Bioaccumulation of metals in human blood in industrially contaminated area

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Abstract

Heavy metals were analyzed in different foods crops, milk, meat and blood samples collected from different age group subjects such as children (1–12 years), adolescent (12–18 years), adults (18–45 years) and old age (above 45 and 55 years for males and females, respectively) from polluted and relatively less polluted areas. The results revealed that the consumption of contaminated food crops, meat and milk have significantly increased the concentrations of selected metals in the human blood. Cu, Zn and Mn concentrations were significantly higher ($p < 0.05$) in the blood samples collected from the polluted area as compared to control area. Old people had accumulated high concentrations of metals as compared to the younger ones within the same area. Males accumulated higher concentrations of metals as compared to females.

Key words: bioaccumulation; heavy metals; blood; contaminated meat; milk; forage grass

Introduction

Wastewater irrigation is a common practice in most parts of the world due to economic advantages and the presence of nutrients (Charry et al., 2008; Jan et al., 2009). Long-term application of wastewater results in substantial build-up of heavy metals in the receiving soils. When the retention capacity of soil for heavy metals decreases due to repeated application of wastewater then, heavy metals leach into the groundwater and soil solution available for plant uptake (Sharma et al., 2008; Jan et al., 2010). Anthropogenic contamination of food crops with heavy metals poses a threat to its quality and safety. Concentrations of heavy metals in soil, atmospheric deposition, climatic conditions, nature of soil and the degree of maturity of plants at the time of harvest are the major factors that affect the uptake and bioaccumulation of heavy metals in vegetables (Khan et al., 2008a). Plant/soil ratios (0.1) for any particular element indicate that the plant is excluding the element from its tissues. Only a portion of source metal is uptaken by the root and then translocate to the leaves, giving a leaf/soil concentration ratio of about 0.2, although the concentration of the particular metals in the soil may be high. Transfer factors > 0.2 indicate that the contamination

of plants caused by anthropogenic activities. Soil pH, soil organic matter, catoin exchange capacity (CEC), and plant genotype can markedly affect metal uptake. The dietary intake of heavy metals through consumption of metal contaminated-food-crops can cause serious health hazards in animals and human beings (Tripathi et al., 2001). The level of metals in blood depends on the bioaccessiblity rate (Khan et al., 2008b) and is considered as an index of biologically active metals in the body reflecting the environmental exposure of a population. Concentration of metal in blood is a significant factor for the public health (Fergusson, 1990). Heavy metals are toxic when accumulate beyond the permissible levels and can cause profound biochemical changes in the body. Children are more sensitive to heavy metals and hence are at more risk than young and older ones. Although some metals, i.e., Cu, Zn and Fe are essential for human beings, chronic metabolic disturbances may results from excessive or deficiency of these metals. High concentration of Cu could induce growth proliferation and cancer, particularly, due to its ability to change between Cu(I) and Cu(II) oxidation states, whereby highly reactive oxygen species are generated, which produce hydroxyl radicals that adversely modify proteins, lipids and nucleic acids (Almeida et al., 2008; Taylor, 1996). Likewise, Ni forms complexes with certain amino acids, peptides and proteins,

which can produce DNA damage or genome alterations, including DNA-protein cross links, DNA strand breaks, chromosomal aberrations (Armendariz and Vulpe, 2003; Kasprzak, 1995). Breast cancer mortalities in different countries were studied to be directly correlated with the estimated dietary intake of Zn, Cr and Cd and inversely correlated with Se (Pasha et al., 2010). Fe is essential for the normal physiological functions in humans, since it is an integral part of many proteins and enzymes. The excessive accumulation of Fe in humans may be associated with an increased risk of cancer (Siddiqui et al., 2006). It causes tissue damage by acting as a catalyst in the conversion of hydrogen peroxide to free-radical ions, which attack cellular membranes, cause DNA strand breaks, inactivate enzymes, depolymerize polysaccharides and initiate lipid per oxidation (Freeman , 2004).

Excessive dietary intake of Pb was linked with the cancers of stomach, small intestine, large intestine, ovary, kidney, lungs, myeloma, all lymphoma and all leukemia (Reddy et al., 2004). Studies conducted regarding the concentrations of metals in the blood and their relationship with environmental exposure provide useful information to the general public. The present study was carried out to investigate the effect of contaminated food chain including crops meat and milk consumption on the blood metals composition of the people from Peshawar and lower Dir,

Pakistan. A correlation between the metal concentration of the food crops, meat, milk and human blood from the study areas was also estimated.

1 Materials and methods

1.1 Study areas

Peshawar is the provincial capital of Khyber pakhtunkhwa and occupies an area of 77 km^2 with a population of more than one million. Kankola is a major food crops producing area situated in the northeast of Peshawar (Fig. 1). Food crops from Kankola are transported to Peshawar. Although the main irrigation source is a canal originated from Shalam River but on the other side a wastewater stream (originated from industrial zone located in Hayatabad) is also used for irrigation purposes due to the lack of public awareness (Jan et al., 2010).

