

COMPARATIVE METAL DISTRIBUTION IN HAIR OF PAKISTANI AND LIBYAN POPULATION AND SOURCE IDENTIFICATION BY MULTIVARIATE ANALYSIS

MUNIR H. SHAH, N. SHAHEEN, A. KHALIQUE, A. A. A. ALRABTI
and M. JAFFAR*

*Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
(*author for correspondence, e-mail: mjaffarqau@hotmail.com, mjaffarqau@yahoo.co.uk)*

(Received 4 December 2004; accepted 4 April 2005)

Abstract. Using nitric acid-perchloric acid wet digestion based FAAS method ten selected metals (Cd, Co, Cr, Fe, K, Mn, Na, Ni, Pb and Zn) were determined in the scalp hair of male Pakistani and Libyan donors ($n = 62$), between 3–54 years age and residing in typical urban areas. The study was taken up to identify sources of metal distribution in two diverse population segments exposed to different environments. Sodium emerged with the highest mean hair concentration of 511.0 $\mu\text{g/g}$ and 496.9 $\mu\text{g/g}$ for Libyan and Pakistani donors respectively, followed by K, at 266.2 $\mu\text{g/g}$ and 242.4 $\mu\text{g/g}$ for the two donor categories. Cadmium showed the lowest mean hair concentration for both Pakistani (0.380 $\mu\text{g/g}$) and Libyan (0.530 $\mu\text{g/g}$) donors. Levels of Na, K, Pb, Cr and Cd were higher in hair of Libyan donors compared with Pakistani counterparts which showed higher comparative levels of Zn, Fe, Co, Ni and Mn. Strong metal-to-metal correlations were found between Na–K ($r = 0.638$) and Cd–Co ($r = 0.574$) for Pakistani donors, while for Libyan donors, Na–K ($r = 0.680$) and Fe–Mn ($r = 0.624$) correlations emerged as strongly significant. Only K was found to have a strong positive correlation ($r = 0.611$) with age for Pakistani donors while this correlation was significantly negative ($r = -0.500$) for Libyan donors, probably arising from individual food habits. Other metals showed no viable relationship with age. Principal Component Analysis (PCA) and Cluster Analysis (CA) of the data evidenced food, industrial emissions and automobile emissions as possible sources of metal distribution. The results of the present study are compared with those reported for subjects from other regions of the world.

Keywords: metals, AAS, hair, source identification, PCA, CA

1. Introduction

The determination of trace metals in human hair has been a subject of continual interest in the biomedical and environmental sciences. Scalp hair has been increasingly used as a bio-monitor for many elements, both toxic and essential, towards assessing environmental exposures and body nutritional status, as well as diagnosis of diseases (Hammer *et al.*, 1971; Flynn *et al.*, 1971; Krishna *et al.*, 1987; Klevay *et al.*, 1987; Ohmori *et al.*, 1989; Ahmed *et al.*, 1990; Wang *et al.*, 1990; Ashraf *et al.*, 1995a; MacPherson and Basco, 2000; Hintz, 2001). Hair trace metal analysis has many advantages over the more conventional kinds of biological matrices such as blood and urine, primarily because of the ease of sample collection, transport and storage. Also, trace element concentrations in hair samples represent an integrated

response over a long time compared with blood and urine levels, which can rapidly fluctuate in response to changing physiological and environmental conditions (Katz and Chatt, 1988; US-EPA, 1979; Laker, 1982). The fact that concentrations of many trace elements in hair are relatively high also facilitates the analysis, with better accuracy (Schroeder *et al.*, 1969; Briggs *et al.*, 1972; Hoffmann, *et al.*, 2000).

Normally, the metal concentration in hair reflects a balanced mineral content of the body reservoir of an individual over a long period of time, only to be significantly changed under conditions of exposure or intake of high amounts of toxic trace metals (Jervis *et al.*, 1977; Golow and Kwaansa-Ansah, 1994; Rivai, 2001; Vishwanathan *et al.*, 2002). Atmospheric exposures originating from specific locations and dietary habits are therefore, responsible for variations in the levels of trace metals in hair (Ashraf *et al.*, 1994; Wilhelm *et al.*, 2002; Khalique *et al.*, 2005). Thus, hair metal analysis becomes important to study the dependence of metal levels on such variates, and that too on a comparative basis so that the contributions from different geographic locations and relevant sources could be correlated (Ashraf *et al.*, 1995b).

Accordingly, the present study envisaged a comparative distribution of selected metals (Cd, Co, Cr, Fe, K, Mn, Na, Ni, Pb and Zn) in the hair of a segment of Pakistani and Libyan population. Male donors from both countries, with matched age groups and belonging to a matched typical urban residence, were selected for the study. Standard multivariate techniques like Principal Component Analysis (PCA) and Cluster Analysis (CA) were used to trace the sources of the metals in hair. The metal levels were examined for a possible mutual correlation also, and were compared with reported metal levels for donors from other regions of the world. It was anticipated that the comparative metal levels in hair of Pakistani and Libyan population segments would bring out distinct sources responsible for the distribution of selected metals to help assess the nutritional status and environmental exposure of the two categories of subjects compared with those from other nations where different environmental and living conditions prevail.

2. Materials and Methods

The study was completed in two phases. Phase 1 was completed during January to April 2002 and phase 2 during April to August 2002. For a comparative outlook, equal number of samples ($n = 62$) were collected during each phase. The sampling locations were Islamabad, Pakistan and Tripoli, Libya. The donor ages ranged between 3–54 years from each location. Only healthy male persons were selected as donors for the study. The details related to a donor's identity, residence, food habits, health status, socio-economic status, and education were recorded on a regular proforma at the time of sampling. Hair samples (~2 g) were cut from the nape of the neck close to the scalp, as strands 3–5 cm long, with a pair of plastic scissors and directly stored in coded zip-locked polythene bags (9 cm × 15 cm).

