Spectrophotometric Quantification of Trace Elements and Toxic Metals in Raw Milk Samples of Peshawar City, Pakistan

Nadir Khan,[1] Rizwan Ullah,[1] Fazal Wahid,[1] Qamar Sultana,[2] Muhammad Rahim[3]*

[1] Department of Chemistry, Government College Peshawar, Khyber Pakhtunkhwa, Pakistan
[2] National Center of Excellence in Physical Chemistry, University of Peshawar, 25120 Pakistan
[3] School of Chemical Sciences, Universiti Sains Malaysia, 11800 Pulau Penang, Malaysia

*Email: kpk566@gmail.com

Abstract: The present study focused on the physicochemical characterization and spectrophotometric determination of the concentrations of trace elements (Zn, Cu, Fe and Mn) and toxic heavy metals (Cd and Pb) in raw milk samples collected from various commercial sites of Peshawar city, Pakistan. Evaluation of the nutritional values as well as the possible health concerns associated with the consumption of contaminated milk shown the average percentage composition of protein, fats, solid non-fats, lactose, salts and added water in the milk sample as 2.78, 4.81, 6.41, 3.05, 0.47 and 31.1 %, respectively. The values of pH, conductivity, density and freezing point were observed as 6.59, 3.51 mS/cm, 1.04 g/mL and -0.3674 ℃ at 26 ℃. The physicochemical characteristics such as percentage protein, fat, lactose, salts, water, solid not fat, total solids, pH, conductivity and density were evaluated and found in the safe limits of the WHO/FAO. The average concentration of trace elements such as zinc and copper were found to be 1.4035 mg/L and 0.2588 mg/L, respectively. The concentration of Zn and Cu was found within permissible limits suggested by WHO. However, the average concentration of Fe, Mn, Cd and Pb were found higher than the permissible limits of WHO. The observed concentrations were: Fe = 1.5553, Mn = 0.4354, Cd = 0.1865 and Pb = 1.1162 mg/L. The presence of the non-essential and hazardous Cd and Pb in milk samples even in lower concentrations may cause severe health problems since these are the most noxious pollutants due to their non-biodegradable and bio-accumulative nature. Over-dose of Fe and Mn also leads to several health issues. A thorough and more precise investigation of raw milk consumption in Peshawar is highly recommended to ascertain the actual reasons and sources in the larger public interests.

Keywords: Nutritional values, raw milk, toxic metals, trace elements.

Introduction

Milk constitutes one of the primary sources of nutrition for both infants as well as other age groups (Walker, Dunshea, & Doyle, 2004). It is considered a complete food source since it is enriched with nutrients of enhanced biological potential including proteins, vitamins, fat, lactose and minerals in significant amounts and proper proportions (Ward & German, 2004). The nutritive value and quality of milk substantially depend upon the small mineral fraction and other basic components it contains. To maintain the desired quality of milk, its mineral contents should be controlled within permissible limits (Miciński et al., 2013). The extent to which various trace elements are found in raw milk is never uniform since it is dependent on several factors including the species and breed of animals, lactating stage of animal, health situation, seasonal variations, the food it consumes and the location of farms where the animals are kept and cared (Dobrzanski, Kolacz, Górecka, Chojnacka, & Bartkowiak, 2005). The raw milk and its processing procedures also determine the elemental levels in various dairy products like skimmed milk, condensed milk, cream, butter, yoghurt and cheese (Cherryl, Chakravarthi, Reddy, & Indira, 2014; Imran, Khan, Hassan, & Khan, 2008; Mahmood & Usman, 2010; Mansour, El-Loly, & Ahmed, 2012; K. Muhammad,

Altaf, Hanif, Anjum, & Tipu, 2009; Rodríguez Rodríguez, Sanz Alaejos, & Díaz Romero, 1999).

Contamination of various food items including raw milk with heavy metals like Cu, Fe, Zn, Mn, Cd and Pb is becoming a serious problem due to their penetration into the food chains (R. Muhammad, Najm, Fazal, Nadir, & Luqman, 2020; Rahim & Mas Haris, 2015). Heavy metals like Cd and Pb are non-essential and toxic elements even at trace levels due to their persistent, bioaccumulative and non-biodegradable nature (Rahim & Mas Haris, 2019; Saqib, Khan, Alam, & Rahim, 2020). The contamination of milk with Cd and Pb is a serious health concern, especially in infants and children as the young absorb these metals more readily than adults (Ahmad, Rahim, & Haris, 2014; Rahim, Imdad, Adnan, Haris, & Nisar, 2014).

