



Evaluation of Heavy Metals in Blood Samples of Both Males and Females of Different Age Groups from Urban Areas of Faisalabad

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The role of minerals is very important in carrying out vital activities of human body but due to paradoxical properties of heavy metals their presence in elevated quantities may cause toxicity. Present study has been carried out to evaluate the concentration of lead, copper, cadmium and chromium in blood samples of both males and females of different age groups from urban areas of Faisalabad by using atomic absorption spectrophotometer. The statistical analysis of present study revealed that concentration of heavy and trace metals in both males and females is independent of age and sex except linear progression in blood lead and cadmium levels with age was observed in males ($P < 0.05$). It is concluded from the this research that the overall high concentrations of heavy and trace metals were observed in all the subjects of urban areas indicating high concentrations of these metals in the environment.

Key Words: Blood evaluation, Toxicity, Lead, Copper, Cadmium, Chromium, Atomic absorption spectrophotometer, Linear progression.

INTRODUCTION

The role of minerals in human body is very important. As mineral balance is required to maintain the normal body functions and disturbance of mineral levels in human body may cause health problems. One of the reasons for this may be due to exposure to toxic metals through a variety of routes. Heavy and trace metals released from industrial operations are carried by air and water and enter into food chain. Being non bio-degradable, passing through food chain, accumulates in different organs of the human body¹. The amount absorbed from the digestive tract depends on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it is distributed in tissues and organs². Over 80 % of worldwide diseases are due to improperly sanitized food and water. The heavy metals are harmful substances that cause severe threats to the environment of modern industrialized countries³. The greatest public health concern is linked with exposure to lead, cadmium, mercury and arsenic⁴. Lead poisoning may be considered a public health problem since its environmental sources are diverse and widespread. Lead poisoning in humans causes several damages in kidneys, brain, reproductive system, central nervous system and sometime cause death⁵. Toxic effects of lead on the kidneys are thought to be responsible for the development of hypertension and its

complications⁶. The selenium concentration in human body decreases after exposure to lead, which may lead to an increased rate of free radical production⁷. The toxic effects of cadmium become detectable only after it exceeds the threshold level of 50-70 g/day⁸. Cigarette smoking is a major source of cadmium exposure. It has been shown that cigarette smoking may cause an increase in blood cadmium levels⁹. The adverse physiological effects of cadmium commonly include depressed growth rate, anaemia, kidney dysfunction due to damage to renal tubules, atherosclerosis and poor mineralization of bones¹⁰. High levels may be considered to be associated with hypertension¹¹. Cadmium and several cadmium containing compounds are known as carcinogenic and can induce many types of cancers¹². Chromium, an essential nutrient has many natural sources including foods. Chromium is essentially required to promote the action of insulin in body tissues. It is also a glucose tolerance factor and its imbalance is related to heart diseases¹³. Chromium is of significant importance in altering the immune response. The reduction of Cr(VI) to Cr(III) results in the formation of reactive intermediates. These intermediaries along with oxidative stress result in oxidative tissue damage. The intermediaries and oxidative stress contributes to the cytotoxicity, genotoxicity and carcinogenicity¹⁴. High levels of chromium during pregnancy may result in the birth of low weight infants and gestational diabetes¹⁵. Copper, a trace element, is essential

for both humans as well as animals. However, due to contaminated water supplies, cigarette smoking and the use of oral contraceptives, most of people may suffer from a low-level copper toxicity which may result in hypertension, heart disease, pre-menstrual syndrome, postpartum depression, schizophrenia and childhood hyperactivity¹⁶. It is reported that if 2-3 mg /L of copper is present in drinking water, it may cause hepatotoxicity in infants¹³.

Toxic metal and micronutrients may interact in the body for example during absorption and excretion of toxic metals, their transport in the body, binding to target proteins, metabolism and sequestration of toxic metals and finally in secondary mechanisms of toxicity such as oxidative stress. Therefore people which are deficient in micronutrients may predispose to toxicity from nonessential metals¹⁷.

