HEALTH RISK ASSESSMENT OF HEAVY METALS IN RAW AND PACKED MILK FROM THE AREAS OF RAWALPINDI AND ISLAMABAD: A COMPARATIVE STUDY

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Abstract
Milk is a complex of biomolecules and is a good source of proteins, fats, sugars, vitamins and minerals. Various types of milk available in the market are solid and liquid milk. Solid milk is available in the form of formula milk and liquid milk is raw and packed milk (branded milk) like GM, OL, Dp, T, H, Mps, DO or and Ns. Milk having different additive components like “micronutrients, vitamin and minerals” play a vital role in our daily life therefore it is by kids, adults and elders all over the world. The chemical composition of raw milk not only focuses on its components, but also the treatment, in the case of quality of the raw materials, packing, storage and conditions are very important. Cow milk is one of the major resources contaminated with different heavy metal atoms. Different samples of milk (raw and packed) were collected from diverse zone of Islamabad. The packed milk like GM, OL, Dp, T, H, Mps, DO or and Ns were collected from different shops of Rawalpindi and Islamabad. Result showed the presence of different types of heavy metals in milk samples. The level of zinc in the raw milk samples was high in the Islamabad city as compared to Rawalpindi. The sequence of heavy metals present in fresh milk were detected as Zn> Cu> Cr> Pb and Cu. In packed milk, level of Chromium was different across the world.

Keywords: Analysis, Health risk, Heavy metals, Packed milk, Raw milk

INTRODUCTION
Milk is a primary source of nutrients all around the world (1). Milk contain variety of very important nutrients which are crucial to maintain healthy life of every individual. Thus, their regular daily consumption has been widely recommended. However, there are evidences that milk might contain varying amount of different toxic contaminants. According to the newest report from WHO one in eight global deaths were linked with air pollution, making it the world largest single environmental health risk (2). From the nutritional point of view, metals contents of milk can be grouped into essential elements (iron, copper and zinc) at low doses and non-essential or toxic ones (lead and cadmium). The presence of the letter, even in low concentration, is available and lead to metabolic disorders with extremely serious consequences (3).

Dairy animals ingest metals while grazing on the pasture and when fed on contaminated concentrate feeds. However, in cow transfer of minerals to milk is highly variable (4). Milk plays an important role in life of human being diet due to the presence of the different items like “micronutrients, vitamin and minerals” The composition of milk differs among species of different animal and depends upon the care provided to the household animals (5). Cow’s Milk contain some of the important elements, such as...
high percentage of fertilizer containing potassium, phosphate, sodium extensive micro elements, chlorine and even heavy metals (6).

Cow milk is one of the major sources of food which is being contaminated with different heavy metals (7). Increased industrialization and extensive use of fertilizers explain the presence of these harmful elements in the nature. Among twenty-three metals, lead (Pb) and cadmium (Cd) are considered the most toxic (8) while chromium (Cr) is known as essential and important in health and metabolism at low concentration (9) but toxic at high concentration (10). They can be absorbed by soil and then transferred in plants that constitute feeding stuff. In final, these elements can be present in animal products like milk and meat (11). It is important to study the presence of toxic heavy metals because due to increased environmental pollution (12). Although minerals and heavy metals elements naturally occurs in milk/dairy products, however they can be added into milk/dairy products via anthropogenic pathways such as transport, industry, power generation and refuse burning (13).

The literature reports highlight the very important role of Milk in our daily diets but it also revealed the presence of different heavy metal elements in different branded packed as well as raw milk. It is therefore important to continually evaluate the quality of the milk. In this context the present research was conducted to investigate five toxic heavy metal contents in different samples of milk (raw and packed) that were collected from diverse zone of Rawalpindi and Islamabad.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING

The different type of raw and packed milk samples (total 31 samples) were obtained from shops of diverse zones of Islamabad (Shzad town farm, ZTBL staff college, local nallah, sqahdara village & tama gra area near to Comsat university) and Rawalpindi areas (Dhok kazim, tharalli, Service road, Koat kokyl & koker road). The packed milk samples were of different brand names (Dairy Omung (DO), Dairy pure (Dp), Good milk (GM1), Haleeb (H), Milk pak (Mp), Nesvita (N), Olper’s (OL2), and Tarang (T)). All the samples were of different quantities i.e. approximately 50-100ml. In each sample, 2 mL of peroxide were added (It help to maintain the preservation of milk for some hour in case of no light.). All the samples were stored at 0’C.