In order to remove any exogenous matter, the hair samples were soaked in 5% (w/v) detergent solution for about 1 hour, with occasional stirring, and were then thoroughly rinsed with distilled water (Yang *et al.*, 2002; Hirano *et al.*, 2001; Williams *et al.*, 2000; Dombovari and Papp, 1998). Subsequently, the washed samples were dried overnight at 50 °C in an electric oven, and later cooled to room temperature in a desiccator having silica gel as the desiccant. For digestion, accurately weighed portion (~0.5000 g) of the hair sample was treated with 10.0 mL of nitric acid (Suprapure, A.A. Grade, 65%), heated at 80 °C for about 10 minutes and then cooled to room temperature, followed by addition of perchloric acid (5 mL, 70%). The digest was heated again until white fumes evolved, marking the completion of the digestion process (Ashraf *et al.*, 1995b). Blanks, without hair sample, were prepared the same way. All reagents used were of ultrahigh purity (certified >99.9%) procured from E-Merck, Germany, or BDH, UK. The Shimadzu Atomic Absorption Spectrophotometer (AA-670) was used for the determination of Cd, Co, Cr, Fe, K, Mn, Na, Ni, Pb, and Zn under optimum analytical conditions in automatic background compensation mode. Triplicate sub-samples of each sample were run separately in order to record average metal concentrations. A parallel comparative check on the accuracy of quantified results was made through the use of standard reference material (NIST SRM 1573a, Tomato Leaves) obtained from the National Institute of Health (NIH), Islamabad, where inter-laboratory comparison of data was also exercised. Table I lists the detection limits and estimated SRM values of selected metals along with certified values. For routine comparative check, two parallel sub-samples of a given sample, processed under similar analytical conditions, were subjected to analysis at the NIH and our laboratory. The two sets of data were

TABLE I
Minimum detection limits of the selected metals and their estimated values in SRM

Metal	Minimum detection limit (ppm)	NIST SRM 1573a	
		Certified value (mg/kg)	Estimated value (mg/kg)
Cd	0.02	1.52	1.503
Co	0.02	0.57	0.578
Cr	0.09	1.99	2.020
Fe	0.1	368	363.6
K	0.04	2.70%	2.73%
Mn	0.05	246	243.5
Na	0.02	136	133.9
Ni	0.1	1.59	1.614
Pb	0.2	—	0.210
Zn	0.02	30.9	31.30

found to agree, with a maximum variation of $\pm 1.5\%$. STATISTICA software was used to conduct the statistical analyses of the data (StatSoft, 1999).

3. Results

Average metal concentrations ($\mu\text{g/g}$, dry weight) along with some basic statistical parameters pertaining to the distribution of the selected metals in scalp hair of Pakistani and Libyan donors are given in Tables II and III respectively. For both categories of donors, Na and K showed the highest metal levels in hair. Sodium showed the maximum level of $971.8 \mu\text{g/g}$ and $937.7 \mu\text{g/g}$, for Pakistani and Libyan donors, with an average of $496.9 \mu\text{g/g}$, and $511.0 \mu\text{g/g}$, respectively. Potassium peaked at $450.3 \mu\text{g/g}$ and $529.8 \mu\text{g/g}$, with average levels of $242.4 \mu\text{g/g}$ and $266.2 \mu\text{g/g}$, respectively for the two categories of donors. For Pakistani donors, amongst the heavy trace metals (Cd, Co, Cr, Ni, and Pb), the maximum average levels were recorded for Pb at $15.97 \mu\text{g/g}$, followed by Co, Cr, Ni and Cd at 3.944 , 3.300 , 2.456 and $0.380 \mu\text{g/g}$, respectively. In comparison, for Libyan donors the average levels for Pb, Cr, Co, Ni and Cd were 24.95 , 3.935 , 3.753 , 1.988 , and $0.530 \mu\text{g/g}$, in that order. The overall average metal concentration distribution pattern in hair of Pakistani donors was: $\text{Na} > \text{K} > \text{Zn} > \text{Fe} > \text{Pb} > \text{Co} > \text{Cr} > \text{Ni} > \text{Mn} > \text{Cd}$, while that for Libyan donors it was: $\text{Na} > \text{K} > \text{Zn} > \text{Pb} > \text{Fe} > \text{Cr} > \text{Co} > \text{Ni} > \text{Mn} > \text{Cd}$. This showed an exchange of Fe–Pb and Co–Cr in the ranking of metal concentrations in the hair of the two classes of donors (Figure 1).

The linear metal-to-metal correlations between metal pairs in individual hair samples were mathematically examined in terms of Pearson correlation coefficient

TABLE II
Basic statistical distribution parameters for age (years)^a and selected metals ($\mu\text{g/g}$, dry weight)^a in scalp hair of Pakistani male donors ($n = 62$)

	Min	Max	Mean	Median	SD	SE	Skewness
Age	3	48	20	17	13	2	1
Cd	0.060	0.719	0.380	0.392	0.186	0.033	-0.012
Co	0.230	9.799	3.944	3.939	2.871	0.516	0.611
Cr	0.130	9.547	3.300	2.684	2.549	0.458	0.936
Fe	8.086	44.20	21.42	18.87	8.648	1.553	1.147
K	119.1	450.3	242.4	225.0	84.05	15.10	0.733
Mn	0.661	5.084	1.933	1.678	0.940	0.169	1.424
Na	221.4	971.8	496.9	486.9	198.3	35.62	0.501
Ni	0.161	8.363	2.456	2.111	1.949	0.350	1.009
Pb	2.454	27.09	15.97	13.74	5.559	0.998	-0.451
Zn	94.0	309.4	226.1	231.5	53.67	9.640	-0.605

^aWhere applicable.

