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Research paper

Evaluation of essential and heavy metal levels in pasteurized and long-life cow milk

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Abstract

In the present investigation, 11 cow's milk samples (pasteurized and long life) were collected from supermarkets in Misurata city, Libya. For the determination of metal contents, the milk samples were subjected to the optimized microwave digestion method using HNO_3 and H_2O_2. The levels of Na, K, Mg, Ca, Fe, Cu, Cr, Mn, Co, Ni, Zn, Cd, and Pb were evaluated by Microwave Plasma - Atomic Emission Spectrophotometer (MP-AES 4100). It was found that potassium is the most concentrated essential metal while zinc is the highest detected heavy metal in all milk samples. The levels of Co and Cd were not detected in all milk samples under investigation (lower than instrumental detection limits). The mean concentrations of the detected metals were as follows: 828.05±0.82, 587.57±0.87, 512.38±0.30, 73.79±0.19, 2.014±0.19, 1.89±0.16, 0.178±0.03, 0.061±0.02, 0.074±0.01, 0.053±0.001, and 0.045±0.001 for: K, Ca, Na, Mg, Zn, Fe, Cu, Mn, Ni, Pb, and Cr ppm, respectively. There is no significant difference in the mean concentrations of Ni, Cr and Pb between the investigated milk samples. Though, the levels of essential and heavy metals observed were similar with other reported values in the literature.

Keywords: *Pasteurized Milk; Long Life Milk; Essential Metals; Heavy Metals.*

1. Introduction

To monitor and support products and methods quality, good feature determinations are essential, both in manufacture, business, and in research. Also, the increasing significance of chemical measurements has impressively forced the enlargement of procedures to enhance the quality of analytical results and to assurance quality to the end consumers [Kira & Maihara 2007].

Heavy metals are incessant chemical pollutants in the world that can produce critical environmental and health risks. They are discharged into the ecosystem from the natural in addition to anthropogenic activities [Abdulkhaliq et al. 2012]. Certain heavy metals like Fe, Zn, Co and Cu are needed to continue suitable metabolic performance in living organisms; others such as Hg, Pb, As and Cd are unnecessary and have no biological function [Khan et al. 2013]. Nevertheless, at extreme levels, both types cause toxicity to living organisms [Belete et al. 2014].

The presence of heavy metals in milk and dairy products is due to subject of the animals to particular circumstances such as consuming pasture or drinking water from polluted resources. Still, heavy metal quantities in the milk be influenced by the level of exposure of the animal to the contaminated position [Amponsah 2014]. The virtually prevalent existence of certain metal contaminants, particularly Pb, Cd, Hg and Ni, enables their access into the food chain and consequently enhances the opportunity of them having toxic influences on people and animals. Even though heavy metals have industrial benefits, their probable toxicity for human and animals is the target of numerous researches. For specific elements, the influences are accumulative and it is required to monitor their levels in consumed food [Elsherif et al. 2017].

Several studies denote the existence of heavy metals in milk and dairy products, and frequently it is required to evaluate the levels of them in food. Cadmium, mercury, and lead remains in milk are of specific interest since milk is mostly consumed by infants and children [Tajkarimi et al. 2008], and the estimation of these metals quantities in milk is especially attended by international organizations.

For performing these estimations, several techniques were utilized such as flame and flameless atomic absorption spectrometry (FAAS and ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), flow injection spectrometry (FIS), fluorescence atomic spectrometry (FAS), capillary zone electrophoresis (CZE), differential pulse anodic stripping voltammetry (DPASV), atomic and stripping potentiometry (ASP) [Cava-Montesinos et al. 2004, Suarez-Luque et al. 2007, Elsherif et al 2012a; 2012b; 2013, 2015].

The purpose of the present work is to evaluate the concentrations of major metals (Na, K, Ca, and Mg) and heavy metals (Fe, Cu, Zn, Co, Ni, Cr, Pb, Cd, and Mn) in eleven long life and pasteurized milk samples available in Misurata city markets and to find out whether the levels of these metals are lower or higher than the recommended concentrations demanded for human consumption; particularly children, and furthermore to attain the extent of contamination.

2. Materials and methods

2.1. Milk samples collection

In the present investigation, the commercially available pasteurized and long life cow's milk samples were purchased from several supermarkets in Misurata city. A one liter of milk product was obtained for each sample. The milk samples of various types examined in the present study are coded and listed in Tables 1.

Table 1: The Type and Code of the Examined Milk Samples

Product type	Symbol
Rayhan full milk	U1
Sohoul full milk	U2
Zahrat full milk	U3
Rabie full milk	U4
Juhayna full milk	U5
Candia full milk	U6
Rayhan skimmed milk	U7
Juhayna skimmed milk	U8
Rawi pasteurized milk	P1
Al Mazraa pasteurized milk	P1
Raw Local milk	R1

*U: long life milk, P: pasteurized milk, R: raw milk.

