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Determination of some Heavy Metals Concentration in some Dairy Products from Three Different Regions of Basrah, Iraq

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Abstract: Ninety samples of cow and buffalo's milk besides their products (cheese and cream), which were collected from three different regions in Basrah city (Basrah centre, Abu Al-Khaseeb and Al-Zubair), were investigated. Levels of some heavy metals (Fe, Cu, Pb, Ni and Cd) were analyzed using an atomic absorption spectrophotometer (AAS). Results showed a trend for three products in which the order in terms of quantities of the levels of Fe, Cu and Pb are: milk > cheese > cream of cows and buffaloes. However, the levels of Ni and Cd were not detected in all samples in this study. Statistical analysis showed that the differences between all different regions were statistically significant (P<0.05). It was also found that the mean levels of Fe in cow and buffalo's products for different regions higher than those recommended by WHO standard and codex. Likewise, the mean contents of Cu and Pb have exceeded the WHO permissible limits.

Keywords: Heavy metals, Contamination, , Dairy products, Atomic Absorption Spectrophotometer.

Introduction

Milk and dairy products are the most important components of the human diet worldwide. This natural food contains both macro- (including proteins, carbohydrates and lipids) and micronutrients vitamins, enzymes and minerals (Perez-Carrera *et al*., 2016). Consequently, many health benefits have been attributed to the consumption of milk and its products (Arianejad *et al*., 2015). However, milk and its products may contain various amounts of heavy metals through manufacturing, packaging processes and increased pollution sources (Anastasio *et al*., 2006; Ogabiela *et al*., 2011; Abdulkhaliq *et al*., 2012).

These heavy metals are considered as the most important contaminations owing to the industrialization of countries and have an impact on their existence in milk and food products, mainly when their levels exceeded the permissible limit, they become toxic, and this poses a serious risk to human health (Meshref *et al*., 2014; Arenas & Trinidad, 2017; Ziarati *et al*., 2018; Abdullah & Hashim, 2019). Contamination by heavy metals in food occurs mainly through the pollution of air, water, and soil.

Unsafe food containing chemical substances or harmful bacteria causes more than 200 diseases ranging from diarrhea to cancers (WHO, 2019). The number of people affected by foodborne illness in the united states each year stands at around 48m, including about 375,000 people who are hospitalized and about 3,000 who die. The cost of each case of foodborne illness ranged from $ 1,626 to $ 1,766 (Scharff, 2012; 2015; Minor *et al*., 2015). There are several studies from different areas, were concerning the Fe, Cu and Pb contaminations in milk and milk products (Enb *et al*., 2009; Abdulkhaliq *et al*., 2012; Belete *et al*., 2014; Meshref *et al*., 2014; Cadar *et al*., 2015; Perez-Carrera *et al*., 2016; Akele *et al*., 2017; Paz *et al*., 2018; Iqbal *et al*., 2019).

Having examined extensively the literature and previous research conducted in this area, there is clear evidence that many gaps exist for fruitful research in a comprehensive study on milk and its products. In particular, there is little published information regarding heavy metals concentrations in milk and its products from cow and buffalo's milk in Iraq, Likewise, there is no data available concerning levels of heavy metal contamination in milk and its products from cows and buffaloes in the southern Iraq, especially in Basrah city. Consequently heavy metals in milk and milk products, their contents and profiles are of great interest not only to the scientists and the milk industry but also to the consuming public.

The present study aimed to quantify toxic heavy metal residues (Fe, Cu, Pb, Ni, and Cd) in cow and buffalo's milk, also their products (cheese and cream) which were collected from three different areas of Basrah city.

Materials & Methods

Materials

A total of 90 samples of milk, cheese, and cream products from cow and buffalo were collected from three different local areas (Basrah centre, Abu Al-Khaseeb and Al-Zubair) in Basrah city in the southern Iraq, during the winter season. Apparatus A phoenix-986 atomic absorption spectrophotometer (AAS), in Atomic lab, College of Agriculture, was used to measure the concentrations of heavy metals in samples.

Methods

The level concentrations of heavy metals (Fe, Cu, Pb, Ni and Cd) in samples were determined by using atomic absorption spectrophotometric (AAS) as described by Abdulkhaliq *et al*. (2012).

