0.0029

BMLS : Baran Mangrol Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Results of **Table 3.8** represents the heavy metal concentration in yoghurt samples of various local shops, which shows the mean concentration of Pb, Cd, Al, As, Fe and Zn are 0.0178, 0.0022, 0.0208, 0.0020, 0.3346 and 0.3290 mg/L respectively.

These result indicate that the concentration of heavy metals in LS are slightly higher than IF.
The pattern for mean concentration of heavy metals are found as follows :
As < Cd < Pb < Al < Zn < Fe
The results of more polluted and less polluted areas are presented in **Table 3.5**, **Table 3.6**, **Table 3.7** and **Table 3.8**, shows that concentration of heavy metals in less polluted areas are lower than the more polluted areas.

3.4.3 BUNDI DISTRICT

3.4.3.1 More Polluted Area (Lakheri)

(a) Individual Farms

Table 3.9: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BnLIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnLIF - Y1	0.0499	0.0074	0.0157	0.0017	0.3808	0.3227
BnLIF - Y2	0.0237	0.0028	0.0463	0.0049	0.4374	0.4086
BnLIF - Y3	0.0264	0.0046	0.0288	0.0054	0.2983	0.3103
BnLIF - Y4	0.0566	0.0045	0.0323	0.0031	0.5122	0.5542
BnLIF - Y5	0.0233	0.0013	0.0624	0.0067	0.4659	0.4323
Minimum	0.0233	0.0013	0.0157	0.0017	0.2983	0.3103
Maximum	0.0566	0.0074	0.0624	0.0067	0.5122	0.5542
Mean	**0.0360**	**0.0041**	**0.0371**	**0.0044**	**0.4189**	**0.4056**
SD	0.0143	0.0020	0.0160	0.0018	0.0738	0.0880
Variance	0.0002	-	0.0003	3E-06	0.0055	0.0078

BnLIF : Bundi Lakheri Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 3.10: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BnLLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnLLS - Y1	0.0345	0.0077	0.0439	0.0024	0.3815	0.3223
BnLLS - Y2	0.0442	0.0031	0.0568	0.0053	0.6369	0.5786
BnLLS - Y3	0.0265	0.0147	0.0591	0.0059	0.4981	0.3104
BnLLS - Y4	0.0571	0.0049	0.0357	0.0038	0.5421	0.4541
BnLLS - Y5	0.0435	0.0016	0.0288	0.0077	0.6659	0.4324
Minimum	0.0265	0.0016	0.0288	0.0024	0.3815	0.3104
Maximum	0.0571	0.0147	0.0591	0.0077	0.6659	0.5786
Mean	**0.0412**	**0.0064**	**0.0449**	**0.0050**	**0.5449**	**0.4196**
SD	0.0103	0.0046	0.0117	0.0018	0.1020	0.0980
Variance	0.0001	-	0.0001	3E-06	0.0104	0.0096

BnLLS : Bundi Lakheri Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The results of IF and LS of Lakheri which is more polluted area of Bundi district, are presented in **Table 3.9** and **Table 3.10**, shows that mean concentration of heavy metals Pb, Cd, Al, As, Fe and Zn are 0.0360, 0.0041, 0.0371, 0.0044, 0.4189 and 0.4056 mg/L respectively in IF while 0.0412, 0.0064, 0.0449, 0.0050, 0.5449 and 0.4196 mg/L respectively in LS. These result indicates that the concentration of heavy metals are found slightly higher in LS as compare to IF.

3.4.3.2 Less Polluted Area (Kapren)

(a) Individual Farms

Table 3.11: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BnKIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnKIF - Y1	0.0097	0.0005	0.0146	BIR	0.3994	0.2224
BnKIF - Y2	0.0151	BIR	0.0236	BIR	0.4236	0.3274
BnKIF - Y3	0.0246	0.0023	0.0126	0.0031	0.2413	0.3147
BnKIF - Y4	0.0123	0.0044	0.0235	BIR	0.2303	0.2971
BnKIF - Y5	0.0291	0.0023	0.0217	0.0037	0.3349	0.4991
Minimum	0.0097	BIR	0.0126	BIR	0.2303	0.2224
Maximum	0.0291	0.0044	0.0236	0.0037	0.4236	0.4991
Mean	**0.0182**	**0.0019**	**0.0192**	**0.0014**	**0.3259**	**0.3321**
SD	0.0074	0.0016	0.0047	0.0017	0.0792	0.0911
Variance	0.0001	-	-	3E-06	0.0063	0.0083

BnKIF : Bundi Kapren Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

The result of the metals concentration of all samples presented in **Table 3.11** indicate that the minimum concentration Pb, Cd, Al, As, Fe and Zn are 0.0097, BIR, 0.0126, BIR, 0.2303, 0.2224 mg/L , maximum concentrations are 0.0291, 0.0044, 0.0236, 0.0037, 0.4236, 0.4991 mg/L respectively and mean concentrations are 0.0182, 0.0019, 0.0192, 0.0014, 0.3259 and 0.3321 mg/L respectively.

(b) Local Shops

Table 3.12: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of BnKLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnKLS - Y1	0.0205	0.0025	0.011	0.0061	0.3563	0.4289
BnKLS - Y2	0.0169	0.0025	0.0232	BIR	0.4337	0.2718
BnKLS - Y3	0.0148	0.0074	0.0239	0.0005	0.3191	0.2982
BnKLS - Y4	0.0293	0.0023	0.0236	0.0033	0.2132	0.3295
BnKLS - Y5	0.0165	0.0013	0.0226	BIR	0.3507	0.3172
Minimum	0.0148	0.0013	0.011	BIR	0.2132	0.2718
Maximum	0.0293	0.0074	0.0239	0.0061	0.4337	0.4289
Mean	**0.0196**	**0.0032**	**0.0209**	**0.0020**	**0.3346**	**0.3291**
SD	0.0052	0.0021	0.0049	0.0024	0.0715	0.0536
Variance	-	-	-	6E-06	0.0051	0.0029

BnKLS : Bundi Kapren Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The result of **Table 3.12** indicates that the concentration of heavy metals in samples collected from local shops are in the range of 0.0148-0.0293, 0.0013-0.0074, 0.011-0.0209, BIR-0.0061, 0.2132-0.4337 and 0.2718-0.4289 mg/L in Pb, Cd, Al, As, Fe and Zn respectively and mean concentrations are 0.0196, 0.0032, 0.0209, 0.0020, 0.3346 and 0.3291 mg/L respectively.

It can be seen from the **Table 3.11** and **3.12** that the concentration of As and Cd are found to be very low and concentration of Pb, Al, Fe and Zn also found with in permissible limits, which are similar for both IF and LS.

3.4.4 JHALAWAR DISTRICT

3.4.4.1 More Polluted Area (Jhalarapatan Kali Sind Thermal Power Plant)

(a) Individual Farms

Table 3.13: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of JJIF

Samples	Pb	Cd	Al	As	Fe	Zn
JJIF - Y1	0.0496	0.0071	0.0136	0.0015	0.3407	0.321
JJIF - Y2	0.0241	0.0031	0.0461	0.0045	0.4373	0.4075
JJIF - Y3	0.0253	0.0047	0.0287	0.0051	0.2932	0.3101
JJIF - Y4	0.0582	0.0043	0.0321	0.0029	0.5117	0.5441
JJIF - Y5	0.0231	0.0012	0.0623	0.0065	0.4649	0.4312
Minimum	0.0231	0.0012	0.0136	0.0015	0.2932	0.3101
Maximum	0.0582	0.0071	0.0623	0.0065	0.5117	0.5441
Mean	**0.0361**	**0.0041**	**0.0366**	**0.0041**	**0.4096**	**0.4028**
SD	0.0148	0.0019	0.0165	0.0017	0.0807	0.0849
Variance	0.0002	-	0.0003	3E-06	0.0065	0.0072

JJIF : Jhalawar Jhalarapatan Individual Farm, SD : Standard deviation

Table 3.13 shows that the concentration of heavy metals in different samples collected from more polluted areas of Jhalarapatan of Jhalawar district. It can be observed that the metal Pb, Cd, Al, Fe and Zn are found slightly higher i.e., 0.0361, 0.0041, 0.0366, 0.4096 and 0.4028 mg/L whereas concentration of Arsenic (As) is observed to be below the permissible limit.

(b) Local Shops

Table 3.14: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of JJLS

Samples	Pb	Cd	Al	As	Fe	Zn
JJLS - Y1	0.0429	0.0067	0.0325	0.0014	0.3805	0.3203
JJLS - Y2	0.0558	0.0121	0.0432	0.0043	0.6349	0.5776
JJLS - Y3	0.0581	0.0037	0.0279	0.0049	0.4971	0.3103
JJLS - Y4	0.0317	0.0039	0.0571	0.0028	0.5411	0.4531
JJLS - Y5	0.0138	BIR	0.0625	0.0067	0.6639	0.4314
Minimum	0.0138	BIR	0.0279	0.0014	0.3805	0.3103
Maximum	0.0581	0.0121	0.0625	0.0067	0.6639	0.5776
Mean	**0.0405**	**0.0053**	**0.0446**	**0.0040**	**0.5435**	**0.4185**
SD	0.0164	0.0040	0.0134	0.0018	0.1016	0.0980
Variance	0.0003	-	0.0002	3E-06	0.0103	0.0096

JJLS : Jhalawar Jhalarapatan Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The value presented in **Table 3.14** have shown similar trend like **Table 3.13.** From these results it is evident that concentration of all metals for the samples collected from the LS are slightly higher than the values of IF. This might be due to the storage containers and effect of packaging.

3.4.4.2 Less Polluted Area (Aklera)

(a) Individual Farms

Table 3.15: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of JAIF

Samples	Pb	Cd	Al	As	Fe	Zn
JAIF - Y1	0.0094	BIR	0.0147	0.0013	0.3984	0.2221
JAIF - Y2	0.0149	0.0032	0.0231	0.0024	0.4232	0.3272
JAIF - Y3	0.0244	BIR	0.0122	0.0002	0.2403	0.3141
JAIF - Y4	0.0121	0.0016	0.0231	BIR	0.2313	0.2961
JAIF - Y5	0.0289	0.0024	0.0215	0.0047	0.3347	0.4987
Minimum	0.0094	BIR	0.0122	BIR	0.2313	0.2221
Maximum	0.0289	0.0032	0.0231	0.0047	0.4232	0.4987
Mean	**0.0179**	**0.0014**	**0.0189**	**0.0017**	**0.3256**	**0.3316**
SD	0.0075	0.0013	0.0046	0.0017	0.0788	0.0911
Variance	0.0001	-	-	3E-06	0.0062	0.0083

JAIF : Jhalawar Aklera Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

The data presented in **Table 3.15** indicates the heavy metal concentration in various yoghurt samples collected from less polluted area Aklera of Jhalawar district. The minimum concentration of Pb, Cd, Al, As, Fe and Zn are 0.0094, BIR, 0.0122, BIR, 0.2313, 0.222 mg/L and the maximum concentrations are 0.0289, 0.0032, 0.0231, 0.0047, 0.4232, 0.4987 mg/L respectively. The mean concentrations are 0.0179, 0.0014, 0.0189, 0.0017, 0.3256 and 0.3316 mg/L respectively. As and Cd are found to be below detection limit and in some samples these metals are not detected.

(b) Local Shops

Table 3.16: Concentration (mg/L) of metals (Mean±SD) in yoghurt sample of JALS

Samples	Pb	Cd	Al	As	Fe	Zn
JALS - Y1	0.0129	0.0042	0.0225	0.0038	0.2805	0.2341
JALS - Y2	0.0238	0.0022	0.0172	0.0037	0.3349	0.3406
JALS - Y3	0.0181	0.0017	0.0199	0.0029	0.4971	0.3103
JALS - Y4	0.0217	BIR	0.0165	BIR	0.3116	0.3981
JALS - Y5	0.0138	0.0032	0.0195	BIR	0.2349	0.4914
Minimum	0.0129	BIR	0.0165	BIR	0.2349	0.2341
Maximum	0.0238	0.0042	0.0225	0.0038	0.4971	0.4914
Mean	**0.0181**	**0.0023**	**0.0191**	**0.0021**	**0.3318**	**0.3549**
SD	0.0043	0.0014	0.0021	0.0017	0.0892	0.0864
Variance	-	-	-	3E-06	0.0080	0.0075

JALS : Jhalawar Aklera Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The data presented in **Table 3.16** indicates that the mean concentration of Pb, Cd, Al, As, Fe and Zn follow same trend as per **Table 3.15**. The order of mean concentration of heavy metals found are as follows: As < Cd < Pb < Al < Fe < Zn

3.5 Graphical Representation

The graphs given below represent the comparison of individual concentration in all four locations.

3.5.1 Pb Concentration

Table 3.17: Lead concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0446	0.0507	0.0152	0.0169
Baran	0.0366	0.0388	0.0181	0.0178
Bundi	0.0360	0.0412	0.0182	0.0196
Jhalawar	0.0361	0.0405	0.0179	0.0181

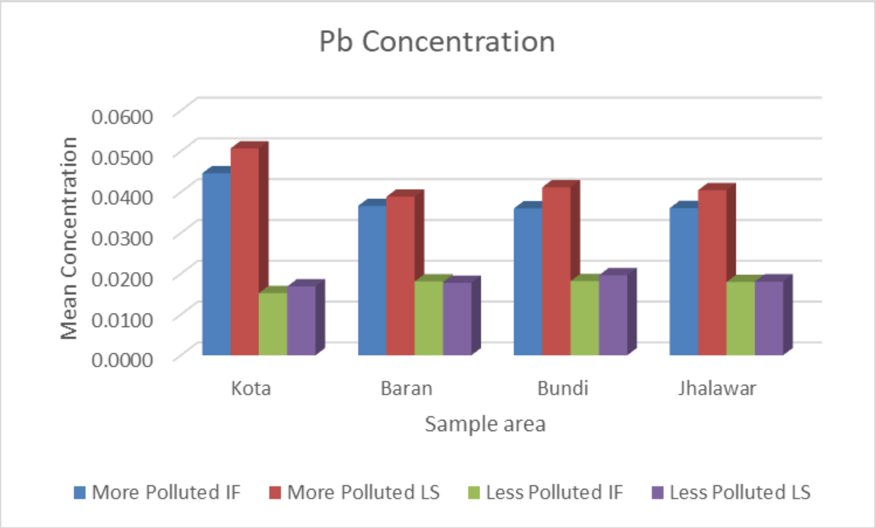


Figure 3.2: Lead concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

3.5.2 Cd Concentration

Table 3.18: Cadmium concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0134	0.0150	0.0013	0.0017
Baran	0.0077	0.0095	0.0020	0.0022
Bundi	0.0041	0.0064	0.0019	0.0032
Jhalawar	0.0041	0.0053	0.0014	0.0023

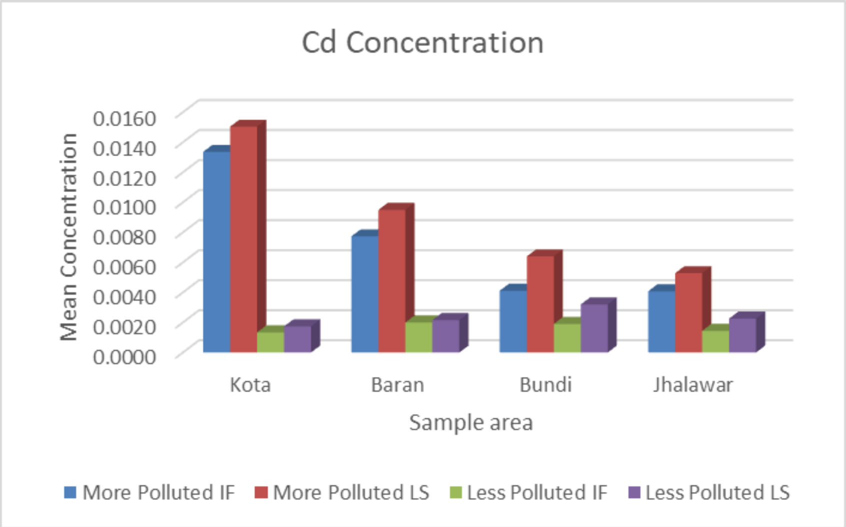


Figure 3.3: Cadmium concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

3.5.3 Al Concentration

Table 3.19: Aluminium concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0561	0.0935	0.0189	0.0195
Baran	0.0504	0.0610	0.0191	0.0208
Bundi	0.0371	0.0449	0.0192	0.0209
Jhalawar	0.0366	0.0446	0.0189	0.0191

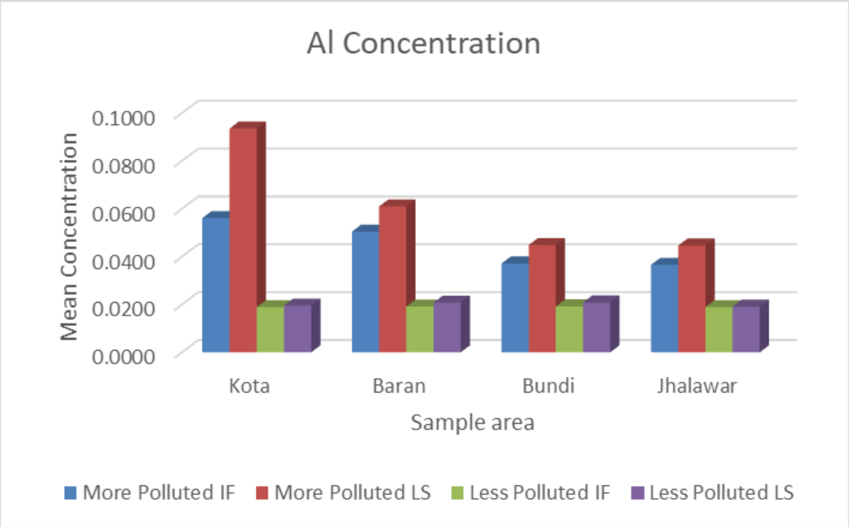


Figure 3.4: Aluminium concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

3.5.4 As Concentration

Table 3.20: Arsenic concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0028	0.0071	BIR	0.0018
Baran	0.0027	0.0048	BIR	0.0020
Bundi	0.0044	0.0050	0.0014	0.0020
Jhalawar	0.0041	0.0040	0.0017	0.0021

BIR : Beyond Instrumental Range

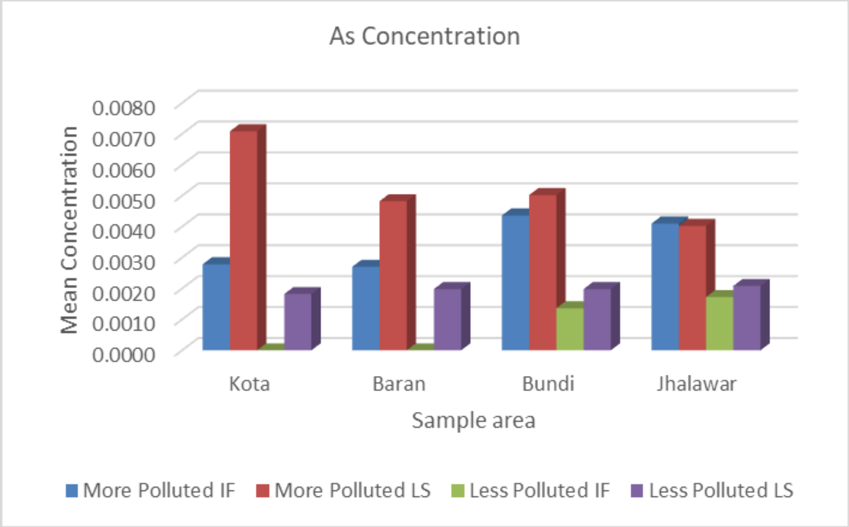


Figure 3.5: Arsenic concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

3.5.5 Fe Concentration

Table 3.21: Iron concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.5237	0.7495	0.3405	0.3592
Baran	0.4188	0.5190	0.3259	0.3346
Bundi	0.4189	0.5449	0.3259	0.3346
Jhalawar	0.4096	0.5435	0.3256	0.3318

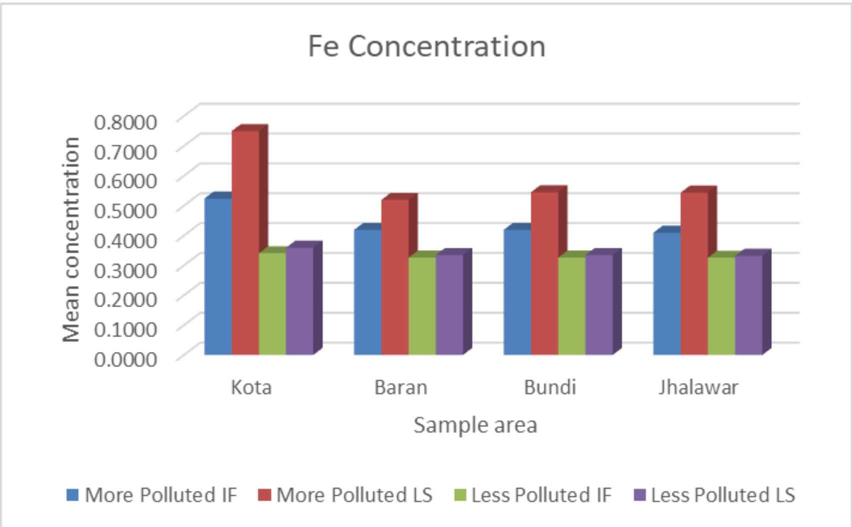


Figure 3.6: Iron concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

3.5.6 Zn Concentration

Table 3.22: Zinc concentration (mg/L) in Yoghurt samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.3256	0.4429	0.3012	0.3187
Baran	0.3455	0.3857	0.3323	0.3290
Bundi	0.4056	0.4196	0.3321	0.3291
Jhalawar	0.4028	0.4185	0.3316	0.3549

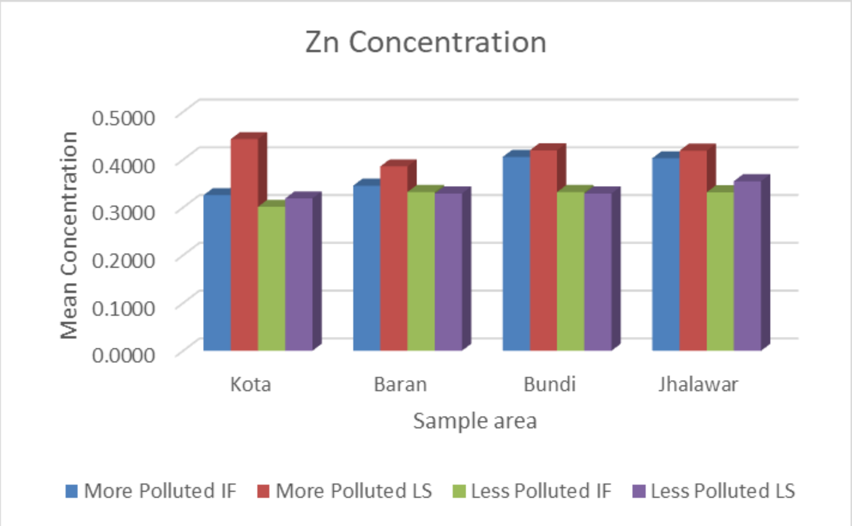


Figure 3.7: Zinc concentration (mg/L) in Yoghurt samples in four different areas of Kota region.

The above given graphs for Pb, Cd, Al, Fe, as it is clearly indicated that there is a significant difference between more polluted and less polluted sites, whereas for the Zn this difference is less. As concentration in less polluted area of Kota and Baran is very less as compared to other sites.

3.6 Effect of processing and packaging in different types of yoghurt with time

To study the effect of processing on yoghurt samples, processed yoghurt, yoghurt drink and flavored yoghurt samples of different brands were taken. To assess the effect of packaging with time in the above mentioned samples, samples were taken out at zero day which refers as the first stage and were immediately digested and assessed. Remaining samples were stored and digested & assessed after 7 days of opening of packaging, this is the second stage. The third and last stage of digestion & assessment was done after 15 days.

Table 3.23: Temporal Analysis of Heavy Metal Concentration in Yoghurt Samples

Metals/Time		Pb	Cd	Al	As	Fe	Zn
0 day	YD	0.0054	0.0006	0.0135	BIR	0.2751	0.2123
	Y	0.0067	0.0018	0.0186	0.0008	0.2341	0.2212
	FY	0.0176	0.0021	0.0211	0.0019	0.3232	0.3021
7 day	YD	0.0159	0.0014	0.0179	0.0006	0.3214	0.3172
	Y	0.0169	0.0022	0.0206	0.0011	0.2935	0.3032
	FY	0.0195	0.0025	0.0241	0.0022	0.3547	0.3142
15 day	YD	0.0162	0.0035	0.0213	0.0014	0.3645	0.3254
	Y	0.0171	0.0031	0.0176	0.0023	0.3245	0.3261
	FY	0.0198	0.0037	0.0256	0.0029	0.3674	0.4215

YD : Yoghurt Drink , Y : Yoghurt , FY : Flavoured Yoghurt, BIR : Beyond Instrumental Range

In the **Table 3.23**, the concentration of heavy metals Pb, Cd, Al, As, Fe and Zn (mg/L) in YD, Y and FY shows at three different validity time i,e,. 0 days, 7th day and 15th day.

Initial Time Point, T$_i$ (At 0 day) : Table 3.23 illustrates that the mean concentration of Pb, Cd, Al, As, Fe and Zn are 0.0054, 0.0006, 0.0135, BIR, 0.2751 and 0.2123 mg/L in YD, 0.0067, 0.0018, 0.0186, 0.0008, 0.2341 and 0.2212 mg/L in Y and 0.0176, 0.0021, 0.0211, 0.0019, 0.3232 and 0.3021 mg/L in FY respectively. So metal concentration in the first stage (0 days) of YD, Y and FY are in the order Fe > Zn > Al > Pb > Cd > As.

Mid Time Point, T$_m$ (At 7th day) : Table 3.23 illustrates that the mean concentration of Pb, Cd, Al, As, Fe and Zn are 0.0159, 0.0014, 0.0179, 0.0006, 0.3214 and 0.3172 mg/L in YD, 0.0169, 0.0022, 0.0206, 0.0011, 0.2935 and 0.3032 mg/L in Y and 0.0195, 0.0025, 0.0241, 0.0032, 0.3547 and 0.3142 mg/L in FY respectively. So metal concentration in the second stage (7 days) of YD is in the order Fe > Zn > Al > Pb > Cd > As. But in Y sample Zn is found slightly higher than Fe.

Final Time Point, T$_f$ (At 15th day) : Table 3.23 illustrates that the mean concentration of Pb, Cd, Al, As, Fe and Zn are 0.0162, 0.0035, 0.0213, 0.0014, 0.3645 and 0.3254 mg/L in YD, 0.0171, 0.0031, 0.0176, 0.0023, 0.3245 and 0.3261 mg/L in Y and 0.0198, 0.0037, 0.0256, 0.0029, 0.3674 and 0.4215 mg/L in FY respectively. So metal concentration in the third stage (15 days) of YD is in the order Fe > Zn > Al > Pb > Cd > As. Whereas in Y and FY samples Zn is found slightly higher than Fe.

From **Table 3.23** it can be clearly seen that there is a slight increase in metal concentration with time. It might be due to the packaging material. Among all these samples, flavored yoghurt has little higher concentrations of metals. It might be due to added fruits and fruit syrups. There are high chances of presence of heavy metals in added fruit and fruit syrup also.

3.7 Statistical Analysis

The one way analysis of variance (ANOVA) is a statistical method for testing of differences in the means of more than two groups. For this study one way ANOVA is performed to determine the statistical evidence and significant difference among the cities taken, the pollution status and the types [13].

To prove the null hypothesis of no difference and difference between the heavy metal concentration means for all pairs of groups, a Tukey – Kramer test was also done.

For the advance statistical modelling and interpretation of data JMP software (John's Macintosh Project) was used [14].

3.7.1 Concentrations of Lead (Pb)

Fit Group

Oneway Analysis of Value by City

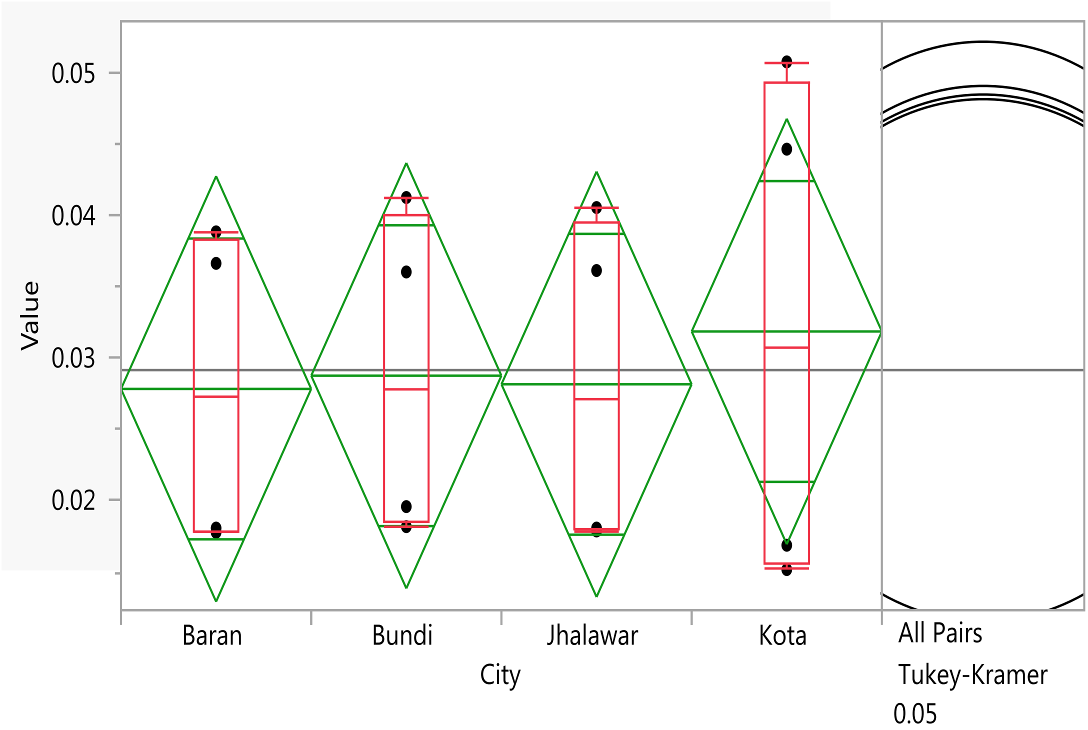


Figure 3.8: Oneway analysis of value by city wise for Pb in yoghurt

Table 3.24: Analysis of variance and ordered differences report by city wise for Pb in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00004061	0.000014	0.0727	0.9735
Error	12	0.00223425	0.000186		
C. Total	15	0.00227486			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	**0.05**

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.02 0 0.02
Kota	Baran	0.0040150	0.0096485	-0.024630	0.0326596	0.9746	
Kota	Jhalawar	0.0036900	0.0096485	-0.024955	0.0323346	0.9801	
Kota	Bundi	0.0030900	0.0096485	-0.025555	0.0317346	0.9881	
Bundi	Baran	0.0009250	0.0096485	-0.027720	0.0295696	0.9997	
Bundi	Jhalawar	0.0006000	0.0096485	-0.028045	0.0292446	0.9999	
Jhalawar	Baran	0.0003250	0.0096485	-0.028320	0.0289696	1.0000	

Oneway Analysis of Value By Pollution status

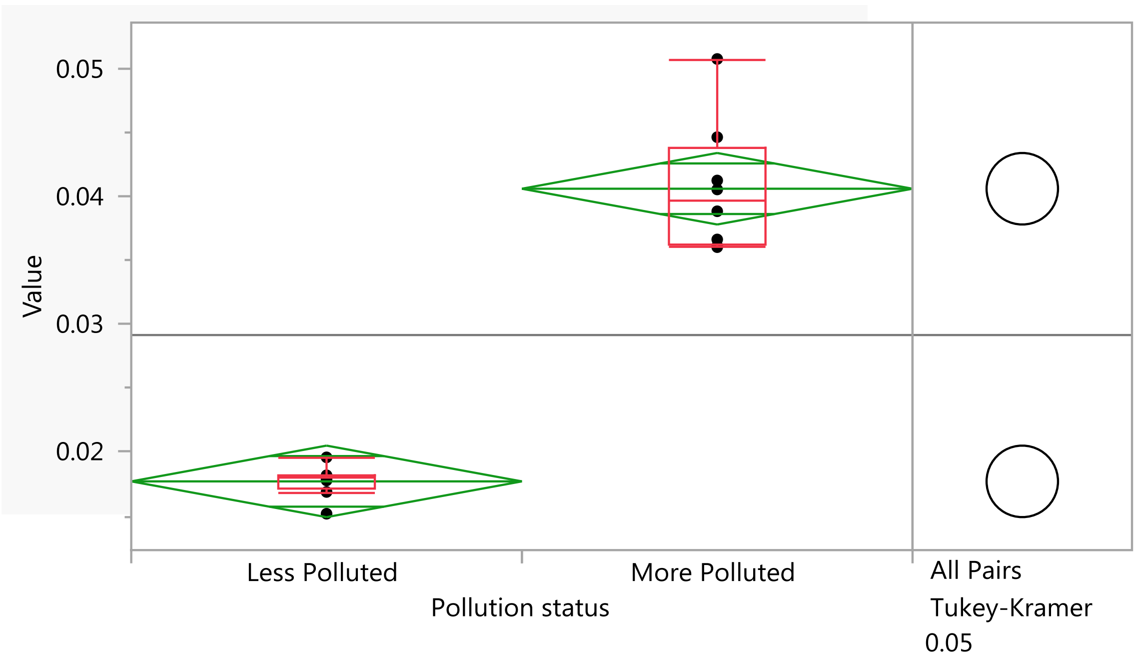


Figure 3.9: Oneway analysis of value by pollution status wise for Pb in yoghurt

Table 3.25: Analysis of variance and ordered differences report by pollution status wise for Pb in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00208529	0.002085	154.0038	<.0001*
Error	14	0.00018957	0.000014		
C. Total	15	0.00227486			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	**0.05**

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.010 0.020
More Polluted	Less Polluted	0.0228325	0.0018399	0.0188864	0.0267786	<.0001*	

Oneway Analysis of Value By Type

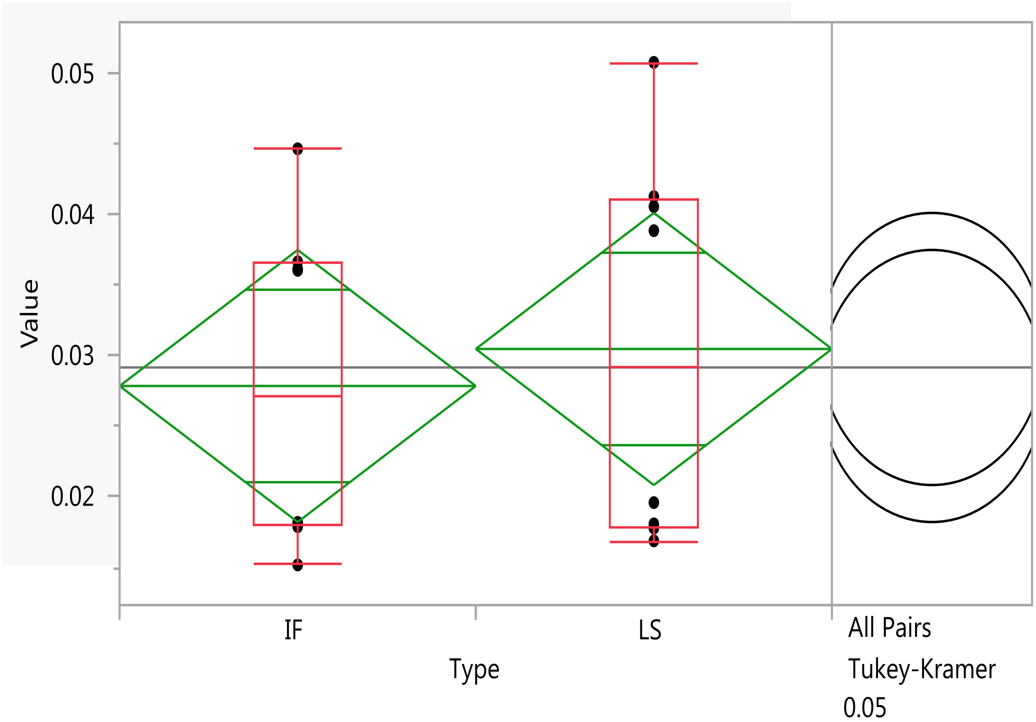


Figure 3.10: Oneway analysis of value by type wise for Pb in yoghurt

Table 3.26: Analysis of variance and ordered differences report by type wise for Pb in yoghurt

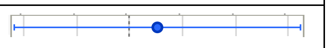
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00002730	0.000027	0.1701	0.6863
Error	14	0.00224756	0.000161		
C. Total	15	0.00227486			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.010 0 0.010
LS	IF	0.0026125	0.0063352	-0.010975	0.0162002	0.6863	

Pooled t test

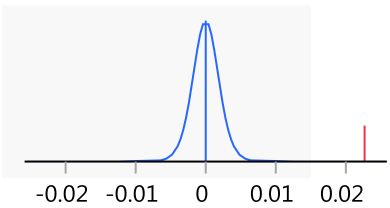


Figure 3.11: More& Less polluted

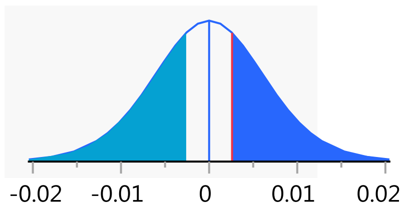


Figure 3.12: LS-IF

To determine citywise statistical interpretation and significant difference for lead concentration in the yoghurt sample one way ANOVA was performed. We use α = 0.05 as the significant level. From **Fig. 3.8,** analysis of variance shows that the difference among Baran, Bundi, Jhalawar and Kota is not significant with p values > 0.9735 which is greater than (α = 0.05) our chosen significant level. So the null hypothesis can't be rejected and from this we can conclude that the Pb concentration among the cities was not significant.

Tukey – Kramer HSD for Pb indicates that all the levels share the common place and from **Fig. 3.8** it is clear that all circles overlap each other which confirms that the mean concentration for cities are significantly indifferent.

Fig. 3.9 represents the pollution type status of Pb. For this p value < 0.0001, which is less than our chosen significant level α = 0.05. It is also clear from the **Fig. 3.9** that both the circles are very far from each other which shows the significant difference between less polluted and more polluted areas. Lower values of less polluted area reveals that this site is comparatively safe and does not contain higher metal concentrations as per RDA standards.

The third variable is location type that is IF and LS within the cities for which probability is 0.6863, which is greater than α = 0.05, showing that the mean values are significantly indifferent but less than city wise pollution level. Tukey – Kramer HSD test also shows that the circles are overlapping each other and there is no significant difference between them.

3.7.2 Concentrations of Cadmium (Cd)

Fit Group

Oneway Analysis of Value By City

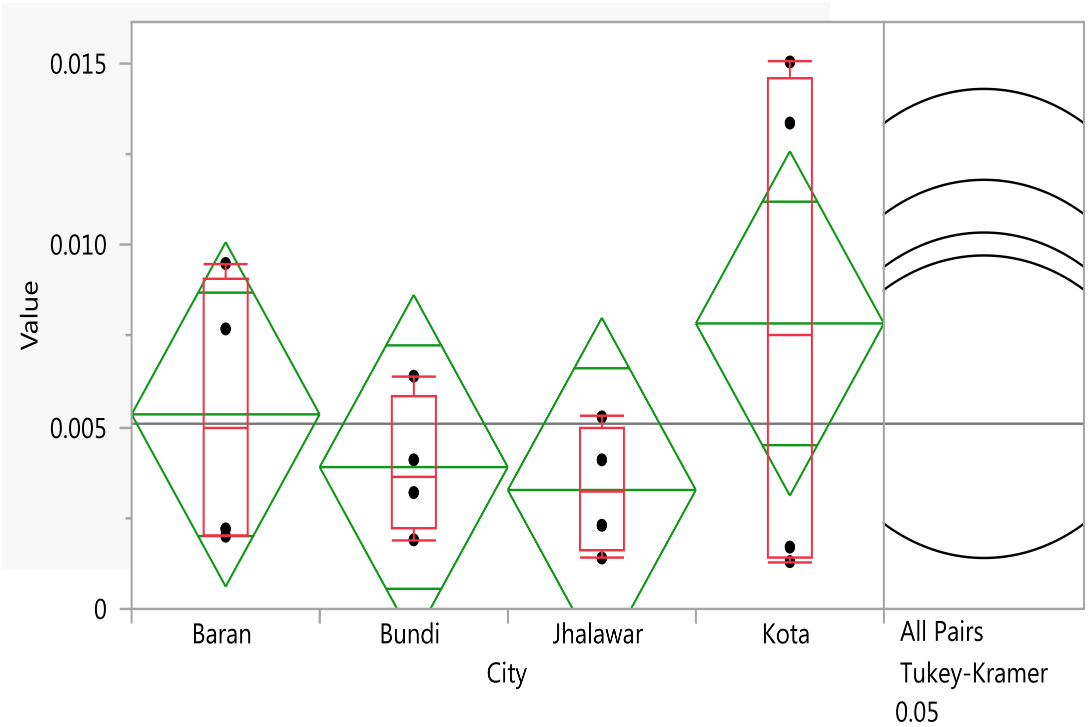


Figure 3.13: Oneway analysis of value by city wise for Cd in yoghurt

Table 3.27: Analysis of variance and ordered differences report by city wise for Cd in yoghurt

Analysis of Variance

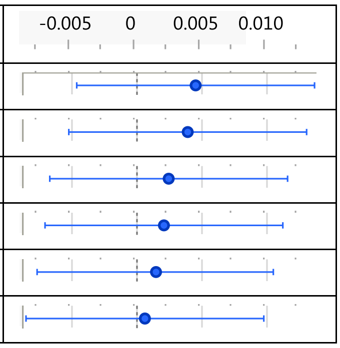
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00004965	0.000017	0.8764	0.4804
Error	12	0.00022662	0.000019		
C. Total	15	0.00027627			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	**0.05**

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value
Kota	Jhalawar	0.0045800	0.0030729	-0.004543	0.0137027	0.4723
Kota	Bundi	0.0039500	0.0030729	-0.005173	0.0130727	0.5885
Kota	Baran	0.0025000	0.0030729	-0.006623	0.0116227	0.8469
Baran	Jhalawar	0.0020800	0.0030729	-0.007043	0.0112027	0.9039
Baran	Bundi	0.0014500	0.0030729	-0.007673	0.0105727	0.9639
Bundi	Jhalawar	0.0006300	0.0030729	-0.008493	0.0097527	0.9968



Oneway Analysis of Value by Pollution status

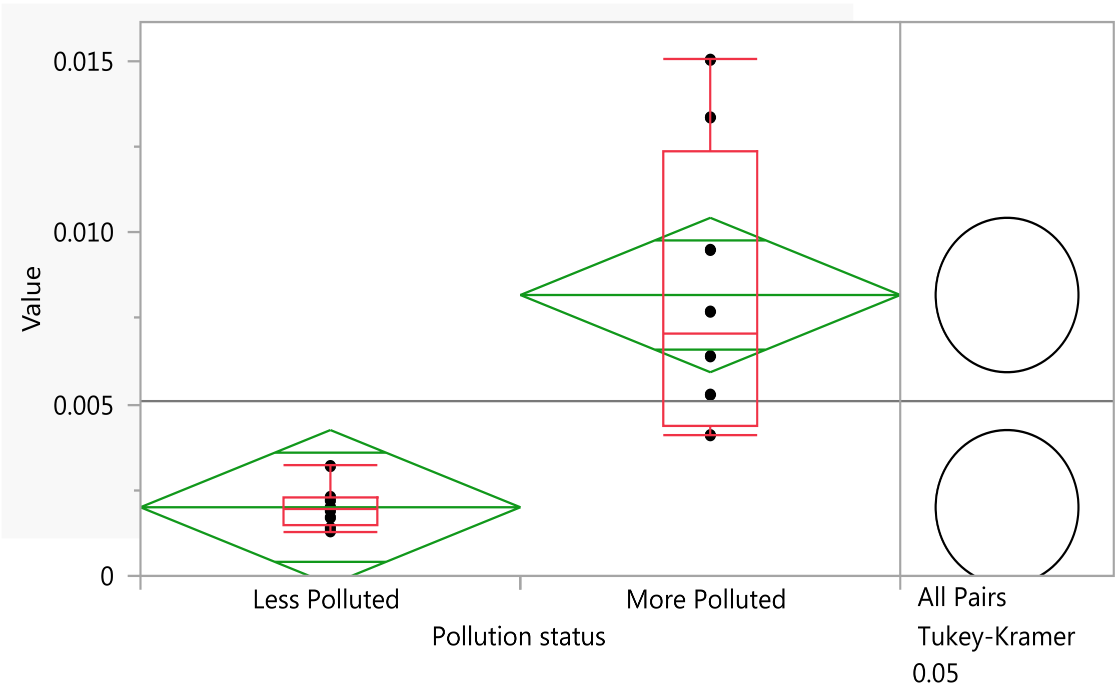


Figure 3.14: Oneway analysis of value by pollution status wise for Cd in yoghurt

Table 3.28: Analysis of variance and ordered differences report by pollution status wise for Cd in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00015302	0.000153	17.3804	0.0009*
Error	14	0.00012326	8.804e-6		
C. Total	15	0.00027627			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.002 0.004 0.006 0.008
More Polluted	Less Polluted	0.0061850	0.0014836	0.0030030	0.0093670	0.0009*	

Oneway Analysis of Value By Type

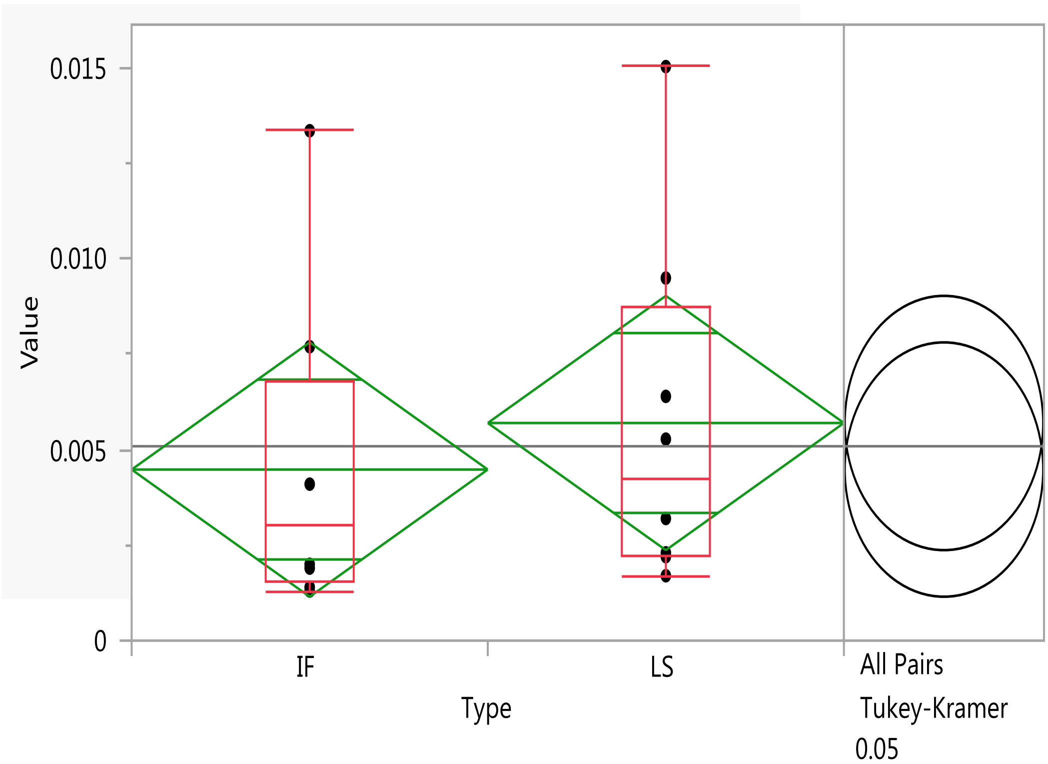


Figure 3.15: Oneway analysis of value by type wise for Cd in yoghurt

Table 3.29: Analysis of variance and ordered differences report by type wise for Cd in yoghurt

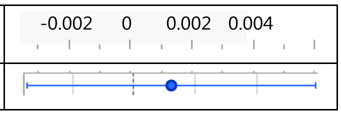
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00000595	5.954e-6	0.3083	0.5875
Error	14	0.00027032	0.000019		
C. Total	15	0.00027627			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	
LS	IF	0.0012200	0.0021971	-0.003492	0.0059323	0.5875	

Pooled t test

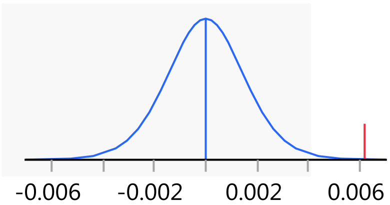


Figure 3.16: More & Less polluted

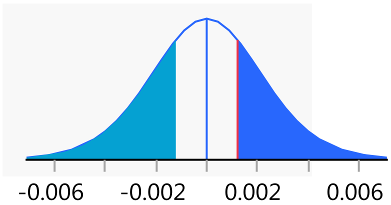


Figure 3.17: LS-IF

Summary of one way ANOVA for Cd

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.4804	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0009	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.5875	Significantly indifferent , Null Hypothesis can't be rejected

Summary Table clearly indicates that city wise and types wise means concentration of Cd is Significantly indifferent whereas according to pollution status this is significantly different. Null hypothesis can't be rejected for first and third variables and can be rejected for second variable. Tukey-Kramer HSD test also support the data.