2.2. Milk sample digestion and analysis

The best microwave digestion method was chosen according to the clearness of digests, nominal digestion program, and smallest reagent amount, ease and lower heating temperature. A 3.0 g of each milk sample was placed in 60 mL Teflon digestion vessel and then volumes of 8 mL of 70 % nitric acid and 2 mL of 30 % hydrogen peroxide were added. The mixture was after that shaken thoroughly and kept for 10 min before closing the vessel. The samples were exposed to the optimized microwave digestion program (MARS6 Microwave Digestion-CEM) for 2 hours at temperatures between 25 – 170^{o} C. After completion the heating program, the sample was cooled to room temperature and then digestion vessels were opened carefully in a fume hood. The digest was transferred into 50 mL volumetric flask and completed to the mark with deionized water. Digestion of reagent blank was similarly done in the same manner as milk samples. The metal concentrations in the digested milk samples were estimated using microwave plasma - atomic emission spectrophotometer (MP-AES 4100) from Agilent – USA.

2.3. Reagents and glass wares

Standard solutions of Na, K, Ca, Mg, Fe, Zn, Mn, Ni, Co, Cd, Cr, Cu, and Pb designed for atomic absorption spectroscopy were bought from Fisher Scientific Company, USA and used for preparing working standard solutions by diluting the stock solution. Nitric acid and hydrogen peroxide were of AR quality (BDH, England). All glass wares (pipette, volumetric flask, measuring cylinder, etc.) were washed before use with deionized water, soaked in nitric acid (10 %), then rinsed with deionized water and finally air dried. The glass ware kept in clean place, to avoid contamination.

3. Results and discussion

The findings of our investigation for 13 elements in 11 milk samples (pasteurized and long life) which were from different brands are provided in Tables 2 and 3. The major metals concentrations in cow's milk samples are given in Table 2, while the heavy metals levels are displayed in Table 3.

Table 2: Major Metal Concentrations in Cow's Milk Samples

Sample	Mg	Ca	K	Na
U1	61.96±0.32	562.7±0.98	798.7±0.72	462.9±0.22
U2	60.95±0.22	518.4±0.78	793.8±0.63	560.3±0.25
U3	72.93±0.14	632.7±0.74	861.7±0.62	624.7±0.52
U4	71.41±0.12	526.9±0.72	769.6±0.83	571.4±0.12
U5	80.44±0.12	583.8±1.12	898.4±0.84	489.5±0.16
U6	80.19±0.32	544.6±0.73	894.7±0.94	512.5±0.55
U7	80.23±0.17	563.7±1.23	901.6±0.93	514.3±0.23
U8	73.76±0.17	638.9±0.93	827.8±0.82	350.4±0.21
P1	73.64±0.12	597.3±0.89	852.9±1.13	556.5±0.31
P2	80.27±0.15	611.6±0.78	893.7±0.78	554.3±0.36
R1	75.92±0.25	682.7±0.68	885.7±0.81	439.4±0.42
Mean	73.79±0.19	587.57±0.87	828.05±0.82	512.38±0.30

Table 3: Heavy Metal Concentrations in Cow's Milk Samples

Sample	Results	Co	Cd	Pb	Cr	Zn	Ni	Mn	Cu	Fe
U1	Mean	ND	ND	0.062	0.036	2.53	0.061	0.089	0.147	1.23
	SD ±	-	-	0.001	0.001	0.13	0.01	0.01	0.04	0.12
U2	Mean	ND	ND	0.064	0.032	1.54	0.052	0.017	0.155	1.26
	SD ±	-	-	0.001	0.001	0.17	0.01	0.04	0.03	0.20
U3	Mean	ND	ND	0.083	0.021	1.24	0.087	0.04	0.165	2.18
	SD ±	-	-	0.001	0.001	0.12	0.01	0.02	0.06	0.14
U4	Mean	ND	ND	0.071	0.042	1.86	0.097	0.051	0.174	1.12
	SD ±	-	-	0.001	0.001	0.32	0.02	0.01	0.02	0.16

U5	Mean	ND	ND	0.031	0.062	2.34	0.068	0.032	0.148	1.87
	SD ±	-	-	0.001	0.001	0.08	0.01	0.01	0.04	0.12
U6	Mean	ND	ND	0.061	0.083	2.31	0.058	0.089	0.188	1.11
	SD ±	-	-	0.001	0.001	0.17	0.01	0.01	0.01	0.22
U7	Mean	ND	ND	0.053	0.040	2.11	0.095	0.067	0.164	4.33
	SD ±	-	-	0.001	0.001	0.11	0.01	0.02	0.01	0.14
U8	Mean	ND	ND	0.051	0.061	2.23	0.057	0.097	0.163	2.17
	SD ±	-	-	0.001	0.001	0.35	0.02	0.01	0.02	0.11
P1	Mean	ND	ND	0.043	0.033	2.03	0.096	0.044	0.185	1.68
	SD ±	-	-	0.001	0.001	0.25	0.01	0.01	0.02	0. 2
P2	Mean	ND	ND	0.032	0.05	1.98	0.086	0.056	0.165	1.57
	SD ±	-	-	0.001	0.001	0.18	0.03	0.03	0.04	0.23
R1	Mean	ND	ND	0.024	0.031	1.98	0.054	0.092	0.305	2.32
	SD ±	-	-	0.001	0.001	0.18	0.02	0.01	0.01	0.13
Mean		-	-	0.053 ±0.001	0.045 ±0.001	2.014 ±0.19	0.074 ±0.01	0.061 ±0.02	0.178 ±0.03	1.89 ±0.16

ND: Not Detected (under detection limits).

