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Spectrophotometric determination of significant minerals in milk samples found in Lahore (Pakistan) for ensuring food safety

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This work was carried out for determination of essential and toxic metals ions in different milk powder samples commonly found in Lahore (Pakistan). Samples were collected from local markets and after dry ashing, they were dissolved in 2M nitric acid for complete mineralization. Then the concentration of metal ions was determined by flame photometry and atomic absorption spectrometry. The concentration levels of cadmium, lead and nickel were found to be below detection limits in all samples, whereas the maximum values of sodium, potassium, calcium, magnesium, iron, and zinc were 48.55, 0.96, 4.94 mg/g, and 768, 4.12 and 3.02 µg/g respectively. Minimum amounts of these metal ions were: 3.69, 0.07, 0.85 mg/g and 214, 0.21, 1.02 µg/g, respectively. The amounts of all metal ions were found to be within safe limits as recommended by WHO.

Key words: Milk samples, powder milk, toxic metals, essential metals, flame emission spectroscopy, atomic absorption spectrometry

INTRODUCTION

Milk is a white fluid created by the mammary organs of vertebrates. It is an essential nourishment for infants of mammals before they can process different sorts of nutrition. Early-lactation drain contains colostrum, which conveys the mother's antibodies to its young and can decrease the danger of numerous ailments. It contains numerous other nutrients [1].

Milk is a perplexing material comprising different components which have a noteworthy role despite the fact that they are present in low amounts. Expanded consciousness of the impact of eating routine on human fitness has encouraged making food items of higher quality which must be rich in supplements and vitamins. Great quality estimations are fundamental to control, keep up items and process quality, in assembling, exchange and research [2]. For healthy nourishment and proper growth of the human body, milk and dairy items have been perceived worldwide as a good source [3]. Enlarged environmental contamination has enhanced the effects of milk uncleanness and doubts about the quality of milk. Because of xenobiotic compounds and environmental pollution milk contamination is registered worldwide which exerts bad influence on public health. Metals of high density and toxicity at low quantity are referred to as *'heavy metals'*. Their harmful effects on humans are constantly growing in the previous few decades [4]. Their importance for human beings cannot be denied, but excessive amounts are detrimental. For example: high amount of iron (II) leads to Alzheimer's or Parkinson's diseases [6]. Cu(II) is essential for respiration and proper growth of body, but its excess leads to muscles injury, lungs irritation, liver cancer ending up to Wilson disease [7]. Zn(II) is a central atom of carbonic anhydrase that controls CO_2 concentration in the body, but its excess leads to rectum cancer [8]. Cr(VI) is enlisted in the top sixteen carcinogenic elements and its excess results in dizziness, abdominal pain, liver CNS and heart tissues toxicity [9]. Ni(II) is a central atom of urease enzyme which plays an important role during amino acid digestion. But its excess leads to renal failure and lungs diseases [10]. Excess of Co(II) causes DNA fragmentation [11]. Cd(II) and Pb(II) are non-essential elements in biological systems [12]. Cd(II) excess leads to gastrointestinal tract irritation, joint pain and *Itai Itai* disease. Pb(II) excess leads to Alzheimer`s, kidney failure, CNS and reproductive systems infections [14]. So, the contents of these metals should be regularly analyzed for continuous monitoring of milk and dairy products for their safe limits [15]. The goal of the current study is to estimate the amount of essential and heavy metals in milk powder and infant milk formulas purchased from different areas of Lahore (Pakistan). The amount measured was compared with the Recommended Dietary Allowance (RDA) given by different international organizations.

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EXPERIMENTAL

Materials and instrumentation

Analytical grade chemicals (Merck, Germany) were used for analysis. For standard solution preparation certified standard solutions (Fluka) were employed. Flame photometer (Corning 410), atomic absorption spectrometer (AAnalyst100), Furnace (Nabertherm, Germany) were used. Five milk powder samples from different localities were collected from various areas of Lahore (Pakistan) and categorized as shown in table 1. Then, after estimating their metal ions concentration, data were analyzed statistically for validation of the results by Microsoft Excel (2010). Average values were reported in the results.