3.7.3 Concentrations of Aluminium (Al)

Fit Group

Oneway Analysis of Value By City

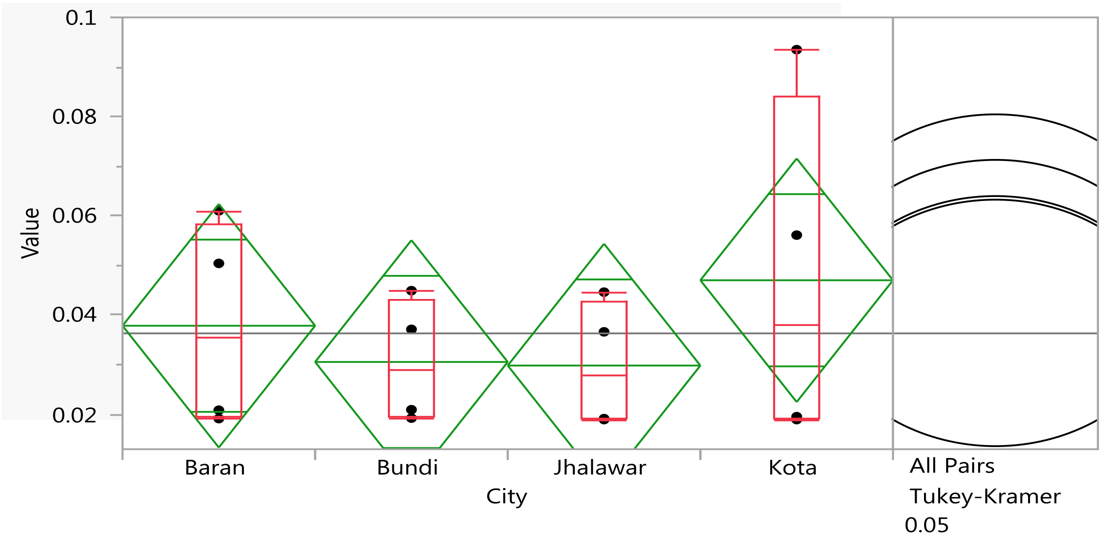


Figure 3.18: Oneway analysis of value by city wise for Al in yoghurt

Table 3.30: Analysis of variance and ordered differences report by city wise for Al in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00076966	0.000257	0.5050	0.6860
Error	12	0.00609596	0.000508		
C. Total	15	0.00686562			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.04 0 0.04
Kota	Jhalawar	0.0172000	0.0159373	-0.030115	0.0645148	0.7081	
Kota	Bundi	0.0164750	0.0159373	-0.030840	0.0637898	0.7337	
Kota	Baran	0.0091750	0.0159373	-0.038140	0.0564898	0.9375	
Baran	Jhalawar	0.0080250	0.0159373	-0.039290	0.0553398	0.9567	
Baran	Bundi	0.0073000	0.0159373	-0.040015	0.0546148	0.9667	
Bundi	Jhalawar	0.0007250	0.0159373	-0.046590	0.0480398	1.0000	

Oneway Analysis of Value By Pollution status

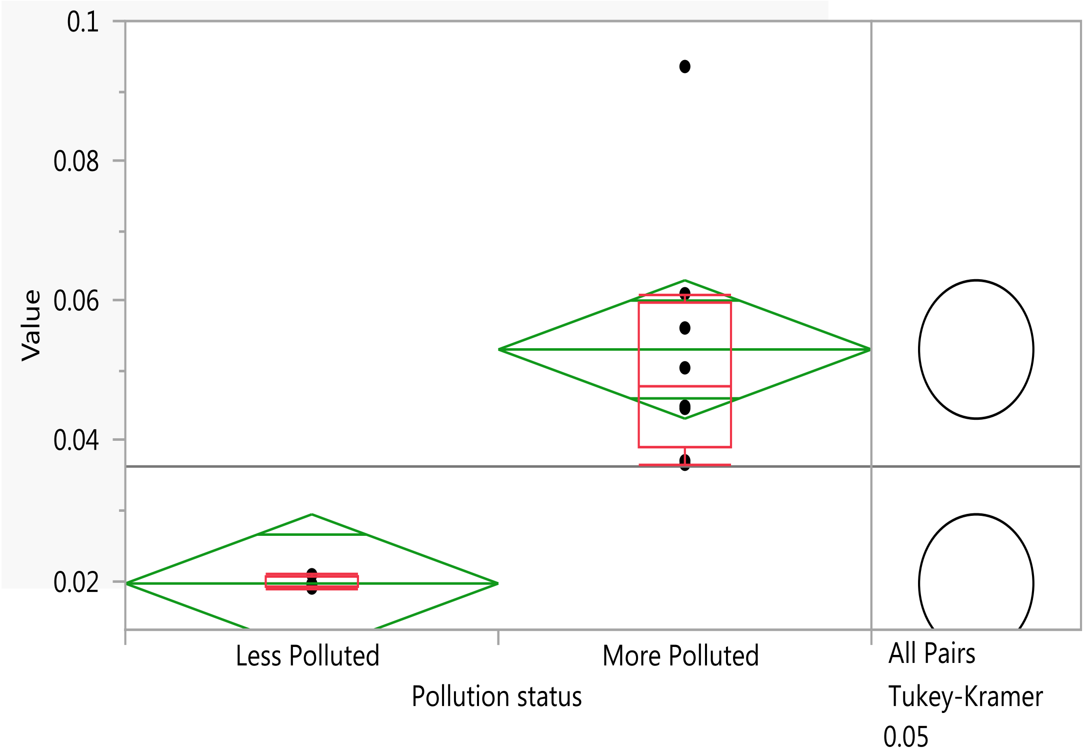


Figure 3.19: Oneway analysis of value by pollution status wise for Al in yoghurt

Table 3.31: Analysis of variance and ordered differences report by pollution status wise for Al in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00448230	0.004482	26.3298	0.0002*
Error	14	0.00238332	0.000170		
C. Total	15	0.00686562			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.01 0.02 0.03 0.04
More Polluted	Less Polluted	0.0334750	0.0065237	0.0194830	0.0474670	0.0002*	

Oneway Analysis of Value By Type

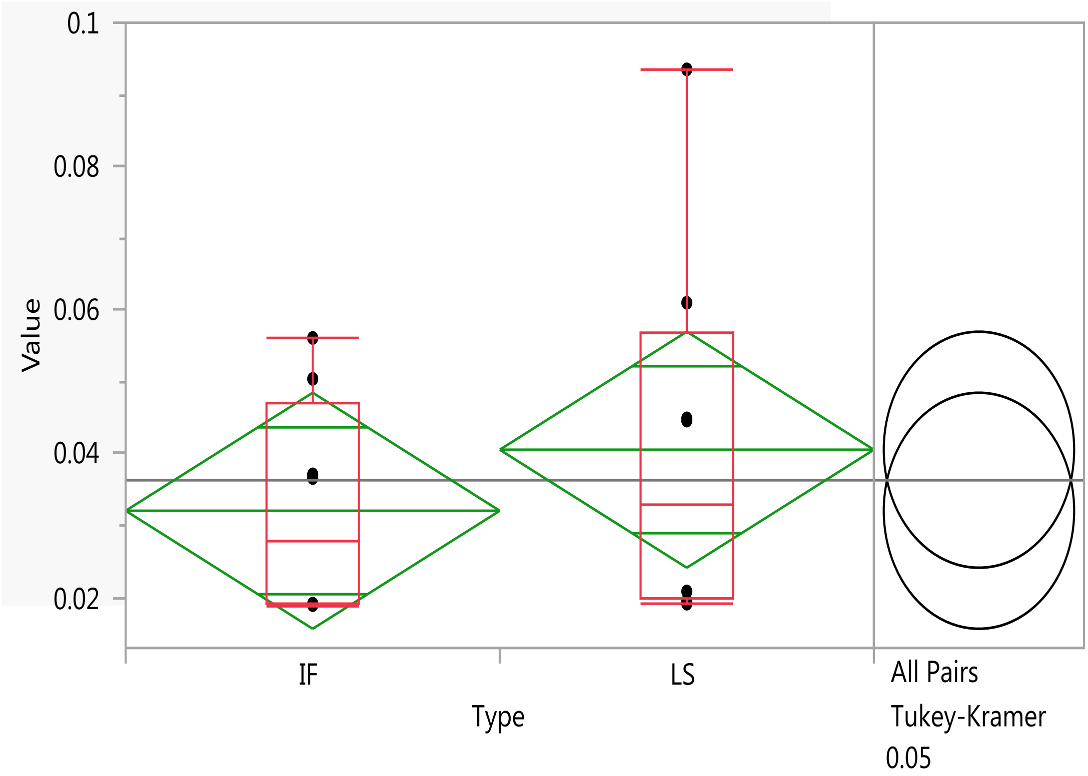


Figure 3.20: Oneway analysis of value by type wise for Al in yoghurt

Table 3.32: Analysis of variance and ordered differences report by type wise for Al in yoghurt

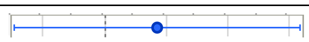
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00028900	0.000289	0.6152	0.4459
Error	14	0.00657662	0.000470		
C. Total	15	0.00686562			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.01 0 0.01 0.02 0.03
LS	IF	0.0085000	0.0108370	-0.014743	0.0317430	0.4459	

Pooled t test

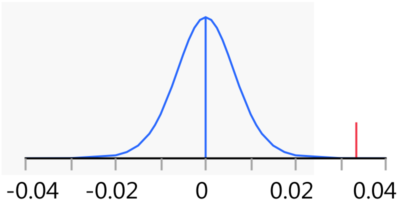


Figure 3.21: More& Less polluted

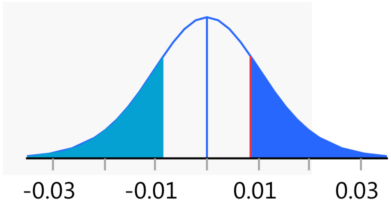


Figure 3.22: LS-IF

Summary of one way ANOVA for Al

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.6860	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0002	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.4459	Significantly indifferent , Null Hypothesis can't be rejected

Analysis of variance for Al also shows the probability > 0.6860 which is greater than α = 0.05 and from **Fig. 3.18** Tukey – Kramer test also shows that the circles are sharing almost same area, which proves that the mean concentration of heavy metals in all the cities are significantly indifferent, the comparison has been made between more polluted and less polluted area with the help of one way analysis. This analysis shows (**Fig. 3.19**) that there is a significant difference between both of them, as a p value > 0.0002 and the circles does not share the same place.

The p value for ANOVA test is 0.4459 which also shows that the concentration of both the places (**Fig. 3.20**) are significantly indifferent.

3.7.4 Concentrations of Arsenic (As)

Fit Group

Oneway Analysis of Value By City

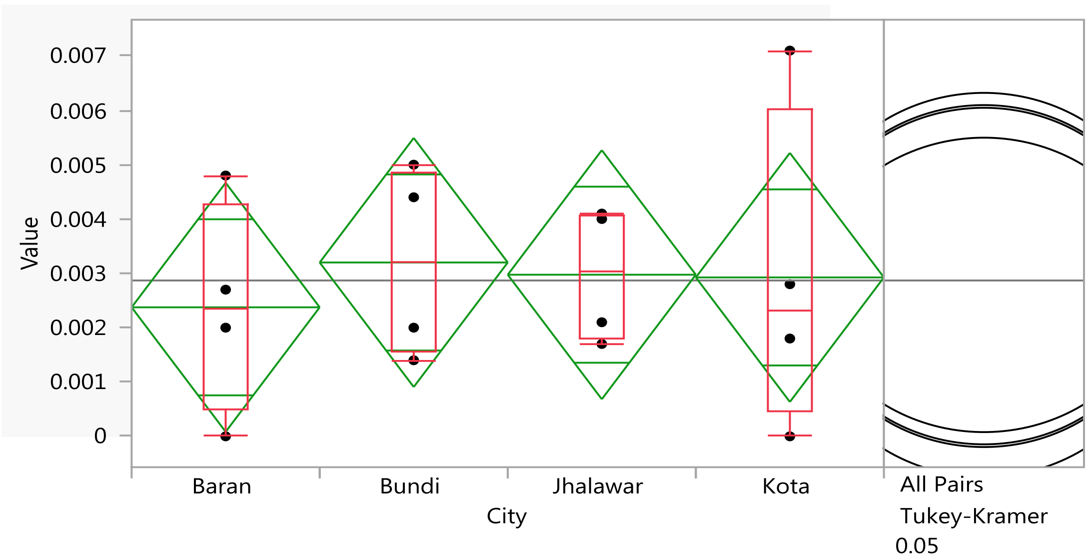


Figure 3.23: Oneway analysis of value by city wise for As in yoghurt

Table 3.33: Analysis of variance and ordered differences report by city wise for As in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00000147	4.9063e-7	0.1109	0.9521
Error	12	0.00005310	4.4252e-6		
C. Total	15	0.00005457			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.004 0 0.004
Bundi	Baran	0.0008250	0.0014875	-0.003591	0.0052410	0.9435	
Jhalawar	Baran	0.0006000	0.0014875	-0.003816	0.0050160	0.9768	
Kota	Baran	0.0005500	0.0014875	-0.003866	0.0049660	0.9819	
Bundi	Kota	0.0002750	0.0014875	-0.004141	0.0046910	0.9976	
Bundi	Jhalawar	0.0002250	0.0014875	-0.004191	0.0046410	0.9987	
Jhalawar	Kota	0.0000500	0.0014875	-0.004366	0.0044660	1.0000	

Oneway Analysis of Value By Pollution status

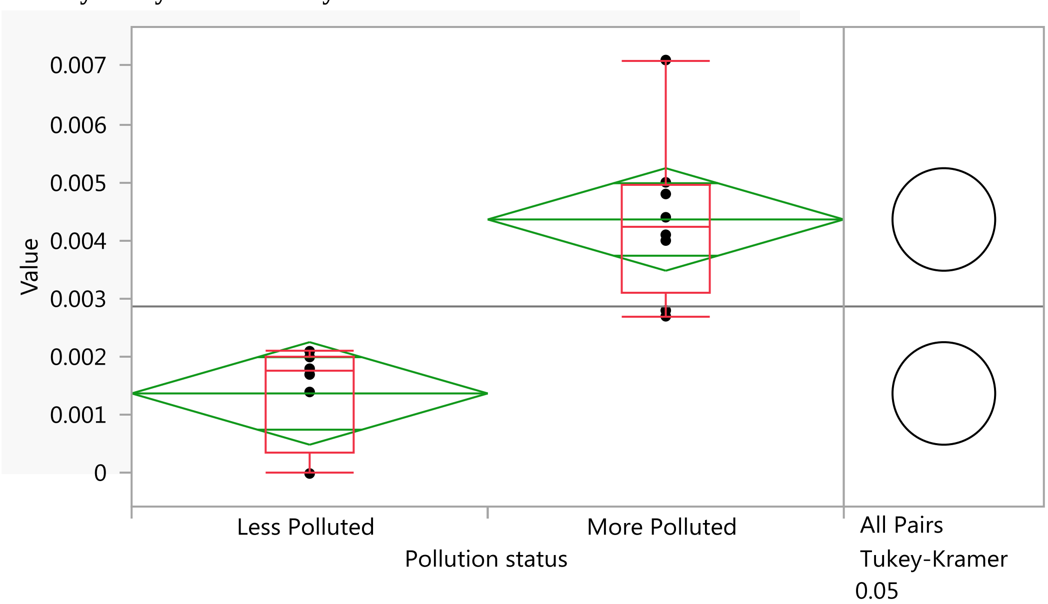


Figure 3.24: Oneway analysis of value by pollution status wise for As in yoghurt

Table 3.34: Analysis of variance and ordered differences report by pollution status wise for As in yoghurt

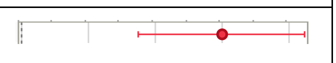
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00003570	0.000036	26.4817	0.0001*
Error	14	0.00001887	1.348e-6		
C. Total	15	0.00005457			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.001 0.002 0.003 0.004
More Polluted	Less Polluted	0.0029875	0.0005805	0.0017424	0.0042326	0.0001*	

Oneway Analysis of Value By Type

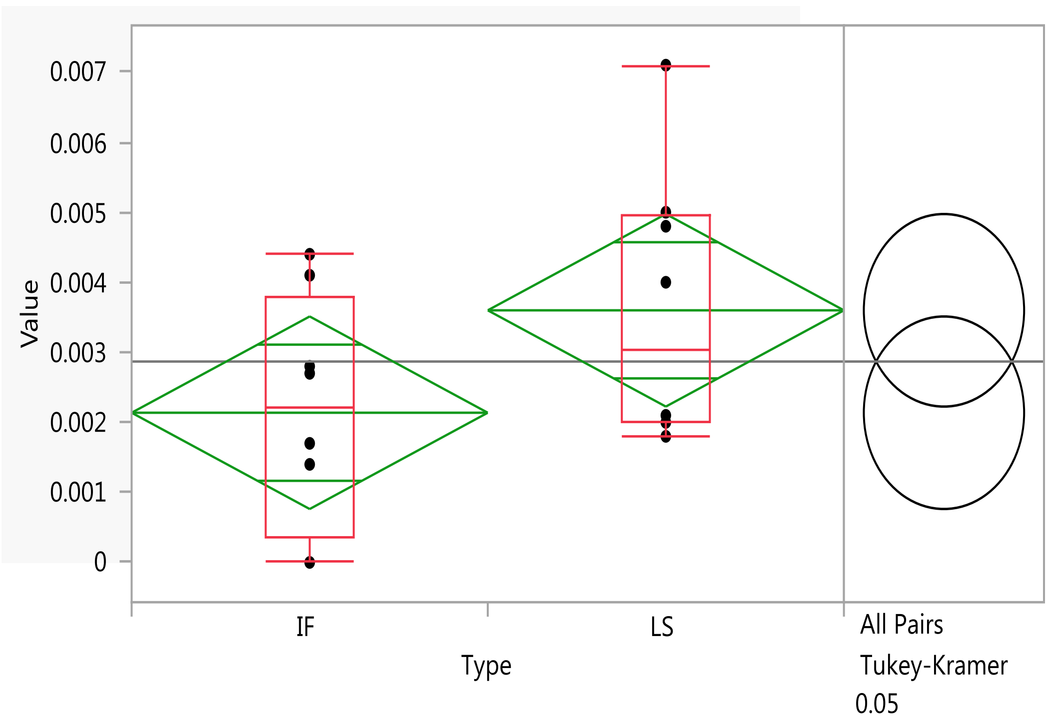


Figure 3.25: Oneway analysis of value by type wise for As in yoghurt

Table 3.35: Analysis of variance and ordered differences report by type wise for As in yoghurt

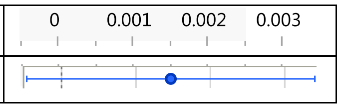
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00000856	8.5556e-6	2.6028	0.1290
Error	14	0.00004602	3.2871e-6		
C. Total	15	0.00005457			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	
LS	IF	0.0014625	0.0009065	-0.000482	0.0034068	0.1290	

Pooled t test

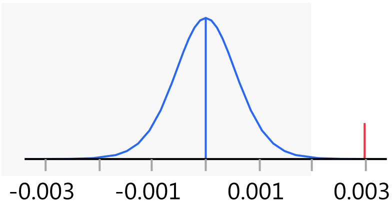


Figure 3.26: More& Less polluted

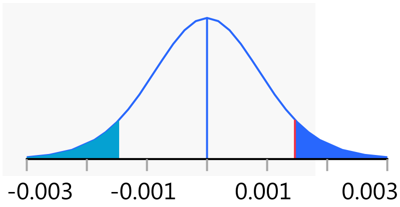


Figure 3.27: LS-IF

Summary of one way ANOVA for As

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.9521	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0001	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.1290	Significantly indifferent , Null Hypothesis can't be rejected

The above given summary table and **Fig. 3.23, 3.24** and **3.25** clearly indicates the First and third variable are significantly indifferent and the second variable is significantly different.

3.7.5 Concentrations of Iron (Fe)

Fit Group

Oneway Analysis of Value By City

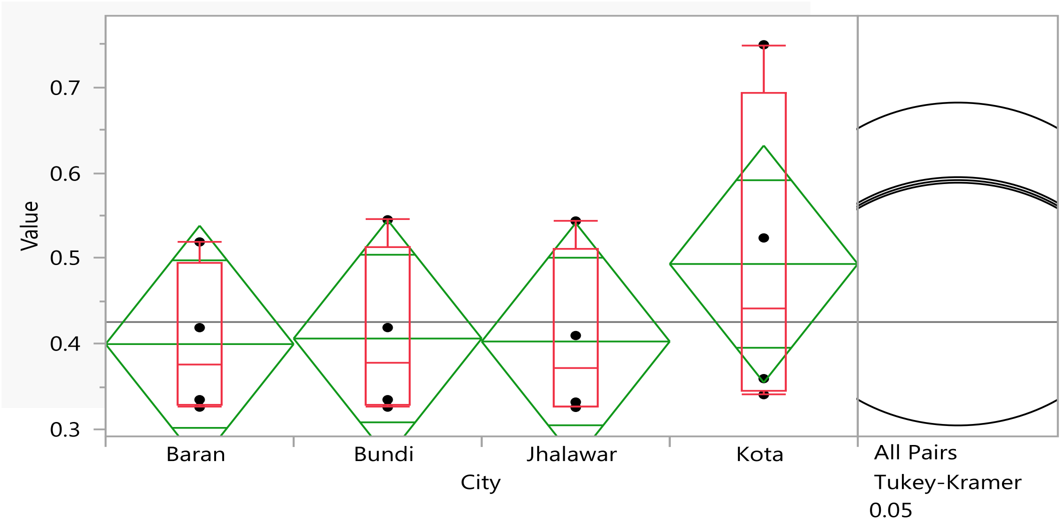


Figure 3.28: Oneway analysis of value by city wise for Fe in yoghurt

Table 3.36: Analysis of variance and ordered differences report by city wise for Fe in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.02463726	0.008212	0.5080	0.6841
Error	12	0.19399193	0.016166		
C. Total	15	0.21862919			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.2 -0.1 0 0.1 0.2 0.3
Kota	Baran	0.0936500	0.0899055	-0.173262	0.3605618	0.7293	
Kota	Jhalawar	0.0906000	0.0899055	-0.176312	0.3575118	0.7481	
Kota	Bundi	0.0871500	0.0899055	-0.179762	0.3540618	0.7689	
Bundi	Baran	0.0065000	0.0899055	-0.260412	0.2734118	0.9999	
Bundi	Jhalawar	0.0034500	0.0899055	-0.263462	0.2703618	1.0000	
Jhalawar	Baran	0.0030500	0.0899055	-0.263862	0.2699618	1.0000	

Oneway Analysis of Value By Pollution status

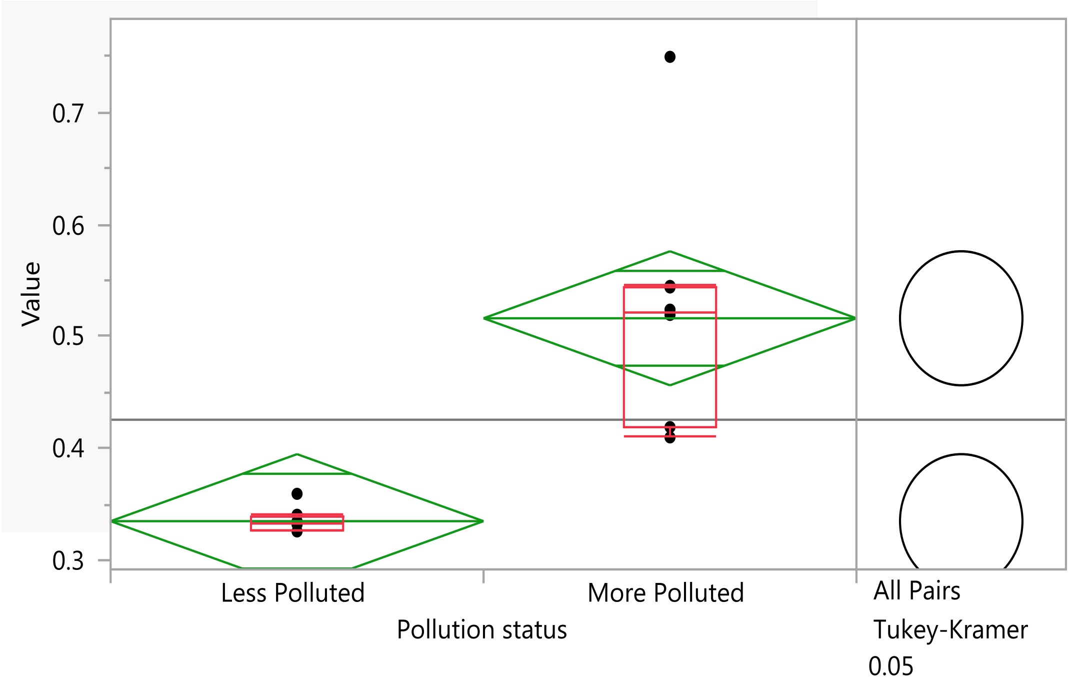


Figure 3.29: Oneway analysis of value by pollution status wise for Fe in yoghurt

Table 3.37: Analysis of variance and ordered differences report by pollution status wise for Fe in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.13137000	0.131370	21.0772	0.0004*
Error	14	0.08725919	0.006233		
C. Total	15	0.21862919			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.05 0.10 0.15 0.20 0.25
More Polluted	Less Polluted	0.1812250	0.0394740	0.0965616	0.2658884	0.0004*	

Oneway Analysis of Value By Type

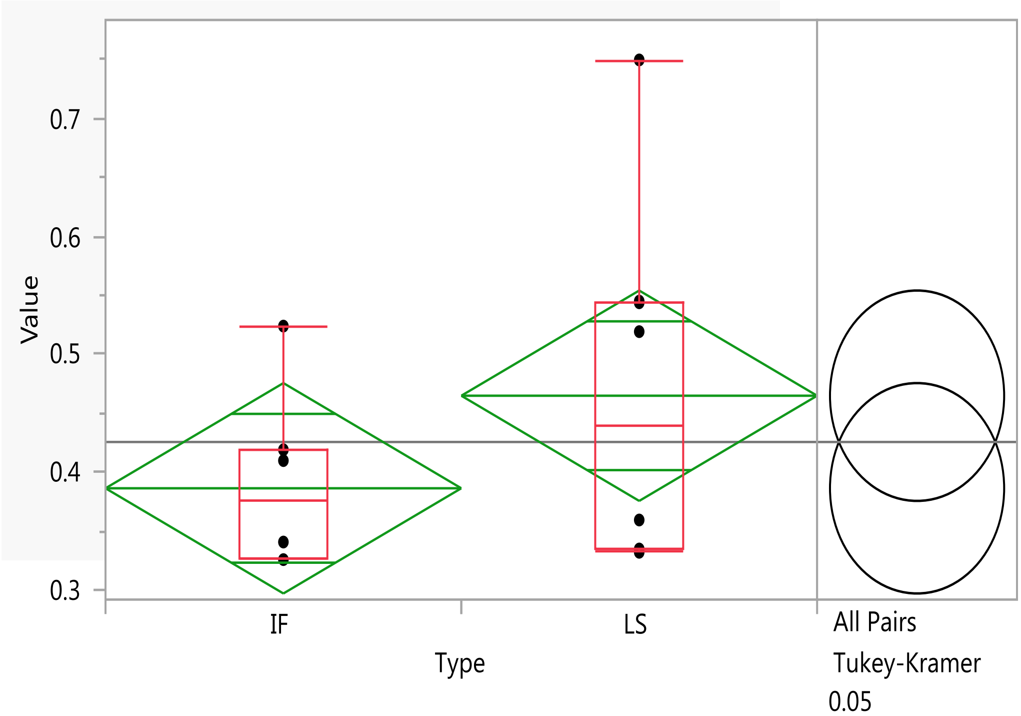


Figure 3.30: Oneway analysis of value by type wise for Fe in yoghurt

Table 3.38: Analysis of variance and ordered differences report by type wise for Fe in yoghurt

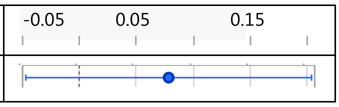
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.02466470	0.024665	1.7803	0.2034
Error	14	0.19396449	0.013855		
C. Total	15	0.21862919			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	
LS	IF	0.0785250	0.0588528	-0.047702	0.2047517	0.2034	

Pooled t test

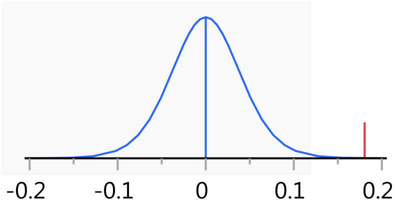


Figure 3.31: More& Less polluted

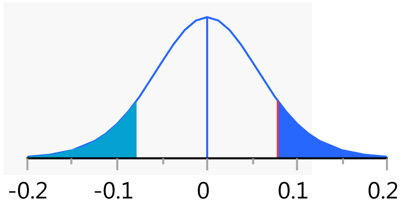


Figure 3.32: LS-IF

Summary of one way ANOVA for Fe

S.No.	Variable	α	p – value	Null Hypothesis
1	**City Wise**	0.05	0.6841	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0004	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.2034	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 3.28 presents the citywise analysis in which we can see that the lower and the higher concentration values are greater in kota as compared to the other three. According to Tukey – Kramer HSD for Baran, Bundi, Jhalawar, the circle shares the same place and for Kota it covers larger area.

Fig. 3.31 and **3.32** shows the graphical representation of pooled t – test for more and less polluted area and LS – IF. The 95 % confidence level has been taken for this test.

3.7.6 Concentrations of Zinc (Zn)

Fit Group

Oneway Analysis of Value By City

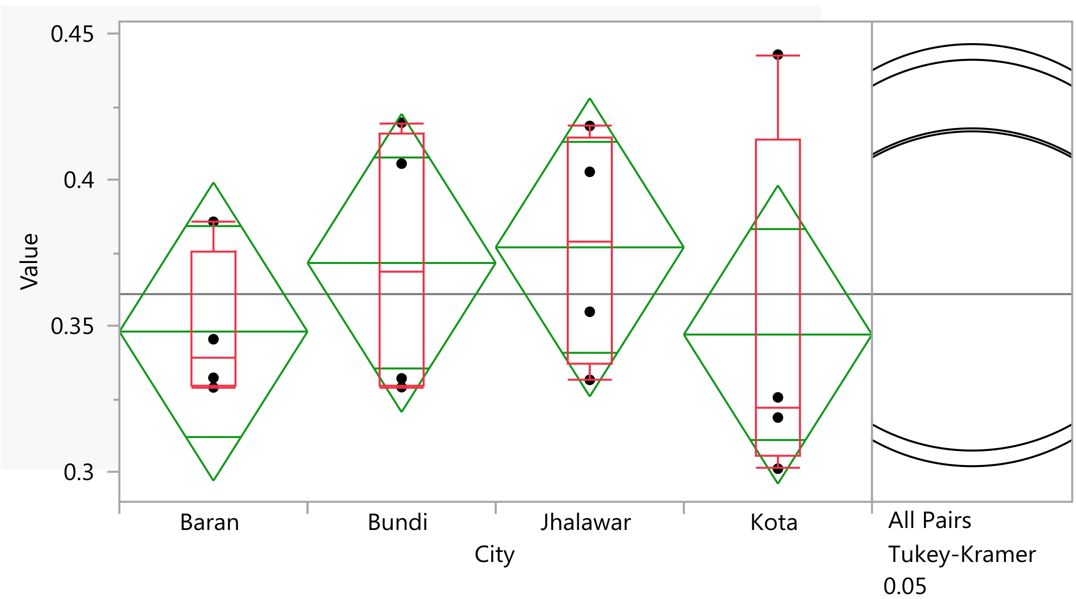


Figure 3.33: Oneway analysis of value by city wise for Zn in yoghurt

Table 3.39: Analysis of variance and ordered differences report by city wise for Zn in yoghurt

Analysis of Variance

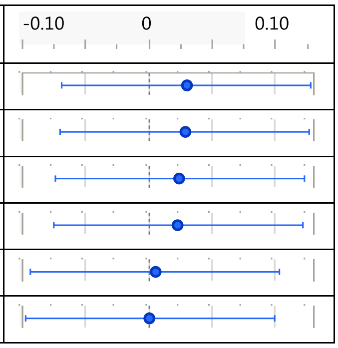
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00290290	0.000968	0.4406	0.7282
Error	12	0.02635218	0.002196		
C. Total	15	0.02925508			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value
Jhalawar	Kota	0.0298500	0.0331362	-0.068525	0.1282249	0.8047
Jhalawar	Baran	0.0288250	0.0331362	-0.069550	0.1271999	0.8201
undi	Kota	0.0245000	0.0331362	-0.073875	0.1228749	0.8794
Bundi	Baran	0.0234750	0.0331362	-0.074900	0.1218499	0.8918
Jhalawar	Bundi	0.0053500	0.0331362	-0.093025	0.1037249	0.9984
Baran	Kota	0.0010250	0.0331362	-0.097350	0.0993999	1.0000



Oneway Analysis of Value By Pollution status

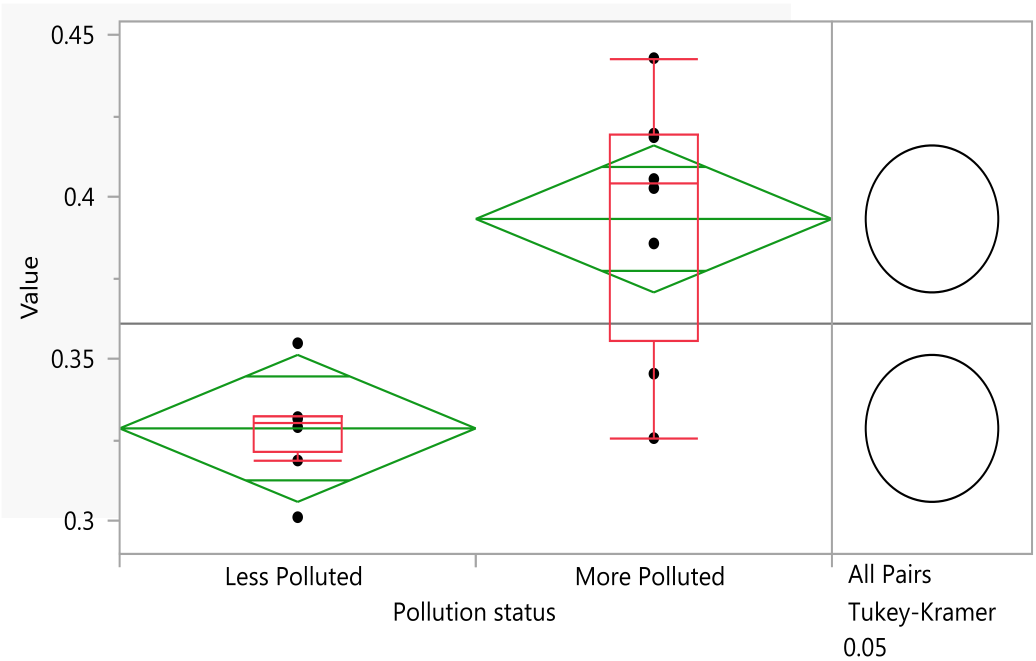


Figure 3.34: Oneway analysis of value by pollution status wise for Zn in yoghurt

Table 3.40: Analysis of variance and ordered differences report by pollution status wise for Zn in yoghurt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.01672496	0.016725	18.6869	0.0007*
Error	14	0.01253012	0.000895		
C. Total	15	0.02925508			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.02 0.04 0.06 0.08
More Polluted	Less Polluted	0.0646625	0.0149583	0.0325800	0.0967450	0.0007*	

Oneway Analysis of Value By Type

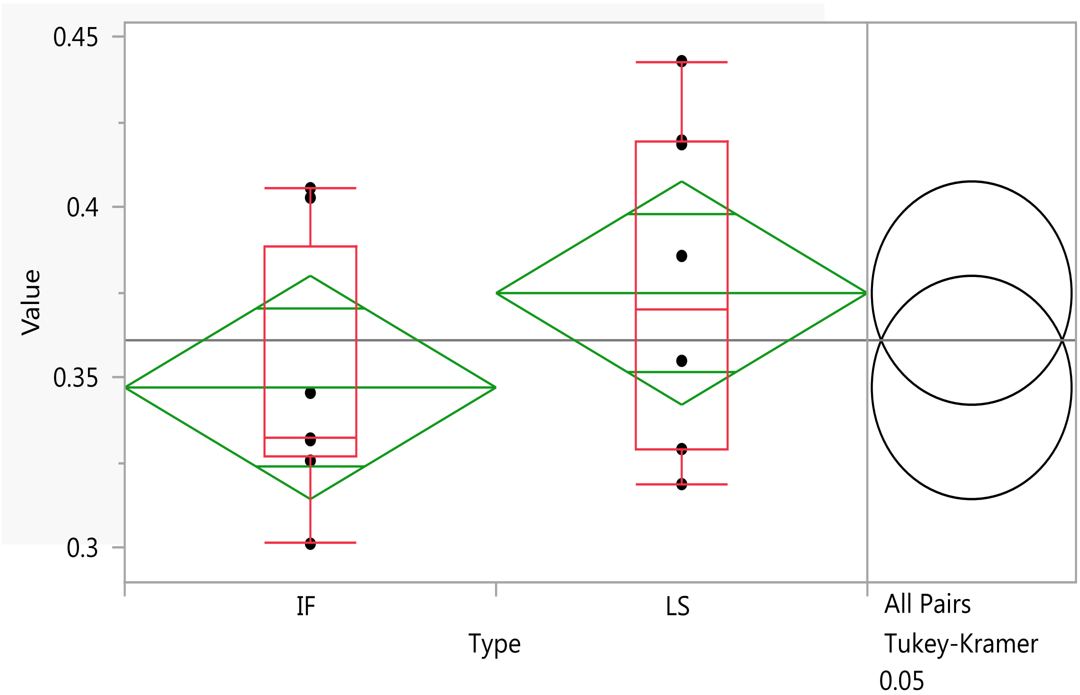


Figure 3.35: Oneway analysis of value by type wise for Zn in yoghurt

Table 3.41: Analysis of variance and ordered differences report by type wise for Zn in yoghurt

Analysis of Variance

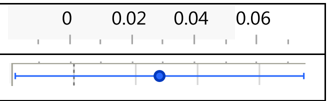
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00307193	0.003072	1.6425	0.2208
Error	14	0.02618315	0.001870		
C. Total	15	0.02925508			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value
LS	IF	0.0277125	0.0216230	-0.018664	0.0740893	0.2208



Pooled t test

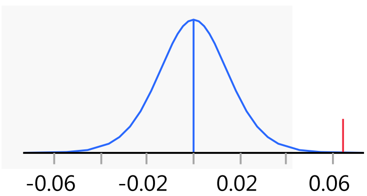


Figure 3.36: More & Less polluted

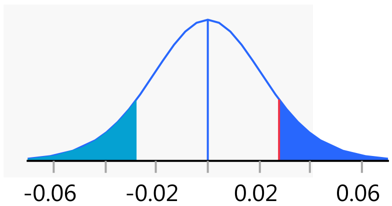


Figure 3.37: LS-IF

Summary of one way ANOVA for Zn

S.No.	Variable	α	p – value	Null Hypothesis
1	City Wise	0.05	0.7282	Significantly indifferent , Null Hypothesis can't be rejected
2	Pollution Status	0.05	0.0007	Significantly different , Null Hypothesis can be rejected
3	Types (IF & LS)	0.05	0.2208	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 3.33 indicates that the pollution level is higher in Bundi and Jhalawar and little bit lower in Baran and Kota. But still from Tukey – Kramer, they all share almost the common place, and their p value is greater than > 0.7282 which is higher than that of our chosen value (α = 0.05). So no significant difference has been found in city wise analysis.

Analysis of variance by pollution status shows the significant difference between the two with the p value is > 0.0007.

One way ANOVA analysis of value by type IF and LS, probability > 0.2208 which shows that the concentration of Zn are significantly indifferent. Tukey – Kramer HSD test also supports the data.

3.8 Correlation Coefficient

In order to predict the possibility of a common sources, as discussed in chapter 2, the strength of correlation of heavy metals in yoghurt samples is calculated using Pearson's correlation coefficient, which is obtained from the overall mean concentrations of heavy metals in all four areas of Rajasthan. The results of correlation analysis between these heavy metals for yoghurt are given in **Table 3.42.**

Table 3.42 : **Correlation coefficients among mean concentration values of metals in yoghurt samples**

Metals	Pb	Cd	Al	As	Fe	Zn
Pb	1					
Cd	0.876835	1				
Al	0.916391	0.949267	1			
As	0.867597	0.719264	0.84586	1		
Fe	0.906499	0.883765	0.951257	0.868708	1	
Zn	0.777364	**0.498845**	0.687582	0.906826	0.765701	1

Table 3.42 clearly indicate that Pearson's coefficient (r) has all positive values. All metals have correlations that are larger than 0.5, indicating a significant correlation between them. Only the correlation between Cd-Zn is less than 0.5. On the basis of these results, we can conclude that there are a number of common factors that are responsible for the heavy metal contamination in the yoghurt samples.

3.9 Conclusion

The heavy metal analysis in Yoghurt samples provided conclusive data regarding concentration labels of all six metals. Data revealed significant levels of Pb, Cd, Fe, Al, Zn in more polluted areas.

Statistical analysis showed that the detected levels of all six metals are significantly higher than the permissible limit ($p < 0.05$) with 95% confidence intervals for one variable (pollution status) for other two it is significantly indifferent.

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CHAPTER - IV

ASSESSMENT OF HEAVY METALS IN BUTTER : INSTRUMENTAL AND STATISTICAL ANALYSIS

This Chapter is divided into four major sections i.e Introduction, Instrumental analysis, Statistical analysis and Conclusion. Instrumental analysis has been done by AAS, JMP is used for statistical analysis.

4.1 Introduction

It is a semi solid emulsion contains fat and proteins. It contains around 81-90 % fat, 10-15% water, 0.5-5% fat free solid and in the case of salted butter 1% of sodium chloride (NaCl).

4.2 Manufacturing Process of Butter

Butter is a dairy product made up by the churning of cream to separate the fat globules from the butter milk. Churning is the procedure used to turn dairy cream into butter. Given **Fig. 4.1** represent the manufacturing process of butter [1,2].

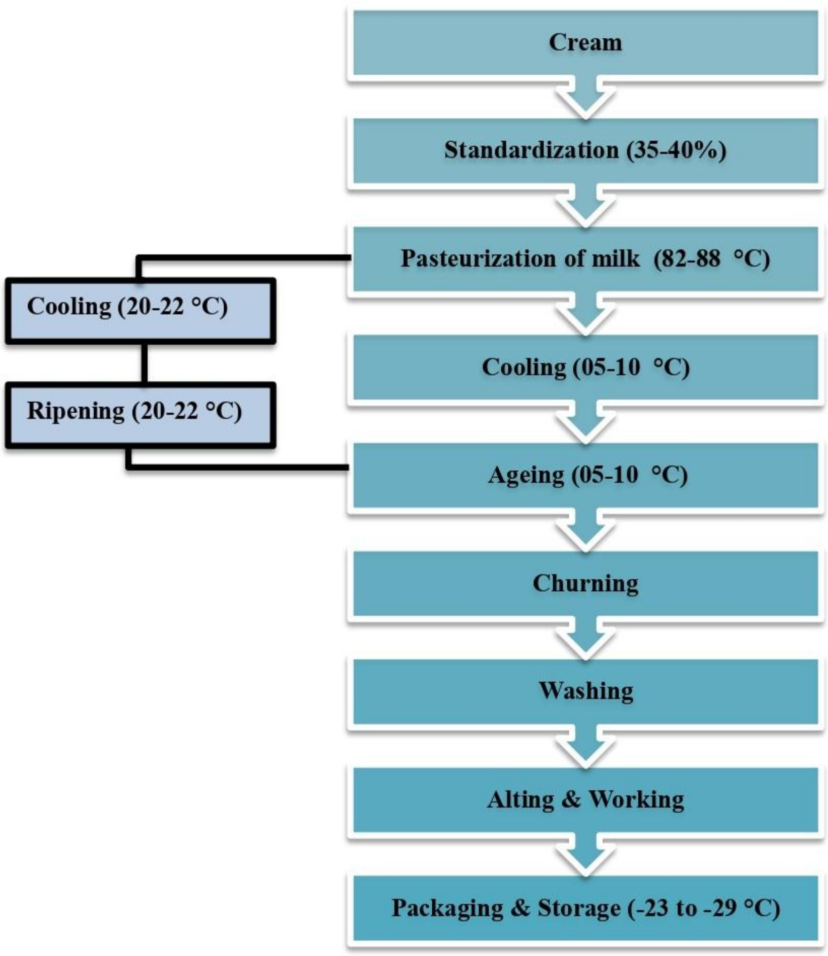


Figure 4.1 Flow diagram of manufacturing of Butter

4.3 Types of Butter

4.3.1 Regular Butter

Regular butter is available in two types i.e., unsalted and salted .It contains 80 % of milk fat.

4.3.2 Clarified Butter

Clarified butter is pure fat having higher smoke point and greater shelf life. When butter is heated slowly it separates into it's 3 components i.e., white milk solid, foam and butter fat.

4.3.3 European Butter

European butter contains higher percentage of butter fat which lies between 80-90 %.

4.3.4 Whipped Butter

Whipped Butter is salted, light and fluffy which is easily spreadable on the snacks. We can make whipped butter by our own at room temperature by just whipping it in mixer to make it aerated and fluffy.

4.3.5 Vegan Butter

Vegan butter is prepared from different types of oil like olive oil, almond oil, vegetable oil and coconut and cashew milk. It's taste is just like the dairy butter.

The quality of food products affected by the exposure of prolonged and mild contamination in our surroundings, which affects the people and animals [3,4]. Excess amount of these toxic metals in butter are hazardous for human health which affect the growth, fertility, nervous system, also cause vomiting, diarrhea, nausea, loss of appetite etc. [5,6]. Heavy metals might be enter in butter via sources like water used in processing, containers and equipment used in the manufacturing procedure, sanitation, packaging and storage processes etc. [7-11]. Thus, it is very important to monitor the concentration of heavy metals in food stuffs as well as butter for both environmental and nutritional toxicological purposes.

Our main study is focused on the Regular Butter. Effect of processing and packaging have also been studied in this chapter.

Investigation of heavy metal concentration in various regions of Kota division, Rajasthan is covered in this chapter. Samples were collected from Kota, Baran, Bundi, and Jhalawar zone. Within each of these zones, there are then two subzones: Less polluted and More polluted (industrial area) were taken into an account. The goal of the current study was to evaluate six specific metals like Pb, Cd, Al, As, Fe, and Zn in 80 Butter samples that were collected from various local shops and individual farms.

4.4 Collection, Digestion & Analysis of Butter

Butter samples were collected from the above given selected zones.

10-gram butter samples (n=5) were collected from each subarea and placed in PTFE containers. For all butter samples, the microwave digestion procedure was employed using the conditions listed in **Table 2.1** in chapter 2. 1 gm of sample was taken and digested in a microwave using 4 ml of 65% HNO_3 and 2 ml of 30% H_2O_2. The resulting mixture was diluted with deionized water and put into a 10 ml volumetric flask. Once the samples were prepared, elemental analysis has performed with the help of AAS. The detailed instrumental analysis results are given below:

4.4.1 Kota District

4.4.1.1 More Polluted Area (Ranpur)

(a) Individual Farms

Table 4.1: Concentration (mg/L) of metals (Mean±SD) in Butter sample of KRIF

Samples	Pb	Cd	Al	As	Fe	Zn
KRIF - B1	0.0434	0.0082	0.0563	0.0061	0.4981	0.2996
KRIF - B2	0.0288	0.0148	0.0479	0.0024	0.5782	0.3161
KRIF - B3	0.0177	0.0092	0.0321	0.0064	0.2959	0.3124
KRIF - B4	0.0344	0.0078	0.0299	BIR	0.4264	0.4212
KRIF - B5	0.0497	0.0067	0.0398	0.0055	0.6389	0.4841
Minimum	0.0177	0.0067	0.0299	BIR	0.2959	0.2996
Maximum	0.0497	0.0148	0.0563	0.0064	0.6389	0.4841
Mean	0.0348	0.0093	0.0412	0.0041	0.4875	0.3667
SD	0.0112	0.0028	0.0099	0.0025	0.1198	0.0732
Variance	0.0001	-	0.0001	6E-06	0.0143	0.0054

KRIF : Kota Ranpur Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

The concentrations of heavy metals in butter samples collected from more polluted area (Ranpur, Kota) are represented in **Table 4.1**

The results show that concentration of Pb, Cd, Al, As, Fe and Zn in above butter samples are varied from 0.0177 – 0.497, 0.0067 – 0.0148, 0.0299 – 0.0563, BIR – 0.0064 , 0.2959 – 0.6389 and 0.2996 – 0.4841 mg/l respectively and mean concentration are found to be 0.0348, 0.0093, 0.0412, 0.0041, 0.4875 and 0.3667 mg/L. The concentration of Pb, Cd, Al, Fe and Zn are found to be slightly higher than permissible limit while concentration of As is found to be below permissible limit.

(b) Local Shops

Table 4.2: Concentration (mg/L) of metals (Mean±SD) in Butter sample of KRLS

Samples/ Heavy metals	Pb	Cd	Al	As	Fe	Zn
KRLS - B1	0.0314	0.0178	0.1189	0.0076	0.9247	0.5364
KRLS - B2	0.0549	0.0133	0.0614	0.0089	0.6872	0.4548
KRLS - B3	0.0261	0.0129	0.0889	0.0074	1.4187	0.3612
KRLS - B4	0.0406	0.0149	0.1197	BIR	0.9798	0.2441
KRLS - B5	0.0516	0.0089	0.1312	0.0085	1.1257	0.5932
Minimum	0.0261	0.0089	0.0614	BIR	0.6872	0.2441
Maximum	0.0549	0.0178	0.1312	0.0089	1.4187	0.5932
Mean	**0.0409**	**0.0136**	**0.1040**	**0.0065**	**1.0272**	**0.4379**
SD	0.0111	0.0029	0.0255	0.0033	0.2414	0.1245
Variance	0.0001	-	0.0007	1E-05	0.0583	0.0155

KRLS : Kota Ranpur Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The results given in **Table 4.2** highlight the concentration of Pb, Cd, Al, As, Fe and Zn .The minimum concentrations of Pb, Cd, Al, As, Fe and Zn are 0.0261, 0.0089, 0.0614, BIR, 0.6872 and 0.2441 while maximum concentrations are 0.0549, 0.0178, 0.1312, 0.0089,1.4187 and 0.5932 mg/L respectively. The mean concentration of Pb, Cd, Al, As, Fe and Zn are found to be 0.0409, 0.136, 0.1040, 0.0065, 1.0272 and 0.4379 mg/L. Above measurements show that metal contamination levels are above the safety threshold.

4.4.1.2 Less Polluted Area (Kaithoon)

(a) Individual Farms

Table 4.3: Concentration (mg/L) of metals (Mean±SD) in Butter sample of KKIF

Samples	Pb	Cd	Al	As	Fe	Zn
KKIF - B1	0.0085	0.0025	0.0325	BIR	0.4835	0.2478
KKIF - B2	0.0151	BIR	0.0174	BIR	0.3781	0.4352
KKIF - B3	0.0092	0.0025	0.0061	BIR	0.3701	0.1268
KKIF - B4	0.0141	0.0011	0.0214	BIR	0.2839	0.3241
KKIF - B5	0.0117	0.0032	0.0155	BIR	0.1923	0.3896
Minimum	0.0085	BIR	0.0061	BIR	0.1923	0.1268
Maximum	0.0151	0.0032	0.0325	BIR	0.4835	0.4352
Mean	**0.0117**	**0.0019**	**0.0186**	**BIR**	**0.3416**	**0.3047**
SD	0.0026	0.0012	0.0086	-	0.0979	0.1091
Variance	-	-	0.0001	-	0.0096	0.0119

KKIF : Kota Kaithoon Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.4: Concentration (mg/L) of metals (Mean±SD) in Butter sample of KKLS

Samples	Pb	Cd	Al	As	Fe	Zn
KKLS - B1	0.0216	0.0021	0.0182	0.0036	0.4321	0.4621
KKLS - B2	0.0148	0.0048	0.0124	0.0036	0.2501	0.3234
KKLS - B3	0.0231	0.0022	0.0181	0.0029	0.3958	0.1718
KKLS - B4	0.0094	0.0017	0.0228	0.0035	0.4115	0.2425
KKLS - B5	0.0256	0.0034	0.0253	BIR	0.3125	0.3916
Minimum	0.0094	0.0017	0.0124	**BIR**	0.2501	0.1718
Maximum	0.0256	0.0048	0.0253	0.0036	0.4321	0.4621
Mean	**0.0189**	**0.0028**	**0.0194**	**0.0027**	**0.3604**	**0.3183**
SD	0.0059	0.0011	0.0044	0.0014	0.0685	0.1032
Variance	-	-	-	2E-06	0.0047	0.0107

KKLS : Kota Kaithoon Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The result of IF and LS of less polluted area, Kaithoon of kota city are presented in **Table 4.3** and **4.4**. Each table shows the metal concentration of butter samples in mg/L. The mean concentration of Pb, Cd, Al, As, Fe and Zn in IF are 0.0117, 0.0019, 0.0186, BIR, 0.3416 and 0.3047 while in LS the mean concentration of Pb, Cd, Al, As, Fe and Zn are 0.0189, 0.0028, 0.0194, 0.0027, 0.3604 and 0.3183 mg/L respectively.

The results of **Table 4.3** and **4.4** indicate that all metal concentrations are relatively higher in samples of local shops as compared to individual farms. Arsenic is totally absent in the samples of KKIF and in KKLS. It is below the detection limit at two places. For KKIF Cd is not detected in some samples.

4.4.2 BARAN DISTRICT

4.4.2.1 More Polluted Area (Chhabra Motipura)

(a) Individual Farms

Table 4.5: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BCIF

Samples	Pb	Cd	Al	As	Fe	Zn
BCIF - B1	0.0498	0.0093	0.0346	0.0041	0.5212	0.4287
BCIF - B2	0.0206	0.0084	0.0876	0.0063	0.5843	0.3244
BCIF - B3	0.0179	0.0105	0.0342	0.0052	0.4211	0.4321
BCIF - B4	0.0268	0.0101	0.0045	0.0043	0.2875	0.2208
BCIF - B5	0.0265	0.0026	0.0149	0.0005	0.3228	0.3543
Minimum	0.0179	0.0026	0.0045	0.0005	0.2875	0.2208
Maximum	0.0498	0.0105	0.0876	0.0063	0.5843	0.4321
Mean	**0.0283**	**0.0082**	**0.0352**	**0.0041**	**0.4274**	**0.3521**
SD	0.0113	0.0029	0.0286	0.0020	0.1131	0.0778
Variance	0.0001	-	0.0008	4E-06	0.0128	0.0061

BCIF : Baran Chhabra Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.6: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BCLS

Samples	Pb	Cd	Al	As	Fe	Zn
BCLS - B1	0.0397	0.0084	0.0348	0.0073	0.3213	0.2988
BCLS - B2	0.0208	0.0083	0.0877	0.0064	0.5745	0.3245
BCLS - B3	0.0477	0.0107	0.0343	BIR	0.4213	0.4322
BCLS - B4	0.0269	0.0063	0.0446	0.0076	0.5876	0.4509
BCLS - B5	0.0267	0.0093	0.0346	0.0058	0.3219	0.3545
Minimum	0.0208	0.0063	0.0343	BIR	0.3213	0.2988
Maximum	0.0477	0.0107	0.0877	0.0076	0.5876	0.4509
Mean	**0.0324**	**0.0086**	**0.0472**	**0.0054**	**0.4453**	**0.3722**
SD	0.0098	0.0014	0.0206	0.0028	0.1167	0.0596
Variance	0.0001	-	0.0004	8E-06	0.0136	0.0036

BCLS : Baran Chhabra Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 4.5 and **4.6** provides the heavy metal concentrations in individual farms and local shops of more polluted area, Chhabra Motipura of Baran district.

