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Assessment of Toxic Metals in Dairy Milk and Animal Feed in Peshawar, Pakistan

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Authors' contributions

This work was carried out in collaboration between all authors. Author BI designed the study, conducted it and wrote the first draft of the manuscript. Author SS helped in laboratory work. Author SA helped in data analysis and writing final draft of manuscript. Author RK overviewed and corrected the final version. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To assess the concentration of toxic heavy metals [Cadmium, Copper, Lead and Nickle] in dairy milk and animal feed of urban and rural areas and correlate them.
Study Design: It was a cross-sectional comparative study.
Place and Duration of Study: The study was conducted on the dairy milk and animal feed in milk farms of Peshawar. The samples were collected during June – July of 2010.
Methodology: The study area was divided into two zones: urban area which was located in the centre of the city and rural area comprising of peri urban villages. Fifty milk samples were collected, 30 from urban and 20 from rural areas. Feed samples were also collected. Milk and feed samples were prepared by wet digestion method using HNO_3 and H_2O_2 as described by Association of Official Analytical Chemists (AOAC) of North America, 1990. This was followed by analysis on Atomic Absorption Spectrometer.
Results: The results showed that the mean concentration of toxic heavy metals under study namely Cadmium, Copper, Lead and Nickle were greater in dairy milk than the maximum residue limits (MRLs) of these elements as given by Joint Expert Committee on Food and Agriculture and World Health Organization. In comparison of urban and rural areas it was found that higher concentration of Cadmium, Copper, and Nickle was found

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in dairy milk samples from urban areas than rural areas but there was no significant difference in Lead concentration of the two areas. Their concentration in urban and rural samples were respectively Cadmium 0.69 and 0.037 with *P*-value <0.001, Copper 1.40 and 0.09 with *P*-value 0.001, Nickel 0.806 and 0.024 with *P*-value <0.001, Lead 2.243 and 2.082 with *P* -value 0.026. However in this study all these elements were within the permissible limits in animal feed except for copper in cotton khal which was 83.3mg kg^{-1} against the MRL of 10 mg kg^{-1} given by National Research Council, NRC, 2005. Also there was no significant difference in the concentration of these elements in feed of urban and rural areas. Correlation study between the concentration of these elements in milk and feed showed positive pearson's correlation coefficient between the two particularly for copper (0.451) in sugarcane khal and (0.341) in wheat grain, Nickle (0.342) in maize, and (0.30) in wheat bran and cotton khal.

Conclusion: The concentrations of toxic metals were higher than the maximum residue limits in milk but were within their normal limits in animal feed suggesting that milk is being contaminated by various other sources but not farm feed. Comparison between urban and rural areas showed higher concentration in urban milk samples as compared to rural except for lead probably because urban farms are located in polluted areas in the centre of the city. However no significant difference was found in animal feed of urban and rural areas because it is supplied from common source.

Keywords: Maximum residue limits; permissable limits; milk; animal feed; heavy metals.

1. INTRODUCTION

Increase in industrialization and urbanization accelerated the input of inorganic contaminants in air, water and soil. Inhalation of air borne pollutants, ingestion of contaminated dust, drinking contaminated water and eating food grown over contaminated soils may cause severe health risks among humans and animals. A significant portion of inorganic contaminants remain within body fat whereas other insignificant portion is excreted from the body [1]. Certain food items are a regular component of human diet and are essential for growth and development. Milk is one such food item, resultantly milk's composition has a direct bearing on human health. However, inorganic and organic contaminants such as heavy metals, pesticides, hormones, dioxins and antibiotics are unlikely to be removed through heating whereas they are considered to be more toxic than biological contaminants when present at high concentration. Heavy metals are known to cause neurotoxicity, nephrotoxicity, fetotoxicity and teratogenicity in humans. May cause disturbance in the blood and cardiovascular system, changes in the detoxification pathways (colon, kidney, liver, skin), release and functioning of various hormone, disturbances in gastrointestinal, reproductive and nervous systems. Consequently changes in the behaviour of mental and neurological functions, alteration in neurotransmitter production and utilization are commonly observed among humans directly exposed to heavy metals contamination [2].

