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Determination of Some Trace Elements in Buffalo's Milk in Tanjaro Area-Sulaimani-Iraq

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Abstract

Buffalo's raw milk subjected to various physical, chemical and biological pollutants due to produce, transporting and marketing processes. The current study was designed to assess the level of some trace elements in buffalo's raw milk. Milk samples were collected from 2 fields located on Tanjaro River during 1 years, the highest mean value of Pb, Cd, Cr, Cu and Zn were 0.075, 0.005, 0.010, 0.126 and 0.723 $mg.kg^{-1}$ were recorded, respectively. The data showed that the most polluted season for Pb was during autumn with the average of 0.051 $mg.kg^{-1}$, while the most polluted season for Cd and Cu was the end summer with the average value 0.004 and 0.103 $mg.kg^{-1}$ respectively, but for the both trace elements Cr and Zn the spring season is the most polluted season with the average of 0.006 and 0.669 $mg.kg^{-1}$ respectively.

Introduction

Milk and milk products are the main components of daily human diet, especially for susceptible groups such as infants, children, school age and old age, due to containing specific proteins, fats, vitamins, minerals and other components that may have an important role in human life *[1]*. Trace metal are found in both plant and animal cells and many of them are needed for human nutrition, some of these metals can cause bad effects when it consumed at higher levels. Many of these metals considered as heavy metals and some of them, such as Cu, Cr, Fe and Zn are essential in very low concentration in order to survival of all forms of life *[2]*, but when they are presented in greater quantities, they can cause metabolic anomalies *[3],* while, the heavy metals like Pb and Cd are already toxic in a very low concentration. Unfortunately, milk could be contaminated with heavy metals from the environment entering the food chain which is come mostly through consumption of plants directly polluted with air or soil particles *[4]*. Soil and plants can be polluted with heavy metals from various sources such as dry or wet atmospheric deposition, agricultural practices (e.g., application of fertilizers), human activities (e.g., pollution from car exhausts mining) water and sewage contamination *[5]*. Dairy animals take up these metals through feed, primarily grass, and usually become contaminated either by grazing a contaminated pasture or by eating contaminated hay or cereals *[6]*. Heavy metals accumulate in tissues of dairy animals and ultimately excrete in milk because of their non-biodegradable and persistent nature *[7; 8]*. The intake of food contaminated with higher levels of these metals can lead to intoxication that can be described as acute or, long-term intoxications; the chemicals producing the latter tend to accumulate in the body during long periods of time, producing illness when the levels reach critical values in certain tissues *[9]*. Tanjaro at Sulaimani governorate in Iraq described as contaminated area, buffalo's milk produced in this area is used for producing many dairy products e.g. cream, yoghurt cheese...etc., and there is no research carried out on this type of milk and milk products produced from it, so the main objective of this study is to determine the level of some trace elements in buffalo's milk in this polluted area.

Materials and Method

A. Milk samples

Sixty four samples (2 litters) of fresh raw buffalo's milk were collected from 2 fields of Tanjaros River during different periods (Apr-May, Jul-Aug, Oct-Nov, 2015 and Jen-Feb. 2016).

Milk samples were collected from the bulk milk "which is taken from more than 20 animals" of the farms directly into carefully washed plastic bottles, all samples stored at -18°C until analysis. Milk used in dairy products was obtained from the same fields.

B. Metals standards

Multi elements standards obtained by Perkin Elmer were used for standardize.

C. Milk composition analysis

Buffalo's milk samples were analysed for moisture, protein and fat content according to the Association of Official Analytical Chemist *[10]*.

D. Ash determination

Ash content was determined by the dry ashing method using muffle furnace based on method by Espie and Mullan (1990) ***[11]***. Five ml samples were ignited in muffle furnace at 250 °C. and gradually the temperature was increase until it reached 500°C and the sample was remained at this temperature for about 7-8 h until the ashing was completed . Sample were removed, cooled and 2 ml of ($HN0_3$) (1N) was added to each sample after filtration the sample volume were completed with deionized water to 50 ml in volumetric flask

E. Determination of trace elements

Trace elements (Pb, Cd, Cu, Zn and Cr) levels in buffalo's milk samples were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy ICP- OES
(Optima 2100, PerkinElmer).