The crops and fodder in the vicinity of Peshawar, which constitute a source of food for lactating animals, are grown on soils irrigated with metal-contaminated water, so the likelihood of their ultimate penetration into raw milk is very greater. In addition, it is observed that continuous long-term exposure to heavy metals by consuming raw milk gets less emphasis in developing countries. Therefore, the present study holds significant importance in terms of b ringing awareness regarding Peshawar city public health hazard. The

quality of raw milk was monitored by measuring protein content, total fats, lactose, salts, added water, solid not fat, total solids, pH, conductivity, density, freezing point, and estimating the levels of Cu, Zn, Fe, Mn, Cd and Pb.

Material and Methods

Samples Collection

A total of 15 samples of raw milk, each measuring 100 mL were collected randomly from local street vendors and dairy shops at targeted locations in Peshawar city, as shown in Figure 1. All milk samples were received from vendors in sterilized plastic bottles and brought to the laboratory in the icebox. The samples were stored in the laboratory in a refrigerator at 40-80 ℃. The sampling sites and their respective codes are shown in Table 1.
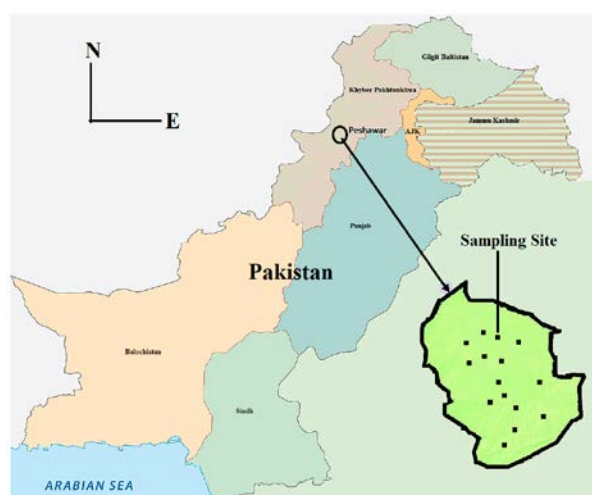

Fig. 1 Map of the study area.

Table 1 Sampling sites and their IDs for raw milk samples

S. No.	Area	Sample ID
1	Kohat Road	M_1
2	Hashnaghari	M_2
3	Faqir Abad No -2	M_3
4	Firdos	M_4
5	Warsak Road	M_5
6	Dabgri Garden	M_6
7	Ramdas Chowk	M_7
8	Namak Mandi	M_8
9	University Road	M_9
10	Faqir Abad No 01	M_{10}
11	Bara Road	M_{11}
12	Fuwara Chowk	M_{12}
13	Gulbahar No – 2	M_{13}
14	Gunj	M_{14}
15	Dalazak Road	M_{15}

Chemicals and Instruments

De-ionized water and analytical grade HNO_3 (CAS: 7697-37-2, and 70 %), HCl (CAS: 7647-01-0, and 37 %) and H_2O_2 (CAS: 7722-84-1 and 30 %) were employed during the experimental work. The standard solution of each metal was prepared from a stock solution of 1000±2 mg/L from Sigma-Aldrich. Stock solutions of manganese,

iron, copper, zinc, cadmium and lead with a concentration of 0.1, 1.0, 5.0 and 10.0 mg/L were prepared by a sequential dilution of their standard solution of 1000 mg/L. The stock solutions were further diluted to obtain a working solution for each metal. The glassware employed during the experimental work was thoroughly rinsed with distilled water, then with diluted (2 M) HNO_3 and finally distilled water again before being air-dried. These were then wrapped up with clean tissue paper to avoid any contamination. The collected milk samples were analyzed for various physicochemical characteristics such as percentage protein, fat, lactose, salts, water, solid not fat, total solids, pH, conductivity, density, freezing point and temperature by using Lactoscan which is an ultrasonic electronic milk analyzer (Adam, 2009; Kanwal, Ahmed, & Mirza, 2004; Mansour et al., 2012; Mohamed, 1981; Roy, Nagpal, & Sadana, 1972; Shojaei & Yadollahi, 2008).

The collected milk samples were analyzed for estimating the levels of six targeted metal using Flame Atomic Absorption Spectrometer Model AA-6300 Shelton USA. The FAAS was provided with a Deuterium background corrector. Various parameters of the instruments like wavelength, slit-width, lamp current and sample energy of each metal were adjusted accordingly before metal content determination. 50 mL of each milk sample was poured separately in a 50 mL clear glass beaker and then dried in column oven at 70 ℃ for 72 hours. After thorough drying, 2 g of each dried milk sample was taken on a porcelain crucible and subjected to digestion at a temperature of 500 ℃ in the oven for 4 hours. The ash so obtained was first allowed to cool and then dissolved in 5 mL of a hydrochloric acid solution of 37 %. The resulting solution was filtered out with the help of an acid-treated filter paper to remove the suspended particles. The filtrate was diluted to final volume of 25 mL (Andrade Korn et al., 2008; Enb, Abou Donia, Abd-Rabou, Abou-Arab, & El-Senaity, 2009; Pedro, De Oliveira, & Cadore, 2006).

