DETERMINATION OF HEAVY METALS LEVEL IN DAIRIES MILK OF QUETTA CITY OF BALOCHISTAN, PAKISTAN

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ABSTRACT: *The main objective of this work presents to point out the accumulation content of toxic heavy metals in samples of milk collected from the various dairy shop of Quetta. Atomic Absorption Spectroscopy was used to assess the content of heavy metals in unprocessed raw dairy milk samples. All milk samples tested for heavy metal residues had levels of Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, and Cd that were above the WHO acceptable limit. The resulting values were further compared to comparable values in the literature as well as Research Triangle Institute (RTI) values established by various international organizations.*

Keywords: Heavy Metals, Milk, and Atomic Absorption Spectroscopy.

1. INTRODUCTION

Milk contains more water than any other element, around 87% for dairy cows. The other elements are dissolved, colloidally dispersed, and emulsified in water ...
Water: 85.5 – 89.5
Lactose: 3.6 – 5.5
Fat: 2.5 – 6.0
Total solids: 10.5 – 14.5

Figure-1. Map of Balochistan reference to Quetta.

Lactose (milk sugar), fat, water, proteins, and minerals are the main components of milk. Pigments, enzymes, vitamins, phospholipids, and gases are all found in tiny amounts in milk. The nutritional content of milk and milk products has been continuously rising, owing to its important role in human health. Figure 2 depicts the chemical structure of milk.

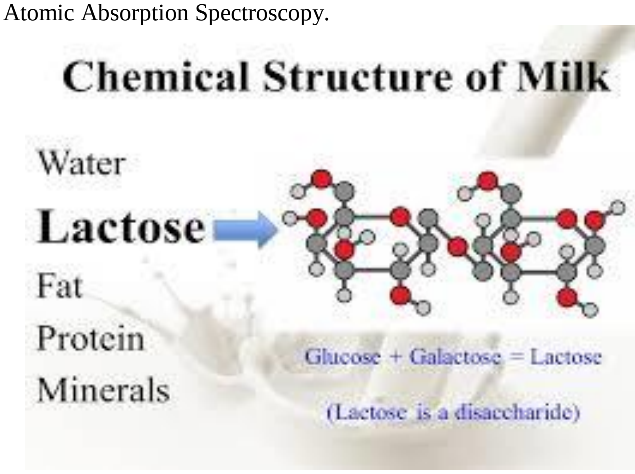

Figure-2. Chemical structure of Milk

The nutritious ingredients or substances present in milk are a source to aid the proper functioning of enzymes [6]. The most significant trace elements present in milk are Ca, Mg, P, K, Na, Cl, Zn, Cu, [7].

Heavy metals, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, and Cd, etc. are also present in milk as essential ingredients as they conduct many metabolic processes; nevertheless, increased access/concentration of heavy metals causes toxicity is a matter of serious health concerns [8]. Metals in milk are a consequence of an animal's exposure to a variety of circumstances, including eating dusty grass and drinking polluted water[9]. When consumed through fodder they enter the tissue of the animal body and are released via milk to humans[10].

Heavy metals are long-lasting pollutants in the environment that may pose significant health and environmental risks. Natural and human activities both release them into the environment [1-4]. Some heavy metals, such as Cu, Fe, and Zn, are required for normal metabolic activity in living organisms, whereas others, such as Pb and Cd, are non-essential and play no function in biology [5-7]. Even necessary metals, in large quantities, may be harmful to living creatures [8, 9].