TABLE III
Basic statistical distribution parameters for age (years)^a and selected metals ($\mu\text{g/g}$, dry weight)^a in scalp hair of Libyan male donors ($n = 62$)

	Min	Max	Mean	Median	SD	SE	Skewness
Age	4	54	24	20	14	3	0
Cd	0.083	1.003	0.530	0.546	0.259	0.047	-0.010
Co	0.177	9.843	3.753	3.09	3.195	0.574	0.877
Cr	0.882	11.01	3.935	3.095	2.461	0.442	1.158
Fe	7.031	38.44	18.62	16.41	7.520	1.351	1.147
K	122.4	529.8	266.2	260.1	120.7	21.68	0.065
Mn	0.537	5.980	1.729	1.405	1.095	0.197	2.348
Na	147.9	937.7	511.0	572.1	282.8	50.79	-0.228
Ni	0.124	6.433	1.988	1.624	1.644	0.295	0.973
Pb	3.834	42.33	24.95	21.46	8.686	1.560	-0.451
Zn	137.5	251.6	190.3	188.2	34.03	6.112	0.080

^aWhere applicable.

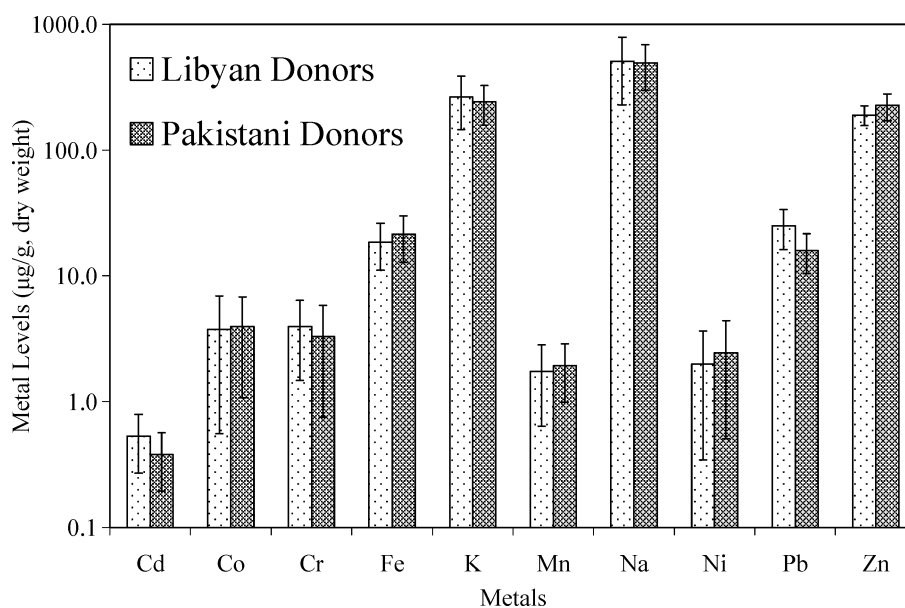


Figure 1. Comparative average metal levels (\pm SD) in the scalp hair of two donor categories.

' r ' which defines the mutual dependence of a pair of variables, metal concentrations in the present study. For hair samples from Pakistani donors strong positive correlations (Table IV, upper diagonal) were found between Na-K ($r = 0.658$), Co-Cd ($r = 0.574$) and Zn-Fe ($r = 0.528$). The counterpart data for Libyan

TABLE IV
 Linear correlation coefficient matrix for metal-to-metal and metal-to-age in scalp hair of Pakistani (upper diagonal) and Libyan (lower diagonal) donors ($n = 62$)

	Age	Cd	Co	Cr	Fe	K	Mn	Na	Ni	Pb	Zn
Age	1	0.086	-0.221	-0.019	0.082	0.611	-0.250	-0.139	0.057	-0.203	0.079
Cd	-0.109	1	0.574	-0.067	0.228	0.241	0.076	0.241	0.024	0.002	-0.183
Co	-0.169	0.332	1	-0.300	-0.160	-0.046	0.272	0.086	0.238	-0.147	0.267
Cr	-0.215	0.386	0.220	1	0.174	0.218	-0.128	0.158	-0.063	0.359	-0.072
Fe	-0.098	0.173	-0.293	-0.392	1	0.206	-0.226	0.109	-0.027	-0.406	0.528
K	-0.500	0.276	-0.003	0.110	0.179	1	-0.138	0.658	0.296	-0.101	0.258
Mn	-0.240	0.304	-0.056	-0.112	0.624	0.137	1	-0.052	-0.086	0.132	0.402
Na	-0.340	-0.414	-0.183	0.111	0.259	0.680	0.246	1	0.063	-0.295	0.121
Ni	0.001	0.494	0.226	-0.384	-0.147	0.176	-0.332	-0.058	1	-0.058	0.048
Pb	0.155	-0.261	-0.056	0.474	-0.513	-0.365	-0.349	-0.158	-0.139	1	-0.109
Zn	0.138	0.188	-0.126	0.161	0.426	0.242	0.406	0.224	-0.360	-0.039	1

Bold values are significant at $p < 0.05$.

donors (Table IV, lower diagonal), showed strong positive correlations between Na–K ($r = 0.680$) and Fe–Mn ($r = 0.624$). In addition, significant correlations also emerged between Cd, Cr, Fe, Pb, Mn and Zn, and other metals, with $r > 0.300$. This situation evidenced a strong interdependence of various metal concentrations in the hair matrix. The linear correlation data on the dependence of selected metals on the age of two categories of donors (Table IV) showed mostly negative or insignificant relationship. Only K exhibited a strong correlation with age ($r = 0.611$) of Pakistani donors while it exhibited significant inverse correlation with age ($r = -0.500$) of Libyan donors.

