TOXICOLOGICAL IMPACT ASSESSMENT OF HEAVY METALS IN HUMAN BLOOD AND MILK SAMPLES COLLECTED IN DISTRICT SHANGLA, PAKISTAN.

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ABSTRACT: Toxic heavy metals were estimated in blood and milk samples of humans. For this purpose, twenty three points were selected and total 230 samples of blood and 134 samples of human's milk were collected from Shangla, Pakistan. Samples were analyzed for heavy metals such as lead, cadmium, chromium, nickel, cobalt, zinc, copper and iron using atomic absorption spectrophotometry. High amount of heavy metals was found in blood of Adult's while low was found in blood of children's. Adult's blood accumulated high amount of heavy metals as compared to blood of children. Interestingly, mean values of lead were found to be higher than cobalt, nickel chromium and cadmium. Similarly, milk samples collected from lactating females at early morning have high amount of lead, cadmium, cobalt, nickel and copper as compared to that of milk samples collected after two hours. Therefore, this study has a strong message for the lactating mothers to drop out initial milk in the early morning and then feed their infants.

Key words: Heavy metals, human milk, blood, lactating Females.

1. INTRODUCTION

All living organisms including human beings depend on their environment for their resources and survival [1]. Human exploitation of their resources may cause numerous problems in the environment. However, environmental pollution is increasing continuously because of rapid industrialization [2]. Industrialization is the major cause of soil, water and air pollution. Industrial wastes contain several types of toxic compounds including heavy metals [3]. The effects of heavy metals(HM) on humans and other living organisms are continuously increasing from the last of few decades [4]. High concentration of HM are toxic which may cause serious diseases including several types of cancers in human [5].

Importance of iron can`t be over emphasize, however high amount of Fe may cause Alzheimer's and Parkinson's diseases [6]. Copper performs very important role in growth while overexposure of Cu may cause hepatic copper (II) overload disease, tissue injury, irritation of lungs and hepatic cancer [7]. High amount of Zn may cause rectum cancer which is the major cause of death in USA [8]. Chromium may cause dizziness, abdominal pain, hepatotoxicity, neurotoxicity, cardiotoxicity, hyperemia, vomiting and ulceration [9]. Inhaling air containing high concentration of Ni may cause lungs diseases and irritate mucosal layer of circulatory system [10]. Overexposure of cobalt may cause DNA fragmentation, activation of caspases and production of high reactive oxygen species such as hydrogen peroxide [11]. Cadmium (Cd) and lead (Pb) along with their compounds are included in black list [12]. Cadmium severely irritate stomach leading to vomiting, diarrhea and may cause death [13]. Like cadmium, lead is also non-essential element and cause several types of diseases such as Alzheimer`s, renal, nervous, reproductive, endocrinal, behavioral development and decreases intelligence quotient (IQ) level of children [14].

2. MATERIALS AND METHOD

2.1. Study area description

Sampling was conducted in district Shangla, Pakistan, located at a latitude of 34^0 53′ 12″ N and longitudes 72^0 36′ 00″ E, with an attitude of 3000 meter. Total area of district Shangla is 1586 km^2 and has population more than 0.5 million. Springs, rivers including rain water are the main sources for agricultural consumption. Shangla has been dominated by peaky and evergreen mountains; therefore, weather may increases the mineral contents of soil and water. The weather is fantastic in summer, temperature may rise to 32.5 ºC and fall to –2.4 ºC in winter with observed relative humidity of 65.9% [15]. Study area has been shown in Fig 1.

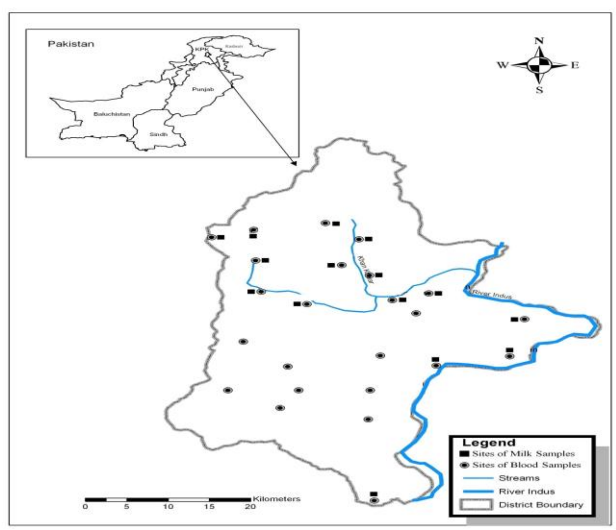

Fig. 1: Map of study area and selected sites.