Dairy farming system here is either mixed farming system or urban and peri-urban dairy farming. Mixed farming is common in rural areas where small number of cattle (up to 10 in numbers) are kept in herds (around 57% are kept in this way) [3]. Cattle are fed on fodder, crop residue, weeds and agro industrial waste products after harvesting. It's a resource poor system. In urban and peri-urban dairy farming cattle are kept in small herds located either in the densely populated towns, near highways or industrial processing units and are fed on agro industrial by- products such as cotton khal, sugarcane khal, maize khal or wheat grains available commercially. Around 32 to 47% are kept in this way [4].

Heavy metals gain entry into the milk through cattle fodder and drinking water (besides air) which in turn are contaminated through soil, sewage sludge used as organic fertilizer, artificial fertilizers, metals used in fungicides and other agricultural chemicals, wastewater from industries used for agriculture, interactions between various metallic structures used in industries making edible items and the food stuff [1]. Food chain transfer of heavy metals takes place near smelting or mining areas, agricultural land, pastures grown over soils irrigated with waste water drainage channels contaminated with industrial, domestic, municipal or other wastes. Cai found that the concentration of Cadmium, Copper and Zinc was greater than their normal content in cattle milk and muscle farmed near Copper and Cadmium smelter [5].

Excessive cadmium exposure leads to liver, renal and bone damage, effects on testis, immune system and cardio vascular system. Cadmium II also causes cancers and mutagenesis [6,7]. Excessive amounts of copper in human body can result in copper deposits in liver and brain with resultant damage [8]. Excess of lead in body effects oxygen supply by inhibition of haemoglobin synthesis, adversely effects bone-marrow, liver, kidneys and male gonads, may lead to gastrointestinal effects, encephalopathy, peripheral neuropathy, chronic irreversible nephropathy, aggressive behaviour, wrist drop. International Agency for Reseach on Cancer (IARC) has placed lead in Group 2B “possible human carcinogen” [9]. Thus these toxic elements are hazardous to human health after prolonged exposure even in small quantities because of bioaccumulation and biomagnification[1]. The objectives of the study were to assess the levels of toxic heavy metals (cadmium, copper, lead and nickle) in dairy milk and animal feed with respect to the maximum residue limits of these elements as given by Joint Expert Committee on Food and Agriculture (JECFA) and World Health Organization (WHO) for any possible harmful effects on humans. The other was to determine the correlation between the concentration of toxic metals in the dairy milk and animal feed and also to compare their levels in urban and rural areas.

2. MATERIALS AND METHODS

It was a cross-sectional comparative study conducted in June – July 2010. The study area was divided into two zones: zone A (urban area) included three areas, area 1, area 2, area 3 which were located in the centre of the city and hence were exposed to pollution (industrial, vehicular etc) while zone B (rural area) included two areas: area 4 & area 5 which were away from pollution. Ten samples were selected from each area thus making a total of fifty (50) samples. The samples were collected by convenience method as some dairy farm owners were not co-operative. About 100mL sample of fresh cattle milk in triplicate was collected. The milk samples were placed in refrigerator for further analysis. The fodder samples were collected from each of the area from which milk samples were collected, since in each area all the farms were being supplied by a single supplier so a total of five fodder samples were analyzed.

Milk samples were prepared by wet digestion method followed by analysis by Atomic Absorption Spectrometry, as described by several researchrs [10] 1mL of raw + 10mL of 1 M HNO_3 and 3mL of H_2O_2, heated at 200ºC for 15 to 20 minutes for dryness. The white ash was re-diluted with 10mL of 1 M HNO_3 and was filtered through Whatmann No 42 and was placed in 20mL glass bottles. Feed samples were prepared by wet digestion method as described by AOAC (1990) [11]. 2g of feed + 10mL M HNO_3 for 24 hrs, heated at 200ºC for