EXPERIMENTAL

To assess the levels of heavy metals (Pb, Cd, Cr and Cu) in blood of human population, 100 blood samples (each about 5 mL) of both males and females were collected from urban areas of Faisalabad in properly labeled disposable syringes with the method of Jacob¹⁸. Trained medical staff was engaged for the collection of blood samples. Blood donors were screened for any sort of disease like hypertension, diabetes, anemia and arthritis and diseased persons and smokers were excluded from this study. All the reference standards used in this study were purchased from Perkin Elmer. Reference standards of 0.5-1.5 ppm conc. were used to obtain calibration curves. Samples preparation for analysis of metal ions was performed using wet digestion method. About 05 mL of blood sample was taken in test tube with equal volume of 69 % nitric acid and 70 % perchloric acid. The tubes were kept at room temperature for over night to let the gases evolve then kept at 40-60 °C to evaporate acid fumes. The temperature of the tubes was further raised to 100-110 °C until 0.3-0.5 mL solution was left. When digestion was completed, clear final volume was made upto 10 mL with distilled deionized water¹⁹. The digested samples were analyzed at least in triplicate. Analysis of heavy metals like lead, cadmium, chromium and copper was performed with atomic absorption spectrophotometer (AAS) Perkin-Elmer Analyst-800. Data obtained after analysis of metal ions was subjected to mean, standard deviation and correlation values. Significance was determined by analysis of variance ANOVA²⁰.

RESULTS AND DISCUSSION

Present study was carried out to estimate the level of heavy and trace metals in human blood samples. The concentration of heavy and trace metals in the various blood samples is shown in Tables 1 and 2 and comparison between males and females has been given by line graphs. The studies were conducted for various age groups of both male and female (15-22, 23-30, 31-38, 39-46, 47-54, 55-62, 63-70 and 71-78 years). Range, mean and standard deviation were determined for each group. Difference of group means were evaluated for significance by 't-test'. The expected 't' values were obtained from the t-distribution tables for 95 % confidence interval ($P = 0.05$)^{21, 22}.

TABLE-1
CONCENTRATION OF LEAD AND CADMIUM
($\mu\text{g/mL}$) IN BLOOD SAMPLES OF MALE AND
FEMALE GROUPS OF VARIOUS AGES.

Age Group		Lead		Cadmium	
		Male	Female	Male	Female
15-22 years	Mean	1.78	1.49	1.68	1.35
	Standard deviation	1.03	1.59	0.72	0.71
23-30 years	Mean	2.85	2.61	2.37	2.17
	Standard deviation	0.75	0.81	0.75	0.72
31-38 years	Mean	3.00	2.73	2.50	2.97
	Standard deviation	0.58	0.81	0.67	1.05
39-46 years	Mean	3.19	2.77	2.84	2.88
	Standard deviation	0.36	1.21	0.58	1.33
47-54 years	Mean	3.18	2.61	3.10	2.74
	Standard deviation	0.56	0.57	0.43	1.16
55-62 years	Mean	3.73	3.01	3.06	2.88
	Standard deviation	0.50	1.38	0.64	1.08
63-70 years	Mean	4.30	3.12	4.00	3.40
	Standard deviation	0.70	1.48	0.26	0.78
71-78 years	Mean	4.30	4.00	3.26	3.03
	Standard deviation	0.26	1.01	0.56	0.86

The results presented in Table-1 list lead and cadmium conc. ($\mu\text{g/mL}$) in blood of males and females. The mean values for all the male subjects are 2.907 and 2.642 while for females it is 2.642 and 2.57 respectively. The minimum value of lead was observed in the youngest group of 15-22 years *i.e.*, 0.01 $\mu\text{g/mL}$ both in males and females and values of cadmium conc. were 1.68 $\mu\text{g/mL}$ for males and 1.35 $\mu\text{g/mL}$ in females. Highest mean value of lead and cadmium was observed in the age group between the ages of 63-78 years that was 4.3 and 4 $\mu\text{g/mL}$ for males and 4 and 3.4 $\mu\text{g/mL}$ for females.