2.2. Collection of samples and pretreatment

Blood samples were collected from twenty three points in the capacity of 5 mL from venous blood using disposable syringes with a 21 G needle. Three drops of EDTA were added to stop blood coagulation. Humans milk samples were collected from lactating females by manual suckling at morning and then after 2 hours with the help of Lady Health Worker. The lactating females provided 10 mL of milk directly into clean polyethylene plastic bottles. All the samples were frozen immediately after collection and stored at 4 ºC until completion of analysis.

2.3. Reagents

Chemicals of analytical grade with high purity of 99.9% (Merck Darmstadt, Germany) were used for analysis. Standard solutions of eight metals were prepared by dilution of 1000 ppm certified standard solutions (Fluka Kamica Busch Switzerland) of the respective metal.

2.3. Analysis of samples

Samples were analyzed for heavy metals using atomic absorption spectrophotometer (Perkin Elmer AAS-700). To ensure precision and accuracy, each sample was analyzed in triplicate and the instrument was continuously calibrated during analysis. The analysis of HM was performed in the Centralized Resource Laboratory, University of Peshawar, Pakistan. The instrument conditions for the respective HM are given in Table1.

Table 1. Instrumental analytical conditions for analysis of selected heavy metals.

Element	Fuel flow (L/minute)	Air flow (L/minute)	Wavelength (nm)	Lamp current (mA)	Detection limits (mg/L)
Fe	2.0	17.0	248.3	30	0.0150
Zn	2.0	17.0	213.9	15	0.0015
Cu	2.0	17.0	324.8	15	0.0015
Cr	2.0	17.0	357.9	25	0.0030
Ni	2.0	17.0	232.0	25	0.0060
Co	2.0	17.0	240.7	30	0.0090
Cd	2.0	17.0	228.8	4.0	0.0008
Pb	2.0	17.0	283.3	10	0.0150

2.4. Statistical analysis

Microsoft® office excel 2010 was used for descriptive calculations while SPSS (statistical package for the social sciences) software Version®–16 was used to determine Cluster Analysis and Correlation in order to assess the significant difference among each point source of HM in blood and milk samples.

3. RESULTS AND DISCUSSION

3.1. Heavy metals in blood and milk samples

The statistical summary of HM in blood and milk samples has shown in Table 2 and 3, respectively. Results indicate that the trend of HM in blood samples is Fe □ Zn □ Cu □ Pb □ Cr □ Ni □ Co □ Cd. The mean values of HM in milk samples collected in early morning were 63.26, 11.91, 3.51, 1.87, 1.76, 0.05, 0.03 and 0.02 ug/L for Fe, Cu, Pb, Cr, Zn, Ni, Cd and Co, respectively. Mean concentrations of HM in milk samples collected at noon were 64.83, 11.34, 2.66, 2.34, 2.05, 0.06, 0.04 and 0.03 ug/L, for Fe, Cu, Cr, Pb, Zn, Ni, Cd and Co, respectively. The statistical treatment indicates that on average, the blood of adults accumulate high amount of HM as compared to blood of children as shown in Fig. 2. High concentrations of Pb, Cd, Co, Ni and Cu were observed in milk samples collected early morning while that of Zn, Cr and Fe were found less in samples collected after 2 hours as shown in Fig. 3.