Dir is divided into two districts namely Upper Dir, and Lower Dir (Fig. 1) with a total population of 767,409. Topographically, Dir is dominated by mountains and hills which are of Hindukush and Hindu Raj ranges. The mountain ranges run from north to south and from northeast to southwest along the northern borders with Chitral District.
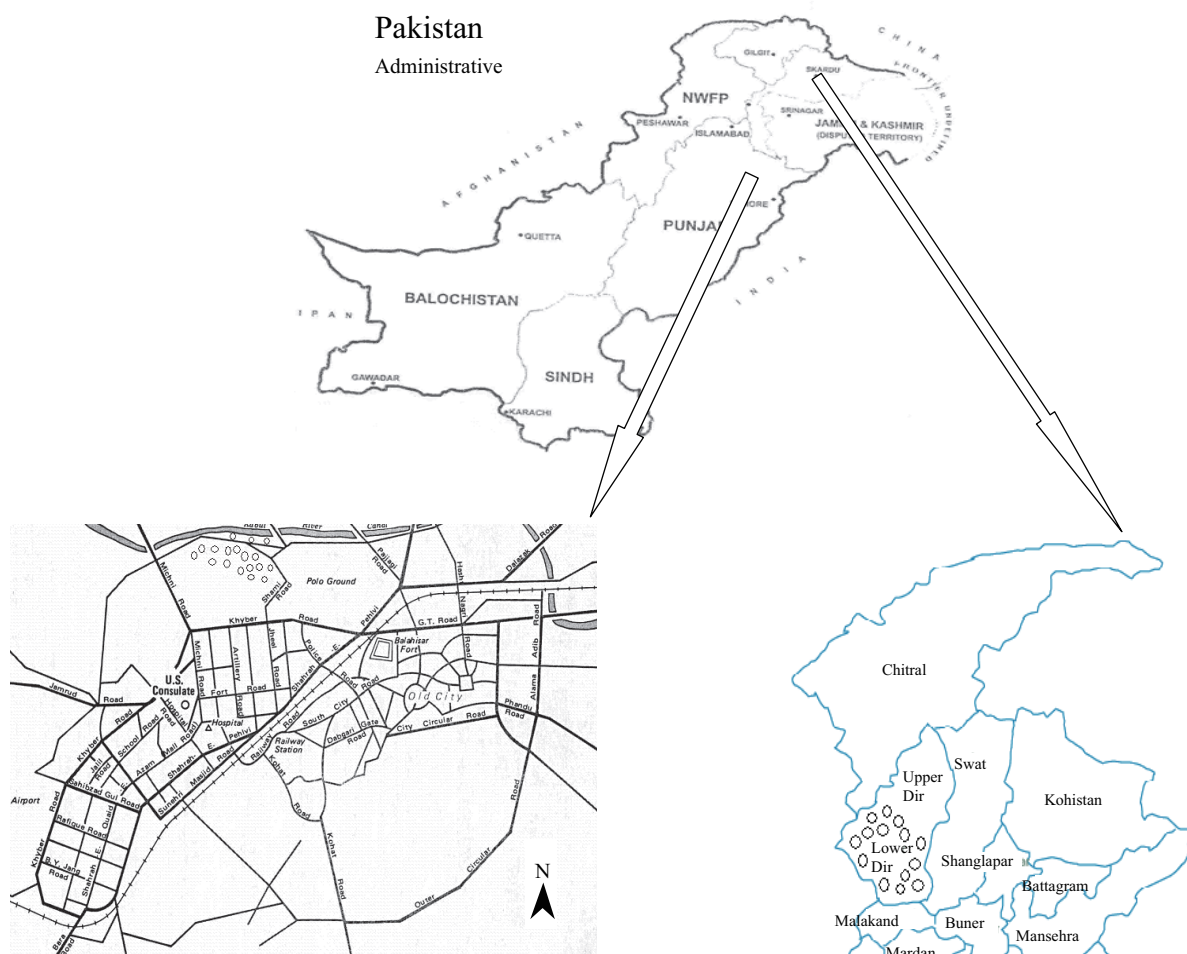

Fig. 1 Location map of the samples collection points in the polluted area and control area.

The main river is Panjkora which enters the district from northeast and flows south west along the boundary of the Bajour Agency up to its con-influence with Swat River. Panjkora River is composed by several streams in the Lower Dir and a main stream form Upper Dir called Dir River. Individual streams in the catchment areas are used as a source of irrigation and River Panjkora is the main irrigation source in the downstream plain areas of Lower Dir (Jan et al., 2010).

1.2 Food crops and forage grass sample

The freshly harvested food crop samples were put in clean plastic bags and transported to the laboratory for analyses. The samples were cleaned with de-ionized water and separated into leaf, stalk, fruit and root. All air-dried sub-samples of vegetables were grounded to fine powder and stored in polythene bags. A major part of agricultural land in the study areas is also used for fodder cultivation. Forage grass samples were collected from the study areas and were pretreated as food crops samples.

Food crop sample (0.5 g) was taken in crucible and was digested with perchloric acid and nitric acid at 1:4 ratio (V/V). After cooling, the digested sample was filtered and made to the final volume of 25 mL using de-ionized water. Precession and accuracy of analysis was also ensured through repeated analysis of the samples against certified reference materials (CRMs) of all metals. Due to the non availability of CRMs of vegetables in our laboratory for quality assurance, recovery studies were conducted using standard spiking methods. The same procedure was adopted for the digestion of forage grass samples.

1.3 Blood sample

A scheme was devised for the collection of human blood samples from both the areas approved by the local ethical committee. The population in each of the study area was divided into different age groups, i.e, children (1–12 years), adolescents (12–18 years), adults (18–45 years) in case of females and 18–55 years incase of males) and old age (above 45 and 55 years for male and female, respectively). Blood samples were collected from children upon their consent as well as of their parents. Blood samples were collected in April, 2009 from the subjects present in different locations. Blood samples (2 mL) were collected by vein puncturing using clean disposable syringes and needles into a haparinized pretreated clean polypropylene tubes and then transported to lab under ice-cold conditions.

Method was first optimized using acid mixture in different proportions. The precession and accuracy of the method was checked by analyzing the Standard Reference Materials (SRMs Human blood, Batch 1701-3, provided by the Pakistan Standards Institution Islamabad) till the results agree within $(94 \pm 7)\%$ of the certified values. The validity of the method was further ascertained by cross method checks, spiked recovery and replicate analysis. Aliquot of 1 mL blood sample was then wet digested with concentrated nitric acid and percholric acid. The digested samples was diluted to the required volume with 0.25% nitric acid.

1.4 Milk and meat sample

Milk samples were collected soon after calving during early hours of the day before milking. After discarding the first 5–6 drops, milk samples (300 mL each) were collected from buffaloes and cows fed on this forage grass in both the areas. Meat samples were purchased from the local markets ensuring that of the cattle fed on understudy forage grasses, packed and stored at –18°C till analysis.

A known volume of milk sample (25 mL) was evaporated to near dryness, wet-ashed and taken up in 10 mL of 0.25% HNO_3.