Table 1. Samples of milk powder.

Sample ID	Sampling area of Lahore	Sample ID	Sampling area of Lahore
1.	Sadar Cantt	2.	Pakistan Mint Stop
3.	Johar Town	4.	Nishat Colony
5.	Wapda Town	6.	DHA phase 5
7.	NFC Society	8.	Taj Bagh
9.	Darogawala	10.	Sui gas society
11.	Bahria Town	12.	Shalimar Link Road
13.	Al-Raheem Garden	14.	PIA housing society
15.	EME Society	16.	Awan Town
17.	Harbanspura	18.	Near General Hospital
19.	Canal Bank Housing Scheme	20.	Mazung Chungi

Procedure and measurement

Chemical treatment of the milk samples was carried out as followed: 1 g of milk powder was taken in a china dish. It was heated until it was converted into black ash. After cooling, 3-5 mL of concentrated nitric acid was added and heated again till all nitric acid was evaporated. Same procedure was repeated till the emitted brown fumes turned to white fumes. Then, the resulting black ash was kept in a furnace (Nabertherm) at 550 ^{0}C, for 2 h for decomposition of organic components of the sample and complete mineralization [15]. The resulting white ash was dissolved in 3-5 mL of 2M HNO_3. After filtration, the filtrate was diluted with distilled water in a 100-mL flask. The instrument was calibrated using standard solutions of metal ions and all samples were analyzed in triplicate. Average values were used for graphical representation of metal ions in various samples.

RESULTS AND DISCUSSION

Milk is the most important diet of human nutrition especially for infants, because it contains a lot of minerals which are required for their proper growth.

In this work the concentration of metal ions Na(I), K(I), Ca(II), Mg(II), Fe(II), Zn(II), Pb(II), Cd(II) and Ni(II) in different brands of milk formula and commercially available milk powder samples obtained from different areas of Lahore (Pakistan) was determined. Some preliminary physico-chemical analyses were done for comparing percentage volatile organic contents and inorganic ash contents of these samples before metal ion determination and results are reported in Table 2.

It indicates that lower moisture contents are found in those samples which are properly packed and sealed in metallic containers, which leads to their long shelf life, whereas open samples and those which are sold as in shopping bags contain higher moisture leading to deterioration by bacterial and fungal growth. Similar trend is observed for ash contents or inorganic contents. Milk samples packed in plastic or cloth bag samples have higher inorganic contents and less volatile organic contents.

After ashing of the milk powder samples, their metal contents were estimated and reported in Table 3. Concentration of essential elements was found to be higher as compared to toxic elements. In most of the samples, no toxic elements were found and the samples are safe for human use. Concentrations of essential metals, like: sodium and calcium, were higher in all samples as compared to other metal ions. In some milk powder samples, lead, cadmium and nickel were detected in minute amounts. Sodium was found in samples in the 4.25-48.55 mg/g range, as is obvious from Fig. 1. Average amount was 11.94 mg/g. Sodium is an important component of extracellular fluids in the organism. Osmotic pressure of body is also controlled by sodium [8-10]. In Addison's diseases, diarrhea, intestinal obstruction and vomiting, hyponatremia occurs (deficiency of sodium in blood serum) while in Cushion's disease hypernatremia occurs [8]. Its deficiency may cause growth retardation [11]. 1500 mg of sodium is required per day.

Potassium was found in all samples in the 0.6-0.96 mg/g range, as shown in Fig. 2. Average value was 0.528 mg/g. It plays a role in acid-base balance, osmotic pressure regulation, nerve impulse conduction, contraction of muscles [12]. Potassium acts as a cofactor in protein synthesis.

Calcium was found in the range of about 0.852-4.942 mg/g and the average value in all samples was 2.43 mg/g, as shown in Fig. 3. Large number of enzymes like adenosine triphosphates (ATPase), succinic dehydrogenase and lipase are activated by calcium. As a constituent of bones and teeth calcium plays an important role. Excess calcium is excreted because extra calcium leads to respiratory and cardiac failure [12].

