**PRESENCE OF HEAVY METALS IN COW’S MILK FROM ZAWIYA MILKY FARMES, USING FAAS ANALYSIS**

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**Abstract**

Heavy metals considered as the most important contamination due to the industrialization and have the influence on its existence in milk and dairy products. Milk is a very important component of human diet. The presence of heavy metals in milk at high concentration may significant health problem. Milk is regarded as a complete food because it contains all of the macronutrients including protein, carbohydrates, fat and vitamins. Milk also has a high concentration of mineral elements (metals) such as sodium, potassium, iron, calcium, magnesium, selenium, copper and zinc. They are critical for proper body growth and maintenance but excess in these metals, particularly, heavy metals cause disturbances and pathological conditions. People nowadays are concerned about food safety issues involving microbial, chemical and physical hazards. Heavy metal residues such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) pose a chemical hazard.

Key words: FAAS, Cattle, Heavy metals, Milk, zawiya

**Introduction**

Milk is an important influencer on human health containing considerable source of proteins, fats, vitamins as well as major minerals and elements [1]. These elements are ubiquitous in the environment widely associated with industrial and human activities such as mining, steel and chemical plants [2]. These can pollute the environment including air, soil, water and subsequently contaminate plants and animals particularly domestic species such as food-producing and dairy animals [3].

Many factors may contribute and lead to milk contamination particularly with trace metals such as those associated with polluted animal feed and water, manufacturing and packaging processes [4,5]. these elements may present in milk as inorganic ions in proteins, peptides, carbohydrates and other molecules [13]. Some of these metals have beneficial health importance acting like enzymatic co-factors that can play vital physiological and protective roles and functions in humans mainly in the immune compromise individuals [7]. These trace metals may become toxic when they exceed their threshold and recommended levels and/or limits up to 40 to 200 folds (Rao005). These limits and their toxicities are dependent on certain risk factors linked to age, sex, element oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms of excretion [14,15]

Contamination in milk is considered as one of the main dangerous aspects that affect humans health and wellbeing within the last few years [16]. This is further accelerated due to rise in environmental pollution and xenobiotic compounds which may significantly damage human health [17, 18].

for instance, the high concertation of these trace elements such as (Mn, CU, Fe, Pb, Cd, and Ni) in any human food or drink will cause several heath problem such as abdominal pain, hepatotoxicity, neurotoxicity, vomiting [19], decreasing of intelligence quotient (IQ) level, Alzheimer’s disease, behavioural disorders [20], tissue injury, irritation of lungs, cancer [21]. They are generated due to over exposure of heavy metals originated from nature and further accumulated in the food chains via bio-transformation, bio-accumulation and biomagnifications [22]. These elements are hardly difficult to be eliminated from cow milk due to the characteristic of lipophilic contaminants to persist in fat compounds [23].

The aim of this investigation was to determine and evaluate selected trace metals in fresh cow's milk collected from local and commercial distributing shops in Zawiya distract between April 2015 - September 2016. The study assessed the concentrations of metals in both cow's milk and commercial milk based on chemical composition using FAAS analysis via microwave digestion as well as the effect of the discussed pollutants factors on their levels.

The advancement of industry and agriculture has resulted in the release of numerous heavy metals into the environment which is harmful to the health of both animals and humans. Animals ingest heavy metals from a variety of sources including soil, water, feed and fodder. Because the mammary gland is the most physiologically active component of an animal that resulted into heavy metals are reflected in milk (Figure 1).



Figure 1.

Source of heavy metal in milk.

**Sample preparation**

**Study area and sample collection**

The study was conducted in the period from April 2015 to September 2016. A total of 33 samples were collected from ten milky farms in different areas of Zawiya district located in North West of Libya, around 40 km west of the capital Tripoli. The cow milk samples were taken from various characteristic healthy cows, including age, weight. The samples were collected from fresh bulk cow milk directly from each farm in clean and dry plastic bottles, all samples were transported in clean cold container and stored in freer at 0 ◦C -5 ◦C until analysis. Other eight commercially available UHT cow milk samples of different brands were collected from different supermarkets in Zawiya city at room temperature. These samples were collected in their one l000 ml paper card and plastic bottle. A total of 80 samples were analyzed.