SAMPLE TREATMENT

The liquid milk samples were treated via wet digestion method prior to their analysis via atomic adsorption spectrophotometer. Accordingly the samples were treated with 65% HNO₃ and 30% per oxide for wet digestion. The temperature for the samples on the hotplate was raised gradually upto 90-120°C until brown fumes from the samples appear. When the brown fumes of the samples turned into white, then samples were allowed to cool at a room temperature. The solution was filtered and the filtrate solution of each sample was transferred to a 25ml volumetric flask and deionized H₂O was added up to the mark. The blank solutions were made in the same way without using any sample. The filterate was stored into the sealed sterilized plastic bottles and properly labeled.

HEAVY METAL ANALYSIS IN THE SAMPLES

Heavy metal concentrations were analyzed in samples through atomic absorption spectrometry (AAS), AAS Vario 6 instrument accordance with Swedish Standard method (SS-EN 028150). The detection limits of heavy metals according to this instrument were in that standardized order, Zn: 0.0014 mg/l, Pb: 0.0013 mg /l, Cu: 0.003 mg/l and Cr: 0.0054 mg/l. The stored filtrated solution for each sample was shaken and their pH was examined which was approximately 4.5 and the range was 4.0-5.1.

STATISTICAL ANALYSIS

Results were analyzed for statistical significance through the Microsoft word graphic. All the values either mean or S.D was described and written in the table form.
RESULTS AND DISCUSSION
HEAVY METALS DETERMINATION IN PACKED AND RAW MILK SAMPLES FROM ISLAMABAD AND RAWALPINDI AREAS

Heavy metals determination can be carried out by using inductively coupled plasma (ICP) spectrometry (14, 15) however the flame Atomic absorption spectrophotometer is a commonly used analytical technique which determines the contamination of heavy metals in milk (16, 17). Therefore, the present study was carried out for determination of five heavy metals (Pb, Cr, Mn, Zn and Cu) in different types of thirty-one milk (raw milk) and eight packed milk samples through atomic Absorption Spectrophotometer (AAS). The packed milk samples were coded according to their brand names, (Dairy Omung (DO₇), Dairy pure (Dp₃), Good milk (GM₁), Haleeb (H₅), Milk pak (Mp₆), Nesvita (N₈), Olper’s (OL₂), and Tarang (T₄). The heavy metals determination results obtained through atomic absorption analysis for the packed milk samples are presented in (Table I).

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Pb (ppm) Mean ± S.D</th>
<th>Cr (ppm) Mean ± S.D</th>
<th>Mn (ppm) Mean ± S.D</th>
<th>Zn (ppm) Mean ± S.D</th>
<th>Cu (ppm) Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM₁</td>
<td>0.0465 ±0.0049</td>
<td>0.0435 ±0.0049</td>
<td>0.0212 ±0.0053</td>
<td>0.5306 ±0.0561</td>
<td>0.4425±0.0147</td>
</tr>
<tr>
<td>OL₂</td>
<td>0.06 ±0.0268</td>
<td>0.049 ±0.0169</td>
<td>0.1048±0.1144</td>
<td>0.8574±0.3741</td>
<td>0.326±0.2867</td>
</tr>
<tr>
<td>DP₃</td>
<td>0.0405 ±0.0063</td>
<td>0.0385 ±0.0049</td>
<td>0.0263±0.0107</td>
<td>0.6734±0.6796</td>
<td>0.7612±0.3659</td>
</tr>
<tr>
<td>T₄</td>
<td>0.042 ±0.0084</td>
<td>0.0415 ±0.0049</td>
<td>0.1908±0.2009</td>
<td>0.7004±0.0400</td>
<td>0.6896±0.0506</td>
</tr>
<tr>
<td>H₅</td>
<td>0.0435 ±0.0360</td>
<td>0.042 ±0.0333</td>
<td>0.0036±0.0015</td>
<td>0.4806±0.0209</td>
<td>0.2778±0.0334</td>
</tr>
<tr>
<td>MP₆</td>
<td>0.0515 ±0.0091</td>
<td>0.0495 ±0.0091</td>
<td>0.0288±0.0015</td>
<td>0.3187±0.1487</td>
<td>0.69±0.47800</td>
</tr>
<tr>
<td>DO₇</td>
<td>0.0895 ±0.0275</td>
<td>0.0875 ±0.0289</td>
<td>0.0667±0.0680</td>
<td>1.0815±0.0445</td>
<td>0.9232±0.0466</td>
</tr>
<tr>
<td>N₈</td>
<td>0.045 ±0.0325</td>
<td>0.0465 ±0.0332</td>
<td>0.042 ±0.02969</td>
<td>0.8369±0.2702</td>
<td>0.4494±0.2047</td>
</tr>
<tr>
<td>Detection limit</td>
<td>0.0013</td>
<td>0.0054</td>
<td>0.0015</td>
<td>0.0014</td>
<td>0.003</td>
</tr>
<tr>
<td>Range</td>
<td>0.018-0.109</td>
<td>0.017-0.108</td>
<td>0.017-0.333</td>
<td>0.193-1.154</td>
<td>0.123-1.028</td>
</tr>
<tr>
<td>WHO</td>
<td>0.01</td>
<td>0.050</td>
<td>0.1</td>
<td>0.03</td>
<td>0.010</td>
</tr>
</tbody>
</table>