TABLE-2
CONCENTRATION OF CHROMIUM AND COPPER
($\mu\text{g/mL}$) IN BLOOD SAMPLES OF MALE AND
FEMALE GROUPS OF VARIOUS AGE.

Age Group		Chromium		Copper	
		Male	Female	Male	Female
15-22 years	Mean	3.03	2.56	2.67	2.72
	Standard deviation	0.81	0.91	1.28	0.53
23-30 years	Mean	2.62	2.75	3.96	4.47
	Standard deviation	0.90	1.04	2.40	1.48
31-38 years	Mean	3.03	3.17	2.76	4.08
	Standard deviation	1.28	1.33	0.49	1.26
39-46 years	Mean	3.43	2.62	3.86	3.44
	Standard deviation	0.90	1.68	1.17	1.10
47-54 years	Mean	2.67	3.06	3.82	3.72
	Standard deviation	1.02	0.70	0.44	0.76
55-62 years	Mean	3.13	2.63	3.23	3.26
	Standard deviation	0.66	0.85	0.45	1.23
63-70 years	Mean	3.05	2.61	3.40	2.73
	Standard deviation	0.18	0.77	0.72	1.46
71-78 years	Mean	4.06	4.30	4.45	4.12
	Standard deviation	0.73	0.55	1.00	1.51

The results presented in Table-2 list chromium and copper conc. ($\mu\text{g/mL}$) in blood of males and females. The mean values of chromium and copper for all the males is 3.05 and 3.432 while for females it is 2.889 and 3.615 respectively. The minimum mean value of chromium was 2.62 $\mu\text{g/mL}$ and value of copper was 2.67 $\mu\text{g/mL}$ for the ages of 15-22 years in male volunteers.

While value of chromium and copper in the age group of 15-22 was 2.56 and 2.72 µg/mL in female volunteers respectively. Highest mean value of chromium and copper between the ages of 71-78 years was 4.06 and 4.45 µg/mL for male volunteers respectively. While for female volunteers value of chromium between the ages of 71-78 years was 4.3 µg/mL and value of copper between the ages of 23-30 years was 4.475 µg/mL.

TABLE-3
ANALYSIS OF SIGNIFICANCE FOR ASSESSING LEVELS OF HEAVY METALS (Pb, Cd, Cr, Cu) IN BLOOD OF DIFFERENT AGE GROUPS OF MALES AND FEMALES

Heavy metals		Lead	Cadmium	Chromium	Copper
Male population	F	12.733	6.441	1.054	1.484
	Sig.	0.000	0.000	0.409	0.199
Female population	F	1.960	2.821	1.001	1.965
	Sig.	0.084	0.017	0.444	0.083

In Table-3 means of different age groups of male and female volunteers were compared for all the heavy metals (Pb, Cd, Cr and Cu). $P > 0.05$ clearly indicates that blood levels of all these heavy metals are independent of age in female subjects. While in males Pb and Cd had $P < 0.05$ indicating that Pb and Cd levels are related with age while non-significant values $P > 0.05$ for Cr and Cu showed that their concentrations are independent of age.

As the subjects were divided into eight groups according to their age and presented by respective alphabets as shown below: Age groups 15-22 years (A), 23-30 years (B), 31-38 years (C), 39-46 years (D), 47-54 years (E), 55-62 years (F), 63-70 years (G) and 71-78 years (H). The results obtained have been presented by line graphs.