The samples were classified according to their collection sources as (i) the popular packaged cow milk was considered as Brand milk (33 samples), (ii) the dairy farm milk (30 samples) collected from the available dairy farms and (iii) the milk samples collected from the small household farmers as domestic cow milk (27 samples). All the samples were collected in a sterile bottle, clean and dray following standard methods and stored in cold freezer until analysis.

 **Analysis and Method**

The analysis started by digest each milk sample using microwave. 10 ml of each milk sample put into Teflon digestion vessel, 10 ml of 69 % w/w HNO3 was added dropwise followed by 6 ml of H2O2 30 % w/v. The mixture was shaken well for 10 min. the vessel was introduced to the microwave after firmly closed, then was fixed inside microwave’s tray. it was heated in two different temperatures, where heated inside the microwave at 60W, 165◦C for 10 min then at 100W, 200 ◦C for 20 min. The hot mixture vessel was moved inside the fume hood for standing until room temperature, the brown colour top skin of fat was removed by separator funnel. The rest solution was transferred into 50 ml Volumatic flask, deionized diluted water was added until mark, then labelled and kept in clean place at room temperature ready for analysis using calibrated Atomic absorption spectrometry. All other samples were digested following same digestion conditions.

A standard solution of each reference heavy metals was prepared for calibration and linearity used proper dilution of their stock standard solution with range of concentrations. For Mn were 0.2, 0.4, 0.6 and 0.8 ppm, for Cu were 1, 2, 3 and 4 ppm, for Pb were 10, 20, 30, 40 ppm.

The concentration of estimated heavy metals in various samples of different districts of Haryana state were analyzed by ‘t’ test and one way ANOVA using SAS system.

Instrument analysis started by introduce each sample to calibrated AAS, the concentration ug/ml for each sample from fresh cow milk was calculated,

**Result**

Results showed variable concentrations of heavy metals in fresh cow milk samples with the highest concentration was for Fe (2.080 ug/ml) followed respectively by Pb (1.800 ug/ml), Mn (1.018 ug/ml), Cu (0.321 ug/ml). (Table and chart 1). In pasteurized milk samples, concentrations of heavy metals were respectively determined as follows: Fe (2.840ug/ml), Pb (0.457ug/ml), Cu (0,055 ug/ml) then Mn (0.216 ug/ml). (Table 1, Chart 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Mean Conc.**  **ug/ml of**  **heavy metal** **Sample location** | Manganese(Mn)  | Iron(Fe) | Copper(Cu) | Lead(Pb) |
| Jodaium | 0.698 | 0.798 | 0.106 | 1.200 |
| Al-Barnawi | 0.359 | 0.696 | 0.004 | 0.866 |
| Delh | 0.441 | 0.996 | 0.300 | 0.410 |
| Span | 0.460 | 0.942 | 0.107 | 0.640 |
| Al-Harsha | 0.813 | 0.832 | 0.321 | 1.800 |
| Bouaissa | 0.432 | 2.080 | 0.257 | 0.500 |
| Surman | 0.568 | 0.694 | 0.132 | 0.826 |
| Sayda Zeinab | 1.018 | 0.928 | 0.242 | 0.325  |
| Tirfasah | 0.869 | 1.070 | 0.182 | 0.820 |
| Binshu’ayb | 0.287 | 0.778 | 0.002 | 1.496 |

**Chart (1)**



Based on the source location, Table 1 and chart 1 showed that the highest concentration of Fe in cow milk was found at Bouaisa (2.080 ug/ml), while the Pb was high at Al-Harsha (1.800 ug/ml) and Binshuayb (1.496 ug/ml). The lowest concentration of Fe at Al-Barnawi (0.696 ug/ml) and at Sayda Zainab (0.325 ug/ml) for Pb. The Mn was high in Sayda Zainab (1.018 ug/ml) and low at Binshuayb (0.287 ug/ml), the Cu was high at Al-Harsha (0.321ug/ml) and low at Binshuayb (0.002 ug/ml).