Magnesium amount was in the range of 214-768 µg/g, as shown in Fig. 4. Its average value in all samples was 598.7 µg/g. Magnesium is the major component of bones and teeth [9]. Magnesium depletion also occurs due to chronic or excessive diarrhea and vomiting [12]. The best sources include leafy green vegetables.

Iron was in the range of 0.21-3.02 µg/g and the average value in all samples was 1.771 µg/g, as shown in Fig. 5. The amount of iron found in a previously studied milk sample was 3.2-12.91 µg/g [13]. In animals and humans, iron is an essential component. Muscle, hemoglobin and myoglobin have iron as a major component. Our food contains considerable amounts of iron which satisfy our needs. Anemia may result due to iron deficiency, which can treated by artificial medication [14]. The largest quantity of iron is present in the liver and spleen. Constipation and vomiting occur by taking orally iron salts [16]. It was observed that iron was in higher amount in infant milk formula than in commercially available milk powders.

Table 2. Physicochemical analysis of milk powder samples.

Sample ID	Powder packaging material	Moisture (%)	Volatile organic contents (%)	Inorganic ash contents (%)
1.	Plastic bag	4.5	82.9	6.4
2.	Metallic container	2.1	86.5	5.2
3.	Cloth bag	6.3	79.9	7.6
4.	Plastic bag	4.1	82.9	6.8
5.	Cloth bag	5.5	81.1	7.2
6.	Metallic container	2.3	86.9	4.6
7.	Metallic container	1.7	87.3	4.8
8.	Metallic container	1.8	86.9	5.1
9.	Metallic container	1.6	88	4.2
10.	Cloth bag	5.4	80.1	8.3
11.	Plastic bag	4.2	82.1	7.5
12.	Cloth bag	5.3	84.3	4.2
13.	Plastic bag	4.3	82.3	7.2
14.	Plastic bag	3.9	82.3	7.6
15.	Plastic bag	3.6	82.6	7.6
16.	Metallic container	1.8	87.2	4.8
17.	Plastic bag	4.1	82.5	7.2
18.	Plastic bag	3.7	82.8	7.3
19.	Plastic bag	3.6	83.9	6.3
20.	Cloth bag	4.6	83.1	6.1
Descriptive Statistical Analysis of Data				
Average		3.72	96.18	6.3
Maximum		1.6	92.3	4.2
Minimum		6.3	88	8.3
Standard deviation (±)		1.42	2.49	1.31

Table 3. Concentration of metal ions in milk powder samples found in Lahore (Pakistan).

Sample ID	Concentration								
	Alkali metals		Alkaline earth metals		Transition metals		Toxic metals		
	Na(I) (mg/g)	K(I) (mg/g)	Ca(II) (mg/g)	Mg (II) (µg/g)	Fe(II) (µg/g)	Zn(II) (µg/g)	Pb(II) (µg/g)	Cd(II) (µg/g)	Ni(II) (µg/g)
1.	16.65	0.07	2.052	214	0.21	1.22	BDL	BDL	BDL
2.	12.96	0.15	2.73	422	3.2	1.11	BDL	0.01	BDL
3.	4.92	0.29	1.06	414	1.1	1.17	BDL	BDL	0.01
4.	3.82	0.47	1.47	324	4.1	2.03	BDL	BDL	BDL
5.	3.69	0.56	1.79	428	3.1	1.71	BDL	0.01	BDL
6.	9.91	0.59	1.76	523	0.41	1.22	BDL	BDL	BDL
7.	14.30	0.65	1.06	514	0.56	1.17	BDL	BDL	BDL
8.	48.55	0.81	0.88	614	0.64	1.71	0.01	BDL	BDL
9.	12.35	0.89	0.85	267	4.12	1.02	BDL	BDL	BDL
10.	16.03	0.96	1.63	718	2.11	1.11	BDL	BDL	BDL
11.	(8.97	0.68	3.53	567	1.11	3.01	BDL	BDL	0.01
12.	4.67	0.32	2.99	522	2.13	2.13	0.01	BDL	BDL
13.	14.80	0.6	4.18	655	2.11	2.11	BDL	BDL	BDL
14.	17.26	0.7	2.58	455	3.02	3.02	BDL	BDL	BDL
15.	5.17	0.19	4.94	567	1.11	1.11	BDL	BDL	BDL
16.	9.01	0.46	3.34	436	1.01	1.75	0.01	BDL	BDL
17.	8.52	0.48	3.02	567	1.11	1.11	BDL	BDL	BDL
18.	4.25	0.36	4.46	345	1.21	1.21	BDL	BDL	BDL
19.	11.61	0.37	3.65	455	1.32	1.32	BDL	BDL	BDL
20.	11.51	0.96	2.47	768	1.74	1.74	BDL	BDL	BDL
Descriptive Statistical Analysis of Data									
Mean	11.94	0.53	2.52	488.75	1.771	1.599	BDL	BDL	BDL
Minimum	3.69	0.07	0.85	214	0.21	1.02	BDL	BDL	BDL
Maximum	48.55	0.96	4.94	768	4.12	3.02	0.01	0.01	0.01
Standard deviation	9.72	0.068	1.52	203.37	1.398	0.37	BDL	BDL	BDL