Milk and its products are essential components of the daily diet, particularly for vulnerable populations including newborns, school-aged children, and the elderly [10, 11]. Milk is a rich source of protein, potassium, vitamin A, vitamin B-12, and niacin, as well as an excellent supply of calcium, vitamin D, riboflavin, and phosphorus [12, 13]. Heavy metals including zinc, lead, cadmium, selenium, sulphur, iodine, and potentially even more hazardous arsenic and cyanide are found in milk [2]. Heavy metal levels in milk must be determined and monitored due to ever-rising environmental contamination since they potentially have a substantial impact on human health [14, 15, 16]. The matter has been investigated further by others [4, 17, 18]. Due to their complex emulsion-like matrices and low metal ion concentration levels, determining trace inorganic components in milk is a difficult job. In the literature, several digestion methods to oxidize organic matrices of various materials have been described [19-21]. Acid digestion methods are the most common sample pre-treatment techniques for elemental analysis in biological and environmental materials, and microwave-induced acid digestion is a well-established approach [22-23].

Indirect photometric chromatography, ion chromatography, flame atomic absorption spectrometry [26=28], furnace atomic absorption spectrometry [29], inductively coupled plasma optical emission spectrometry [30-31], potentiometric stripping [32], capillary zone electrophoresis [33], differential scanning capillary zone electrophoresis [34], differential scanning capillary zone electrophoresis [35], differential scanning capillary zone The Flame Atomic Absorption Spectrophotometer was utilized in this research [38-39].

Details of some heavy metals present in milk areas

LEAD

The majority of natural elements found on the earth are lead (Pb). Its physical features include a low melting point and high malleability, making it applicable to many industrial uses. In terms of use, it is rated sixth among metals (as shown in the table below)[13]. Its use is related to more than 900 industries, including the mining, smelting, refining, and manufacture of batteries[14-15]. It is also used in agriculture, insecticides, and gasoline, as well as to improve fuel quality. Increased discharge of effluent from factories next to rivers is causing the volume of effluent in water bodies to increase rapidly because of industrialization [13]. Additionally, sewage sludge contamination has occurred owing to a number of other issues, including agricultural usage of sewage sludge from water polluted by both urban pollution and chemicals used for agriculture. In addition to polluting our groundwater supplies, these activities have combined to give us some unpleasant results. In short, it is very popular due to its accessibility, and therefore it is well-known as a significant environmental and occupational danger in urban environments.

COPPER

Copper is a trace element that has a variety of roles in proteins and enzymes that help the cell to grow and multiply. Copper is taken up via certain high-affinity plasma membrane copper transporters or low-affinity permeases, which are spread across the membrane in a carefully regulated manner [20]. Higher copper consumption has been related to an increased risk of lead absorption in recent studies. A direct consequence of its overabundance was the production of very reactive oxidizing species (such hydroxyl radicals), which caused damage to DNA [23].

ZINC

Zinc is an important trace element for microbes, plants, and animals' growth and development as a catalytic, structural, and regulatory ion [24]. Zinc consumption in humans varies between 2.5 and 10 milligrams per day[25]. It's a component of the zinc-dependent hormone thermic, which is necessary for thymus activities including T-cell maturation and differentiation [26]. This hormone has a high capacity for dislodging Zn from Zn-metalloproteinase. It inhibits $HgCl_2$'s impact on brain development [27]. On the one hand, when Zn supplementation seems to protect against oxidative damage to iron in the event of iron supplementation, long-term or higher-dose Zn therapy has been combined with copper depletion [28-29]. As a result, in order to avoid problems, a balanced strategy to supplementing these metal ions is required.

CADMIUM

Cadmium is a very poisonous element that has no recognized physiological purpose in the body. Cadmium poisoning is linked to a wide range of health problems, including major killer illnesses including heart disease, cancer, and diabetes. Many metallic enzymes are displaced by cadmium, and many of the symptoms of cadmium poisoning may be traced back to a zinc shortage caused by cadmium. It accumulates in the kidneys, liver, and other organs and is believed to be more hazardous than lead or mercury. It is poisonous at a hundredth of the amounts of lead, mercury, aluminum, or nickel. Cadmium poisoning is becoming more common nowadays for a variety of causes. A zinc deficiency in many frequently consumed meals is one of the main causes. Zinc, a cadmium-protective mineral, is becoming more scarce in soil and, as a result, in food. Zinc intake is significantly reduced by food processing and consuming processed meals. Cadmium exposure is also rising as a result of its usage as a coating for iron, steel, and copper. It's also found in copper alloys, rubber and plastic stabilizers, cigarette papers, fungicides, and a variety of other goods. These businesses often use this metal to contaminate water, air, and food.