The results given in table 1 indicated that in GM₁ sample all the five examined heavy metals were detected, where the concentration of Zn was high (0.5306ppm) as compared to other heavy metals such as Pb (0.0465ppm), Cr (0.0435ppm), Mn (0.0212ppm) and Cu (0.4425ppm). In OL₂ packed milk sample also Zn Cr, Mn, Pb and Cu were identified in different concentration where the Zn concentration (0.85745ppm) was recorded as the highest compared to Cr, Mn, Pb and Cu (0.049ppm) showed the lowest concentration. DP₃ sample also shown the presence of Zn Cr, Mn, Pb and Cu where the highest concentration was for Cu (0.7612ppm) while the lowest was detected for Mn (0.026365ppm). All the examined five metals (Zn, Cr, Mn, Pb & Cu) were observed in T₄ sample, where Zn was detected in highest concentration (0.7004ppm) and Cr (0.0415ppm) was observed in lowest concentration. H₅, MP₆ and N₈ samples also showed the presence of all the examined heavy metals (Zn, Cr, Mn, Pb & Cu). In H₅ samples, the highest concentration was observed Zn metal (0.4806ppm) while lowest was for Mn metal i.e., 0.03628ppm. The highest concentration of heavy metal in MP₆ was detected for Cu (0.69ppm) while lowest was observed for Mn i.e. 0.028875ppm. In N₈ milk samples, the highest concentration was detected for Zn i.e., 0.8369ppm while the lowest concentration was observed for Mn i.e. 0.042ppm. The DO₇ samples shown the presence of Zn, Cr, Mn, Pb & Cu where Zn showed highest concentration (1.0815ppm) and Mn (0.0667ppm) showed the lowest concentration.
Overall results for the packed milk samples indicated that highest concentration of Pb was observed in DO7 (0.0895±0.0275ppm) and the lowest concentration was found in T4 (0.042±0.0084ppm) samples. It was also observed that the highest concentration of Cr was found in DO7 (0.0875±0.0289ppm) while its lowest concentration was in DP3 (0.0385±0.0049ppm). The concentration of Mn was highest in T4 sample (0.1908±0.2090) while H5 showed lowest concentration of Mn (0.0036±0.0015ppm). The Zn (1.0815±0.0445ppm) concentration was found highest in DO7 sample where MP6 showed its lowest concentration (0.3187±0.1487ppm). Among the examined heavy metals, Cu ranges from 0.123-1.028ppm with its highest concentration in DO7 sample (0.9232±0.0466ppm) and lowest (0.2778±0.0334ppm) in H5 sample.

The graph given in Figure 1 inferred that Zn were present in all samples in high concentration compared to others but its highest concentration was observed in DO7 sample. In some samples the concentrations of Cu were above the detection limit.

The raw milk samples from Islamabad (n=21) and Rawalpindi areas (n=13) were also subjected to Atomic absorption spectrophotometer for the selected heavy metals determination, and the results obtained are presented in (Table II).

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Pb(ppm) Mean ± S.D</th>
<th>Cr(ppm) Mean ± S.D</th>
<th>Mn(ppm) Mean ± S.D</th>
<th>Zn(ppm) Mean ± S.D</th>
<th>Cu(ppm) Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>*In-In</td>
<td>0.0639±0.0234</td>
<td>0.0643±0.0236</td>
<td>0.0428±0.0366</td>
<td>1.3343±0.8356</td>
<td>0.8515±0.5141</td>
</tr>
<tr>
<td>*Rn – Rn</td>
<td>0.0649±0.0197</td>
<td>0.0582±0.0171</td>
<td>0.0464±0.0286</td>
<td>0.7556±0.7453</td>
<td>0.5214±0.3507</td>
</tr>
<tr>
<td>Range</td>
<td>0.027-0.102</td>
<td>0.102-0.109</td>
<td>0.016-0.168</td>
<td>0.1235-3.045</td>
<td>0.101-2.6</td>
</tr>
<tr>
<td>Detection limit</td>
<td>0.0013</td>
<td>0.0054</td>
<td>0.0015</td>
<td>0.0014</td>
<td>0.003</td>
</tr>
<tr>
<td>WHO</td>
<td>0.01</td>
<td>0.050</td>
<td>0.1</td>
<td>0.03</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*In-In represents Islamabad while Rn-Rn represents Rawalpindi area samples