*BDL= Below detection limit

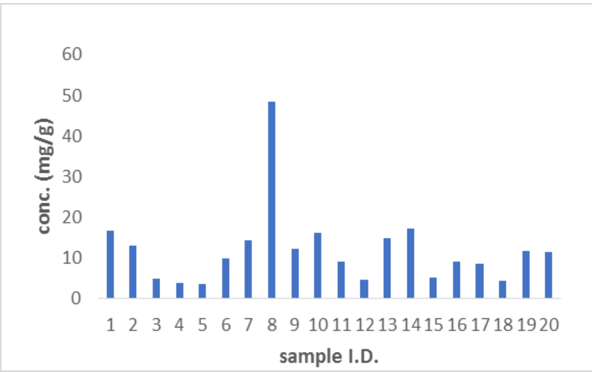

Fig. 1. Concentration of sodium in samples.

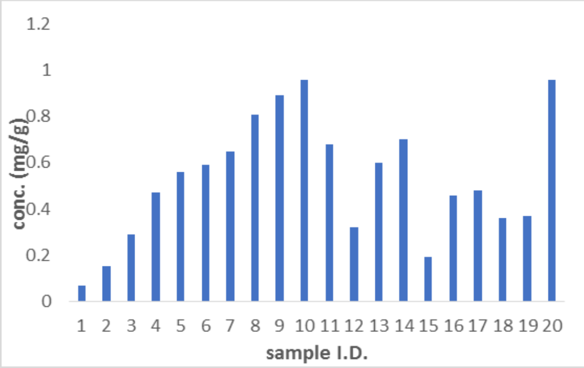

Fig. 2. Concentration of potassium in samples**.**

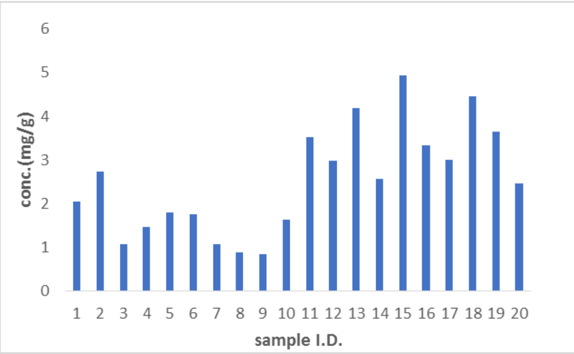

Fig. 3. Concentration of calcium in samples

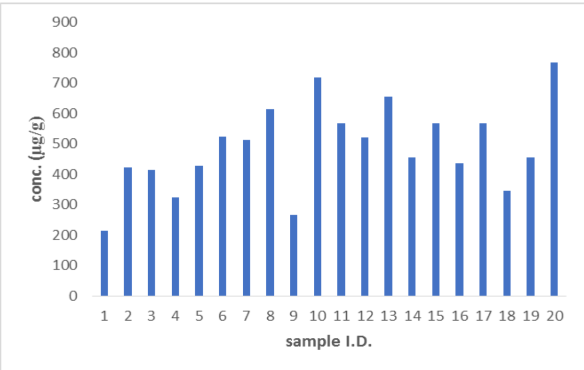

Fig. 4. Concentration of magnesium in samples.