15 - 20 min, cooled 5 to 10 minutes, + 10mL of aqua regea (HNO_3: HCl, 3:1), re-heated to ensure dryness, finally cooled for 5 to 10 minutes & diluted with 20mL of distilled water, filtered through Whatmann filter paper No. 42 and stored in plastic bottles. The fodder extract was analyzed for heavy metals by Atomic Absorption Spectrometer. Each standard solution was measured 3 times and the mean was plotted. A blank solution of distilled water was used to check accuracy of the standard solutions and it was run after every 10 samples. The metal content was calculated using the formula:-

Concentration (mg L^{-1} or ppm) =

$$\frac{\text{Concentration of the element through AAS (ppm) x sample volume}}{\text{ample weight}}$$

Chemicals and reagents: All were analytical grade of Merck-Germany.

Precision and quality control: A whole standard reference powder milk sample (NIST SRM 8435) was used to compare the content of heavy metals in milk and feed samples of this study. Analytical precision is defined as relative standard deviation (RSD) in percentage and is obtained using a formula: standard deviation/mean of sample. Accuracy was estimated through certified standard values. To ensure the quality of results triplicate sub samples were analyzed and blank and standard samples were also run for calibration of atomic absorption spectrometer. Standard curve with linear regression and better relative standard deviations that can be employed to determine heavy metals content was calibrated. Periodic calibration of standards after 10 samples was performed in order to evaluate the accuracy, calibration and reliability of atomic absorption spectrometer. The accuracy was examined through quality control test in which standard reference measurement was compared with the sample measurement and the atomic absorption spectrometer reading was used unless the recovery of elements in the sample was ± 15 at 15%, which is the lower limit for determination and standard deviation of triplicate samples less than 15% [12].

Statistical analysis was done by using Statistical Package for Social Sciences version 16. During analysis mean concentrations of the toxic metals in milk were determined and comparison between the urban and rural areas was done using t-test, taking a p-value of less than 0.05 as significant. Descriptive statistics were calculated for the concentrations of toxic metals in milk and fodder. Pearson's correlation coefficient was calculated to determine the correlation between the concentrations of elements in milk and animal fodder.

The research protocol was approved by the review board of the University of Peshawar and funded by them as well. The data was analysed by the stastistician of Khyber Medical College.

3. RESULTS

The results showed that the mean concentration of toxic heavy metals under study namely Cadmium, Copper, Lead and Nickle were greater in dairy milk than the maximum residue limits of these elements as given by Joint Expert Committee on Food and Agriculture [13] and World Health Organization [Table 1]. However in this study all these elements were within the permissible limits in animal feed except for copper in cotton khal which was 83.3 mg kg-1 against the MRL of 10 mg kg^{-1} given by National Research Council, NRC, 2005 [14] [Graph 1]. Correlation study between the concentration of these elements in milk and feed showed positive correlation between the two [Table 2]. When comparison was done between urban and rural areas it was found that higher concentration of Cadmium, Copper and Nickel were found in dairy milk samples from urban areas as compared to rural areas but there was no significant difference in lead concentration of the two areas Table 3. Also there was no significant difference in the concentration of these elements in animal feed of urban and rural areas [Table 4].

Table 1. Mean concentration of toxic heavy metals (mg L^{-1}) in dairy milk and their maximum residue limits [MRLs]