Meat sample was homogenized separately and 5–10 g of fresh homogenate was weighed into quartz dishes and evaporated to dryness in oven at 100°C then the dried sample was ashed in the muffle furnace at 450°C for overnight. Ashed sample was cooled to room temperature and was added 0.5 mL of concentrated nitric acid and was re-evaporated and then ashed in muffle furnace. The ash was then dissolved in 0.5 mL concentrated nitric acid and diluted to 20 mL with deionized water.

1.5 Analysis

Analytical grade chemicals were purchased and used for sample preparation and analyses. Solutions were prepared in double de-ionized water. For each metal, calibration standards were prepared from the stock solution.

Heavy metals (Zn, Cu, Mn, Ni, Pb, Cd and Cr) in the prepared samples were analyzed using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS AAS–700 Perkin Elmer, USA). Hollow Cathode Lamps (HCL) were used as a source of radiation for each metal. The basic instrumental parameters for each metals are given in Table 1.

Table 1 Instrumental analytical conditions for analysis of selected metals

Metal	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Limit of detection (µg/L)
Zn	213.9	0.7	15	0.020
Cd	228.8	0.7	4	0.002
Pb	283.3	0.7	30	0.050
Ni	232.0	0.2	25	0.070
Cu	324.8	0.7	15	0.014
Cr	357.9	0.7	25	0.004
Mn	279.5	0.2	20	0.005

2 Results

2.1 Distribution of heavy metals in food crops and forage grass

Heavy metal concentrations in the edible part of food crops grown on wastewater irrigated soil and control soil are given in Tables 2 and 3.

The data show that Zn concentration ranged from 38.38 to 296.26 mg/kg in food crops grown on wastewater irrigated soil, in background from 2.23 to 95.44 mg/kg and in control from 30.54 to 89.34 mg/kg. *Brassica rapa, Spinacia oleracae* L., *Pisum sativum, Hebiscus esculan-*

Table 2 Mean metal concentrations (mg/kg) in food crops irrigated with wastewater

Vegetables	Zn	Cd	Pb	Ni	Cu	Cr	Mn
Brassica rapa	108.43 (6.34)	0.06 (0.01)	0.26 (0.00)	58.44 (3.11)	55.98 (3.22)	2.10 (0.01)	135.46 (7.43)
Spinacia oleracae L.	194.23 (6.23)	0.07 (0.00)	0.14 (0.01)	63.46 (3.05)	36.22 (2.30)	1.98 (0.01)	134.88 (6. 33)
Brassica oleracae botrytis	74.14 (3.44)	0.10 (0.00)	0.28 (0.01)	50.64 (3.01)	48.42 (2.44)	1.82 (0.00)	75.74 (3.22)
Pisum sativum	122.54 (7.23)	0.11 (0.00)	0.27 (0.01)	53.43 (2.05)	54.73 (3.54)	1.53 (0.01)	91.98 (5.22)
Lycopersicum esculantum	98.65 (4.34)	0.06 (0.01)	0.24 (0.01)	66.48 (4.40)	62.53 (4.42)	2.20 (0.01)	144.14 (6.00)
B. compestris	81.38 (4.22)	0.04 (0.00)	0.22 (0.00)	46.72 (2.00)	52.62 (3.21)	1.77 (0.00)	150.44 (5.55)
Hebiscus esculantus	118.41 (6.20)	0.20 (0.01)	0.26 (0.00)	54.12 (2.47)	54.76 (2.01)	1.55 (0.01)	74.22 (3.22)
B. oleracae capitita	68.51 (3.22)	0.07 (0.00)	0.10 (0.01)	52.54 (3.12)	61.24 (3.23)	1.63 (0.01)	61.86 (2.23)
Triticum aesativum L. grain	70.43 (3.42)	0.04 (0.00)	0.18 (0.00)	50.44 (3.04)	45.56 (3.44)	1.28 (0.00)	107.16 (5.33)
Mentha viridis	72.55 (3.76)	0.12 (0.00)	0.27 (0.01)	28.32 (2.21)	66.46 (4.00)	1.93 (0.00)	119.86 (5.33)
Coriandum sativum	192.54 (7.13)	0.09 (0.01)	0.28 (0.01)	46.38 (2.30)	65.45 (3.04)	0.93 (0.00)	156.24 (6.00)
Oryza sativa L. grain	78.43 (3.11)	0.08 (0.01)	0.24 (0.00)	56.56 (5.62)	65.33 (4.42)	0.78 (0.00)	66.72 (3.45)
Lactuca sativum	67.13 (3.22)	0.06 (0.00)	0.16 (0.00)	45.65 (3.10)	58.65 (4.05)	1.45 (0.00)	144.54 (6.44)
Portulaca oleracae	166.44 (7.32)	0.11 (0.01)	0.13 (0.01)	52.34 (3.23)	75.15 (5.31)	0.98 (0.00)	70.2 (3.44)
Allium sativum	79.22 (3.11)	0.12 (0.00)	0.13 (0.01)	32.33 (2.11)	60.65 (3.22)	1.65 (0.01)	145.96 (7.34)
Allium	76.26 (3.00)	0.09 (0.00)	0.25 (0.00)	55.54 (3.00)	67.54 (4.43)	1.75 (0.00)	104.06 (5.39)
Daucus carota	146.44 (6.40)	0.08 (0.00)	0.13 (0.00)	49.31 (3.45)	54.44 (3.23)	2.01 (0.01)	148.84 (6.23)
Malva neglecta	288.47 (7.22)	0.04 (0.00)	0.26 (0.00)	29.55 (1.14)	61.76 (3.11)	1.73 (0.01)	87.42 (3.67)
Solanum tuberosum	296.26 (6.11)	0.12 (0.01)	0.22 (0.01)	61.54 (3.53)	78.72 (4.53)	2.11 (0.01)	96.38 (3.45)
Zea mays	38.38 (2.11)	0.05 (0.00)	0.21 (0.02)	46.57 (2.14)	46.34 (2.11)	1.49 (0.00)	87.48 (3.40)
WHO/FAO guidelines 2001	100	0.1	0.3	67	73	2.30	500
Mean background values	56	0.03	0.07	29	40	0.331	70

Data in parenthesis represent standard deviation.

