MILK: Carrier of Heavy Metals from Crops through Ruminant Body to Human Beings

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Summary: Exposure of heavy metals to humans is higher today than ever before in modern history due to continuously increasing industrialization around the globe. Industrial wastes are rich in heavy metals and these wastes are discharged near agricultural fields or mixed with soil, from where these metals are taken up by the crops and are finally transported to humans. Due to this increasing threat of heavy metals contamination in food, it is necessary to analyze the food before consumption. Content of selected metals (Cd, Cr, Cu, Pb and Zn) in cow milk is determined in this study. To investigate the possible pathways of these metals to reach in milk; fodder supplied to these cows was analyzed besides analysis of soil samples on which this fodder was grown. Pearson correlation among metal contents in soil-forage and forage-milk was also determined to check the route of transfer of these metals from soil to forage and from forage to milk. It was found that a strong correlation ($p < 0.5$) exists for Cr, Cd, Cu and Zn. This shows that these metals are mainly transferred through soil. However, a weak correlation was found for Pb, which shows that Pb is introduced into forage through some other source (automobile exhaust etc.). A comparison of present study is also done with previously reported work from other countries on metal contents in milk and findings of both the studies were in good agreement mutually.

Key Words: Heavy metals, Milk, Forage, Soil, Pearson correlation, Atomic Absorption Spectrometer.

Introduction

Since environmental pollution is increasing day by day, it has become necessary to study exposure of heavy metals [1]. Heavy metals present in soil, sediments and water accumulate in plants and are finally transferred to the bodies of grazing animals [2]. Soil contains essential as well as non-essential elements in varying range [3]. Concentration of these elements in soil is dependent upon many factors including nature of soil, fertilizers, fungicides, insecticides [4, 5] and composition of irrigation water [6]. Metal profile of soil gets changed with the change in these factors. Commonly found metals in soil include, Al, Fe, Mn, Cu, Cr, Cd, Zn, Se, Ni, Ag, Pb and Hg. These metal contents are taken up from the soil by the crops grown on it [7], and finally enter in food chain of animals [8]. As food is the major source of heavy metals accumulation, so human beings are quite conscious about concentration of metals in food items. They prefer food rich in nutrition and safe for health i.e. having metal concentration within safe limit as prescribed by WHO. Milk is a nutritionally complete natural food. Cow milk is particularly considered good for health [9], as it is rich source of vitamins, proteins, carbohydrates and minerals [10]. Some of these elements (Cr, Cu, Ni and Zn) are considered essential but toxic at higher level [11], while other elements including Cd, Pb, Hg and As are very toxic even at trace level [12]. These metals can cause several diseases, particularly in infants because children are more susceptible to effects of these metals [13]. For example, lead is reported to be causing anemia, hypertension and renal disorder; cadmium is responsible of nausea, vomiting and renal failure; while arsenic causes skin problems, prostate and lung cancer, diseases of respiratory and gastrointestinal tract. Mercury is the source of nausea, bloody diarrhea, attacks nervous system and is carcinogenic for animals and humans [14]. So it is necessary to analyze the level of these metals in milk and to suggest possible pathway; they follow to reach from soil to cow milk. Main source of heavy metals in organisms like cow is either through respiratory system or food [15]. Forage supplied to the animals is considered major contributor of mineral contents. Forage plants accumulate these contents from soil and water used for irrigation purposes. Cow, when graze on this fodder, collects these elements in its body. So finally these elements reach the blood [16] and accumulate in milk. Because mammary glands of cow are very active part of its body [17], so milk of cow can be used to determine the transfer of these elements from soil to milk and finally to human beings. These animals can also be used as potential

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indicators of environmental contaminants due to their proximity with human beings [18]. Metal analysis of milk samples is done in Pakistan [19-21] but its correlation with soil and forage samples are yet not reported, as per our knowledge. So aim of this work is to determine the metal contents in milk, forage and soil samples as well as to study their transfer factor from soil to forage and finally to milk.