F. Statistical Analysis

The data were statistically analysed according to the methods of analysis of variance as a general test, factorial experiment with three replications and conducted in Complete Randomized Design (CRD) by using the XLSAT program ver. 7.5.2. All possible comparisons among the means were carried out by using Least Significant Difference (LSD) test at a significant level of (0.05) after they show their significance in the general test.

Results and Discussion

A. Chemical compositions of Buffalo's milk samples

The compositions of buffalo's milk was used in this study are shown in table 1. The average of fat content varied between (5.9-5.5%), while the average of protein varied between (2.4 - 2.8%), these results were higher than that detected by Han et al (2012) *[12]* and Rafiq *et al*.(2016) *[13]*. On the other hand, the overall milk composition was agreed with that mentioned by Enb *et al*. (2009) *[14]*. for buffalo's milk composition.

Many factors have been reported that they affect the milk composition such as: nutrition, genetics, diseases, lactation stages and seasons *[15]*.

Table-1: Chemical composition of buffalo's milk samples in Tanjaro area

Item	*Composition Range (%)*	*Composition (Mean %)*
Moisture	*86.37 - 86.30*	*86.33*
Total solid	*13.7 - 13.63*	*13.67*
Total protein	*2.4 - 2.8*	*2.6*
Fat	*5.9 - 5.5*	*5.7*
Solid Not Fat	*7.8-7.13*	*7.97*
Ash	*0.72 - 0.68*	*0.7*

B. Trace elements:

Lead (Pb)

Lead residue concentration of raw buffalo's milk samples collected from 2 fields in four seasons is shown in Table 2. Statistical analysis has shown significant differences ($p \leq 0.05$) between raw milk samples of different locations and different collecting month during 1 year.

It is noticeable that the highest mean value (0.075 mg.kg^{-1}) was recorded in the field A in August, while the lowest mean value (0.015 mg.kg^{-1}) was also recorded in the same field in January. These results were more than the maximum lead concentration recommended by IDF limit (MRL) (1991) *[16]* which should not exceed 0.02 mg.kg^{-1}. Also, the level of Pb appeared to be higher in all samples than normal raw polluted milk range (0.001-0.005 mg.kg^{-1}) as recommended by IDF standard (1991) *[16]*. These high levels are commonly attributed to the contamination of agricultural soils, atmospheric depositions in contaminated areas, or irrigation with sewage or contaminated water.

In relation to fields, the data in the table 3 showed was a significant effect for field on lead concentration. The concentration of Pb from field A (0.040mg.l^{-1}) is higher significantly ($p \leq 0.05$) than the concentration of it in field B (0.035mg.l^{-1}).

The results in this study are higher than that reported by Ataroe *et al*. (2008) *[17]* (0.0089mg.kg^{-1}) and lower than those reported by Farid and Baloch (2012)) *[18]* (0.11 mg.kg^{-1}).

On the other hand, the effect of field on heavy metals residue in milk was studied by some researchers, El Sayed *et al*. (2011) *[19]* showed that the milk samples taken from different region were significantly differing in their heavy metal content and the same results were obtained by Alani & Al-Azzawi (2015) *[20]* in their study on some heavy metals concentrations in raw milk collected from different locations in Iraq, which they reported that the sites had a significant effect on lead concentration.

Table-2: Metal residual concentration (mg kg^{-1}) in buffalo's milk samples collected from 2 fields during 4 different seasons.