For major metals in milk samples, the mean concentration of K was the highest (828.05 ± 0.82 ppm) followed by Ca (587.57 ± 0.87 ppm), Na (512.38 ± 0.30 ppm), and the last one was Mg (73.79 ± 0.19 ppm). The trend for heavy metals observed for milk samples were as follows: Zn (2.014 ± 0.19 ppm) ~ Fe (1.89 ± 0.16 ppm), Cu (0.178 ± 0.03 ppm), Ni (0.074 ± 0.01 ppm) ~ Mn (0.61 ± 0.02 ppm), and finally Pb (0.053 ± 0.001 ppm) ~ Cr (0.045 ± 0.001 ppm). The Co and Cd concentrations were lower the instrument detection limits in all examined milk samples. From the detected heavy metals, the highest mean concentration obtained was for Zn which is similar to Belete et. al. (2014) and Farid et. al. (2004) findings.

3.1. Sodium levels

In Fig. 1, the sodium levels for milk samples under investigation are shown which ranged from 350.4 to 624.6 ppm. Though, there are no limits for sodium levels specified by the Libyan standard specifications for milk samples but our results were similar to those reported by Qin et al. (361 – 571 ppm) (2009), Gabryszuk et al. (357 – 490 ppm) (2008), and Gaucheron (391 – 644 ppm) (2005). Sodium (Na) consumption should be examined in young children because their excretory system is less effective to eliminate Na than that of adults (Michaelsen et al. 2000). Accordingly, extra sodium may cause in early health difficulties for the young children, along with influencing their long-term health condition. While Na sources may vary over time, indications propose that main sources of nutritional Na for primary school children aged 4–13 years are processed food like meat and poultry products, cereal and cereal products, and milk products. Whole milk, powder milk, flavoured milk, diary milk products provides the most sodium (Marcio et al. 2016).

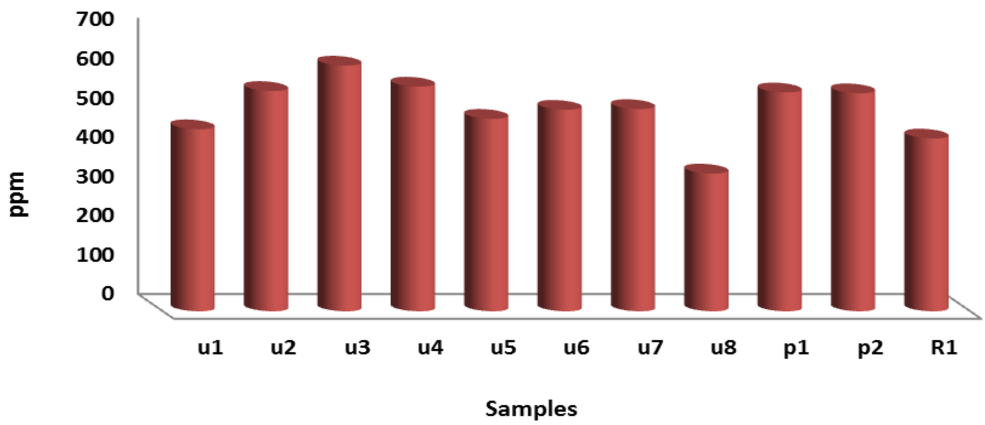

Fig. 1: Sodium Contents in Milk Samples.

3.2. Potassium levels

As shown in Fig. 2, it is observed that potassium contents for investigated milk samples were ranged from 769.6 – 901.6 ppm which is related to previous studies, Qin et al. (908 – 117 ppm) (2009), Gabryszuk et al. (856 – 978 ppm) (2008), and Gaucheron (1212 – 1681 ppm) (2005). Also, there are no limits for potassium levels specified by the Libyan standard specifications for milk samples. Moreover, we observed that K contents were higher than the other essential minerals. Sodium and potassium levels in the body are 1.4 g/kg and 2 g/kg, respectively. Potassium is the highest widespread cation in the intracellular fluid. It controls the osmotic pressure inside the cell and additionally in the activation of a number of glycolytic and respiratory enzymes. Milk and yogurt, besides nuts, are also excellent sources of potassium (Ateteallah et al. 2017).