CHROMIUM

Chromium is required for fat and carbohydrate metabolism. Chromium promotes the production of fatty acids and cholesterol, which is important for brain function and other bodily functions. The breakdown (metabolism) of insulin is also aided by chromium. Impaired glucose tolerance may be a sign of chromium insufficiency. It is found in elderly individuals with type 2 diabetes and in babies who are malnourished in protein calories. Supplementing with chromium may help control certain problems, but it is not a replacement for other treatments. Toxicity is uncommon due to chromium's poor absorption and high elimination rates. This AAS research study on heavy metals and their detection in milk accessible in Quetta, Balochistan, Pakistan will provide as a foundation for future researchers and scientists, as well as offer useful data for analysis.

2. EXPERIMENTAL

COLLECTION OF SAMPLES

The sample was obtained in a sanitary way from several dairy stores in Quetta at various times without additional contamination. Table-1 shows how they were labeled based on their source, provenance, and period.

Table 1

Sr no	Source	time
1	Adil dairy shop	6:45 $_{pm}$
2	Rehmat Ulla dairy shop	7:00 $_{pm}$
3	Al Madina Dairy shop	7:30 $_{pm}$
4	Jan Muhammad dairy shop	7:35 $_{pm}$
5	Jadoon milk shop	7:40 $_{pm}$
6	Masha Allah dairy ghar	8:00 $_{pm}$
7	Shah Jee dairy shop	8:15 $_{pm}$

SAMPLE DILUTION:

For atomic absorption spectrophotometer examination, the milk sample must be brought into a clear solution (AAS). Because the sample was too thick for analysis in its diluted condition, 5ml of it was placed in a beaker and diluted with 10 ml of water.

PRECIPITATION:

Following sample dilution, 0.1 trichloroacetic acid was added to precipitate the protein contained in milk, and the sample was centrifuged for 20 minutes.

DIGESTION:

For examination by the Atomic Absorption spectrophotometer, the sample produced from the fraction of centrifugation must be placed into clear solution. Nitric acid, sulphuric acid, and chloric acid were used to digest the milk first[30-31].

Figure-2. Instrumentation of the Atomic Absorption/

PREPARATION OF ANALYTE SOLUTION FOR AAS:

Fisher Scientific Company in the United States provided atomic absorption spectroscopic standard solutions for Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, and Cd. Diluting the stock solution yielded working standard solutions. All borosilicate glassware (conical flask, volumetric flask, watch glass, pipette, measuring cylinder, etc.) was used (England). When necessary, de-ionized water was utilized, and the Atomic Absorption instrumentation is shown in Figure-2 as following.

A Double Beam, Four Lamp Torrent Spectrometer with a Deuterium Background Corrector, and a Temperature Programmable Graphite Tube Furnace Assembly was used in this investigation (GTA 96). The atomization process was modified in order to improve the furnace's heating capabilities. Previous temperature and drying time values had been defined for every piece. Everything listed here was inputted into the AAS's computer. We used standards from Fisher Scientific to calibrate the AAS equipment with various aqueous solutions of elements.

For mercury, zinc, cadmium, chromium, copper, and lead, calibration curves were developed through linear regression analysis of standard solution concentrations with absorbance values. A new calibration curve was created for each element every time new milk samples were prepared for analysis. The results were gathered by performing at least three measurements on each standard solution, following which each mean was displayed.

The AAS machine's sensitivity was evaluated using a 10 ppb standard. Solution of lead (Pb). Several studies yielded a mean absorption value of with a relative, found to accord well with the manufacturer's claimed value. A 1.6 percent standard deviation (RSD) was used. After wet digestion, a ten (10) l aliquot of materials was injected. with the aid of an auto-sampler, into the graphite tube of the AAS, and The elemental concentration was determined.