Experimental

Sample Collection

Samples of raw cow milk were collected from 13 different farms located in different regions of Sargodha, Pakistan. Milking was done during morning time and samples were collected in polyethylene bottles properly washed with deionized water to avoid any contamination. Milk samples were stored at -20°C before analysis. Forage supplied to these cows was also collected from all the farms and stored in plastic bags. Soil samples, from fields of forage grown, were obtained from the depth of 0-30 cm and stored in bags. Forage and soil samples were collected from 10 different places for each cow farm.

Analytical Procedure

Individual milk sample (10 ml) was digested in microwave (2-5 min) using 5 ml of ultrapure nitric acid (65%) and 1ml of hydrogen peroxide (35%). Volume was made up to 20 ml with deionized water. Soil samples were dried at 60° C in an oven for 48 h and then digested using HNO_3 and H_2O_2. Forage samples were washed properly with distilled water and then with deionized water to remove soil or any other surface contaminants. It was cut into small size pieces and after drying, acid digestion was performed by process as described above [22].

Reagents and Glassware

All the reagents used were of Analytical grade and procured from Merck (Darmstadt, Germany) or Sigma Aldrich (Milwaukee, WI, USA). For preparation of standard solutions of Cd, Cr, Cu, Pb and Zn, ultrapure deionized water was used to avoid any contamination. For sample digestion, spectroscopy grade HNO_3 and H_2O_2 were used. All the glassware was properly washed with ultrapure water.

Instrumentation

Metal analysis was performed on Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Specifications used during analysis are given in Table-1.

Table-1: Optimized set of conditions for analysis of milk, soil and forage samples on Graphite Furnace Atomic Absorption Spectrometer (Model AA-6300, Schimadzu Japan)

Parameters	Cd	Cr	Cu	Pb	Zn
Wave length (nm)	228.8	357.9	324.8	283.3	213.9
Lamp current (mA)	8	10	6	10	8
Slit width (nm)	0.7	0.7	0.7	0.7	0.7
Dry Temperature (°C)	150	150	150	150	150
Liquefaction Temperature (°C)	250	250	250	250	250
Ashing Temperature (°C)	500	800	800	800	450
Atomization Temperature (°C)	2200	2300	2300	2400	1800
Cleaning Temperature (°C)	2400	2500	2500	2500	2400

Background correction (BGC)-D_2 Lamp, Sample volume = 20 µL,
Type of Graphite Furnace Used = Plate Type

Results and Discussion

Metal contents found in soil, forage and milk samples (Mean values and standard deviation) are given in Table-2-6 respectively. Metals selected for the analysis include Cd, Cr, Cu, Pb and Zn and their level in soil samples were found in the range of 0.41-0.59, 1.21-1.41, 6.8-7.9, 1.18-1.46 and 30.0-35.5 µg/ g, respectively. Concentrations of all the metals was found within the normal range except for Cd, which was found higher in samples collected from 3 dairy farms. These dairy farms were located near industrial areas. So this higher concentration of Cd may be attributed to the discharge of untreated industrial effluent into the fields nearby agricultural plots. Cd is recently reported to be the most important contaminant discharged by the industries in modern time [23]. Forage samples were also analyzed and amount of Cd, Cr, Cu, Pb and Zn was found to be in the range of 0.22-0.55, 1.1-1.37, 6.5-7.3, 1.16-1.46 and 30.1-36.1 µg/g, respectively.

Table-2: Amount (µg/g) of heavy metals (Cd, Cr, Cu, Pb and Zn) in soil samples used to grow forage in various cow farms of Sargodha region.