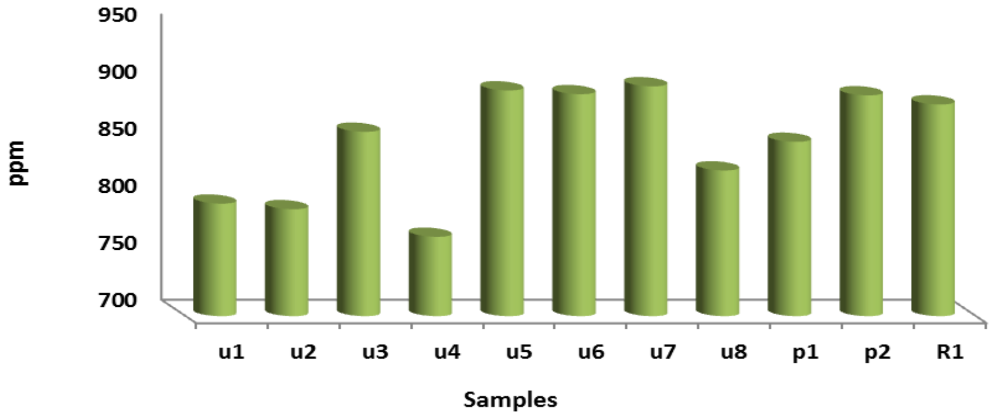

Fig. 2: Potassium Contents in Milk Samples.

3.3. Calcium levels

Fig. 3 presents calcium levels for each milk sample. And according to the Table 2., Ca concentrations were in the range of 518.4 – 682.7 ppm. The highest concentration of Calcium was found in the sample R1 while the lower concentration was in U2. Generally, The Ca contents of our milk samples were lower than the values reported by other authors (970 – 1650 ppm) (Cashman et al. 2006, Sola-Laranaga et al. 2009). Still, the concentrations of Ca in our milk samples were comparable with those reported by Gabryszuk et al. (567 – 706 ppm) (2008). Calcium is an fundamental macronutrient for humans, which presents about 2 % of human body weight in adults. This mineral has primarily a structural role in bones and teeth, and regulates many essential biological functions. In recent times, the concern in calcium has focused on its role in avoiding osteoporosis. The bioavailability of calcium in milk is thought to be fundamental (Ateteal-lah et al. 2017).

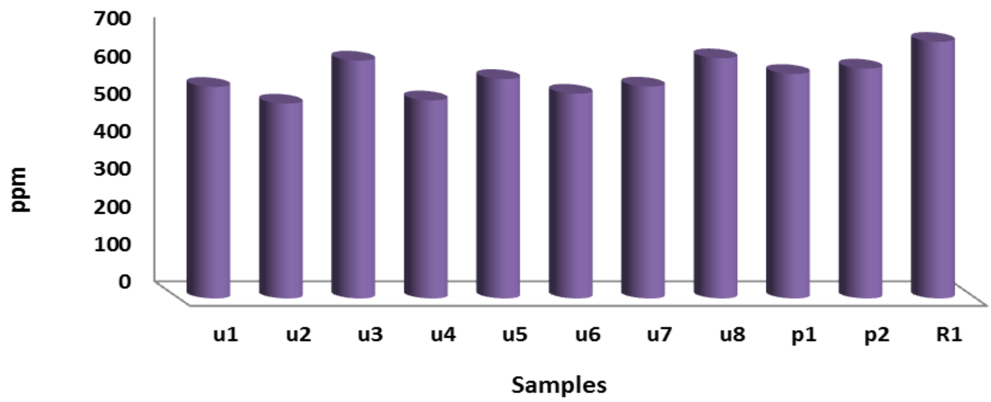

Fig. 3: Calcium Contents in Milk Samples.

3.4. Magnesium levels

The Mg levels were ranged from 60.95 to 80.44 ppm. The results of Mg concentrations are displayed in Fig. 4. Concentrations of Mg in our milk samples were compared with other studies. In Qin et al. (2009) study, the concentrations of Mg was ranged between 117 - 125 ppm. Malbe et al. (2010) reported that the range of Mg concentrations in milk samples is between 96 - 125 ppm. In Moreno et al. (1994) study, Mg content was in the range of 98 - 121 ppm. Magnesium being a main element involved for the organizing of various body tasks. Mg Lowers muscle straining, lower pain related to migraine headaches and better sleep & bad neurological complaint like anxiety and depression. Magnesium is required for above 300 biochemical reactions in the body. It assists to take care of regular nerve and muscle function, maintains a healthy immune system, saves the heart beat stable, and supports bones stay strong. It also aids control blood glu-cose levels and assists in the creation of energy and protein. Mainly nutritional magnesium originates from vegetables, like dark green, leafy vegetables (Lutfullah et al. 2014).

