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Intake of Heavy Metals through Milk and Toxicity Assessment

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Authors' Contributions
MR and SA designed the study. AI conducted experimental work, compiled the data and wrote the article. MAS helped in analysis of metals. AF assisted in experimental work and writing of article. AM collected samples and statistically analyzed the data. MR supervised the study.

Key words
Heavy metals, Threat, Health, Toxicity, Milk, Vulnerable, Infants.

ABSTRACT

In the present study five heavy metals were analyzed in the milk samples of cow, buffalo and goat. The level of Pb was found to exceed the maximum permissible limit (0.02 µg/g) set by Codex Alimentarius Commission in 53% milk samples. In 44% milk samples the level of Cd was found to exceed the permissible limit (0.0026 µg/g) set by International Dairy Federation (1979). The mean levels of Ni, Cu and Co were found in the normal ranges. The data for estimated daily intake of heavy metals through milk showed that infants are most prone towards heavy metal toxicity due to the higher rate of milk consumption.

INTRODUCTION

Heavy metals in milk are reported from all around the globe but most of the studies reporting heavy metals level higher than the permissible limits are from the developing countries like Pakistan (Lutfullah *et al*., 2014), Iran (Rezaei *et al*., 2014), Egypt (Abdelkhalek *et al*., 2015) and Nigeria (Ogabiela *et al*., 2011). The heavy metals may enter inside the animal body through feed, drinking water or sometime through vapors or dermal contact (Batool *et al*., 2016; Raikwar *et al*., 2008). In case of processed milk the heavy metals may also enter through different machines involved in the processing and distribution (Anetta *et al*., 2012; Riaz *et al*., 2015).

The toxic impacts of heavy metals on human health include skeletal damages, renal failure, cell damages, osteoporosis, cancer of lungs and blood, hormonal disturbances, gastrointestinal problems and anemia (Ismail *et al*., 2015; Arora *et al*., 2008; Kanumakala *et al*., 2002; Kumar *et al*., 2007). The toxic impacts of heavy metals on human health proliferate due to the ubiquitous, non thermo degradable and non biodegradable nature of heavy metals (Ismail *et al*., 2014).

The aim of current study was to assess the level of heavy metals in milk. In the present study, five heavy metals including lead (Pb), cadmium (Cd), cobalt (Co), nickel (Ni) and copper (Cu) were analyzed in milk samples obtained directly from the farms of four districts of Punjab province. Moreover, the estimated daily intake (EDI) of these metals through consumption of milk by various age groups was also calculated.

MATERIALS AND METHODS

Sample collection

Milk samples of three lactating animals including cows, buffalos and goats were collected during July 2014 to February 2015 from four districts of Punjab Province including Dera Ghazi Khan, Sahiwal, Rahim Yar Khan and Bahawalnagar. A total of 540 milk samples were collected (goat: 120, cow: 240, buffalo: 180). The milk samples were collected from the farms situated near the cities. The samples were collected in clean glass bottles directly from the farms to avoid the chances of after milking contamination. The samples were stored in ice during transportation and were immediately sent to the laboratories of Department of Food Science and Technology, Bahauddin Zakariya University, Multan where stored at -20˚C until further analysis.

Sample digestion and analysis

Wet digestion method of Richards (1968) was

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employed for the digestion of milk samples for which 10 ml of HNO_3 was added to 1 ml of milk in a 100 ml conical flask and heated at 80˚C for 20 min. After cooling down to room temperature 5ml of perchloric acid was added to the flask and heated at 180˚C until the volume was reduced to 2-3 ml. The digested milk samples were diluted with deionized water upto 50 ml. For metal analysis the prepared samples were loaded on atomic absorption spectrophotometer by using a mixture of air and acetylene for flame production.

Instruments and chemicals

The chemicals used during experiments were purchased from Merck chemicals (Darmstadt, Germany) and were of analytical grade while the standard solutions of various metals were supplied by CPA chemicals (CPA chemicals ltd., Stara Zagora, Bulgaria). Sample drying and digestion were performed through hot air oven (Memmert UNB 200, Munich, Germany) and hot plate (Lab Tech EH 35A plus, Beijing, China), respectively. Flame atomic absorption spectrophotometer (Thermo Scientific 3000 Series, Waltham, MA, USA) was used for the quantification of heavy metals in milk samples.