Fields	*Seas.*	*Mon.*	*Lead (Pb)*	*Cadmium (Cd)*	*Chromium (Cr)*	*Copper (Cu)*	*Zinc (Zn)*
Field A	*Winter*	*Jan.*	*0.015±0.001*	*ND*	*0.002± 0.00*	*0.098±0.007*	*0.031±0.085*
		Feb.	*0.016±0.001*	*ND*	*0.001±0.00*	*0.126±0.05*	*0.230±0.047*
	Spring	*Apr.*	*0.025±0.009*	*ND*	*0.002±0.00*	*0.063±0.003*	*0.615±0.012*
		May	*0.028±0.002*	*ND*	*0.002±0.00*	*0.052±0.005*	*0.500±0.113*
	Summer	*Jul.*	*0.039±0.007*	*ND*	*0.002± 0.00*	*0.061± 0.00*	*0.400± 0.00*
		Aug.	*0.075±0.001*	*0.004± 0.00*	*0.003± 0.00*	*0.107±0.022*	*0.330±0.091*
	Autumn	*Oct.*	*0.065±0.006*	*0.003± 0.00*	*0.004± 0.00*	*0.105±0.020*	*0.435±0.031*
		Nov.	*0.060±0.001*	*0.004± 0.00*	*0.006± 0.00*	*0.067±0.008*	*0.426±0.017*
Field B	*Winter*	*Jan.*	*0.052±0.004*	*0.005± 0.00*	*0.007± 0.00*	*0.049±0.005*	*0.461±0.009*
		Feb.	*0.026±0.001*	*0.005±0.00*	*0.007±0.00*	*0.058±0.007*	*0.484±0.013*
	Spring	*Apr.*	*0.017±0.009*	*0.005±0.00*	*0.010±0.00*	*0.060±0.039*	*0.723±.085*
		May	*0.028±0.006*	*0.004±0.00*	*0.005±0.00*	*0.067±0.012*	*0.345±0.018*
	Summer	*Jul.*	*0.039±0.005*	*0.003±0.00*	*0.004±0.00*	*0.105±0.065*	*0.324±0.043*
		Aug.	*0.038±0.001*	*0.003± 0.00*	*0.003± 0.00*	*0.099±0.011*	*0.211±0.088*
	Autumn	*Oct.*	*0.039±0.008*	*0.004± 0.00*	*ND*	*0.051±0.002*	*0.280±0.013*
		Nov.	*0.039±0.005*	*0.003± 0.00*	*ND*	*0.095±0.070*	*0.164±0.005*
L.S.D (p≤0.05)			*0.005*	*0.0004*	*0.0006*	*0.0514*	*0.089*

The differences in lead concentration in different fields may be due to fact the field A is located near the traffic road and the lead is toxic mineral associated with traffic pollution. Fuel combustion with lead additives plays an important role in atmospheric pollution, especially in high traffic density areas where it can remain for a long time *[21; 22]*.

The effect of the seasons (represented by the months) on lead concentration in buffalo's raw milk showed in table 4. There are the significant differences ($p \leq 0.05$) for lead concentration among the seasons; the highest concentration was in summer (August), while the lowest lead concentration was in winter (February). The results in this study are higher than that reported by Qin *et al.* (2009) *[23]* which was 0.035 $mg.kg^{-1}$ and lower than those mentioned by Roy *et al.* (2009) *[24]* and Ayar *et al.* (2009) *[22]* which were 0.09 and 0.11 $mg.kg^{-1}$ respectively. The similar effect for season on lead concentration was noticed by El Sayed *et al.* (2011) *[19]* and the highest mean concentration values of Pb was in May–June, while higher concentration of Pb in winter milk samples was finding by Younus *et al.* (2013) *[25]*. Heavy metal residues have been reported to be increased in plants parts during summer season due to water evaporation from these parts of plants leaving behind relatively higher metal concentration *[26]*, and these differences may be attributed to area and the level of water in tanjaro river differences.
Regarding to the effect of Pb on human health, it has been reported that this metal, even at low concentration, causes harms in the hematological and neurological systems *[27; 28]*. Pb can also affect the cardiovascular system and kidneys *[28; 29]*. Lung and prostate cancers *[30; 31]*, in addition to kidney and bone diseases *[31; 32]*, were reported to be linked to Cd exposure.

Table- 3: Metal residual concentration ($mg.kg^{-1}$) in buffalo's milk samples collected from the fields.

Fields	*Lead (Pb)*	*Cadmium (Cd)*	*Chromium (Cr)*	*Copper (Cu)*	*Zinc (Zn)*
Field A	*0.040±0.022*	*0.004± .001*	*0.003± 0.001*	*0.085±0.032*	*0.404±0.123*
Field B	*0.035±0.010*	*0.004± 0.001*	*0.006 ±0.003*	*0.073±0.038*	*0.373±0.176*
L.S.D $_{(p \leq 0.05)}$	*0.002*	*0.0001*	*0.0002*	*0.0181*	*0.032*

Cadmium (Cd)