Minimum concentration of Pb, Cd, Al, As, Fe and Zn are 0.0179, 0.0026, 0.0045, 0.0005, 0.2875, 0.2208 and maximum concentrations are 0.0498, 0.0105, 0.0876, 0.0063, 0.5843, 0.4321 and mean concentration are 0.0283, 0.0082, 0.0352, 0.0041, 0.4274 and 0.3521 mg/L respectively of IF, while **Table 4.6** represent the results of LS, in which minimum concentration of Pb, Cd, Al, As, Fe and Zn are 0.0208, 0.0063, 0.0343, BIR, 0.3213, 0.2988 and maximum concentrations are 0.0477, 0.0107, 0.0877, 0.0076, 0.5876, 0.4509 and mean concentration are 0.0324, 0.0086, 0.0472, 0.0054, 0.4453 and 0.3722 mg/L respectively.

The concentration of all metals are expressed in mg/L. The data of tables indicate the moderate levels of contamination in more polluted area.

4.4.2.2 Less Polluted Area (Mangrol)

(a) Individual Farms

Table 4.7: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BMIF

Samples	Pb	Cd	Al	As	Fe	Zn
BMIF - B1	0.0215	BIR	0.0211	BIR	0.4384	0.3924
BMIF - B2	0.0122	0.0046	0.0214	BIR	0.3204	0.3222
BMIF - B3	0.0198	BIR	0.0228	BIR	0.2723	0.2849
BMIF - B4	0.0143	0.0066	0.0148	BIR	0.2288	0.3273
BMIF - B5	0.0215	BIR	0.0159	BIR	0.3462	0.2398
Minimum	0.0122	BIR	0.0148	BIR	0.2288	0.2398
Maximum	0.0215	0.0066	0.0228	BIR	0.4384	0.3924
Mean	**0.0179**	**0.0022**	**0.0192**	**BIR**	**0.3212**	**0.3133**
SD	0.0039	0.0028	0.0032	-	0.0711	0.0505
Variance	-	-	-	-	0.0051	0.0025

BMIF : Baran Mangrol Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.8: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BMLS

Samples	Pb	Cd	Al	As	Fe	Zn
BMLS - B1	0.0223	0.0034	0.0254	BIR	0.3454	0.1725
BMLS - B2	0.0166	0.0046	0.0233	BIR	0.2986	0.3744
BMLS - B3	0.0276	0.0039	0.0158	0.0029	0.3519	0.2551
BMLS - B4	0.0164	BIR	0.0231	BIR	0.3543	0.4011
BMLS - B5	0.0168	BIR	0.0172	0.0032	0.2871	0.4262
Minimum	0.0164	BIR	0.0158	BIR	0.2871	0.1725
Maximum	0.0276	0.0046	0.0254	0.0032	0.3543	0.4262
Mean	**0.0199**	**0.0024**	**0.0210**	**0.0012**	**0.3275**	**0.3259**
SD	0.0044	0.0020	0.0038	0.0015	0.0286	0.0966
Variance	-	-	-	2E-06	0.0008	0.0093

BMLS : Baran Mangrol Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 4.7 and **4.8** provide an overview on heavy metal concentration for less polluted area, Mangrol of Baran district. The mean concentration of Pb, Cd, Al, As, Fe and Zn of IF are 0.0179, 0.0022, 0.0192, BIR, 0.3212, 0.3133 mg/L respectively, whlie mean concentration of Pb, Cd, Al, As, Fe and Zn of LS are found 0.0199, 0.0024, 0.0210, 0.0012, 0.3275 and 0.3259 mg/L respectively.

The mean concentration of Pb, Cd, Al, As, Fe and Zn in individual farms as well as in local shops of less polluted areas are found to be in the order of Fe > Zn > Al > Pb > Cd > As .

Concentrations of Pb, Al, Fe and Zn are relatively higher in samples of IF and LS of more polluted areas but the concentration of Cd and As are found to be below the permissible limit in samples of both areas. Result of **Table 4.7** reveals that As is not found in all butter samples collected from BMIF while Cd is also not detected in some butter samples collected from BMIF and BMLS.

4.4.3 BUNDI DISTRICT

4.4.3.1 More Polluted Area (Lakheri)

(a) Individual Farm

Table 4.9: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BnLIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnLIF - B1	0.0301	0.0094	0.0347	0.0041	0.3215	0.2987
BnLIF - B2	0.0209	0.0085	0.0876	0.0059	0.5846	0.3443
BnLIF - B3	0.0178	0.0038	0.0045	0.0081	0.4215	0.4321
BnLIF - B4	0.0271	0.0004	0.0047	0.0038	0.2875	0.3211
BnLIF - B5	0.0265	0.0028	0.0147	0.0057	0.3228	0.3547
Minimum	0.0178	0.0004	0.0045	0.0038	0.2875	0.2987
Maximum	0.0301	0.0094	0.0876	0.0081	0.5846	0.4321
Mean	**0.0245**	**0.0050**	**0.0292**	**0.0055**	**0.3876**	**0.3502**
SD	0.0045	0.0034	0.0312	0.0015	0.1082	0.0453
Variance	-	-	0.0010	2E-06	0.0117	0.0021

BnLIF : Bundi Lakheri Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

Local Shops

Table 4.10: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BnLLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnLLS - B1	0.0299	0.0093	0.0347	0.0044	0.3214	0.5978
BnLLS - B2	0.0205	0.0111	0.0875	0.0065	0.6841	0.3241
BnLLS - B3	0.0373	0.0087	0.0643	0.0081	0.4213	0.4319
BnLLS - B4	0.0371	0.0105	0.0046	0.0047	0.5869	0.3206
BnLLS - B5	0.0261	0.0024	0.0147	0.0061	0.3229	0.3539
Minimum	0.0205	0.0024	0.0046	0.0044	0.3214	0.3206
Maximum	0.0373	0.0111	0.0875	0.0081	0.6841	0.5978
Mean	**0.0302**	**0.0084**	**0.0412**	**0.0060**	**0.4673**	**0.4057**
SD	0.0065	0.0031	0.0309	0.0013	0.1453	0.1041
Variance	-	-	0.0010	2E-06	0.0211	0.0108

BnLLS : Bundi Lakheri Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The results of all samples of IF and LS of for more polluted area, Lakheri of Bundi district are presented in **Table 4.9** and **Table 4.10**, which provide an overview on heavy metal concentration. The mean concentration pattern shows the similar trend as Baran.

Table 4.9 indicate the concentration of Pb, Cd, Al, As, Fe and Zn are ranges from 0.0178-0.0301, 0.0004-0.0094, 0.0045-0.0876, 0.0038-0.0081, 0.2875-0.5846 and 0.2987-0.4321 mg/L respectively, and mean concentration obtained are 0.0245, 0.0050, 0.0292, 0.0055, 0.3876 and 0.3502 mg/L respectively.

The results of LS indicated in **Table 4.10**, which shows that minimum concentration of Pb, Cd, Al, As, Fe, Zn are 0.0205, 0.0024, 0.0046, 0.0044, 0.3214, 0.3206, maximum concentrations are 0.0373, 0.0111, 0.0875, 0.0081, 0.6841, 0.5978 and mean concentration are 0.0302, 0.0084, 0.0412, 0.0060, 0.4673 and 0.4057 respectively.

On comparing the results of both individual farms and local shops of Lakheri, Bundi, it is found that there is a slight increase in the mean concentration of local shops than individual farm. It is revealed from the analysis that concentration of Aluminium is slightly higher than the concentration of zinc in LS.

4.4.3.2 Less Polluted Area (Kapren)

(a) Individual Farms

Table 4.11: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BnKIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnKIF - B1	0.0221	0.0028	0.0213	0.0034	0.4383	0.3923
BnKIF - B2	0.0167	0.0024	0.0209	BIR	0.2206	0.3221
BnKIF - B3	0.0205	0.0026	0.0227	0.0021	0.2721	0.2847
BnKIF - B4	0.0133	BIR	0.0197	0.0041	0.2289	0.3271
BnKIF - B5	0.0121	0.0019	0.0201	0.0004	0.3461	0.2397
Minimum	0.0121	BIR	0.0197	BIR	0.2206	0.2397
Maximum	0.0221	0.0028	0.0227	0.0041	0.4383	0.3923
Mean	**0.0169**	**0.0019**	**0.0209**	**0.0020**	**0.3012**	**0.3132**
SD	0.0039	0.0010	0.0010	0.0016	0.0817	0.0505
Variance	-	-	-	3E-06	0.0067	0.0025

BnKIF : Bundi Kapren Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.12: Concentration (mg/L) of metals (Mean±SD) in Butter sample of BnKLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnKLS - B1	0.0213	0.0037	0.0252	BIR	0.3451	0.1722
BnKLS - B2	0.0219	0.0052	0.0231	0.0043	0.2989	0.3943
BnKLS - B3	0.0196	0.0013	0.0137	0.0019	0.3516	0.2552
BnKLS - B4	0.0147	0.0029	0.0223	0.0032	0.3541	0.4013
BnKLS - B5	0.0216	0.0015	0.0229	0.0022	0.2873	0.4263
Minimum	0.0147	0.0013	0.0137	BIR	0.2873	0.1722
Maximum	0.0219	0.0052	0.0252	0.0043	0.3541	0.4263
Mean	**0.0198**	**0.0029**	**0.0214**	**0.0023**	**0.3274**	**0.3299**
SD	0.0027	0.0014	0.0040	0.0014	0.0284	0.0990
Variance	BIR	BIR	BIR	2E-06	0.0008	0.0098

BnKLS : Bundi Kapren Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The results of IF and LS of less polluted area, Kapren of Bundi district are presented in **Table 4.11** and **Table 4.12**. Each table shows the concentration of metal in mg/L. The mean concentration of Pb, Cd, Al, As, Fe and Zn in butter samples for IF are 0.0169, 0.0019, 0.0209, 0.0020, 0.3012 and 0.3132 and the same for LS are 0.0198, 0.0029, 0.0214, 0.0023, 0.3274 and 0.3299 mg/L respectively.

4.4.4 JALAWAR DISTRICT

4.4.4.1 More Polluted Area (Jhalarapatan Kali Sind Thermal Power Plant)

(a) Individual Farms

Table 4.13: Concentration (mg/L) of metals (Mean±SD) in Butter sample of JJIF

Samples	Pb	Cd	Al	As	Fe	Zn
JJIF - B1	0.0337	0.0081	0.0311	0.0022	0.3205	0.2977
JJIF - B2	0.0366	0.0067	0.0202	BIR	0.5836	0.3233
JJIF - B3	0.0043	0.00073	0.0172	0.0051	0.4205	0.4319
JJIF - B4	0.0045	BIR	0.0273	0.0018	0.2873	0.3201
JJIF - B5	0.0137	0.0019	0.0262	0.0037	0.3218	0.3537
Minimum	0.0043	BIR	0.0172	BIR	0.2873	0.2977
Maximum	0.0366	0.0081	0.0311	0.0051	0.5836	0.4319
Mean	**0.0186**	**0.0035**	**0.0244**	**0.0026**	**0.3867**	**0.3453**
SD	0.0140	0.0033	0.0050	0.0017	0.1081	0.0468
Variance	0.0002	-	-	3E-06	0.0117	0.0022

JJIF : Jhalawar Jhalarapatan Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.14: Concentration (mg/L) of metals (Mean±SD) in Butter sample of JJLS

Samples	Pb	Cd	Al	As	Fe	Zn
JJLS - B1	0.0189	0.0073	0.0323	0.0034	0.3201	0.5218
JJLS - B2	0.0191	0.0071	0.0861	0.0063	0.6821	0.3121
JJLS - B3	0.0353	0.0034	0.0631	0.0071	0.4202	0.4209
JJLS - B4	0.0332	0.0095	0.0021	0.0037	0.5829	0.3201
JJLS - B5	0.0223	0.0014	0.0117	0.0051	0.3119	0.3509
Minimum	0.0189	0.0014	0.0021	0.0034	0.3119	0.3121
Maximum	0.0353	0.0095	0.0861	0.0071	0.6821	0.5218
Mean	**0.0258**	**0.0057**	**0.0391**	**0.0051**	**0.4634**	**0.3852**
SD	0.0071	0.0029	0.0315	0.0014	0.1466	0.0783
Variance	-	-	0.0010	2E-06	0.0215	0.0061

JJLS : Jhalawar Jhalarapatan Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 4.13 and **4.14** provides the heavy metal concentrations in individual farms and local shops of more polluted area, Jhalarapatan of Jhalawar district. These areas are situated in the proximity of industries where emission of waste disposal is higher.

The mean concentration of Pb, Cd, Al, As, Fe, and Zn of IF are 0.0186, 0.0035, 0.0244, 0.0026, 0.3867, and 0.3453 mg/L respectively. The outcomes indicate that metal contamination is wide-spread in the area under study. Similarly **Table 4.14** indicate the mean concentration of Pb, Cd, Al, As, Fe, and Zn of LS are 0.0258, 0.0057, 0.0391, 0.0051, 0.4634 and 0.3852 mg/L respectively.

From the results it can be seen that metal ion concentration in IF and LS are found in the order of Fe > Zn > Al > Pb > Cd > As. These results also illustrate that there is a slight increase the mean concentration of metals of local shops. And also the analysis shows a packing increase in metal concentration.

4.4.4.2 Less Polluted Area (Aklera)

(a) Individual Farms

Table 4.15: Concentration (mg/L) of metals (Mean±SD) in Butter sample of JAIF

Samples	Pb	Cd	Al	As	Fe	Zn
JAIF - B1	0.0203	0.0029	0.0123	BIR	0.4373	0.3921
JAIF - B2	0.0123	BIR	0.0263	0.0011	0.3205	0.3219
JAIF - B3	0.0112	0.0027	0.0217	0.0022	0.2722	0.2845
JAIF - B4	0.0101	BIR	0.0165	BIR	0.2286	0.3272
JAIF - B5	0.0067	0.0026	0.0203	0.0003	0.3463	0.2387
Minimum	0.0067	BIR	0.0123	BIR	0.2286	0.2387
Maximum	0.0203	0.0029	0.0263	0.0022	0.4373	0.3921
Mean	**0.0121**	**0.0016**	**0.0194**	**0.0007**	**0.3210**	**0.3129**
SD	0.0045	0.0013	0.0047	0.0008	0.0708	0.0507
Variance	-	-	-	7E-07	0.0050	0.0026

JAIF : Jhalawar Aklera Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 4.16: Concentration (mg/L) of metals (Mean±SD) in Butter sample of JALS

Samples	Pb	Cd	Al	As	Fe	Zn
JALS - B1	0.0213	0.0023	0.0181	BIR	0.4311	0.4218
JALS - B2	0.0147	0.0029	0.0123	0.0026	0.2503	0.3031
JALS - B3	0.0119	0.0021	0.0262	BIR	0.3954	0.2986
JALS - B4	0.0092	BIR	0.0227	0.0032	0.4112	0.2412
JALS - B5	0.0251	0.0031	0.0251	0.0031	0.3122	0.3191
Minimum	0.0092	BIR	0.0123	BIR	0.2503	0.2412
Maximum	0.0251	0.0031	0.0262	0.0032	0.4311	0.4218
Mean	**0.0164**	**0.0021**	**0.0209**	**0.0018**	**0.3600**	**0.3168**
SD	0.0059	0.0011	0.0051	0.0015	0.0682	0.0588
Variance	-	-	-	2E-06	0.0046	0.0035

JALS : Jhalawar Aklera Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 4.15 and **Table 4.16** indicates the results of metal concentration in butter samples of less polluted area, Aklera of Jhalawar district. Each row represents the heavy metal concentration of particular sample of that zone. Minimum, maximum, mean, SD and variance are also given for both IF and LS.

From the **Tables 4.15** it is observed that minimum concentration of Pb, Cd, Al, As, Fe and Zn are 0.0067, BIR, 0.0123, BIR, 0.2286, 0.2387 and maximum concentration are 0.0203, 0.0029, 0.0263, 0.0022, 0.4373 and 0.2921 respectively. The mean concentration of Pb, Cd, Al, As, Fe, and Zn of IF are 0.0121, 0.0016, 0.0194, 0.0007, 0.3210, and 0.3129 respectively. Whereas the minimum concentration of Pb, Cd, Al, As, Fe and Zn of LS are obtained 0.0092, BIR, 0.0123, BIR, 0.2503, 0.2412 while maximum concentration are 0.0251, 0.0031, 0.0262, 0.0032, 0.4311 and 0.4218 respectively. The mean concentration of Pb, Cd, Al, As, Fe, and Zn of LS are 0.0164, 0.0021, 0.0209, 0.0018, 0.3600, and 0.3168 respectively.

On comparing the results of both IF and LS, it is observed that metal concentration in samples of LS are slightly higher then IF, but all metal concentrations are found below the permissible limit.

4.5 Graphical Representation:

4.5.1 Pb Concentration

Table 4.17 : Lead concentration(mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0348	0.0409	0.0117	0.0189
Baran	0.0283	0.0324	0.0179	0.0199
Bundi	0.0245	0.0302	0.0169	0.0198
Jhalawar	0.0186	0.0258	0.0121	0.0164

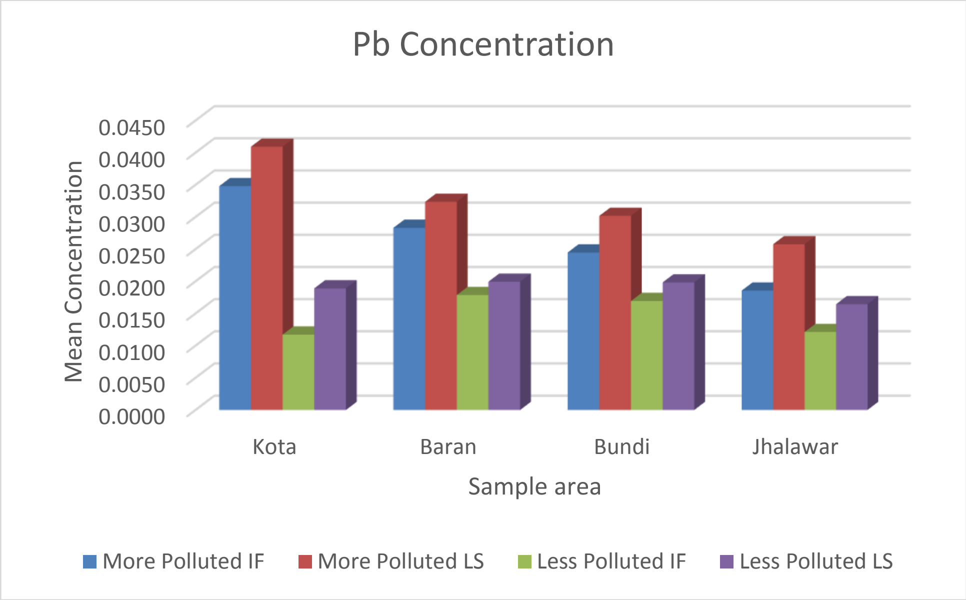


Figure 4.2 : Lead concentration(mg/L) in butter samples in four different areas of Kota region

4.5.2 Cd Concentration

Table 4.18 : Cadmium concentration(mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0093	0.0136	0.0019	0.0028
Baran	0.0082	0.0086	0.0022	0.0024
Bundi	0.0050	0.0084	0.0019	0.0029
Jhalawar	0.0035	0.0057	0.0016	0.0021

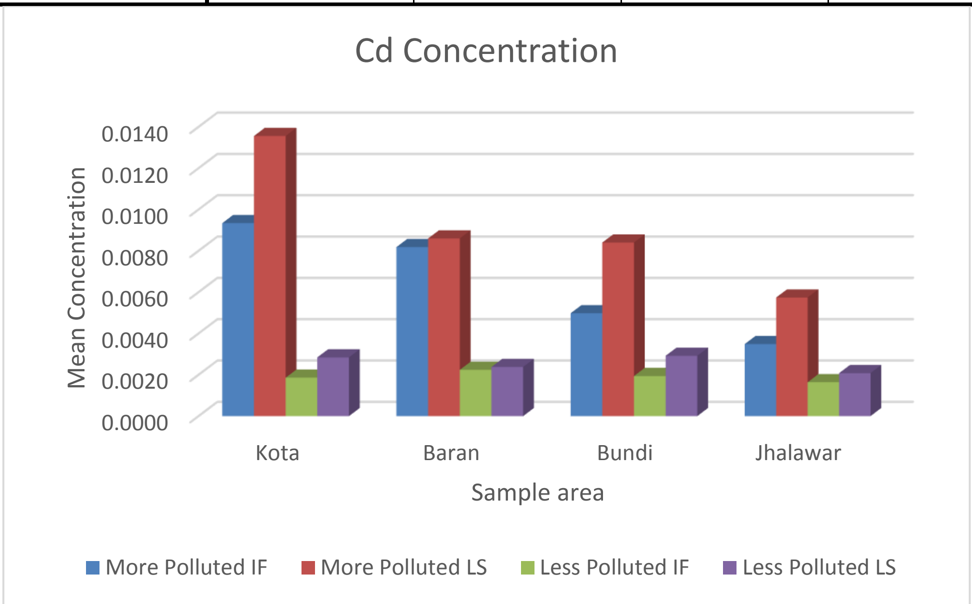


Figure 4.3 : Cadmium concentration(mg/L) in butter samples in four different areas of Kota region

4.5.3 Al Concentration

Table 4.19 : Aluminium concentration (mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0412	0.1040	0.0186	0.0194
Baran	0.0352	0.0472	0.0192	0.0210
Bundi	0.0292	0.0412	0.0209	0.0214
Jhalawar	0.0244	0.0391	0.0194	0.0209

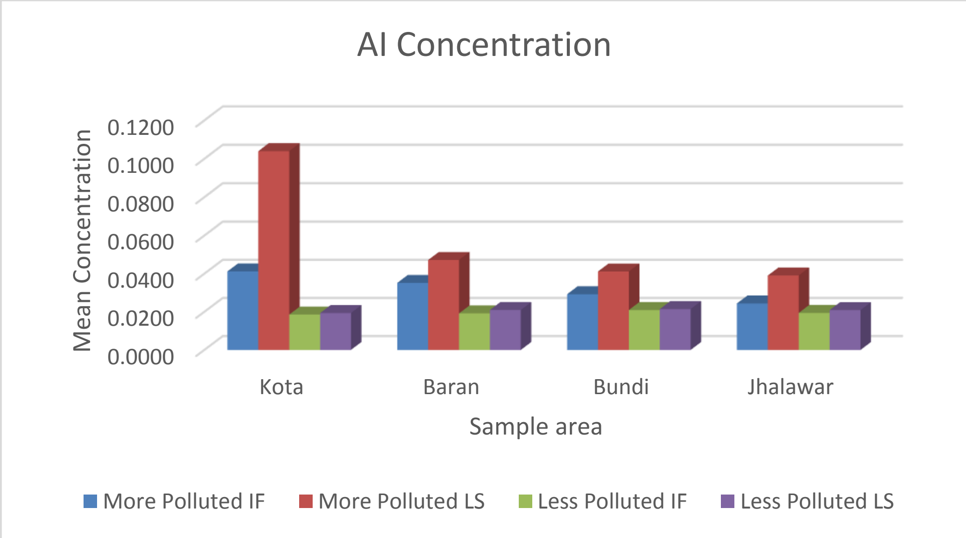


Figure 4.4 : Aluminium concentration (mg/L) in butter samples in four different areas of Kota region

4.5.4 As Concentration

Table 4.20: Arsenic concentration(mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0041	0.0065	BIR	0.0027
Baran	0.0041	0.0054	BIR	0.0012
Bundi	0.0055	0.0060	0.0020	0.0023
Jhalawar	0.0026	0.0051	0.0007	0.0018

BIR : Beyond Instrumental Range

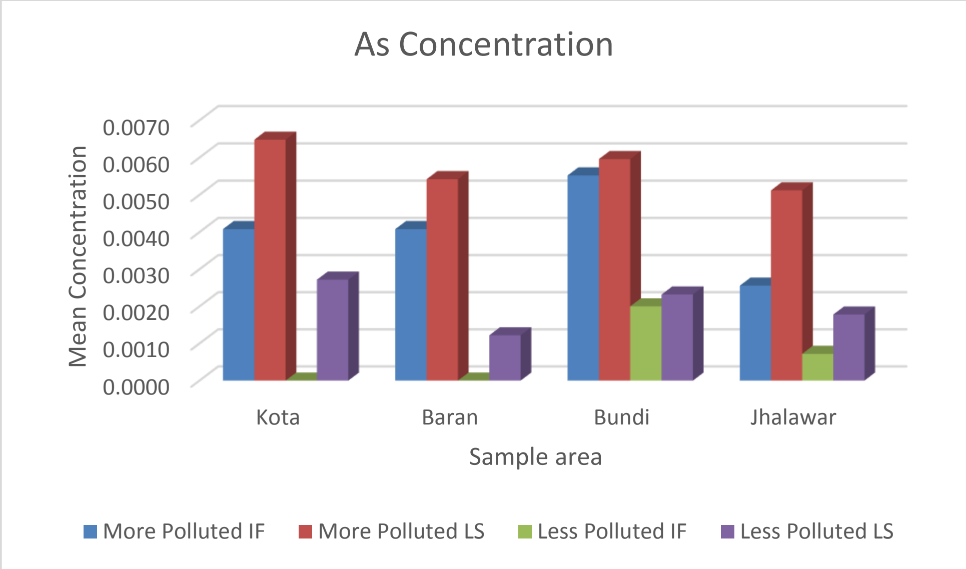


Figure 4.5 : Arsenic concentration(mg/L) in butter samples in four different areas of Kota region

4.5.5 Fe Concentration

Table 4.21: Iron concentration(mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.4875	1.0272	0.3416	0.3604
Baran	0.4274	0.4453	0.3212	0.3275
Bundi	0.3876	0.4673	0.3012	0.3274
Jhalawar	0.3867	0.4634	0.3210	0.3600

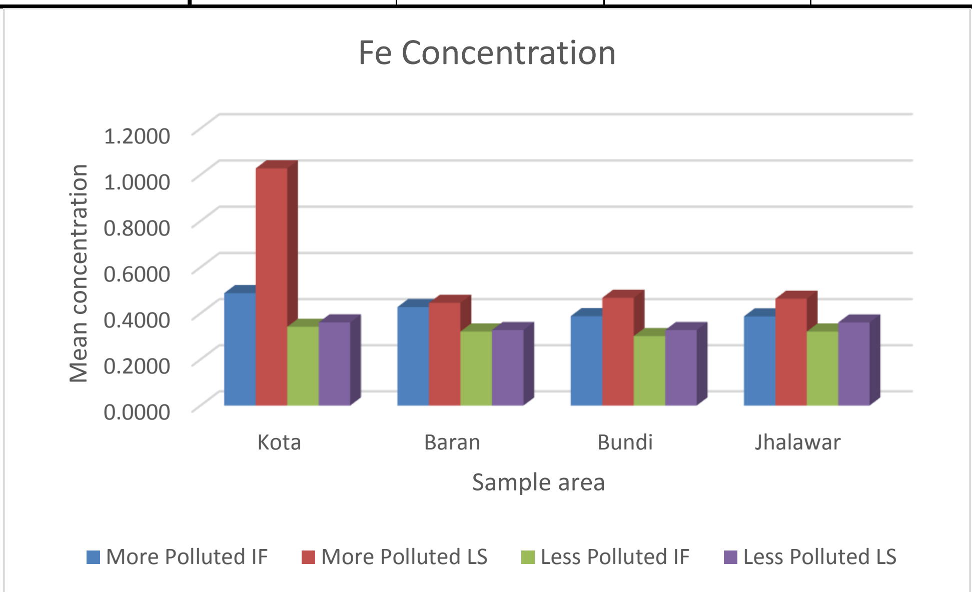


Figure 4.6 : Iron concentration(mg/L) in butter samples in four different areas of Kota region

4.5.6 Zn Concentration

Table 4.22 : Zinc concentration(mg/L) in butter samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.3667	0.4379	0.3047	0.3183
Baran	0.3521	0.3722	0.3133	0.3259
Bundi	0.3502	0.4057	0.3132	0.3299
Jhalawar	0.3453	0.3852	0.3129	0.3168

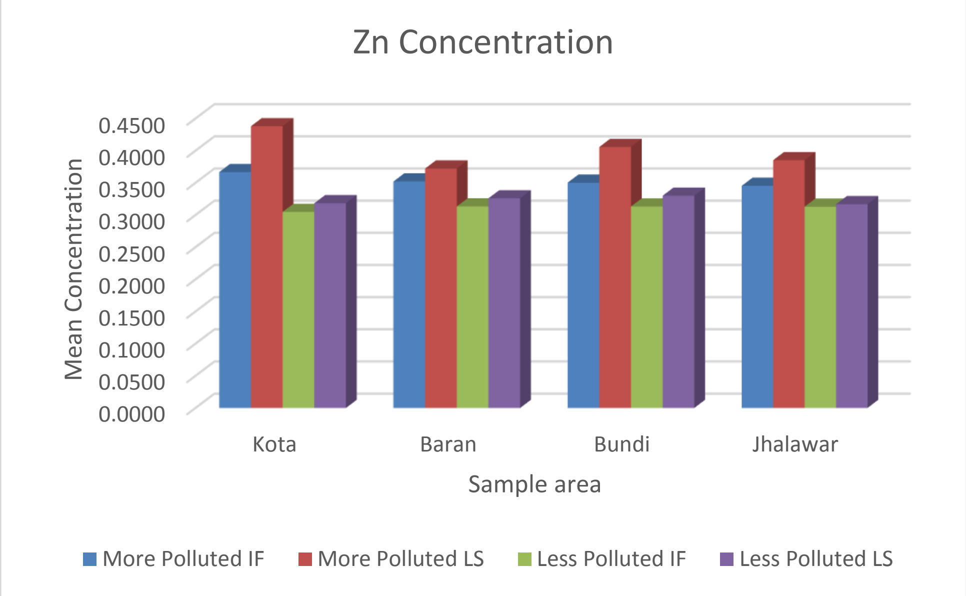


Figure 4.7 : Zinc concentration(mg/L) in butter samples in four different areas of Kota region

Fig. 4.1 demonstrated the Pb levels in butter samples of all four places. Figure shows that there is a noticeable increase in Pb levels of polluted area. From **Fig. 4.2** it can be clearly seen that Cd levels are highest in Kota. Similarly a significant difference can be seen for Al and Fe levels of Kota from other places. Arsenic levels are similar for all places though it is found in very low concentration. The difference observed for Zn concentration among all areas are very minimal.

4.6 Effect of processing and packaging in different types of Butter samples with Time :

Packaging material can leach harmful chemicals into food and beverages. The interaction between packaging materials and products can affect the quality and shelf life of food. So it is important to monitor and to ensure that packaging and processing preserves the safety of the product throughout its intended shelf life [12-14].

So our main aim is to assess the potential risk associated with packaging material and processing methods and to take a step forward in minimizing the risk and ensuring food safety.

For this Butter samples of three different brands were taken and analyse the heavy metal concentration with respect to time. On the basis of shelf life of butter, first sample was taken out at 0 day of each brand which is immediately digested. Second and third sample was taken out after a month and three months respectively from the date of opening the packing. The table given below shows the results of three different brands after analysing with AAS.

Table 4.23 Temporal Analysis of Heavy Metal Concentration in Different Butter Brands

Butter Sample	Days	Pb	Cd	Al	As	Fe	Zn
Brand 1	0	0.0105	BIR	0.0129	BIR	0.2904	0.2652
	30	0.0124	0.0009	0.0143	0.0010	0.2985	0.2751
	90	0.0164	0.0027	0.0221	0.0032	0.3687	0.3147
Brand 2	0	0.0098	0.0015	0.0154	BIR	0.2845	0.2354
	30	0.0109	0.0015	0.0167	0.0014	0.2985	0.2557
	90	0.0165	0.0039	0.0206	0.0018	0.3471	0.3025
Brand 3	0	0.0132	0.0005	0.0099	0.0009	0.3254	0.2458
	30	0.0135	0.0018	0.0184	0.0013	0.3447	0.2485
	90	0.0174	0.0042	0.0258	0.0020	0.3681	0.3167

BIR : Beyond Instrumental Range

Table 4.23 indicate the results of heavy metal concentration of Pb, Cd, Al, As, Fe and Zn (mg/L) in different brands of butter (Brand 1 , Brand 2 and Brand 3) at three different storage time i.e., 0 days, 30 days and 90 days.

Initial Time Point, T_i (At 0 day) : Table 4.23 revealed that the mean concentrations of Pb, Cd, Al, As, Fe and Zn at 0 day are 0.0105, BIR, 0.0129, BIR, 0.2904 and 0.2652 mg/L in brand 1, 0.0098, 0.0015, 0.0154, BIR, 0.2845 and 0.2354 mg/L in brand 2 and 0.0132, 0.0005, 0.0099, 0.0009, 0.3254 and 0.2458 mg/L in brand 3.

Mid Time Point, T_m (At 30^{th} day): Table 4.23 illustrate that the mean concentration of Pb, Cd, Al, As, Fe and Zn at 30 days are 0.0124, 0.0009, 0.0143, 0.0010, 0.2985 and 0.2751 mg/L in brand 1, 0.0109, 0.0015, 0.0167, 0.0014, 0.2985 and 0.2557 mg/L in brand 2 and 0.0135, 0.0018, 0.0184, 0.0013, 0.3447 and 0.2485 mg/L in brand 3 respectively.

Final Time Point, T_f (At 90^{th} day): Table 4.23 illustrate that the mean concentration of Pb, Cd, Al, As, Fe and Zn are found 0.0164, 0.0027, 0.0221, 0.0032, 0.3687 and 0.3147 mg/L in brand 1, 0.0165, 0.0039, 0.0206, 0.0018, 0.3471 and 0.3025 mg/L in brand 2 and 0.0174, 0.0042, 0.0258, 0.0020, 0.3681 and 0.3167 mg/L in brand 3 respectively.

The results of **Table 4.23** indicate that some changes in concentration have occured with period of time. From these data it is clear that there is no significant changes occur between 0 to 30 days. In case of brand 2 there is no change in Cd concentration was observed but it changes after 90 days. A significant change in metal concentration is observed after 90 days..

These variation in metal concentration in butter samples of different brands might be due to contamination by various factors such as manufacturing process, equipment used for processing, storage, packaging and transportation.

4.7 Statistical Analysis

4.7.1 Concentrations of Lead (Pb)

Fit Group

Oneway Analysis of Value By City

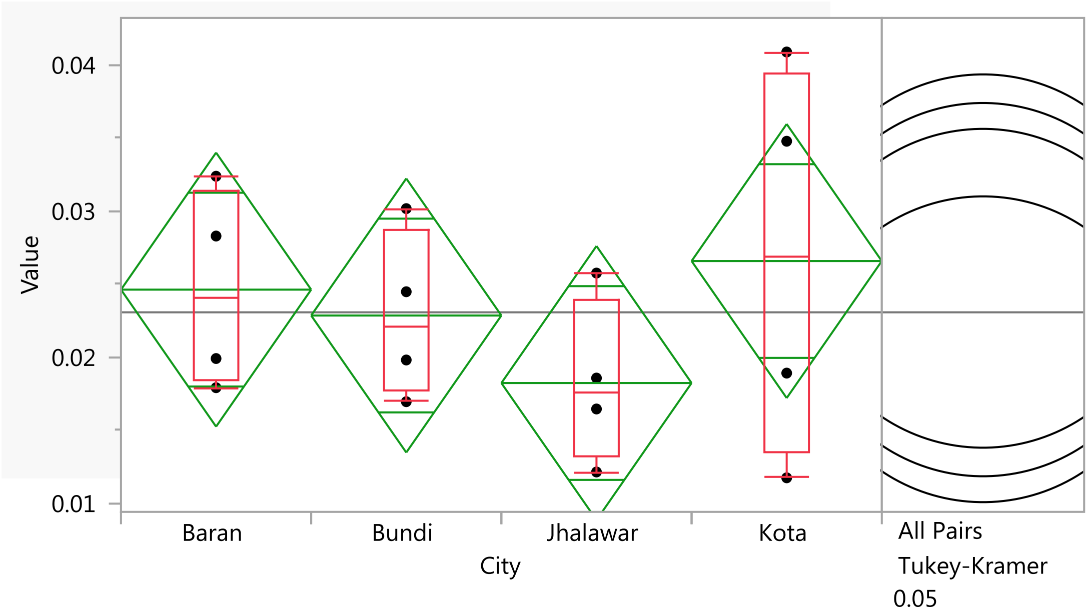


Figure 4.8: Oneway analysis of value by city wise for Pb in butter

Table 4.24: Analysis of variance and ordered differences report by city wise for Pb in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00015338	0.000051	0.6874	0.5768
Error	12	0.00089248	0.000074		
C. Total	15	0.00104585			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	-0.01 0 0.01 0.02
Kota	Jhalawar	0.0083650	0.0060981	-0.009739	0.0264690	0.5387	
Baran	Jhalawar	0.0064050	0.0060981	-0.011699	0.0245090	0.7244	
Bundi	Jhalawar	0.0046300	0.0060981	-0.013474	0.0227340	0.8710	
Kota	Bundi	0.0037350	0.0060981	-0.014369	0.0218390	0.9261	
Kota	Baran	0.0019600	0.0060981	-0.016144	0.0200640	0.9879	
Baran	Bundi	0.0017750	0.0060981	-0.016329	0.0198790	0.9910	

Oneway Analysis of Value By Pollution status

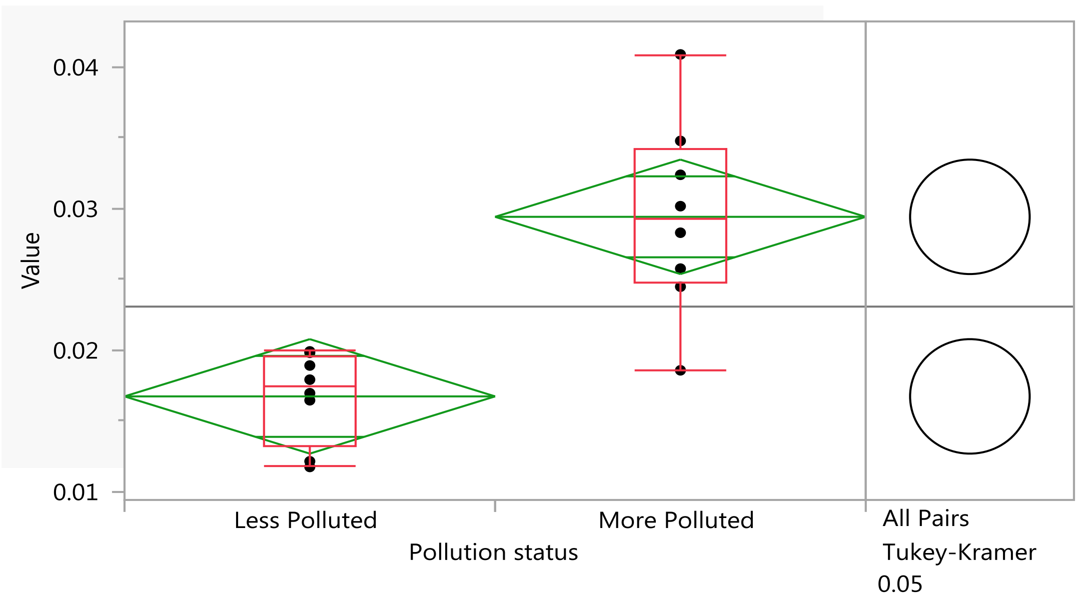


Figure 4.9: Oneway analysis of value by pollution status wise for Pb in butter

Table 4.25: Analysis of variance and ordered differences report by pollution status wise for Pb in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00064618	0.000646	22.6345	0.0003*
Error	14	0.00039968	0.000029		
C. Total	15	0.00104585			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std. Err Dif.	Lower CL	Upper CL	p-Value	0 0.005 0.010 0.015
More Polluted	Less Polluted	0.0127100	0.0026715	0.0069801	0.0184399	0.0003*	

Oneway Analysis of Value By Type

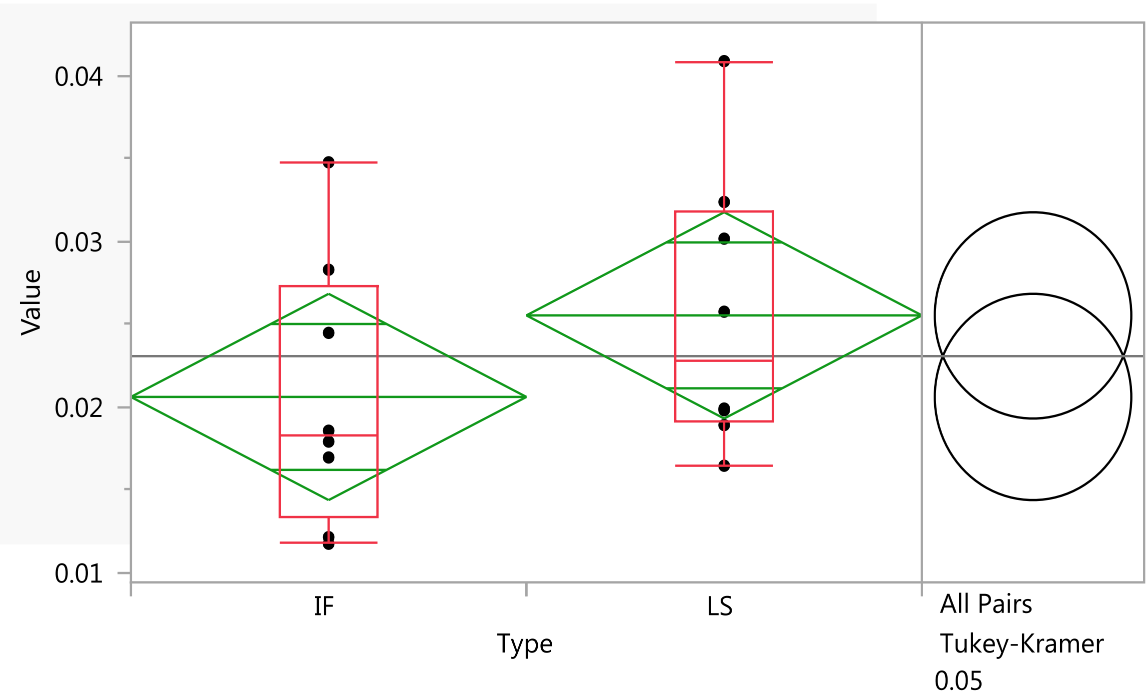


Figure 4.10: Oneway analysis of value by type wise for Pb in butter

Table 4.26: Analysis of variance and ordered differences report by type wise for Pb in butter

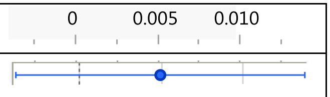
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00009742	0.000097	1.4380	0.2504
Error	14	0.00094844	0.000068		
C. Total	15	0.00104585			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0049350	0.0041154	-0.003892	0.0137616	0.2504	

Pooled t test

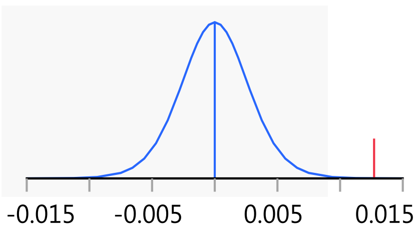


Figure 4.11: More & Less polluted

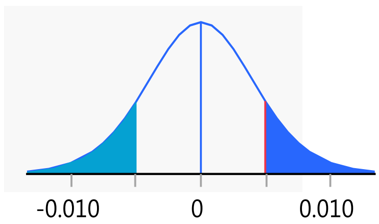


Figure 4.12: LS-IF

Summary of one way ANOVA for Pb

S.No.	Variable	α	p - value	Null Hypothesis
1	City Wise	0.05	0.5768	Significantly indifferent , Null Hypothesis can't be rejected
2	Pollution Status	0.05	0.0003	Significantly different , Null Hypothesis can be rejected
3	Types (IF & LS)	0.05	0.2504	Significantly indifferent , Null Hypothesis can't be rejected

To determine statistical evidence and singnificant difference for lead concentration in the butter sample one way ANOVA was performed.

One way analysis of variance From **Fig. 4.8** shows that the citywise difference among Baran, Bundi, Jhalawar and Kota is not significant with p-values > 0. 5768 which is greater than our chosen significant level (α = 0.05). So the null hypothesis can't be rejected and from this we can conclude that the difference in Pb concentration among the cities was not significant.

Tukey – Kramer HSD for Pb indicates that all the levels share the common place and from **Fig. 4.8** it is clear that all circles overlap each other which confirms that the mean concentration for cities are significantly indifferent.

Fig. 4.9 represents the pollution type status of Pb. For this p value > 0.0003, which is less than our chosen significant level α = 0.05. From the **Fig. 4.9** it is also clear that both the circles are very far from each other which shows the significant difference between less polluted and more polluted areas. Lower values of less polluted area reveals that this site is comparatively safe and does not contain higher metal concentrations as per RDA standards.

The third variable is location type that is IF and LS within the cities for which probability is 0.2504, which is greater than α = 0.05, showing that the mean values are significantly indifferent but less than city wise pollution level. Tukey – Kramer HSD test also that shows that the circles are overlapping each other and there is no significant difference between them.

4.7.2 Concentrations of Cadmium (Cd)

Fit Group

Oneway Analysis of Value By City

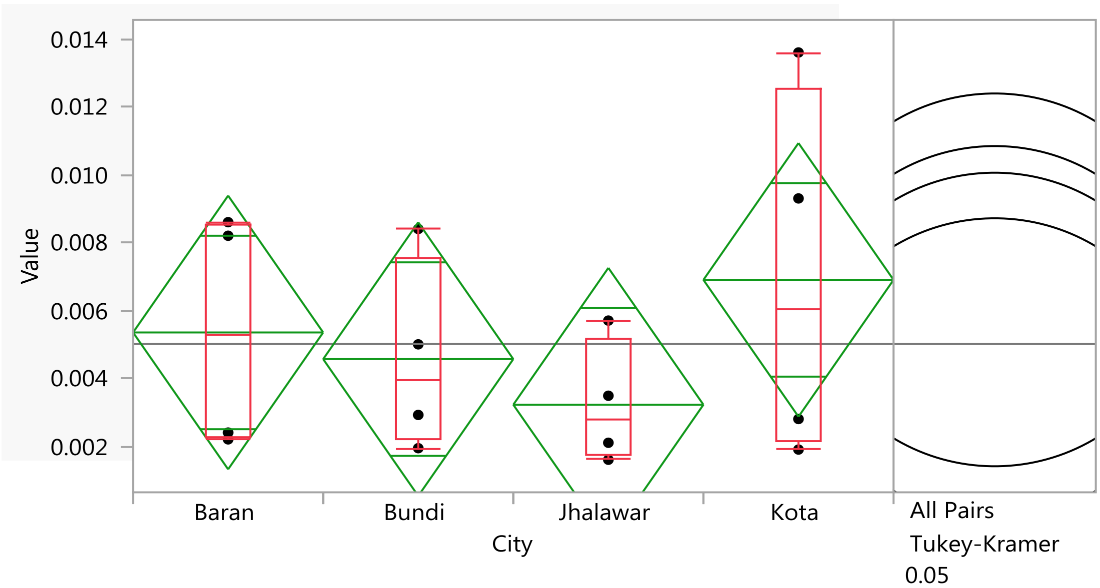


Figure 4.13: Oneway analysis of value by city wise for Cd in butter

Table 4.27: Analysis of variance and ordered differences report by city wise for Cd in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00002834	9.446e-6	0.6896	0.5756
Error	12	0.00016436	0.000014		
C. Total	15	0.00019270			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.005 0 0.005 0.010
Kota	Jhalawar	0.0036785	0.0026170	-0.004091	0.0114477	0.5194	
Kota	Bundi	0.0023350	0.0026170	-0.005434	0.0101042	0.8090	
Baran	Jhalawar	0.0021285	0.0026170	-0.005641	0.0098977	0.8470	
Kota	Baran	0.0015500	0.0026170	-0.006219	0.0093192	0.9325	
Bundi	Jhalawar	0.0013435	0.0026170	-0.006426	0.0091127	0.9543	
Baran	Bundi	0.0007850	0.0026170	-0.006984	0.0085542	0.9901	

Oneway Analysis of Value By Pollution status

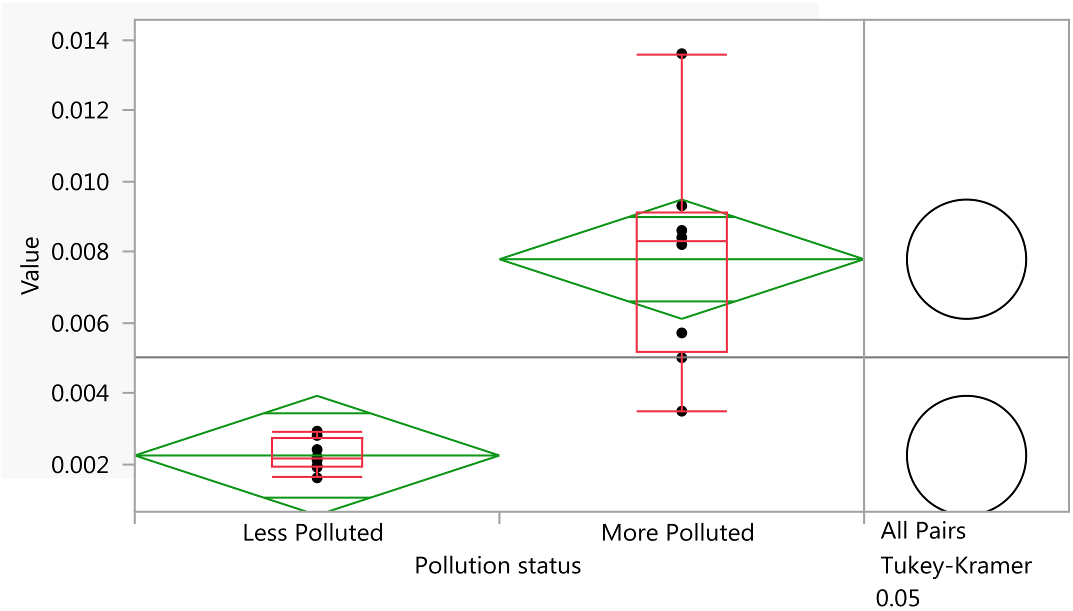


Figure 4.14: Oneway analysis of value by pollution status wise for Cd in butter

Table 4.28: Analysis of variance and ordered differences report by pollution status wise for Cd in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00012335	0.000123	24.9034	0.0002*
Error	14	0.00006935	4.953e-6		
C. Total	15	0.00019270			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.002 0.004 0.006
More Polluted	Less Polluted	0.0055533	0.0011128	0.0031665	0.0079400	0.0002*	

Oneway Analysis of Value By Type

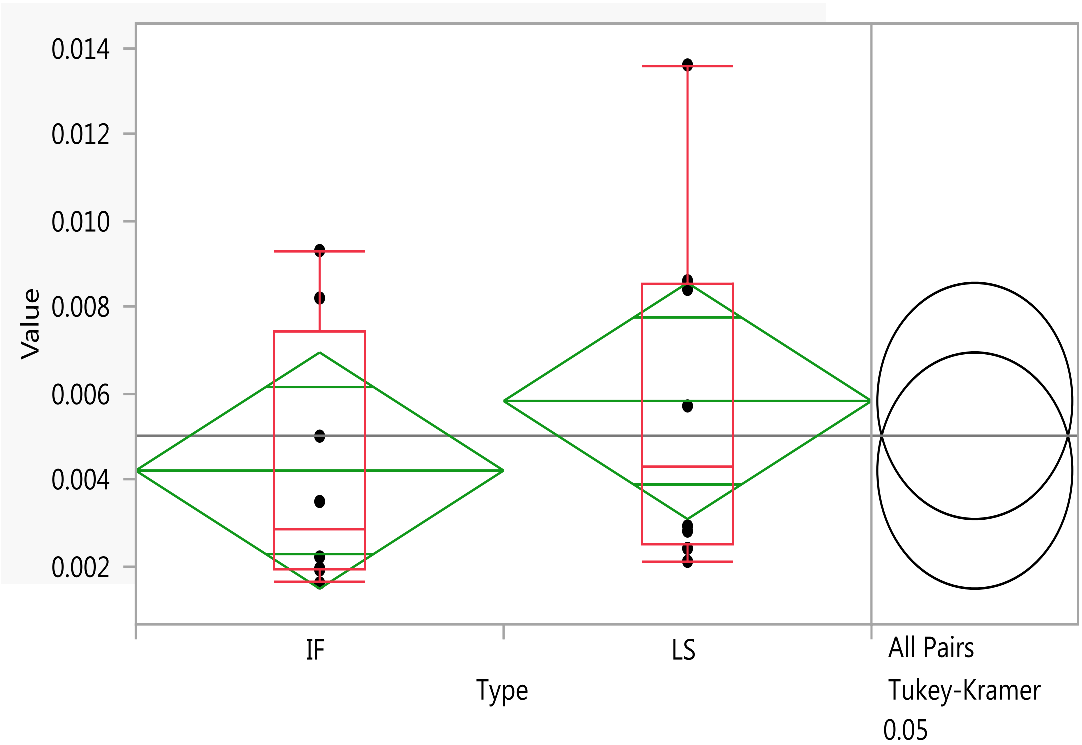


Figure 4.15: Oneway analysis of value by type wise for Cd in butter

Table 4.29: Analysis of variance and ordered differences report by type wise for Cd in butter

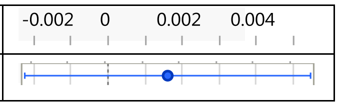
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00001039	0.000010	0.7979	0.3868
Error	14	0.00018231	0.000013		
C. Total	15	0.00019270			

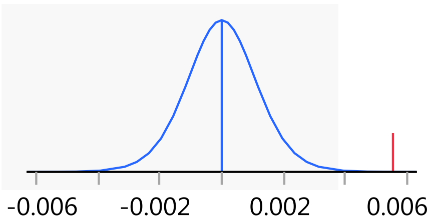
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0016117	0.0018043	-0.002258	0.0054816	0.3868	

Pooled t test



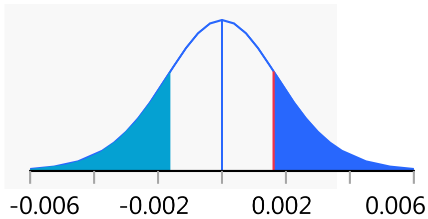


Figure 4.16: More & Less polluted **Figure 4.17: LS-IF**

Summary of one way ANOVA for Cd

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.5756	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0002	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.3868	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 4.13 presents the citywise analysis in which we can see that the lower and higher concentration value are greater in comparison to other three. According to Tukey – Kramer HSD Baran, Bundi, Jhalawar, the circle shares the same place and for Kota

it covers larger area. According to **Fig. 4.14**, less and more polluted areas are significantly different with the p value > 0.0002 which is less than our chosen value (α = 0.05). One way ANOVA for analysis of value by type IF and LS, probability > 0.3868 which shows that the concentration of Cd are significantly indifferent. Tukey – Kramer HSD test also supports the data. **Fig. 4.16** and **4.17** shows the graphical representation of pooled t – test for more and less polluted area and LS – IF.