Quality control

For measuring the limit of detection (LOD) twelve blanks were digested in the same way as the milk samples and their concentrations for Pb, Cd, Co, Ni and Cu were recorded by analyzing through flame atomic absorption spectrophotometer. The LOD values for each element were recorded as three times the standard deviation (SD) of twelve blanks (3.3 SD/b). The LOD values recorded for Pb, Cd, Co, Ni and Cu were 0.5, 0.1, 0.2, 0.7 and 3.2 µg/kg, respectively. The recovery percentages for various elements were calculated by spiking known amounts of standard solutions in milk samples. The concentration of various elements in non spiked milk samples were measured as control. Analyses were performed in triplicate and their mean values were recorded as final concentrations. The experiments were repeated if found with higher than the limit of 1% repeatability. Following formula was used for measuring the recovery percentages of spiked milk samples:

$$\text{Recovery \%} = \frac{\text{Conc. spiked milk} - \text{concentration in non spiked milk}}{\text{spiked amount}} \times 100$$

The recovery percentages for Pb, Cd, Co, Ni and Cu were found as 94.5, 95.3, 92.8, 96.4, 97.1%, respectively.

Estimated daily intake through milk

The estimated daily intake (EDI) values of heavy metals through milk were calculated by adopting the procedure proposed by Cano-Sancho *et al.* (2010). The daily intake values of milk for different age groups were calculated through a food frequency questionnaire. For male and females, five different age groups were selected for calculating the values of EDI. Following formula was used for calculating the EDI values of heavy metals through milk:

$$\text{EDI} = \frac{\text{Milk intake (kg/day)} \times \text{Heavy metal content in milk (ug/kg)}}{\text{Average individual weight (kg)}}$$

Statistical analysis

For the statistical evaluation of data the software Statistix 8.1 (Statistix Inc., Florida, USA) was used. One way analysis of variance (ANOVA) was used for comparison purposes followed by the least significant difference (LSD) test. The probability level selected for statistically significant differences was $P < 0.05$. Mean and SD values were calculated by using Microsoft Excel (2007 version).

RESULTS AND DISCUSSION

Heavy metals concentrations in the milk samples from four different districts of Punjab Province of Pakistan are presented in Table I. The statistical analysis showed significant differences in the concentrations of various heavy metals within different districts and animal species. The heavy metal concentrations in milk samples of different animal species were in the order of cow > buffalo > goat while in case of area wise distribution, metal concentrations were in the order of Rahim Yar Khan > Bahawalnagar > DG Khan > Sahiwal.

Pb is one of the most toxic heavy metals and its level in milk and milk products is increasing day by day due to the uncontrolled urbanization and industrialization (Swarup *et al*., 2005). Milk samples in this study were found to have Pb concentrations in the range of 0.007 – 0.041 µg/g with a mean concentration of 0.021 µg/g. Highest Pb concentration was detected in buffalo milk samples from Sahiwal while the least was recorded in the goat milk samples from Rahim Yar Khan. The maximum permissible limit for Pb in milk given by the Codex Alimentarius Commission (2011) is 0.02 µg/g. A comparison of our results with this standard limit showed 53% milk samples were exceeding the permissible limit. Sayed *et al*. (2011) measured the level of Pb in milk samples from Egypt and found the mean level of 0.327 µg/g, which is much higher as compared to present study. The mean level of Pb reported in cow milk samples from Iran (Najarnezhad and Akbarabadi, 2013) is 0.012 µg/g which is slightly lower as compared to our study and a much lower Pb level in milk is reported in Korea (0.004 µg/g) by Khan *et al*. (2014)

Table I.- Heavy metals (µg/g) in milk samples from different districts of Punjab Province.