Results and Discussion

Physiochemical Parameters

The physicochemical characteristics of raw milk samples such as percentage protein content, fat content, non-fat solids, lactose, salts, added water and total solids were determined. The PH, conductivity, density, freezing point and temperature of the raw milk were also observed as summarized in Table 2. The average values along with WHO suggested limits are shown in Figure 2.

Protein Content (%)

The protein content of the collected samples varied between 1.67 to 3.51 %, with an average value of 2.78 %. The WHO suggested limit is 3.4 % which is much

Table 2 Physicochemical parameters of the collected raw milk samples of Peshawar city.

Area	ID	%Protein	%Fat	%SNF	%Lactose	%Salts	%Added water	pH	Conductivity mS/cm	Density g/mL	Freezing Point ºC	Temperature ºC
Kohat Road	M₁	2.22±0.0	3.13±0.5	4.85±0.8	2.13±0.0	0.34±0.0	54.03±0.0	6.64±0.0	3.23±0.0	1.01±0.0	-0.239±0.2	25.6±0.0
Hashnaghari	M₂	2.96±0.0	8.12±0.1	6.59±0.1	2.88±0.0	0.46±0.0	33.07±1.5	6.4±0.1	3.45±0.0	1.03±0.0	-0.348±0.0	27.4±0.7
Faqir Abad No -2	M₃	2.89±0.1	5.59±0.1	6.37±0.0	2.60±0.0	0.45±0.0	36.92±0.1	6.78±0.0	4.13±0.0	1.00±0.0	-0.328±0.1	32.0±1.0
Firdos	M₄	3.26±0.0	5.50±0.2	7.15±0.0	3.14±0.0	0.51±0.0	28.65±0.7	6.7±0.1	3.37±0.0	1.12±0.0	-0.371±0.1	27.1±0.9
Warsak Road	M₅	3.36±0.1	5.50±0.7	7.38±0.0	3.24±0.0	0.52±0.0	26.15±0.9	6.6±0.0	3.64±0.0	1.02±0.0	-0.384±0.0	27.3±0.7
Dabgri Garden	M₆	2.88±0.1	5.35±0.3	7.91±0.0	4.80±0.0	0.66±0.0	00.00±0.3	6.54±0.0	3.74±0.0	1.01±0.0	-0.559±0.1	25.5±2.5
Ramdas Chowk	M₇	2.01±0.0	3.61±0.1	5.53±0.0	3.33±0.0	0.46±0.0	28.65±3.6	6.7±0.0	3.22±0.2	1.09±0.0	-0.371±0.1	25.7±1.0
Namak Mandi	M₈	3.51±0.1	3.71±0.0	7.64±0.0	3.37±0.0	0.55±0.0	24.61±0.0	6.65±0.0	4.01±0.0	1.00±0.0	-0.392±0.0	25.8±3.0
University Road	M₉	3.05±0.0	5.21±0.0	6.69±0.1	2.94±0.0	0.47±0.0	33.84±0.1	6.78±0.0	3.19±0.0	1.05±0.0	-0.344±0.1	27.6±0.7
Faqir Abad No 01	M₁₀	1.67±0.0	2.98±0.1	3.68±0.1	1.62±0.0	0.26±0.0	31.26±0.0	6.08±0.0	3.60±0.0	1.06±0.0	-0.346±0.1	27.4±0.1
Bara Road	M₁₁	3.24±0.2	4.73±0.2	7.08±0.3	3.11±0.0	0.50±0.0	30.00±0.0	6.63±0.1	3.90±0.0	1.02±0.0	-0.364±0.0	25.4±1.0
Fuwara Chowk	M₁₂	2.59±0.1	4.07±0.9	5.67±0.0	2.49±0.0	0.40±0.0	45.19±0.6	6.67±0.0	3.20±0.0	1.10±0.0	-0.285±0.2	25.6±0.0
Gulbahar No – 2	M₁₃	2.52±0.0	4.29±0.0	5.53±0.1	2.43±0.0	0.39±0.0	46.63±0.3	6.5±0.1	3.31±0.0	1.07±0.1	-0.278±0.0	27.5±0.1
Gunj	M₁₄	2.48±0.4	4.13±0.0	5.45±0.0	2.39±0.0	0.38±0.0	47.50±0.1	6.60±0.1	3.36±0.0	1.03±0.0	-0.273±0.0	27.4±5.1
Dalazak Road	M₁₅	3.13±0.3	6.30±0.1	8.59±0.3	5.31±0.3	0.72±0.0	00.00±0.0	6.7±0.0	3.28±0.0	1.02±0.0	-0.629±0.3	22.8±1.2
		3.4	4.0	13.0	4.8	0.8	---	6.5	4.75	1.03	-0.540	---