4.7.3 Concentrations of Aluminium (Al)

Fit Group

Oneway Analysis of Value By City

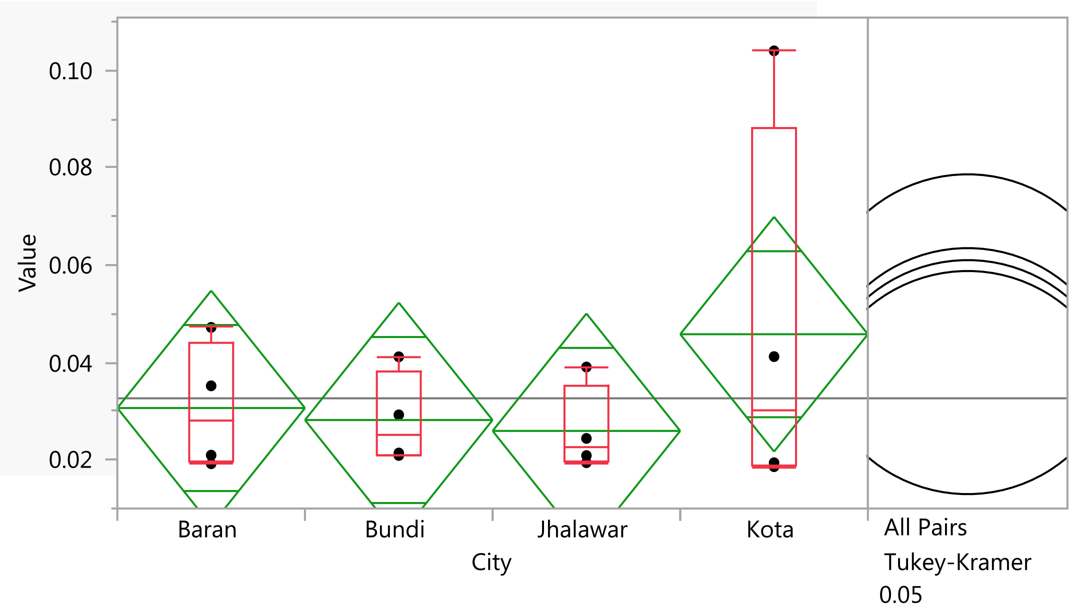


Figure 4.18: Oneway analysis of value by city wise for Al in butter

Table 4.30: Analysis of variance and ordered differences report by city wise for Al in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00096805	0.000323	0.6590	0.5928
Error	12	0.00587599	0.000490		
C. Total	15	0.00684404			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.04 0 0.04
Kota	Jhalawar	0.0198600	0.0156471	-0.026593	0.0663133	0.5979	
Kota	Bundi	0.0176250	0.0156471	-0.028828	0.0640783	0.6811	
Kota	Baran	0.0151600	0.0156471	-0.031293	0.0616133	0.7692	
Baran	Jhalawar	0.0047000	0.0156471	-0.041753	0.0511533	0.9901	
Baran	Bundi	0.0024650	0.0156471	-0.043988	0.0489183	0.9985	
Bundi	Jhalawar	0.0022350	0.0156471	-0.044218	0.0486883	0.9989	

Oneway Analysis of Value By Pollution status

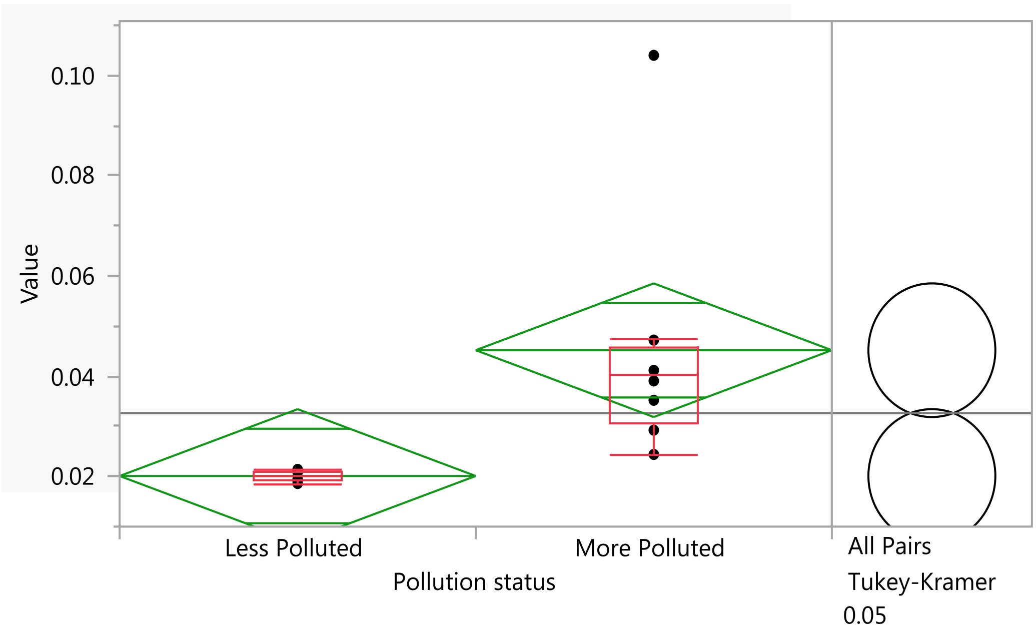


Figure 4.19: Oneway analysis of value by pollution status wise for Al in butter

Table 4.31: Analysis of variance and ordered differences report by pollution status wise for Al in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00251753	0.002518	8.1464	0.0127*
Error	14	0.00432651	0.000309		
C. Total	15	0.00684404			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.01 0.02 0.03 0.04
More Polluted	Less Polluted	0.0250875	0.0087897	0.0062354	0.0439396	0.0127*	

Oneway Analysis of Value By Type

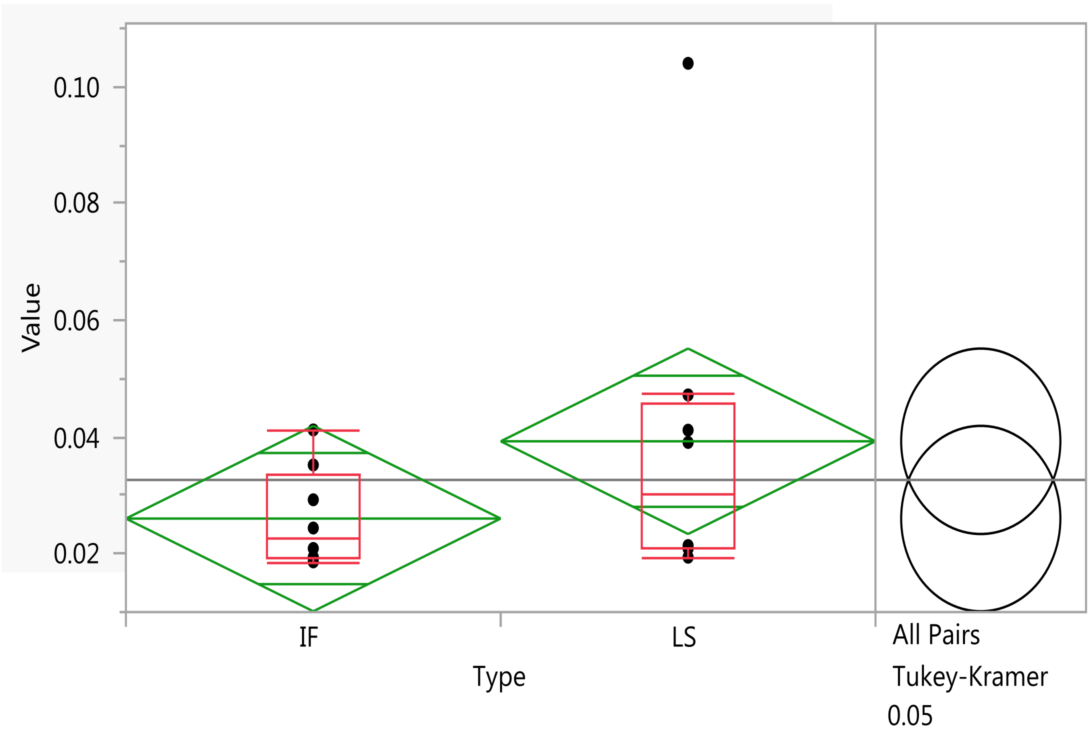


Figure 4.20: Oneway analysis of value by type wise for Al in butter

Table 4.32: Analysis of variance and ordered differences report by type wise for Al in butter

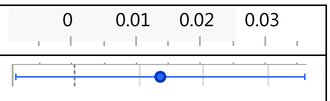
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00070093	0.000701	1.5974	0.2269
Error	14	0.00614312	0.000439		
C. Total	15	0.00684404			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0132375	0.0104737	-0.009226	0.0357014	0.2269	

Pooled t test

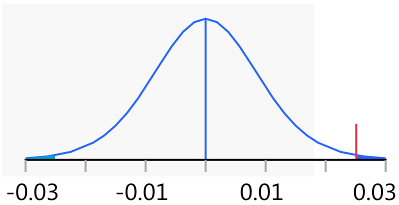


Figure 4.21: More & Less polluted

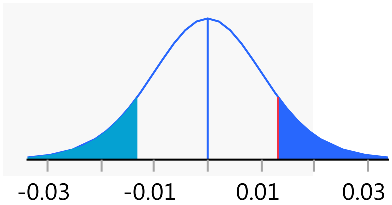


Figure 4.22: LS-IF

Summary of one way ANOVA for Al

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.5928	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0127	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.2269	Significantly indifferent , Null Hypothesis can't be rejected

For all three variables, statistical interpretation, one-way analysis of variance and Tukey – Kramer HSD was carried out for aluminium in butter and results are shown in **Fig. 4.18, 4.19** and **4.20**, which clearly indicates that First and the third variable are significantly indifferent and the second variable is significantly different. Null hypothesis can't be rejected for first and third variables and can be rejected for second variable. Tukey-Kramer HSD test also supports the data.

4.7.4 Concentrations of Arsenic (As)

Fit Group

Oneway Analysis of Value By City

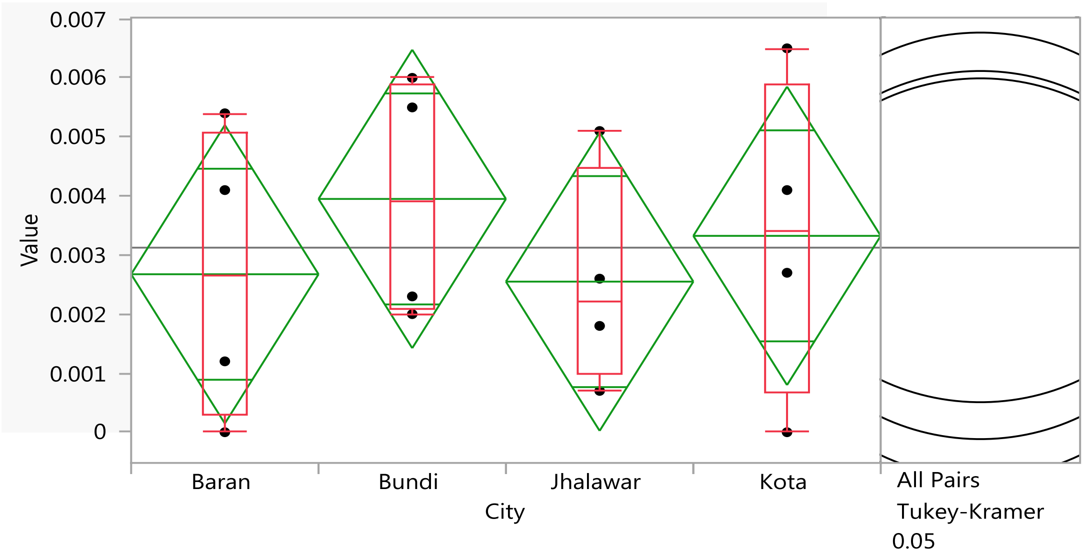


Figure 4.23: Oneway analysis of value by city wise for As in butter

Table 4.33: Analysis of variance and ordered differences report by city wise for As in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00000502	1.6717e-6	0.3108	0.8172
Error	12	0.00006454	5.3779e-6		
C. Total	15	0.00006955			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Bundi	Jhalawar	0.0014000	0.0016398	-0.003468	0.0062683	0.8280
Bundi	Baran	0.0012750	0.0016398	-0.003593	0.0061433	0.8631
Kota	Jhalawar	0.0007750	0.0016398	-0.004093	0.0056433	0.9637
Kota	Baran	0.0006500	0.0016398	-0.004218	0.0055183	0.9779
Bundi	Kota	0.0006250	0.0016398	-0.004243	0.0054933	0.9803
Baran	Jhalawar	0.0001250	0.0016398	-0.004743	0.0049933	0.9998

Oneway Analysis of Value By Pollution status

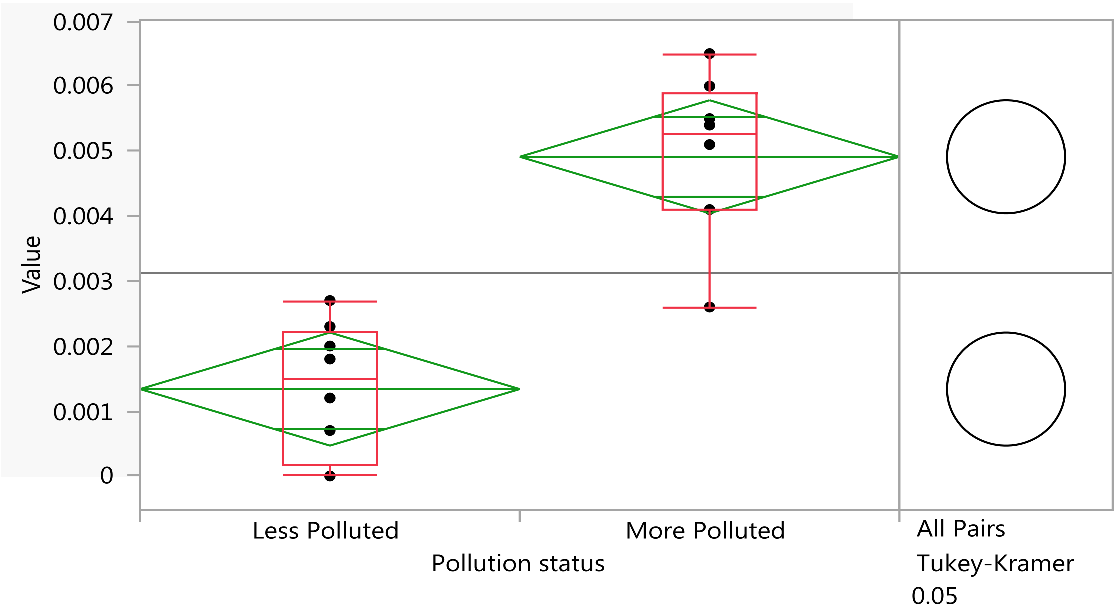


Figure 4.24: Oneway analysis of value by pollution status wise for As in butter

Table 4.34: Analysis of variance and ordered differences report by pollution status wise for As in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00005112	0.000051	38.8395	<.0001*
Error	14	0.00001843	1.316e-6		
C. Total	15	0.00006955			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.001 0.002 0.003 0.004
More Polluted	Less Polluted	0.0035750	0.0005736	0.0023447	0.0048053	<.0001*	

Oneway Analysis of Value By Type

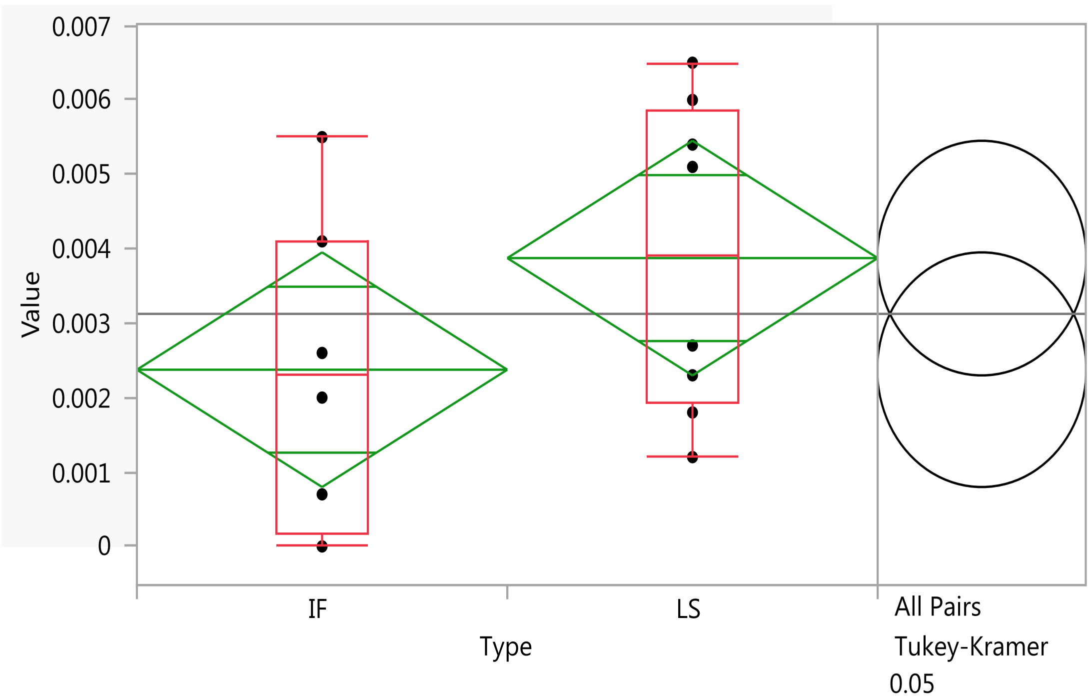


Figure 4.25: Oneway analysis of value by type wise for As in butter

Table 4.35: Analysis of variance and ordered differences report by type wise for As in butter

Analysis of Variance

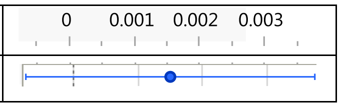
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00000900	0.000009	2.0809	0.1711
Error	14	0.00006055	4.325e-6		
C. Total	15	0.00006955			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
LS	IF	0.0015000	0.0010398	-0.000730	0.0037302	0.1711



Pooled t test

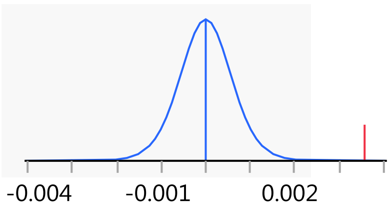


Figure 4.26: More & Less polluted

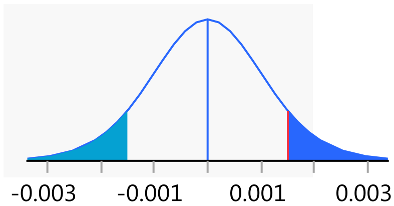


Figure 4.27: LS-IF

Summary of one way ANOVA for As

S.No.	Variable	α	p - value	Null Hypothesis
1	City Wise	0.05	0.8172	Significantly indifferent , Null Hypothesis can't be rejected
2	Pollution Status	0.05	0.0001	Significantly different , Null Hypothesis can be rejected
3	Types (IF & LS)	0.05	0.1711	Significantly indifferent , Null Hypothesis can't be rejected

Summary Table clearly indicates that city wise and types wise means concentration of As is Significantly indifferent whereas according to pollution status, this is significantly different. Null hypothesis can't be rejected for first and third variables and can be rejected for second variable. Tukey-Kramer HSD test also support the data.

4.7.5 Concentrations of Iron (Fe)

Fit Group

Oneway Analysis of Value By City

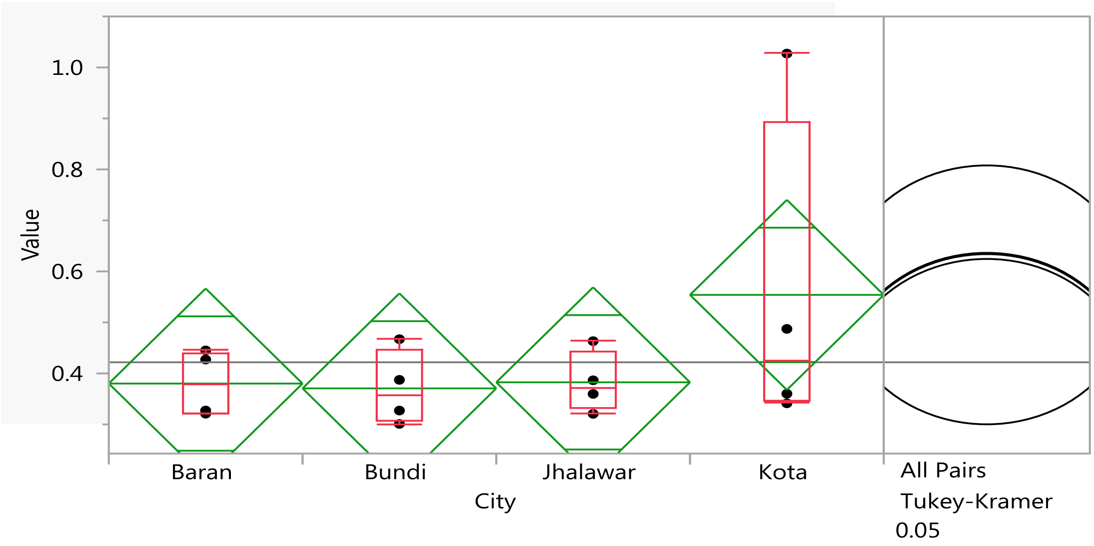


Figure 4.28: Oneway analysis of value by city wise for Fe in butter

Table 4.36: Analysis of variance and ordered differences report by city wise for Fe in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.09342297	0.031141	1.0651	0.4002
Error	12	0.35084264	0.029237		
C. Total	15	0.44426561			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.2 0 0.2 0.4
Kota	Bundi	0.1833000	0.1209068	-0.175648	0.5422484	0.4585	
Kota	Baran	0.1738250	0.1209068	-0.185123	0.5327734	0.5015	
Kota	Jhalawar	0.1713800	0.1209068	-0.187568	0.5303284	0.5128	
Jhalawar	Bundi	0.0119200	0.1209068	-0.347028	0.3708684	0.9996	
Baran	Bundi	0.0094750	0.1209068	-0.349473	0.3684234	0.9998	
Jhalawar	Baran	0.0024450	0.1209068	-0.356503	0.3613934	1.0000	

Oneway Analysis of Value By Pollution status

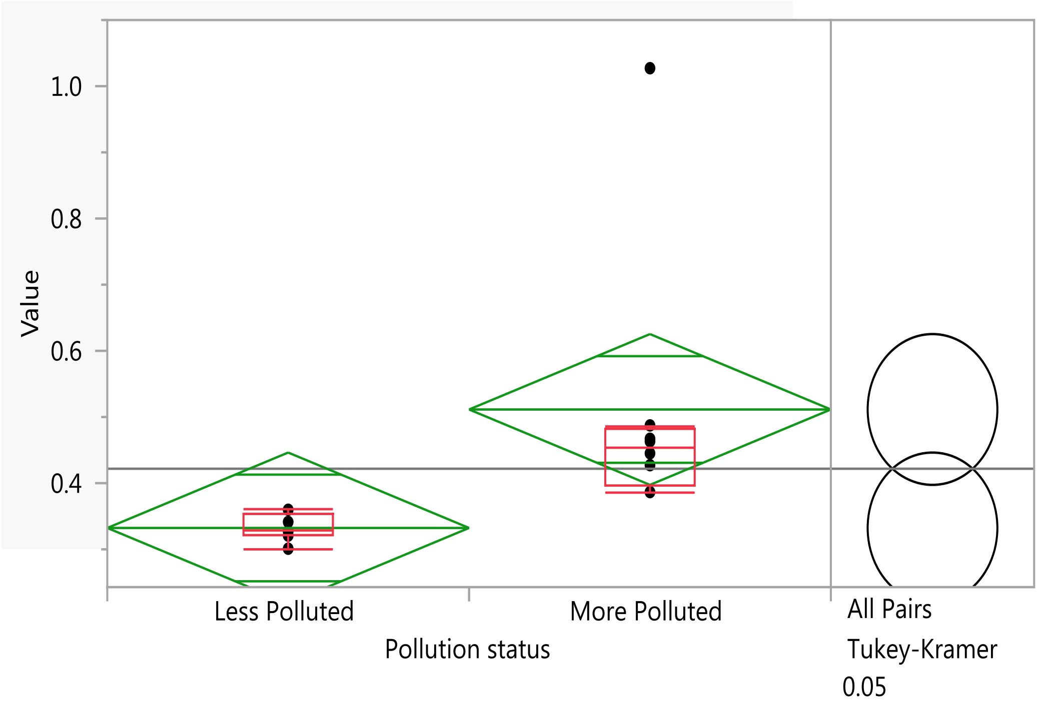


Figure 4.29: Oneway analysis of value by pollution status wise for Fe in butter

Table 4.37: Analysis of variance and ordered differences report by pollution status wise for Fe in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.12819622	0.128196	5.6783	0.0319*
Error	14	0.31606939	0.022576		
C. Total	15	0.44426561			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.1 0.2 0.3
More Polluted	Less Polluted	0.1790225	0.0751272	0.0178907	0.3401543	0.0319*	

Oneway Analysis of Value By Type

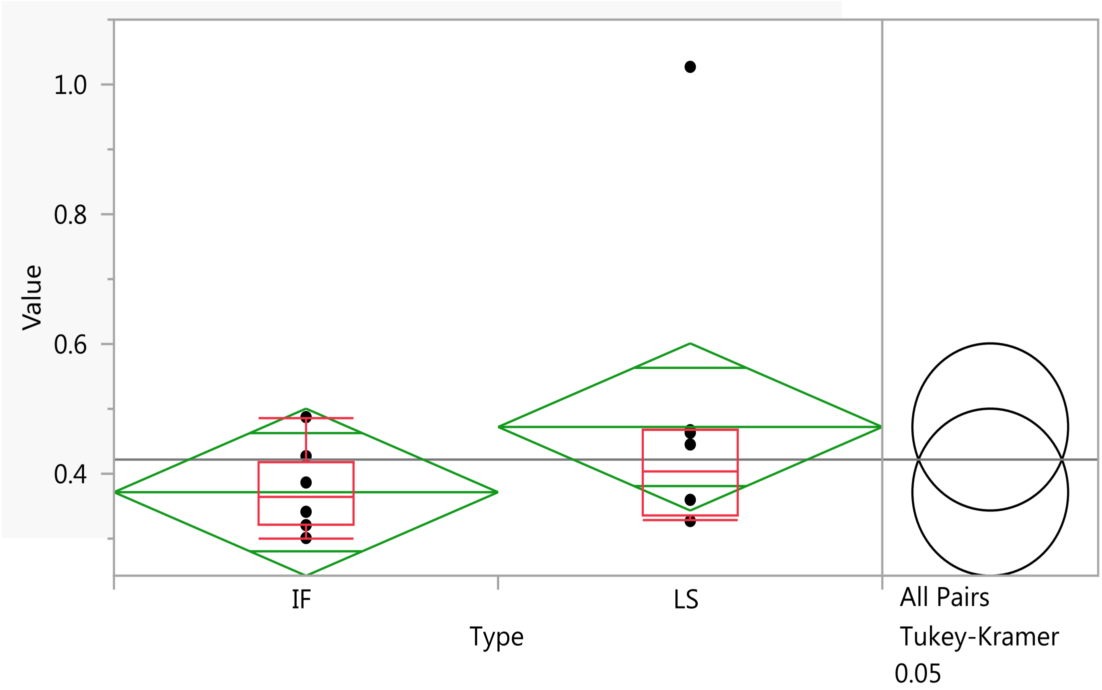


Figure 4.30: Oneway analysis of value by type wise for Fe in butter

Table 4.38: Analysis of variance and ordered differences report by type wise for Fe in butter

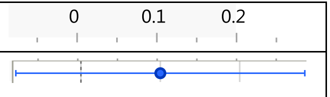
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.04043920	0.040439	1.4020	0.2561
Error	14	0.40382641	0.028845		
C. Total	15	0.44426561			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.1005475	0.0849187	-0.081585	0.2826800	0.2561	

Pooled t test

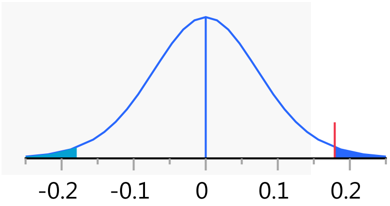


Figure 4.31: More & Less polluted

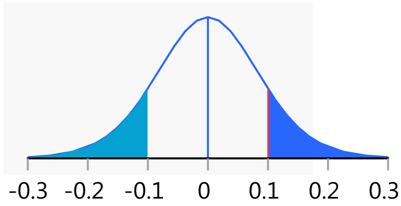


Figure 4.32: LS-IF

Summary of one way ANOVA for Fe

S.No.	Variable	α	p - value	Null Hypothesis
1	City Wise	0.05	0.4002	Significantly indifferent , Null Hypothesis can't be rejected
2	Pollution Status	0.05	0.0319	Significantly different , Null Hypothesis can be rejected
3	Types (IF & LS)	0.05	0.2561	Significantly indifferent , Null Hypothesis can't be rejected

Analysis of variance for Fe also shows the probability > 0. 4002 which is greater than α = 0.05 and from **Fig. 4.28** Tukey – Kramer test also shows that the circles are sharing almost same area, which proves that the mean concentration of heavy metal s in all the cities are significantly indifferent , the comparison has been made between more polluted and less polluted area with the help of one way analysis. This analysis shows (**Fig. 4.29**) that there is a significant difference between both of them, as p value > 0. 0319 . The p value for ANOVA test is 0.2561 which also shows that the concentration of both the places IF and LS (**Fig. 4.30**) are significantly indifferent.

4.7.6 Concentrations of Zinc (Zn)

Fit Group

Oneway Analysis of Value By City

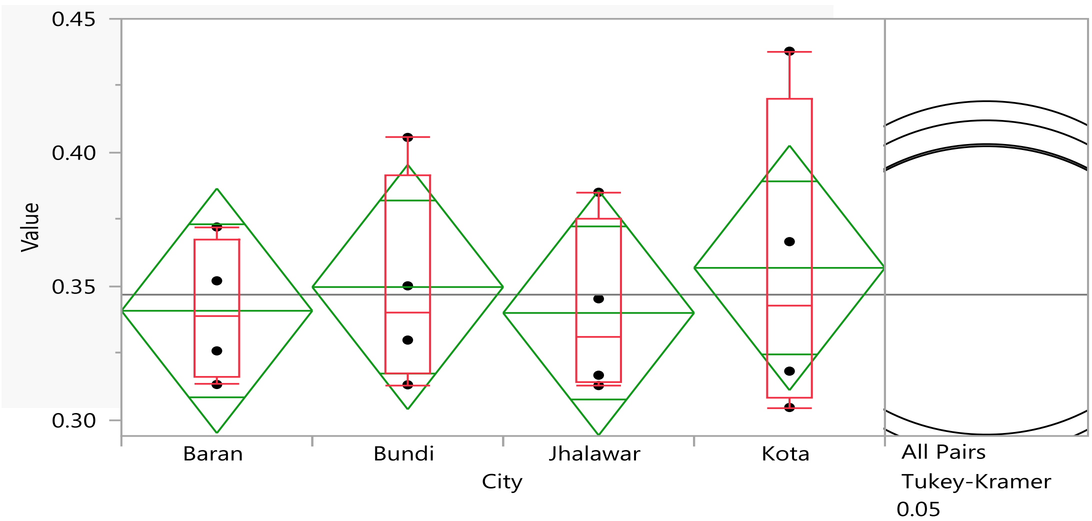


Figure 4.33: Oneway analysis of value by city wise for Zn in butter

Table 4.39: Analysis of variance and ordered differences report by city wise for Zn in butter

Analysis of Variance

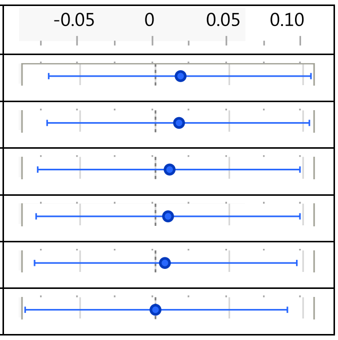
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00076628	0.000255	0.1448	0.9310
Error	12	0.02116426	0.001764		
C. Total	15	0.02193054			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Kota	Jhalawar	0.0168550	0.0296959	-0.071306	0.1050162	0.9398
Kota	Baran	0.0160450	0.0296959	-0.072116	0.1042062	0.9474
Bundi	Jhalawar	0.0096900	0.0296959	-0.078471	0.0978512	0.9874
Bundi	Baran	0.0088800	0.0296959	-0.079281	0.0970412	0.9902
Kota	Bundi	0.0071650	0.0296959	-0.080996	0.0953262	0.9948
Baran	Jhalawar	0.0008100	0.0296959	-0.087351	0.0889712	1.0000



Oneway Analysis of Value By Pollution status

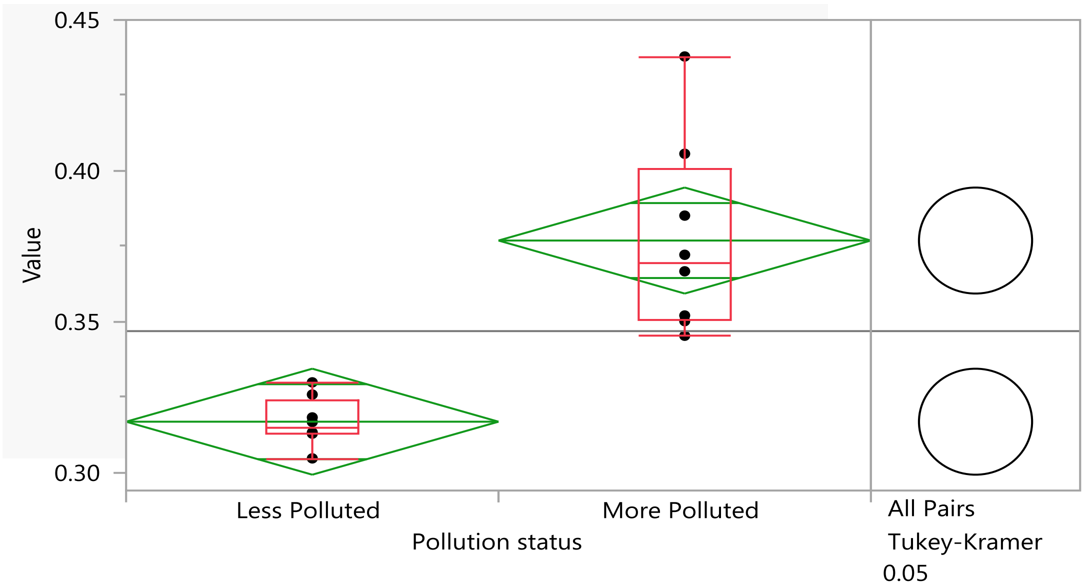


Figure 4.34: Oneway analysis of value by pollution status wise for Zn in butter

Table 4.40: Analysis of variance and ordered differences report by pollution status wise for Zn in butter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.01441801	0.014418	26.8687	0.0001*
Error	14	0.00751253	0.000537		
C. Total	15	0.02193054			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.02 0.04 0.06 0.08
More Polluted	Less Polluted	0.0600375	0.0115824	0.0351957	0.0848793	0.0001*	

Oneway Analysis of Value By Type

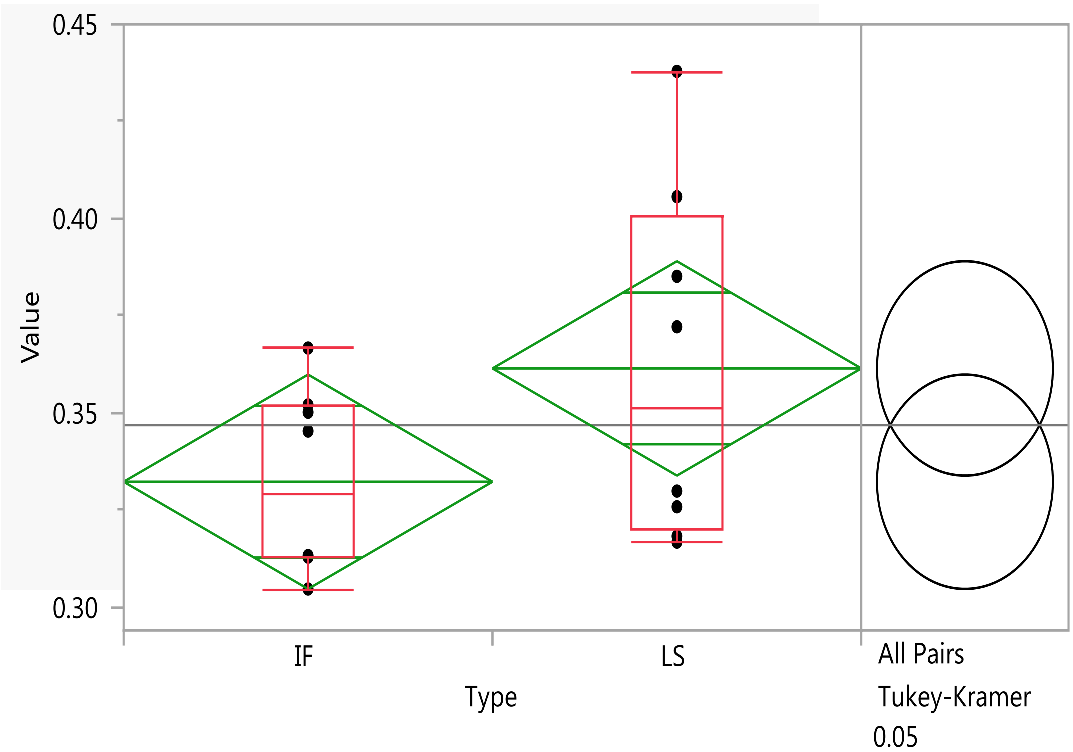


Figure 4.35: Oneway analysis of value by type wise for Zn in butter

Table 4.41: Analysis of variance and ordered differences report by type wise for Zn in butter

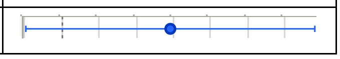
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00340297	0.003403	2.5714	0.1311
Error	14	0.01852757	0.001323		
C. Total	15	0.02193054			

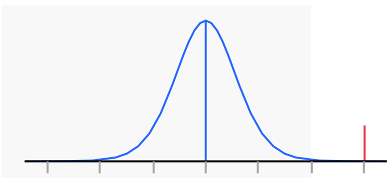
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.02 0.04 0.06
LS	IF	0.0291675	0.0181893	-0.009845	0.0681796	0.1311	

Pooled t test



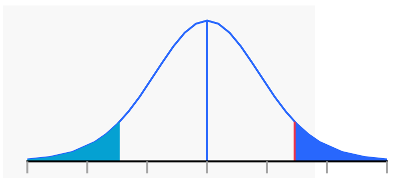


Figure 4.36: More & Less polluted

Figure 4.37: LS-IF

Summary of one way ANOVA for Zn

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.9310	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0001	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.1311	Significantly indifferent , Null Hypothesis can't be rejected

Fig.4.33 indicates that the Analysis of variance for Zn shows the probability > 0.9310 which is greater than α = 0.05 and from **Fig. 4.33** Tukey – Kramer test also shows that the circles are sharing almost same area, which proves that the mean concentration of heavy metals in all the cities are significantly indifferent. The analysis of variance in **Fig. 4.34** shows a significant difference between more and less polluted areas, as a p value > 0.0001 and the circles does not share the same place.

From **Fig. 4.35** one way ANOVA for analysis of value by type IF and LS shows the probability > 0.1311 which indicate that the concentration of Zn are significantly indifferent. Tukey – Kramer HSD test also support the data.

4.8 Correlation Coefficient

The results of correlation analysis between heavy metals for butter are given in **Table 4.42**

Table 4.42 : Correlation coefficients among mean concentration values of metals in butter samples

	Pb	**Cd**	**Al**	**As**	**Fe**	**Zn**
Pb	1					
Cd	0.97231	1				
Al	0.854364	0.900974	1			
As	0.866132	0.845472	0.726593	1		
Fe	0.784935	0.844639	0.981244	0.650819	1	
Zn	0.903121	0.918738	0.885864	0.893588	0.837885	1

Table 4.42 clearly indicate that Pearson's coefficient (r) has all positive values. All metals have correlations that are larger than 0.5, indicating a significant correlation between them. A strong correlation is found between Cd-Pb, Pb-Zn, Cd-Al, Cd-Zn and Al-Fe which is above 0.9. On the basis of these results, it can be concluded that there are a number of common factors that are responsible for the heavy metal contamination in the butter samples.

4.9 Conclusion

From the study of butter samples of Kota, Baran , Bundi and Jhalawar districts, it is concluded that among all four districts maximum concentration levels of heavy metals are found in Kota. Between local shops and individual farms, samples collected from local shops are more contaminated. It might be due to the transportation or container used by the local shop keeper.

For statistical evidence, One way ANOVA and Tukey- Kramer HSD were applied on all metals. Among these variables two of them, city wise and type wise, the sum of means were statistically indifferent, Whereas for pollution status it was significantly different. Tukey- Kramer also supports the data.

From the observations of effect of packaging with time, it is concluded that heavy metal concentration has gradually been increased with time.

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CHAPTER - V

ASSESSMENT OF HEAVY METALS IN CHEESE : INSTRUMENTAL AND STATISTICAL ANALYSIS

This Chapter is divided into four major sections i.e Introduction, Instrumental analysis, Statistical analysis and Conclusion. Instrumental analysis has been done by AAS, JMP software is used for statistical analysis.

5.1 Introduction

Cheese is a versatile dairy product made from milk. It's production involves coagulating milk, separating the curd (solid) from whey (liquid) and then processing and aging the curd.

The three main components are milk, a coagulant and bacterial cultures.

5.2 Manufacturing Process of Cheese

Cheese is made mostly from milk of cows, buffalo, sheep, goat or a blend of these milks. The flow chart of manufacturing process of cheese is given in **Fig. 5.1** [1,2].

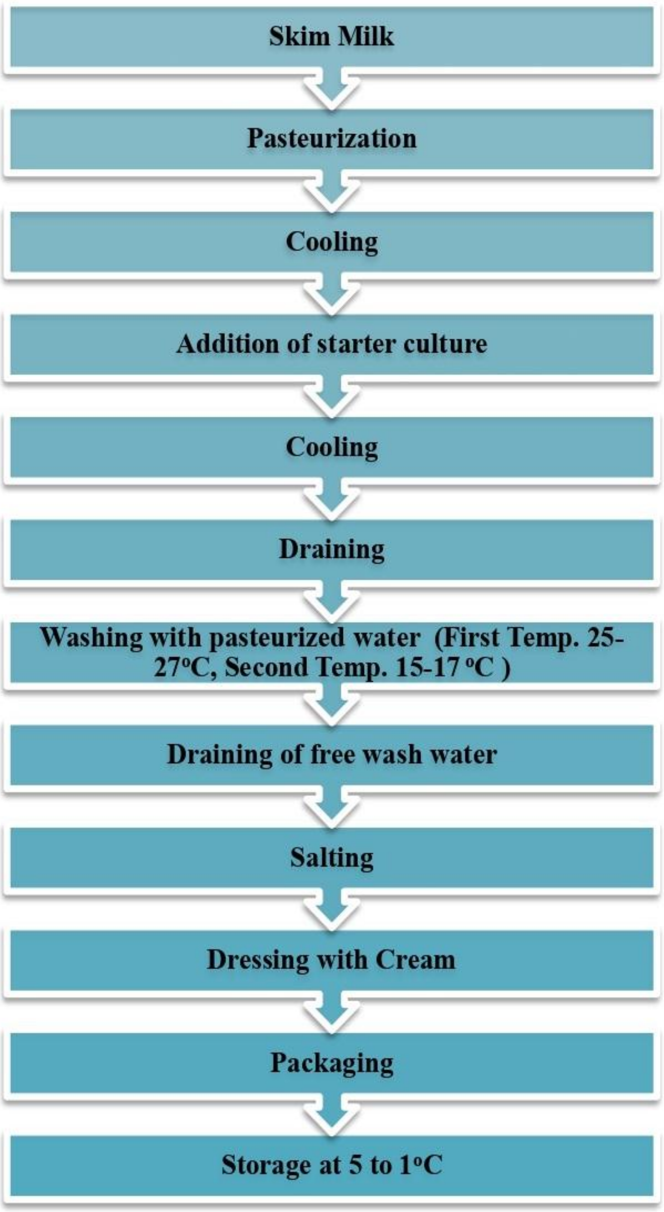


Figure 5.1: Flow Chart of manufacturing Cheese

5.3 Types of Cheese

A number of factors affect the variety of cheese, including the surrounding environment, types and species of milk-producing animals and the production techniques used etc [3-5].

5.3.1 Cottage Cheese

Cottage cheese commonly named as paneer, is mild in flavour and creamy in texture. This cheese is highly nutritious, protein rich and low in fat. This is an excellent source of some necessary nutrients like calcium, vitamin b12 and Selenium.

5.3.2 Cheddar Cheese

A mild cheese made from curdled cow's milk. For Cheddaring process whey is removed. In this process moisture content is reduced which allows the acidity to come into it.

5.3.3 Feta Cheese

Feta cheese is prepared from 100% sheep's milk and 30% goat's milk. It is dipped in to brine for several days to make it's taste more rich. It is available in the market in the plastic containers filled with brine. Feta cheese does not melt quickly.

5.3.4 Mozzarella Cheese

Mozzarella Cheese is a common dairy product and traditional staple of Italy made up from buffalo and cow's milk. It has unique smooth texture and for acidic flavour brine is used.

5.3.5 Parmesan Cheese

This type of cheese is rich and nutty in flavour. This is a hard and has granular texture. It is also known as king of cheese.

5.3.6 Swiss Cheese

Swiss cheese is made up of wisconsin milk of cows who feed on grass. It has a distinct appearance having holes on it. These holes are air pockets which appears when cheese release water.

5.3.7 Gouda Cheese

The oldest dutch cheese made up of cow's milk which is rich in vitamin K . This yellow colour cheese is soft and creamy and easily served in slices.

5.3.8 American Cheese

This cheese is commonly used in America and available in slices. It has mild flavour, creamy texture and having a smooth consistency which is achieved by the addition of emulsifier.

5.3.9 Burrata Cheese

Burrata is also an Italian cheese, which is prepared by blending mozzarella with cream. It has a delicate flavour, smooth texture with little sweetness.

5.3.10 Chhena Cheese

Chhena is made up from cow and buffalo milk and used in India for making sweets. It has soft and crumbled texture. It is made up from adding lemon juice and vinegar in curdled milk.

Quality of these dairy products especially in more polluted areas easily affected by heavy metal contamination through various factors like soil, water, manufacturing, packaging, storage etc. and cause a serious risk to humans health [6,7]. Various heavy metals like Lead, Cadmium, Copper, Zinc etc. have been observed in dairy products [8,9]. When consumptions of these metals are higher than recommended values than they shows hazardous effect on human health. Lead and cadmium are carcinogenic elements which causes cardiovascular diseases and also shows negative impact on blood, skeletal, nervous system [10-14].

This chapter includes the determination of heavy metal concentration in cheese and to assess the health risk to consumers. Our main study is focused on the Regular Cheese. Effect of extra added flavours, processing and packaging have also been studied in this chapter.

The investigation of heavy metals in Cheese in various regions of Kota division of Rajasthan is covered in this chapter. Samples were collected from Kota, Baran, Bundi, and Jhalawar zone. Each zone, has two subzones i.e., Less polluted and More polluted (industrial area). The goal of the current study was to evaluate six specific metals like Pb, Cd, Al, As, Fe, and Zn in 80 Cheese samples that were collected from various local shops and individual farms.

5.4 Collection, Digestion & Analysis of Cheese

Cheese samples were collected from the above given selected zones.
5 samples of cheese were collected from each subzone in PTFE containers. Microwave digestion method was used to digest all cheese samples. In this method 1 gm of each sample was digested with 4 ml of 65% HNO_3 and 2 ml of 30% H_2O_2 in microwave oven using the condition as per given in table 2.1 in chapter 2. Resulting solution was transferred into 10ml volumetric flask and diluted with deionised water. After preparing the samples, elemental analysis has been done by AAS.

The detailed instrumental analysis results are given below :

5.4.1 KOTA DISTRICT

5.4.1.1 More Polluted Area (Ranpur)

(a) Individual Farms

Table 5.1 . Concentration (mg/L) of metals (Mean ± SD) in Cheese samples of KRIF

Samples	Pb	Cd	Al	As	Fe	Zn
KRIF - C1	0.0124	0.0112	0.0446	0.0067	0.3981	0.4947
KRIF - C2	0.0279	0.0158	0.0398	0.0054	0.7914	0.3561
KRIF - C3	0.0453	0.0069	0.0216	0.0043	0.5245	0.4108
KRIF - C4	0.0217	0.0097	0.0578	0.0047	0.5951	0.3357
KRIF - C5	0.0192	0.0054	0.0495	0.0069	0.4824	0.3272
Minimum	0.0124	0.0054	0.0216	0.0043	0.3981	0.3272
Maximum	0.0453	0.0158	0.0578	0.0069	0.7914	0.4947
Mean	**0.0253**	**0.0098**	**0.0427**	**0.0056**	**0.5583**	**0.3849**
SD	0.0112	0.0036	0.0121	0.0010	0.1329	0.0621
Variance	0.0001	-	0.0001	1E-06	0.0177	0.0039

KRIF : Kota Ranpur Individual Farm, SD : Standard deviation

(b) Local Shops

Table 5.2. Concentration (mg/L) of metals (Mean±SD) in Cheese sample of KRLS

Samples/ Heavy metals	Pb	Cd	Al	As	Fe	Zn
KRLS - C1	0.0372	0.0124	0.0599	0.0061	1.2607	0.4884
KRLS - C2	0.0389	0.0154	0.0811	0.0009	0.7221	0.5518
KRLS - C3	0.0496	0.0179	0.1954	0.0084	0.9547	0.6947
KRLS - C4	0.0364	0.0123	0.1167	0.0092	0.4984	0.3579
KRLS - C5	0.0297	0.0125	0.0887	0.0089	1.0179	0.4999
Minimum	0.0297	0.0123	0.0599	0.0009	0.4984	0.3579
Maximum	0.0496	0.0179	0.1954	0.0092	1.2607	0.6947
Mean	**0.0384**	**0.0141**	**0.1084**	**0.0067**	**0.8908**	**0.5185**
SD	0.0064	0.0022	0.0472	0.0031	0.2606	0.1088
Variance	-	-	0.0022	1E-05	0.0679	0.0118

KRLS : Kota Ranpur Local Shops, SD : Standard deviation

Table 5.1 and **5.2** provide the heavy metal concentrations in individual farms and local shops of more polluted area of Kota region. These areas are situated in the proximity of industries where emission of waste disposal in higher.