**Table (2) and chart (2) show the heavy metals concentration in ug/ml for the pasteurized milk**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Mean Conc.**  **ug/ml of**  **heavy metal** **Pasteurized milk Brand** | Manganese(Mn)  | Iron(Fe) | Copper(Cu) | Lead(Pb) |
| Lactel (French) | 0.216 | 2.320 | 0.162 | 0.314 |
| Delice (Tunisian) | 0.218 | 2.000 | 0.055 | 0.150 |
| Almarai (KSA) | 0.243 | 1.920 | 0.081 | 0.381 |
| Juhayna (Egyptian) | 0.269 | 1.900 | 0.054 | 0.457 |
| MUH (German) | 0.270 | 2.160 | 0.108 | 0.300 |
| Mocitos (Spanish) | 0.299 | 2.840 | 0.187 | 0.152 |
| Granrolo (Italian) | 0.378 | 2.31 | 0.189 | 0.153 |

**Chart 2**

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Further results of the pasteurized milk samples showed that iron concentration was high in most samples compared to the raw cow milk, but the highest value was for Mocitos brand (2.840 ug/ml) and the lowest was for Juhayna brand (1.90 ug/ml). The Pb was high for Juhayna (0.457 ug/ml) and low for Delice (0.150 ug/ml) and for Mocitos (0.152 ug/ml), even the allowed intake for human body by WHO is (0.035 ug/ml) [24]. (table 1,2 and chart 1,2),

**Discussion**

The existence of trace elements and heavy metals in dairy products especially milk has been recorded in various countries and regions [11,15,16,19,21]. Increased concentrations of heavy metals in body result in low fitness, reproduction problems, immunity decline and occurrence of cancerous and teratogenic diseases [12]. Pb persist in the body and exert its toxic effect by combining with one or more reactive groups essential for physiological functions of the cells thus causing cellular disturbances or clinical manifestation [9]. The major clinical signs in animals and humans for Pb and Cu poisoning are their direct effects on haematopoiesis, reduced integrity of red blood cells membrane leading to intravascular haemolysis, anemia and dehydration [18]. It may be attributed to contamination due to exposure of lactating cow to environmental pollution or consumption of contaminated feeding stuffs and waste water [6]. Therefore, determination of the heavy metals residual levels in milk could be indicator of the degree of pollution of the environment where the milk was produced [11].

Iron (Fe) is a component of blood and many enzymes. It is involved in blood metabolism and oxygen transport in milk contains that has a small amount of iron. This study showed Fe was varied from 0.696 ug/ml to 2.08 ug/ml for cow milk and 1.900 mg/ml to 2.84 ug/ml for pasteurized milk. Recommended maximum values of Fe 0.037 ug/ml by IDF-International Dairy Federation [8]. It advised to be in average values/concentration in spite of there is higher concentration than this value of cow milk in different countries [25]. The over existing of Fe in cow milk might be the fed with hay from pasture rich in iron between March to May or contaminated drinking water.

Copper (Cu) is a component of enzymes used in iron metabolism. Gakhar et al. [10] find that this heavy metal plays an important role in pre-venting mastitis in cow milk that contains a small amount of copper. Some researchers found the amounts of Cu in the milk of individual varied from 0.2 to 0.8 ug/ml [20]. The IDF-International Dairy Federation [8] Recommended maximum values of heavy metals in cow milk for Cu is 0.01 ug/ml. In current study the concentration values of Cu in cow milk was in average and varied from 0.002-0.321 ug/ml and for pasteurized milk was from 0.054-0.189 ug/ml, it is in average regarding several researches [20].

Manganese (Mn) can be existing in several chemical formulas such as MnCl2, Mn2O4, MnO2 and KMnO4. The Mn in current study was in average and varied from 0.287 – 1.018 ug/ml for cow milk and 0.216 – 0.378 ug/ml for pasteurized samples WHO was recommended maximum daily intake of Mn 0.07 – 10.0 mg/day [26]

**Conclusion**

In conclusion, results showed that most of the milk samples contained metals and at average to higher concentrations than the recommended levels. Such revelation showed the public health hazards that such sources implicate on humans and the environment. National and local authorities should take appropriate action to control and apply appropriate standards. Advanced and high standards and quality in cow milk including animal feed and drink, milk process, transportation, storage and manufacturing are extremely required and further investigation are needed. Heavy metals do not naturally arise in milk as a consequence of human activities such as industrial and agricultural processes, but they can naturally occur in milk as a result of human activities such as industrial and agricultural processes.

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