higher than the average value of our result. The observed values are in close association with the results already reported in the literature, especially those reported by Cherryl et al. (2014). Low protein contents have already been concluded by Imran et al. (2008), Asif and Sumaira (2010) and Hossain and Dev (2013). A survey of relevant literature reveals that high protein contents have been suggested by Fundora et al. (2001) and Braun and Stefanie (2008). A possible reason for lower protein value may be the addition of water to milk which leads to a decline in the percentage of solids not fat (SNF) including protein (Braun & Preuss, 2008; Fundora, Lezcano, Montejo, Pompa, & Enriquez, 2001; Hossain & Dev, 2013; Imran et al., 2008; Mahmood & Usman, 2010).

Fats Content (%)

Fats are an important constituent of milk. As our body cannot prepare fats, therefore, we need fats from our environment. Whereas, milk is one of the important natural sources of fats. The fats content of the collected milk samples were found in the range of 2.98 to 8.12 %, with an average value of 4.81 %. Whereas, WHO has suggested a minimum of 4 % of fats for raw milk. As such, the average observed value is 1.2 times higher than the suggested limit. Furthermore, the observed values are in a near match to those of Roy et al. (1972). Relatively greater fat contents have been reported by Adam (2009) and Mansour et al. 2012 (Adam, 2009; Mansour et al., 2012; Roy et al., 1972).

Solids Non-Fats (%)

Solid non-fat (SNF) refers to components other than fats such as lactose, casein, protein, minerals and vitamins. The raw milk samples were analyzed for SNF. The analyzed samples contained different amounts of solid non-fat content. The minimum solid non-fats content was found to be 3.68 % in sample M₁₀ while the maximum content was 8.59 % in sample M₁₅. The average SNF content was found to be 6.41 % which is 2.03 times lower than WHO suggested values. The observed results are in a close match with those suggested earlier by Abdel-Sabou (Abdel-Sabour,

2007), while somewhat higher results recorded by Aoyama and Abdel- Hameid (Abdel-Hameid, 2002; Aoyama, Arai, & Sasano, 1992).

Lactose Content (%)

Lactose is a disaccharide sugar found in milk. It is composed of galactose and glucose units and has the molecular formula $C_{12}H_{22}O_{11}$. Chemically lactose is β-D-galactopyranosyl-(1→4)-D-glucose. Lactose is a natural sugar that is widely used in the food and pharmaceutical industries. Lactose content in the analyzed milk samples ranged from 1.62 % in sample M₁₀ to 5.31 % in sample M₁₅. The average lactose content was observed to be 3.05 % which is 1.57 times lower than the WHO limits. The observed findings are in close agreement with those already concluded by Paul (Paul, Dinn, Kannangara, & Fisher, 1998).
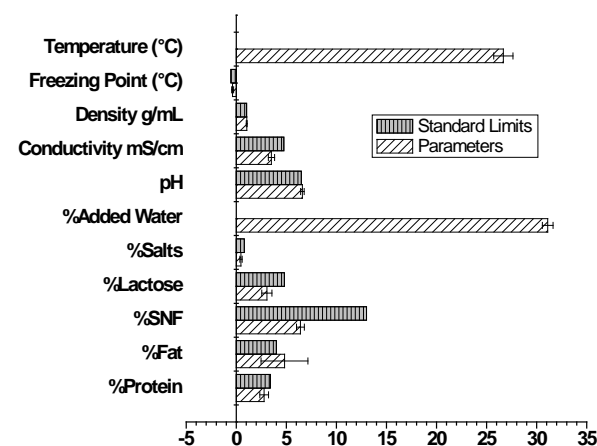

Fig. 2. Physico-chemical parameters of the raw milk samples collected from Peshawar city.

Salts Content (%)

A varying amount of salts was found in the investigated milk samples. The most common salts found in milk are phosphates, sulphates, citrates, chlorides, carbonates and bicarbonates of sodium, calcium, potassium and magnesium. These milk salts are crucially important for a key biological role. The

minimum salts content was found to be 0.26 % in a sample of M_{15} while the maximum content was 0.72 % in samples of M_{10}. As such, the mean observed value of percentage salts content was found to be 0.47 % which is 1.70 times lower than the WHO permissible level.

Density

Among the collected milk samples, sample M_{10} was found to have the minimum density of 1.00 g/mL while sample M_8 sample has the maximum density of 1.12 g/mL with the mean value of 1.04 g/mL. Such results are closely matching the observations made in a

Table 3 Heavy metal contents (mg/L) of the raw milk samples of the Peshawar city.