Table 5.1 indicate the results of IF i.e., minimum concentration of Pb, Cd, Al, As, Fe and Zn are 0.0124, 0.0054, 0.0216, 0.0043, 0.3981 and 0.3272 and maximum concentrations are 0.0453, 0.0158, 0.0578, 0.0069, 0.7914 and 0.4947 respectively. The mean concentration of Pb, Cd, Al, As, Fe and Zn for both location are 0.0253, 0.0098, 0.0427, 0.0056, 0.5583 and 0.3849 respectively. While **Table 5.2** indicate the results of LS of more polluted area, in which minimum concentrations of Pb, Cd, Al, As, Fe and Zn are 0.0297, 0.0123, 0.0599, 0.0009, 0.4984 and 0.3579 and maximum concentrations are 0.0496, 0.0179, 0.1954, 0.0092, 1.2607 and 0.6947 and the mean

concentrations are found to be 0.0384, 0.0141, 0.1084, 0.0067, 0.8908 and 0.5185 respectively. The amount of heavy metal in both the places expressed in mg/L.
From the results, it can be seen that the order of metal ion concentration is Fe > Zn > Al > Pb > Cd > As, in both locations of more polluted area.

All metal concentrations are relatively higher in the samples of local shops as compare to individual farms . The data in both the tables indicate the moderate levels of contamination but their values are exceeded the permissible limits set by regulatory bodies like WHO, FSSAI etc.

5.4.1.2 Less Polluted Area (Kaithoon)

(a) Individual Farms

Table 5.3. Concentration (mg/L) of metals (Mean±SD) in Cheese sample of KKIF

Samples	Pb	Cd	Al	As	Fe	Zn
KKIF - C1	0.0215	0.0025	0.0321	0.0033	0.2651	0.1782
KKIF - C2	0.0174	0.0006	0.0111	0.0012	0.2546	0.4323
KKIF - C3	0.0141	0.0012	0.0247	0.0026	0.3611	0.3428
KKIF - C4	0.0243	0.0038	0.0071	BIR	0.4115	0.3541
KKIF - C5	0.0119	0.0022	0.0207	BIR	0.3431	0.2451
Minimum	0.0119	0.0006	0.0071	BIR	0.2546	0.1782
Maximum	0.0243	0.0038	0.0321	0.0033	0.4115	0.4323
Mean	**0.0178**	**0.0021**	**0.0191**	**0.0014**	**0.3271**	**0.3105**
SD	0.0046	0.0011	0.0091	0.0013	0.0594	0.0889
Variance	-	-	0.0001	2E-06	0.0035	0.0079

KKIF : Kota Kaithoon Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 5.4. Concentration (mg/L) of metals (Mean±SD) in Cheese sample of KKLS

Samples	Pb	Cd	Al	As	Fe	Zn
KKLS - C1	0.0261	0.0024	0.0236	0.0032	0.2135	0.2182
KKLS - C2	0.0197	0.0028	0.0245	0.0017	0.3781	0.4323
KKLS - C3	0.0109	0.0032	0.0141	BIR	0.6201	0.3428
KKLS - C4	0.0217	0.0025	0.0464	0.0024	0.2319	0.3741
KKLS - C5	0.0222	0.0036	0.0198	0.0028	0.3323	0.2451
Minimum	0.0109	0.0024	0.0141	BIR	0.2135	0.2182
Maximum	0.0261	0.0036	0.0464	0.0032	0.6201	0.4323
Mean	**0.0201**	**0.0029**	**0.0257**	**0.0020**	**0.3552**	**0.3225**
SD	0.0051	0.0004	0.0110	0.0011	0.1459	0.0800
Variance	-	-	0.0001	1E-06	0.0213	0.0064

KKLS : Kota Kaithoon Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Kaithoon is referred to as less polluted in comparison of Ranpur. **Table 5.3** and **5.4** provides the heavy metal concentrations in individual farms and local shops of less polluted area, Kaithoon of Kota region.

Table 5.3 indicate the concentration of Pb, Cd, Al, As, Fe and Zn which ranges 0.0119 - 0.0243, 0.0006 - 0.0038, 0.0071 - 0.0321, BIR - 0.0033, 0.2546 - 0.4115, 0.1782 - 0.4323 and mean concentration are 0.0178, 0.0021, 0.0191, 0.0014, 0.3271, 0.3105 respectively for IF. While mean concentration of Pb, Cd, Al, As, Fe and Zn of LS are found to be 0.0201, 0.0029, 0.0257, 0.0020, 0.3552, 0.3225 respectively. The amount of heavy metal in both the places expressed in mg/L.

The mean concentration of Pb, Cd, Al, As, Fe and Zn in individual farms as well as local shops are found to be in the order of Fe > Zn > Al > Pb > Cd > As .Arsenic is found to be below detection limit in most of the samples of IF and LS.

5.4.2 BARAN DISTRICT

5.4.2.1 More Polluted Area (Chhabra Motipura)

(a) Individual Farms

Table 5.5 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BCIF

Samples	Pb	Cd	Al	As	Fe	Zn
BCIF - C1	0.0349	0.0126	0.0293	0.0047	0.3333	0.2988
BCIF - C2	0.0383	0.0087	0.0216	0.0066	0.4388	0.3245
BCIF - C3	0.0244	0.0091	0.0478	0.0009	0.3567	0.4319
BCIF - C4	0.0048	0.0033	0.0362	0.0041	0.5806	0.4211
BCIF - C5	0.0152	0.0123	0.0255	0.0057	0.4532	0.3546
Minimum	0.0048	0.0033	0.0216	0.0009	0.3333	0.2988
Maximum	0.0383	0.0126	0.0478	0.0066	0.5806	0.4319
Mean	**0.0235**	**0.0092**	**0.0321**	**0.0044**	**0.4325**	**0.3662**
SD	0.0124	0.0034	0.0092	0.0019	0.0872	0.0524
Variance	0.0002	-	0.0001	4E-06	0.0076	0.0027

BCIF : Baran Chhabra Individual Farm, SD : Standard deviation

(b) Local Shops

Table 5.6 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BCLS

Samples	Pb	Cd	Al	As	Fe	Zn
BCLS - C1	0.0235	0.0054	0.0317	0.0033	0.3214	0.3211
BCLS - C2	0.0434	0.0137	0.0283	0.0004	0.5846	0.5205
BCLS - C3	0.0235	0.0179	0.0497	0.0098	0.4216	0.4245
BCLS - C4	0.0196	0.0021	0.0695	0.0066	0.6879	0.3209
BCLS - C5	0.0366	0.0123	0.0237	0.0034	0.3231	0.4325
Minimum	0.0196	0.0021	0.0237	0.0004	0.3214	0.3209
Maximum	0.0434	0.0179	0.0695	0.0098	0.6879	0.5205
Mean	**0.0293**	**0.0103**	**0.0406**	**0.0047**	**0.4677**	**0.4039**
SD	0.0091	0.0057	0.0169	0.0032	0.1460	0.0756
Variance	0.0001	-	0.0003	1E-05	0.0213	0.0057

BCLS : Baran Chhabra Local Shops, SD : Standard deviation

Table 5.5 and **Table 5.6** provide an overview on heavy metal concentration for more polluted area, Chhabra Motipura of Baran district. The mean concentration pattern shows the similar trend as Kota. It can be seen from the tables that for individual farms mean concentration of Pb is 0.0235 where as for local shops it is 0.0293 mg/L. Like wise for Cd in BCIF it is 0.0092 and for BCLS it is 0.0103 mg/L. On comparing the results of both individual farms and local shops of chhabra Motipura, it is found that there is a slight increase in the mean concentration of local shops.

5.4.2.2 Less Polluted Area (Mangrol)

(a) Individual Farms

Table 5.7 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BMIF

Samples	Pb	Cd	Al	As	Fe	Zn
BMIF - C1	0.0134	0.0044	0.0264	BIR	0.3236	0.1333
BMIF - C2	0.0244	0.0007	0.0132	BIR	0.228	0.3383
BMIF - C3	0.0134	BIR	0.0168	0.0007	0.3519	0.2454
BMIF - C4	0.0115	0.0025	0.0184	BIR	0.3543	0.4039
BMIF - C5	0.0187	0.0023	0.0119	0.0013	0.2231	0.4232
Minimum	0.0115	BIR	0.0119	BIR	0.2231	0.1333
Maximum	0.0244	0.0044	0.0264	0.0013	0.3543	0.4232
Mean	**0.0163**	**0.0020**	**0.0173**	**0.0004**	**0.2962**	**0.3088**
SD	0.0047	0.0015	0.0051	0.0005	0.0587	0.1075
Variance	-	-	-	3E-07	0.0034	0.0116

BMIF : Baran Mangrol Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 5.8 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BMLS

Samples	Pb	Cd	Al	As	Fe	Zn
BMLS - C1	0.0253	0.0028	0.0318	BIR	0.3421	0.2224
BMLS - C2	0.0185	0.0029	0.0123	BIR	0.2341	0.3012
BMLS - C3	0.0132	0.0023	0.0232	BIR	0.4203	0.2943
BMLS - C4	0.0113	0.0019	0.0278	0.0013	0.3328	0.3223
BMLS - C5	0.0235	0.0031	0.0146	0.0014	0.2292	0.4873
Minimum	0.0113	0.0019	0.0123	BIR	0.2292	0.2224
Maximum	0.0253	0.0031	0.0318	0.0014	0.4203	0.4873
Mean	**0.0184**	**0.0026**	**0.0219**	**0.0005**	**0.3117**	**0.3255**
SD	0.0055	0.0004	0.0075	0.0007	0.0721	0.0876
Variance	-	-	0.0001	4E-07	0.0052	0.0077

BMLS : Baran Mangrol Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The result of heavy metals concentration of 10 samples of individual farms and local shops of less polluted area, Mangrol of Baran district are presented in **Table 5.7** and **5.8**.

Results in **Table 5.7** and **5.8** revealed that mean concentration of Pb, Cd, Al, As, Fe, Zn in IF are 0.0163, 0.0020, 0.0173, 0.0004, 0.2962, 0.3088 and in LS are 0.0184, 0.0026, 0.0219, 0.0005, 0.3117 and 0.3255. The amount of heavy metal in both the places expressed in mg/L. From the results it can be seen that metal ion concentration in IF and LS are found in the order of Zn > Fe > Al > Pb > Cd > As.

On comparing the values of mean concentration of individual farms of chhabra motipura and mangrol a significant difference is observed. A possible reason for this observed difference could be higher anthropogenic activities in chhabra motipura.

5.4.3 BUNDI DISTRICT

5.4.3.1 More Polluted Area (Lakheri)

(a) Individual Farms

Table 5.9 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BnLIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnLIF - C1	0.0319	0.0047	0.0218	0.0035	0.3334	0.3213
BnLIF - C2	0.0366	0.0088	0.0285	0.0005	0.4389	0.2206
BnLIF - C3	0.0049	0.0064	0.0295	0.0097	0.3565	0.4246
BnLIF - C4	0.0109	0.0046	0.0193	0.0067	0.3207	0.3211
BnLIF - C5	0.0149	0.0025	0.0238	0.0036	0.4535	0.4327
Minimum	0.0049	0.0025	0.0193	0.0005	0.3207	0.2206
Maximum	0.0366	0.0088	0.0295	0.0097	0.4535	0.4327
Mean	**0.0198**	**0.0054**	**0.0246**	**0.0048**	**0.3806**	**0.3441**
SD	0.0123	0.0021	0.0039	0.0031	0.0550	0.0783
Variance	0.0002	-	-	1E-05	0.0030	0.0061

BnLIF : Bundi Lakheri Individual Farm, SD : Standard deviation

(b) Local Shops

Table 5.10 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BnLLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnLLS - C1	0.0236	0.0057	0.0491	0.0043	0.3213	0.4987
BnLLS - C2	0.0233	0.0039	0.0213	0.0064	0.5842	0.3243
BnLLS - C3	0.0337	0.0078	0.0475	0.0081	0.6213	0.4316
BnLLS - C4	0.0198	0.0083	0.0458	0.0039	0.6876	0.3213
BnLLS - C5	0.0067	0.0123	0.0249	0.0053	0.3229	0.3542
Minimum	0.0067	0.0039	0.0213	0.0039	0.3213	0.3213
Maximum	0.0337	0.0123	0.0491	0.0081	0.6876	0.4987
Mean	**0.0214**	**0.0076**	**0.0377**	**0.0056**	**0.5075**	**0.3860**
SD	0.0087	0.0028	0.0120	0.0015	0.1549	0.0690
Variance	0.0001	-	0.0001	2E-06	0.0240	0.0048

BnLLS : Bundi Lakheri Local Shops, SD : Standard deviation

The results of IF and LS of more polluted area, Lakheri of Bundi district are presented in **Table 5.9** and **5.10** . **Table 5.9** shows the mean concentration of Pb, Cd, Al, As, Fe, Zn are 0.0198, 0.0054, 0.0246, 0.0048, 0.3806, 0.3441 mg/L respectively in IF while table 5.10 represent the mean concentration of Pb, Cd, Al, As, Fe, Zn are 0.0214, 0.0076, 0.0377, 0.0056, 0.5075, 0.3860 mg/L respectively in LS. From both locations metals concentrations are found in order of Fe > Zn > Al > Pb > Cd > As. Results in **Table 5.9** and **5.10** illustrate that all cheese samples except As contain the metal concentration above the recommended permissible limit.

5.4.3.2 Less Polluted Area (Kapren)

(a) Individual Farms

Table 5.11 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BnKIF

Samples	Pb	Cd	Al	As	Fe	Zn
BnKIF - C1	0.0103	0.0046	0.0263	BIR	0.3234	0.2193
BnKIF - C2	0.0115	0.0008	0.0231	0.0019	0.2281	0.3113
BnKIF - C3	0.0095	0.0035	0.0201	0.0071	0.3511	0.1844
BnKIF - C4	0.0111	0.0016	0.0183	BIR	0.3541	0.3294
BnKIF - C5	0.0108	0.0045	0.0117	0.0019	0.2234	0.4874
Minimum	0.0095	0.0008	0.0117	BIR	0.2234	0.1844
Maximum	0.0115	0.0046	0.0263	0.0071	0.3541	0.4874
Mean	**0.0106**	**0.0030**	**0.0199**	**0.0022**	**0.2960**	**0.3064**
SD	0.0007	0.0015	0.0049	0.0026	0.0584	0.1056
Variance	-	-	-	7E-06	0.0034	0.0112

BnKIF : Bundi Kapren Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 5.12 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of BnKLS

Samples	Pb	Cd	Al	As	Fe	Zn
BnKLS - C1	0.0251	0.0027	0.0315	0.0042	0.3423	0.2223
BnKLS - C2	0.0186	0.0065	0.0171	0.0003	0.2342	0.3013
BnKLS - C3	0.0033	0.0025	0.0134	0.0013	0.4204	0.2944
BnKLS - C4	0.0159	0.0017	0.0226	0.0041	0.3327	0.3124
BnKLS - C5	0.0276	0.0032	0.0197	0.0027	0.2293	0.4874
Minimum	0.0033	0.0017	0.0134	0.0003	0.2293	0.2223
Maximum	0.0276	0.0065	0.0315	0.0042	0.4204	0.4874
Mean	**0.0181**	**0.0033**	**0.0209**	**0.0025**	**0.3118**	**0.3236**
SD	0.0085	0.0017	0.0061	0.0015	0.0721	0.0878
Variance	0.0001	-	-	2E-06	0.0052	0.0077

BnKLS : Bundi Kapren Local Shops, SD : Standard deviation

The results of IF and LS of less polluted Kapren of Bundi district are presented in **Table 5.11** and **Table 5.12**. The minimum , maximum, mean concentration, SD and variance of Pb, Cd, Al, As, Fe and Zn in cheese samples of all location are given. Each table shows the concentration of metal in mg/L.

The mean concentration of Pb, Cd, Al, As, Fe, Zn are 0.0106, 0.0030, 0.0199, 0.0022, 0.2960, 0.3064 respectively for IF while the mean concentration of Pb, Cd, Al, As, Fe and Zn for LS are 0.0181, 0.0033, 0.0209, 0.0025, 0.3118 and 0.3236 respectively.

For all four places As concentration is found to be below permissible limit which reduces the threat to environment and human health. Though As concentration is very very low but if it accumulates then it will be alarming.

5.4.4 JHALAWAR DISTRICT

5.4.4.1 More Polluted Area (Jhalarapatan Kali Sind Thermal Power Plant)

(a) Individual Farms

Table 5.13 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of JJIF

Samples	Pb	Cd	Al	As	Fe	Zn
JJIF - C1	0.0329	0.0037	0.0471	BIR	0.3324	0.3203
JJIF - C2	0.0269	0.0098	0.0203	0.0064	0.4369	0.2216
JJIF - C3	0.0071	0.0044	0.0465	0.0041	0.3575	0.4236
JJIF - C4	0.0055	0.0016	0.0451	0.0029	0.3217	0.3201
JJIF - C5	0.0129	0.0026	0.0239	0.0043	0.4525	0.4317
Minimum	0.0055	0.0016	0.0203	BIR	0.3217	0.2216
Maximum	0.0329	0.0098	0.0471	0.0064	0.4525	0.4317
Mean	**0.0171**	**0.0044**	**0.0366**	**0.0035**	**0.3802**	**0.3435**
SD	0.0109	0.0029	0.0119	0.0021	0.0542	0.0776
Variance	0.0001	0.0000	0.0001	4E-06	0.0029	0.0060

JJIF : Jhalawar Jhalarapatan Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local Shops

Table 5.14 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of JJLS

Samples	Pb	Cd	Al	As	Fe	Zn
JJLS - C1	0.0232	0.0047	0.0212	BIR	0.3203	0.4986
JJLS - C2	0.0253	0.0032	0.0281	0.0007	0.4842	0.3241
JJLS - C3	0.0427	0.0068	0.0385	0.0092	0.6203	0.4306
JJLS - C4	0.0178	0.0062	0.0191	0.0057	0.6871	0.3203
JJLS - C5	0.0065	0.0093	0.0228	0.0032	0.3224	0.3532
Minimum	0.0065	0.0032	0.0191	BIR	0.3203	0.3203
Maximum	0.0427	0.0093	0.0385	0.0092	0.6871	0.4986
Mean	**0.0231**	**0.0060**	**0.0259**	**0.0038**	**0.4869**	**0.3854**
SD	0.0118	0.0021	0.0070	0.0034	0.1501	0.0691
Variance	0.0001	-	-	1E-05	0.0225	0.0048

JJLS : Jhalawar Jhalarapatan Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

The concentration of each heavy metal is measured in ppm. Mean concentration of Pb, Cd, Fe and Zn of JJLS are significantly higher than JJIF, while As in JJLS is little higher than JJIF. And a reverse pattern can be seen for Al as samples for JJIF contains more Al than JJLS.

5.4.4.2 Less Polluted Area (Aklera)

(a) Individual Farms

Table 5.15 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of JAIF

Samples	Pb	Cd	Al	As	Fe	Zn
JAIF - C1	0.0132	0.0013	0.0234	0.0011	0.3424	0.2224
JAIF - C2	0.0077	0.0054	0.0221	0.0015	0.2332	0.3012
JAIF - C3	0.0185	0.0011	0.0202	0.0061	0.4201	0.2442
JAIF - C4	0.0113	0.0012	0.0153	BIR	0.3321	0.2254
JAIF - C5	0.0178	BIR	0.0127	0.0011	0.2283	0.4871
Minimum	0.0077	BIR	0.0127	BIR	0.2283	0.2224
Maximum	0.0185	0.0054	0.0234	0.0061	0.4201	0.4871
Mean	**0.0137**	**0.0018**	**0.0187**	**0.0020**	**0.3112**	**0.2961**
SD	0.0040	0.0019	0.0041	0.0021	0.0724	0.0996

JAIF : Jhalawar Aklera Individual Farm, SD : Standard deviation, BIR : Beyond Instrumental Range

(b) Local shops

Table 5.16 . Concentration (mg/L) of metals (Mean±SD) in Cheese sample of JALS

Samples	Pb	Cd	Al	As	Fe	Zn
JALS - C1	0.0218	0.0051	0.0201	BIR	0.2641	0.2224
JALS - C2	0.0114	0.0025	0.0143	0.0017	0.2536	0.3012
JALS - C3	0.0101	0.0049	0.0178	0.0016	0.3616	0.2942
JALS - C4	0.0215	0.0022	0.0311	BIR	0.4105	0.3214
JALS - C5	0.0121	0.0033	0.0165	0.0015	0.3331	0.4871
Minimum	0.0101	0.0022	0.0143	BIR	0.2536	0.2224
Maximum	0.0218	0.0051	0.0311	0.0017	0.4105	0.4871
Mean	**0.0154**	**0.0036**	**0.0200**	**0.0010**	**0.3246**	**0.3253**
SD	0.0052	0.0012	0.0059	0.0008	0.0592	0.0876
Variance	-	-	--	6E-07	0.0035	0.0077

JALS : Jhalawar Aklera Local Shops, SD : Standard deviation, BIR : Beyond Instrumental Range

Table 5.15 and **5.16** presents the results of metal concentration in cheese samples of less polluted area, Aklera of Jhalawar district. Each row represents the heavy metal concentration of particular sample of that zone. Minimum, maximum, mean, SD and variance are also given for all zones.

The mean concentration of Pb, Cd, Al, As, Fe and Zn for IF are 0.0137, 0.0018, 0.0187, 0.0020, 0.3112, 0.2961 while for LS the mean concentration are 0.0154, 0.0036, 0.0200, 0.0010, 0.3246, 0.3253 respectively. The amount of heavy metal in both the places expressed in mg/L.

There is a difference in the mean concentration of individual farms and local shops of particular zone. The values are higher for local shops. This might be possible due to the container used and transportation of samples by local shopkeepers.

5.5 Graphical Representation

Column plots are drawn below for all six metals for all areas have shown below.

5.5.1 Pb Concentration

Table 5.17 : Lead concentration(mg/L) in cheese samples in four different areas of Kota region

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0253	0.0384	0.0178	0.0201
Baran	0.0235	0.0293	0.0163	0.0184
Bundi	0.0198	0.0214	0.0106	0.0181
Jhalawar	0.0171	0.0231	0.0137	0.0154

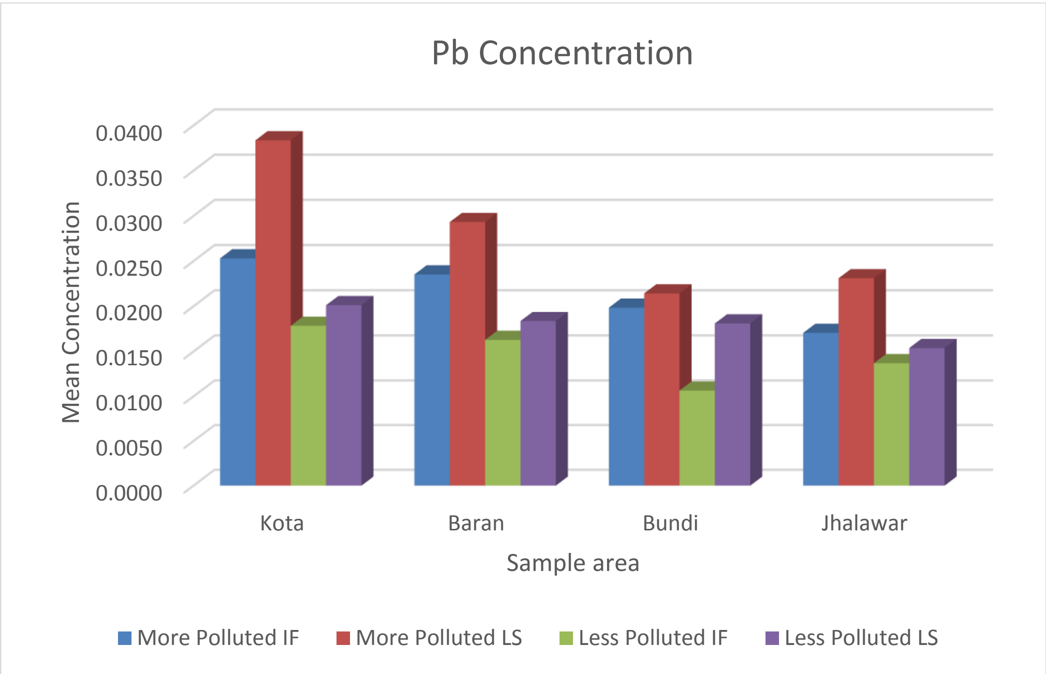


Figure 5.2 : Lead concentration(mg/L) in cheese samples in four different areas of Kota region

5.5.2 Cd Concentration

Table 5.18 : Cadmium concentration(mg/L) in cheese samples in four different areas of Kota region.

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0098	0.0141	0.0021	0.0029
Baran	0.0092	0.0103	0.0020	0.0026
Bundi	0.0054	0.0076	0.0030	0.0033
Jhalawar	0.0044	0.0060	0.0018	0.0036

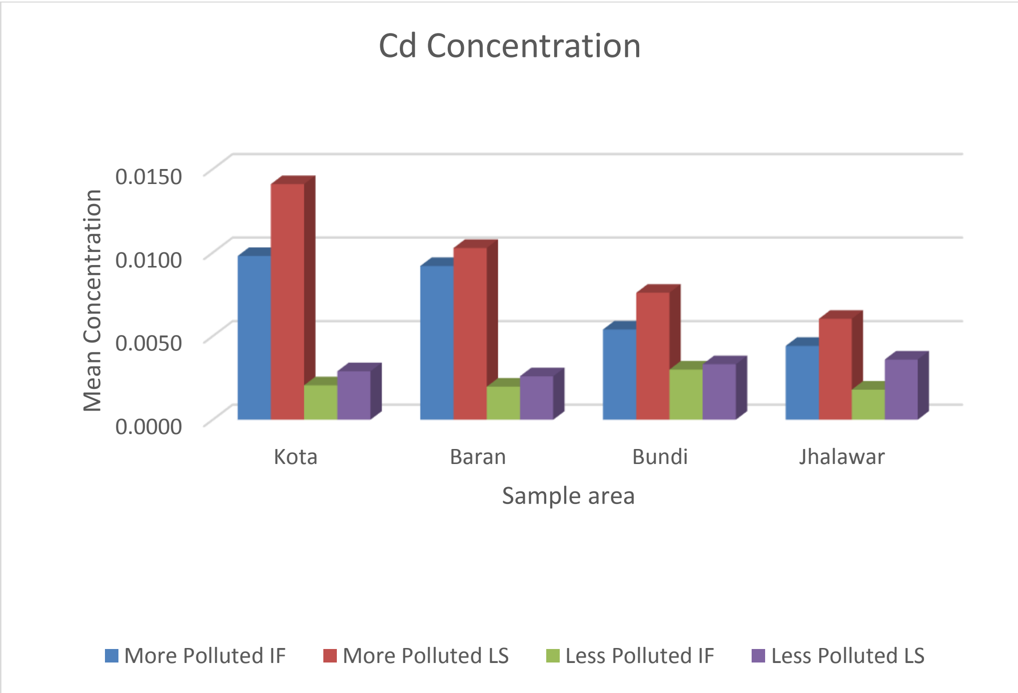


Figure 5.3 : Cadmium concentration(mg/L) in cheese samples in four different areas of Kota region.

5.5.3 Al Concentration

Table 5.19 : Aluminium concentration(mg/L) in cheese samples in four different areas of Kota region.

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0427	0.1084	0.0191	0.0257
Baran	0.0321	0.0406	0.0173	0.0219
Bundi	0.0246	0.0377	0.0199	0.0209
Jhalawar	0.0366	0.0259	0.0187	0.0200

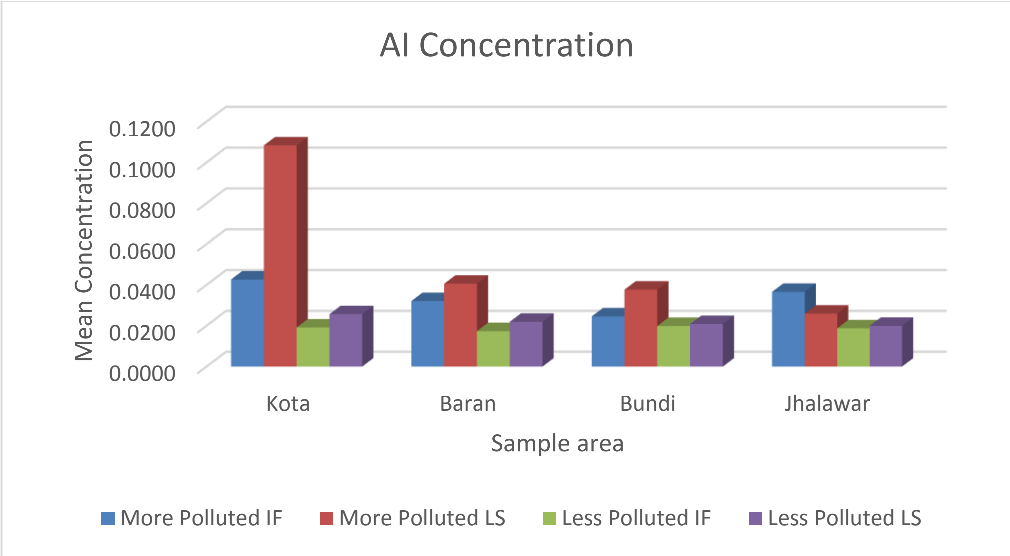


Figure 5.4 : Aluminium concentration(mg/L) in cheese samples in four different areas of Kota region

5.5.4 As Concentration

Table 5.20: Arsenic concentration(mg/L) in cheese samples in four different areas of Kota region.

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.0056	0.0067	0.0014	0.0020
Baran	0.0044	0.0047	0.0004	0.0005
Bundi	0.0048	0.0056	0.0022	0.0025
Jhalawar	0.0035	0.0038	0.0012	0.0012

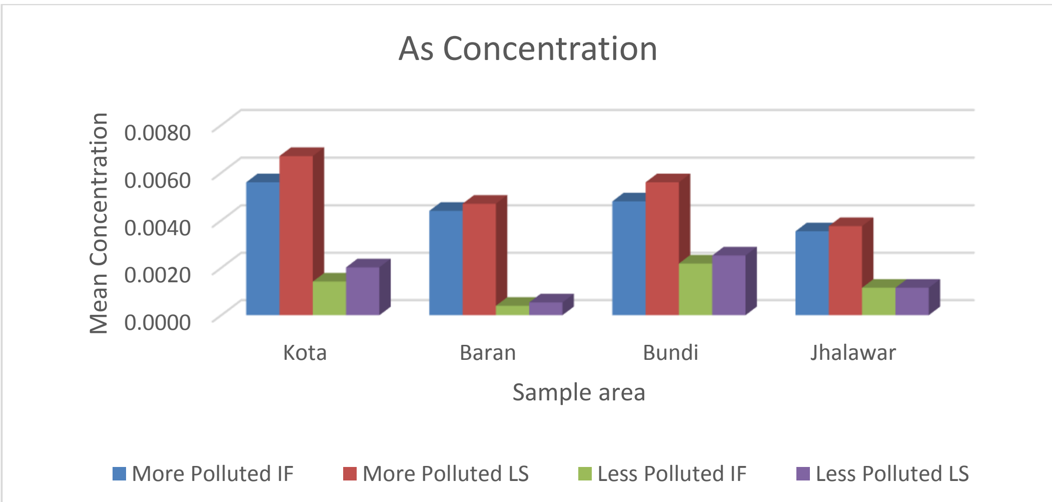


Figure 5.5 : Arsenic concentration(mg/L) in cheese samples in four different areas of Kota region

5.5.5 Fe Concentration

Table 5.21: Iron concentration(mg/L) in cheese samples in four different areas of Kota region.

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.5583	0.8908	0.3271	0.3552
Baran	0.4325	0.4677	0.2962	0.3117
Bundi	0.3806	0.5075	0.2960	0.3118
Jhalawar	0.3802	0.4869	0.3112	0.3246

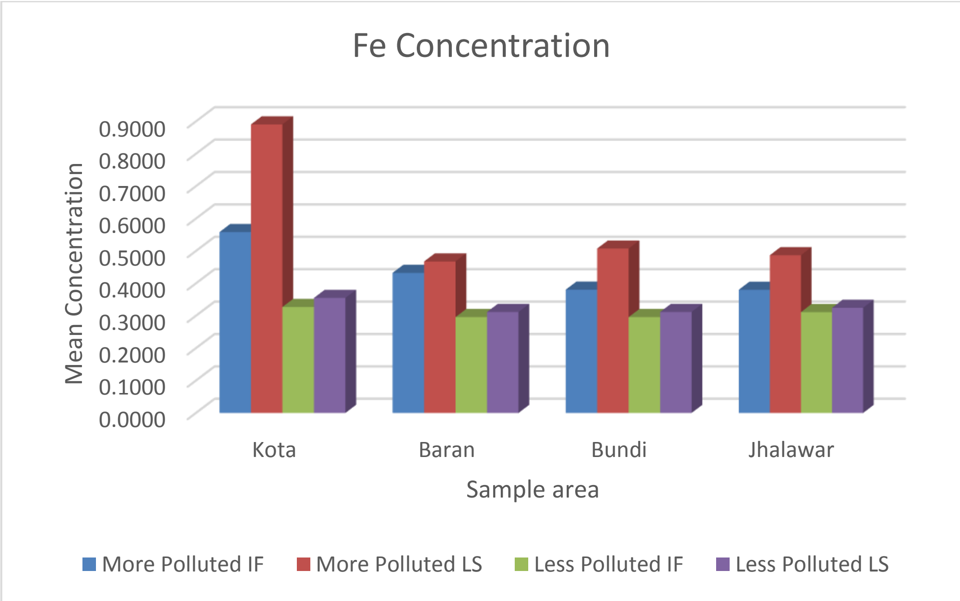


Figure 5.6 : Iron concentration(mg/L) in cheese samples in four different areas of Kota region.

5.5.6 Zn Concentration

Table 5.22: Zinc concentration(mg/L) in cheese samples in four different areas of Kota region.

Area	More Polluted		Less Polluted	
	IF	LS	IF	LS
Kota	0.3849	0.5185	0.3105	0.3225
Baran	0.3662	0.4039	0.3088	0.3255
Bundi	0.3441	0.3860	0.3064	0.3236
Jhalawar	0.3435	0.3854	0.2961	0.3253

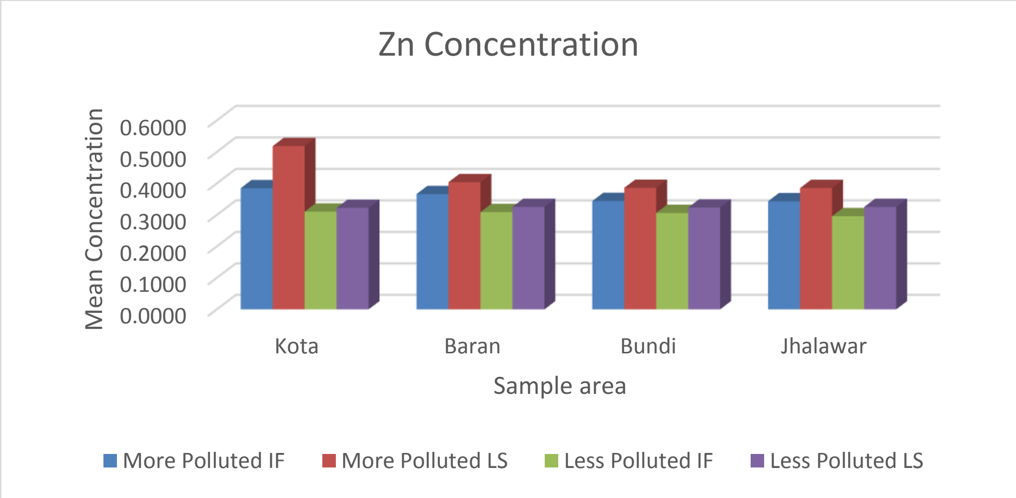


Figure 5.7 : Zinc concentration(mg/L) in cheese samples in four different areas of Kota region

It can be seen from the graph of Arsenic and Cadmium that in all four areas mean concentrations are below the detection limits.

The bar graphs are plotted to compare individual metal concentration with respect to sample areas. The x – axis represents the sampling sites and y-axis represents the metal concentration. The high peaks depict the high concentrations. For Pb the highest peak is found in LS of Kota. There is a noticeable upward trend in local shops of more polluted area for all metals.

The lowest lead concentration was recorded in IF situated in less polluted area of Bundi. Area wise order of cadmium concentration in Kota > Baran > Bundi > Jhalawar.

For Al Baran, Bundi, Jhalawar have almost similar peaks. As concentration shows very very low peak for less polluted area of Baran district.

Fe is higher for Kota and similar for other three areas. Peaks for Zn are almost similar though highest value 0.5185 mg/L was observed for local shops of more polluted area of Kota district.

The spike in more polluted areas of all district could be attributed to industrial discharge from the nearby manufacturing plants.

5.6 Effect of processing and packaging in different types of Cheese samples with Time

Cheese samples of different brands (brand 1, brand 2 and brand 3) were collected from different market locations and retail stores in Rajasthan . All samples were collected were analysed at three stages of validity, which were as follows:

Initial Time Point, T_i (At 0 day)

Mid Time Point, T_m (At 30th day)

Final Time Point, T_f (At 90th day)

Table 5.23 : Temporal analysis of heavy metal concentration of in different Cheese brands

Cheese Sample	Days	Pb	Cd	Al	As	Fe	Zn
Brand 1	0	0.0142	BIR	0.0163	BIR	0.2199	0.2142
	30	0.0167	0.0024	0.0196	0.0015	0.2541	0.2612
	90	0.0218	0.0024	0.0244	0.0023	0. 3457	0.3251
Brand 2	0	0.0138	0.0014	0.0177	BIR	0.2275	0.2432
	30	0.0174	0.0019	0.0213	0.0012	0.2751	0.2923
	90	0.0198	0.0022	0.0323	0.0022	0.3749	0.3164
Brand 3	0	0.0134	BIR	0.0162	BIR	0.2123	0.2211
	30	0.0155	0.0016	0.0186	0.0021	0.3432	0.3121
	90	0.0205	0.0022	0.0211	0.0036	0.3547	0.3283

Results summarized in **Table 5.23** shows the change in concentration of heavy metal (Pb, Cd, Al, As, Fe and Zn) in different brands of cheese (Brand 1, Brand 2 and Brand 3) with storage time i.e., 0 days, 30 days and 90 days.

Initial Time Point, Ti (At 0 day) : Table 5.23 revealed that the mean concentration of Pb, Cd, Al, As, Fe and Zn at 0 day are found 0.0142, BIR, 0.0163, BIR, 0.2199 and 0.2142 mg/L in brand 1, 0.0138, 0.0014, 0.0177, BIR, 0.2275 and 0.2432 mg/L in brand 2 and 0.0134, BIR, 0.0162, BIR, 0.2123 and 0.2211 mg/L in brand 3.

Mid Time Point, Tm (At 30th day) : From the **Table 5.23** illustrate that the mean concentration of Pb, Cd, Al, As, Fe and Zn at 30 days are found 0.0167, 0.0024, 0.0196, 0.0015, 0.2541 and 0.2612 mg/L in brand 1, 0.0174, 0.0019, 0.0213, 0.0012, 0.2751 and 0.2923 mg/L in brand 2 and 0.0155, 0.0016, 0.0186, 0.0021, 0.3432 and 0.3121 mg/L in brand 3 respectively.

Final Time Point, Tf (At 90th day): From the **Table 5.23** illustrate that the mean concentration of Pb, Cd, Al, As, Fe and Zn are found 0.0218, 0.0024, 0.0244, 0.0023, 0.3457 and 0.3251 mg/L in brand 1, 0.0198, 0.0022, 0.0323, 0.0022, 0.3749 and 0.3164 mg/L in brand 2 and 0.0205, 0.0022, 0.0211, 0.0036, 0.3547 and 0.3283 mg/L in brand 3 respectively.

The data of **Table 5.23** indicate the effect of storage on heavy metals concentration. We observed that there is significant difference in the concentration of metals occurs and it gradually increases during storage period from 0 to 30 to 90 days.

Concentration of As is not found in brand 1, 2 and 3 at 0 days but after 30th and 90th days there is significant changes observed in As concentration . Similarly in brand 1 and 2, Cd was also not detected but after 30th and 90th days the level of Cd changes. So migration of As and Cd in cheese sample might be possible due to packaging type as well as their storage.

It is also evident from the result that the concentration of metals in all samples of brand 1, 2 and 3 recorded at 0 day and 30th day, did not exceed the permissible limits but in case of Pb, Al and Fe at 90th days there is a slight increase in the concentration of metal than permissible limit. Cd, As and Zn are found below the permissible limit in all samples at all stages.

5.7 Statistical Analysis

5.7.1 Concentrations of Lead (Pb)

Fit Group

Oneway Analysis of Value By City

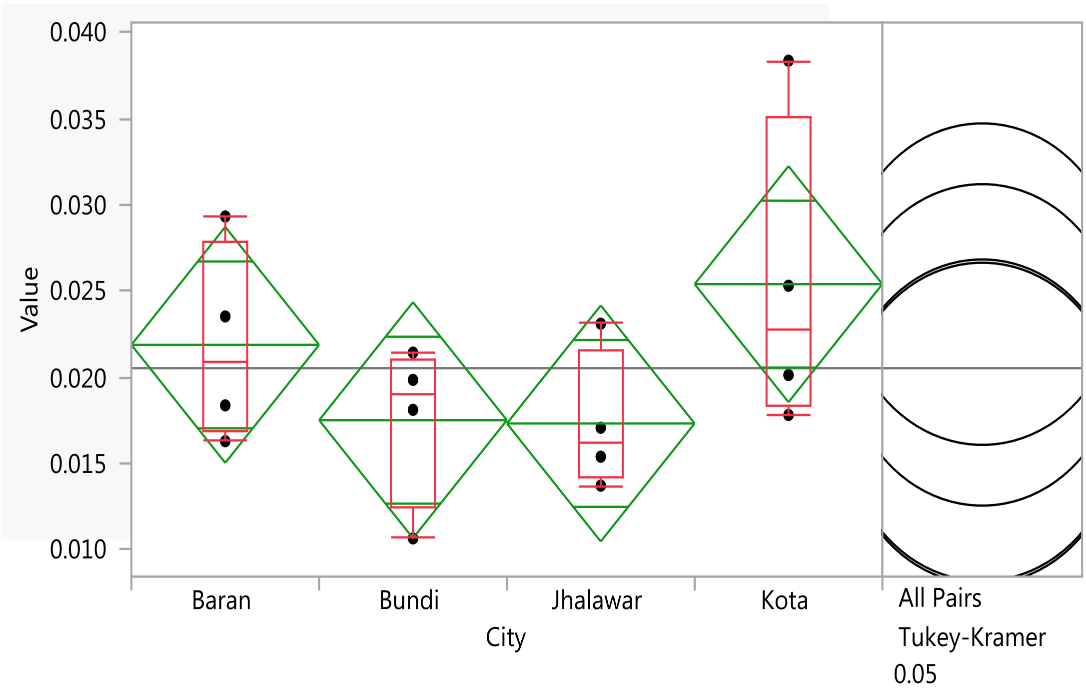


Figure 5.8 : Oneway analysis of value by city wise for Pb in cheese

Table 5.24: Analysis of variance and ordered differences report by city wise for Pb in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00018005	0.000060	1.5194	0.2598
Error	12	0.00047401	0.000040		
C. Total	15	0.00065406			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.01 0 0.01 0.02
Kota	Jhalawar	0.0080850	0.0044441	-0.005109	0.0212788	0.3116	
Kota	Bundi	0.0078950	0.0044441	-0.005299	0.0210888	0.3302	
Baran	Jhalawar	0.0045600	0.0044441	-0.008634	0.0177538	0.7380	
Baran	Bundi	0.0043700	0.0044441	-0.008824	0.0175638	0.7614	
Kota	Baran	0.0035250	0.0044441	-0.009669	0.0167188	0.8562	
Bundi	Jhalawar	0.0001900	0.0044441	-0.013004	0.0133838	1.0000	

Oneway Analysis of Value By Pollution status

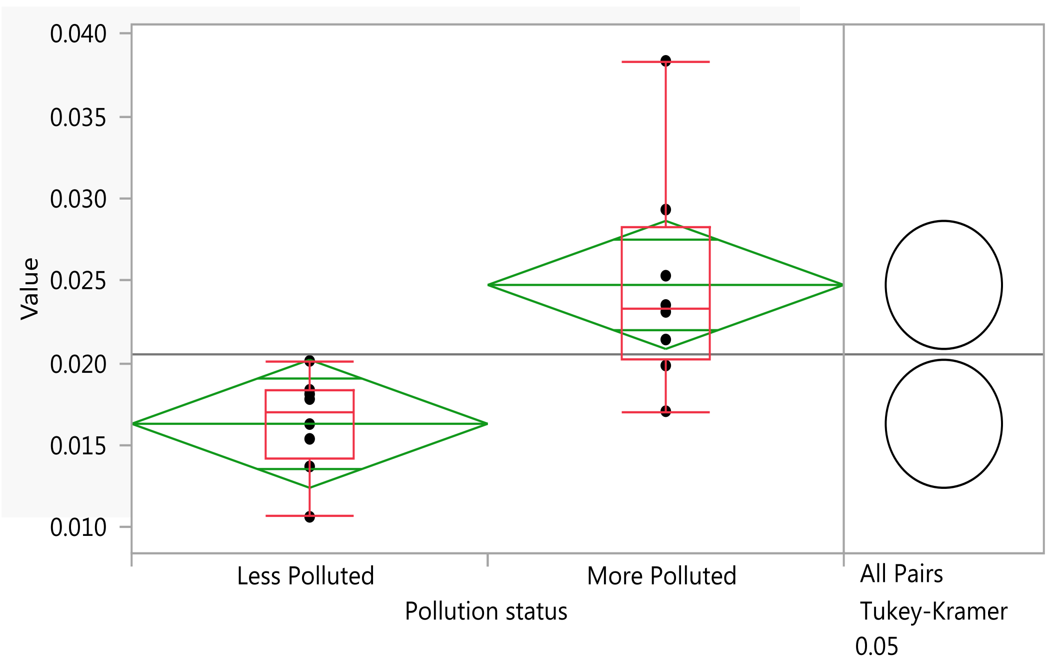


Figure 5.9 : Oneway analysis of value by pollution status wise for Pb in cheese

Table 5.25 Analysis of variance and ordered differences report by pollution status wise for Pb in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00028510	0.000285	10.8182	0.0054*
Error	14	0.00036896	0.000026		
C. Total	15	0.00065406			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.004 0.008 0.012
More Polluted	Less Polluted	0.0084425	0.0025668	0.0029372	0.0139478	0.0054*	

Oneway Analysis of Value By Type

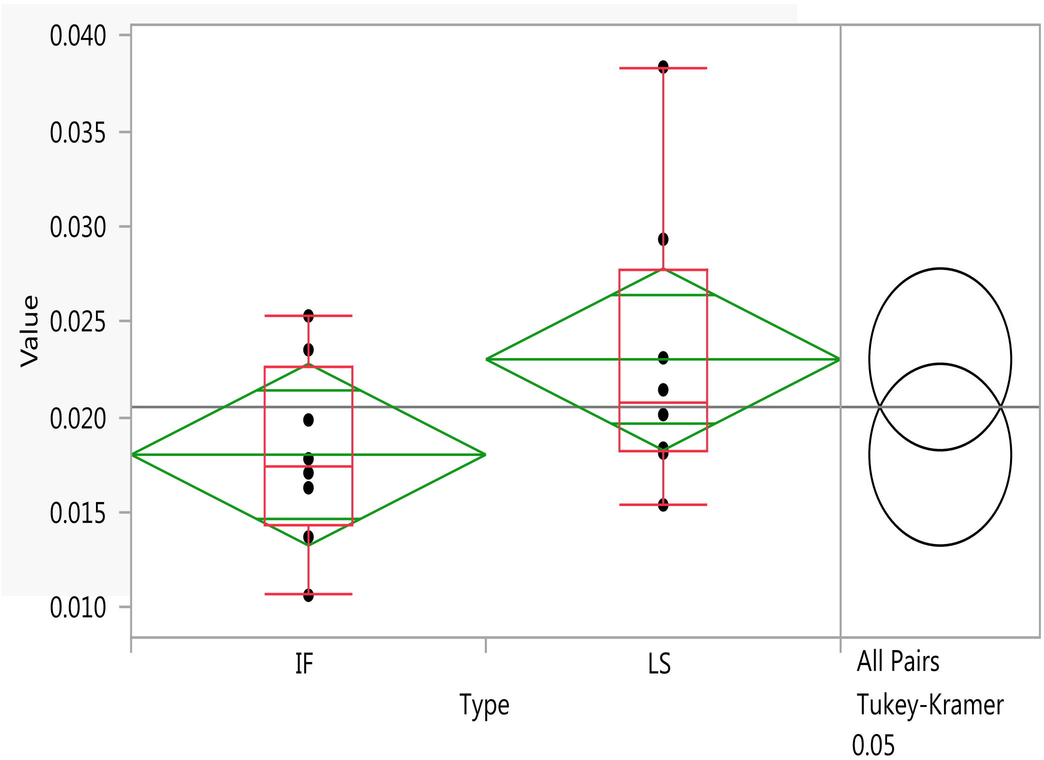


Figure 5.10 : Oneway analysis of value by type wise for Pb in cheese

Table 5.26 Analysis of variance and ordered differences report by type wise for Pb in cheese

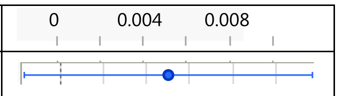
Analysis Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00010010	0.000100	2.5298	0.1340
Error	14	0.00055396	0.000040		
C. Total	15	0.00065406			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0050025	0.0031452	-0.001743	0.0117482	0.1340	

Pooled t test

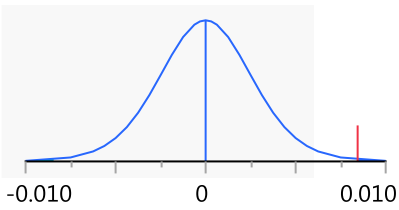


Figure: 5.11 More and Less polluted

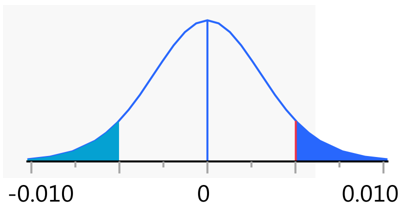


Figure: 5.12 LS-IF

Summary of one way ANOVA for Pb

S.No.	Variable	α	p – value	Null Hypothesis
1	**City Wise**	0.05	0.2598	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0054	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.1340	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 5.8 presents the citywise analysis which indicates that the pollution level is higher in Kota and Baran and little bit lower in Bundi and Jhalawar. But still from Tukey – Kramer HSD, they all share almost the common place and their p value is greater than 0.2598 which is higher than that of our chosen value (α = 0.05). So no significant difference has been found in city wise analysis. Analysis of variance by

pollution status shows the significant difference between the two with the p value is > 0.0054. One way ANOVA for analysis of value by type IF and LS, probability > 0.1340 which shows that the concentration of Pb are significantly indifferent. Tukey – Kramer HSD test also supports the data. **Fig. 5.11** and **5.12** shows the graphical representation of pooled t - test for more and less polluted area and LS – IF.