3. RESULTS AND DISCUSSION

For trace elements, the mean concentrations (respectively) were found to be between 0.001 and 0.232 mg/l, 0.001 and 3.260 mg/l, 0.001 and 0.108 mg/l, 0.001 and 0.122 mg/l, 0.001 and 0.098 mg/l, and 2.086 and 5.498 mg/l. Only 5% of the samples had the lowest Cr content, while 90% of the samples had an increasing amount of metal. According to the tests, a quantity that was in the range of 13 to 14 times the set reference point was considered to be "very high." But its long-term toxicity has been extensively studied, and in contrast, has shown no long-term effects. About a fifth of the samples had just 5% Fe content, while 95% of the samples had over 16 times the average amount of Fe. Additionally, 90% of the evaluations obtained a better result than the required benchmark score. Using a dairy herd's milking equipment, which is typically constructed of non-recycled metal alloys such as steel and aluminum.

While it is well recognized that Co compounds and Co ions may cause cancer [27], only 5% of samples contained Co in them, and the highest measurement was more than double the normal safety limit (Table 3).

Table 2 . Ranges of intrinsic factors in groups derived from dendrograms at temperatures between 20.0 and 4.0°C.

Group code	pH	Conductivity (mS/cm)	Salinity (o/oo)	T.D.S. (g/l)	D.O (mg/l)	Specific gravity
Adil dairy shop	6.50-6.78	3.55-4.28	1.95-2.88	2.22-2.88	0.25-0.90	1.017-1.024
Rehmat Ulla dairy shop	5.72-6.68	5.75-6.70	1.60-2.30	1.55-2.25	0.39-1.04	1.017-1.033
Al Madina Dairy shop	5.75-6.54	4.87-5.70	3.30-3.80	3.23-3.72	0.63-0.99	1.028-1.033
Reference	6.3-6.9[a]	4-5[b]	--	--	--	1.023-1.040[c]

Table 3. Dendrogram-derived concentration ranges for heavy metals (mg/l)

Group	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Adil dairy shop	0.003-0.224	0.002-0.458	0.080-0.918	0.002-0.103	0.002-0.118	0.002-0.036	2.864-4.011	2.866-4.010	0.002-0.409
Rehmat Ulla dairy shop	0.046-0.202	0.001-0.396	0.001-0.083	0.001-0.034	0.001-0.010	0.001-0.025	2.086-2.720	0.001-0.042	0.001-0.295
Al Madina Dairy shop	0.028-0.174	0.001-0.384	0.658-2.100	0.002-0.054	0.000-0.062	0.004-1.688	3.830-5.498	0.000-0.053	0.000-1.428
Jan Muhammad dairy shop	0.001-0.098	0.001-0.002	3.163-3.260	0.018-0.032	0.001	0.030-0.070	3.890-4.462	0.001-0.028	0.154-0.170
Refe-rence*	0.017	0.026	0.210	0.0005	0.025	0.052	4.200	--	--

Figure 3 and Table 3 show that over half of the samples had relatively low Ni contents, while the maximum value was 3 to 4 times higher than the standard limit. Likewise, about 20% of the samples had quantities of vitamin D that were above the reference level but were below the adult recommendation for maximum daily intake (1.0 mg/day). Ni levels in the bovine milk in Mumbai, India were almost similar (0.010-0.131 mg L-1) [8]. By taking over the duties of 13 key metal ions, Ni works as an adversary [28].

The results showed that Cu levels were found at the lowest concentration in 20% of samples, while the maximum value was almost double the standard concentration. This is according to Table 3. However, just 15% of outcomes came out better than the reference. Another important cause of copper contamination in milk is how much cattle are drinking: according to [8], it is more than before. Only 5% of samples had the lowest amount of zinc in it, while 90% of the samples surpassed the regulatory maximum limit for zinc (Table 3).