Samples were heated in an oven at 60 °C until a constant weight was achieved. The dried samples were grated and stored in a glass vial. Briefly, 2g of milk and dairy product samples were digested with concentrated nitric and perchloric acid mixture (HNO_3: $HClO_3$ = 3:3 $v.v^{-1}$) (Abdulkhaliq *et al*., 2012). The samples were incubated for 18 h in the laboratory, placed in a water bath at 70 °C for 30 min, digested at 100–400 °C to obtain a clear solution. Samples were cooled and diluted to 25 mL by adding ionized water. Working standard solutions of Fe, Cu, Pb, Ni and Cd were prepared by dilution of standard solution to achieve analytical concentrations.

Data analysis

Levels of heavy metals were expressed as mean ± standard error (SE), minimum and maximum values. A one-way analysis of variance (ANOVA) was performed, using SPSS v24, and Duncan's multiple range tests were used to determine significant differences in the measured elements at $p < 0.05$.

Results & Discussion

The main problem with heavy metals is their ability to bio-accumulate. Heavy metals residues in milk and milk products are of particular concern as milk is largely consumed by humans around the world (Enb *et al*., 2009; Licata *et al*., 2012). The level concentrations of three metals (Fe, Cu and Pb) in milk and milk products for the studying area are shown in (Tables 1 and 2). However, Ni and Cd were not detected. These results seem to concur with the findings of Lante *et* *al*. (2006) who found that Cd was absent in milk samples. Likewise, Belete *et al*. (2014) concluded that the levels of Ni and Cd were not detected in the cow's milk of Borena Zone, Ethiopia even though they were reported in other studies.

All three metals (Fe, Cu and Pb) were detected at the highest levels in all samples examined, and their concentrations were in the order of Fe > Cu > Pb for heavy metals in cow and buffalo's products (Figs. 1- 3). The results indicate that the level of Fe was the highest in milk and its products. The level of Fe in milk, cheese and cream for the cow's samples was in the range of 2.89 - 6.34, 2.53-3.60 and 0.04-1.96 $mg.L^{-1}$ with mean levels of 4.54±1.45, 2.96 ± 0.45 and 0.83 ± 0.84 $mg.L^{-1}$ respectively (Table 1), whereas the level of Fe for the buffalo's samples was in the range of 1.70 -2.64, 1.60- 2.36 and 0.06- 2.16 $mg.L^{-1}$ with mean levels of 2.15± 0.38, 1.98± 0.31 and 1.10± 0.88 $mg.L^{-1}$ respectively (Table 2). Moreover, for these three products of cows and buffaloes, there is a trend in which the order in terms of quantities of the level of Fe, Cu and Pb is: milk > cheese > cream (see Tables 1 and 2; Figs. 1- 3).

The range of Fe levels in milk and milk product is of a similar order to what has been reported elsewhere, Abdulkhaliq *et al*. (2012) found the highest mean level in the milk of Fe (3.2-12.91 $\mu g\ .g^{-1}$) followed by Cu (0.62-0.85 $\mu g\ .g^{-1}$), Cd (0.022-0.057 $\mu g\ .g^{-1}$) and Pb (ND-0.93 $\mu g\ .g^{-1}$). However, the lowest level of Fe is found in white cheese; these findings would seem to be consistent with those obtained in the present study (see Figs. 1- 3). Another study of trace metal concentrations in Ethiopia by Akele *et al*. (2017) reported that the range of Cu (0.8-1.532 $\mu g\ .g^{-1}$), Pb (0.120-0.186 $\mu g\ .g^{-1}$)and Cd (0.244-0.330). In the present study, the range of Fe levels in buffalo's milk was found to be less than cow's milk (see fig. 1) which is in agreement with what has been reported by Ahmad *et al*. (2017).

Comparing the level of Fe in cow products (milk) of Basrah centre, Abu Al-Khaseeb and Al-Zubair; it was noted that Abu Al-Kaseeb region had the largest amount of Fe (6.32 $mg.L^{-1}$) followed by Al-Zubair region (4.39 $mg.L^{-1}$) and lowest in Basrah centre (2.90 $mg.L^{-1}$) (Fig. 1). The results showed different patterns of Fe, Cu and Pb distribution in milk samples of various regions of Basrah city. High concentrations of elements in some regions may result from different contamination of the environment by factories and industrial waste (Al-Halfy & Al-Tamimi, 2013; Najarnezhad & Akbarabadi, 2013). These results seem to concur with the findings of Malhat *et al*. (2012) who reported higher levels of Fe, Cu, and Pb in cow's milk. A study by Al-Dabbagh (2013) found that the levels of Pb and Cu contents in milk of buffaloes, cows, goats and sheeps in Mosul city were 0.623, 0.590, 0.5661 and 1.323 $mg.kg^{-1}$ for Pb and 0.191, 0.640, 0.152 and 0.593 $mg.kg^{-1}$ for Cu respectively, these values are higher than those obtained in the present study.