Table 3 Mean metal concentrations (mg/kg) in food crops collected from control area

Vegetables	Zn	Cd	Pb	Ni	Cu	Cr	Mn
Brassica rapa	88.54 (3.23)	ND	0.23 (0.01)	53.44 (2.44)	18.22 (1.32)	1.12 (0.00)	73.24 (3.12)
Spinacia oleracae L.	72.45 (3.17)	0.02 (0.00)	0.19 (0.00)	50.67 (2.44)	31.43 (2.21)	1.53 (0.01)	88.14 (3.40)
B. oleracae botrytis	64.43 (3.30)	ND	0.20 (0.01)	37.43 (2.04)	35.65 (2.43)	0.92 (0.00)	25.24 (2.51)
Pisum sativum	64.41 (2.33)	0.02 (0.00)	0.09 (0.00)	41.65 (2.30)	63.42 (3.54)	0.77 (0.00)	50.68 (2.34)
Lycopersicum esculantum	65.64 (3.40)	0.01 (0.00)	0.21 (0.02)	56.65 (3.16)	39.56 (3.43)	1.43 (0.02)	85.44 (3.23)
B. compestris	54.43 (2.64)	0.05 (0.00)	0.15 (0.00)	28.46 (2.44)	41.56 (3.44)	1.62 (0.02)	23.56 (2.43)
Hebiscus esculantus	68.54 (3.10)	1.05 (0.01)	0.23 (0.01)	44.67 (2.03)	37.57 (2.11)	1.66 (0.02)	22.86 (1.42)
B. oleracae capitita	46.33(2.11)	ND	0.17 (0.00)	38.54 (2.04)	52.33 (3.17)	0.83 (0.00)	19.22 (1.00)
Triticum aesativum L grain.	52.22 (2.03)	ND	0.08 (0.00)	40.44 (2.40)	18.44 (1.16)	1.44 (0.02)	75.66 (3.63)
Mentha viridis	44.45 (2.43)	0.06 (0.00)	0.24 (0.01)	23.74 (1.13)	22.54 (1.05)	1.74 (0.03)	89.78 (3.23)
Coriandum sativum	84.67 (5.33)	ND	0.09 (0.00)	38.67 (2.30)	53.65 (3.08)	1.22 (0.03)	95.24 (4.34)
Oryza sativa L. grain	30.54 (1.22)	0.03 (0.01)	0.11 (0.00)	56.44(4.03)	43.44 (3.33)	0.88 (0.00)	98.56 (4.64)
Lactuca sativum	56.67 (2.11)	ND	0.06 (0.00)	45.66 (2.33)	33.21 (2.01)	1.75 (0.01)	33.82 (2.43)
Portulaca oleracae	74.41 (3.04)	0.07 (0.00)	0.08 (0.01)	21.65 (1.19)	46.32 (2.22)	1.11 (0.01)	28.02 (2.41)
Allium sativum	52.44 (2.45)	0.04 (0.00)	0.12 (0.00)	24.75 (1.10)	51.55 (4.35)	1.30 (0.05)	30.99 (2.11)
Allium	56.71 (3.70)	0.01 (0.00)	0.06 (0.01)	39.53 (3.42)	49.16 (3.13)	1.54 (0.04)	26.38 (2.33)
Daucus carota	64.56 (3.11)	0.03 (0.01)	0.05 (0.00)	30.32 (2.00)	34.63 (3.00)	1.32 (0.06)	32.82 (2.11)
Malva neglecta	89.34 (5.65)	ND	0.13 (0.00)	17.47 (1.16)	45.65 (2.54)	0.78 (0.00)	43.55 (3.23)
Solanum tuberosum	72.41 (3.19)	0.06 (0.00)	0.19 (0.00)	49.64 (2.43)	50.45 (3.30)	1.65 (0.00)	13.03 (1.11)
Zea mays	28.55 (2.31)	ND	0.06 (0.00)	32.38 (2.16)	26.55 (2.03)	1.32 (0.01)	36.08 (2.43)
WHO/FAO guidelines 2001	100	0.1	0.3	67	73	2.30	500

Data in parenthesis indicate standard deviation. ND: not detected.

tum, Corriandum sativum, Portulaca oleracae, Daucus carota, Mentha viridis and *Solanum tuberosum* accumulated significantly higher concentration of Zn compared to control areas, and WHO/FAO permissible limits. In wastewater irrigated vegetables Cd concentration ranged from 0.04 to 0.20 mg/kg in background from 0.01 to 0.07 mg/kg and in control from 0.01 to 1.05 mg/kg. *M. viridis, Allium sativum, P. oleracae, S. tuberosum* and *P. sativum* have high concentration of Cd. Pb concentration in wastewater irrigated food crops ranged from 0.10 to 0.28 mg/kg, in background from 0.07 to 0.25 mg/kg and in control from 0.06 to 0.24 mg/kg. *H. escluantus, B. oleracae botrytis, C. sativum, M. viridis, P. staivum, B. rapa* and *Malva neglecta* were found to have high Pb concentration. Ni concentration ranged from 29.55 to 66.48

mg/kg in food crops grown in wastewater irrigated soil, in background from 18.24 to 58.26 mg/kg and in control from 17.47 to 56.65 mg/kg. Cu concentration ranged from 36.22 to 78.72 mg/kg in wastewater irrigated food crops, in background from 20.21 to 66.34 mg/kg and in control from 18.22 to 63.42 mg/kg. Only two species *S. tuberosum* and *Portulaca oleracae* accumulated high concentration of Cu. Cr concentration in wastewater irrigated food crops ranged from 0.93 to 2.10 mg/kg, in background from 0.79 to 1.92 mg/kg and in control from 0.77 to 33.82 mg/kg. Mn concentration in food crops grown on wastewater irrigated soil ranged from 61.86 to 156.24 mg/kg in background from 16.14 to 102.22 mg/kg and in control from 13.03 to 98.56 mg/kg.