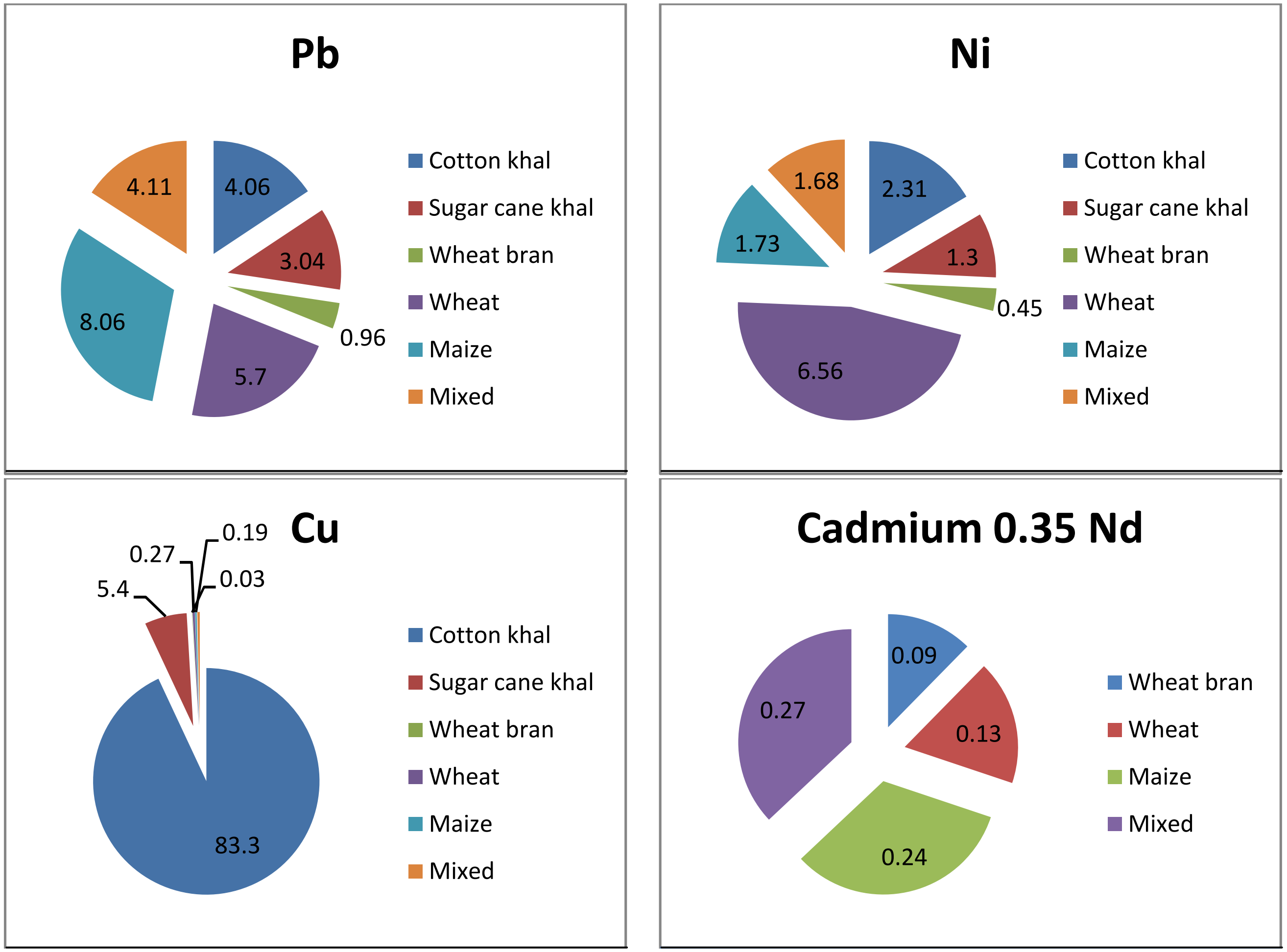
Elements	Mean urban	Mean rural	Milk MRL mgL^{-1}(JECFA 1989)
Cd	0.69	0.037	0.057- 0.071
Cu	1.40	0.09	0.01
Ni	0.806	0.024	0.01
Pb	2.243	2.082	0.025

Table 2. Correlation between toxic heavy metal content of dairy milk and animal feed