To assist in the visualization of these results, the levels of Cu and Pb in the cow and buffalo's milk and their products for three different regions mentioned are presented in the following histograms (Figs. 2 and 3), the levels of Cu and Pb in buffalo's milk were significantly higher than in cow's milk. This finding appears to agree with Najarnezhad *et al*. (2015).

Table (1): Levels of Fe, Cu, and Pb ($mg.L^{-1}$) in cow products samples from three different areas in Basrah city (n = 15 for each sampling site).

		Basrah centre		Abu Al-Khaseeb		Al-Zubair	
Metal		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Fe	Milk	2.90	± .01c	6.32	± .02a	4.39	± .02b
	Cheese	2.77	± .01b	3.57	± .02a	2.55	± .02c
	Cream	1.95	± .01a	.05	± .01c	.48	± .00b
Cu	Milk	.37	± .01a	.35	± .03b	.03	± .01c
	Cheese	.22	± .02b	.35	± .02a	.00	± .00c
	Cream	.01	± .01b	.01	± .01a	.00	± .00c
Pb	Milk	.00	± .00c	.05	± .02b	.29	± .01a
	Cheese	.00	± .00c	.01	± .00b	.12	± .02a
	Cream	.00	± .00b	.00	± .00b	.09	± .01a

- Means within each product in the same row with different subscripts have significantly differed at $p < 0.05$

Table (2): Levels of Fe, Cu, and Pb ($mg.L^{-1}$) in buffalo products samples from three different areas in Basrah city (n = 15 for each sampling site).

		Basrah centre		Abu Al-Khaseeb		Al-Zubair	
Metal		**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Fe	**Milk**	2.63	± .01[a]	1.72	± .02[c]	2.10	± .02[b]
	Cheese	2.34	± .02[a]	1.61	± .01[c]	1.99	± .00[b]
	Cream	2.14	± .01[a]	.07	± .01[c]	1.09	± .01[b]
Cu	**Milk**	1.85	± .01[a]	.43	± .01[c]	.54	± .01[b]
	Cheese	.37	± .01[b]	.40	± .02[a]	.03	± .01[c]
	Cream	.11	± .01[a]	.01	± .01[b]	.01	± .00[c]
Pb	**Milk**	.00	± .00[c]	.35	± .01[b]	.67	± .01[a]
	Cheese	.00	± .00[b]	.01	± .00[b]	.23	± .01[a]
	Cream	.00	± .00	.00	± .00	.00	± .00

- Means within each product in the same row with different subscripts have significantly differed at $p < 0.05$