The cadmium residual concentration of buffalo's raw milk collected from 2 fields in four seasons is shown in Table 2. A significant difference ($p \leq 0.05$) has been shown among raw milk samples of different locations and different collecting month during 1 year.
It is clear that the highest mean value (0.005 $mg.kg^{-1}$) of cadmium content was recorded in field B in winter, whereas the lowest mean value (0.003 $mg.kg^{-1}$) was recorded in both fields during season's summer and autumn.
However, it is of particular interest to note that the presence of Cd in all samples studied not in dangerous concentrations and these results were less than the maximum Cadmium concentration recommended by Codex Alimentarius Commission (2015) *[33]* which should not exceed than the 0.05 $mg.kg^{-1}$.
Cadmium is a highly toxic metal. It affects the human body through both acute and chronic actions, some parts of the human body are more sensitive to cadmium toxicity such as kidneys, lungs, and bones. Cadmium is also classified as a human carcinogen, particularly causing lung cancer *[34; 35]*. The results in this study are lower than 0.006 $mg.kg^{-1}$ which is reported by Roy et al (2009) *[24]*.
The effect of field on heavy metals residue in milk was studied by some researchers, Licata *et al.*(2004) *[36]* showed that the milk samples taken from various farm in Calabria (Italia) had a significant differences on Cd concentration and the same results were obtained by Rahimi (2013) *[37]* who showed that the concentration of Cd in buffalo's milk samples taken from different region of Iran was affected significantly by the location.
The effect of the seasons (represented by the months) on cadmium concentration in buffalo's raw milk showed in table 4. There were significant differences ($p \leq 0.05$) for cadmium concentration among the seasons; the highest concentration was in winter (0.005 $mg.kg^{-1}$) and the lowest cadmium concentration was in summer (0.003 $mg.kg^{-1}$). These results was not agreed with El Gendy *et al.* (2007) *[38]* who reported that the different Cd concentration between samples collected during cold and warm months were not significant. While Shahbazi (2016) *[39]* showed that the concentration of Cd in winter was higher significantly ($p \leq 0.05$) than that recorded in summer.

Moreover, the industrial effluents drained into sewerage water may be having more Cd residual contents during the particular season *[40]*. It has been proved that Cd accumulates in plants of contaminated industrial areas, and in this way, it enters the animals to accumulate in tissues, and it is secreted in the milk *[21; 41]*.

Chromium (Cr)

The chromium residue concentration of raw buffalo's milk samples collected from 2 fields in four seasons is shown in Table 2. Statistical analysis has shown significant differences ($p \leq 0.05$) among raw milk samples of different fields and different collecting month during 1 year. The highest mean value (0.01 $mg.kg^{-1}$) was recorded in field B in April, while the lowest mean value (0.001$mg.kg^{-1}$) was recorded in the field A during February. Our results were not exceeds Maximum Residual Limit recommended by IDF standard (1979) *[42]* (0.01$mg.kg^{-1}$).

Table- 4: Metal residual concentration (mg.kg-1) in buffalo's milk samples collected from different seasons.

Seas.	*Mon.*	*Lead (Pb)*	*Cadmium (Cd)*	*Chromium (Cr)*	*Copper (Cu)*	*Zinc (Zn)*
Winter	*January*	*0.034±0.020*	*0.005±0.002*	*0.005±0.002*	*0.073±0.027*	*0.383±0.085*
	February	*0.021±0.005*	*0.005±0.00*	*0.004±0.003*	*0.092±0.051*	*0.360±0.142*
Spring	*April*	*0.021±0.007*	*0.005±0.00*	*0.006±0.004*	*0.063±0.024*	*0.669±0.080*
	May	*0.028±0.001*	*0.004±0.002*	*0.005±0.001*	*0.060±0.012*	*0.421±0.110*
Summer	*July*	*0.039±0.004*	*0.003±0.001*	*0.003±0.001*	*0.083±0.047*	*0.357±0.045*
	August	*0.056±0.020*	*0.003±0.00*	*0.003±0.00*	*0.103±0.013*	*0.271±0.103*
Autumn	*October*	*0.052±0.014*	*0.004±0.001*	*0.004±0.001*	*0.078±0.032*	*0.356±0.090*
	November	*0.049±0.011*	*0.003±0.002*	*0.006±0.003*	*0.081±0.047*	*0.295±0.148*
L.S.D (p≤0.05)		*0.004*	*0.0003*	*0.0004*	*0.0363*	*0.063*

In relation to samples fields, the data showed that there was a significant effect for field on chromium concentration (table 3). The concentration of Cr from field B (0.006$mg.kg^{-1}$) is higher significantly ($p \leq 0.05$) than the concentration in location A (0.003 $mg.kg^{-1}$).