An important aspect of the present study was to employ the standard multivariate techniques to find out the possible sources of the metals in the scalp hair. The ‘Principal Component Analysis’ and ‘Cluster Analysis’ were used to this effect for source identification of the selected metals. The Principal Component Loadings, using varimax normalized rotation, on metal distribution in the hair of Pakistani donors indicated five factors extracted with eigen values >1 , commutatively explaining more than 71% of total variance (Table V). The counterpart data for the Libyan donors yielded four factors with eigen values >1 , contributing more than 75% of total variance (Table V). Clustering behaviour of the selected metals is presented in the form of a dendrogram in Figure 2 for the two donor categories. The concentrations of selected metals in the hair of donors belonging to the present study were compared with counterpart data from other parts of the world, as shown in Table VI.

4. Discussion

The present study brings out data related to the metal distribution in hair in terms of parameters such as spread around mean metal concentrations, possible correlations, origin identification and comparative evaluation of the two classes of donors.

Tables II and III present basic distribution parameters pertaining to selected metals in hair samples of Pakistani and Libyan male donors, respectively. The macronutrient Na recorded the highest average concentration in the hair of the two donor categories, followed by K. On a comparative scale, the mean concentration level of Zn remained close to that of K. It, therefore, indicated that Na, K, and Zn constituted a major quantitative proportion of metals in the hair of both donor categories.

Almost all of the metals exhibited substantial spread around the mean concentration levels, duly indicated by notably high standard deviation and standard error values in the case of Na, K, Zn, Fe and Pb. These metals, therefore, showed random distribution, and as such did not expectedly follow the normal Gaussian distribution, as reflected by the skewness factor reported in the tables. The study, therefore, evidenced that the individual hair samples from the two donor categories were quite specific in terms of their metal contents that varied quite randomly.

TABLE V
 Significant Principal Component Loadings (>0.3) using varimax normalized rotation on the dataset of selected metals in scalp hair ($n = 62$)

Metal	Pakistani donors					Libyan donors				
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 1	Factor 2	Factor 3	Factor 4	
Cd	0.643	-	-	-	-	-	0.308	-	0.408	
Co	0.779	-	-	-	-	-	-	-	0.503	
Cr	-	-	0.610	-	-	-	0.843	-	0.414	
Fe	-	-	-	0.566	-	-	-	0.591	-	
K	-	0.846	-	-	-	0.845	-	-	-	
Mn	0.315	-	-	0.543	-	-	-	0.503	0.341	
Na	-	0.728	-	-	-0.310	0.801	-	-	-0.423	
Ni	-	-	-	-	0.869	-0.565	-	-	0.454	
Pb	-	-	0.608	-	-	-	0.701	-	-	
Zn	-	-	-	0.472	-	0.737	-	0.372	-0.651	
Eigen values	1.950	1.667	1.340	1.203	1.021	2.776	2.151	1.431	1.157	
% total variance	19.50	16.67	13.40	12.03	10.21	27.76	21.51	14.31	11.57	
Cumulative Eigen value	1.950	3.618	4.957	6.160	7.181	2.776	4.927	6.358	7.515	
Cumulative%	19.50	36.18	49.57	61.60	71.81	27.76	49.27	63.58	75.15	