Regardless of one's age, growth stage, or metal deficiency, having low zinc levels may cause a range of medical problems. However, the bulk of the samples (70 percent) had lower zinc levels than the reference limit. A study by Prasad has shown that teenagers with zinc deficiency have both developmental and sexual maturation issues [29].

Trace quantities of the potentially hazardous elements cadmium and lead were discovered to be in the range of 0.001 to 0.053 mg/l and 0.001 to 1.428 mg/l, respectively.

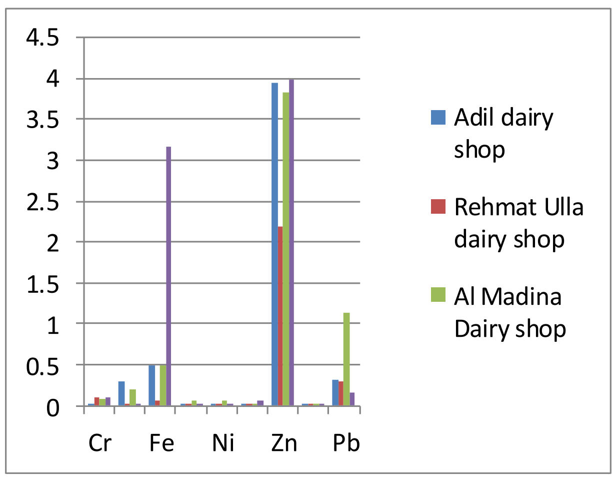

Figure-3: Heavy metal concentration ranges (mg/l) in groups determined from Dendrogram

The acceptable limit of 0.010 ppm set by [30] is much below the maximum concentrations that were found in samples at a level that was more than five to six times lower than the lowest values found. Nearly two-thirds of the samples had higher Cd than allowed. In [31], it was shown that the contaminants' level was affected by the exposure to a contaminated environment and chemical additions, such as colorants and stabilizers.

One-fifth of the samples had the lowest amounts of Pb found, whereas, above the EC's limit of 0.02 mg/l, the most concentrated Pb levels were found in over 50% of the samples [32]. Laboratory animals with levels of milk, fasting, low calcium, vitamin D, and iron have been found to have a greater lead absorption [33], and the same results have been shown in people.

4. CONCLUSION

Many industrial waste sources and sewage pipelines in the city of Quetta in Pakistan are responsible for the high levels of hazardous heavy metals found in the milk of cows reared there [9]. Variation in the amount of trace and heavy metals in milk samples from different dairies in Quetta city is a point to ponder for the quality control management authority in the Quetta region as the levels of heavy metals are far above the WHO standards.,

This variation is also a matrix of the pollution level of environments in various localities of Quetta city where animals graze.

5. RECOMMENDATIONS

● To keep the public safe, metal levels mandated by regulatory bodies must be followed to the letter [36].

● Eating more food on a daily basis causes more metal exposure because of the volume of the food being eaten. Daily, 113 grams of milk is consumed by each of Mumbai's residents. Drinking milk every day is common in the United States and Spain, where a study has shown that people there consume 224g and 124g, respectively [9]. In Ethiopia, daily milk intake is very low. Based on the country's yearly 19 kg per capita consumption, it is estimated to be 53 mL per day [49]. A yearly amount is converted to daily values for daily consumption projections. Whole milk is just one-tenth of a gram per milliliter denser than water. Using these figures, 53 g is 53 mL of full-fat milk. The heavy metals intake calculated in the table is based on an estimated daily milk consumption of 53 mL. The RDA (Recommended Dietary Allowance), as determined by different international organizations, is included in the fourth column. [9]

.● It was discovered that daily intakes of some ingredients were lower than recommended/permissible limits, as designated by different international organizations. FAO and WHO recommends daily Cr consumption be only slightly lower than the RDA, while daily Mn, Cu, and Zn consumption should be even lower than that. Regardless, the fact remains that in Ethiopia, adults drink milk very sparingly, and the unavailability of certain metals in other common Ethiopian meals may be mitigated via substitution.

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