The results given in (Table II) for the raw milk samples, the concentration of the Pb in Islamabad area samples was 0.0639±0.0234ppm (range 0.027-0.102ppm) and in Rawalpindi area sample it concentration was detected as highest concentration 0.0649±0.0197ppm (range 0.027-0.102ppm). The results indicated that Cr concentration was in a range of 0.025 to 0.109ppm for Islamabad area samples and 0.036-0.079ppm for the Rawalpindi area samples where its highest concentration was observed in Islamabad samples (0.0643±0.0236ppm) and lowest in Rawalpindi samples (0.0582±0.0171ppm). In case of Mn concentration, the mean± standard deviation concentration of Mn in Islamabad area samples was
0.0428±0.0366ppm (range 0.019-0.168ppm) and in Rawalpindi area: 0.0464±0.0286ppm (range 0.016-0.168ppm) where the highest concentration of the Mn was detected in Rawalpindi area samples as compare to the Islamabad area samples. The mean concentration of Zn concentration in Islamabad area sample was 1.3343±0.8356ppm (range 0.202-3.054ppm) and in Rawalpindi area it concentration was 0.7556±0.7453ppm (range 0.1235-3.045ppm). It indicates that Zn was high in raw milk area while lowest in Rawalpindi area samples. The mean±SD concentration for Cu was highest in Islamabad area samples was 0.8515±0.5141ppm (range 0.246-2.6ppm) and lowest 0.5214±0.3507ppm (range 0.101-2.6ppm) in Rawalpindi area samples. Overall in raw milk samples the concentration of Zn was highest compared to the other examined heavy metals (Pb, Cr, Cu and Mn) as depicted in Fig. 2.

Fig. 2. Heavy metals detected in raw milk.

The mean concentration of heavy metals in cow and buffalo milk samples is different across the world. The Mean ± S.D of Zn 1.3343±0.8356 in raw milk was highest in Islamabad as compared to Rawalpindi. The heavy metals detected in cow fresh milk were in sequence Zn> Cu> Pb and Cu and the Mean ± S.D of these metals were 1.3343±0.8356 ppm> 0.8515±0.5141 ppm> 0.0643±0.0236 ppm> 0.0639±0.0234 ppm and 0.0428±0.0366 ppm from Islamabad, but from Rawalpindi the heavy metals were detected in raw milk that is in sequence 0.7556±0.7453 ppm> 0.5214±0.3507 ppm> 0.0582±0.0171 ppm> 0.0649±0.0197 ppm and 0.0464±0.0286 ppm. According to Meshref et al., work Total 77 milk samples were collected from different farmers from the capital city (Beni-Suef governorate) of Egypt, they determined the concentration of heavy metal in milk i.e., Pb 0.044–0.751 ppm> Cd 0.008–0.179 ppm> Cu 0.002–1.692 ppm (18).

Tona et al. (2013) worked on 40 samples of cow milk and reported that different types of heavy metals were detected in cow milk samples. They determine the concentration of Pb and Cd. The concentration of Pb and Cd were 0.0025ppm to 0.0061 ppm while the normal range was 0.0125 to 0.0175 ppm. They reported that presence of these heavy metals in milk are toxic for human health (19).

Zodape et al. (2012) work on 15 samples of cow milk of different brands collected from various locations of Mumbai city. They determined the concentration of five heavy metals, namely Copper, Zinc, Chromium and Lead. The mean concentration of copper was found between 37.290ppm and 0.039ppm; these values are lower than the tolerable limits set by Food and Nutrition Board (1980). The mean higher and lower concentrations of chromium was found 0.175 ppm and 0.013 ppm respectively and lead mean concentration was found between 5.904 ppm and 0.139 ppm and these values are higher than previously reported values in literature (20).