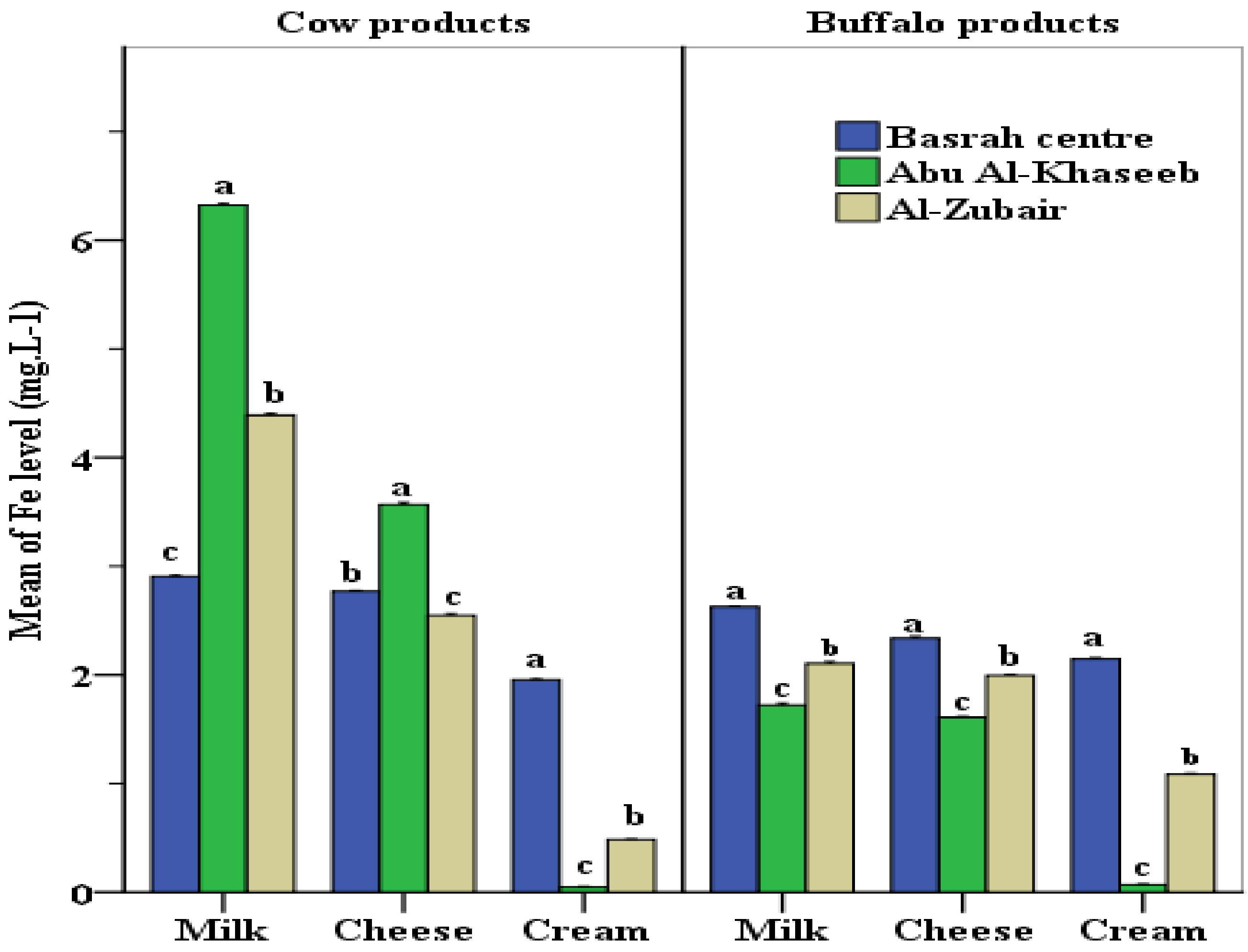

Fig. (1): Overall mean ±SE of Fe level, as measured by AAS in dairy products from Cows and Buffaloes (n = 5; error bar = ± 2 SD) in the Southern Iraq, Basrah. Different letters within each product represent significant differences (*P* <0.05).