Dairy Farms	Cd	Cr	Cu	Pb	Zn
01	0.5±0.1	1.34±0.11	7.1±3.2	1.35±1.01	33.1±4.3
02	0.57±0.22	1.34±0.19	7.5±1.8	1.29±1.13	33.5±5.5
03	0.49±0.31	1.37±0.17	6.9±2.3	1.4±1.1	36.7±7.5
04	0.54±0.31	1.36±1.01	7.2±1.2	1.32±0.17	34.0±2.5
05	0.59±0.22	1.41±0.15	7.6±2.1	1.2±0.6	35.5±4.6
06	0.41±0.21	1.37±0.75	7.3±1.4	1.19±0.35	33.9±7.1
07	0.29±0.17	1.21±1.10	6.7±3.6	1.16±1.01	35.9±4.5
08	0.56±0.21	1.39±0.51	7.5±1.5	1.45±0.17	32.0±3.9
09	0.49±0.22	1.25±1.03	7.1±2.2	1.46±1.13	31.1±4.5
10	0.53±0.41	1.31±1.20	7.3±3.5	1.36±1.11	32.5±3.5
11	0.58±0.21	1.36±1.14	7.7±2.5	1.24±1.20	36.1±6.8
12	0.46±0.13	1.38±0.16	7.0±0.1	1.18±0.28	33.6±7.1
13	0.54±0.14	1.33±0.17	7.2±3.7	1.36±1.12	30.3±6.3

*Ten milk samples were taken from each dairy farm, and each sample was individually analyzed and data is reported as Mean (n=10) ± Standard Deviation (n=10).

Table-3: Amount (μg/g) of heavy metals (Cd, Cr, Cu, Pb and Zn) in forage samples used as cow feed in various cow farms of Sargodha region.

Dairy Farms	Cd	Cr	Cu	Pb	Zn
01	0.41±0.21	1.17±0.75	6.6±1.4	1.19±0.35	32.9±7.1
02	0.53±0.41	1.31±1.02	7.3±3.5	1.36±1.11	30.5±3.5
03	0.22±0.20	1.26±1.40	6.7±2.5	1.24±1.20	36.1±6.8
04	0.42±0.21	1.17±0.50	6.5±1.5	1.45±0.71	32.0±3.9
05	0.54±0.31	1.36±1.10	7.2±1.2	1.32±0.37	30.0±2.5
06	0.37±0.22	1.31±0.91	7.0±1.8	1.29±1.03	33.5±5.5
07	0.25±0.02	1.1±0.5	7.0±2.1	1.2±0.6	35.5±4.6
08	0.54±0.14	1.33±0.17	7.2±3.7	1.36±1.02	30.3±6.3
09	0.47±0.22	1.25±1.03	7.1±2.2	1.46±1.03	31.1±4.5
10	0.46±0.13	1.28±0.16	6.8±0.1	1.18±0.80	31.6±7.1
11	0.55±0.12	1.34±0.11	7.1±3.2	1.35±1.01	30.1±4.3
12	0.49±0.31	1.37±0.17	6.9±2.3	1.4±1.1	31.7±7.5
13	0.29±0.17	1.21±1.10	6.8±3.6	1.16±1.10	34.1±4.5

*Ten milk samples were taken from each dairy farm, and each sample was individually analyzed and data is reported as Mean (n=10) ± Standard Deviation (n=10).

Table-4: Amount (μg/l) of heavy metals (Cd, Cr, Cu, Pb and Zn) in raw cow milk from various cow farms of Sargodha region

Dairy Farms	No. of animals	Cd	Cr	Cu	Pb	Zn
01	10	0.11±0.10	1.20±0.10	33.5±11.1	2.5±1.1	3177±130.5
02	10	0.18±0.02	1.9±1.1	43.1±5.3	1.9±0.5	3035±110.5
03	12	0.10±0.01	1.3±0.9	37.5±3.9	2.2±1.2	3150±105
04	15	0.12±0.11	1.6±0.9	37.5±1.2	1.3±1.1	3105±112
05	15	0.19±0.15	2.0±1.5	44.5±10.5	2.7±1.3	3005±150.5
06	13	0.11±0.10	1.0±0.5	35.0±6.6	2.6±1.7	3160±112.7
07	10	0.09±0.07	1.25±1.02	33.0±6.9	2.0±1.2	3180±145.6
08	11	0.2±0.1	1.7±0.9	38.5±5.5	1.95±0.70	2950±147.6
09	15	0.17±0.12	1.75±1.03	40.5±9.5	2.6±1.9	3037±145
10	13	0.13±0.11	1.3±0.7	41.0±10.5	1.6±1.2	3095±135
11	13	0.21±0.2	1.86±1.4	45.7±9.5	2.4±1.3	2945±136
12	15	0.17±0.12	1.8±0.7	44.6±0.1	1.8±1.2	3051±160
13	15	0.09±0.04	1.35±0.7	35.5±4.8	1.8±1.1	3165±155