Cadmium	Pearson's R	*P* value
Mixed feed	0.136	0.34
Maize	0.134	0.35
Wheat grain	0.012	0.94
Wheat bran	0.173	0.23
Sugarcane khal	0.163	0.26
Cotton khal	0.093	0.52
Copper		
Mixed feed	0.030	0.84
Maize	0.014	0.92
Wheat grain	0.341	0.012
Wheat bran	0.269	0.06
Sugarcane khal	0.451	0.01
Cotton khal	0.134	0.35
Lead		
Mixed feed	0.260	0.06
Maize	0.153	0.28
Wheat grain	0.002	0.98
Wheat bran	0.023	0.87
Sugarcane khal	0.083	0.56
Cotton khal	0.179	0.21
Nickel		
Mixed feed	0.099	0.49
Maize	0.342	0.01
Wheat grain	0.054	0.71
Wheat bran	0.289	0.04
Sugarcane khal	0.092	0.52
Cotton khal	0.272	0.05

Table 3. Comparison between mean concentration of toxic heavy metals (mg L^{-1}) in dairy milk of urban and rural areas

		Areas (frequency)			
		Urban (n=30)	**Rural (n=20)**	**t-values**	***P*-values**
Metals in milk	Cd	0.69±0.071	0.037±0.013	3.53	<0.001
	Cu	1.40±0.009	0.09±0.004	2.16	0.001
	Ni	0.806±0.006	0.024±0.005	5.73	<0.001
	Pb	2.243±0.283	2.082±0.167	1.36	0.026



Graph 1. Mean concentration of Toxic Heavy Metals (mg L-1) in various animal feed

Table 4. Comparison between mean concentration (mg kg^{-1}) of toxic heavy metals in animal feed of urban and rural areas

		Areas (frequency)			
		Urban (n=30)	**Rural (n=20)**	**t-values**	***P*-values**
Cotton khal	Cd	0.39±0.113	0.033±0.015	1.53	0.09
	Cu	83.40±0.583	83.09±0.693	1.16	0.09
	Ni	2.806±0.024	2.024±0.014	1.73	< 0.001
	Pb	2.243±0.027	2.082±0.028	1.38	< 0.001
		Urban (n=30)	**Rural (n=20)**	**t-values**	***P*-values**
Sugar cane khal	Cd	0.39 ±0.253	0.033±0.016	1.60	<0.001
	Cu	5.40±0.117	5.09 ±0.098	1.08	0.13
	Ni	1.806±0.045	1.024±0.061	1.59	0.17
	Pb	3.243±0.113	3.082±0.094	1.40	< 0.001
		Urban (30)	**Rural (20)**	**t-values**	***P*-values**
Wheat bran	Cd	0.09±0.245	0.07±0.015	1.53	0.08
	Cu	0.03±0.239	0.06±0.014	1.14	0.17
	Ni	0.46±0.067	0.24±0.239	1.82	0.08
	Pb	0.92+0.091	0.082±0.013	1.23	0.15
		Urban (30)	**Rural (20)**	**t-values**	***P*-values**
Wheat in fodder	Cd	0.14±0.226	0.17±0.217	1.64	0.09
	Cu	0.27±0.211	0.29±0.224	1.27	0.11
	Ni	6.56±0.072	6.46±0.071	1.44	0.27
	Pb	5.43±0.078	5.82±0.045	1.37	0.25
		Areas (f)			
		Urban (30)	**Rural (20)**	**t-values**	***P*-values**
Maize in fodder	Cd	0.24±0.219	0.37±0.224	1.53	0.13
	Cu	0.19±0.202	0.23±0.253	1.16	0.13
	Ni	1.83±0.022	1.24±0.052	1.73	0.12
	Pb	8.06±0.046	8.82±0.022	1.65	0.16
		Urban (30)	**Rural (20)**	**t-values**	***P*-values**
Mixed fodder	Cd	0.27±0.214	0.037±0.016	1.47	0.19
	Cu	0.34±0.214	0.09±0.018	1.16	0.24
	Ni	1.68±0.018	0.024±0.016	1.60	0.31
	Pb	4.11± 0.048	4.52±0.192	1.11	0.21