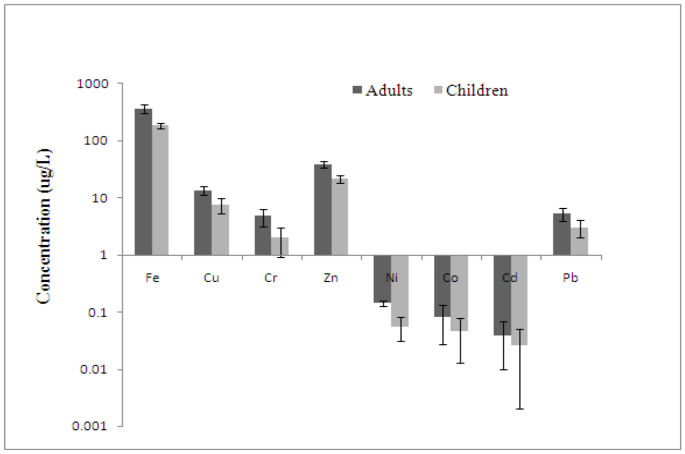

Fig. 2: Mean concentration of heavy metals in blood samples.

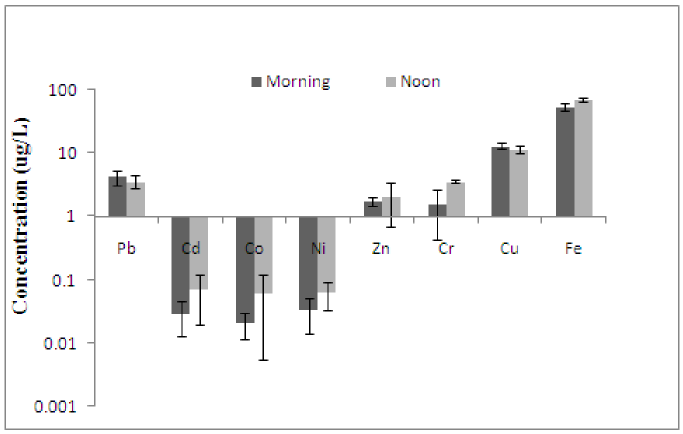

Fig. 3: Concentration of toxic heavy metals in milk samples.

3.2. Statistical techniques

Cluster analysis (CA) divided the blood samples into four groups as presented in Fig. 4, similarly CA of complete linkage method was applied to milk samples and were divided into five groups on the basis of the concentration of heavy metals as shown in Fig. 5. Correlation provides valuable information of HM sources and pathways. The correlation co-efficient of heavy metals in blood samples showed that some of heavy metals indicate strong correlation like Pb-Ni (r = – 0.410), Cd–Cu (r = –0.551) and Ni–Zn (r = – 0.432). Milk samples indicated a strong correlation between zinc and chromium i.e. Zn–Cr (r = – 0.510).

Table 2: Distribution of selected heavy metals in blood samples (n^b=230)

	Blood (Children)					**Blood (Adults)**				
Elements	Min:	Max:	Mean	Median	SD^b	Min:	Max:	Mean	Median	SD^b
Fe	145.608	225.749	188.209	189.205	21.046	286.491	420.206	371.856	388.253	41.215
Cu	3.072	11.288	7.683	7.906	2.320	6.538	16.592	13.527	13.905	2.340
Cr	0.356	3.851	1.993	1.734	1.059	0.793	7.829	4.816	4.694	1.604
Zn	15.618	27.093	21.644	22.107	3.286	25.904	47.492	39.533	39.863	5.402
Ni	0.006	0.305	0.056	0.035	0.077	0.022	0.887	0.143	0.064	0.201
Co	0.005	0.173	0.046	0.042	0.033	0.041	0.456	0.081	0.062	0.083
Cd	0.006	0.106	0.026	0.017	0.024	0.011	0.152	0.039	0.037	0.029
Pb	0.926	4.751	3.089	3.109	1.067	3.007	7.805	5.299	5.264	1.404

All values are in ug/L; [a] Number of blood samples; [b] Standard deviation)

Table 3: Statistical analysis of heavy metals in milk samples (n^a = 134)