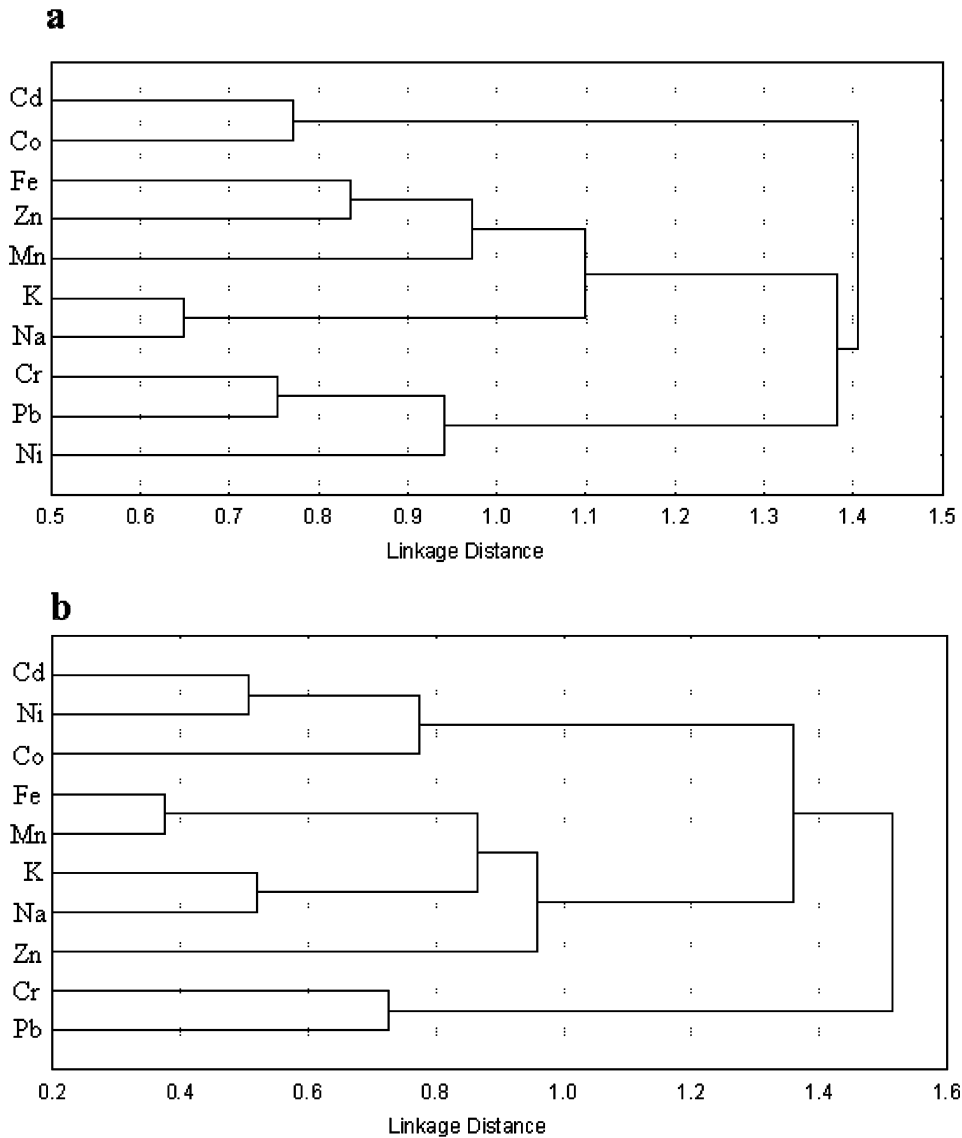


Figure 2. Dendrogram of the metals showing the clustering behaviour of metals in the scalp hair of (a) Pakistani and (b) Libyan male donors.

For Pakistani donors, the mean hair metal concentration levels followed the distribution pattern: Na > K > Zn > Fe > Pb > Co > Cr > Ni > Mn > Cd, while for Libyan donors the pattern changed to: Na > K > Zn > Pb > Fe > Cr > Co > Ni > Mn > Cd. As reported earlier, Na, K, and Zn are considered to play consistent and vital metabolic roles in human body (Fierke, 2000; Goyer, 1997; Food and Nutrition Board, 1989). Therefore, their pattern of distribution

TABLE VI
Comparison of mean hair metal levels ($\mu\text{g/g}$, dry weight) from the present study with those reported for other regions of the world

Location	Cd	Co	Cr	Fe	K	Mn	Na	Ni	Pb	Zn
Islamabad, Pakistan ^a	0.380	3.944	3.300	21.42	242.4	1.933	496.9	2.456	15.97	226.1
Tripoli, Libya ^a	0.530	3.753	3.935	18.62	266.2	1.729	511.0	1.988	24.95	190.3
Chakwal, Pakistan ^b	1.154	1.247	2.085	82.66	–	4.015	–	2.374	–	207.9
Bombay and New Delhi, India ^c	0.32	0.051	1.02	36.0	14.1	2.23	17.3	0.35	13.2	211
Fukui, Japan ^e	0.28	0.18	0.23	15.0	12.9	2.4	153	2.70	3.62	114
Moncton, Canada ^c	0.503	0.043	0.35	18.8	9.7	3.2	32.6	0.26	5.38	248
Scranton, USA ^c	0.97	0.047	0.234	13.6	4.84	7.32	31.9	1.01	5.35	124
Poznan, Poland ^c	0.31	0.022	0.27	22.1	1.46	0.82	3.08	0.52	2.52	160
Czech Republic ^d	0.23	–	0.40	–	–	–	–	–	2.05	128
Sindh, Pakistan ^e	1.1	–	4.9	32.0	108.0	2.4	1099.3	3.5	15.4	183.5
Italy ^f	0.21	–	–	17.87	–	0.41	–	0.70	8.10	144.2
Irian Jaya, Indonesia ^g	0.6	–	–	242	–	–	–	6.4	15.7	228

^aPresent study; ^bKhalique *et al.* (2005); ^cTakagi *et al.* (1986); ^dBenes *et al.* (2003); ^eKazi *et al.* (2000); ^fCaroli *et al.* (1992); ^gPonzetta *et al.* (1998).

matches with the two donor categories. As far as trace elements such as Pb, Cr, and Cd were concerned, their levels were relatively higher in the hair of Libyan donors, while Co, Mn and Ni were found at elevated levels in the hair of Pakistani donors (Figure 1). The case of Pb needs special mention since its enhanced levels were recorded for both donor categories, specifically so for Libyan donors. This finding indicated some major source of Pb in the area which could not be any other than vehicular emission since leaded gasoline fuel is still in use in Tripoli, Libya. In Pakistan, although Pb free gasoline was introduced in the year 2000, with implementation of some measures of quality by the EPA, Pakistan (Qadir, 2002), a proper legislative control is still awaited. The status of atmospheric quality is thus being threatened by Pb, posing health hazard to the local inhabitants. A similar threatening situation emerged for other toxic metals which showed elevated ambient levels in the atmosphere, frequently exceeding the stipulated safe limits laid down by world health agencies (Shah *et al.*, 2004a,b).

Table IV summarizes the correlation data relating to the relevant pairs of variables. Strong positive correlation between the age of Pakistani donors and K was noted. This kind of behaviour could originate on cumulative basis from their dietary habits, especially through the use of rock salt as table salt for everyday use. On the other hand, the inverse correlation between K and the age of Libyan donors showed the lack of such a dietary source. The data also revealed strong positive correlation between Na–K and significant correlation between Cr–Pb for the two donor groups. These two sets of positive correlations, pertaining to macronutrient pair Na–K and

the toxic metal pair Cr–Pb, could be attributed to different origins. The first pair could be traced in the dietary input and the specific internal metabolism of the body, while the second one could be assigned to some external sources affecting the body with Cr and Pb, the latter necessarily were not derived from regular food or water. It is, therefore, inferred that both Pb and Cr emerged from ambient air rich in automotive exhaust (Shah *et al.*, 2004a).