5.7.2 Concentrations of Cadmium (Cd)

Fit Group

Oneway Analysis of Value By City

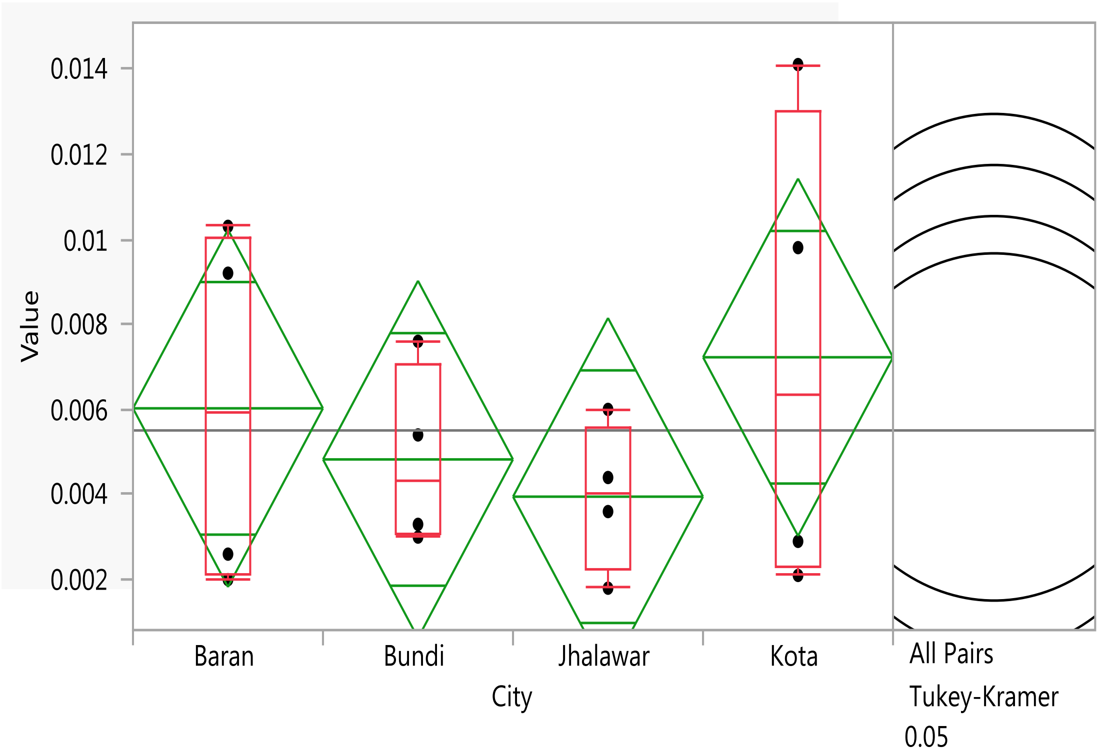


Figure 5.13 : Oneway analysis of value by city wise for Cd in cheese

Table 5.27: Analysis of variance and ordered differences report by city wise for Cd in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00002444	8.146e-6	0.5492	0.6582
Error	12	0.00017799	0.000015		
C. Total	15	0.00020243			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.005 0 0.005 0.010
Kota	Jhalawar	0.0032750	0.0027233	-0.004810	0.0113599	0.6368	
Kota	Bundi	0.0024000	0.0027233	-0.005685	0.0104849	0.8145	
Baran	Jhalawar	0.0020750	0.0027233	-0.006010	0.0101599	0.8699	
Kota	Baran	0.0012000	0.0027233	-0.006885	0.0092849	0.9702	
Baran	Bundi	0.0012000	0.0027233	-0.006885	0.0092849	0.9702	
Bundi	Jhalawar	0.0008750	0.0027233	-0.007210	0.0089599	0.9880	

Oneway Analysis of Value By Pollution status

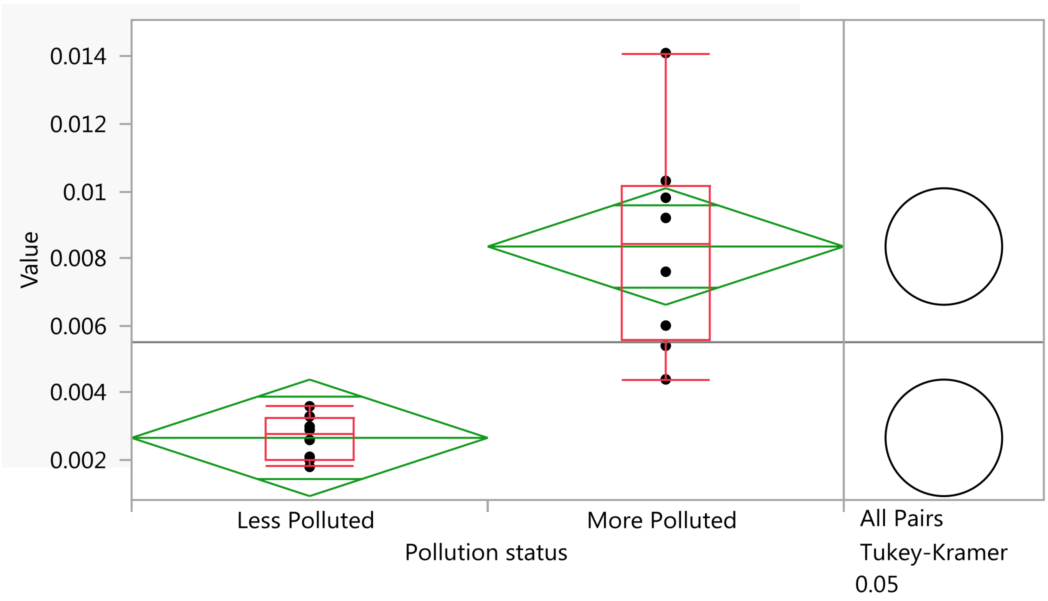


Figure 5.14 : Oneway analysis of value by pollution status wise for Cd in cheese

Table 5.28: Analysis of variance and ordered differences report by pollution status wise for Cd in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00012939	0.000129	24.8015	0.0002*
Error	14	0.00007304	5.217e-6		
C. Total	15	0.00020243			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.002 0.004 0.006
More Polluted	Less Polluted	0.0056875	0.0011420	0.0032381	0.0081369	0.0002*	

Oneway Analysis of Value By Type

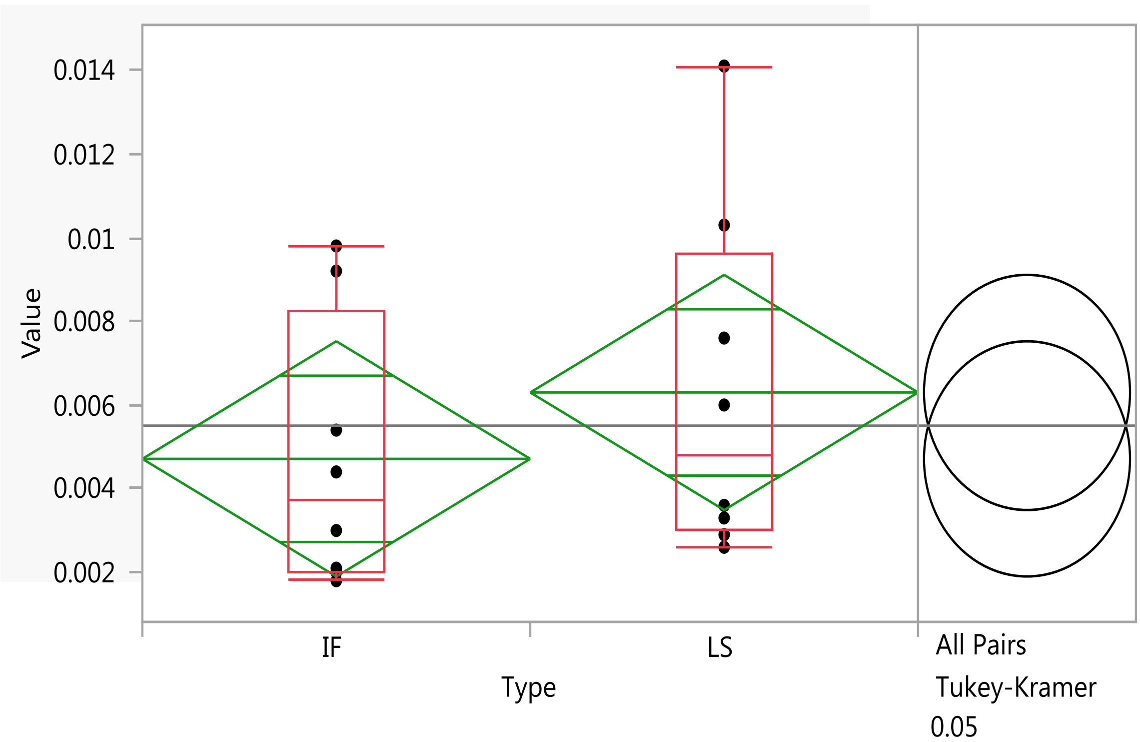


Figure 5.15 : Oneway analysis of value by type wise for Cd in cheese

Table 5.29: Analysis of variance and ordered differences report by type wise for Cd in cheese

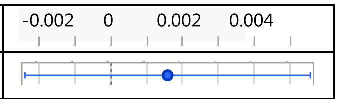
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00001008	0.000010	0.7337	0.4061
Error	14	0.00019235	0.000014		
C. Total	15	0.00020243			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0015875	0.0018533	-0.002387	0.0055625	0.4061	

Pooled t test

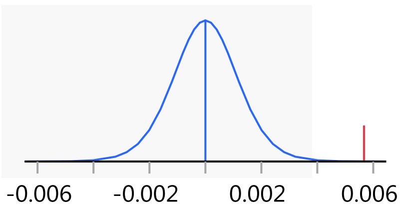


Figure: 5.16 More and Less polluted

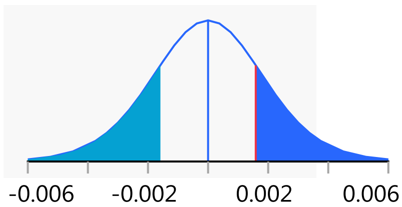


Figure: 5.17 LS-IF

Summary of one way ANOVA for Cd

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.6582	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0002	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.4061	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 5.13, 5.14 and **5.15** and the above given summary table clearly indicates the results of analysis of variance. The mean comparison using Tukey-Kramer HSD indicate that the circles are overlapping each other for first and third variables indicating

no significant difference between them, where as Tukey-Kramer HSD for second variable shows the circles that are very far from each other indicating significant difference between more and less polluted areas . Null hypothesis can't be rejected for first and third variables and can be rejected for second variable.

5.7.3 Concentrations of Aluminium (Al)

Fit Group

Oneway Analysis of Value By City

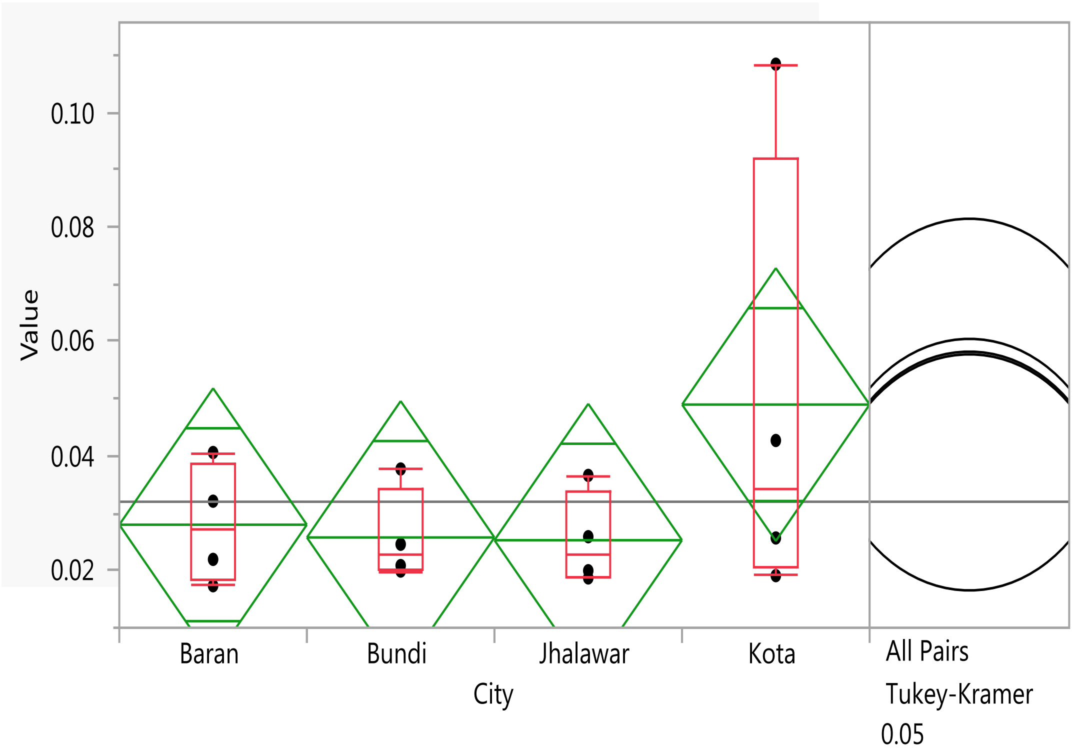


Figure 5.18 : Oneway analysis of value by city wise for Al in cheese

Table 5.30: Analysis of variance and ordered differences report by city wise for Al in cheese

Analysis of Variance

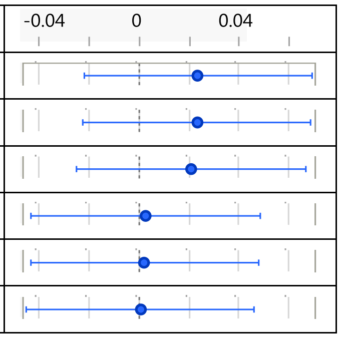
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00155168	0.000517	1.0823	0.3936
Error	12	0.00573472	0.000478		
C. Total	15	0.00728640			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Kota	Jhalawar	0.0236800	0.0154579	-0.022211	0.0695715	0.4500
Kota	Bundi	0.0232050	0.0154579	-0.022686	0.0690965	0.4665
Kota	Baran	0.0209750	0.0154579	-0.024916	0.0668665	0.5472
Baran	Jhalawar	0.0027050	0.0154579	-0.043186	0.0485965	0.9980
Baran	Bundi	0.0022300	0.0154579	-0.043661	0.0481215	0.9989
Bundi	Jhalawar	0.0004750	0.0154579	-0.045416	0.0463665	1.0000



Oneway Analysis of Value By Pollution status

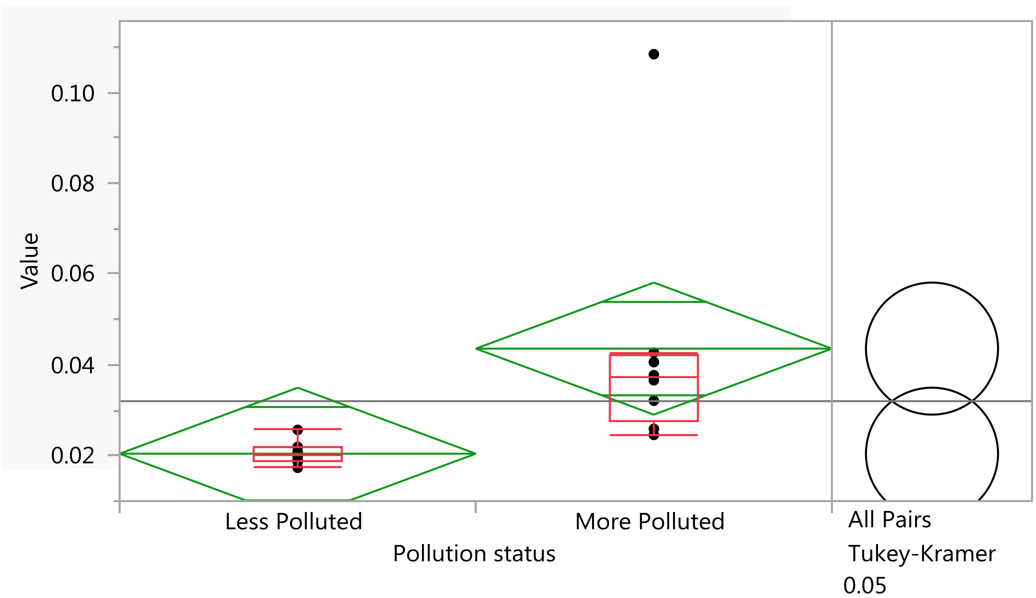


Figure 5.19 : Oneway analysis of value by pollution status wise for Al in cheese

Table 5.31: Analysis of variance and ordered differences report by pollution status wise for Al in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00214184	0.002142	5.8286	0.0300*
Error	14	0.00514456	0.000367		
C. Total	15	0.00728640			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.01 0.02 0.03 0.04
More Polluted	Less Polluted	0.0231400	0.0095847	0.0025828	0.0436972	0.0300*	

Oneway Analysis of Value By Type

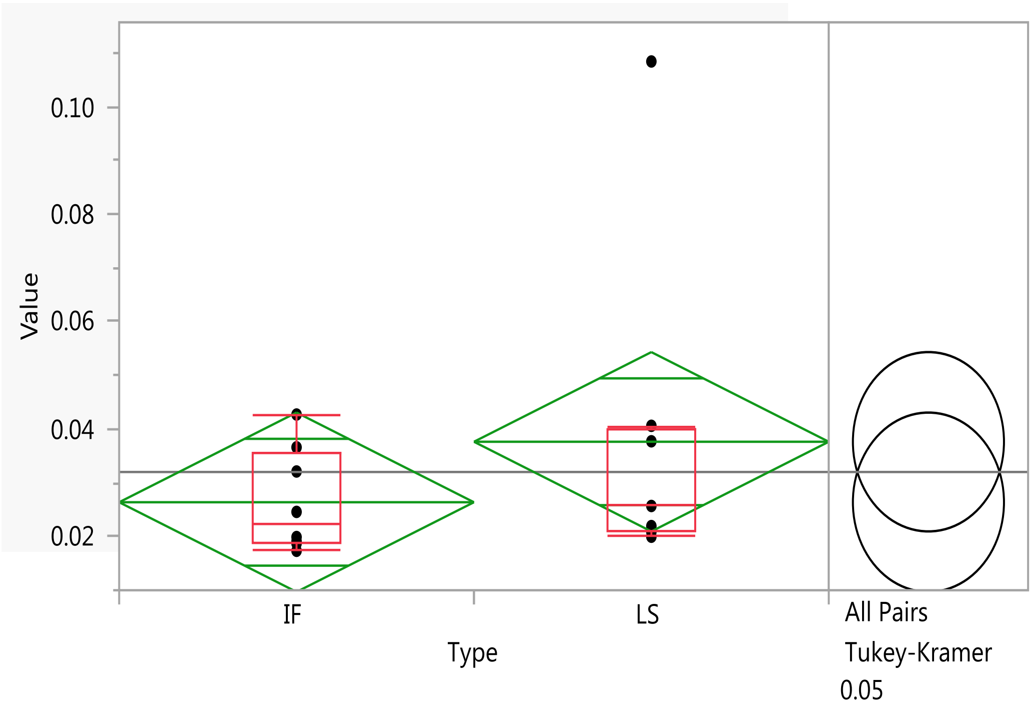


Figure 5.20 : Oneway analysis of value by type wise for Al in cheese

Table 5.32: Analysis of variance and ordered differences report by type wise for Al in cheese

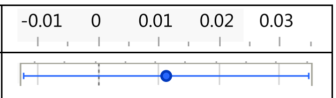
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00050670	0.000507	1.0463	0.3237
Error	14	0.00677970	0.000484		
C. Total	15	0.00728640			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0112550	0.0110030	-0.012344	0.0348541	0.3237	

Pooled t test

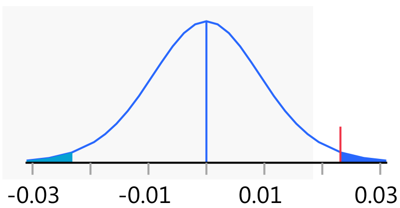


Figure: 5.21 More and Less polluted

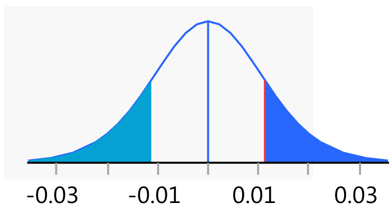


Figure: 5.22 LS-IF

Summary of one way ANOVA for Al

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.3936	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0300	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.3237	Significantly indifferent , Null Hypothesis can't be rejected

The graphical representation has been given for Al concentration in cheese for all four cities in **Fig. 5.18**, in which we can see that the lowest as well as the highest concentration value are greater in kota in comparison to other three. Analysis of variance for Al also shows the probability > 0.3936 which is greater than α = 0.05 and according to Tukey – Kramer HSD, Baran , Bundi , Jhalawar, the circle shares almost same place and for Kota it covers larger area. which proves that the mean concentration of heavy metals in all the cities are significantly indifferent. The comparison has been made between more polluted and less polluted area with the help of one way analysis. This analysis shows (**Fig. 5.19**) that there is a significant difference between both of them, as a p value > 0.0300 . The results of ANOVA test in **Fig. 5.20** shows that the p value is 0.3237, which also shows that the concentration of both the places are significantly indifferent.

5.7.4 Concentrations of Arsenic (As)

Fit Group

Oneway Analysis of Value By City

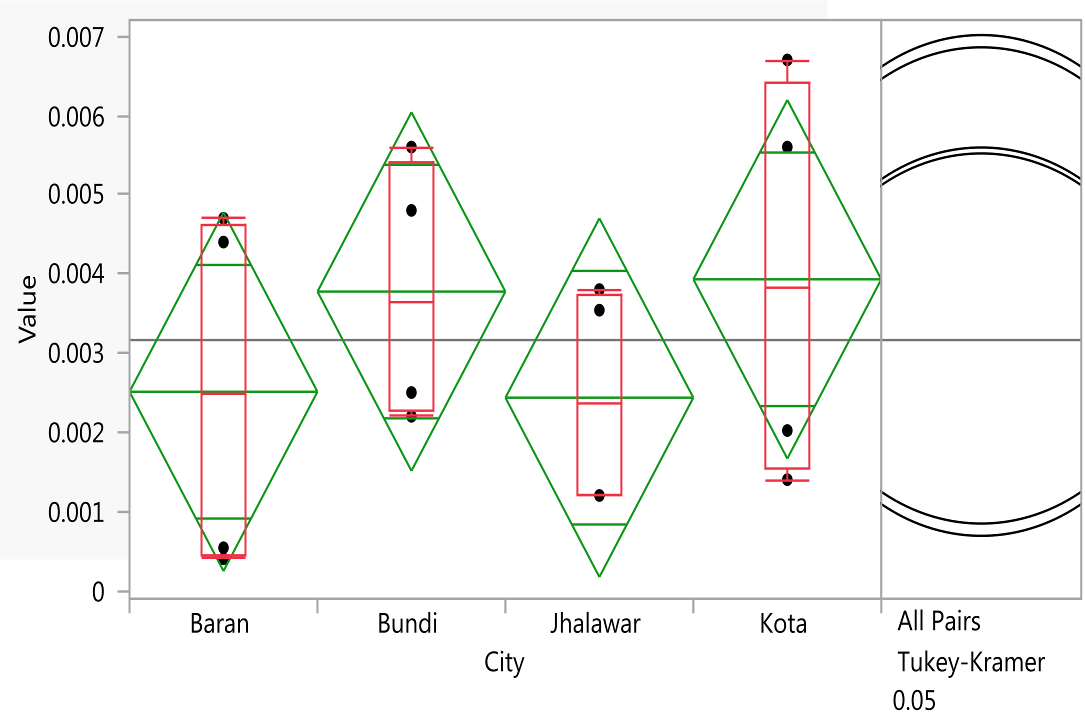


Figure 5.23 : Oneway analysis of value by city wise for As in cheese

Table 5.33: Analysis of variance and ordered differences report by city wise for As in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00000768	2.559e-6	0.5924	0.6318
Error	12	0.00005183	4.3195e-6		
C. Total	15	0.00005951			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.004 0 0.004
Kota	Jhalawar	0.0014950	0.0014696	-0.002868	0.0058580	0.7428	
Kota	Baran	0.0014200	0.0014696	-0.002943	0.0057830	0.7706	
Bundi	Jhalawar	0.0013400	0.0014696	-0.003023	0.0057030	0.7991	
Bundi	Baran	0.0012650	0.0014696	-0.003098	0.0056280	0.8246	
Kota	Bundi	0.0001550	0.0014696	-0.004208	0.0045180	0.9996	
Baran	Jhalawar	0.0000750	0.0014696	-0.004288	0.0044380	0.9999	

Oneway Analysis of Value By Pollution status

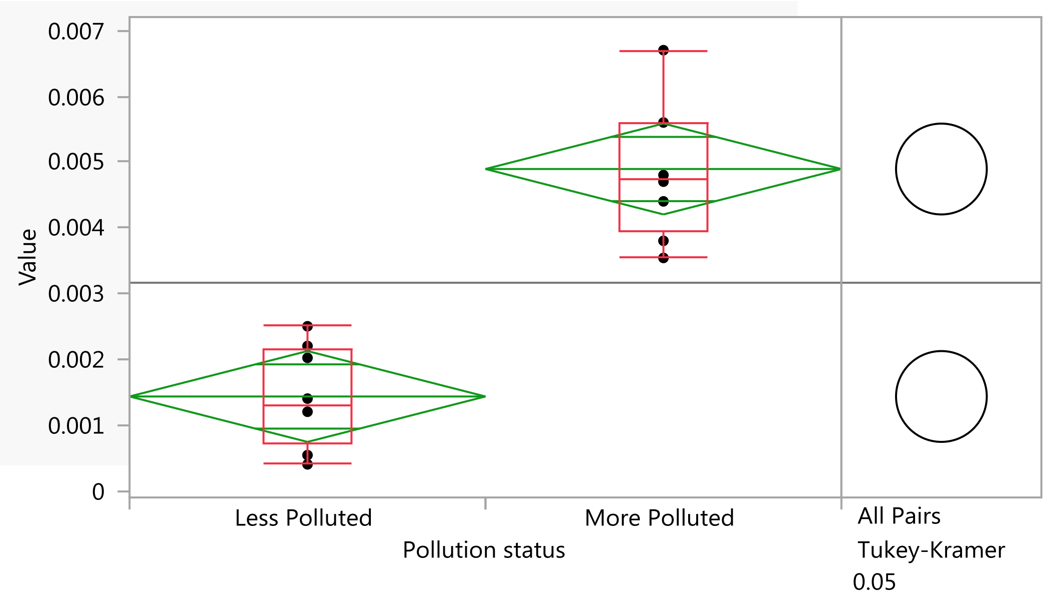


Figure 5.24 : Oneway analysis of value by pollution status wise for As in cheese

Table 5.34: Analysis of variance and ordered differences report by pollution status wise for As in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.00004789	0.000048	57.6711	<.0001*
Error	14	0.00001162	8.303e-7		
C. Total	15	0.00005951			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.001 0.002 0.003 0.004
More Polluted	Less Polluted	0.0034600	0.0004556	0.0024828	0.0044372	<.0001*	

Oneway Analysis of Value By Type

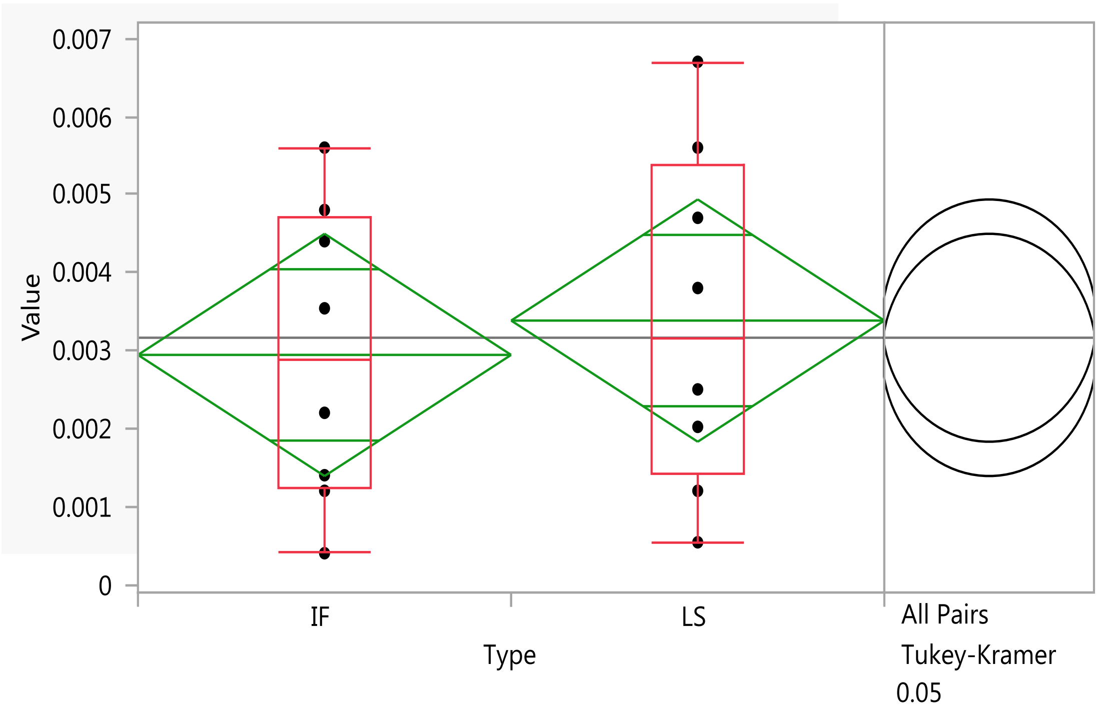


Figure 5.25 : Oneway analysis of value by type wise for As in cheese

Table 5.35: Analysis of variance and ordered differences report by type wise for As in cheese

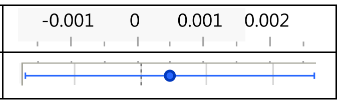
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00000077	7.744e-7	0.1846	0.6740
Error	14	0.00005874	4.1955e-6		
C. Total	15	0.00005951			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0004400	0.0010241	-0.001757	0.0026366	0.6740	

Pooled t test

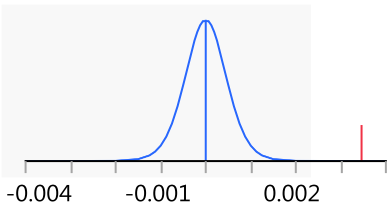


Figure: 5.26 More and Less polluted

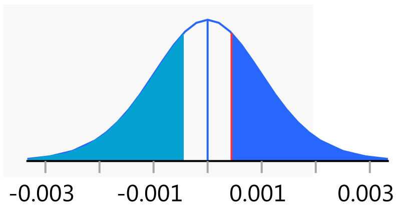


Figure: 5.27 LS-IF

Summary of one way ANOVA for As

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.6318	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0001	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.6740	Significantly indifferent , Null Hypothesis can't be rejected

One way ANOVA was performed for the determination of significant difference in Arsenic in cheese samples for different variables, whose results are shown in above summary table. **Fig. 5.23, 5.24** and **5.25** and the results of this summary table clearly indicate that the difference in mean concentration among city wise and types wise are significantly indifferent whereas according to pollution status this is significantly different. **Fig. 5.26** and **5.27** shows the graphical representation of pooled t – test for more and less polluted area and LS – IF.

5.7.5 Concentrations of Iron (Fe)

Fit Group

Oneway Analysis of Value By City

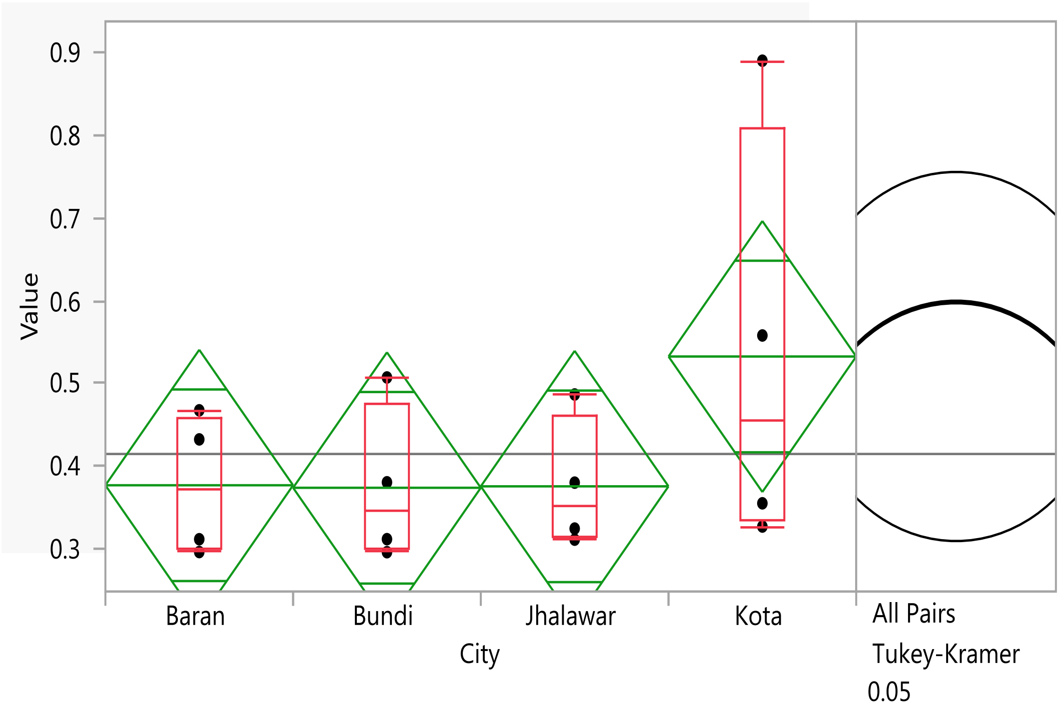


Figure 5.28 : Oneway analysis of value by city wise for Fe in cheese

Table 5.36: Analysis of variance and ordered differences report by city wise for Fe in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.07422047	0.024740	1.0925	0.3898
Error	12	0.27174796	0.022646		
C. Total	15	0.34596844			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	-0.3 -0.1 0.1 0.3
Kota	Bundi	0.1588750	0.1064088	-0.157032	0.4747818	0.4709	
Kota	Jhalawar	0.1571250	0.1064088	-0.158782	0.4730318	0.4799	
Kota	Baran	0.1558100	0.1064088	-0.160097	0.4717168	0.4867	
Baran	Bundi	0.0030650	0.1064088	-0.312842	0.3189718	1.0000	
Jhalawar	Bundi	0.0017500	0.1064088	-0.314157	0.3176568	1.0000	
Baran	Jhalawar	0.0013150	0.1064088	-0.314592	0.3172218	1.0000	

Oneway Analysis of Value By Pollution status

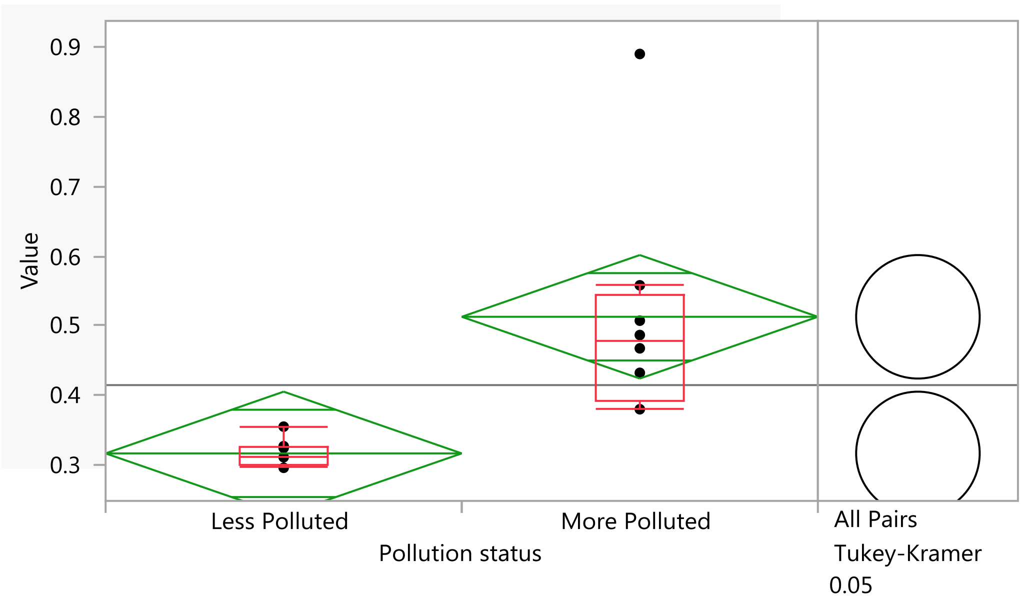


Figure 5.29 : Oneway analysis of value by pollution status wise for Fe in cheese

Table 5.37: Analysis of variance and ordered differences report by pollution status wise for Fe in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.15419758	0.154198	11.2570	0.0047*
Error	14	0.19177086	0.013698		
C. Total	15	0.34596844			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.1 0.2 0.3
More Polluted	Less Polluted	0.1963400	0.0585191	0.0708291	0.3218509	0.0047*	

Oneway Analysis of Value By Type

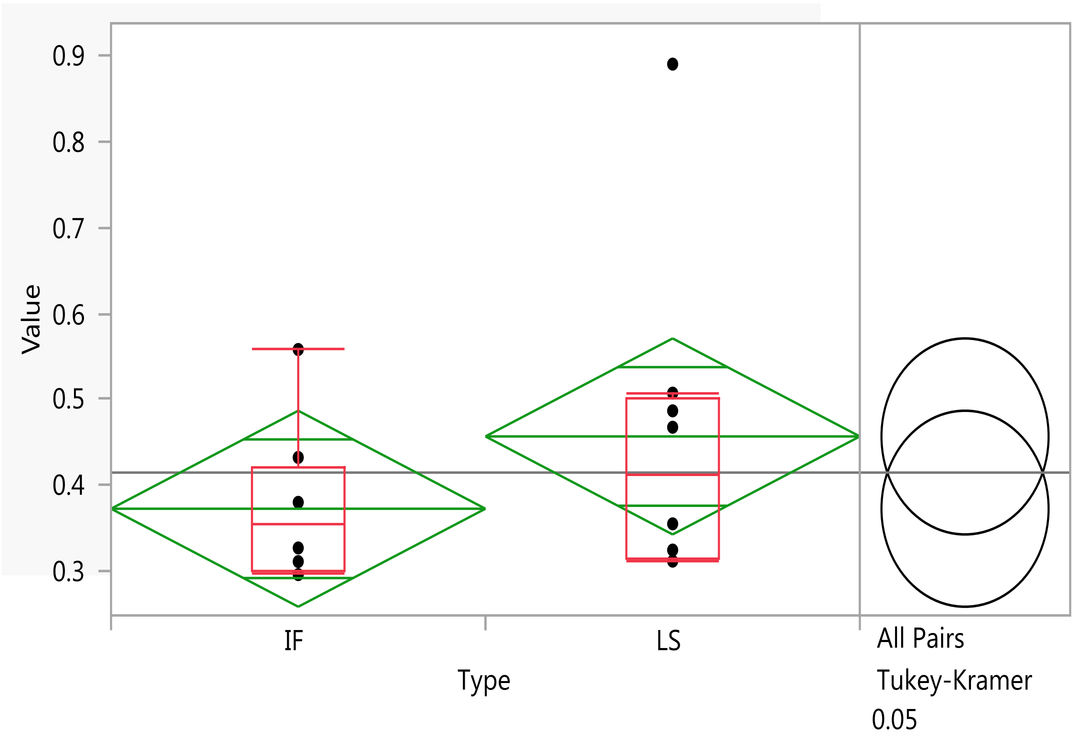


Figure 5.30 : Oneway analysis of value by type wise for Fe in cheese

Table 5.38: Analysis of variance and ordered differences report by type wise for Fe in cheese

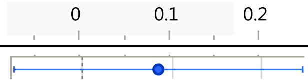
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.02839225	0.028392	1.2516	0.2821
Error	14	0.31757619	0.022684		
C. Total	15	0.34596844			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0842500	0.0753061	-0.077265	0.2457654	0.2821	

Pooled t test

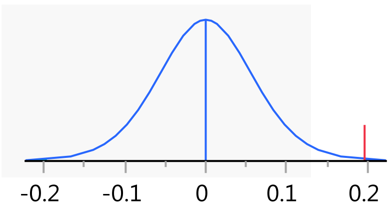


Figure: 5.31 More and Less polluted

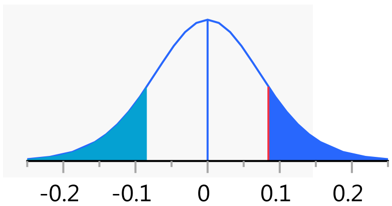


Figure: 5.32 LS-IF

Summary of one way ANOVA for Fe

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.3898	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0047	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.2821	Significantly indifferent , Null Hypothesis can't be rejected

Fig. 5.28 indicates that the pollution level is higher in Kota and lower in Baran, Bundi and Jhalawar, but still from Tukey – Kramer HSD, they all share almost the

common place, and their p values are greater than > 0.3898 which is higher than that of our chosen value ($\alpha = 0.05$). So no significant difference has been found in city wise analysis. Analysis of variance by pollution status shows in **Fig. 5.29**, which shows the significant difference between the two with the p value is > 0.0047. One way ANOVA for analysis of value by type IF and LS have shown in **Fig. 5.30**, according to this probability > 0.2821 which shows that the concentration of Fe in cheese is significantly indifferent. Tukey – Kramer HSD test also supports the data.

5.7.6 Concentrations of Zinc (Zn)

Fit Group

Oneway Analysis of Value By City

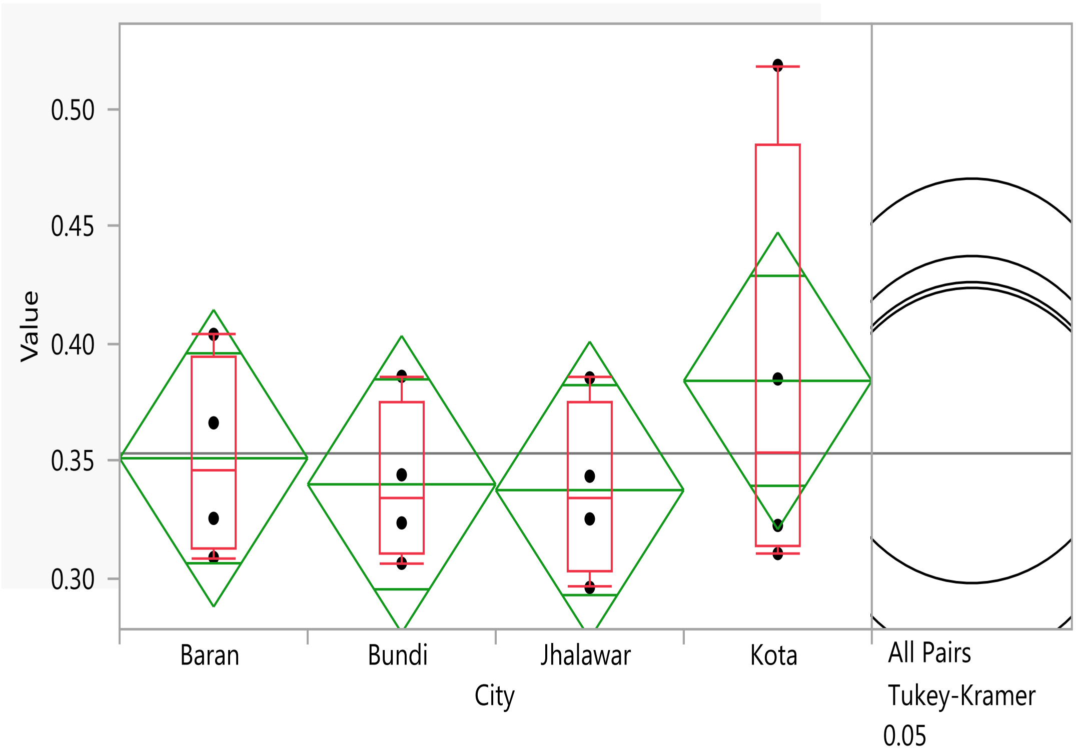


Figure 5.33 : Oneway analysis of value by city wise for Zn in cheese

Table 5.39: Analysis of variance and ordered differences report by city wise for Zn in cheese

Analysis of Variance

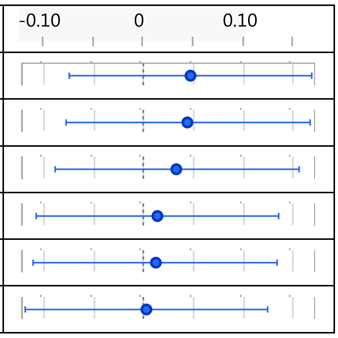
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
City	3	0.00551532	0.001838	0.5452	0.6607
Error	12	0.04046376	0.003372		
C. Total	15	0.04597908			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.96880	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Kota	Jhalawar	0.0465650	0.0410608	-0.075336	0.1684665	0.6767
Kota	Bundi	0.0441000	0.0410608	-0.077801	0.1660015	0.7110
Kota	Baran	0.0329950	0.0410608	-0.088906	0.1548965	0.8515
Baran	Jhalawar	0.0135700	0.0410608	-0.108331	0.1354715	0.9869
Baran	Bundi	0.0111050	0.0410608	-0.110796	0.1330065	0.9927
Bundi	Jhalawar	0.0024650	0.0410608	-0.119436	0.1243665	0.9999



Oneway Analysis of Value By Pollution status

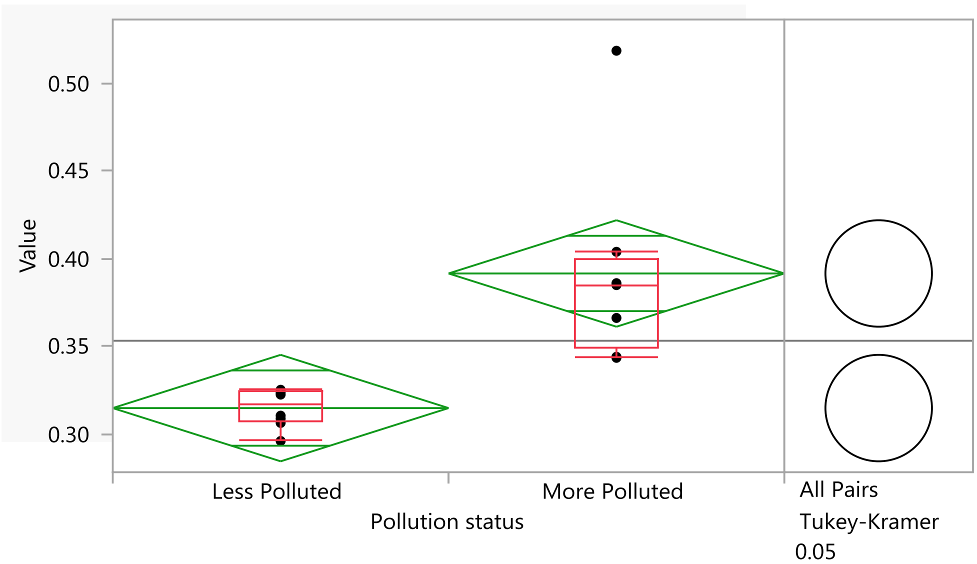


Figure 5.34 : Oneway analysis of value by pollution status wise for Zn in cheese

Table 5.40: Analysis of variance and ordered differences report by pollution status wise for Zn in cheese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pollution status	1	0.02354997	0.023550	14.6996	0.0018*
Error	14	0.02242911	0.001602		
C. Total	15	0.04597908			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	0 0.02 0.04 0.06 0.08 0.10
More Polluted	Less Polluted	0.0767300	0.0200130	0.0338064	0.1196536	0.0018*	

Oneway Analysis of Value By Type

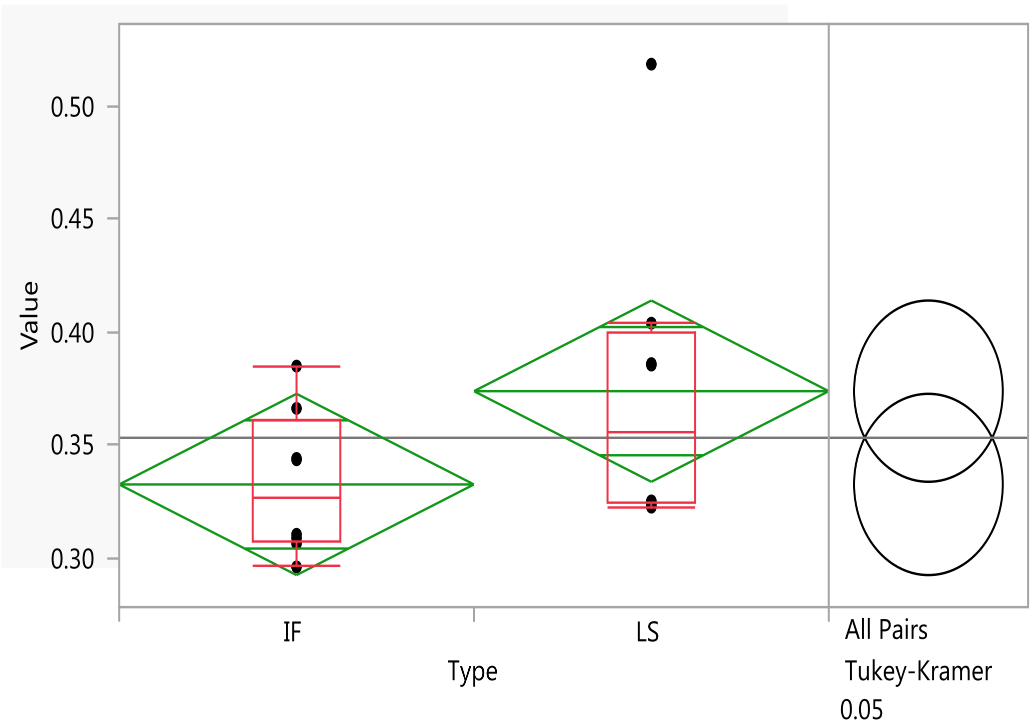


Figure 5.35 : Oneway analysis of value by type wise for Zn in cheese

Table 5.41: Analysis of variance and ordered differences report by type wise for Zn in cheese

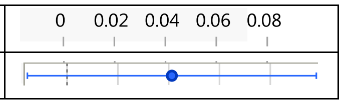
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type	1	0.00681615	0.006816	2.4366	0.1408
Error	14	0.03916293	0.002797		
C. Total	15	0.04597908			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.14479	0.05

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
LS	IF	0.0412800	0.0264450	-0.015439	0.0979989	0.1408	

Pooled t test

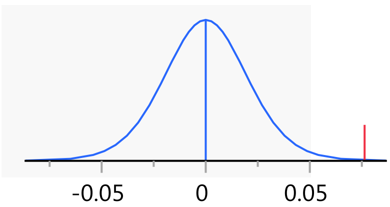


Figure: 5.36 More and Less polluted

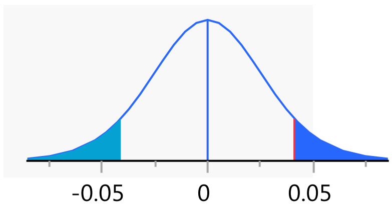


Figure: 5.37 LS-IF

Summary of one way ANOVA for Zn

S.No.	Variable	α	p - value	Null Hypothesis
1	**City Wise**	0.05	0.6607	Significantly indifferent , Null Hypothesis can't be rejected
2	**Pollution Status**	0.05	0.0018	Significantly different , Null Hypothesis can be rejected
3	**Types (IF & LS)**	0.05	0.1408	Significantly indifferent , Null Hypothesis can't be rejected

The above given summary table and **Fig. 5.33**, **5.34** and **5.35** clearly indicates the First and third variable i.e., city wise and types wise with p value > 0.6607 and > 0.1408 respectively are significantly indifferent and the second variable is i.e., pollution wise status with probability > 0.0018, is significantly different. Tukey – Kramer HSD test also supports the data. **Fig. 5.36** and **Fig. 5.37** shows the graphical representation of pooled t – test for more and less polluted area and LS – IF.

5.8 Correlation Coefficient

The results of correlation analysis between these heavy metals for Cheese are given in **Table 5.42**

Table 5.42 : Correlation coefficients among mean concentration values of metals in cheese samples

Metals	Pb	Cd	Al	As	Fe	Zn
Pb	1					
Cd	0.908568	1				
Al	0.8653	0.837117	1			
As	0.764323	0.900715	0.721352	1		
Fe	0.909994	0.902243	0.95362	0.819901	1	
Zn	0.944839	0.935641	0.933602	0.830805	0.974499	1

Table 5.42 clearly indicate that Pearson's coefficient (r) has all positive values. All metals have correlations that are larger than 0.7, indicating a strong correlation between them. On the basis of these results, we can conclude that there are many common factors which are responsible for the heavy metal contamination in the cheese samples.

5.9 Conclusion

On the basis of the results of One way Anova conducted on heavy metal concentrations in cheese samples, it is concluded that there are no statistically significant differences in heavy metal concentration among the cities and the types IR and LS as p-values is greater than the significant level of 0.05. So we fail to reject the null hypothesis, on the other hand significant difference is found for the test conducted on pollution status. Tukey-Kramer clearly shows the difference in pollution status of more and less polluted areas.