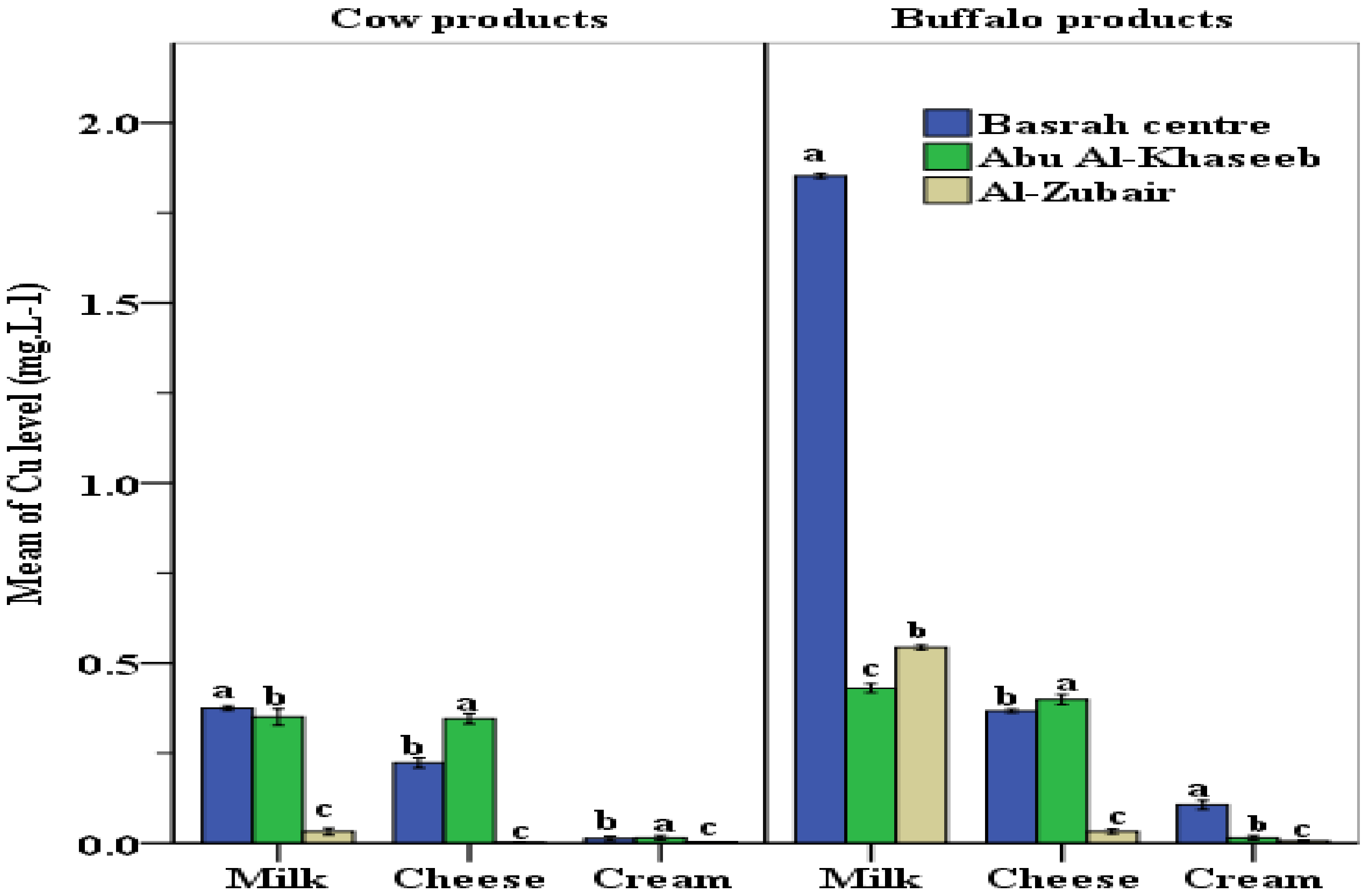

Fig. (2): Overall mean (±SE) of (Cu) level, as measured by AAS in dairy products from Cows and Buffaloes (n = 5; error bar = ± 2 SD) in the Southern Iraq, Basrah.

Different letters within each product represent significant differences (*P* <0.05)