As regards to the field, El Sayed *et al.* (2011) *[19]* showed that the buffalo's milk samples taken from different region were significantly differ in Cr contents. The same results was also attained by Kodric *et al.* (2011) *[43]*.

On the other side, the effect of the seasons (represented by the months) on Cr concentration in buffalo's raw milk also showed in table 4. There were the significant differences ($p \leq 0.05$) for Cr concentration among the seasons; the highest concentration was in spring, while the lowest concentration was in summer.

Younus *et al*. (2013) *[25]* showed that the Cr residual in milk sample from dairy farm located near a wastewater drainage duct were higher during summer season. The result may be attribute to chromium deposits have been found in much higher concentration in shoots, leaves, nodes and the tender parts of plant during summer *[26; 44]*.

Copper (Cu)

Copper residue concentration of raw buffalo's milk samples collected from 2 fields in four seasons is shown in Table (2). According to the data, both regions and seasons had a significant ($p \leq 0.05$) effect on Cu residue levels. It is noticeable that the highest copper content (0.1260 $mg.kg^{-1}$) was recorded in field A in February, while the lowest mean value (0.0486 $mg.kg^{-1}$) was recorded in field B in January.

As shown in table (3) fields had non-significant effect on Cu residue concentration in buffalo's raw milk samples, but Among the seasons, the significant highest mean concentration levels were found in summer, while the lowest copper concentration was in spring (table 4), these levels were found to be below the IDF limits 1979 which is 0.1 $mg.kg^{-1}$.

The seasonal effect on copper concentration is recorded also by Yokus et al. (2004) *[45]* who showed that the seasonal variations affected on serum Cu concentration in sheep milk and the highest concentration of copper was found in October, while the lowest concentration was in April, while Younus *et al*. (2013) *[25]* showed that the effect of season on copper concentration during summer was higher than winter and it was

1.354 mg.kg^{-1} and 0.5 mg.kg^{-1}respectively at urban milk shops, contrasting with this study Shahbazi et al. (2016) *[39]* found that the concentration of Cu (433±150 µg.kg^{-1}) in winter was significantly higher ($p \leq 0.05$) than summer (368±142 µg.kg^{-1}).

The results recorded higher values than data reported by some authors such as Licata et al. (2004) *[36]* who reported that the concentration of copper in milk from cows bred on various farms in Calibria/Italy was 1.98 µg.kg^{-1}, and Florea, et al. (2006) *[3]* who reported that the average content of copper in raw milk was 14.43 µg.100g^{-1}.

Possible contamination of milk with copper can occur from animal feed, high copper content of water and copper bearing and copper alloys used in equipment *[46]*.

Zinc (Zn)

Zing concentration value of raw buffalo's milk samples collected from 2 fields in four seasons is shown in Table (2). The average mean of zinc concentrations ranged from 0.1641–0.7234 mg.kg^{-1}. It is noticeable that both regions and seasons had significant differences ($p \leq 0.05$) effect on Zn levels. The highest mean value (0.723 mg.kg^{-1}) was recorded in field B in April, while the lowest mean value (0.164mg.kg^{-1}) was also recorded in field B in milk sample in November. According to the data obtained, fields had non-significant effect (table 3) but season has significant effect on Zn residues levels in buffalo's raw milk (table 4).

The effect of season on Zn residual was studied by some researchers, Reykdal et al. (2011) *[47]* showed that there was no significant effect between pasteurized milk samples in winter between January and March (0.389 mg.kg^{-1}) and summer between June and August (0.433 mg.kg^{-1}). While, other study obtained the significant difference of zinc residual between winter (0.504±0.179 µg.kg^{-1}) and summer (0.438±0.178 µg.kg^{-1}) season *[39]*. These values were lower than those reported by Hamed (2005) *[48]* who reported that the average mean of zinc concentrations ranged from 0.222-3.533 mg.kg^{-1}.

A possible cause for milk contamination by zinc is the use of this metal in producing cans and processing equipment *[48]*.

Conclusion

According to the results of the present study both seasons and fields have significant effects on the levels of all trace elements in buffalo's raw milk samples. In relation to seasonal effects Pb and Cu showed the highest level during summer, while Cr, Zn and Cd showed the highest level during spring. On the other hand, concerning to the effects of the locations, the toxic elements (Pb and Cd) more affects by the locations compared to the other elements studied.

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