For Pakistani donors, some strong correlations were also shown by other pairs of metals, such as, Co–Cd, and Zn–Fe. These metals could originate from the industrial/metallurgical emissions. The role of food in this regard is too naive, even in the case of Fe or Zn. A similar correlation situation existed for the metal pair, Zn–Mn ($r > 0.40$) for the two groups. The origin of Fe–Zn and Mn–Zn could be traced again to the dietary habits of the donors. However, these observed attributes to food chain or atmospheric exposure needed proper quantitative source identification to label some specific origin for the observed significant correlations.

Accordingly, source identification of the metals was conducted using the Principal Component Analysis (PCA) and Cluster Analysis (CA) (Hopke, 1992; Jobson, 1991). The PCA is a powerful tool for evolving better mutual relationships among the existing variables in a given system and for revealing groups or clusters within the data that have common origin. It reduces dimensionality of the linearly correlated data-set by using a smaller number of linearly independent, but new variables. These new variables are principal components/factors, each of which is a linear combination of the originally correlated variables. The CA classifies a set of observations into two or more mutually exclusive unknown groups, based on combination of internal variables. The purpose to use CA in the present work was to discover a system of observations where number of groups/variables shared properties in common. It was thus cognitively easier to predict properties based on group membership when they all shared common origin. The findings of CA are displayed in Figure 2(a and b), which show strong interaction between Na–K, Pb–Cr, Cd–Co, Fe–Mn–Zn, for both categories. Therefore, the CA was in good agreement with the linear correlation study described above and was quite helpful in identifying the origin of these interlinked metals distributed in the scalp hair.

Additional confirmation on source apportionment of selected metal data was exercised through Principal Component Loadings, employing varimax normalized rotation (Table V). Accordingly, five factors were extracted as groups of various metals, with eigen values >1 against the cumulative total variance of more than 71% for Pakistani donors. Here Cd and Co formed a common factor (Factor 1), with significant contribution of Mn, at a total variance of 19.50% finding their origin from the industrial/metallurgical units. Sodium and K at a total variance of 16.67% contributed to Factor 2, which could be attributed to the internal macronutrient reservoir of the body. Factor 3 incorporated a viable 13.40% of total variance in favour of Pb and Cr, which were assigned to automotive exhaust, duly supported by

correlation and cluster analysis. Factor 4 had higher loadings for Fe, Zn and Mn at 12.03% of total variance, which showed the contribution of essential metals finding their way in the dietary habits of donors, mainly taken as a function of food chain. Factor 5 described an isolated case of Ni having 10.21% of the total variance, again stemming from the dietary habits of donors, specially the continued consumption of hydrogenated oil used as a cooking medium in almost all Pakistani homes. The hydrogenated oil was reported earlier as having high residual Ni content (Yaqub *et al.*, 2000). In comparison, for Libyan donors, four factors were extracted by Principal Component loadings using varimax normalized rotation on the data set with a cumulative variance of more than 75%. Factor 1 with the total variance of 27.76% showed the higher loadings for K, Na and Zn which could be attributed to the internal macronutrient reservoir of the body. Lead and Cr along with significant contribution of Cd at 21.51% of total variance accounted for factor 2 originating from the automotive exhaust, as discussed above for the other group. Factor 3 exhibited the higher loadings for Fe, Mn and significant contribution from Zn at 14.31% total variance originated from the dietary habits of the donors. The last factor (Factor 4) at 11.57% of total variance revealed higher loadings in favour of Cd, Co, and Ni with significant contribution from Cr and Mn, and could be attributed to the industrial and metallurgical emissions in Tripoli, Libya. These findings were also supported by the cluster analysis shown in Figure 2a and b, in total agreement with the results of PCA. Moreover, the correlation results too were in good agreement individually with PCA and CA.

The overall metal distribution data of the present study on bi-national donors were compared with the counterpart data from other regions of the world (Table VI). Data in the table showed higher hair metal concentrations with the only exception of Mn, for the present study subjects as compared with the corresponding subjects listed in the table. The hair Cd levels found in the present study were comparable to those reported for donors from Bombay and New Delhi (India), Fukui (Japan), Moncton (Canada), Poznan (Poland) (Takagi *et al.*, 1986), Czech Republic (Benes *et al.*, 2003), Italy (Caroli *et al.*, 1992) and Irian Jaya (Indonesia) (Ponzetta *et al.*, 1998), but lower than those reported for Scranton (USA) (Takagi *et al.*, 1986), Sindh (Pakistan) (Kazi *et al.*, 2000) and Chakwal (Pakistan) (Khalique *et al.*, 2005). The present study based Co and Cr levels were several-fold higher in comparison with the reported levels. The current levels of these metals were found lower than the corresponding levels from within Pakistan (Kazi *et al.*, 2000). Further, the Fe levels were found to be comparable with those listed in Table VI except those pertaining to Irian Jaya, Indonesia (Ponzetta *et al.*, 1998) and Chakwal, Pakistan (Khalique *et al.*, 2005) which showed enhanced levels. The Na and K hair contents of the present study groups were far higher than those reported in literature, with the only exception of those for Sindh, Pakistan (Kazi *et al.*, 2000). The observed differences could have emerged due to variation in food habits or levels of these elements in drinking water. The estimated Mn levels were lower by 1.2 to 4-fold than for the other reported groups, except those for Poznan, Poland

(Takagi *et al.*, 1986) and Italy (Caroli *et al.*, 1992). Comparable Ni levels were found in relation to Chakwal, Pakistan (Khalique *et al.*, 2005), Sindh, Pakistan (Kazi *et al.*, 2000) and Fukui, Japan (Takagi *et al.*, 1986). However, these levels remained higher than other groups. Enhanced Pb levels were observed in the present study groups than other reported groups except for Irian Jaya, Indonesia (Ponzetta *et al.*, 1998), Sindh, Pakistan (Kazi *et al.*, 2000) and Bombay and New Delhi, India (Takagi *et al.*, 1986), while the Zn levels were comparable with all other reported groups. The observed higher concentrations of toxic trace metals could be attributed to long-term exposure of the industrial and automobile emissions, as well as geographic locations of the subjects, compared with other subjects residing in different geographic locations of the world. The present bi-national study, on the whole, evidenced increased levels of Co, Cr, Na, K, Ni and Pb originating from a diversity of environmental exposure, different food habits and geographic locations of various population segments residing in Islamabad, Pakistan and Tripoli, Libya.