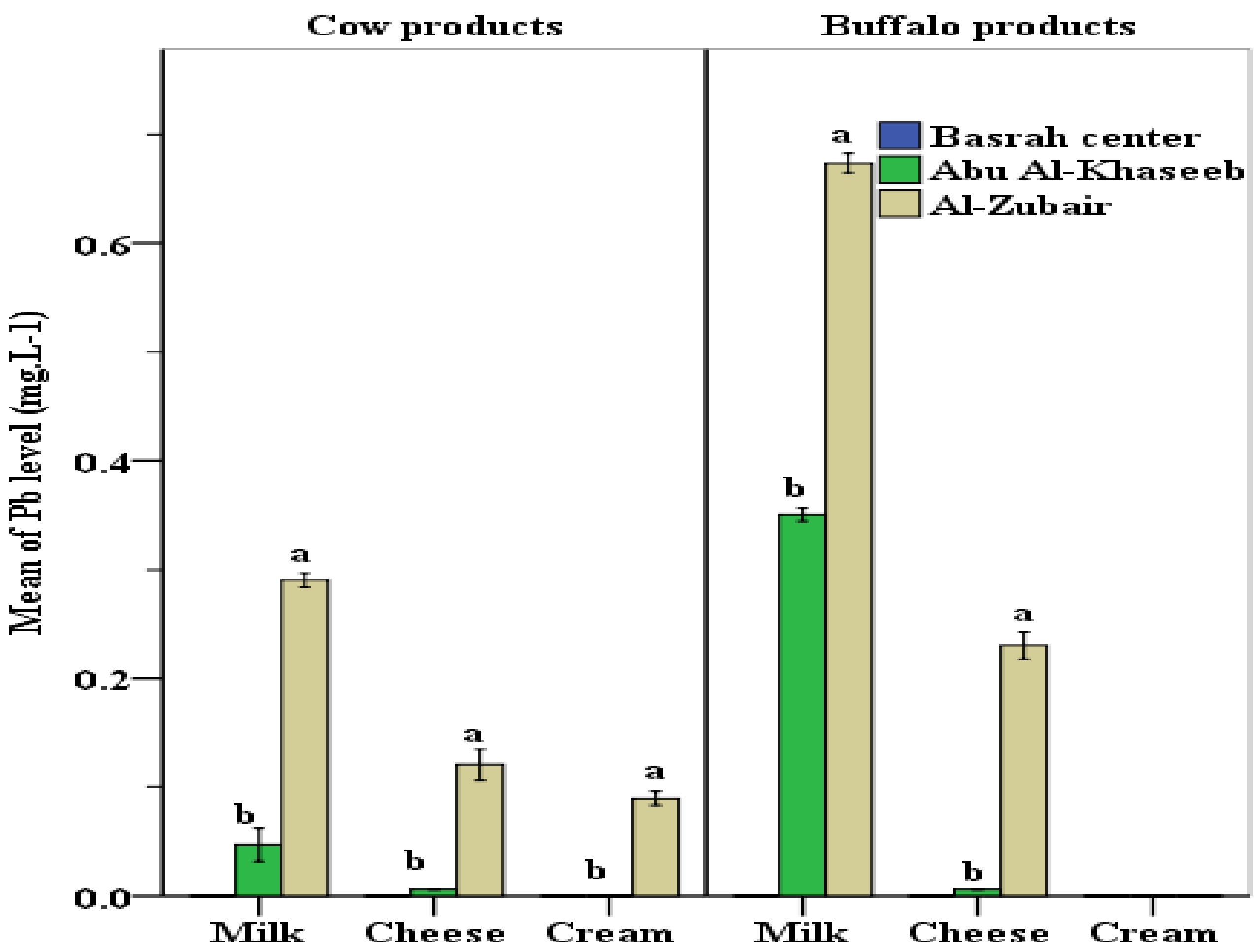

Fig. (3): Overall mean (±SE) of (Pb) level, as measured by AAS in dairy products from Cows and Buffaloes (n = 5; error bar = ± 2 SD) in the southern Iraq, Basrah.

Different letters within each product represent significant differences (*P* <0.05).

Results and observations from this study are not entirely in agreement with findings of previous studies; neither are the latter in complete agree with one another. For instance, Abdulkhaliq *et al*. (2012) examined the levels of metals (Fe, Cu, Pb and Cd) by using GAAS in160 samples of cow's milk and dairy products i.e. cheese in West Bank, Palestine.

Their results indicated the mean concentrations of heavy metals in different liquid milk samples were: 8.23, 0.62, 0.20 µg. g^{-1} and 35.71 µg. g^{-1} for Fe, Cu, Pb and Cd respectively. Consequently, the levels were decreased in the order of Fe > Cu > Pb > Cd. These authors concluded that the level concentrations of Pb and Cd in milk and its products (apart from white cheese) exceeded the WHO permissible limits. The higher levels could be associated with contamination throughout manufacturing processing and environmental pollution (Al-Manhal, 2013). These findings would seem to be consistent with those obtained in the present study. Indeed, some studies are in agreement with those reported in the literature from other countries. In their papers that the levels of Fe, Cu, Pb, Cd and Ni in cow and buffalo's milk and their products were unsafe for consumers (Licata *et al*., 2012; Malhat *et al*., 2012; Temiz & Soylu, 2012; Tona *et al*., 2013; El-Ansary & El-Leboudy, 2015; Ojezele *et al*., 2017), while others have reported no significant effect cause any adverse health concerns to consumers (Najarnezhad & Akbarabadi, 2013; Belete *et al*., 2014; Cadar *et al*., 2015; Najarnezhad *et al*., 2015; Perez-Carrera *et al*., 2016; Ahmad *et al*., 2017; Akele *et al*., 2017). More reviews providing in-depth discussions can be found in recent papers (Noori *et al*., 2016; Ziarati *et al*., 2018).

Samples of Results showed that the lowest values of heavy metals studied were obtained for Basrah centre milk samples, while the highest was found for those collected from Abu Al-Khaseeb region due to the nature of these areas, variations in human activities and using sewage water for agricultural irrigation (Ogabiela *et al*., 2011; Al-Hassen *et al*., 2013).