4. DISCUSSION

Cadmium, Copper, and Nickel levels in this study were 0.69, 1.40 and 0.806 in urban samples, which is significantly greater ($p<0.05$) than in rural samples wherein the concentrations were 0.037, 0.09 and 0.024. However there was no significant difference in the concentration of Lead. These levels were also higher than the maximum permissible levels for Cadmium, Copper, Nickel and Lead in milk which are 0.057, 0.01, 0.01 and 0.025 respectively [13]. The difference in the concentration of toxic elements in milk samples of urban than rural areas was most likely because urban farms were exposed to various sources of pollution either directly or indirectly thence effecting the milk supplies. Those areas from which samples with high concentration of toxic elements were found, were either close to main roads with heavy vehicular traffic, resultingly pollutants settling on pastures and effecting milk. In most of the areas the infrastructure of the farms was poorly developed so that clean drinking water was not available and water from a nearby drainage channel was made available in the farms. These drainage channels contained various toxic elements from domestic, municipal, agricultural and industrial waste water. Moreover in these farms some of the feed was provided to the cattle on farms while most of the time the cattle were grazed outside in peri-urban fields which are also irrigated with waste water drainage channels. In this way toxic elements inadvertantly entered into milk supplies even if their special diet does not contain high concentrations of heavy metals.

The results of this study were in agreement with the findings of several research studies conducted world over e.g Simsek and colleagues [15] conducted a study in 2000 in which he found that Copper in cattle milk farmed near the pharmaceutical industrial unit was 0.96 mg L^{-1} and was 0.58mg L^{-1} in the dairy cattle milk farm in rural areas. This was probably because of dumping of untreated industrial effluents to the soil and water. Debashis [16] reported in 2009 that lead content in milk samples collected from farms near industrial processing units was greater than that of milk samples of rural farms. Swarup [17] reported that Lead content was significantly greater ($p<0.05$) in urban milk samples than rural milk samples. Dwivedi [18] reported that Lead and Cadmium contamination in milk samples collected from farms near Lead Zinc smelter was greater than milk samples collected from farms away. Jan and collegues [19] noted in 2011 that the mean content of Chromium, Copper, Nickel and Lead was two folds greater in the milk of dairy cattle drinking wastewater than drinking clean water. Chromium and Nickel were high because of discharge of industrial wastewater into rivers and irrigation channels whereas elevated levels of Copper and Lead in dairy cattle milk was most probably because of corrosion of water carrying pipelines and exhausts from heavy traffics.

Licata [20] studied the concentrations of toxic elements in dairy milk of Italy in 2004. He found that the content of Arsenic, Cadmium, Chromium, Copper and Lead was 37.90, 0.02, 2.03, 1.98 and 1.32µg kg^{-1} and concluded that greater content of Arsenic and Lead in milk was more likely because of drinking contaminated water. Awan [21] reported that the concentration of Lead varied between 0.04 - 0.019 mg kg^{-1} and between 0.081- 0.143mg kg^{-1} in raw and pasteurized milk samples of dairy cattle which was greater than WHO maximum residue limits of Lead (0.025 mg kg^{-1}) probably because of eating Lead contaminated diet. Anastasio et al. [22] have also reported elevated levels Cadmium and Lead in milk in Italy.

It is generally believed that the level of essential nutrients and toxic metals in milk primarily depends on their content in the feed. Animal feed with elevated levels of these elements causes an increase in their level in milk also. Cattle feed induced toxicity of some of the heavy metals in milk has been reported world over. Khattak [23] reported that the average content of Cadmium and Copper was 4.1 and 122mgL^{-1} in cow milk because cow's feed was prepared from the forages irrigated with industrial and municipal wastewater. Raj [24] reported that grasses used as a fodder for cattle are found to be high in Cadmium and Lead than their recommended levels for animal feed and this resulted in high levels in milk. Moreover toxic metals are known to produce long term toxic effects on human body because they are stored in adipose tissue of body and show bioaccumulation and biomagnifications [1]. Therefore their presence in milk even in small amounts may be detrimental in the long run.