HEALTH RISKS ASSOCIATE WITH THE DETECTED HEAVY METALS

CHROMIUM

The mean and standard deviation of Chromium were unlike crossways in the world. In GM1, OL2, DP3, T4, H5, MP6, DO7 and NS milk samples the Mean and SD of Cr were in sequence detected as 0.0435±0.0049> 0.049±0.0169> 0.0385±0.0049> 0.0415±0.0049> 0.042±0.0353, 0.0495±0.0091> 0.0875±0.0289 and 0.0465±0.0332. Cr play vital function in boosting “insulin” but Cr shortage causes lot of diseases like cardiovascular disease as well as impaired fertility (21, 22), lung cancer, acute renal tubular necrosis and renal failure (23, 24).
LEAD

The Mean concentration and S.D of Pb in GM₁, OL₂, DP₃, T₄, H₅, MP₆, DO₇ and N₈ milk samples were observed during this research in order 0.0465± 0.0049, 0.06± 0.0268, 0.0405± 0.0063, 0.042± 0.0084, 0.0435± 0.0360, 0.0515± 0.0091, 0.0895± 0.0275 and 0.045± 0.0325. Lead does not break down in the environment and this potent neurotoxin can harm the nervous system as well as produce reproductive complications and kidney failure especially in young children. Lead is particularly vulnerable to abdominal pain, constipation, headache, irritability, memory problems, in ability to have children and tingling in the hand and feet, hearing damage and various behavioural disorders (25, 26).

ZINC

The Mean concentration and S.D of Zn heavy element in GM₁, OL₂, DP₃, T₄, H₅, MP₆, DO₇ and N₈ milk samples were observed during this study in order 0.5306±0.0561, 0.8574±0.3741, 0.6734±0.6796, 0.7004±0.0209, 0.3187±0.1487, 1.0815±0.0445 and 0.8369±0.2702. Zinc deficiency in the newborn may be manifested by dermatitis, loss of hair, impaired healing, susceptibility to infections, and neuropsychological abnormalities. Other chronic clinical disorders such as ulcerative colitis and the mala Absorption syndrome, chronic renal disease and haemolytic anaemia are also associated with zinc deficiency (27).

COPPER

Mean concentration and S.D of Cu heavy element in GM₁, OL₂, DP₃, T₄, H₅, MP₆, DO₇ and N₈ milk samples were observed as 0.4425±0.0147, 0.3262±0.2867, 0.7612±0.3659, 0.6896±0.0506, 0.2778±0.0334, 0.69±0.47800, 0.9232±0.0466 and 0.4494±0.2047. According to Hussain et al. reported the concentration of Cu in different samples of branded milk was 0.21ppm, 0.18ppm, 0.14ppm, 0.13ppm and 0.13ppm determined through atomic absorption (28). Exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anaemia, immune toxicity and developmental toxicity (29).

MANGANESE

The Mean and S.D of manganese in this research were observed in various packed milk such as GM₁, OL₂, DP₃, T₄, H₅, MP₆, DO₇ and N₈ milk samples through Atomic absorption spectrometry in sequence 0.0212±0.0053, 0.1048±0.1144, 0.0263±0.0107, 0.1908±0.2009, 0.0036±0.0015, 0.0288±0.0015, 0.0667±0.0680 and 0.042±0.02969. Excess manganese interferes with the absorption of dietary iron long-term exposure to excess levels may result in iron deficiency anaemia. Increased manganese intake impairs the activity of copper metallo enzymes.

CONCLUSION

A total 31 raw milk samples and 6 brands (n=2) of packed milk were collected from Rawalpindi and Islamabad region where heavy metal contents were determined in the samples. The heavy metals detected in the studied packed milk the order follows as, Zn>Cu>Pb>Mn and Cr while in the fresh or raw milk samples follow the order as, Zn> Cu> Pb> Cr and Mn. The mean concentration of Zn and Cu in fresh milk and packed milk were found higher than the WHO permissible limits. Consequently, consumption of adulterated milk by consumers will result health problems. There is a need to set legal and/or permissible limits at country level for heavy metals in milk. Risk of contamination, mode of transportation, storage and appropriate strategies are required at farms or in the industry situated near waste water drainage area. Special attention should be given to heavy metals as once they are present in concentrations greater than the acceptable daily intake, it may be difficult to reduce them to an acceptable level during processing. Milk used for human consumption should be monitored regularly and tested for contamination by toxic heavy metals, as well as for levels of essential trace elements. Since milk is one of the important sources of food,
but milk can cause different types of diseases due to toxicity of heavy metals. It is therefore recommended that some precautions must be followed to save our lives from toxicity of heavy metals;

1. Before use of raw milk, it should be sterilized.
2. Care of packaging should be done by suppliers.

   Must check and avoid feed of cows through which heavy metals can penetrate into the milk and thus heavy metals can absorb in human body through milk consumption.

Acknowledgment:
The authors are thankful to HEC Pakistan, Quaid e Azam University, Islamabad-Pakistan and SBK Women University for providing Lab, chemicals, analysis and conducive research environment.

Conflict of interest:
The authors declare that they have no conflicts of interest.

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