Mean concentrations of metals in forage grass are given

Table 4　Metal concentrations in fodder grass (μg/g) and plant soil transfer coefficient values

Metal	Normal range	Transfer factor	Fodder grass values from study area	Transfer coefficient values from study area
Cu	5.00–20.00	0.00–0.10	13.70–25.00 (22.00)	0.65–0.72
Zn	1.00–100.00	0.00–10.00	15.00–185.00 (167.00)	0.40–0.51
Cr	0.03–14.00	0.00–0.10	17.30–29.40 (25.00)	0.62–0.81
Ni	0.00–50.00	0.00–1.00	8.00–16.70 (15.00)	0.25–0.32
Pb	5.00–10.00	0.00–0.20	49.00–88.00 (82.00)	0.19–0.29
Mn	5.00–25.00	0.01–0.10	32.00–44.00 (38.00)	0.56–0.76

Data in parenthesis represent the mean concentration.

in Table 4. Their concentration were within normal ranges. Cu, Zn, Cr, Ni, Pb and Mn concentrations were in the range of 13.70–25.00, 15.00–185.00, 17.30–29.40, 8.00–16.70, 49.00–88.00 and 32.00–44.00 μg/g respectively. While the transfer coefficient values for Cu, Zn, Cr, Ni, Pb and Mn were in the range of 0.65–0.72, 0.40–0.51, 0.62–0.81, 0.25–0.32, 0.19–0.29 and 0.56–0.76, respectively.

2.2 Distribution of heavy metals in meat and milk samples

The statistical parameters of metal distribution in meat and milk samples collected from contaminated and control areas are presented in Table 5. Meat samples collected from control area, mean concentrations of Cu, Zn, Cr, Ni, Pb, and Mn were 68.28, 18.68, 3.91, 0.10, 12.18 and 29.00 μg/L, respectively, while in milk mean concentration of these metals were 9.96, 0.49, 5.94, 0.04, 5.34 and 26.24 μg/L, respectively. In case of meat samples collected from polluted area Cu, Zn, Cr, Ni, Pb, and Mn mean concentrations were 91.57, 45.26, 7.34, 0.74, 53.13 and 55.07 μg/L, respectively, while in milk samples were 14.88, 0.88, 8.44, 0.30, 13.23 and 37.45 μg/L, respectively.

2.3 Distribution of heavy metals in the blood samples

The basic statistical parameters of the selected trace metals distribution in the blood of the subjects from the polluted and control areas are given in the Tables 6 and 7. From the Table 6, it is clear that the mean concentrations of Cu Zn, Cr, Ni, Pb, Mn and Fe in male children's blood from control area were 1.32, 5.97, 0.45, 0.09, 0.02, 1.57, 290.90 μg/L respectively, while in female children these values were 0.29, 3.63, 0.17, 0.03, 0.06, 1.55 and 369.55 μg/L, respectively. In adolescent males, mean concentrations of Cu Zn, Cr, Ni, Pb, Mn and Fe were 1.48, 6.73, 0.48, 0.06, 0.03, 1.39 and 304.71 μg/L, respectively, while in female

these values were 1.00, 12.04 0.31, 0.01, 0.06, 1.82 and 359.73 μg/L, respectively. In case of adult males, the mean concentrations of Cu, Zn, Cr, Ni, Pb, Mn and Fe were 1.35, 7.84, 0.33, 0.12, 0.03, 1.50 and 310.11 μg/L respectively, while in female these values were 0.18, 8.32, 0.26, 0.02, 0.18, 1.82 and 378.26 μg/L, respectively.

In old age males, mean concentration of Cu Zn, Cr, Ni, Pb, Mn and Fe were 0.80, 9.54, 0.37, 0.05, 0.04, 2.22 and 347.55 μg/L respectively, while in females these values were 0.23, 6.77, 0.08, 0.01, 0.24, 1.17 and 318.78 μg/L, respectively. From the Table 7, it is clear that the mean concentrations of Cu Zn, Cr, Ni, Pb, Mn and Fe in male children' blood from polluted area were 2.52, 10.71, 0.05, 0.02, 0.02, 1.70 and 402.75 μg/L respectively, while in female children these values were 2.35, 8.84, 0.18, 0.02, 0.01, 1.28 and 455.95 μg/L, respectively. In adolescent males Cu Zn, Cr, Ni, Pb, Mn and Fe mean concentrations were 3.72, 22.48, 0.61 , 0.05, 0.06, 3.20 and 425.61 μg/L respectively and in females these values were 5.17, 19.38, 0.31, 0.12, 0.04, 2.07 and 431.04 μg/L, respectively. In case of male adults, mean concentrations of Cu, Zn, Cr, Ni, Pb, Mn and Fe were 7.03, 30.95, 0.48, 0.05, 0.013, 5.39 and 452.17 μg/L respectively, while in female these values were 4.49, 30.40, 0.18, 0.07, 0.05, 3.91 and 684.81 μg/L, respectively. In old age males, Cu Zn, Cr, Ni, Pb, Mn and Fe mean concentrations were 14.62, 46.77, 2.79, 0.04, 0.20, 8.92 and 354.12 μg/L, respectively while in females these values were 14.34, 42.57, 0.38, 0.08, 0.12, 6.76 and 406.51 μg/L, respectively. The data was statistically analyzed using SPSS software for Windows (version–16). MNOVA, Cluster analysis (CA) and various correlation techniques were applied for the interpretation of the data.