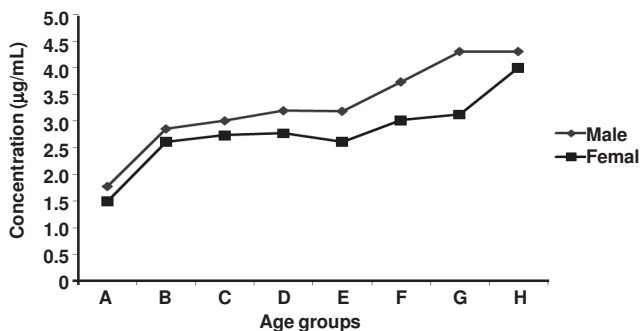


Fig. 1. Concentration of lead (mg/mL) in blood samples of males and females

Results showed that blood lead levels of subjects were significantly higher. The highest concentration of lead (3.7-5.1 µg/mL) was observed in blood samples of male subjects within age group of 63-70 years while among blood samples of female subjects highest concentration (3.1-5.1 µg/mL) was observed in age group of 71-78 years. Statistical analysis showed positive correlation between blood lead levels and age in males ($P < 0.05$) and independent of age in females ($P > 0.05$). However blood lead levels are independent of sex ($P > 0.05$).

The highest concentration of cadmium (3.7-4.2 µg/mL) was observed in blood samples of male volunteers within age group of 63-70 years while among blood samples of female

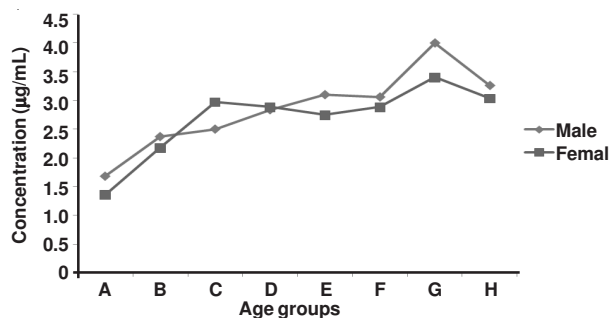


Fig. 2. Concentration of Cadmium (mg/mL) in blood samples of males and females:

volunteers highest concentration (0.3-4.5 µg/mL) was observed in age group of 39-46 years. Statistical analysis showed positive correlation between blood cadmium levels and age in males ($P < 0.05$) and independent of age in females ($P > 0.05$). However blood cadmium levels are independent of sex ($P > 0.05$).

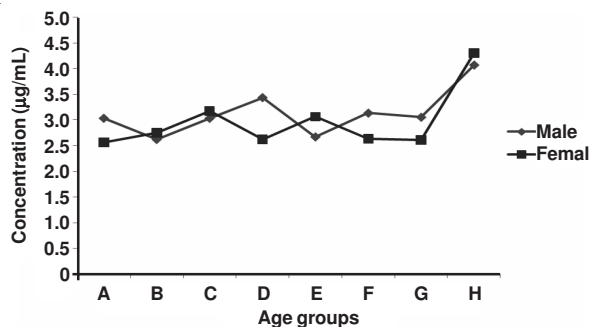


Fig. 3. Concentration of chromium (mg/mL) in blood samples of males and females.

The highest concentration of chromium (3.5-4.9 µg/mL) was observed in blood samples of males within age group of 71-78 years while among blood samples of females highest concentration (1.2-5.1 µg/mL) was observed in age group of 31-38 years. However statistical analysis showed that blood chromium levels are independent of age and sex ($P > 0.05$).

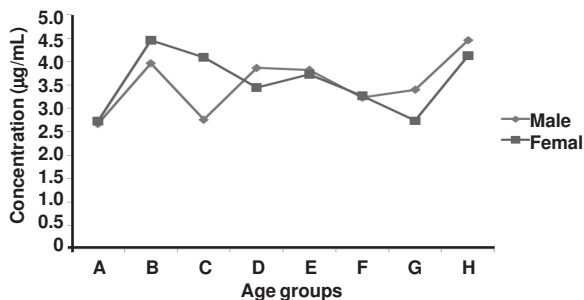


Fig. 4. Concentration of copper (µg/mL) in blood samples of males and females.