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CHAPTER – VI

COMPARISON OF RESULTS AND HEALTH RISK ASSESSMENT

This chapter includes the comparison of results with RDA values. To assess health risk, estimated daily intake, metal pollution index and health risk index are calculated

6.1 Introduction

The recommended dietary allowance (RDA) refers to the daily intake level of a nutrient considered sufficient to meet the requirements of nearly all healthy individuals in a particular life stage and gender group [1,2]. RDA's are part of the dietary reference intakes (DRI's) a set of nutrient based reference values established by health authorities [3,4]. So the main purpose is to provide guidelines for adequate nutrient intake and maintain optimal health. RDA's are developed on the basis of certified evidence and updated periodically.

The aim of our study was to evaluate the nutritional value of dairy products. To determine the concentration level of heavy metals in yoghurt, butter and cheese, the experimental data is compared with authorized, globally standardized data or RDA values [5,6].

6.2 Comparison of Yoghurt Results with RDA

The results of mean concentration of heavy metal in yoghurt samples are compared with RDA (recommended dietary allowance) values. Results shows that how the mean concentration of heavy metals differ from RDA values, established by various national and international organizations. It should be observed that the concentrations of heavy metals in the samples from each sites vary, which indicate that the heavy metals depend on the places where animals graze, drink water, manufacturing processes, storing, packaging and transportation also.

Comparing the results of overall mean concentrations of heavy metals across all selected areas with RDA values are illustrated in the tables given below.

Table 6.1: Overall mean concentration of toxic metals (Pb, Cd, As) in yoghurt samples of more polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0476**	138.00	**0.0026**	**0.0142**	446.15	**0.01**	**0.0049**	Nil
Baran	**0.02**	**0.0377**	88.50	**0.0026**	**0.0086**	231.54	**0.01**	**0.0038**	Nil
Bundi	**0.02**	**0.0386**	93.00	**0.0026**	**0.0053**	102.31	**0.01**	**0.0047**	Nil
Jhalawar	**0.02**	**0.0383**	91.50	**0.0026**	**0.0047**	80.00	**0.01**	**0.0041**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.2: Overall mean concentration of toxic metals (Al, Fe, Zn) in yoghurt samples of more polluted area

Metals/ Area	Al			Fe			Zn		
	RL	**AMC**	**% Increase**	**RL**	**AMC**	**% Increase**	**RL**	**AMC**	**% Increase**
Kota	**0.02**	**0.0748**	274.00	**0.37**	**0.6366**	72.05	**0.328**	**0.3843**	17.16
Baran	**0.02**	**0.0557**	178.50	**0.37**	**0.4689**	26.73	**0.328**	**0.3656**	11.46
Bundi	**0.02**	**0.0410**	105.00	**0.37**	**0.4819**	30.24	**0.328**	**0.4126**	25.79
Jhalawar	**0.02**	**0.0406**	103.00	**0.37**	**0.4765**	28.78	**0.328**	**0.4107**	25.21

RL : Recommended Level, AMC : Actual Mean Concentration

Comparison and Analysis :

Lead (Pb) :

Actual Mean Concentration (AMC) for Kota, Baran, Bundi and Jhalawar are given in **Table 6.1** and percentage increase calculated are 138, 88.5, 93 and 91.5 % respectively.
Recommended Level (RL) : 0.02 mg/L
Analysis : The AMC of lead has surpassed the level by more than 100%, which lead to serious health issues including neurological damage and developmental delays in children and adults.

Cadmium (Cd) :

AMC : All four locations exceeded the RL.
RL : 0.0026 mg/L
Analysis : The percentage increase in all four locations are more than 60 %, which leads to the conclusion that over the time continuous exposure can damage kidneys and risk of bone demineralization.

Arsenic (As) :

AMC : The actual Mean Concentration of As in yoghurt samples of Kota, Baran, Bundi and Jhalawar are below the permissible limits.
RL : 0.01 mg/L
Analysis : Low Arsenic AMC, indicating that the exposure is within the safe limits but continuous monitoring is essential as Arsenic accumulate in the body over the period of time, leading to health hazards.

Aluminium (Al) :

AMC : Exceeded the permissible limit in all four places.
RL : 0.02 mg/L
Analysis : AMC of Al in yoghurt samples stepped up more than 100 % of permissible limit. Higher concentration of Al leads to the abnormal formation of RBCs and also affects the parathyroid gland.

Iron (Fe) :

AMC : Exceeded the permissible limit in yoghurt samples of all four locations.

RL : 0.37 mg/L

Analysis : The percentage increase in yoghurt samples of more polluted area of Kota, Baran, Bundi and Jhalawar are 72.05 %, 26.73 %, 30.24 % and 28.78 % respectively. Excessive iron can be stored in major organs which may lead to organ failure. It can also cause arthritis, heart disease, diabetes etc.

Zinc (Zn) :

AMC : Exceeded the permissible limits in all four location i.e., polluted area of Kota, Baran, Bundi and Jhalawar.

RL : 3.28 mg/L

Analysis : Higher doses of zinc i.e., 10 to 15 times higher than the RDA may cause anemia, damage of pancreas, decrease of HDL etc. In our study the percentage increase than RDA for Kota, Baran, Bundi and Jhalawar are 17.16 %, 11.46 %, 25.79 % and 25.21 % respectively.

Table 6.3: Overall mean concentration of toxic metals (Pb, Cd, As) in yoghurt samples of less polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0160**	Nil	**0.0026**	**0.0015**	Nil	**0.01**	**0.0009**	Nil
Baran	**0.02**	**0.0179**	Nil	**0.0026**	**0.0021**	Nil	**0.01**	**0.0010**	Nil
Bundi	**0.02**	**0.0189**	Nil	**0.0026**	**0.0026**	Nil	**0.01**	**0.0017**	Nil
Jhalawar	**0.02**	**0.0180**	Nil	**0.0026**	**0.0019**	Nil	**0.01**	**0.0019**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.4: Overall mean concentration of toxic metals (Al, Fe, Zn) in yoghurt samples of less polluted area

Metals/ Area	Al			Fe			Zn		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0192**	Nil	**0.37**	**0.3499**	Nil	**0.328**	**0.3099**	Nil
Baran	**0.02**	**0.0200**	Nil	**0.37**	**0.3302**	Nil	**0.328**	**0.3307**	0.82
Bundi	**0.02**	**0.0200**	Nil	**0.37**	**0.3303**	Nil	**0.328**	**0.3306**	0.79
Jhalawar	**0.02**	**0.0190**	Nil	**0.37**	**0.3287**	Nil	**0.328**	**0.3433**	4.66

RL : Recommended Level, AMC : Actual Mean Concentration

In less polluted area of all four locations, the actual mean concentrations of metals are within the limits and not exceeded the RDA values except for the Zn. In the less polluted area of Baran, Bundi and Jhalawar, there is a percentage increase in Zn by 0.82 %, 0.79 % & 4.66 %.

6.3 Comparison of Butter Results with RDA

Table 6.5: Overall mean concentration of toxic metals (Pb, Cd, As) in butter samples of more polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0379**	89.50	**0.0026**	**0.0115**	342.31	**0.01**	**0.0053**	Nil
Baran	**0.02**	**0.0303**	51.50	**0.0026**	**0.0084**	223.08	**0.01**	**0.0048**	Nil
Bundi	**0.02**	**0.0273**	36.50	**0.0026**	**0.0067**	157.69	**0.01**	**0.0057**	Nil
Jhalawar	**0.02**	**0.0222**	11.00	**0.0026**	**0.0046**	76.92	**0.01**	**0.0038**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.6: Overall mean concentration of toxic metals (Al, Fe, Zn) in butter samples of more polluted area

Metals/ Area	Al			Fe			Zn		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0726**	263.00	**0.37**	**0.7574**	104.70	**0.328**	**0.4023**	22.65
Baran	**0.02**	**0.0412**	106.00	**0.37**	**0.4364**	17.95	**0.328**	**0.3621**	10.40
Bundi	**0.02**	**0.0352**	76.00	**0.37**	**0.4275**	15.54	**0.328**	**0.3779**	15.21
Jhalawar	**0.02**	**0.0317**	58.50	**0.37**	**0.4251**	14.89	**0.328**	**0.3653**	11.37

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.7: Overall mean concentration of toxic metals (Pb, Cd, As) in butter samples of less polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0153**	Nil	**0.0026**	**0.0024**	Nil	**0.01**	**0.0014**	Nil
Baran	**0.02**	**0.0189**	Nil	**0.0026**	**0.0023**	Nil	**0.01**	**0.0006**	Nil
Bundi	**0.02**	**0.0184**	Nil	**0.0026**	**0.0024**	Nil	**0.01**	**0.0022**	Nil
Jhalawar	**0.02**	**0.0143**	Nil	**0.0026**	**0.0019**	Nil	**0.01**	**0.0013**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.8: Overall mean concentration of toxic metals (Al, Fe, Zn) in butter samples of less polluted area

Metals/ Area	Al			Fe			Zn		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	**0.02**	**0.0190**	Nil	**0.37**	**0.3510**	Nil	**0.328**	**0.3115**	Nil
Baran	**0.02**	**0.0201**	0.50	**0.37**	**0.3243**	Nil	**0.328**	**0.3196**	Nil
Bundi	**0.02**	**0.0212**	6.00	**0.37**	**0.3143**	Nil	**0.328**	**0.3215**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

The comparative tables i.e., **Table 6.5, 6.6, 6.7** and **6.8** for butter shows that in polluted area, all the metals except arsenic exceeded the permissible limits. The percentage increase for Pb, Cd, Al, Fe and Zn ranges in between 11 – 89.5 %, 76.92 – 342.31 %, 58.5 – 263 %, 14.89 – 104.7 % and 11.37 – 22.65 % respectively.

And for non-polluted area the metal concentration in most of the places within the limits. Only Aluminium concentration in Baran and Bundi exceeded the permissible limits with the percentage increase of 0.50 % and 6.00 % respectively.

6.4 Comparison of Cheese Results with RDA

Table 6.9: Overall mean concentration of toxic metals (Pb, Cd, As) in cheese samples of more polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	0.02	0.0318	59.00	0.0026	0.0120	361.54	0.01	0.0062	Nil
Baran	0.02	0.0264	32.00	0.0026	0.0097	273.08	0.01	0.0046	Nil
Bundi	0.02	0.0206	3.00	0.0026	0.0065	150.00	0.01	0.0052	Nil
Jhalawar	0.02	0.0201	0.50	0.0026	0.0052	100.00	0.01	0.0037	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.10: Overall mean concentration of toxic metals (Al, Fe, Zn) in cheese samples of more polluted area

Metals/ Area	Al			Fe			Zn		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	0.02	0.0755	277.50	0.37	0.7245	95.81	0.328	0.4517	37.71
Baran	0.02	0.0363	81.50	0.37	0.4501	21.65	0.328	0.3850	17.38
Bundi	0.02	0.0312	56.00	0.37	0.4440	20.00	0.328	0.3650	11.28
Jhalawar	0.02	0.0313	56.50	0.37	0.4335	17.16	0.328	0.3644	11.10

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.11: Overall mean concentration of toxic metals (Pb, Cd, As) in cheese samples of less polluted area

Metals/ Area	Pb			Cd			As		
	RL	AMC	% Increase	RL	AMC	% Increase	RL	AMC	% Increase
Kota	0.02	0.0190	Nil	0.0026	0.0025	Nil	0.01	0.0017	Nil
Baran	0.02	0.0173	Nil	0.0026	0.0023	Nil	0.01	0.0005	Nil
Bundi	0.02	0.0144	Nil	0.0026	0.0032	23.08	0.01	0.0024	Nil
Jhalawar	0.02	0.0145	Nil	0.0026	0.0027	3.85	0.01	0.0012	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

Table 6.12: Overall mean concentration of toxic metals (Al, Fe, Zn) in cheese samples of less polluted area

Metals/ Area	Al			Fe			Zn		
	RL	**AMC**	**% Increase**	**RL**	**AMC**	**% Increase**	**RL**	**AMC**	**% Increase**
Kota	**0.02**	**0.0224**	12.00	**0.37**	**0.3411**	Nil	**0.328**	**0.3165**	Nil
Baran	**0.02**	**0.0196**	Nil	**0.37**	**0.3039**	Nil	**0.328**	**0.3172**	Nil
Bundi	**0.02**	**0.0204**	2.00	**0.37**	**0.3039**	Nil	**0.328**	**0.3150**	Nil
Jhalawar	**0.02**	**0.0194**	Nil	**0.37**	**0.3179**	Nil	**0.328**	**0.3107**	Nil

RL : Recommended Level, AMC : Actual Mean Concentration

The results of analysis of cheese samples are presented in **Table 6.9, 6.10, 6.11** and **6.12**. The AMC for polluted areas of all four locations are exceeded the RDA values for all metals except for Arsenic and for non-polluted areas AMC's are within the limits except for one or two locations i.e., in Bundi and Jhalawar percentage increase in Cd are 23.08 and 3.85 % respectively and there is a little increase observed i.e., 12 % and 2 % in Al concentration of Kota and Bundi respectively.

The purpose of this study is to evaluate the percentage increase across the various samples i.e., yoghurt, butter and cheese from RDA values. The means of metal concentration showed the similarities, which suggests that the places having greater anthropogenic activities have greater metal ion concentration.

6.5 Distribution of Metals in Yoghurt

Distribution of metals (Pb, Cd, Al, As, Fe and Zn) in all 80 Yoghurt samples have shown in **Fig. 6.1** and **Fig. 6.2.**

Distribution

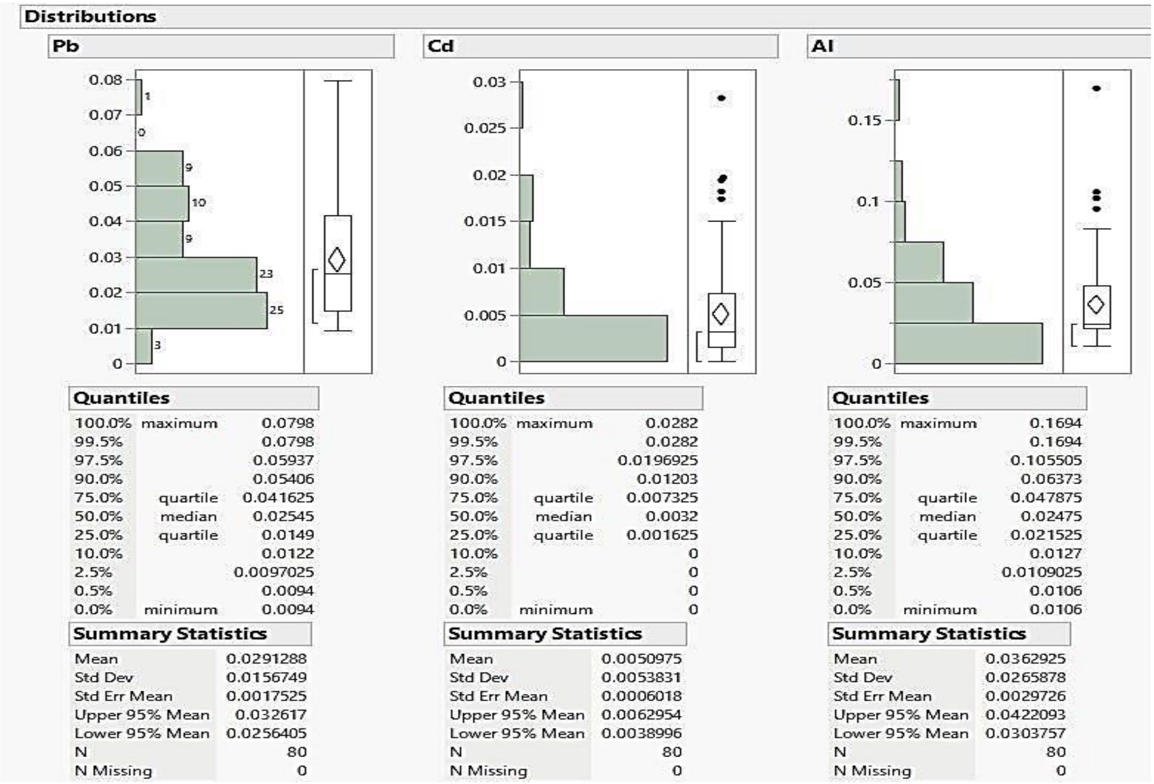

Figure 6.1: Distribution of Pb, Cd, and Al content in 80 Yoghurt Samples

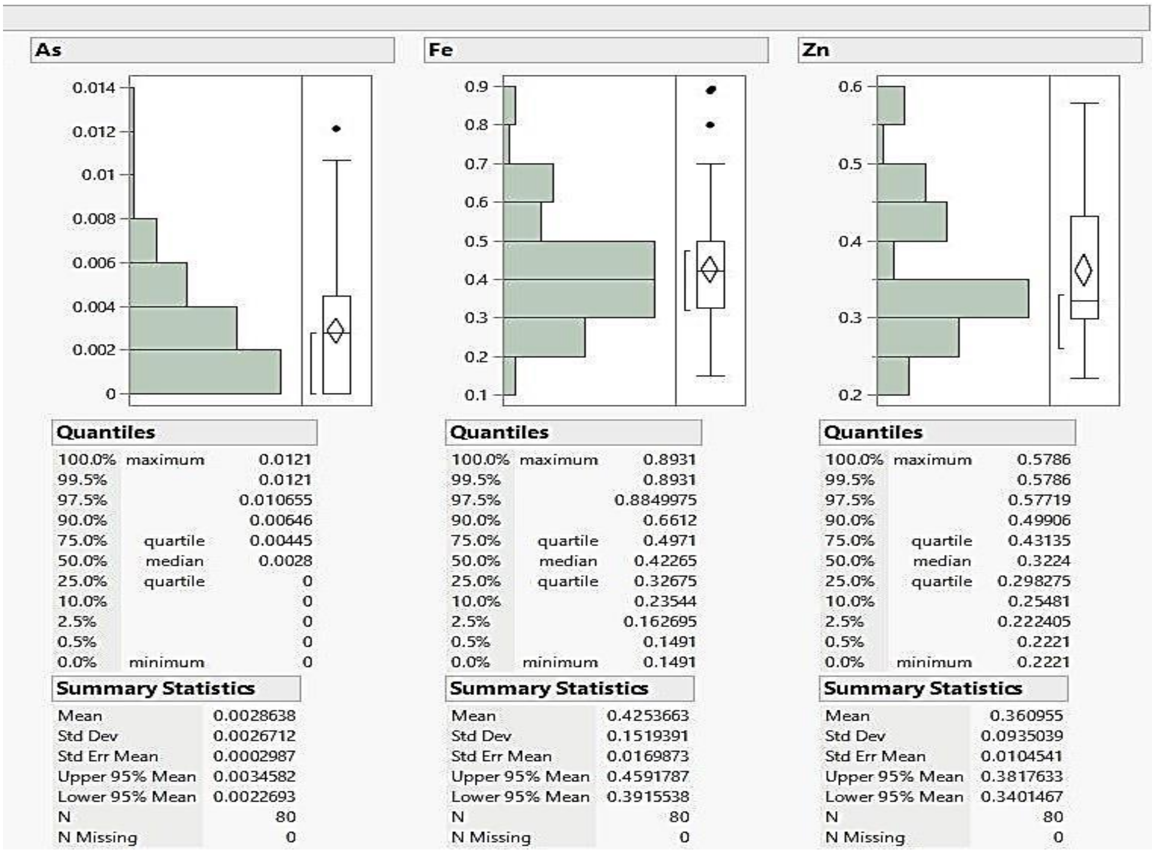

Figure 6.2: Distribution of As, Fe, and Zn content in 80 Yoghurt Samples

Multivariate Correlation

The multivariate correlation coefficient quantifies the strength of relationship between the outcome variable and a set of predictor variables. It is denoted by R and it ranges from 0-1, where 0 indicates no linear relationship and 1 indicates a perfect linear relationship. Higher the value of R stronger will be the relationship between predictor and outcome variable.

From scatter plot matrix which shows the pair wise relationship between different variables represents the positive correlation among all metals.

A strong correlation can be seen between Cd-Al, Al-Fe (**Table 6.13**), which is greater than R = 0.7.From the results it is concluded that heavy metals in all 80 samples of yoghurt collected from above mentioned all four districts are affected by almost similar sources.

Table 6.13: Multivariate Correlation between metal in all yoghurt samples

	Pb	**Cd**	**Al**	**As**	**Fe**	**Zn**
Pb	**1.0000**	**0.4866**	**0.3633**	**0.3006**	**0.4438**	**0.3514**
Cd	**0.4866**	**1.0000**	**0.7110**	**0.3687**	**0.5418**	**0.1404**
Al	**0.3633**	**0.7110**	**1.0000**	**0.5923**	**0.7585**	**0.3717**
As	**0.3006**	**0.3687**	**0.5923**	**1.0000**	**0.5967**	**0.4132**
Fe	**0.4438**	**0.5418**	**0.7585**	**0.5967**	**1.0000**	**0.3967**
Zn	**0.3514**	**0.1404**	**0.3717**	**0.4132**	**0.3967**	**1.0000**

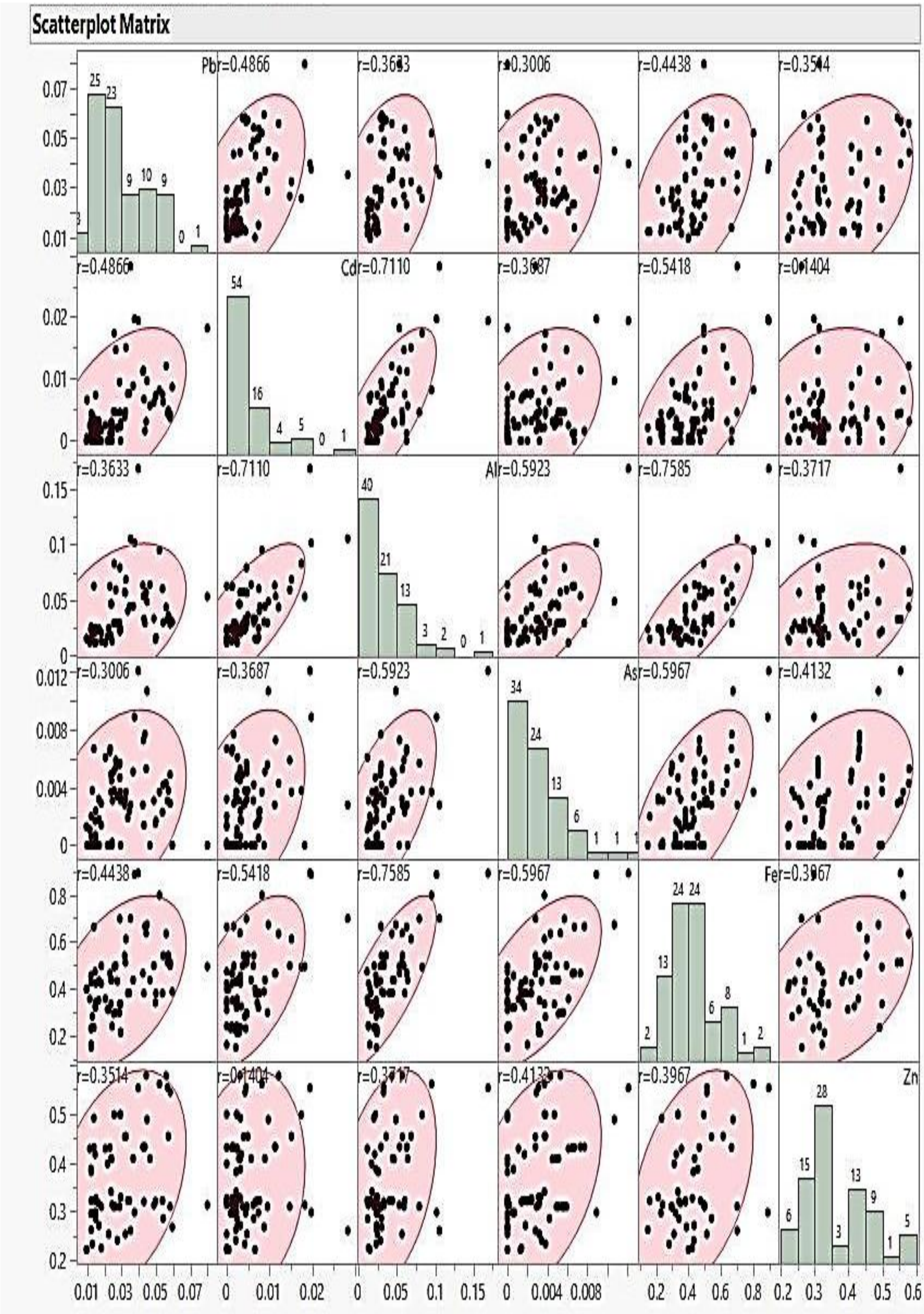

Figure 6.3: Multivariate correlation between metals in all Yoghurt samples

6.6 Distribution of metals in Butter

Distribution of metals (Pb, Cd, Al, As, Fe and Zn) in all 80 Butter samples have shown in **Fig. 6.4**and **Fig. 6.5.**

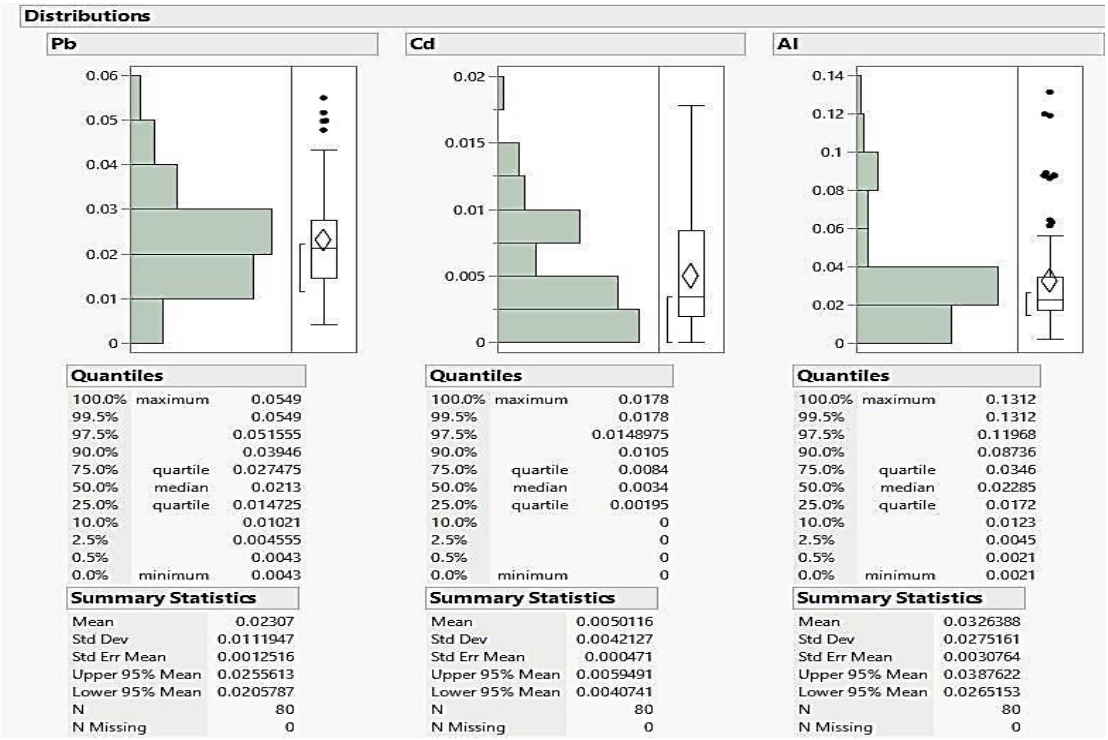

Figure 6.4: Distribution of Pb, Cd, and Al content in Butter Samples

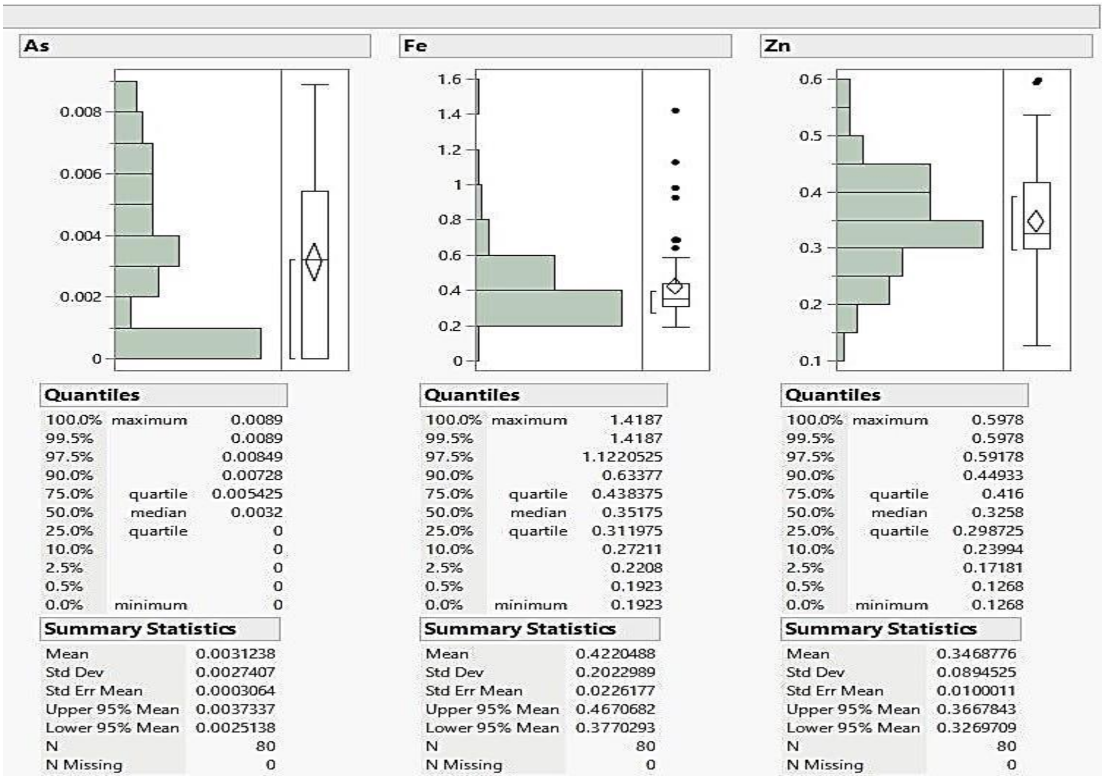

Figure 6.5: Distribution of As, Fe, and Zn content in Butter Samples

Multivariate Correlation for Butter samples

A positive correlation is observed between all metals in 80 butter samples collected from different areas. A strong correlation is observed between Al-Fe (R = 0.7).

Table 6.14 : Multivariate correlation between metal in all butter samples

	Pb	Cd	Al	As	Fe	Zn
Pb	**1.0000**	**0.6222**	**0.3994**	**0.4035**	**0.4631**	**0.3539**
Cd	**0.6222**	**1.0000**	**0.6328**	**0.4767**	**0.6189**	**0.2417**
Al	**0.3994**	**0.6328**	**1.0000**	**0.4848**	**0.7718**	**0.2760**
As	**0.4035**	**0.4767**	**0.4848**	**1.0000**	**0.4327**	**0.3776**
Fe	**0.4631**	**0.6189**	**0.7718**	**0.4327**	**1.0000**	**0.2475**
Zn	**0.3539**	**0.2417**	**0.2760**	**0.3776**	**0.2475**	**1.0000**

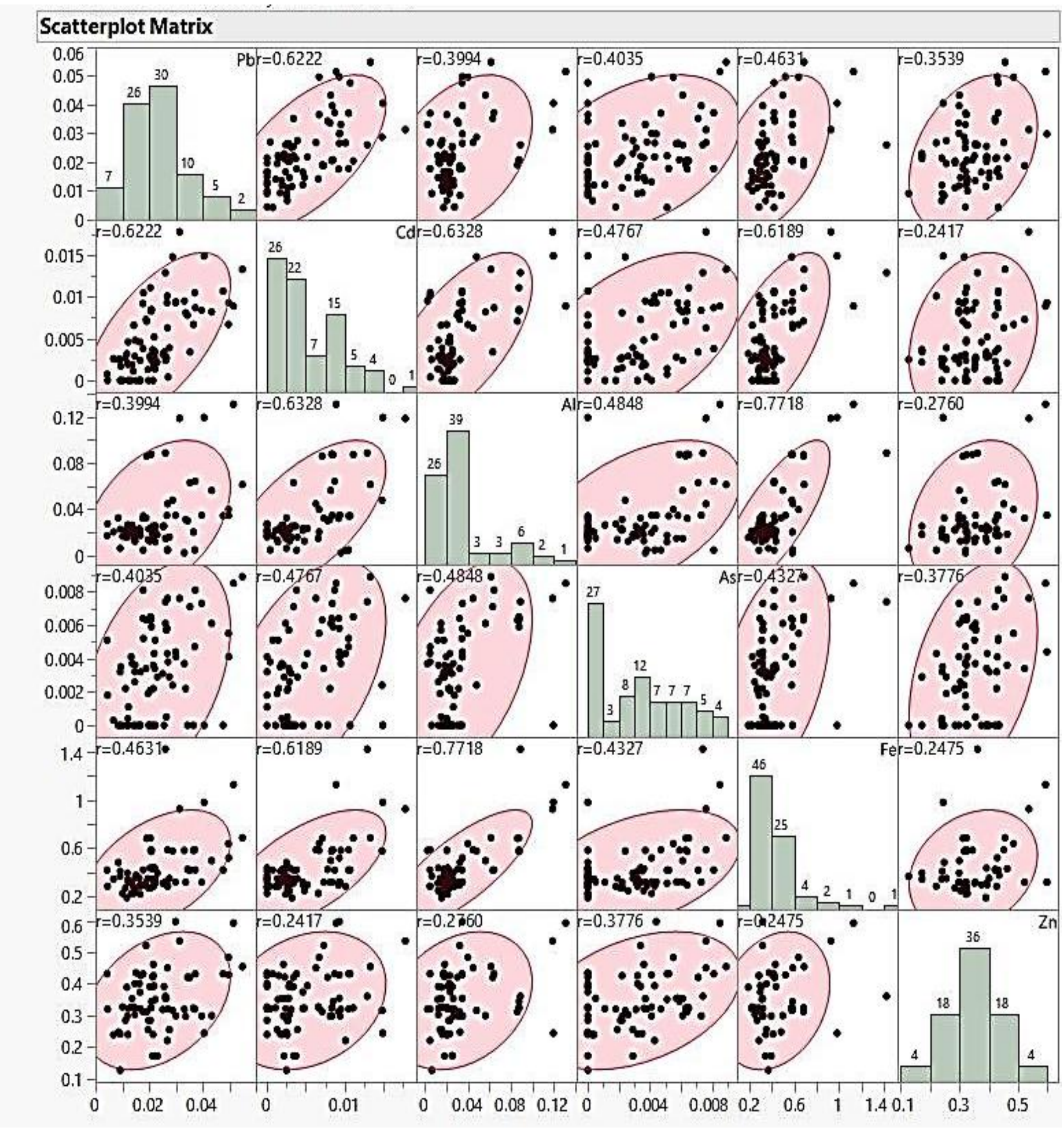

Figure 6.6 : Multivariate correlation between metals in all Butter samples

6.7 Distribution of metal in Cheese

Distribution of metals (Pb, Cd, Al, As, Fe and Zn) in all 80 Cheese samples have shown in **Fig. 6.7** and **Fig. 6.8.**

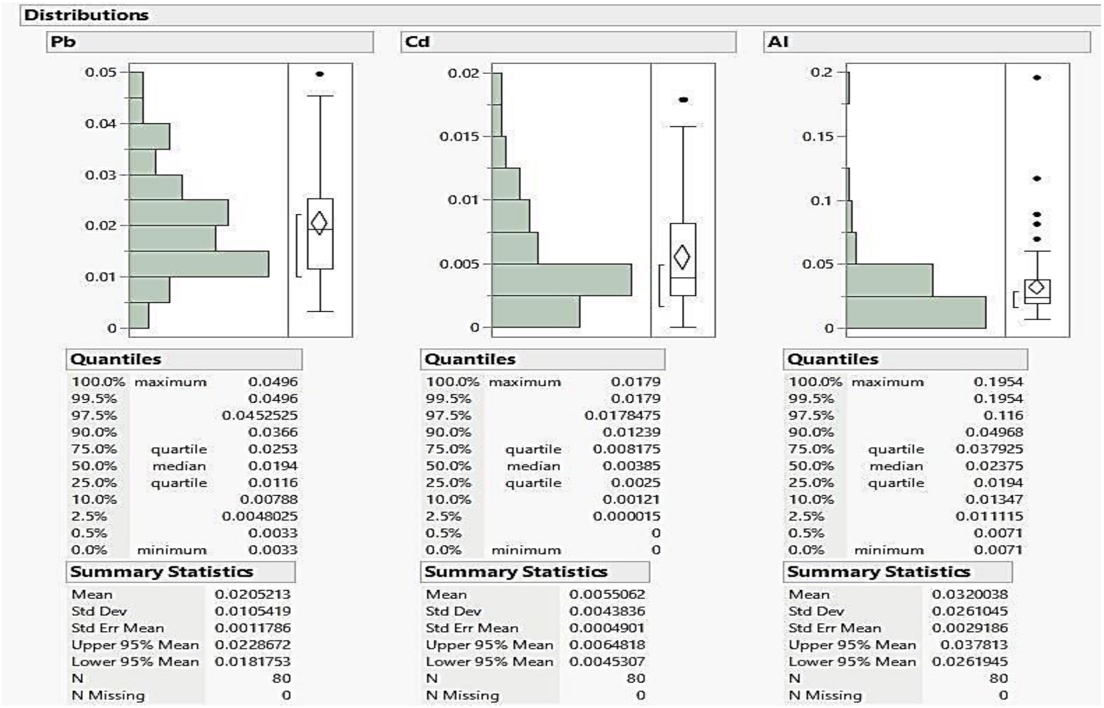

Figure 6.7 Distribution of Pb, Cd, and Al content in Cheese Samples

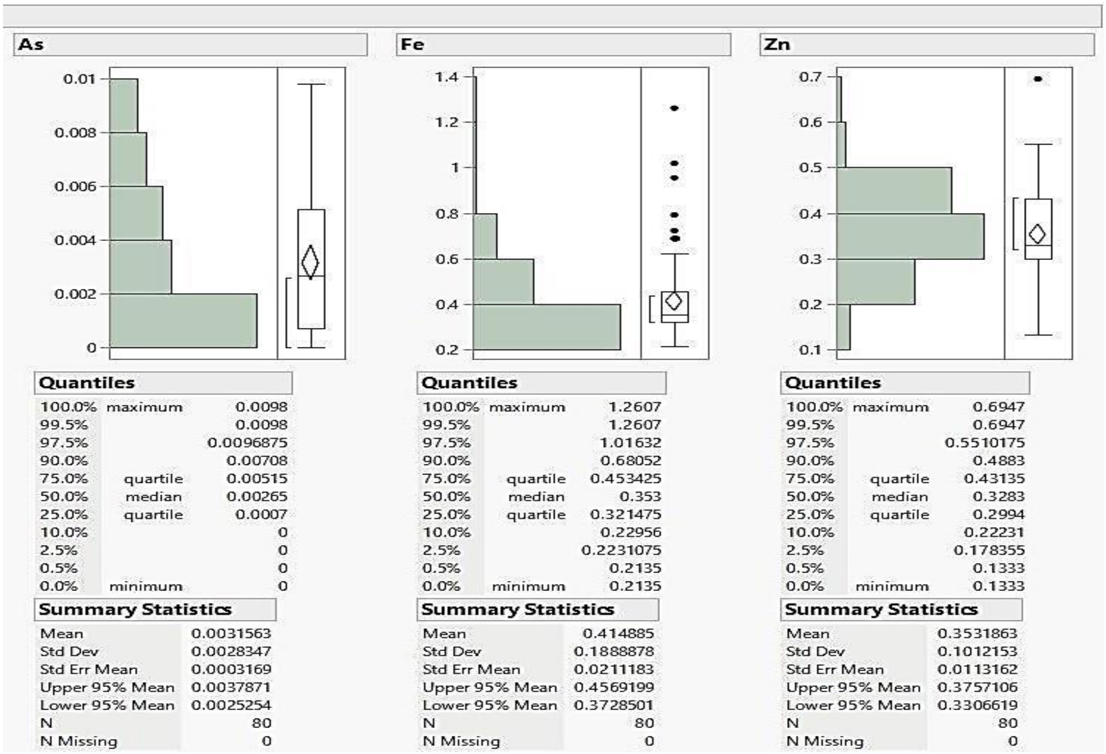

Figure 6.8: Distribution of As, Fe, and Zn content in Cheese Samples

Multivariate Correlation for Cheese samples

Multivariate correlation between all six metals for 80 cheese samples are presented in **Table 6.15** showing positive correlation.

Table 6.15 : Multivariate correlation between metal in all cheese samples

	Pb	Cd	Al	As	Fe	Zn
Pb	**1.0000**	**0.5265**	**0.4600**	**0.2471**	**0.4331**	**0.3177**
Cd	**05265**	**1.0000**	**0.5686**	**0.5301**	**0.5509**	**0.4809**
Al	**0.4600**	**0.5686**	**1.0000**	**0.4887**	**0.5970**	**0.4360**
As	**0.2471**	**0.5301**	**0.4887**	**1.0000**	**0.4735**	**0.2570**
Fe	**0.4331**	**0.5509**	**0.5970**	**0.4735**	**1.0000**	**0.3606**
Zn	**0.3177**	**0.4809**	**0.4360**	**0.2570**	**0.3606**	**1.0000**

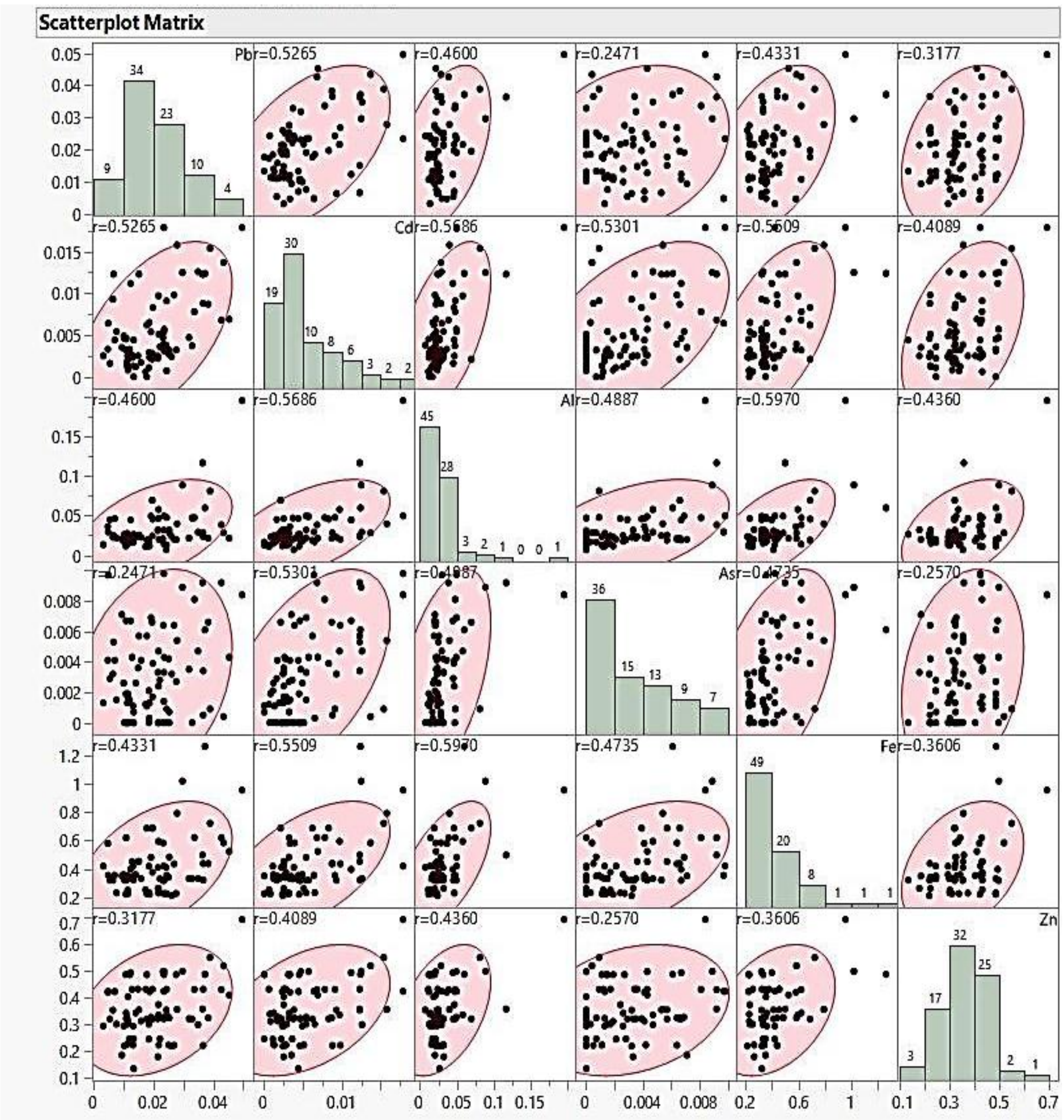

Figure 6.9 : Multivariate correlation between metals in all Cheese samples

6.8 Group Distribution

Distribution of metals (Pb, Cd, Al, As, Fe and Zn) in all 240 samples of Yoghurt, Butter and cheese are represented by **Fig. 6.10** and **Fig. 6.11.**

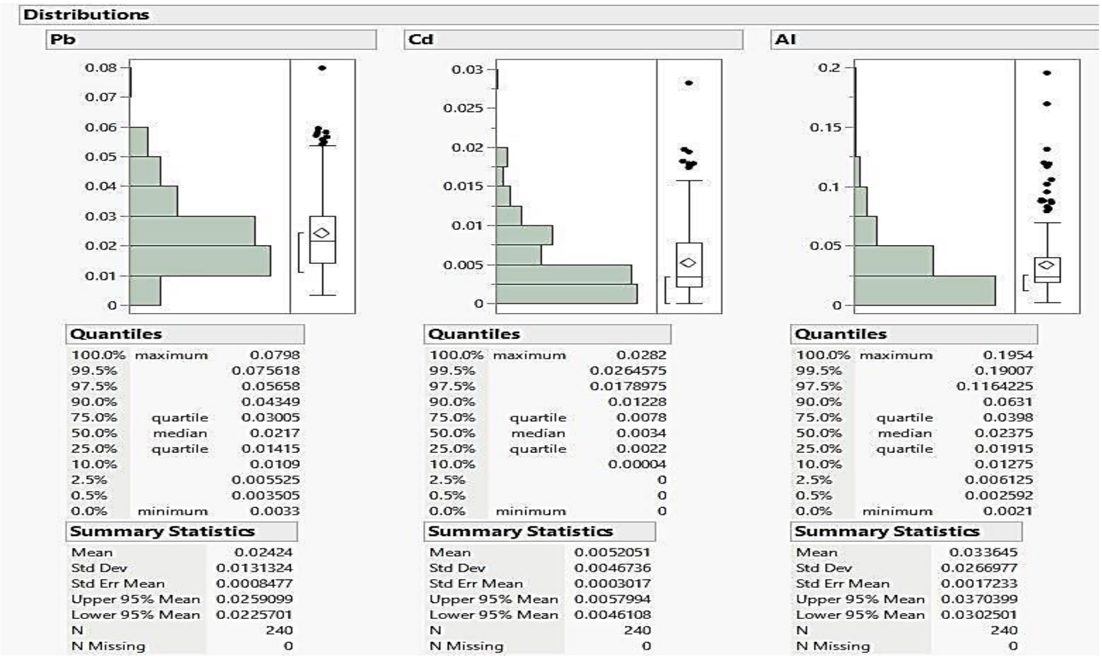

Figure 6.10: Distribution of Pb, Cd, and Al content in 240 Dairy Samples (Yoghurt, Butter and cheese)

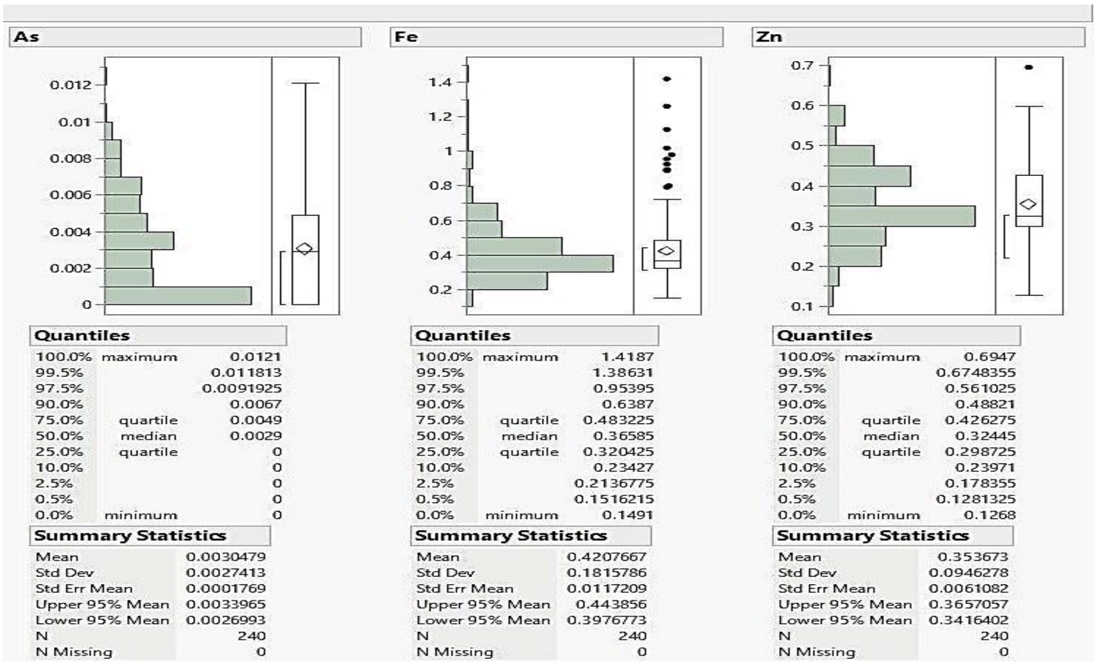

Figure 6.11 Distribution of As, Fe, and Zn content in 240 Dairy Samples (Yoghurt, Butter and cheese)

Table 6.16 shows the group statistics of yoghurt, butter and cheese samples, which includes 240 samples collected from Kota, Baran, Bundi and Jhalawar. A positive correlation is found among all metals and a strong correlation can be seen between Al- Fe (r = 0.7040). From the results it is concluded that heavy metals in 240 samples are depending on almost similar sources.