The concentration of Cadmium, Copper, Nickel and Lead in animal fodder in this study were ("0.35, 83.3, 2.31, 4.06 in cotton khal") ("not detected, 5.4, 1.30, 3.04 in sugarcane khal"), ("0.09, 0.03, 0.45, 0.96 in wheat bran"), ("0.13, 0.27, 6.56, 5.7 in wheat wholegrain"), ("0.24, 0.19, 1.73, 8.06 in maize"), ("0.27, 0.34, 1.68, 4.11 in mixed feed"). From this it appears that the concentration of toxic elements in animal feed of this study were mostly within the range given by NRC, 2005 wherein the maximum residue limit for Cadmium, Copper, Nickel and Lead are 0.5, 10, 50 and 30 mg kg^{-1} for animal feed except for Cadmium in cotton khal which is 83.3 mg kg^{-1}. This suggests that the source of contamination of these elements in milk was not feed alone, but some other factors, as mentioned above were also responsible for this. Most important is the drinking water of cattle from waste water drainage channels containing industrial and sewage waste which is also supported by various local studies. In a study conducted in Peshawar on heavy metal concentration in canal water and sewage water it appeared that Lead was high in 67% sewage water samples and 45% canal water samples whereas Cadmium was high in 33% sewage water and 13% canal water samples [25]. In another study on drinking water it was found that Nickle was 0.097 to 0.250 mg /l against permissable value of 0.02 and Cadmium was 0.019 to 0.087mg /l against permissable value of 0.003 [26]. Average values of heavy metals in sewage water used for irrigation of vegetables in Peshawar were found to be (mg L^{-1}): Copper 1.03, Nickle 0.61, Lead 0.45 (32% above safe level), Cadmium 0.27 (100% above safe level) [27]. Heavy metals in the dust samples showed that Cd and Cr are added into environment with a rate of 0.08 and 0.52mg m^{-2} $month^{-1}$, respectively at 50 m distance from brick kiln chimney [28].

5. CONCLUSIONS

The concentrations of toxic metals in milk were higher than the maximum permissible limits of these metals in milk as given by WHO / JECFA and moreover the concentrations in milk samples of urban areas were greater than in the milk samples of rural areas since urban areas are more exposed to pollution. However the content of these elements in animal feed was within the permissible limits as given by Joint Expert Committee on Food and Agriculture (JECFA) and World Health Organization (WHO) and moreover it was not significantly different in the animal feed of the two areas because feed is being supplied by common sources to all farms. This suggests that milk contains toxic elements but they are probably not coming from water and feed taken on the farm, instead there are other sources of their entry in animal body like grazing in open fields and drinking water from waste water drainage channels.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reilly C. Metal Contamination of Food. The Metals We Consume. 2nd Ed. Elsevier Science Publishers Limited. 1991;1-20.
2. Hu XY, He JZ, Gao YZ. Soil remediation in mining and control to acid water. Protect Measure Soil Environment. 2002;3:135-140.
3. Sarwar M, Khan MA, Mahr-un-Nisa, Iqbal Z. Dairy industry in Pakistan. A Scenario. International Journal of Agriculture and Biology. 2002;3:420-428.
4. Livestock Census. Agriculture Census Organization, Statistic Division, Govt of Pakistan, Gulberg, Lahore; 1996.