Sample ID	Zn	Cu	Fe	Mn	Cd	Pb
M_1	0.4405±0.0	0.1037±0.0	0.9576±0.1	0.7070±0.0	0.3011±0.0	0.8256±0.0
M_2	1.5573±0.0	0.5717±0.0	1.8975±0.0	0.4711±0.0	0.3253±0.0	1.6683±0.1
M_3	1.9933±0.0	0.1104±0.0	0.9076±0.0	0.7495±0.1	0.2575±0.0	0.2792±0.0
M_4	0.0896±0.0	0.4460±0.0	2.5589±0.0	0.4550±0.0	0.5373±0.0	1.4439±0.0
M_5	3.0378±0.0	0.2992±0.0	4.9774±0.1	0.9246±0.0	0.6780±0.1	0.8836±0.0
M_6	1.8911±0.0	0.4187±0.0	1.3769±0.0	0.8148±0.0	0.2108±0.0	0.5305±0.0
M_7	0.8249±0.0	0.1690±0.0	1.3481±0.0	0.3262±0.0	0.0993±0.0	0.9022±0.0
M_8	1.0845±0.2	0.2299±0.0	0.6246±0.0	0.6299±0.1	0.0916±0.0	0.8946±0.0
M_9	0.9423±0.0	0.2205±0.0	1.6883±0.0	0.4026±0.0	0.0039±0.0	1.8026±0.1
M_{10}	0.6198±0.0	0.2923±0.0	0.7174±0.0	0.3364±0.0	0.0981±0.0	0.9650±0.0
M_{11}	0.6429±0.0	0.3452±0.0	0.9558±0.0	0.1126±0.0	0.1396±0.0	2.2655±0.0
M_{12}	2.3165±0.1	0.2989±0.0	1.8668±0.0	0.2764±0.0	0.0116±0.0	1.3495±0.0
M_{13}	1.4933±0.1	0.2606±0.0	0.8612±0.0	0.1001±0.0	0.0618±0.0	0.8332±0.0
M_{14}	2.2153±0.0	0.2959±0.0	1.0131±0.0	0.0912±0.0	0.0313±0.0	0.7917±0.0
M_{15}	1.9034±0.0	0.2654±0.0	1.5789±0.0	0.1332±0.0	0.0612±0.0	1.3080±0.0
WHO/FAO	05-15	0.05-1.5	0.3	0.05	0.003	0.001

Added Water (%)

Most people add water to raw milk to increase its quantity and to earn more money. Therefore, the raw milk samples were analyzed for added w ater. The minimum water content was found to be 24.61 % in sample M_8 while the maximum content was 54.03 % in samples M_1. The samples M_6 and M_{15} showed the minimum amount of water. The average added water was found to be 31.10 % which decreased the percentage composition of protein, SNF, lactose and salts in the milk samples. However, similar results have been reported by Abdel (Abdel-Sabour, 2007).

Samples pH

The normal pH of milk is usually ranging between 6.4 to 6.8. The mean value of pH was found to be 6.59 which is slightly greater than the WHO level. The pH of the collected milk samples was found slightly in the acidic range. It ranged from 6.08 of sample M_{10} to 6.78 of sample M_3. Almost similar pH values have been reported by Kanwal (Kanwal et al., 2004).

Conductivity

The conductivity of the collected samples varied from 3.19 mS/cm of sample M_9 to the highest of 4.13 mS/cm of sample M_3. The average value of the conductivity of milk was observed to be 3.51 mS/cm which is 1.35 times lower than the permissible limit. A similar study carried out by Bastola had observed conductivity of 4.0- 5.5 mS/cm in their raw milk samples (Bastola, 2016).

similar study conducted by Musallam (Musallam et al., 2017).

Freezing point

Freezing points of the studied milk samples were found in the range of -0.63 to -0.27 ºC with an average of -0.35 ºC while the suggested freezing point is -0.54 ºC. The increase in freezing point indicates that water has been added. These results are closely related to the results of the Egyptian regulated standard (-0.53 to -0.56 ºC).

The concentration of trace elements and toxic metals

Analysis of samples through FAAS revealed the concentration of six targeted metals including Zn, Cu, Fe, Mn, Cd and Pb in the collected milk samples of Peshawar city which are reported in Table 3 and Figure 3.

Zinc (Zn)

The Zn content of the raw milk samples was found in the range of 0.4405 to 3.0378 mg/L with an average concentration of 1.4035 mg/L. Among the fifteen analyzed samples, M_5, M_{12} and M_{14} contained the highest concentrations of zinc. The zinc content in the entire samples was within the permissible limit of the WHO. Similar results have been suggested earlier by Santos for Brazilian commercial milk (Santos, De Nadai Fernandes, Tagliaferro, & Bacchi, 2008). The results show that the average concentration of Zn was found within the permissible limit suggested by WHO/FAO.