Table 6.16: Multivariate correlation between metal in all dairy products

	Pb	Cd	Al	As	Fe	Zn
Pb	1.0000	0.5024	0.3974	0.2841	0.4174	0.3336
Cd	0.5024	1.0000	0.6347	0.4497	0.5547	0.2568
Al	0.3974	0.6347	1.0000	0.5150	0.7040	0.3641
As	0.2841	0.4497	0.5150	1.0000	0.4876	0.3410
Fe	0.4174	0.5547	0.7040	0.4876	1.0000	0.3277
Zn	0.3336	0.2568	0.3641	0.3410	0.3277	1.0000

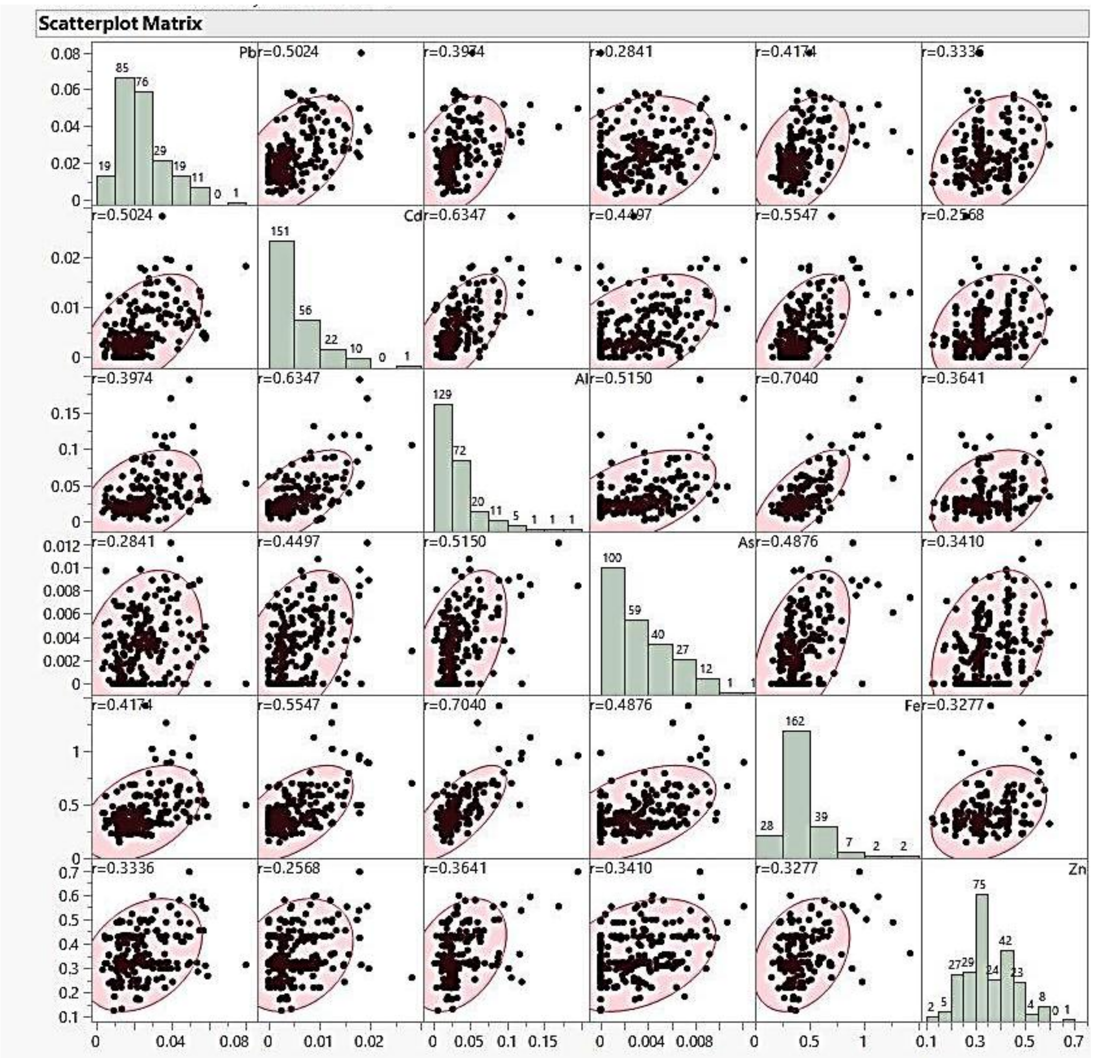

Figure 6.12 : Multivariate correlation between metals in all 240 dairy products

6.9 Health Risk Assessment

Estimated daily intake refers to the estimated amount of substances that a person consumes on daily basis. The related term health risk assessment is a quantitative measure used to evaluate the potential risk posed to health by various factors.

Our whole study is based on the assessment of heavy metals in three dairy products that is yoghurt, butter and cheese. Assessment has been done on the basis of average consumption of dairy products per day, which is 125 g/day for yoghurt, 15 g/day for butter and 28 g/day for cheese by adults [7] .

Estimated Daily Intake (EDI) and Health Risk Assessment (HRI)

Estimated Daily Intake (EDI) and Health Risk Index (HRI) are calculated to assess the health risk for consumers, due to intake of dairy products in all studied areas [8-10].

Table 6.17: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Yoghurt

Metals/ Area	Pb		Cd		As	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	6.63E-05	1.89E-02	1.64E-05	1.64E-02	6.08E-06	1.52E-03
Baran	5.80E-05	1.66E-02	1.11E-05	1.11E-02	4.95E-06	1.24E-03
Bundi	5.98E-05	1.71E-02	8.14E-06	8.14E-03	6.63E-06	1.66E-03
Jhalawar	5.86E-05	1.67E-02	6.80E-06	6.80E-03	6.21E-06	1.55E-03

Table 6.17 shows the estimated daily intake and health risk index of highly toxic metals. For all four district HRI ranges between 1.66E-02 to 1.89E-02 for Pb, 6.80E-03 to 1.64E-02 for Cd and 1.24E -03 to 1.66E-03 for As. Highest HRI values for Kota indicates the higher pollution level and so as highest potential risk to health.

Table 6.18: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Yoghurt

Metals/ Area	Al		Fe		Zn	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	9.79E-05	9.79E-05	1.03E-03	1.47E-03	7.23E-04	2.41E-03
Baran	7.88E-05	7.88E-05	8.32E-04	1.19E-03	7.25E-04	2.42E-03
Bundi	6.36E-05	6.36E-05	8.46E-04	1.21E-03	7.74E-04	2.58E-03
Jhalawar	6.21E-05	6.21E-05	8.39E-04	1.20E-03	7.85E-04	2.62E-03

Table 6.18 represents the EDI and HRI for Al, Fe and Zn. As from the table it is clear that intake of Fe through yoghurt is maximum in comparison to other metals. The highest value order for the same among the districts are Kota > Baran > Bundi >

Jhalawar. It is evident from both the tables that HRI for all metals does not exceeded from one which indicates no risk for consumers.

Table 6.19: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Butter

Metals/ Area	Pb		Cd		As	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	6.65E-06	1.90E-03	1.73E-06	1.73E-03	8.30E-07	2.08E-04
Baran	6.16E-06	1.76E-03	1.34E-06	1.34E-03	6.70E-07	1.68E-04
Bundi	5.71E-06	1.63E-03	1.14E-06	1.14E-03	9.88E-07	2.47E-04
Jhalawar	4.56E-06	1.30E-03	8.09E-07	8.09E-04	6.36E-07	1.59E-04

Table 6.20: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Butter

Metals/ Area	Al		Fe		Zn	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	1.14E-05	1.14E-05	1.39E-04	1.98E-04	8.92E-05	2.97E-04
Baran	7.66E-06	7.66E-06	9.51E-05	1.36E-04	8.52E-05	2.84E-04
Bundi	7.05E-06	7.05E-06	9.27E-05	1.32E-04	8.74E-05	2.91E-04
Jhalawar	6.49E-06	6.49E-06	9.57E-05	1.37E-04	8.50E-05	2.83E-04

Table 6.19 and **6.20** shows the EDI and HRI values for butter. On comparing EDI among all metals Fe is consumed maximum through butter and lowest consumed metal is As.

Tables revealed that among all four districts health risk index is found to be maximum in Kota, only HRI for As is maximum in Bundi.

The trend of HRI for Fe is Kota > Jhalawar > Baran > Bundi having values 1.98E-04 > 1.37E-04 > 1.36E-04 > 1.32E-04 and for As the order is Bundi > Kota > Baran > Jhalawar having values 2.47E-04 > 2.08E-04 > 1.68E-04 > 1.59E-04 respectively.

Table 6.21: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Cheese

Metals/ Area	Pb		Cd		As	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	1.19E-05	3.39E-03	3.37E-06	3.37E-03	1.84E-06	4.59E-04
Baran	1.02E-05	2.92E-03	2.81E-06	2.81E-03	1.17E-06	2.93E-04
Bundi	8.17E-06	2.33E-03	2.25E-06	2.25E-03	1.76E-06	4.40E-04
Jhalawar	8.08E-06	2.31E-03	1.85E-06	1.85E-03	1.12E-06	2.81E-04

Table 6.22: Estimated daily intake (EDI) and health risk index (HRI) of toxic metals in Cheese

Metals/ Area	Al		Fe		Zn	
	EDI	HRI	EDI	HRI	EDI	HRI
Kota	2.28E-05	2.28E-05	2.49E-04	3.55E-04	1.79E-04	5.98E-04
Baran	1.31E-05	1.31E-05	1.76E-04	2.51E-04	1.64E-04	5.46E-04
Bundi	1.20E-05	1.20E-05	1.75E-04	2.49E-04	1.59E-04	5.29E-04
Jhalawar	1.18E-05	1.18E-05	1.75E-04	2.50E-04	1.58E-04	5.25E-04

Table 6.21 and **6.22** shows the EDI and HRI results for cheese. These tables clearly indicate the highest HRI values for all metals in Kota district.

The above mention results explain the highest potential risk for all metals in kota district. Though these values of HRI are not exceeded for all metals than 1, demonstrates that there is no potential health risk for consumers.

Overall EDI & HRI

Table 6.23 : Overall Estimated daily intake (EDI) and health risk index (HRI)

Metal	Kota		Baran		Bundi		Jhalawar	
	EDI	HRI	EDI	HRI	EDI	HRI	EDI	HRI
Pb	2.83E-05	8.08E-03	2.48E-05	7.08E-03	2.46E-05	7.02E-03	2.37E-05	6.78E-03
Cd	7.16E-06	7.16E-03	5.10E-06	5.10E-03	3.84E-06	3.84E-03	3.15E-06	3.15E-03
As	2.92E-06	7.29E-04	2.26E-06	5.66E-04	3.12E-06	7.81E-04	2.66E-06	6.64E-04
Al	4.41E-05	4.41E-05	3.32E-05	3.32E-05	2.75E-05	2.75E-05	2.68E-05	2.68E-05
Fe	4.72E-04	6.74E-04	3.68E-04	5.25E-04	3.71E-04	5.30E-04	3.70E-04	5.28E-04
Zn	3.31E-04	1.10E-03	3.25E-04	1.08E-03	3.40E-04	1.13E-03	3.43E-04	1.14E-03

Table 6.23 represents the collective results of EDI and HRI for all dairy products taken for study.

6.10 Metal Pollution Index (MPI)

The Metal Pollution Index (MPI) is a quantitative measure used to evaluate the cumulative impact of multiple metals contaminant in a sample. It provides a simple value that reflects the combined concentration of multiple metals. This is the geometric mean of different concentrations which reduces the impact of extremely high and low concentration providing a balanced representation of overall metal pollution. High MPI values indicate greater level of metal pollution.

Table 6.24 : MPI results for Kota

Metals/Samples	Pb	Cd	Al	As	Fe	Zn	MPI
Yoghurt	**0.0318**	**0.0079**	**0.0470**	**0.0029**	**0.4932**	**0.3471**	**0.0422**
Butter	0.0266	0.0069	0.0458	0.0033	0.5542	0.3569	0.0418
Cheese	0.0254	0.0072	0.0490	0.0039	0.5328	0.3841	**0.0437**

Table 6.25 : MPI results for Baran

Metals/Samples	Pb	Cd	Al	As	Fe	Zn	MPI
Yoghurt	0.0278	0.0054	0.0378	0.0024	0.3996	0.3481	0.0348
Butter	0.0246	0.0054	0.0306	0.0027	0.3803	0.3409	0.0332
Cheese	0.0219	0.0060	0.0280	0.0025	0.3770	0.3511	0.0325

Table 6.26 : MPI results for Bundi

Metals/Samples	Pb	Cd	Al	As	Fe	Zn	MPI
Yoghurt	0.0287	0.0039	0.0305	0.0032	0.4061	0.3716	0.0341
Butter	0.0229	0.0046	0.0282	0.0040	0.3709	0.3497	0.0336
Cheese	0.0175	0.0048	0.0258	0.0038	0.3740	0.3400	0.0316

Table 6.27 : MPI results for Jhalawar

Metals/Samples	Pb	Cd	Al	As	Fe	Zn	MPI
Yoghurt	0.0281	0.0033	0.0298	0.0030	0.4026	0.3770	0.0325
Butter	0.0182	0.0032	0.0259	0.0025	0.3828	0.3400	0.0280
Cheese	0.0173	0.0040	0.0253	0.0024	0.3757	0.3375	0.0282

Table 6.24 shows the MPI results of all three dairy products for Kota. The highest MPI was found for cheese followed by yoghurt and butter. From **Table 6.25**, **6.26**, **6.27** for Baran, Bundi and Jhalawar suggest the higher MPI for yoghurt which seems to be different from the results of Kota.

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CONCLUSION

The objective of this study was to evaluate the concentration of heavy metals in dairy samples (yoghurt, cheese and butter) using atomic absorption spectrophotometer. It also aimed to minimize the analysis time by using microwave digestion.

The analysis revealed that all six metals are found in yoghurt, cheese and butter samples not only in the areas of great anthropogenic activities but also in the areas which seems to be less contaminated. The primary objective was to assess the heavy metals in water , soil and fodder samples and their results indicate substantial contamination. Our study provides valuable insight into translocation of heavy metals from soil to fodder and fodder to milk, though it is very low and bio accumulation factor (BAF) was also found to be less than one, from which it can be concluded that there is only absorption and no accumulation of heavy metals occur in plant and soil.

Among the districts highest contamination level of heavy metals is found in Kota district.

A significant impact of industrialization and urbanization can be seen from the study as the results of heavy metal concentration of more polluted areas are exceeded the permissible limits set by the various regulatory bodies. Heavy metal concentration in all the samples of less polluted areas were found to be below the permissible limit.

For statistical evidence one way ANOVA test was performed followed by Tukey- Kramer HSD using jmp software. Analysis was done city wise (Kota, Baran, Bundi and Jhalawar), pollution status wise (more and less polluted) and type wise (IF and LS). No significant difference was observed in city wise and type wise analysis. On performing Tukey-Kramer all circles share the same place. But a significant difference has been observed in pollution status wise analysis.

This study aimed to examine the effect of processing and packaging with time. The investigation revealed a little increase in metal concentration over the period of time. From that it can be concluded that leaching of metals might be occur from the packing materials, though there is a very little increase in the concentrations.

This study also aimed to evaluate the concentration of heavy metals in various samples and assess whether these levels fall with in established safety limits. On comparing our results with RDA values, the data indicated that all measured values for more polluted areas are exceeded the permissible limit and the values for less polluted areas are below the permissible limits.

This study aimed to assess the health risk associated with heavy metal exposure using Health Risk Index (HRI) and Metal Pollution Index (MPI) to evaluate potential health risk on human health.

The analysis revealed that HRI values for all six metals were below the threshold indicating a low risk to human health while MPI levels indicated a moderate level of pollution across the sample sites suggesting that there is a contamination but it is not critical.

This study indicates that according to HRI and MPI, immediate risk to human health is low but ongoing monitoring and effective measures can be taken to prevent future risk and long term safety.

Recommendations

1. More sampling and analysis must be done and find out the trends in heavy metal concentrations with time.
2. Regulatory standards for permissible limit should be reviewed and updated time to time.
3. Educate people by organizing awareness programmes and training programmes for framers regarding environmental pollutions and ways to reduce the exposure.
4. Waste management should be improved and pretreatment of industrial waste must be done before releasing it into an environment.
5. Safe packaging materials must be used.
6. Hygiene , sanitation protocols and processing facilities in dairy farms must be followed.

By implementing these recommendations, it is possible to manage the heavy metal pollution in dairy products, improve consumers health and get a more safer and sustainable environment.

SUMMARY

SUMMARY

Dairy products are good source of macro and micro elements, it also contains a small quantity of other metals, that are harmful to humans even if they are present in small amount. Dairy products get contaminated by various natural phenomenon and different anthropogenic activities. Due to land pollution and water pollution, soil gets contaminated which is a major caused of heavy metals contamination in plants and crops.

Animals get contamined who graze on such crops as heavy metals are absorbed in the animal tissues. Due to this milk get contaminated so as dairy products. Some other factors like manufacturing procedure, handling, packaging, storage and transportation are also responsible for heavy metal contamination in dairy products. As a by-product of mammary gland, milk may contain a variety of xenobiotics. Due to which heavy metals are present in milk products can cause a serious risk to human health. Due to various and dynamic industries in Kota division are expected to have lead to a higher level of heavy metal contamination.

The current study was carried out on dairy products (Yoghurt, Butter and Cheese) samples which were collected from individual farms and local shops of more polluted and less polluted areas of the Kota, Baran, Bundi and Jhalawar. The objective of the work is to find the levels of heavy metals in dairy products in Kota division, Rajasthan, India and aims at finding out whether levels are below or above the optimum level needed for human and also the risk associated with human health.

Main Objectives of the Research Work

1. To assess the hazardous concentration of heavy metal in soil, water, and fodder samples in order to examine the translocation of metals from these sources to dairy products.
2. To investigate the concentration of heavy metals in various samples of yoghurt, butter and cheese, collected from the different areas of Kota division, Rajasthan.
3. To determine the effect of manufacturing procedures and packaging material on dairy products.
4. To compare the recommended dietary allowance (RDA) values established by several national and international organizations with the metal ion concentration found in dairy products.
5. To analyze the data using statistical analysis, correlation analysis.
6. To evaluate the health risk analysis via estimated daily intake (EDI), metal pollution index (MPI), and health risk index (HRI).

   The thesis **“Microwave Assisted Digestion of Milk Based Dairy Product for Determination of Heavy Metals with Atomic Absorption Spectrophotometer”** is divided into six chapters.

Structure of Thesis: The present work includes issues related to the contamination of toxic metals in the dairy products (yoghurt, butter and cheese) of more and less polluted areas of the Kota division, Rajasthan. From the structural point of view, the thesis consists of six chapters in the following manner:

Chapter I includes a basic overview of the topic and present developments in the research field. This chapter also covers the origins of the research problem and the scope of the work. The elements found in dairy products and their functions in the human body are covered in this chapter. This chapter also explains the production and use of milk and dairy products. The biological role of heavy metals in this ecosystem, along with their characteristics and classification, are elucidated in this chapter.

The source of heavy metal contamination and its hazardous effect are outlined in this chapter. It also describes how they came to the animal body. It also describes the impact of some heavy metals on human health and their toxicity mechanism. This chapter also includes studies on milk and dairy products contamination by different researchers all over the world.

Chapter II includes a detailed description of various chemicals and equipment used for the study, also describes the study area or sample collection sites and their characteristics designed for the research work, which includes the individual farms and local shops of less polluted area having less anthropogenic activities and more polluted areas where anthropogenic activities are found to be more.

This chapter also includes the recovery test for various wet, dry and microwave digestion methods to ensure the method validation parameters. The highest recovery valued method is adopted for the whole study.

This chapter includes details of different parameters like EDI (estimated daily intake), MPI (metal pollution index), and HRI (health risk index) to assess human health risk. EDI is calculated by using metal concentration, dairy products consumed by a person and the average body weight of an adult in India. Metal pollution index is obtained by calculating the geometrical mean of concentrations of metals. The health risk index is calculated as a ratio of estimated daily intake and the oral reference dose R_fD. HRI indicates potential health risk when it is ≥ 1 (equal or higher than 1).

Formulas to analyze the statistical parameter regarding concentration minimum, maximum, arithmetic mean standard deviation, variance and detailed instrumental analysis also given in this chapter. The results of elemental concentrations of heavy metals in Soil, Water, Fodder and milk samples that were collected from the same collection points are also reported in this chapter. This analysis is carried out to check the translocation of metals from these sources to dairy products.

Chapter III include a complete data representation, and statistical analysis of yoghurt samples taken from all selected locations of Kota division. Selection sites are chosen where untreated city effluent is used as the source of irrigation. Mostly city effluents in the selected areas are contaminated with industrial effluents. For the entire study six metals (Pb, Cd, Al, As, Fe, and Zn) are used that are often utilized in industries. Out of these metals some metals are extremely dangerous even if they are present in small amount.

The elemental concentrations of selected heavy metals are assessed by Atomic Absorption Spectrophotometer (Model: Shimadzu-6300AA) in yoghurt samples collected from the individual farms and local shops of more and less polluted areas of Kota, Baran, Bundi and Jhalawar. The result trend shows that the mean concentration of all the metals is maximum in the yoghurt samples taken from local shops of more polluted areas and lowest in sample of individual farms of less polluted areas.

The manufacturing and packaging effect is also analyzed with time in this chapter. To assess the effect of packaging with time in different yoghurt samples (yoghurt drink, yoghurt, flavored yoghurt). Samples were taken out at 0, 7 and 15 days. Results clearly indicate that there is a slight increase in metal concentration with time. It might be due to the packaging material and due to added fruits as fruit syrup.

Correlation matrix and data processing will be done through MS Excel 2016 software and JMP software. For this study one way ANOVA is performed to determine the statistical evidence and significant difference among the cities taken, the pollution status and the types. To prove the null hypothesis of no difference and difference between the heavy metal concentration, means for all pairs of groups, a Tukey – Kramer test was also done.

One way ANOVA for Al in Yoghurt

S.No.	Variable	α	p - value	Null Hypothesis
1	City Wise	0.05	0.6860	Significantly indifferent , Null Hypothesis can't be rejected
2	Pollution Status	0.05	0.0002	Significantly different , Null Hypothesis can be rejected
3	Types (IF & LS)	0.05	0.4459	Significantly indifferent , Null Hypothesis can't be rejected

Analysis of variance for Al also shows the probability > 0.6860 which is greater than α = 0.05 and Tukey – Kramer test also shows that the circles are showing almost same area, which proves that the mean concentration of heavy metals in all the cities are significantly indifferent, the comparison has been made between more polluted and less polluted area with the help of one way analysis. This analysis shows that there is a significant difference between both of them, as a P - value > 0.0002. The P – value for ANOVA test is 0.4459 which also shows that the concentration of both the places (IF and LS) are significantly indifferent.

Chapter IV comprises a complete data representation, and statistical analysis of butter samples taken from all same selection sites of Kota division and similar six metals (Pb, Cd, Al, As, Fe, and Zn) are used for the study. The elemental concentrations are assessed by Atomic Absorption Spectrophotometer (Model: Shimadzu-6300AA) in Butter samples. The result trend shows that the mean concentration of all the metals is maximum in the samples taken from local shops of more polluted areas and lowest in the sample of individual farms of less polluted areas.

The manufacturing and packaging effect in butter samples of different brands are also analyzed with time. From the results it observed that there are some changes in concentration occurred with period of time. Correlation matrix and data processing will be done through MS Excel 2016 software and JMP software. Pearson's correlation coefficient (r) is calculated in order to explore any inter-relations between studied heavy metals in butter samples.

For this study one way ANOVA is performed to determine the statistical evidence and significant difference among the cities taken, the pollution status and the types. For all three variables, statistical interpretation one-way analysis of variance and Tukey – Kramer HSD test carried out for heavy metals in butter and results clearly indicates the city wise and type wise variable are significantly indifferent while the pollution status wise variable is significantly different. Null hypothesis can't be rejected for city wise and type wise and can be rejected for pollution status wise. Tukey-Kramer HSD test also support the data.

Chapter V comprises a complete data representation and statistical analysis of cheese samples collected from same selection sites . The concentrations of Pb, Cd, Al, As, Fe, and Zn in the cheese samples were determined using an Atomic Absorption Spectrophotometer (Model: Shimadzu-6300AA). The results shows almost same trend like yoghurt and butter samples. To assess the manufacturing and packaging effect with time in cheese of different brand were taken. The results clearly indicate the significant difference occurs in the concentration of metals during storage period of 0 to 30 to 90 days.

Correlation matrix and data processing will be done through MS Excel 2016 software and JMP software. Pearson's correlation coefficient (r) is calculated in order to explore the inter-relations between studied heavy metals in cheese samples.

For this study one way ANOVA is performed to determine the statistical evidence and significant difference among the cities taken, the pollution status and the types. To prove the null hypothesis of no difference and difference between the heavy metal concentration means for all pairs of groups, a Tukey – Kramer test was also done.

Analysis of variance for Al also shows the probability > 0.3936 which is greater than $\alpha = 0.05$ and according to Tukey – Kramer, Baran, Bundi, Jhalawar. Table shows the mean concentration of heavy metals in all the cities are significantly indifferent. The comparison has been made between more polluted and less polluted area with the help of one way analysis, which shows that there is a significant difference between both of them, as a p value is > 0.0300 .

The results of ANOVA test in types wise (IF and LS) shows that the p value is 0.3237, which also shows that the concentration of both the places are significantly indifferent.

Chapter VI includes the results of mean concentration of heavy metal in yoghurt, butter and cheese samples are compared with RDA (recommended dietary allowance) values. Results shows that how the mean concentration of heavy metals differ from RDA values, established by various national and international organizations. It is observed that the concentrations of heavy metals in the samples from each sites vary, which indicate that the heavy metal concentration is dependent on the places where animals graze and drink water. Manufacturing, storage, packaging and transportation also affects the concentrations as well.

In this Chapter, we calculate health risk assessment for potentially toxic metals Pb, Cd, and As. To evaluate the risk associated with human health due to the consumption of yoghurt, butter and cheese in all studied areas, Estimated daily intake (EDI), Hearth Risk Index (HRI), and Metal Pollution index (MPI) are calculated.

The result trend shows that the Estimated daily intake of Pb, Cd, As, Al, Fe and Zn ranges between 2.37E-05 to 2.83E-05, 3.15E-06 to 7.16E-06, 2.66E-06 to 3.12E-06, 2.68E-05 to 4.41E-05, 3.68E-04 to 4.72E-04 and 3.25-04 to 3.43E-04 respectively.

The Hearth Risk Index (HRI) values of Pb, Cd, As, Al, Fe and Zn range between 6.78E-03 to 8.08E-03, 3.15E-03 to 7.16E-03, 5.66E-04 to 7.81E-04, 2.68E-05 to 4.41E-05, 5.25E-04 to 6.74E-04 and 1.08E-03 to 1.14E-03. It has to be noted that none of the above six metals have HRI greater than unity for all locations.

This study, therefore, indicates that Pb, Cd, As, Al, Fe and Zn are frequently found in dairy products not only in more polluted areas but also in the areas which seem to be less polluted. This could be due to soil, fodder contamination, and climatic factors which contaminates the environment vegetation, and due to packaging and storage.

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Comparison of Dry, Wet and Microwave Digestion Procedures for the Determination of Heavy Metal in Yoghurt Samples

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***Abstract:** This work proposes a novel technique for utilizing the most suitable digestion process to determine concentrations of heavy metals such as Pb, Cd, Al, As, Zn, and Fe in Yoghurt samples using AAS. Conventional digestion techniques often involve time-consuming and tedious procedures, which leads to the loss of analyte and reduced accuracy. In this study, we propose the utilization of microwave-assisted digestion, a rapid and efficient technique that offers complete digestion of the samples and minimizes the risk of analyte loss.*

In order to get optimal efficiency and precision, this study outlines the optimization of the microwave-assisted digestion parameters, such as power, time, and reagent concentrations. The proposed method was validated by calculating the recovery % among dry, wet and microwave digestion methods. Results show that among all, the recovery percentage was found to be highest in the microwave digestion method.

***Keywords:** Heavy metals, yoghurt, AAS, Microwave digestion.*

I. INTRODUCTION

Dairy products are a necessary component of the human diet, a number of environmental and processing conditions may cause heavy metal contamination in them [1,2]. Heavy metals such as lead, cadmium, and mercury may enter in dairy products from many different kinds of sources including soil, water, and agricultural procedures [3]. When heavy metals present in excess, including lead, cadmium, mercury, and arsenic can be extremely harmful to one's health [4]. In order to protect the public's health and uphold customer confidence, milk and dairy product quality and safety must be ensured. Because heavy metals are poisonous and can build up in the food chain, they constitute one of the biggest potential risks [5-7].

For the purpose of protecting the public's health, precise and effective techniques for heavy metal analysis in dairy products are thus necessary [8]. One common characteristic of traditional methodologies for heavy metal analysis in dairy products is that it takes more time for sample preparation.

This may result in insufficient digestion and complicate to precisely measuring the metal concentration by the use of atomic absorption spectrophotometry (AAS). To overcome these challenges, a novel approach utilizing microwave-assisted digestion has been developed with the aim of increasing the efficiency of dairy product digestion for precise heavy metal detection via AAS [9-11]. This new approach has been proposed that utilizes microwave-assisted digestion, which is an advanced technique for sample preparation that breaks down complex matrices fast and efficiently with the help of microwave energy [12, 13].

Compared to traditional methods, this technology ensures complete and thorough digestion of dairy samples in a fraction of the time by considerably speeding up the digestive process. As a result, there is a significantly lower chance of incomplete digestion, which enhances accuracy and precision in the heavy metal analysis that follows using AAS. For the detection of heavy metals, the atomic absorption spectrophotometry method provides good sensitivity and selectivity [14-16]. It is based on the idea of detecting the wavelengths of light that metal atoms in a vapour state absorb. Heavy metal concentrations in the dairy samples that have been digested can be precisely and reliably measured by measuring the quantity of light absorbed.

The main aim of this study is to compare the digestion techniques to analyse six heavy metals (Pb, Cd, Al, As, Fe and Zn) in Yoghurt samples collected from different areas of Jhalawar district, Rajasthan. In this paper, we report microwave digestion as the best technique among dry, wet and microwave digestion methods. A detailed methodology of the innovative microwave-assisted digestion approach and its applications are mentioned.

II. MATERIALS AND METHODS

A. Materials: Yoghurt Samples Collection

A total of 20 Yoghurt samples were collected from more polluted and less polluted sites in Jhalawar district. Individual farms and local shops in these areas were taken into account. All the Yoghurt samples were collected in PTFE bottles and kept at -20°C in a deep freezer until analysis.

B. Digestion Reagents

1) Concentrated Nitric Acid (65% HNO_3)
2) Hydrogen peroxide (30% H_2O_2)
3) Deionized or Distilled Water
4) To prepare calibrated standards, a stock standard solution containing 1000 mg L^{-1} of each element was utilized. Just before analysis, the calibrated solutions were made from the stock solution using deionized water.

C. Digestion Techniques

The dairy product samples were subjected to three different types of digesting processes: dry, wet, and microwave digestion. The digestion procedures are given below.

1) Dry Digestion

One gram of Yoghurt sample was placed in a porcelain crucible and dried in a furnace at 100 ˚C. A gradual increase in temperature from 100˚C to 500˚C was made. After ashing the material for around seven or eight hours, a white or grey ash residue was obtained. 5 mL of 65% v/v HNO_3 was used to dissolve the residue and the mixture was slowly heated to further dissolve the residue. After that, the mixture was poured into a 10-mL volumetric flask and brought to volume. A blank digest was carried out in the same way.

2) Wet Digestion

One gram of Yoghurt sample was treated with 5 ml of nitric acid (65% HNO_3) and 2 ml of hydrogen peroxide (30% H_2O_2) and digested at 90ºC temperature on an electric hot plate. After that, the temperature increased up to 120ºC until brown fumes disappeared, which indicated the completion of oxidation of organic matter. The organic matrix of Yoghurt was destroyed and left the elements into a clear solution, after cooling, the clear solution was filtered into a volumetric flask of 25 ml capacity and made to the mark with DI water, and finally, the milk samples were ready to analyze by AAS.

3) Microwave Digestion

One gram of yoghurt sample was digested with 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) in the microwave digestion system. The digestions of samples were carried out at different conditions summarized in Table 1. Resulted solution was transferred into 10 mL volumetric flask and diluted with deionized water. A blank digest was carried out in the same way. All sample solutions were clear.

TABLE I
Microwave Digestion Conditions

Step	Time (min)	Power (W)
1	2	200
2	2	200
3	4	400
4	6	400
5	8	600
6	8	VENT

4) Method Validation

To get the best method among the dry, wet and microwave digestion methods, we conduct a recovery test in which a known concentration (spiked concentration) was added to the sample. The quantities for the spike chosen were only 5-8% to ensure that there would be no significant change from the amount normally present in the main sample. After analyzing these samples by AAS the recovery percentages were calculated by the using of following equation[17]:

$$\% \, recovery = \frac{\text{Conc. in spike sample} - \text{Conc. in sample}}{\text{Amount spiked in sample}} \times 100$$

The results are shown in Table 2 and were obtained using the above procedure to determine the various recovery percentages for various approaches.

TABLE II

Comparison of heavy metal contents (mg/L) in yoghurt samples determined by AAS after digestion using three different methods, n = 5

Metal	Dry ashing M1	Recovery (%)	Wet ashing M2	Recovery (%)	Microwave M3	Recovery (%)
Pb	0.1121	91.95	0.1125	94.63	0.1129	97.31
Cd	0.0268	82.61	0.0272	88.41	0.0278	97.10
Al	0.0886	91.96	0.0891	93.30	0.0898	95.17
As	0.0283	87.5	0.0284	89.58	0.0286	93.75
Fe	1.1901	91.26	1.2008	93.17	1.2189	96.40
Zn	0.8242	89.20	0.8269	90.14	0.8427	95.64

Table 2 shows that the recovery percentage for microwave digestion is greater than that of both dry and wet digestion methods. In dry digestion method it ranges from 82.61 % - 91.96 %, in wet digestion method it ranges from 88.41 % - 94.63 % where is in microwave digestion method it ranges from 93.75 % - 97.31 %. The approximate time required for dry, wet and microwave digestions were 8 hr, 3 hr and 30 min, respectively. In light of these results, the microwave digestion procedure was found to be the best digestion method and chosen for the whole study.

5) Advantages of the Proposed Method

By using this suggested approach, labs may effectively analyze several dairy product samples for the presence of heavy metals, ensuring accordance to food safety regulations and protecting the general public's health. The benefits of the suggested approach are shown in Figure 1 below.

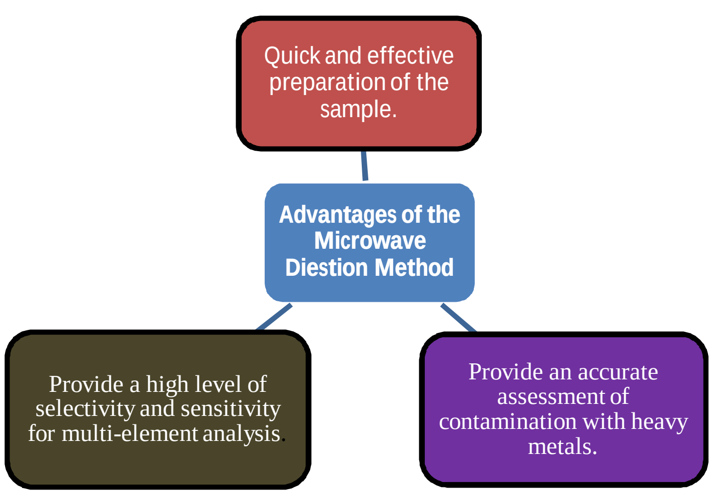

Figure 1: Advantages of proposed method

III. RESULTS AND DISCUSSION

After selecting the digestion technique, study is carried out on yoghurt samples collected from Jhalawar district. Two areas were selected on the basis of more and less pollution. Individual farms and local shops from both the areas were taken for heavy metals analysis. Table no. 3 shows, the concentrations of Pb, Cd, Al, As, Fe and Zn in yoghurt samples analyzed with the help of AAS.

TABLE III

Concentration of metals (mean ± SD) in yoghurt samples of IF and LS from more and less polluted areas of Jhalawar district.

Heavy Metals/ Area	More polluted		Less polluted	
	IF	LS	IF	LS
Pb	0.0369±0.0143	0.0446±0.0134	0.0189±0.0046	0.0191±0.0021
Cd	0.0041±0.0019	0.0053±0.0040	0.0014±0.0013	0.0023±0.0014
Al	0.0366±0.0165	0.0405±0.0164	0.0179±0.0075	0.0181±0.0043
As	0.0041±0.0017	0.0040±0.0018	0.0017±0.0017	0.0021±0.0017
Fe	0.4096±0.0807	0.5435±0.1016	0.3256±0.0788	0.3318±0.0892
Zn	0.4028±0.0849	0.4185±0.0980	0.3316±0.0911	0.3549±0.0864

IF : Individual farms , LS : Local shops

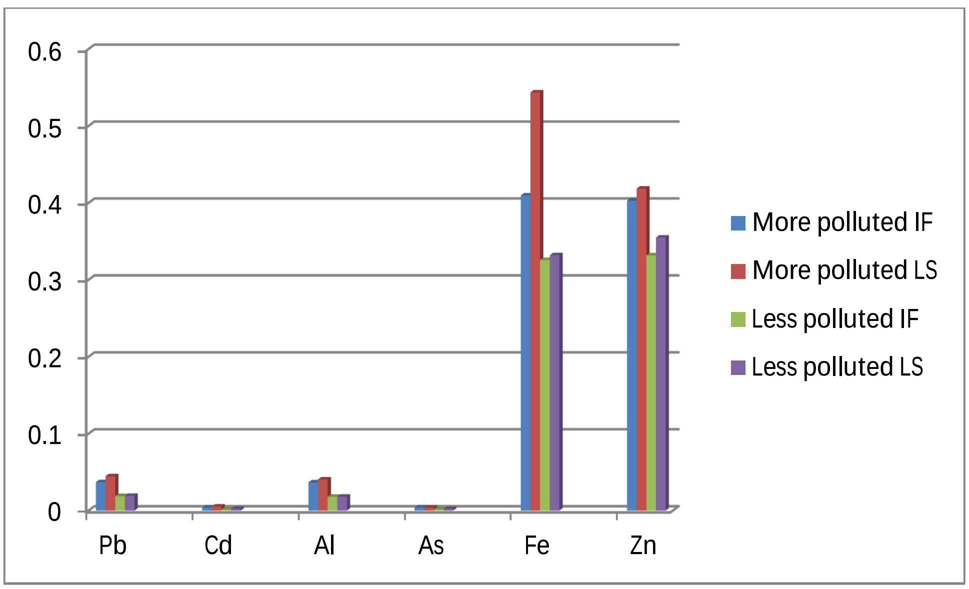

Figure 2: Graphical representation of Mean Concentration of metals in yoghurt samples of IF and LS from more and less polluted areas of Jhalawar district

Table no.3 reveals that the mean concentration of metals are higher in the samples collected from local shops than that of individual farms in both the areas . For more polluted area , mean concentration of lead in IF is 0.0369 mg/L whereas for LS it is 0.0446 mg/L . On the other hand these values for less polluted areas 0.0189 mg/L and 0.0191 mg/L respectively. Similar trend can be seen for all metals.

The permissible limit for Pb , Cd , Al , As , Fe and Zn are 0.02 mg/L , 0.003 mg/L, 0.02 mg/L, 0.01 mg/L , 0.37 mg/L and 0.328 mg/L respectively . On comparing our results with above RDA values, it reveals that mean concentrations of yoghurt in more polluted areas slightly exceeded the maximum permissible limit . Whereas the mean concentration in less polluted areas are within the permissible limit. But concentration of Arsenic was found to be below the detection limit in all samples.

IV. CONCLUSIONS

Based on analysis following conclusions are drawn: The dry and wet digestion methods are more time consuming and complicated than the microwave digestion method. The microwave heating provides complete sample digestion and reduces the overall analysis time providing better and safer method for sample preparation. The combination of microwave digestion with AAS provides high accuracy and sensitivity for heavy metal analysis in yoghurt samples. The results shows that the mean concentration of heavy metals is found comparatively higher in more polluted areas. The above study clearly indicates that the concentration of heavy metals in yoghurt influenced by the anthropogenic activity.

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Microwave Assisted Digestion for Cadmium Analysis in Yoghurt Samples Using Atomic Absorption Spectrophotometer

Aarti Bansal[1], Monika Dakshene[2, *], Ram Bilas Meena[3]

Abstract

This paper presents an innovative approach for the digestion of dairy products aimed at determining heavy metal concentrations using atomic absorption spectrophotometry. The present study was conducted to assess the concentration of cadmium in different Yoghurt samples. A total of 80 random samples of dairy products (20 each of sour Yoghurt, sweet Yoghurt, flavoured Yoghurt, and homemade Yoghurt) were collected from different areas of Kota city. For the analysis of heavy metals, a microwave-assisted digestion method was employed for the digestion of the collected samples, which were further analyzed by atomic absorption spectrophotometry. The results obtained revealed that the cadmium content (mean ± SD) in sour, sweet, flavoured, and homemade Yoghurt samples was found to be 0.0201 ± 0.0038, 0.0169 ± 0.0052, 0.0347 ± 0.0096, and 0.0050 ± 0.0041 mg/L respectively. According to these findings, the highest value of cadmium was found in flavoured Yoghurt. The study revealed that the cadmium levels in the samples exceeded the maximum permitted level of 0.0026 mg/L set by the World Health Organization (WHO). This study provides a valuable contribution to the field of food safety analysis, offering a reliable and efficient method for monitoring heavy metal contamination in dairy products and ensuring consumer protection.

Keywords: Yoghurt, heavy metals, atomic absorption spectrophotometry, microwave digestion

INTRODUCTION

The most essential food for human nutrition, consumed by both adults and children worldwide, are milk and dairy products [1]. Dairy products manufactured from milk are regarded as almost complete diet as they are an excellent source of proteins, lipids, vitamins, minerals, and carbohydrates that body requires for nutrition [2,3]. Safety of dairy products are compromised when they come into contact with harmful environmental pollutants. The purest form of milk and its products must be created, but they are contaminated by certain human activities, environmental pollutants, and the processing techniques used [4,5].

The main causes of heavy metal pollution in soil and water are industrial effluent and environmental contamination. Plants absorb heavy metals from contaminated soils that have been caused by human activity, and these metals then build up in their tissues [6]. These metals are also accumulated in the tissues and milk of animals that feed on these plants and drink contaminated water. Heavy metals are present in food when contaminated milk and its derivatives are consumed. Heavy metals can also find their way into milk and its products through food, water, production processes, and packaging

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[7]. Drinking milk contaminated by various sources might be detrimental to one's health.These heavy metals can have extremely harmful effects on consumers and cause a range of health conditions when their concentrations are higher than the maximum permitted limits [8,9].

One of the heavy metal, Cadmium has widespread application in the battery, PVC stabilizer, alloy, and pigment sectors [10]. Cadmium is used extensively in a number of industrial processes, including the manufacturing of fertilizers, nonferrous metals, iron and steel, cement, and fossil fuels [11].Organic matter in soils has a considerable adsorptive capacity for cadmium. Cadmium can be quite harmful when it is found in soils because food will absorb more of it [12]. One of the metals that is most dangerous to humans is cadmium [13]. Long-term exposure to radiation causes normal cells to change into cancerous cells [14]. Increased amounts of cadmium reduce sperm count and cause infertility [15]. Cardiovascular disease is brought on by cadmium exposure [16]. The metabolic pathways for vitamin D are impacted by cadmium [17]. Kidney injury results from elevated blood levels of cadmium [18]. Urinary cadmium has numerous harmful effects on various tissues, including the mammary glands, the lungs, periodontal tissues, excessive blood pressure, and diabetes [19].when people come into contact with cadmium that is found in the air, water, soil, or food, even at low concentration causes health problems [20].

For the purpose of protecting the public's health, precise and effective techniques for Cadmium analysis in dairy products are thus necessary [21,22]. Time-consuming sample preparation is a common component of conventional methods for Cadmium analysis. This can lead to inadequate digestion and make it more difficult to accurately determine the Cd concentration using atomic absorption spectrophotometry (AAS) [23]. In order to address these issues, a novel strategy that makes use of microwave-assisted digestion has been created with the goal of improving dairy product digestion efficiency for accurate heavy metal analysis using AAS [24].

Microwave-assisted digestion, a sophisticated sample preparation method that uses microwave energy to quickly and effectively break down complicated matrices, is used in the novel methodology that has been suggested [25]. Using this technique dairy samples are thoroughly and completely digested in a fraction of the time and accelerates the digestion process. Therefore, the possibility of incomplete digestion is significantly decreased, which improves the precision and accuracy of the analysis of heavy metals followed by AAS.

The novel microwave-assisted digestion method and its use in the analysis of different Yoghurt samples will be discussed in this study. The effectiveness and accuracy of the suggested method are demonstrated by the results of the heavy metal analysis using AAS, which makes it a potential instrument for routine monitoring and safety assessment in the dairy industry [26, 27].

This would ensure adherence to food safety laws and ultimately protect public health. To determine this approach's wider application and possible influence on food safety practices, more validation and investigation into various dairy product matrices and heavy metal contamination are necessary [28]. The goal of the study was to identify the hazardous and necessary heavy metal Cadmium (Cd) present in different Yoghurt samples like sour, sweet and flavoured Yoghurt as well as homemade Yoghurt that were collected from a number of locations in Kota.

MATERIALS AND METHODS

1. *Sample Collection:* Total of 80 Yoghurt samples, sour Yoghurt (20 samples), sweet Yoghurt (20 samples),flavoured Yoghurt (20 samples) and homemade Yoghurt (20 samples) were collected from different areas of Kota. Sour, sweet and flavoured Yoghurt samples were collected from the locally available dairy farms and homemade traditional Yoghurt samples were collected from the home of farmers who regularly prepare Yoghurts for themselves. All the Yoghurt samples were collected in PTFE bottles and kept at -20°C in a deep freezer until analysis.

2. Reagents and Chemicals
   i. Nitric acid (HNO_3, ≥ 65% purity) and hydrogen peroxide (H_2O_2, ≥ 30% purity) were obtained from chemical suppliers.
   ii. Deionized water was used for dilution and preparation of acid solutions.
   iii. *Sample Digestion:* One gram of sample was digested with 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) in microwave digestion system. The digestion of samples were carried out at different conditions are summarized in Table 1.

Table 1. Microwave Digestion Conditions.

Step	Time (min)	Power (W)
1	2	200
2	2	200
3	4	400
4	6	400
5	8	600
6	8	VENT

Resulted solution was transferred in to 10 ml volumetric flask and and diluted with deionized water. A blank digest was carried out in the same way.

   iv. *Sample Analysis:* All digested samples were analyzed by using the Atomic Absorption Spectrophotometer for the quantitative determination of Cadmium (Cd) concentration in the sample. Calibration standards were regularly analyzed to ensure the stability of the instrument.
   v. *Data Analysis*
      - *Statistical Analysis:* - Statistical analysis was carried out for each sample. Mean, standard deviation and variance were calculated.
      - *EDI (Estimated Daily Intake):* - The EDI values were determined by multiplying the concentration of cadmium in Yoghurt by the average daily consumption rate, which is divided by the average body weight of an adult [2].

$$EDI = \frac{DCR_Y \times C_M}{BW}$$

Here DCR_Y represents the daily consumption rate of Yoghurt (g day^{-1}).,which is 125 g day^{-1} for adult people.
C_M indicates the mean concentration of metal in Yoghurt samples (mg/L).

While BW represents the average body weight of adult people. The average body weight for adults is 60 kg [29].

      - *HRI (Health Risk Index):* The health risk index of identified heavy metals is determined by calculating the ratio of estimated daily intake and reference dose expressed as R_fD [29].

$$HRI = \frac{EDI}{R_f D}$$

The reference dose for Cd is 0.001mg/kg/BW/day [29].
The value of HRI shows potential health risk when it is equal or higher than one (≥ 1.())

RESULTS AND DISCUSSION

1. *Determination of heavy metals in Yoghurt samples:* The concentrations of Cd were analyzed in 80 Yoghurt samples which are commonly consumed by the people of Kota city. The concentrations of Cd in sour, sweet, flavoured and homemade Yoghurt samples are presented in Table 2. Concentration of Cadmium in homemade Yoghurt ranges from 0.0021 to 0.0121 mg/L, which is found to be the lowest among all types of yoghurt while the maximum concentration of

Cd was found in flavoured Yoghurt which ranges from 0.0221 to 0.0445 mg/L. The results from samples show that of sour, sweet, flavoured and homemade Yoghurt contained (mean±SD) of Cd, 0.0201±0.0038, 0.0169±0.0052, 0.0347±0.0096 and 0.0050±0.0041 mg/L respectively. According to these data, the highest value of Cd was found in flavoured Yoghurt.

Table 2. Cadmium concentration (in mg/L) in different Yoghurt samples.

Sample	**Sour Yoghurt N=20**	**Sweet Yoghurt N=20**	**Flavoured Yoghurt N=20**	**Homemade Yoghurt N=20**
Min	0.0158	0.0108	0.0221	0.0021
Max	0.0259	0.0226	0.0445	0.0121
Mean	0.0201	0.0169	0.0347	0.0050
SD	0.0038	0.0052	0.0096	0.0041
Variance	1.42E-05	2.7592E-05	9.2362E-05	1.6883E-05

N= Number of samples, SD : Standard Deviation

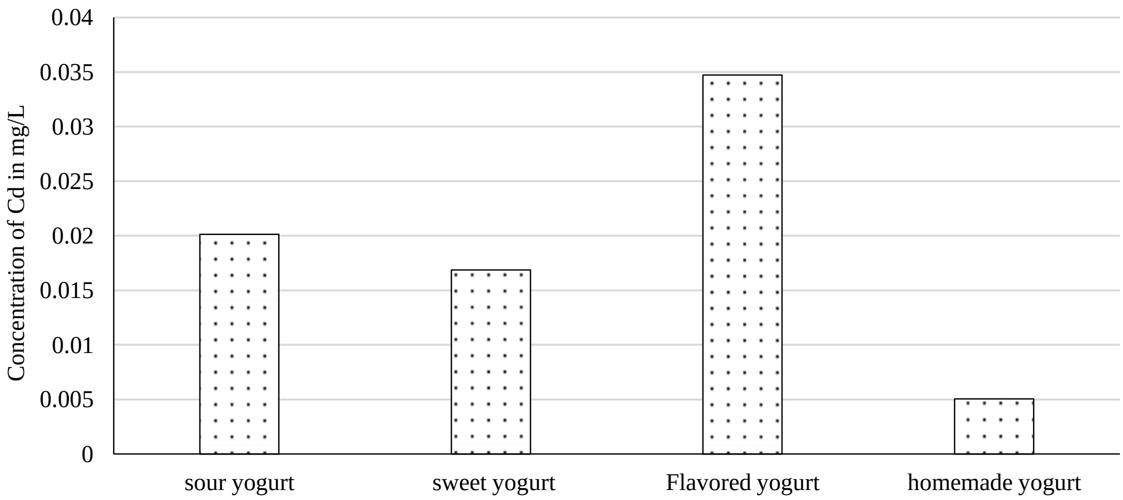

Figure 1. Average concentration of Cadmium (mg/L) in different Yoghurt samples.

The permissible limit of Cd intake set by the WHO (World Health Organization) is 0.0026 mg/L. On comparing our results with observed values, it reveals that concentrations of cadmium were found significantly higher than the maximum limit by WHO. Variation in these concentration ranges depends on several factors, including the source of milk, type of Yoghurt, environmental conditions, and production methods.

The Estimated Daily Intake (EDI) for Cd was calculated by taking DCR_Y (daily consumption rate of Yoghurt), M_C (metal concentration) and BW (average body weight). The EDI values of heavy metals in sour, sweet, flavoured and homemade Yoghurt are shown in Table 3.

Table 3. Estimated daily intake of Cadmium through sour, sweet,flavoured and homemade Yoghurt samples.

Yoghurt sample	**Sour Yoghurt**	**Sweet Yoghurt**	**Flavoured Yoghurt**	**Homemade Yoghurt**
EDI	4.19E-05	3.52E-05	7.22E-05	1.041E-05

EDI- Estimated daily Intake in mg/kg bw/day.

In this study, EDI was calculated only for adult people. and the highest EDI was detected for flavoured Yoghurt (7.22E-05) while the lowest EDI was detected for homemade Yoghurt (1.041E-05). The EDI values indicated that cadmium is consumed regularly below the tolerance range.

To assess the health hazards due to Cd intake HRI is calculated with the help of EDI values and R_fD (reference dose). The HRI values of heavy metals in sour, sweet, flavoured and homemade Yoghurt are shown in Table 4.

Table 4. Health risk index of Cadmium through sour, sweet,flavoured and homemade Yoghurt samples

Yoghurt sample	Sour Yoghurt	Sweet Yoghurt	Flavoured Yoghurt	Homemade Yoghurt
HRI	0.0419	0.0352	0.0722	0.01041

HRI- Health Risk Index

The value of HRI for each sample is found to be less than one, which clearly indicates that there is no such harm in consuming Yoghurt. So this study proposing that the different areas of Kota were not exposed to dangerous health risks from the intake of different types of Yoghurt.

CONCLUSION

The results showed that a highest concentration of Cd was found in flavoured Yoghurt whereas lowest concentration of Cd was found in homemade Yoghurt samples. The higher Cd concentration in flavoured Yoghurt may be due to additional added ingredients like fruits, syrups, and additives. The processing and packaging methods may also be responsible for Cd contamination in flavoured Yoghurt. Although the concentration of Cd is found under the safe limit but long long-term exposure especially to children is a matter of concern. The results of this study provide reassurance that the consumption of sweet, sour, and homemade Yoghurt in the Kota Region is generally safe with respect to Cd exposure.

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This is to certify that Prof. / Dr. / Mr./Mrs./Ms. ......Aarti Bansal Research Scholar......of College /University .....Govt. college kota, Kota (Raj)...............participated as a Resource person / Registered Delegate in **NATIONAL SEMINAR ON RECENT TRENDS, ISSUES AND CHALLENGES IN ENVIRONMENTAL AND BIODIVERSITY CONSERVATION** Organized by Govt. P. G. College Pratapgarh (Raj.) in Collaboration with Govind Guru Tribal University, Banswara, Rajasthan, INDIA during 19-20 February, 2021. He / She attended the seminar and Presented paper entitled / delivered invited talk on the topic ......Environmental impacts on heavy metal Concentration in dairy products and health risk assessment.......

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Principal
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