5. Conclusions

The present study showed a marked hair metal concentration dependence on geographic location, environmental exposure and dietary habits of the donors. The observed variations in metal concentrations in hair of two donor groups reflected different food habits and geographic location as causatives that collectively affected individual variability and metabolic activity. In addition, environmental exposure emerged as a critical covariate that was found to overload the city atmosphere of Tripoli and Islamabad due to Pb pollution arising from automobile exhaust emissions, as had been reported for other mega cities of the world. Multivariate techniques of PCA and CA provided evidence to the acclaimed three distinctly different sources of metals in the scalp hair of both donor categories: dietary habits or food; industrial emissions and vehicular exhausts. Data from the present study showed a currently substantial build-up in metal levels compared with the earlier reported results, except Fe, Mn and Zn. The present data could act as baseline information for a relevant futuristic environmental pollution abatement programme.

Acknowledgments

One of us (A.A.A. Alrabti) is grateful to the foreign affair mission to Pakistan for support to pursue advanced studies in Quaid-i-Azam University under Research Assistantship. The financial support from Quaid-i-Azam University, Islamabad, under the title URF, for conducting this study is gratefully acknowledged. Thanks are also due to National Institute of Health (NIH), Islamabad, for inter-laboratory data comparison facility and provision of standard reference material.

References

- Ahmed, A. F. M. and Elmubarak, A. H.: 1990, 'Assessment of trace elements in hair of Saudi Arabian suburban adult male population', *Environ. Technol.* **12**, 387–392.
- Ashraf, W., Jaffar, M., Anwer, K. and Ehsan, U.: 1994, 'Age- and sex-based comparative distribution of selected metals in the scalp hair of an urban population from two cities in Pakistan', *Environ. Poll.* **87**, 61–64.
- Ashraf, W., Jaffar, M., Mohammad, D. and Iqbal, J.: 1995a, 'Utilization of scalp hair for evaluating epilepsy in male and female groups of Pakistani population', *Sci. Total Environ.* **164**, 69–73.
- Ashraf, W., Jaffar, M. and Mohammad, D.: 1995b, 'Comparison of trace metal levels in the hair of Pakistani urban and rural adult male population', *Int. J. Environ. Studies* **47**, 63–68.
- Benes, B., Sladka, J., Spevackova, V. and Smid, J.: 2003, 'Determination of normal concentration levels of Cd, Cr, Cu, Hg, Pb, Se, and Zn in hair of the child population in the Czech Republic', *Cent. Eur. J. Publ. Health* **4**, 184–186.
- Briggs, M. H. and Briggs, M.: 1972, 'Trace substances in human hair', *Experientia* **28**, 406–408.
- Caroli, S., Senofonte, O., Violante, N., Fornarelli, L. and Powar, A.: 1992, 'Assessment of reference values for elements in hair of urban normal subjects', *Microchem. J.* **46**, 174–183.
- Dombovari, J. and Papp, L.: 1998, 'Comparison of sample preparation methods for elemental analysis of human hair', *Microchem. J.* **59**, 187–193.
- Fierke, C.: 2000, 'Function and mechanism of Zn', *J. Nutr.* **130**, 1437s–1446s.
- Flynn, A., Fratianne, R. B., Hill, O. A. and Strain W. H.: 1971, 'Malversation in hair analysis', *Am. J. Clin. Nutr.* **24**, 893–899.
- Food and Nutrition Board, National Research Council: 1989, *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, DC, USA.
- Golow, A. A. and Kwaansa-Ansah, E.: 1994, 'Comparison of lead and zinc levels in the hair of pupils from towns in the Kumasi municipal area of Ghana', *Environ. Contam. Toxicol.* **53**, 325–331.
- Goyer, R. A.: 1997, 'Toxic and essential metal interactions', *Ann. Rev. Nutr.* **17**, 37–50.
- Hammer, D. J., Finklea, J. F., Hendricks, R. H. and Shy, C. M.: 1971, 'Hair trace metals and environmental exposure', *Am. J. Epidemiol.* **93**, 84–92.
- Hintz, H. F.: 2001, 'Hair analysis as an indicator of nutritional status', *J. Equine Vet. Sci.* **21**(4), 199.
- Hirano, Y., Yamamura, K., Oguma, K. and Harda, K.: 2001, 'Direct determination of tin in human hair by graphite furnace atomic absorption spectrometry using hair ashing in graphite tube', *Anal. Sci.* **17**, i1295–i1298.
- Hoffmann, K., Becker, K., Friedrich, C., Helm, D., Krause, C. and Seifert, B.: 2000, 'The German Environmental Survey 1990/1992 (GerES II): Cadmium in blood, urine and hair of adults and children', *J. Expo. Anal. Environ. Epidemiol.* **10**(2), 126–135.
- Hopke, P. K.: 1992, Factor and Correlation Analysis of Multivariate Environmental Data, in: C. N. Hewitt (ed), *Methods of Environmental Data Analysis*, Elsevier Applied Sciences, London, UK, pp. 139–180.
- Jervis, R. E., Tiefenbach, B. and Chattapodhyay, A.: 1977, 'Scalp hair as a monitor of population exposure to environmental pollutants', *J. Radioanal. Chem.* **37**, 751–760.
- Jobson, J. D.: 1991, *Applied Multivariate Data Analysis*, Springer-Verlag, New York, NY, USA.
- Katz, S. A. and Chatt, A.: 1988, *Hair Analysis: Application in Biomedical and Environmental Sciences*, VCH Publishers, New York, USA.
- Kazi, S., Ali, S. S., Furrakh, F., Kazi, T. G. and Kazi, G. H.: 2000, 'Comparison of metal ions in biological samples of Schizophrenic patients and controlled subjects', *Eur. Clin. Lab.* (Application note), 13–14.
- Khalique, A., Ahmad, S., Anjum, T., Jaffar, M., Shah, M. H., Shaheen, N., Tariq, S. R. and Manzoor, S.: 2005, 'A comparative study based on gender and age dependence of selected metals in scalp hair', *Environ. Monit. Assess.* **104**(1–3), 45–57.