Copper (Cu)

The Cu content of the raw milk samples ranged from 0.1037 to 0.5717 mg/L with an average concentration of 0.2588 mg/L. The amount of Cu in all analyzed samples was found in the permissible range of WHO standards. do-Nascimento has reported the concentration of Cu as 0.62 mg/kg in milk samples of buffalo (do Nascimento et al., 2010).

Iron (Fe)

The Fe content of the collected milk samples varied between 0.6246 to 4.9783 mg/L. The average concentration of Fe in all samples studied was 1.5553 mg/L. The iron content in all the samples was within the permissible limit of the WHO. These findings are comparable to those investigated by do-Nascimento (do Nascimento et al., 2010) but showing a decreasing trend than that was concluded by Sikirić (Sikirić, Brajenović, Pavlović, Havranek, & Plavljanić, 2003).

Manganese (Mn)

Mn content of the raw milk samples was observed in the range of 0.0912 to 0.9246 mg/L showing an average concentration of 0.4354 mg/L. The manganese content in all the samples was within the permissible limit of the WHO. Comparable conclusions have been suggested by Tassew (Tassew Belete & Rao, 2014).

Cadmium (Cd)

The Cd content of the collected milk samples was observed between 0.0039 to 0.6780 mg/L showing a mean value of 0.1865 mg/L. The mean value of cadmium in the studied samples is closely matching the conclusions made by Licata (Licata et al., 2004).

Lead (Pb)

The results indicated an average value of 1.1162 mg/L. The Pb content of different samples ranged between 0.2794 to 2.2655 mg/L. Similar results have also been reported by Licata (Licata et al., 2004) and slightly higher values suggested at California by Bruhn (Bruhn & Franke, 1976).

A higher concentration of Fe, Mn, Cd and Pb were observed in the investigated raw milk samples. The level of these metals in almost all the samples studied exceeded the recommended maximum permissible levels of the WHO. The presence of the non-essential and hazardous Cd and Pb in milk samples even in lower concentrations may cause severe health problems. Cadmium and lead are the most noxious pollutants and may enter into the food chain through aerial deposition and contamination of soil, waters, foods and plants.
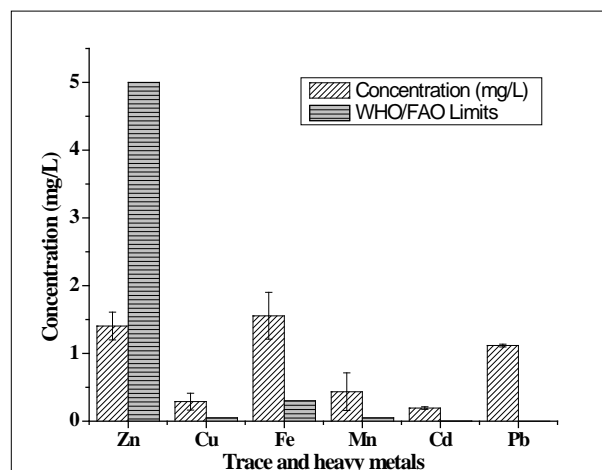

Fig. 3. The concentration of trace elements and heavy metals in raw milk samples of Peshawar city

Conclusion

The study of physicochemical characterization and spectrophotometric determination of heavy metals Zn, Cu, Fe, Mn, Cd and Pb in the raw milk samples was conducted in the industrial sites of the district Peshawar, Khyber Pakhtunkhwa, Pakistan. The average percentage composition of protein, fats, solid non-fats, lactose, salts and added water in the milk samples were found to be 2.78, 4.81, 6.41, 3.05, 0.47 and 31.1 %, respectively. The values of pH, conductivity, density and freezing point were observed as 6.59, 3.51 mS/cm, 1.04 g/mL and -0.3674 °C at 26 °C. On average, the results of the physicochemical characterization revealed that all parameters were found lower than the permissible limits suggested by WHO. This might be due to the addition of water. Moreover, the results indicated that on average 31.1 % water has been added to the collected samples by the sellers. The percentage composition of the raw milk constituents was decreased with the addition of water. The addition of water is the main reason for the lower concentration of percentage protein, fats, solid non-fats, lactose and salts in the raw milk samples.

The concentration of zinc and copper in the raw milk samples was found to be 1.40 and 0.29 mg/L, respectively. Results of the trace elements indicated that their concentrations are within the safe range. The trace elements such as zinc and copper were within the permissible limits of the WHO and consumption of such milk may contribute little to the total zinc and copper intake. Whereas the concentration of iron, manganese, cadmium and lead was observed as 1.56, 0.43, 0.19 and 1.11 mg/L, respectively in the raw milk samples. The average concentrations of Fe, Mn, Cd and Pb were found higher than the permissible limits of WHO. The level of these metals in almost all the samples studied exceeded the recommended maximum permissible levels. The presence of the non-essential and hazardous Cd and Pb in milk samples even in lower concentrations may cause severe health problems. Cadmium and lead are the most noxious

pollutants and may enter into the food chain through aerial deposition and contamination of soil, waters, foods and plants.