5. Cai O, Long ML, Zhu M, Zhou QZ, Zhang L and Liu J. Food chain transfer of cadmium and lead to cattle in a lead–zinc smelter in Guizhou, China. Environmental Pollution. 2009;157(11):3078-3082.
6. Browning E. Toxicity of Industrial Metals. London: Butterworths; 1969.
7. U.S. Environmental Protection Agency. Updated mutagenicity and carcinogenicity assessment of cadmium: Addendum to the health assessment document for cadmium. Report No. EPA/600/8-83/025F, Office of Health and Environmental Assessment, office of Research and Development, Washington, DC; 1985.
8. Karpel JT, Peden WH. Copper deficiency in long term parenteral nutrition. Pediatrics. 1972;80:32-36.
9. World Health Organization. Environmental Lead Criteria 3: Lead (WHO Geneva); 1976.
10. Qin LQ, Wang XP, Li W, Tong X, Tong WJ. The minerals and heavy metals in cow's milk from China and Japan. Health Sciences. 2009;55(2):300-305.
11. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC, Association of Official Analytical Chemists; 1990.
12. Siddiqui S. Spatial distribution of trace and major elements in the hilly soils of Lesser Himalayan Muzaffarabad-Pakistan. Soil Science. 2011;176(7):372-383.
13. JECFA: 33. Report: 1989. Evaluation of certain food additives and contaminants. WHO Technical Report Series 776. Genf. T6 `10JECFA:33.Report: 1993. Evaluation of certain food additives and contaminants; 1989. WHO Technical Report Series 837.
14. National Research Council. Committee on Minerals and Toxic Substances in Diets and Water for Animals, National Research Council (US) Subcommittee on Mineral toxicity in animals. Mineral tolerance of animals. 2nd ed. National Academic Press Washington DC. 2005;496.
15. Simsek O, Gultekin R, Oksuz O, Kurultay S. The effect of environmental Pollution on the heavy metal content of raw milk. Food Nahrung. 2000;4(5):360-363.
16. Debashis R, Bharathidhasan S, Veena M, Harjit K and Neelam K. Heavy Metal Contents in Cow and Buffalo Milk Samples from Haryana. Indian Journal of Animal Nutrition. 2009;26(1):29-33.
17. Swarup RC, Patra R, Naresh P, Kumar P and Shekhar P. Blood lead levels in lactating cows reared around polluted localities, transfer of lead into milk. Science of the Total Environment. 2005;347:106-110.
18. Dwivedi S K, Swarup D, Dey S, Patra RC. Lead poisoning in cattle and Buffalo near primary lead-zinc smelter in India. Veterinary Human Toxicology. 2001;43:93-94.
19. Jan FA, Ishaq M, Khan S, Shakirullah M, Asim SM and Ahmad I. A comparative study of bioaccumulation of some essential and toxic metals in human's blood through different food chains in industrially contaminated area and relatively less polluted area. Environmental Sciences. 2011;23(10):89-94.
20. Licata P, Trombetta D, Cristani M, Giofre F, Martino D, Calo M and Niccari F. Levels of toxic and essential metals in samples of bovine milk from various dairy farms in Calabria Italy. Environmental International. 2004;30(1):1-6.
21. Awan HN, Tahir HA and Rafique U. Determination of zinc and lead in raw and processed milk. Rawal Medical Journal. 2005;30(2):76-78.
22. Anastasio A, Caggiano R, Macchiato M, Cattellani P, Ragosta M, Paino et al. Heavy Metal Concentration in Dairy products from sheep milk collected in two regions of Southern Italy. Acta Veterinaria Scandinavica. 2006;4(7):69-74.
23. Khattak RA, Haq MU, Puno HK, Saif MS. Forages induced dairy metal contamination. Journal of Chemical Society of Pakistan. 2004;26(3):279-284.

24. Raj BG, Patnaik MC, Babu SP, Kalakumar B, Singh MV, Shylaja J. Heavy metal contaminants in water-soil-plant-animal contionuum due to pollution of Musi river around Hyderabad in India. Indian Journal of Animal Sciences. 2006;76:131-133.
25. Mussarat M, Bhatti A, Khan F. Concentration of metals in sewage and canal Water used for irrigation in Peshawar. Sarhad Journal of Agriculture. 2007;23:2.
26. Khan SA, Din Z, Ihsanullah & Zubair A. Levels of selected heavy metals in drinking water of Peshawar city. International Journal of Science and Nature. 2011;2(3):648-652.
27. Perveen S, ihsanullah I, Shah Z, Nazif W, Shah SS, Shah H. Study on Accumulation of Heavy Metal in Vegetables Receiving Sewage Water. J. Chem. Soc. Pak. 2011;33(2):220.
28. Ismail M, Muhammad D, Khan FU, Munsif F, Ahmad T, Ali S, Khalid M, Haq NU, Ahmad M. Effect of brick kiln's emissions on heavy metal (CD and CR) content of contiguous soil and plants. Sarhad J. Agric. 2012;28(3):403-409.

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