	Morning					**Noon**				
Elements	Max	Mini	Mean	Median	SD^b	Max	Mini	Mean	Median	SD
Pb	6.035	< BDL	3.511	4.058	2.030	5.783	<BDL^c	2.341	2.025	1.798
Cd	0.062	< BDL	0.028	0.027	0.019	0.139	< BDL	0.037	0.025	0.036
Co	0.024	< BDL	0.022	0.018	0.023	0.086	< BDL	0.027	0.015	0.034
Ni	0.104	0.008	0.047	0.048	0.026	0.142	0.006	0.056	0.057	0.035
Zn	3.581	0.948	1.762	1.637	0.617	4.826	< BDL	2.052	2.104	1.447
Cr	2.993	< BDL	1.867	1.984	0.901	3.805	0.905	2.661	2.644	0.763
Cu	8.572	15.724	11.909	12.064	2.188	14.167	8.573	11.337	11.59	1.653
Fe	76.285	46.206	63.264	65.803	9.849	74.504	46.283	64.829	67.785	9.998

All values are in ug/L; [a] Number of milk samples; [b] Standard deviation; [c] Below detection level

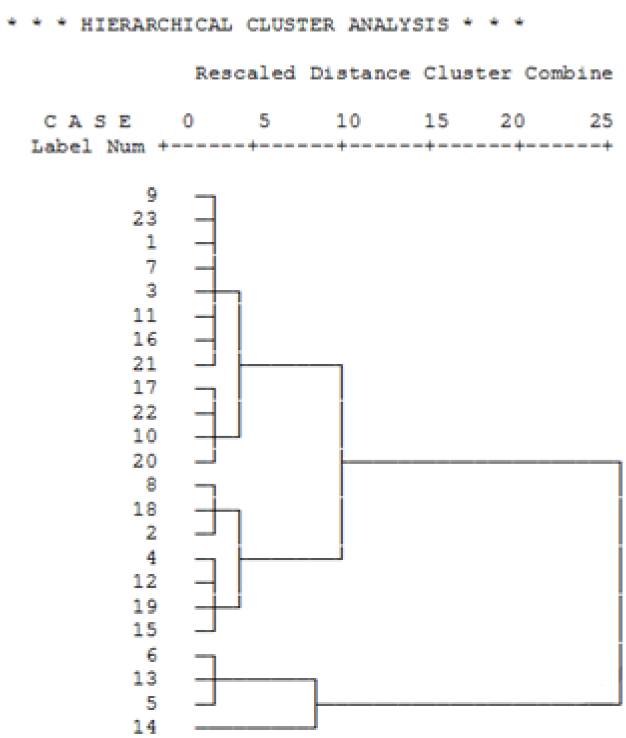

Fig. 4: Dendrogram for the classification of blood samples.

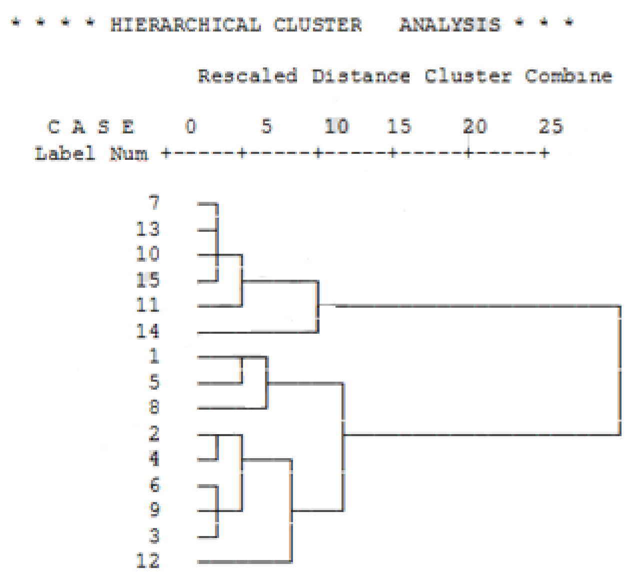

Fig. 5: Dendrogram for the classification of milk samples.

4. CONCLUSION

The study was carried out to estimate the concentrations of toxic heavy metals in blood and humans milk samples. Samples were collected from twenty three points and analyzed for selected heavy metals in the Centralize Resource Laboratory (CRL), University of Peshawar, and Pakistan. The results show that blood of adults has greater amount of heavy metals as compare to the children. Human's milk collected in early morning has greater amount of Pb, Cd, Co, Ni and Cu and less amount of Zn, Cr and Fe. Therefore, this study shows a strong message to the lactating mothers to drop out initial milk in early morning and then feed their infants.

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