Area	Animal type	Pb	Cd	Co	Ni	Cu
DG Khan	Goat	0.009±0.002^{fg}	0.0023±0.003^{cd}	0.131±0.002^{c}	0.013±0.001^{gh}	0.091±0.002^{b}
DG Khan	Cow	0.014±0.003^{efg}	0.0041±0.001^{b}	0.087±0.025^{g}	0.044±0.018^{d}	0.076±0.011^{d}
DG Khan	Buffalo	0.024±0.001^{cd}	0.0071±0.001^{a}	0.103±0.024^{e}	0.049±0.020^{d}	0.082±0.009^{cd}
Sahiwal	Goat	0.021±0.002^{cde}	0.0013±0.000^{de}	0.093±0.002^{fg}	0.035±0.003^{e}	0.043±0.004^{e}
Sahiwal	Cow	0.033±0.006^{b}	0.0026±0.001^{bcd}	0.041±0.009^{i}	0.018±0.004^{fg}	0.048±0.005^{e}
Sahiwal	Buffalo	0.041±0.005^{a}	<0.0001	0.067±0.008^{h}	0.026±0.005^{f}	0.021±0.007^{f}
Rahim Yar Khan	Goat	0.007±0.002^{g}	0.0041±0.002^{b}	0.099±0.002^{ef}	0.067±0.001^{bc}	0.141±0.003^{a}
Rahim Yar Khan	Cow	0.014±0.003^{efg}	0.0033±0.004^{bc}	0.187±0.006^{a}	0.083±0.008^{a}	0.093±0.022^{b}
Rahim Yar Khan	Buffalo	0.018±0.001^{de}	0.0043±0.002^{b}	0.143±0.004^{b}	0.017±0.007^{fg}	0.087±0.019^{c}
Bahawalnagar	Goat	0.016±0.002^{ef}	<0.0001	0.063±0.003^{h}	0.009±0.003^{h}	0.018±0.003^{f}
Bahawalnagar	Cow	0.028±0.007^{bc}	0.0014±0.001^{de}	0.117±0.030^{d}	0.073±0.009^{b}	0.041±0.003^{e}
Bahawalnagar	Buffalo	0.024±0.009^{cd}	0.0023±0.001^{cd}	0.009±0.027^{j}	0.062±0.006^{c}	0.082±0.004^{c}
Total Mean		0.021	0.003	0.095	0.041	0.068

Pb, Lead; Cd, Cadmium; Co, Cobalt; Ni, Nickel; Cu, Copper.

and in Spain (0.005 µg/g) by Sola-Larrañaga and Navarro-Blasco (2009). The mean level of Pb reported in Hungary (0.023 µg/g) by Poti et al. (2012) is almost in line with our study.

Cd toxicity in humans may lead to kidney failure as well as liver and skeletal disorders (Zaidan et al., 2013). The mean Cd level of milk samples detected in present study was 0.003 µg/g with a range of <0.0001 – 0.0071 µg/g (Table I). The maximum limit for Cd in milk reported by International Dairy Federation (1979) is 0.0026 µg/g. Comparing our results with this limit, 44% milk samples were found to exceed the limit.

The mean Cd level of milk samples is also higher than the maximum permissible limit. The mean levels of Cd reported in bovine milk from Egypt by Enb *et al*. (2009) and from Nigeria by Ogabiela *et al*. (2011) are 0.086 µg/g and 0.131 µg/g, respectively, which are much higher as compared to the current findings. The Cd level reported from Poland (Pilarczyk *et al*., 2013) and Croatia (Bilandžić *et al*., 2011) are 0.004 and 0.003 µg/g respectively, which are almost in line with our study. However, a very low Cd level is reported in Spain (0.0004 µg/g) and Korea (0.002 µg/g) by Sola-Larrañaga and Navarro-Blasco (2009) and Khan *et al*. (2014), respectively, indicating a better control of Cd in these countries.

Co being a part of vitamin B_{12} is considered an essential element for normal human growth. However, in excess amounts it can disturb the reproductive system and thyroid glands (Nordberg *et al*., 2007) and is also reported as a probable carcinogenic compound by IARC (1991). The mean Co concentration in milk samples found in the present study was 0.095 µg/g and was in the range of 0.009 – 0.187 µg/g. The mean concentration of Co detected in present study is much lower than the earlier reported by Patra *et al*. (2008) (0.19 µg/g) from India however, it is higher than reported from Korea (0.006 µg/g) and Spain (0.005 µg/g) by Khan *et al*. (2014) and Rey-Crespo *et al*. (2013), respectively.

Ni being a cofactor for a number of hormones and enzymes is considered as essential element for humans. However, the excessive intake may result in cell damage, impaired reproductive system, altered hormonal and enzymatic activities, oxidative stress and neurotoxicity (Nordberg *et al*., 2007; Doreswamy *et al*., 2004; Das *et al*., 2008). The mean Ni concentration found in the present study was 0.041 µg/g with a range of 0.009 – 0.083 µg/g (Table II). The upper intake level of Ni through dietary sources is 0.1-1 µg/g (Food and Nutrition Board, 2001). None of the milk samples was found to exceed this limit. Therefore, milk only is not able to breach the upper intake level for Ni in the people of sampling districts. The mean level of Ni in milk samples reported from Korea (Khan *et al*., 2014) and Nigeria (Ogabiela *et al*., 2011) were 0.153 and 2.631 µg/g, respectively, which are much higher as compared to our findings. The level of Ni reported in milk samples from France (Noël *et al*., 2012) and Spain (Rey-Crespo *et al*., 2013) is 0.055 and 0.026 µg/g, respectively; these findings are in complete agreement with our results.