Cheese is a dairy product that is the basis of the diet of millions of people around the world (Paz *et al*., 2018). Heavy metals are one of the factors that affect the process of cheese making and eventually cause contamination that can pass in the food chain in different ways (Ziarati *et al*., 2018). Anastasio *et al*. (2006) observed highest residual of mean levels of Pb in Ricotta, Fresh and mature cheese (0.391 ± 0.212, 0.47 ± 0.365 and 0.58 ± 0.271 µg .g^{-1}) respectively. These authors in their results indicated that the milk products from the two regions of Italy are safe for consumers. Similarly, Tona *et al*. (2013) found that the level of Pb within the maximum residue limit (MRLs) in the soft cheese samples. However, Abdulkhaliq *et al*. (2012) found the lowest concentrations of heavy metals in white cheese. A study by Arafa *et al*. (2014) reported that the levels of Fe, Cu and Pb in kareish cheese were in the range of 1.76 - 14.74, 0.002- 0.53 and 0.19-0.65 with mean concentrations of 3.93 ± 0.67, 0.87 ± 0.26 and 0.43 ± 0.029 ppm respectively. Likewise, in the present study the levels of Fe, Cu and Pb in cheese were in the range of 2.53 -3.60, 0.00- 0.37 and 0.00-0.14 with mean concentrations of 2.96 ± 0.45, 0.19 ± 0.15 and 0.04 ± 0.06 mg.L^{-1} respectively (see Table 1; Figs. 1-3). The quality of equipment for cheese processing may affect the content of heavy metals at the end of the product (Levkov *et al*., 2017).

The decrease of heavy metals (Fe, C and Pb) due to the loss of these metals into whey and scalding water. Furthermore, the curdling the milk the most important contamination step (Yuzbasi *et al*., 2009). These authors found that the levels of Fe was 5.7 ± 1.1 $mg.kg^{-1}$ and Cu 1.5 ± 0.1 $mg.kg^{-1}$ contents in Kasar cheese entail more of a technological hazard rather than a nutritional risk as they may cause raised catalytic oxidation of cheese lipids.

Consumption of cheese in Basrah city is close to risk, though bioaccumulation of Fe, Cu, and Pb through the food chain and intake from another foodstuff would also be of concern. The current study provides significant information on the levels of trace elements (Fe and Cu) and heavy metal (Pb) in milk and milk products.

Conclusion

The purpose of this study to examine the level of five heavy metals from cows and buffaloes products commonly consumed in different areas in the southern Iraq. These metals determined using AAS. Samples were purchased from the different local areas in Basrah city. In conclusion, the results of the present study showed that all samples exceeded the maximum limit established by the WHO standard and codex. The levels of Fe, Cu and Pb in milk and milk products in different regions of Basrah city have to be periodically monitored by the responsible governments. Finally, the results of this study suggest that consumers may advantage from knowing the permissible level of heavy metals in milk and milk products. Further studies are needed to increase and improve our understanding of fate as well as the availability of these metals in food. It could be concluded that there is a high hazard of exposure to heavy metals that have been studied in Basrah city. It's highly recommended that food companies should have a fully implemented and effective food safety plan based on Codex Alimentarius HACCP principles.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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تحديد تركيز بعض المعادن الثقيلة في بعض منتجات الالبان لثلاثة مناطق مختلفة في البصرة، العراق

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المســتخلص: فحصت 90 عينة من حليب البقر والجاموس ومنتجاتهما من الجبن والقشطة. جمعت العينات من ثلاثة مناطق مختلفة في محافظة البصرة هي مركز البصرة وابو الخصيب والزبير. حددت مستويات بعض المعادن الثقيلة مثل الحديد والنحاس والرصاص والنيكل والكوبلت باستعمال جهاز قياس الامتصاص الذري. اظهرت النتائج ان تراكيز الحديد والنحاس والرصاص كانت بحسب الترتيب الاتي الحليب <الجبن> القشطة لكلا النوعين من حليب الابقار والجاموس بينما كانت مستويات النيكل والكادميوم ضئيلة جدا في جميع العينات. واظهرت نتائج التحليل الاحصائي وجود فروقات معنوية عند $(0.05) \leq p$ بين تراكيز هذه العناصر ولجميع المناطق. بينت النتائج ان متوسط مستويات الحديد والنحاس والرصاص في منتجات الابقار والجاموس للمناطق المختلفة المدروسة اعلى من الحدود الموصي بها من قبل منظمة الصحة العالمية.

الكلمات المفتاحية: معادن ثقيلة ، تلوث، منتجات الالبان، مقياس طيف الامتصاص الذري.