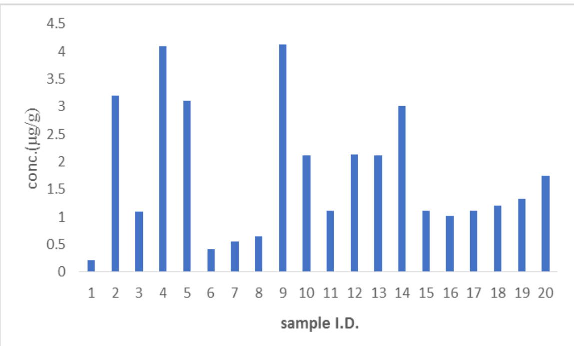

Fig. 5. Concentration of iron in samples.

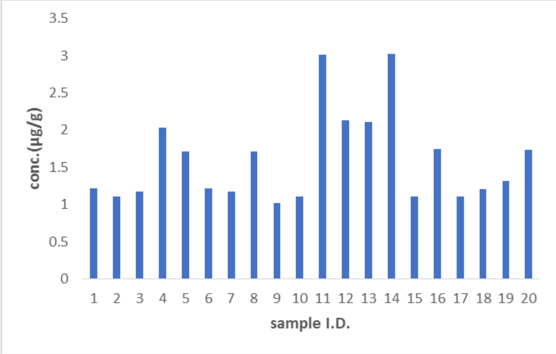

Fig. 6. Concentration of zinc in samples.

Zinc was present in the range of 1.02-3.01 µg/g and the average value of zinc was 1.59 µg/g, as shown in Fig. 6. It is important for proper working of carbonic anhydrase enzyme which regulates carbon dioxide concentration in blood. Its excess, however, leads to "fever foundry". The concentration of zinc was lower than that found in a previous study which was 2.016 µg/g [17].

Lead is the most dangerous metal for all living organisms, mostly for plants, animals and specifically microorganisms. Main source of lead are the lead mines, fuel combustion sewage sludge applications and farm yard manure. Continuous contact with lead can result in anemia, pale skin, paralysis, nausea, and vomiting. The nervous system may also be affected by prolonged exposure, resulting in severe headache, coma and death. Fertility lost and birth defects may also occur due to prolonged exposure to lead [18]. In all milk powder samples, Pb(II) was not found except for samples collected from Taj Bagh and Shalimar Link Road area. So, all samples of milk were safe to use. Lungs and kidney may be damaged due to prolonged inhalation [19]. Due to nickel exposure, itching of the fingers, hands, and forearms may occur. Respiratory effects, lung and nasal cancer had been linked to nickel inhalation [20]. Nickel was almost absent in all milk samples except for samples collected from Johar Town and Bahria Town. Serious health problems may cause cadmium [21], like: kidney and heart failure [22]. Chronic poisoning leads to muscle, lungs and skeleton damage disorders in humans [23]. Fortunately, cadmium was also absent in most of the milk samples found in Lahore, except for those samples collected from Pakistan Mint stop and Wapda Town.

CONCLUSION

It was found in the present study that packaging of milk powder samples in plastic, cloth or metallic containers had great effect on their composition. Samples packed in metallic containers have less moisture and more nutritional elements as compared to those packed in plastic or cloth. The concentration levels of cadmium, lead and nickel were below detection limits in all samples, whereas the maximum values of sodium, potassium, calcium, magnesium, iron, zinc were 48.55, 0.96, 4.94 mg/g, and 768, 4.12 and 3.02 μg/g, respectively. Minimum amounts of these metal ions were: 3.69, 0.07, 0.85 mg/g and 214, 0.21, 1.02 μg/g, respectively. All samples contained essential metals in considerable amounts, especially the most important nutrients while toxic metals, like Pb(II), Cd(II) and Ni(II) were absent in most of the samples. Hence, all these milk samples were safe for drinking and usage in dairy food products.

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