- Klevay, L. M., Bistrrian, B. R., Fleming, C. R. and Newmann, C. G.: 1987, 'Hair analysis in clinical and experimental medicine', *Am. J. Clin. Nutr.* **46**, 233–236.
- Krishna, P. and Shrestha, A. O.: 1987, 'Trace elements in hair of epileptic and normal subjects', *Sci. Total Environ.* **67**, 215–225.
- Laker, M.: 1982, 'On determining trace element levels in man: The use of blood and hair', *Lancet* **2**(8292), 260–262.
- MacPherson, A. and Bacso, J.: 2000, 'Relationship of hair calcium concentration to incidence of coronary heart disease', *Sci. Total Environ.* **255**(1–3), 11–19.
- Ohmori, S. and Kobayashi, T.: 1989, 'Catching conditions of a disease with non-destructive activation analysis of hair', *J. Radioanal. Nucl. Chem.* **133**, 271–278.
- Ponzetta, M. T., Nardi, S., Calliari, I. and Lucchese, M.: 1998, 'Trace elements in human scalp hair and soil in Irian Jaya', *Biol. Trace Elem. Res.* **62**, 199–212.
- Qadir, N. F.: 2002, 'Air Quality Management in Pakistani Cities: Trends and Challenges', *Better Air Quality in Asian and Pacific Rim Cities*, Hong Kong Convention and Exhibition Centre, December 2002, pp. 16–18.
- Rivai, I. F.: 2001, 'Heavy metals in human hair related to age groups and automotive pollution levels of Bandarlampung city, Indonesia', *Bull. Environ. Contam. Toxicol.* **66**(4), 443–448.
- Ryabukhin, Y. S.: 1977, 'Activation Analysis of Hair as an Indicator of Contamination of Man by Environmental Trace Element Pollutant', *Report IAEA/RI/4 IH*, 8.
- Schroeder, H. A. and Nason, A. P.: 1969, 'Trace metals in human hair', *J. Invest. Dermatol.* **53**, 71–78.
- Shah, M. H., Shaheen, N. and Jaffar, M.: 2004a, 'Screening of urban aerosol particulate composites for selected metal distribution and their dependence on meteorological parameters', *Ann. Chim. Rome* **94**(11), 805–815.
- Shah, M. H., Shaheen, N., Jaffar, M. and Saqib M.: 2004b, 'Distribution of lead in relation to size of airborne particulate matter in Islamabad, Pakistan', *J. Environ. Manage.* **70**(2), 95–100.
- Takagi, Y., Matsuda, S., Imai, S., Masuda, T., Vinson, J. A., Mehra, M. C., Puri, B. K. and Kaniewski, A.: 1986, 'Trace elements in human hair: An international comparison', *Bull. Environ. Contam. Toxicol.* **36**, 793–800.
- US-EPA: 1979, 'Toxic Trace Metals in Mammalian Hair and Nails', *U.S. Environmental protection Agency* (EPA-600 4-79-049).
- Vishwanathan, H., Hema, A., Deepa Edwin, Usha Rani, M. V.: 2002, 'Trace metal concentration in scalp hair of occupationally exposed autodriver', *Environ. Monit. Asses.* **77**, 149–154.
- Wang, Y., Qin, J., Wu, S. and Linbao, Y.: 1990, 'Study of the relation of Se, Fe, Sr, Pb, Zn, Cu and Ca to liver cancer mortality from analysis of scalp hair', *Sci. Total Environ.* **91**, 191–198.
- Wilhelm, M., Pesch, A., Rostek, U., Begerow, J., Schmitz, N., Idel, H. and Ranft, U.: 2002, 'Concentrations of lead in blood, hair and saliva of German children living in three different areas of traffic density', *Sci. Total Environ.* **297**, 109–118.
- Williams, T. M., Apostol, A. N. and Miranda, C. R.: 2000, 'Assessment by hair analysis of mercury exposure among mining impacted communities of Mindanao and Palawan, The Philippines', *Environ. Geochem. Health* **22**, 19–31.
- Yang, L., Wang, W., Hou, S., Williams, W. P. and Peterson, P. J.: 2002, 'Arsenism clinical stages and their relation with hair arsenic concentration of residents of Bayinmaodao rural district, Inner Mangolia, China', *Environ. Geochem. Health* **24**, 337–348.
- Yaqub, S., Jaffar, M. and Masud, K.: 1999, 'Current status of nickel contamination of local vegetable ghee', *Pak. J. Sci. Ind. Res.* **42**, 126–129.