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

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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 ºC at 26 ºC. 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 (Dobrzenski, 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 (Cherry, Chakravarthi, Reddy, & Indira, 2014; Imran, Khan, Hassan, & Khan, 2008; Mahmood & Usman, 2010; Mansour, El-Loly, & Ahmed, 2012; K. Muhammad, Altaf, Hanif, Anjum, & Tipu, 2009; Rodriguez Rodriguez, Sanz Alaejos, & Diaz 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 great. 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 bringing 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 °C. The sampling sites and their respective codes are shown in Table 1.

Chemicals and Instruments
De-ionized water and analytical grade HNO₃ (CAS: 7697-37-2, and 70 %), HCl (CAS: 7647-01-0, and 37 %) and H₂O₂ (CAS: 7722-84-1 and 30 %) were employed during the experimental work. The stock solutions of each metal was prepared from 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) HNO₃ 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 °C 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 °C 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

Physicochemical 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
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 Cherrell 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. (2013). A possible reason for lower protein value may be the addition of artificial milk powder which leads to a decline in the percentage of solids not fat (SNF) including protein. The analyzed samples contained different levels of SNF. The analyzed samples ranged from 1.62 % in sample M 10 to 5.31 % in sample M 15. 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). 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 M 10 while the maximum content was 8.59 % in sample M 15. 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-Hame (Aoyama, 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 M 10 to 5.31 % in sample M 15. 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.](image-url)
The pH of the collected milk samples was found to be 6.59, which is slightly greater than the WHO level of 6.4 to 6.8. The mean value of pH was found to be 6.47%, which is 1.70 times lower than the WHO permissible level.

| Sample ID | Zn (mg/L) | Cu (mg/L) | Fe (mg/L) | Mn (mg/L) | Cd (mg/L) | Pb (mg/L) |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| M₁        | 0.4405±0.0 | 0.1037±0.0 | 0.9576±0.1 | 0.7070±0.0 | 0.301±0.0 | 0.8256±0.0 |
| M₂        | 1.5573±0.0 | 0.5717±0.0 | 1.8975±0.0 | 0.4711±0.0 | 0.3253±0.0 | 1.6683±0.1 |
| M₃        | 1.9933±0.0 | 0.1104±0.0 | 0.9076±0.0 | 0.7495±0.1 | 0.2575±0.0 | 0.2792±0.0 |
| M₄        | 0.0896±0.0 | 0.4460±0.0 | 2.5589±0.0 | 0.4550±0.0 | 0.5373±0.0 | 1.4439±0.0 |
| M₅        | 3.0378±0.0 | 0.2992±0.0 | 4.9774±0.1 | 0.9246±0.0 | 0.6780±0.1 | 0.8836±0.0 |
| M₆        | 1.8911±0.0 | 0.4187±0.0 | 1.3769±0.0 | 0.8148±0.0 | 0.2108±0.0 | 0.5305±0.0 |
| M₇        | 0.8249±0.0 | 0.1690±0.0 | 1.3481±0.0 | 0.3262±0.0 | 0.0993±0.0 | 0.9022±0.0 |
| M₈        | 1.0845±0.2 | 0.2299±0.0 | 0.6246±0.0 | 0.6299±0.1 | 0.0916±0.0 | 0.8946±0.0 |
| M₉        | 0.9423±0.0 | 0.2205±0.0 | 1.6883±0.0 | 0.4026±0.0 | 0.0039±0.0 | 1.8026±0.1 |
| M₁₀       | 0.6198±0.0 | 0.2923±0.0 | 0.7174±0.0 | 0.3364±0.0 | 0.0981±0.0 | 0.9650±0.0 |
| M₁₁       | 0.6429±0.0 | 0.3452±0.0 | 0.9558±0.0 | 0.1126±0.0 | 0.1396±0.0 | 2.2655±0.0 |
| M₁₂       | 2.3165±0.1 | 0.2989±0.0 | 1.8686±0.0 | 0.2764±0.0 | 0.0116±0.0 | 1.3495±0.0 |
| M₁₃       | 1.4933±0.1 | 0.2606±0.0 | 0.8612±0.0 | 0.1001±0.0 | 0.0618±0.0 | 0.8332±0.0 |
| M₁₄       | 2.2153±0.0 | 0.2959±0.0 | 1.0131±0.0 | 0.0912±0.0 | 0.0313±0.0 | 0.7917±0.0 |
| M₁₅       | 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   | 0.05-15    | 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 water. The minimum water content was found to be 24.61% in sample M₁₀, while the maximum content was 54.03% in sample M₁₀. The samples M₆ and M₁₃ 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).

### Conductivity

The conductivity of the collected samples varied from 3.19 mS/cm of sample M₀ to the highest of 4.13 mS/cm of sample M₃. 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).

### Density

Among the collected milk samples, sample M₁₀ was found to have the minimum density of 1.00 g/mL while sample M₈ 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 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 °C with an average of -0.35 °C while the suggested freezing point is -0.54 °C. 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 °C).

### 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, M₁, M₁₂, and M₁₄ 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 Sikiric (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.

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 ºC at 26 ºC. 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).

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