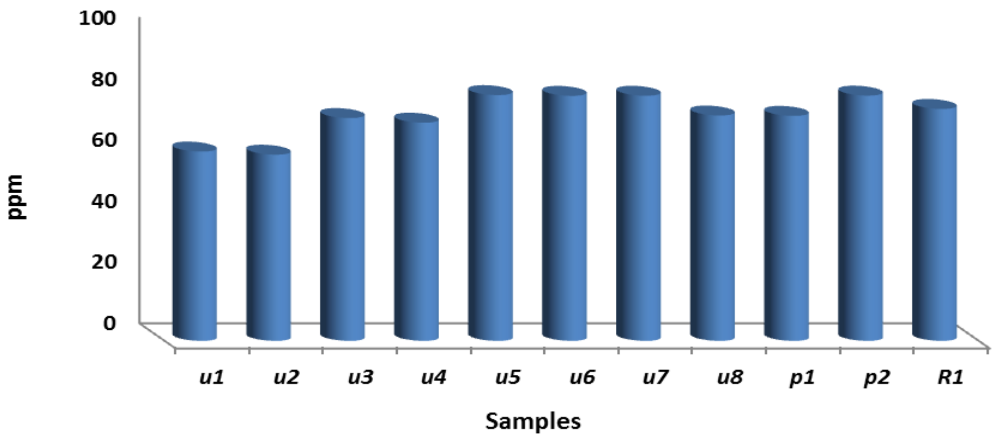

Fig. 4: Magnesium Contents in Milk Samples.

3.5. Iron levels

The results of iron levels in the present study are shown in Fig. 5. The Fe concentration in the analyzed milk samples was ranged from 1.11 to 4.33 ppm. Though, the present values of Fe concentration is lower than the corresponding values of Malhat et al. (1.84 – 50.00 ppm) (2012). This shows that the cow's milk sample is a poor source of iron. However, our results were comparable with other studies (Qin et al. 2009, Birghila et al. 2008, Ogabiela et al. 2011). Various studies has revealed that consumption of cow's milk in nature constantly play as a risk factor for anemia in children (Hadler et al. 2004, Assis et al. 2004, Oliveira et al. 2005). In the study of Hadler et al. (2004) detected that the consumption of fresh cow's milk had a positive relation with anemia occurrence in children aged 6 to 12 months. The authors describe that an enlarged ingestion of cow's milk, whose iron level is lowered and has minimal bioavailability, can lessen the total amount of iron included in the diet or replacement other probable sources of this nutrient. Improved milk, enhanced with iron, though, decreases the risks of anemia by enhancing the concentration of iron in the milk (Oliveira et al. 2005).

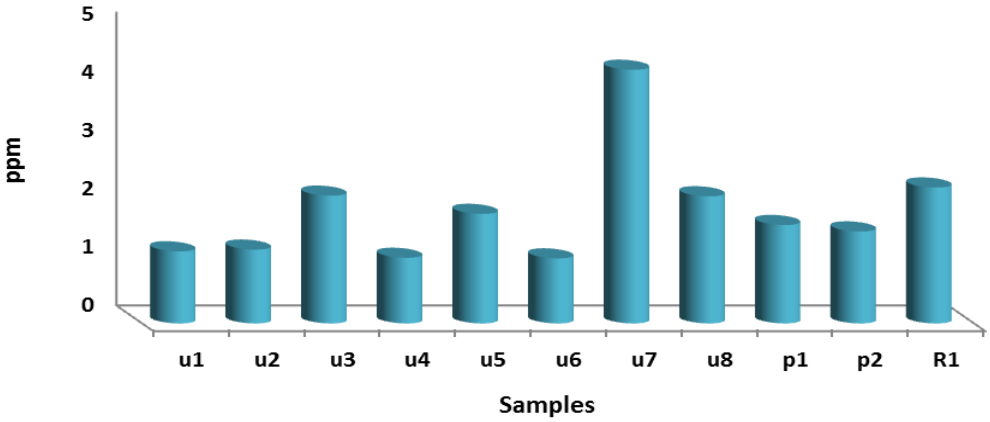

Fig. 5: Iron Contents in Milk Samples.

3.6. Zinc levels

The zinc contents in the examined milk samples are presented in Fig. 6. The results demonstrated that the Zn contents were in the range of 1.24 – 2.53 ppm in the milk samples. These concentrations were lower than those determined in previous studies (Lante et al. 2004, Enb et al. 2009). However, Zn levels are in the line with those reported by Onianwa et al. (1999). Ingesting unreasonably extent of zinc into the body through food, water, or dietary complements can influence health. The suggested dietary allowances for zinc is 11 mg/day for men and 8 mg/day for women. If huge amounts of zinc (10–15 times greater than the suggested) are obtained; even for a short time, stomach cramps, nausea, and vomiting may happen. Feeding elevated levels of zinc for a number of months may initiate anemia, harm the pancreas, and reduce levels of high-density lipoprotein (HDL) cholesterol (National Academies Press (US) 2001).

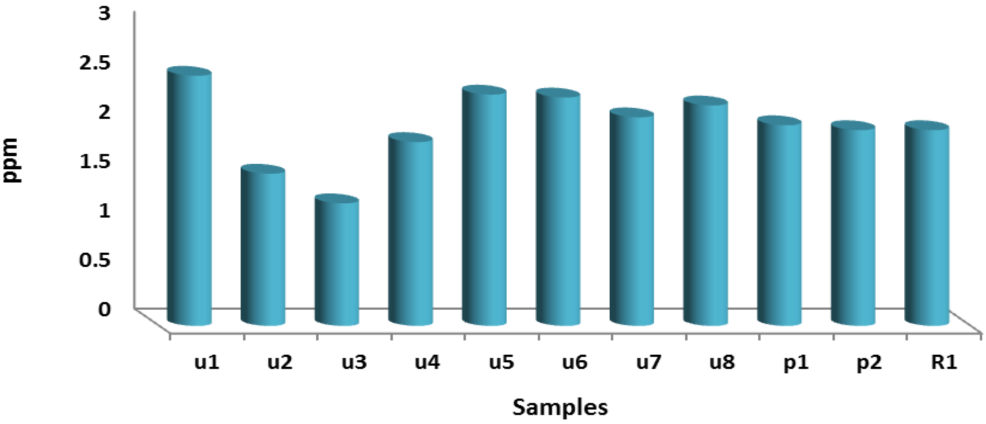

Fig. 6: Zinc Contents in Milk Samples.