Recommendations

The estimation of heavy metals in food products must be ensured before consumption since their presence beyond the daily recommended requirements may pose very serious health risks.

A strong recommendation is made to conduct more reliable and accurate confirmatory testing such as NIR to ensure quality results.

The concentration of more heavy metals should be evaluated using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Acknowledgements

References

Abdel-Hameid, K. G. (2002). Studies on the sanitary condition of raw milk in Qena Governorate. MVSc, Thesis, Faculty of Veterinary Medicine, Assiut, University, Egypt.

Abdel-Sabour, R. (2007). Detection of Milk Adulteration In Assiut Governorate, MVSc, Thesis, Faculty of Veterinary Medicine, Assiut, University, Egypt.

Adam, A. A. H. (2009). Milk adulteration by adding water and starch at Khartoum state. *Pakistan Journal of Nutrition*, **8**, 439-440.

Ahmad, N., Rahim, M., Haris, M. R. H. M. (2014). Toxicological Impact Assessment of Heavy Metals in Human Blood and Milk Samples Collected in District Shangla, Pakistan. *Science International*, **26**, 223-226.

Andrade Korn, M. D. G., da Boa Morte, E. S., Batista dos Santos, D. C. M., Castro, J. T., Barbosa, J. T. P., Teixeira, A. P., Fernandes, A. P., Welz, B., dos Santos, W. P. C., Nunes dos Santos, E. B. G. (2008). Sample preparation for the determination of metals in food samples using spectroanalytical methods—a review. *Applied Spectroscopy Reviews*, **43**, 67-92.

Aoyama, H., Arai, Y., Sasano, M. (1992). Quality of raw milk produced in Hokkaido prefecture [Japan] during the period from 62nd year of Showa (1987) to 3rd year of Heisei (1991). *Japanese Journal of Dairy and Food Science (Japan)*,

Bastola, K. P. (2016). Quality of Raw Milk Supplied to Organized Milk Collection Centers of Western Chitwan. *ABC Research Alert*, **4**,

Braun, P. G., Preuss, S. E. (2008). Nutritional composition and chemico-physical parameters of water buffalo milk and milk products in Germany. *Milchwissenschaft*, **63**, 70-72.

Bruhn, J., Franke, A. (1976). Lead and cadmium in California raw milk. *Journal of dairy science*, **59**, 1711-1717.

Cherryl, D., Chakravarthi, M., Reddy, Y., Indira, D. (2014). Physiochemical quality of raw milk samples in Andhra Pradesh. *Indian Journal of Animal Production and Management*, **30**,

Do Nascimento, I. R., de Jesus, R. M., dos Santos, W. N., Souza, A. S., Fragoso, W. D., dos Reis, P. S. (2010). Determination of the mineral composition of fresh bovine milk from the milk-producing areas located in the State of Sergipe in Brazil and evaluation employing exploratory analysis. *Microchemical Journal*, **96**, 37-41.

Dobrzanski, Z., Kolacz, R., Górecka, H., Chojnacka, K., Bartkowiak, A. (2005). The content of microelements and trace elements in raw milk from cows in the Silesian region. *Polish Journal of Environmental Studies*, **14**, 685.

Enb, A., Abou Donia, M., Abd-Rabou, N., Abou-Arab, A., El-Senaity, M. (2009). Chemical composition of raw milk and heavy metals behavior during processing of milk products. *Global Veterinaria*, **3**, 268-275.

Fundora, O. G., Lezcano, M., Montejo, O., Pompa, A., Enriquez, N. (2001). A comparative study of milk composition and stability of Murrah river buffaloes and Holstein cows grazing star grass. *Revista Cubana de Ciencia Agricola (Cuba)*,

Hossain, M. B., Dev, S. R. (2013). Physiochemical characteristics of various raw milk samples in a selected dairy plant of Bangladesh. *International Journal of Engineering*, **1**, 2305-8269.

Imran, M., Khan, H., Hassan, S. S., Khan, R. (2008). Physicochemical characteristics of various milk samples available in Pakistan. *Journal of Zhejiang University Science B*, **9**, 546-551.

Kanwal, R., Ahmed, T., Mirza, B. (2004). Comparative analysis of quality of milk collected from buffalo, cow, goat and sheep of Rawalpindi/Islamabad region in Pakistan. *Asian Journal of Plant Sciences*, **3**, 300-305.

Licata, P., Trombetta, D., Cristani, M., Giofre, F., Martino, D., Calo, M., Naccari, F. (2004). Levels of "toxic" and "essential" metals in samples of

bovine milk from various dairy farms in Calabria, Italy. *Environment International*, **30**, 1-6.