*Ten milk samples were taken from each dairy farm, and each sample was individually analyzed and data is reported as Mean (n=10) ± Standard Deviation (n=10).

Table-5: Pearson correlation between metal contents in soil and forage samples.

		Metal contents in forage				
		Cd	Cr	Cu	Pb	Zn
Metal contents in soil	Cd	0.631861	0.520671	0.140144	0.266285	-0.68496
	Cr	0.393343	0.677836	0.012066	0.200579	-0.35935
	Cu	0.773275	0.630589	0.541075	0.255429	-0.80192
	Pb	-0.00192	-0.10867	-0.16794	0.060467	-0.05877
	Zn	-0.15725	0.045299	0.011406	-0.02287	0.219535

Table-6: Pearson correlation between metal contents in forages and milk samples.

		Metal contents in milk				
		Cd	Cr	Cu	Pb	Zn
Metal contents in forage	Cd	0.708767	0.750875	0.754243	0.10428	-0.85666
	Cr	0.750103	0.554537	0.792842	0.247808	-0.71205
	Cu	0.693179	0.551647	0.493198	0.39904	-0.64803
	Pb	0.6308	0.673538	0.520092	-0.01345	-0.61915
	Zn	-0.89439	-0.77311	-0.74438	-0.08018	0.860045

Pearson correlation among the metal contents in soil-forage and forage-milk was also determined to check the route of transfer of these metals from soil to forage and from forage to milk. It was found that a strong correlation ($p < 0.5$) exists for Cr, Cd, Cu and Zn. This shows that these metals are mainly transferred through soil. However, a weak (0.060467) correlation was found for Pb, which shows that Pb is introduced into forage through some other source (automobile exhaust etc.).

Milk samples, collected from these dairy farms, were analyzed for the same metals. Concentration range for these metals was found to be 0.009-0.2, 1.0-2.0, 33.0-45.7, 1.3-2.7 and 2945-3180 μg/ l for Cd, Cr, Cu, Pb and Zn respectively. Amount of all these metals was found within the safe limit as recommended by EPA Pakistan. Pearson correlation between forage and milk samples was also determined. Strong correlation was found for all metal contents except Pb, which exhibited negative correlation (-0.01345).This negative correlation shows that Pb is not transferred through this route. Pb is mainly transferred to the animals through air with automobiles exhaust along roadsides, because it is used as antiknocking agent in petrol. Results show that when amount of Cd increases in certain samples, Zn level falls down in those samples. This may be due to the fact that both of these elements have similar properties. So when Cd level rises, it replaces Zn in substrate samples [24].

So results show that concentration of metals in milk of grazing animals, fed on locally produced fodder, reflects the level of metals in soil, on which this fodder is grown. These results are also supported by previous studies [25] as shown in Table-7, which reflects heavy metal contents determined from dairy products of different countries.

Table-7: Comparison of heavy metals in cow milk sample (μg/l) from different countries [26-31].

Country	Cd	Cr	Cu	Pb	Zn	Reference
India	0.07	-----	43.2	1.70	3177	Tripathi et al.1999
Turkey	1.7	-----	-----	10.3	-----	Ayar et al. 2009
Germany	0.1	-----	40.3	1.8	3730	Ostapczuk et al.1987.
Italy	0.47	2.7	67.0	20.0	4801	Liacata et al. 2004
Croatia Iran	3.02 9.51	-----	38.0	2.30 1.93	5100	Pavlovic et al. 2004 Rahimi 2013
Pakistan	0.143846	1.539231	39.22308	2.103846	3081.154	Present Study

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