The highest concentration of copper (1.5-9.3 µg/mL) was observed in blood samples of male subjects within age group of 23-30 years while among blood samples of female subjects highest concentration (1.8-7.2 µg/mL) was observed in age group of 23-30 years. However statistical analysis showed that blood copper levels are independent of age and sex ($P > 0.05$).

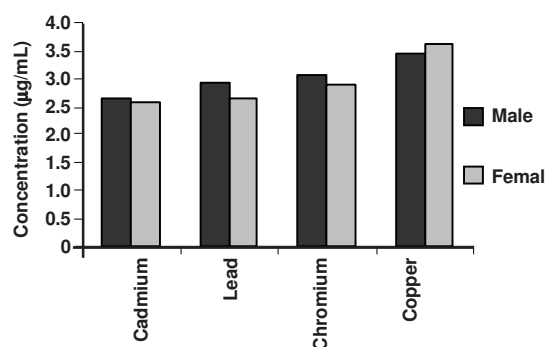


Fig. 5. Overall level of metals (lead, cadmium, chromium and copper) in blood samples of male and female subjects from urban areas of Faisalabad.

Above mentioned graph shows that among all the blood samples of male subjects the mean value of copper was highest (3.432 µg/mL) followed by chromium (3.05 µg/mL), lead (2.907 µg/mL) and cadmium (2.642 µg/mL) and among all the blood samples of female subjects, the same pattern of decreasing concentrations of metals was observed.

The statistical analysis of present study revealed that concentration of heavy and trace metals in both male and female subjects is independent of age and sex. However, linear progression in blood lead and cadmium levels with age was observed in males ($P < 0.05$). Decreased concentration of lead in blood was observed in females (2.642 µg/mL) than in males (2.907 µg/mL) (Tables 1 and 2). This observation is in accordance with the results of Sartor and Rondita (1980) who have reported that the blood lead increased with age at an almost constant rate for the residents of polluted areas²³.

Lead is a highly toxic element since the excretion rate of lead from the human body is slow and it tends to deposit finally in the brain. Consequently it is important to prevent lead spreading and detect its traces before its effects become serious²⁴.

A much less difference in concentration of copper in the blood of both male and female subjects was observed. It complies with the results of Wasowicz *et al.*²⁵ who indicated that Cu and Zn contents are not influenced by sex.

Similarly, less difference in concentration of chromium was observed for males and females in present study. Normal range of chromium is 1-5 mcg/L in human beings¹³. While excessive levels of chromium that are usually limited to industrial settings and dietary chromium intake may lead to serious health problems in the general population²⁶.

Deficiency or excess of a trace element may cause disorders in the absorption, distribution, metabolism and elimination of other trace elements. For example iron deficiency can increase absorption of lead and cadmium²⁷.

The main intake sources of essential and non-essential elements in the human body are air, water and food. Therefore considerable attention has been directed towards assessing the accumulation and understanding of toxic effects of certain trace elements in human body. So in order to reduce the concentration of metals in blood, hair and other body tissues the level of metals in environment must be reduced. Otherwise the flow of metals into the body through food chain will continue and

the levels of non-essential metals will increase and finally reach to the toxic level.

Recommendations

- Public awareness programmes should be held frequently to disseminate the information about toxic effects rendered by heavy metals and suggesting the ways to reduce them.
- Toxic metal exposure usually remains unrecognized, so it is important to monitor the level of heavy metals at least once in a year in all age groups of both sexes.
- Lead content in the environment can be reduced by using fuels of high quality, lead free petrol, proper maintenance of vehicles and encouraging mass transit.
- All the edibles should be properly anatomized to curb the presence of any content of toxic metals.