Cu is an essential element for skin and blood vessel's strength, for the production of myelin and hemoglobin and for the proper functioning of enzyme systems (Harris, 2001; Osredkar and Susta, 2011). However, the excessive

Table II.- Estimated daily intake of heavy metals in Punjab Province.

Age group (Years)		No. of consumers	Mean weight (kg)	Milk intake (kg/day)	µg/kg/day				
					Pb	Cd	Co	Ni	Cu
1-3	Male	32	11.43	0.521	0.946	0.150	4.330	1.884	3.126
	Female	21	9.52	0.342	0.745	0.118	3.413	1.485	2.464
4-5	Male	24	20.21	0.587	0.603	0.095	2.759	1.201	1.992
	Female	22	16.24	0.321	0.410	0.065	1.878	0.817	1.356
6-9	Male	19	30.71	0.312	0.211	0.033	0.965	0.420	0.697
	Female	21	25.21	0.286	0.235	0.037	1.078	0.469	0.778
10-15	Male	26	48.76	0.242	0.103	0.016	0.471	0.205	0.340
	Female	24	41.85	0.204	0.101	0.016	0.463	0.201	0.334
16 >	Male	102	62.54	0.234	0.078	0.012	0.355	0.155	0.257
	Female	98	54.61	0.182	0.069	0.011	0.317	0.138	0.229

For abbreviations see Table I. Tolerable upper intake levels for Pb and Cd are 3.57 and 0.8-1.0 µg/kg/day, respectively while the upper intake levels for Cu, Ni and Cu are not established yet so they are compared with their normal reported ranges in milk.

intake of Cu may lead to immunity disorders, dermatitis, impaired nervous system, gastrointestinal and neurological problems (Storelli et al., 2007; Barn et al., 2014).The mean Cu concentration detected in present study was 0.068 µg/g and was in the range of 0.018 – 0.141 µg/g (Table I). The maximum limit for Cu in milk proposed by IDF (1979) is 0.01 µg/g. Comparing our results with this limit 100% milk samples were found to exceed the permissible limit. However, this limit seems to be outdated as the normal range for Cu in milk proposed by Puls (1994) is 0.1 – 0.9 µg/g, none of the milk samples was found to exceed this normal range.

The mean concentration of Cu found in present study is almost in line with the earlier reported from Spain (Sola-Larrañaga and Navarro-Blasco, 2009) and Kazakhstan (Konuspayeva *et al*., 2011). In Egypt, Malhat *et al*. (2012) reported 1.451 µg/g Cu in milk, while in Croatia the level of Cu in milk samples reported by Bilandzˇic´ *et al*. (2011) is 0.917 µg/g, these values are much higher as compared to our findings.

The data for EDI of various heavy metals is presented in Table II and was in the order of Co > Cu > Ni >Pb> Cd. Maximum EDI values were found for infants (1-3 year) while the least were recorded for the adults (>16 year), due to the highest and lowest intake levels of milk by infants and adults, respectively. The heavy metal found with highest daily intake was Co (4.33 µg/kg/day), while the least was found for Cd (0.011 µg/kg/day). The EDI values for Pb and Cd for adults reported from Egypt by Salah *et al*. (2013) are 64.4 and 158.5 µg/kg/day, respectively, which are several folds higher as compared to our findings. In Saudi Arabia, the EDI values of Pb, Cd and Cu through milk were calculated for adults and were found as 0.3-0.4, 0.4-0.6 and 4.4-5.9 µg/day (Farid *et al*., 2004). The EDI values for Pb and Cu are almost in line with the present study while those for Cd are higher than our findings. The tolerable upper intake level for Pb and Cd are 3.57 and 0.8-1.0 µg/kg/day, respectively (Tripathi *et al*., 1999). Although, the EDI values for Pb and Cd calculated in the present study are lower than the upper limits but still they have the potential to result in serious problems as other dietary and non dietary factors also contribute in the calculation for total daily intake of heavy metals.

CONCLUSION

Increased urbanization and industrialization has resulted in elevated level of heavy metals in milk and milk products. Milk samples in present study were found to have Pb and Cd levels higher than the permissible limits, while the levels of Co, Ni and Cu were in normal or safe zones. The EDI values were also found in the normal ranges.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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