3.7. Copper levels

As it can be observed from the Fig. 7, the Cu content found in the cow milk varies in its quantity from 0.305 to 0.147 ppm. There are no limits for copper levels specified by the Libyan standard specifications for milk samples. Increased copper levels in milk leads to vitamin C deficiency and also it is a catalyst for oxidation and fatty taste in milk. Copper poisoning may lead to infections and cancers in the lung and breast (Zheng et al. 2007). Cu levels are in our study were lower than those reported by other authors (Licata et al. 2004, Nasr et al. 2007, Goncalves et al. 2008). The minimal levels of Cu could be as a result of Zn included in food that disturbs with the copper absorption system, describing the occurrence of low levels of this metal in milk (Doull’s 2000) . However, our results were comparable to those reported by Malhat. et al. (2012).

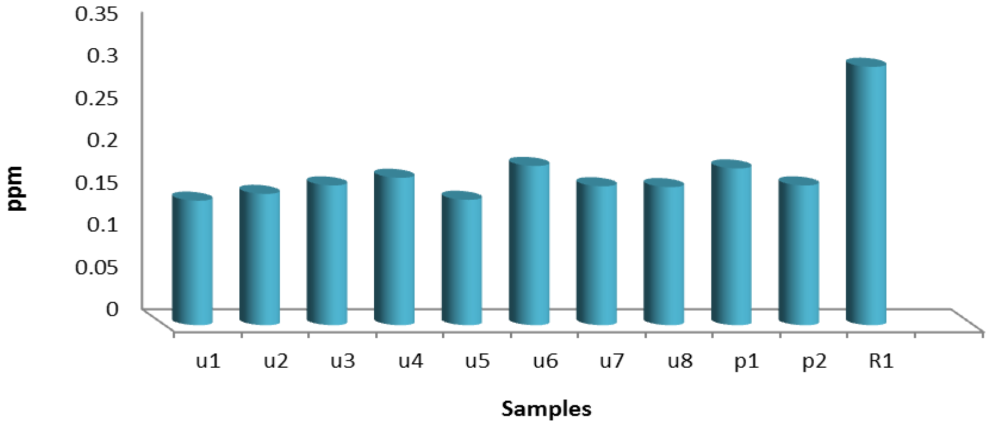

Fig. 7: Copper Contents in Milk Samples.

3.8. Manganese levels

Manganese concentrations in the examined milk samples are shown in Fig. 8. Our Mn concentrations in the cow milk samples have ranged from 0.017 to 0.097 ppm with an average of 0.061 ± 0.02 ppm. In Ogabiela et al. study (2011) to estimate the mineral content of fresh milk in Challawa industrial region in Kano Nigeria, the mean manganese concentration was 0.179 ± 0.13 ppm while its concentration in the region of Zaria Kaduna had an average of 0.219 ± 0.09 ppm. In the study conducted by Birghila et al. (2008) to estimate the minor and major elements in cow milk, mean manganese concentration in fresh milk was 0.08 ppm while in pasteurized milk was between 0.04 – 0.09 ppm which were similar to our results. Manganese is an important trace metal that is naturally existing in many foods and offered as a dietary supplement. Manganese is a cofactor for various enzymes, containing manganese superoxide dismutase, arginase, and pyruvate carboxylase. Manganese concentrations are 3 to 10 mcg/L in breast milk and 30 to 100 mcg/L in cow's milk–based infant formulas. Soy-based infant formulas have elevated manganese concentrations, 200 to 300 mcg/L, than milk-based formulas (Buchman et al 2014).

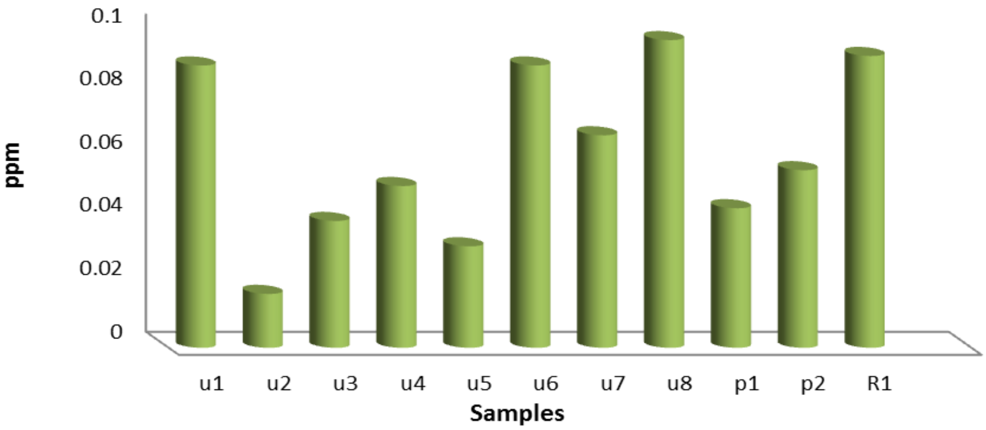

Fig. 8: Manganese Contents in Milk Samples.