Mahmood, A., Usman, S. (2010). A comparative study on the physicochemical parameters of milk samples collected from buffalo, cow, goat and sheep of Gujrat, Pakistan. *Pakistan Journal of Nutrition*, **9**, 1192-1197.

Mansour, A. I. A., El-Loly, M. M., Ahmed, R. O. (2012). A preliminary detection of physical and chemical properties, inhibitory substances and preservatives in raw milk. *Reading*, **4**, 0.14.

Miciński, J., Kowalski, I. M., Zwierzchowski, G., Szarek, J., Pierożyński, B., Zabłocka, E. (2013). Characteristics of cow's milk proteins including allergenic properties and methods for its reduction. *Polish Annals of Medicine*, **20**, 69-76.

Mohamed, A. (1981). Study on the status of raw market milk, M. Sc. Thesis, Fac. Vet. Med., Alex. Univ., Egypt.

Muhammad, K., Altaf, I., Hanif, A., Anjum, A., Tipu, M. (2009). Monitoring of hygienic status of raw milk marketed in Lahore City, Pakistan. *The Journal of Animal & Plant Sciences*, **19**, 74-77.

Muhammad, R., Najm, U. S., Fazal, W., Nadir, K., Luqman, I. A. (2020). Analysis of Toxic Heavy Metal Content of the Most Widely Consumed Fruits. *Journal of Physical Science*, **31**, 61-73.

Musallam, H. M., Almozogai, H. M., Amkabis, S. S., Aoag, M. A., Hassan, T. M., Elhefian, E. A., Asseid, F. M. (2017). Physicochemical Characteristics of Various Milk Samples. *Nova Journal of Medical and Biological Sciences*, **6**, 1-3.

Paul, J., Dinn, N., Kannangara, T., Fisher, L. (1998). Protein content in dairy cattle diets affects ammonia losses and fertilizer nitrogen value. *Journal of Environmental Quality*, **27**, 528-534.

Pedro, N. A., De Oliveira, E., Cadore, S. (2006). Study of the mineral content of chocolate flavoured beverages. *Food Chemistry*, **95**, 94-100.

Rahim, M., Imdad, U., Adnan, K., Haris, M. R. H. M., Nisar, A. (2014). Spatial Distribution and Risk Assessment of Heavy Metals From Drinking Water in District Shangla. *Science International-(Lahore)*, **26**, 1625-1630.

Rahim, M., Mas Haris, M. R. H. (2015). Application of biopolymer composites in arsenic removal from aqueous medium: A review. *Journal of Radiation Research and Applied Sciences*, **8**, 255-263.

Rahim, M., Mas Haris, M. R. H. (2019). Chromium (VI) removal from neutral aqueous media using banana trunk fibers (BTF)-reinforced chitosan-based film, in comparison with BTF, chitosan, chitin and activated carbon. *SN Applied Sciences*, **1**, 1180.

Rodríguez Rodríguez, E., Sanz Alaejos, M., Díaz Romero, C. (1999). Chemometric studies of several minerals in milks. *Journal of agricultural and food chemistry*, **47**, 1520-1524.

Roy, N., Nagpal, D., Sadana, T. (1972). Electrical conductance of milk from buffalo and cattle of Indian origin. 1. Correlation with fat and SNF. *Milchwissenschaft*,

Santos, L., De Nadai Fernandes, E., Tagliaferro, F., Bacchi, M. (2008). Characterization of Brazilian commercial milks by instrumental neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry*, **276**, 107-112.

Saqib, N. U., Khan, A., Alam, I., Rahim, M. (2020). Glass beads immobilized doped TiO 2 NPs with enhanced adsorption efficiency for arsenic (III) from aqueous solution. *SN Applied Sciences*, **2**, 1-11.

Shojaei, Z., Yadollahi, A. (2008). Physicochemical and microbiological quality of raw, pasteurized and UHT milks in shops. *Asian journal of scientific research*, **1**, 532-538.

Sikirić, M., Brajenović, N., Pavlović, I., Havranek, J., Plavljanić, N. (2003). Determination of metals in cow's milk by flame atomic absorption spectrophotometry. *Czech J. Anim. Sci*, **48**, 481-486.

Tassew Belete, A. H., Rao, V. M. (2014). Determination of Concentrations of Selected Heavy Metals in Cow's Milk: Borena Zone, Ethiopia. *Journal of Health Science*, **4**, 105.

Walker, G., Dunshea, F., Doyle, P. (2004). Effects of nutrition and management on the production and composition of milk fat and protein: a review. *Australian Journal of Agricultural Research*, **55**, 1009-1028.

Ward, R. E., German, J. B. (2004). Understanding milk's bioactive components: a goal for the genomics toolbox. *The Journal of nutrition*, **134**, 962S-967S.