MNOVA (A multivariate statistical technique) was applied to find-out the the significant difference between the blood metal concentrations of different age groups of the

Table 5　Statistical parameters of metal concentrations (μg/L) in meat and milk samples collected from different areas in Peshawar and lower Dir

	Location/sample	Cu	Zn	Cr	Ni	Pb	Mn
Dir	Mean	68.28	18.68	3.91	0.10	12.18	29.00
Meat (n = 20)	Range	5.43–169.55	0.78–2.37	3.38–15.24	0.06–0.16	13.69–59.93	4.32–22.56
	Std. deviation	12.44	0.01	0.02	0.00	3.24	1.15
Dir	Mean	9.96	0.49	5.94	0.04	5.34	26.24
Milk (n = 24)	Range	8.35–12.66	0.35–1.27	25.64–73.00	0.16–0.93	0.01–9.23	17.75–47.57
	Std. Deviation	1.35	0.00	5.45	0.02	0.05	2.11
Peshawar	Mean	91.57	45.26	7.34	0.74	53.13	55.07
Meat (n = 20)	Range	37.35–120.42	1.35–2.77	4.89–8.57	0.02–0.05	4.64–81.78	15.81–5.80
	Std. deviation	6.98	0.08	0.11	0.00	4.23	4.04
Peshawar	Mean	14.88	0.88	8.44	0.30	13.23	37.45
Milk (n = 26)	Range	7.66–22.69	0.39–0.78	36.47–49.80	0.23–0.38	0.12–17.34	29.84–42.57
	Std. deviation	2.53	0.09	5.04	0.08	1.23	3.72

n: number of samples taken.

Table 6 Statistical parameters of metal concentrations (µg/L) in blood of people of different age groups from lower Dir

Individuals		Cu	Zn	Cr	Ni	Pb	Mn	Fe
n = 70	Mean	1.32	5.97	0.45	0.09	0.02	1.57	290.90
Children (male)	Range	0.12–3.54	1.23–16.71	0.13–1.5	0.00–0.41	0.00–0.04	1.09–2.10	198.47–405.87
	Std. Deviation	0.012	0.02	0.00	0.00	0.00	0.04	15.64
n = 65	Mean	1.48	6.73	0.41	0.06	0.03	1.39	304.71
Adolescent (male)	Range	0.12–4.21	1.25–12.34	0.00–0.87	0.00–0.24	0.00–0.07	0.85–2.17	124.46–428.70
	Std. Deviation	0.06	0.16	0.00	0.00	0.00	0.00	17.21
n = 63	Mean	1.35	7.84	0.33	0.12	0.03	1.50	310.11
Adults (male)	Range	1.20–3.58	1.45–14.86	0.09–0.91	0.00–0.74	0.00–0.08	0.98–2.66	241.14–431.57
	Std. Deviation	0.08	0.64	0.08	0.02	0.00	0.01	13.36
n = 55	Mean	0.80	9.54	0.37	0.05	0.04	2.22	347.55
Old age (male)	Range	0.01–2.15	1.54–18.42	0.15–1.04	0.00–0.15	0.00–0.15	0.95–8.80	204.92–204.92
	Std. Deviation	0.02	0.56	0.06	0.00	0.00	0.04	19.87
n = 70	Mean	0.30	3.63	0.17	0.03	0.06	1.55	369.55
Children (female)	Range	0.12–1.24	1.25–1.12	0.00–0.45	0.00–0.09	0.00–0.30	0.57–2.18	237.41–542.21
	Std. Deviation	0.02	0.09	0.07	0.01	0.00	0.02	12.36
n = 65	Mean	1.00	12.04	0.31	0.01	0.06	1.82	359.73
Adolescent (female)	Range	0.12–1.54	11.11–14.87	0.09–1.24	0.00–0.02	0.00–0.12	0.58–2.87	235.84–456.26
	Std. Deviation	0.07	1.87	0.04	0.00	0.00	0.01	16.93
n = 63	Mean	0.18	8.32	0.26	0.02	0.18	1.82	378.26
Adults (female)	Range	0.12–0.25	0.12–11.20	0.08–1.25	0.00–0.10	0.00–1.47	1.11–2.41	308.40–423.87
	Std. Deviation	0.05	1.40	0.04	0.00	0.05	0.04	39.62
n = 55	Mean	0.23	6.77	0.08	0.01	0.24	1.17	318.78
Old age (female)	Range	0.12–0.46	0.15–11.54	0.00–0.17	0.00–0.01	0.00–0.10	0.56–1.84	248.65–472.65
	Std. Deviation	0.00	0.03	0.00	0.00	0.00	0.00	11.66

n: number of samples taken.

Table 7 Statistical parameters of metal concentrations (µg/L) in blood of people of different age groups from Peshawar

Individuals		Cu	Zn	Cr	Ni	Pb	Mn	Fe
n=70	Mean	2.52	10.71	0.05	0.02	0.02	1.70	402.75
Children (male)	Range	0.12–7.12	0.12–17.56	0.00–0.16	0.00–0.07	0.00–0.07	0.94–2.41	238.54–519.64
	Std. Deviation	0.06	1.47	0.01	0.00	0.00	0.08	15.64
n = 65	Mean	3.72	22.48	0.61	0.05	0.06	3.20	425.61
Adolescent (male)	Range	1.57–8.42	0.66–33.74	0.00–1.82	0.00–0.210	0.00–0.24	1.32–5.16	211.84–596.37
	Std. Deviation	0.01	2.08	0.03	0.00	0.00	0.07	12.75
n = 63	Mean	7.03	30.95	0.48	0.05	0.13	5.39	452.17
Adults (male)	Range	2.34–14.16	0.99–58.95	0.03–1.62	0.00–0.31	0.00–0.24	0.00–7.62	357.15–581.24
	Std. Deviation	0.02	2.06	0.07	0.00	0.00	0.01	18.57
n = 55	Mean	14.62	46.77	2.79	0.04	0.20	8.92	354.12
Old age (male)	Range	2.48–34.35	29.35–87.72	0.05–18.68	0.00–0.12	0.00–0.35	6.25–13.43	212.02–506.78
	Std. Deviation	1.07	3.09	0.02	0.00	0.00	1.04	18.47
n = 70	Mean	2.35	8.84	0.18	0.02	0.01	1.28	455.95
Children (female)	Range	0.86–5.21	0.12–20.41	0.02–0.78	0–0.04	0–0.04	0.75–2.08	358.45–530.94
	Std. Deviation	0.01	1.05	0.05	0.00	0.00	0.04	16.83
n = 65	Mean	5.17	19.38	0.31	0.12	0.04	2.07	431.04
Adolescent (female)	Range	0.72–12.61	8.67–34.71	0.00–1.86	0.00–0.72	0.00–0.12	1.05–3.16	264.12–538.94
	Std. Deviation	0.018	1.14	0.02	0.00	0.00	0.014	14.65
n = 63	Mean	4.49	30.40	0.18	0.07	0.05	3.91	684.81
Adults (female)	Range	1.50–11.88	22.55–45.05	0.00–0.55	0.00–0.64	0.00–0.72	3.12–5.58	247.68–348.64
	Std. Deviation	0.047	2.30	0.00	0.00	0.00	0.01	15.22
n = 55	Mean	14.34	42.57	0.38	0.08	0.12	6.76	406.51
Old age (female)	Range	5.81–0.76	1.44–81.36	0.00–1.40	0.00–0.53	0.00–0.12	4.32–10.52	203.84–596.36
	Std. Deviation	1.03	2.03	0.00	0.00	0.00	0.02	15.42