3.9. Nickel levels

From Fig. 9, we notice that the nickel content in the milk samples ranged from (0.052 - 0.097 ppm). The lowest level of nickel was in the sample U2 while the highest was detected in the sample U4. In the study conducted by Enb et al. (2009), nickel concentrations in bovine milk samples ranged from (0.002 -0.009 ppm) which were lower than the results found in our current study. While in the Birghila et al. (2008) study to estimate the minor and major elements in milk samples using ICP- AES, the mean Ni concentration in fresh milk was 0.04 ppm whereas in pasteurized milk was 0.04 ppm. Nickel is found in both human and cow's milk at levels stated to range from 0.001 to over 0.1 ppm, although concentrations in USA studies indicate levels in the region of 0.015 ppm (EU, 2004).

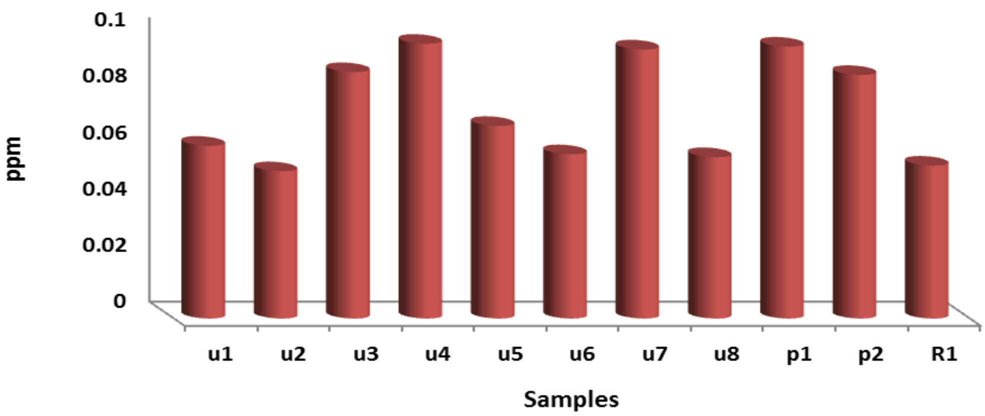

Fig. 9: Nickel Contents in Milk Samples.

3.10. Chrome levels

Distribution of Cr concentrations in the milk samples are depicted in Fig. 10. The mean concentration of Cr was 0.045 ± 0.001 ppm (range: 0.021 – 0.083 ppm). In Birghila et al. (2008), the Cr levels in fresh and pasteurized milk samples were observed to be similar to our values (0.04 and 0.05 ppm). Whereas in the studies conducted by Enb et al. (2009), Licata et al. (2004), and Flynn (1992), the levels were lower compared to the present study. Though Cr and other microelements are necessary to continue the metabolic systems of human body, they can cause poisoning at elevated level (Qin et al. 2009). It can initiate stomach upsets and ulcers, convulsions, kidney and liver injury, and even death. The poisonousness of chromium relates to its chemical formula, with hexavalent chromium (VI) compounds having a toxic, mutagenic and even carcinogenic nature, and the trivalent chromium (III), which occurs in foods having a low toxicity. Acceptance limit of Cr in milk is 0.3 ppm (Qin et al. 2009). The recommended value for chromium (III) is 50-200 µg per day (Belete et al. 2014).

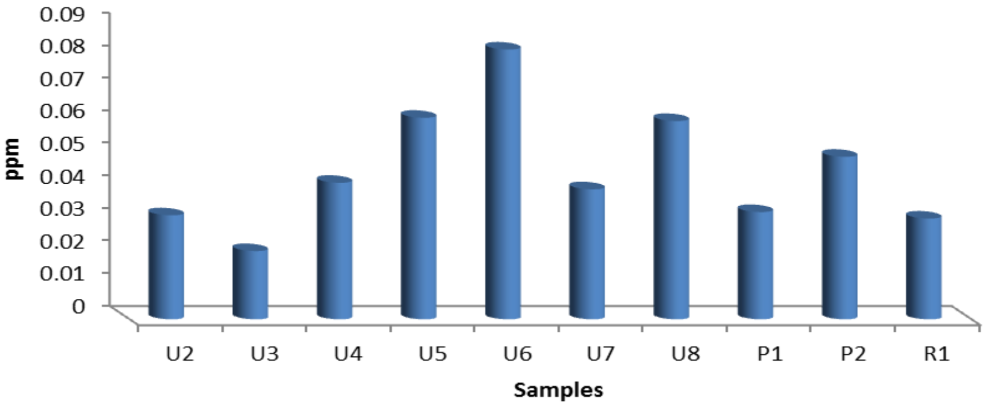

Fig. 10: Chrome Contents in Milk Samples.