REFERENCES

1. G. Wu, H. Kang, X. Zhang, H. Shao, L. Chu and C. Ruan, *J. Hazard. Mater.*, **174**, 8 (2010).
2. H. Howard, in ed: M. McCally, Life support: The Environment and Human Health, The MIT Press, Cambridge, USA, p. 65 (2002).
3. E. Callender, Heavy Metals in the Environment—Historical Trends, Treatise on Geochemistry, Chapter 9.03, pp. 67-105 (2007).
4. L. Jarup, C.G. Elinder, B. Dahlberg and B. Persson, *Br. Med. Bull.*, **68**, 167 (2003).
5. M.J. Ahmed and M.A. Mamun, *Talanta*, **55**, 43 (2001).
6. R. Sohaila, N. Khalid, J.H. Zaidi, S. Ahmad and M.Z. Iqbal, *J. Zhejiang Univ. Sci. B.*, **7**, 732 (2006).
7. C. Jin, Y. Li, Y.L. Li, Y. Zou, G.L. Zhang, M. Normura and G.Y. Zhu, *J. Nucl. Instrum. Methods Phys. Res. B: Beam Interact. Mater. Atoms*, **266**, 3607 (2008).
8. I.H. Bukhari, M.N. Hassan, A. Haleem and Maqbool M. Bhatti, *Res. J. Agric. Biol. Sci.*, **1**, 190 (2005).
9. L. Jarup, M. Berglund, C.G. Elinder, G. Nordberg and M. Vahter, *Scand. J. Work Environ. Health*, **24**, 1 (1998).
10. S. Soisungwan, J.R. Baker, S. Urbenjapol, M.H. Elkins, P.E.B. Reilly, D.J. Williams and M.R. Moore, *Toxicol. Lett.*, **137**, 65 (2003).
11. S.K. Bakshi, K.P. Chawla, R.N. Khandekar and R. Raghunath, *J. Assoc. Physic. (India)*, **42**, 449 (1994).
12. National Toxicology, 11th report on Carcinogens, Retrieved 21/04 (2008).
13. L. Marry, Basic Skills in Interpreting Laboratory Data, ASHP Inc., 7272 Wisconsin Avenue, Bethesda, edn. 4, pp. 142-147 (2009).
14. S. Richa, R.K. Upreti, P.K. Seth and U.C. Chaturvedi, *FEMS Immunol. Med. Microbiol.*, **34**, 1 (2002).
15. A. Aharoni, B. Tesler, Y. Palfi, J. Dori and M. Sharf, *Am. J. Clin. Nutr.*, **55**, 104 (1992).
16. E.T. Tuula, *J. Orthomol. Med.*, **10**, 149 (1995).
17. M.A. Peraza, F. Ayala, D.S. Barber, E. Casarez and L.T. Rael, *Environ. Health Perspect.*, **106**, 203 (1998).
18. R.A. Jacob, in ed: N.W. Tietz, Trace Elements, Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, edn. 15, p. 965 (1986).
19. I. Fatima, J.H. Zadi, M. Asif, S. Ahmad and I.H. Quershi, *Environ. Pollut.*, **24**, 225 (1997).
20. R.G. Steel and J.H. Torrie, Principles and Procedures of Statistics, McGraw Hill Book Co., Inc. New York. (1980).
21. J.C. Miller and J.N. Miller, Statistics for Analytical Chemistry, John Wiley & Sons, New York. (1984).
22. K.S. Subramanian, *Atom. Spectrosc.*, **8**, 7 (1987).
23. J.L. Annett, J.L. Pirkle, D. Mauc, J.W. Neese, D.D. Dayse and M.G. Kovar, *New Engl. J. Med.*, **308**, 1373 (1983).
24. K.A. Essa, *East. Mediter. Health J.*, **5**, 798 (1999).
25. W. Wasowicz, J. Gromadzinska and K. Ryzdzyński, *Int. J. Occup. Med. Environ. Health*, **14**, 223 (2001).
26. A.A. Richard, *Sci. Total Environ.*, **86**, 75 (2003).
27. J.N. Morrison and J. Quarterman, *Biol. Trace Elem. Res.*, **14**, 1115 (1987).