n: number of samples taken.

two areas, as well as within the same area and also between male and female subjects of the study area. Comparing male children from the two areas, a significant difference was found for the concentration of Cr ($p = 0.006$) and Fe ($p = 0.007$), while in female children there was a significant difference for the concentrations of Cu ($p = 0.001$) and Zn ($p = 0.001$). No significant variation was noted for the concentrations of Mn, Pb and Ni. Comparing the metal concentrations of adolescent's blood of the polluted and control area, a significant difference for the concentrations of Cu ($p = 0.013$), Zn ($p = 0.000$), Mn ($p =$ 0.000) and Fe ($p = 0.024$) was noticed while in females a significant variation for Cu ($p = 0.012$) and Zn ($p = 0.008$) concentrations was observed. Adult males were found to be significantly different with respect to Cu ($p = 0.001$), Zn ($p = 0.004$), Pb ($p = 0.010$), Mn ($p = 0.000$) and Fe ($p= 0.001$) concentrations while female adults were with respect to Cu ($p = 0.000$), Zn ($p = 0.000$) and Mn ($p = 0.000$) concentrations. Old age males of the two areas were significantly different with respect to Cu ($p = 0.001$), Zn ($p = 0.000$) and Mn ($p = 0.000$) concentrations and females with respect to Cu ($p = 0.000$), Zn ($p = 0.000$) and Mn (p

= 0.000) concentrations. We also statistically compared the metal concentrations in the blood of different age groups within the same area, significant variation was found in the concentrations of some metals. In order to find out which group has significantly high concentration of a particular metal we applied post-hoc test that is the extended form of MNOVA. The results revealed that Zn concentration was relatively higher in different age groups compared to Cu and Mn concentrations. A huge variation in Cu concentrations was observed between the old age people as compared to adults, adolescent and children which indicated that old age people have accumulated higher concentrations of metal compared to other age groups. Pb concentration was found higher in old age's blood samples ($p = 0.008$) as compared to adults, adolescent and children. Mn concentration was also found significantly different in different age groups of the same area, with higher concentration in the old age followed by adults, adolescent and children respectively. For other metals, i.e., Cr, Ni, Fe no any significant variation was found ($p > 0.05$). Comparing female subjects of different age groups of the same area, there were found significant difference in Cu ($p = 0.011$), Zn ($p = 0.000$) and Mn ($p = 0.001$) concentrations. Adults males and females were found significantly different in their Cu concentrations. Old age males and female subjects were found to be different in Mn ($p = 0.008$) concentration. In order to find out the effects of the food crops, meat and milk consumption on the blood metal composition we statistically correlated the metal concentration in both the medias. Data showed some positive correlation between the metal pairs in case of foods and blood, i.e., between Cu and Zn ($r = 0.591$), Mn and Cu ($r = 0.412$) Ni and Pb ($r = 0.408$), Cr and Ni ($r = 0.419$) while a negative correlation was also found between Cu and Cr ($r = -0.583$) Mn and Pb ($r = -0.580$) Zn and Cr ($r = 0.460$) Cu and Pb ($r = -0.523$) and Zn and Pb ($r = 0.431$). Meat and milk samples collected from the two areas were also found to have statistically different concentration of metals, with higher concentration in the samples from polluted area.

2.4 Cluster analysis

Cluster analysis (hierarchal cluster method) was applied to classify the individuals from polluted and control areas into groups based on the mean metal concentrations in their blood. It classified individuals from both the areas into four groups (Fig. 2). The first group comprised of male children, adolescent and adults from control area, second group had female children, adolescent, adults and old age males from control and polluted area, third group had children, adolescent and adults males and female children and adolescent from polluted area and the fourth group comprised of adults females from the polluted area.

3 Discussion

Transfer factor is a convenient way of quantifying relative differences in bioavailability of metals to plants (Popko et al., 2003; Ismail, 2004). In this study, the transfer co-efficient values were found higher for Cu, Zn, Mn and
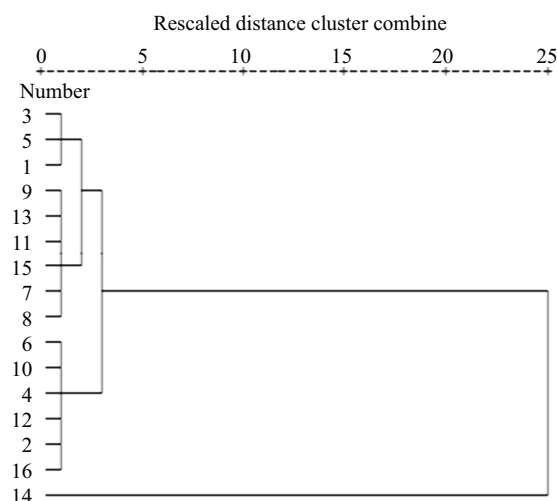
Fig. 2 Dendogram for the classification of individual from the polluted area and control area based on the metals as variables.