3.11. Lead levels

From Fig. 11, we notice that the lead content in the studied milk samples ranged from (0.024 - 0.083 ppm). The highest concentration was in sample U3, while the lowest was in sample R1. The concentrations of Pb in Krishna et al. (2015) study for long life and pasteurized milk samples were in the ranges 0.0442 – 0.0575 ppm which were comparable to the present study. The Pb contents of Italian fresh milk samples were reported to be 0.0095 ppm which is lower than our results. Also, the results of Rodriguez et al. (2001) for the determination of fresh cow milk were in Spain lower than the present study. The level of lead in our current study was higher than the FAO / WHO limit (0.0205 ppm). The occurrence of Pb in milk and dairy products may be as a result of environmental sources (atmospheric deposition, waste disposal, vehicle exhausts, urban effluent etc.). Lead may have destroying effects on the nervous system of infants. Buildup of lead in bones due to earlier exposure is circulated into the blood along with calcium and excreted into breast milk. Exposure to lead is related with anxiety and depression in adults. Additionally, it harmfully impacts the growth of newborns (Bahmani et al. 2018).

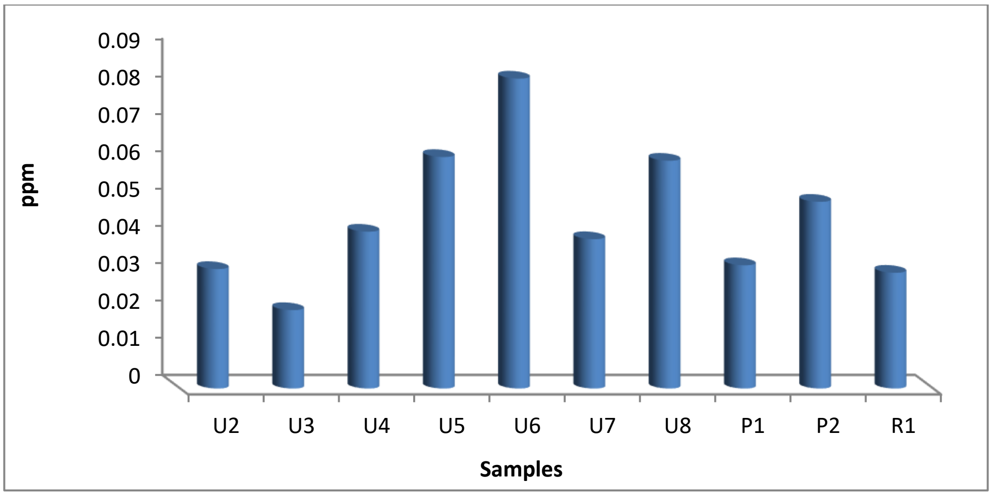

Fig. 11: Lead Contents in Milk Samples.

3.11. Cobalt and cadmium levels

In all examined milk samples, the levels of Co and Cd were lower than the instrumental detection limits. Milk and eggs have about 0.004- 0.005 ppm; dairy products such as cheese and butter are rather rich in cobalt (0.02 ppm). Eggs and milk comprise even larger quantities of vitamin B12 irrelevant cobalt (about 70 and 95 %, respectively) demonstrating excretion of absorbed soluble cobalt (Scientific Opinion 2009). The occurrence of Cd in milk and dairy products may be owing to either natural or anthropogenic sources (fertilizers and atmospheric deposition in soils). It is regard as the most critical pollutant in current days. Cd and Pb are among the metals that have affected the most concern in terms of undesirable effects on human health. This is because they are easily transported through food chains and are not known to provide any fundamental biological function (Arafa et al. 2014).

4. Conclusion

The current paper provides significant information on the levels of essential (Na, K, Ca, and Mg) and heavy metals (Fe, Zn, Cu, Mn, Cr, Co, Ni, Cd, and Pb) in long life and pasteurized milk samples. For major metals, potassium was the metal detected at highest levels with an average concentration of 828.05±0.82 ppm followed by calcium with a mean of 587.57±0.87 ppm. While for minor metals, zinc and iron had the highest concentration with an average of: 2.014±0.19 and 1.89±0.16 ppm, respectively. Cobalt and cadmium were not detected in all examined milk samples. The outcomes of our study obviously show that the ingesting of milk and dairy products relate to an important quantity of the reasonable daily intake of K, Ca, Na, Mg, and Zn. Fresh milk are poor source of Fe, Cu, Mn, Cr, and Ni. Consumption of fresh milk is approximately free of risks, but bioaccumulation of lead and other heavy metals via the food chain and consumption from other food stuff should also be of interest. More consideration should be provided to heavy metals as once they are occurred in concentrations greater than the acceptable daily intake.

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