Cr. This is due to high mobility and phytoavailability of these metals, which is a reflection of their relatively poor sorption in soils. In contrast, metals such as Ni, and Pb have low transfer coefficients because they are strongly bound, usually to the soil colloids (Kabata-Pendias and Pendias, 1992). The results of our study (Table 4) are in good agreement with the earlier two hypotheses which clearly indicated high concentrations of Zn, Cu, and Mn in plants and high transfer coefficient values as compared to Ni and Pb. In case of control samples, which is generally free from anthropogenic contamination, the metal concentrations and their transfer co-efficients were found very low. High concentration of Cu, Cr, Pb and Mn were observed in milk samples. Buffalo milk was found to have high concentrations of metals as compared to cow's milk which may be ascribed to the high fat content in buffalo's milk, which helps in metal retention due to the formation of bioactive (lipophilic) complexes (Leeuwen and Pinheiro, 2001; Charry et al., 2008).

Huge variation in the minimum, maximum and mean metal concentrations was observed in the blood samples collected from both study areas. Higher mean concentrations were found for Cu, Zn and Mn as compared to Cr, Ni, and Pb and this is in good agreement with the mean metal concentrations in different food crops from polluted and control areas. Most metals showed random distribution. In case of the polluted and control areas, the order of distribution of trace metals was found as Zn>Cu>Mn>Cr>Ni>Pb. The concentrations of the Zn, Cu and Mn in the blood samples from the polluted area was found nearly two folds higher as compared to control area, indicating the large input of the selected metals through consumption of contaiminated food. This clearly indicated the effect of the food crops, milk and meat consumption on the metal composition of the blood. The gender-wise and age-wise distribution of trace metals in the blood of the subjects showed that overall concentration of metals in the subjects from the polluted area was higher than the control. However, their concentrations were found within the safe limits and may not pose any risk. Random distribution of trace metal concentrations was noticed in the blood

of males and females subjects. Significant differences in concentrations were observed for Cu, Zn and Mn in the blood samples from two areas as well as between different age groups. Male children from the polluted area were found to have significantly higher concentration of Cr while female children had high Cu and Zn concentration as compared to control area. Adolescent males from polluted area accumulated significantly higher concentration of Cu, Zn, Mn and Fe while females accumulated higher concentrations of Cu and Zn as compared to control area. There was also noticed a significantly higher concentration of Cu, Mn, Zn and Pb in adult males and females from the polluted area. Old aged people from both the areas were found to have accumulated significantly higher concentrations of Cu, Zn Mn and Pb as compared to adults, adolescent and children. For other metals, i.e., Cr, Ni, Fe no any significant variations were found. There was found no significant variation in metals concentration in blood male and female subjects of different age groups.

In order to find out food crops, meat and milk as the possible sources of contamination of the human blood along with the other sources, correlation study was performed between the metal concentrations in food crops, meat, milk and blood. Strong correlation was observed between Cu and Zn, followed by Mn-Zn, Pb-Ni, Cr-Ni and Ni-Cr metal pairs, while Cr and Pb was found to be negatively correlated with Cu, Zn and Mn. The correlation study further strengthened by the linear regression analysis which shows the dependence of different metal pairs in the form of equations (Table 8). Cluster analysis also classified the individuals from both the areas into four groups based on their mean metal concentrations in their blood. Individuals having nearly the same total metal concentrations fall in the same group. The correlation and regression study clearly indicated that the consumption of metal contaminated food increased the concentrations of metals in the blood. Statistical comparison also revealed that old age males and females have accumulated higher concentrations of the these metals in their blood as compared to adults, adolescents and children which may be due to slow accumulation of these metals in their bodies. In a few cases of the polluted area, the participants had a variety

of health risk. Irritation of the skin with black rashes was the symptom which may be attributed to exposure to Pb Zn and Cr concentrations (Muchuweti et al., 2006; Oleiver, 1997).

4 Conclusions

From the present study it can be concluded that the consumption of contaminated food crops, meat and milk has significantly increased the concentrations of trace metals in human blood as compared to the control area, indicating that these food chains may be one of the major pathways of exposure and sources of contamination of human blood with metals. This was further strengthened by correlation and regression study between metal concentrations in the food crops, meat, milk and the blood, where some positive correlation between Cu-Zn, Mn-Zn, Cr-Ni, Ni-Pb, and Cr-Pb metal pairs, while some negative correlations between Cr-Pb with Mn, Cu and Zn were observed in the form regression equations. Overall metal concentrations in males were higher as compared to females which may be due to diet habits and body mass along with other factors. Old age people had accumulated higher concentrations of trace metals as compared to younger ones due to slow accumulation of the metals in their bodies.

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Table 8 Significant linear correlation for selected metals in food crops and blood samples collected orm different localities in polluted and control areas

Regression equation	Correlation (r)
$Y_{Cu} = 66.738 + 0.585X_{Zn}$	0.591
$Y_{Zn} = 25.443 + 0.645X_{Cu}$	0.587
$Y_{Cr} = 1.325 - 0.0495X_{Cu}$	−0.538
$Y_{Cr} = 1.325 - 0.248X_{Pb}$	−0.429
$Y_{Ni} = 26.851 + 0.429X_{Cr}$	−0.419
$Y_{Cr} = 26.851 + 0.760X_{Pb}$	0.488
$Y_{Pb} = 0.250 - 0.991X_{Mn}$	−0.598
$Y_{Pb} = 0.250 - 0.665X_{Zn}$	−0.431
$Y_{Mn} = 50.994 + 0.803X_{Zn}$	0.612
$Y_{Cr} = 0.873 - 0.532X_{Mn}$	−0.330
$Y_{Ni} = 61.580 - 0.964X_{Cr}$	−0.402
$Y_{Ni} = 61.580 + 0.662X_{Pb}$	0.412

r-Values $\geqslant 0.330$ or $r = -0.330$ are significant at $p < 0.05$.
X, Y mean the concentrations of metals.

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