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HUMAN SCALP HAIR AS A BIOMARKER TO ASSESS LEAD AND COPPER EXPOSURE AMONG INDIVIDUALS BELONGING TO THREE DIFFERENT OCCUPATIONAL GROUPS

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Abstract: The primary aim of the present study is to screen individuals exposed to lead (Pb) and copper (Cu) in the workplace. An effort has also been made to document human exposure to these two elements in the environment. The concentrations of lead (Pb) and copper (Cu) have been determined in human scalp hair (HSH) from 355 individuals. The samples comprised individuals of three occupational groups, namely, motor mechanics, leather tannery workers and printers (compositors and machine men). Samples were also collected from their respective controls and from individuals from a rural agricultural population. The hair samples have been initially washed and acid-digested. Flame atomic absorption spectrophotometry (Flame-AAS) has been used to determine the elemental content. Known factors influencing trace element concentrations in HSH have been controlled. The main statistical tests included one way analysis of variance and Tukey test. Elevated concentrations of both Pb and Cu have been obtained in the scalp hair of individuals comprising the three occupational groups as compared with their respective controls. There were also statistically significant differences between the control samples. It can be concluded that there has been exposure to Pb in both the occupational sector and the environmental sector. This exposure to Pb can be attributed to the usage of Pb-based compounds by these individuals at the place of work in the first case and to environmental-Pb in the second. Since Cu is a metabolically essential element, the diet and the nutritional status of the individuals assume great importance.

Keywords: Lead, Copper, Human Hair, Occupational, Environmental

INTRODUCTION

Anthropological research in different areas of public health has gained interest over the years. In fact, according to Goodenough (1963), It is quite prudent to assume that anthropology has long-standing interests in human biocultural development generally, and particular interests in public health and medicine. Human exposure to different toxic trace elements (e.g., lead, arsenic, mercury and cadmium) and pollutants have been a significant public health concern over the years (e.g., Sanna et al. 2003; Paoliello and De Capitani, 2005; Hussein et al. 2008; Obeng-Gyasi 2019; Goswami et al. 2020). There has been also significant contributions by biological anthropologists in this field, both from India and abroad (e.g., Sen and Chaudhuri, 1996; Sen and Das Chaudhuri, 1996a; Fitzgerald et al., 1998; Sen, 1998; Denham et al. 2005; Sen and Chaudhuri, 2007; Sen and Chaudhuri, 2008; Sen, 2008, Schell and Gallo, 2010; Schell et al. 2006, 2010, 2016; Schell, 2020; Sen et al. 2021). As a matter of fact, a definition of environmental pollution has also been advanced by

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a biological anthropologist. According to this definition, pollution is “a material or a form of energy that is unwanted, usually because it is believed to be detrimental to health and well-being” (Schell et al. 2006).

The toxic trace element lead (Pb) has been very widely studied in humans mainly due to its widespread deleterious effects (Jarup, 2003; Moreira and Moreira, 2004; Papanikolaou et al. 2005; Wani et al. 2015). This element is incorporated into bone and teeth where it can remain for years (Rabinowitz, 1991). It affects metal transport, gene regulation and energy metabolism in the cellular and sub-cellular level (Sarkar et al. 2005). It is also a potential inhibitor of many important enzymes and is strongly toxic to the peripheral and central nervous system (Bressler and Goldstein, 1991; Zawadzki et al. 2006; Scinicariello et al. 2007). The essential trace element copper (Cu) is a redox active metal and is an essential nutrient for all species studied to date (Uriu-Adams and Keen, 2005). It plays a significant role in the normal metabolic functioning of the human body and is a major constituent of many enzymes and proteins (Schumann et al. 2002; Mercer and Llanos, 2003). This element is also required for a wide variety of enzymatic and other processes within the developing foetus ? However, high concentrations of copper can also cause deleterious effects (e.g., Turnlund et al. 2004; Kasperczyk et al. 2016).

Human scalp hair (HSH) is a biological material that can be easily collected by a non-invasive technique, easily stored and transported. Given these attributes, HSH appears to be an attractive material for the purpose of biomonitoring populations and individuals (Chen et al. 2005; Mandal et al. 2003; Wilhelm and Idel, 1996), even though there is an extensive ongoing debate about its limitations as a biomarker of elemental exposure (Barbosa et al.2005). Studies have successfully utilized HSH as an important tool for the assessment of Pb body burden and have reported elevated concentrations of this element in the scalp hair of individuals working in different occupations (Strumylaite et al. 2004; Babalola et al. 2005; Cespón-Romero and Yebra-Biurrun, 2007; Soleo et al. 2012; Vinnikov et al. 2018). HSH has also been used to document environmental exposure to Pb with the residents of industrial/urban areas exhibiting elevated HSH Pb levels over those from agricultural/rural areas (Buononato et al. 2016; Sanna et al. 2008; Zaida et al. 2007; Hasan et al. 2004).

Although researchers have continued to lay emphasis on anthropometric measurements and indices to assess the nutritional status of populations (Mondal and Sen, 2010; Tigga et al. 2015; Roy and Sen, 2021)), recent nutritional studies have focussed on the determination of essential trace elements in selected body tissues. Efforts have now been made to determine the status of different essential trace elements among populations (Takyi, 2004). Studies have subsequently indicated that the determination of HSH Cu concentrations can be utilized as a measure of Cu content (Gibson et al. 1985; Sakai et al. 2000; Kaluza et al. 2001).

There have been some studies from India in the broad field of trace elements in HSH, apart from those studies by Sen and Sen and Chaudhuri, cited earlier. .

The majority of these studies have been on the association of certain diseases with different trace elements (Bhattacharya et al. 2016; Pradeep et al. 2014; Lakshmi Priya et al. 2011; Sukumar and Subramanian, 2007; Srinivas et al. 2001). Given the recent widespread importance on biomonitoring studies, studies are now focusing on the assessment of occupational and environmental exposure to toxic trace elements using HSH (Ghosh, 2007; Vishwanathan et al. 2002; Majumdar et al. 1999). Although some occupational groups such as radiographers and auto drivers have been studied, there are many occupational groups in India that are potentially exposed to toxic elements among whom biomonitoring studies need to be initiated. Three such occupational groups that are potentially exposed to Pb are motor mechanics, leather tannery workers and printers. Studies dealing with human environmental exposure to Pb are also far lesser in number. There is a scarcity of studies that assess environmental exposure to Pb.

Biomonitoring of human exposure to trace elements reflects an individual's current body burden to such elements. The body burden is a function of recent and/or past exposure and this is where correct selection and measurement of biomarkers of trace element exposure is of prime significance. A biomarker basically is an objective biological measure that is utilized to assess health or make a diagnosis of disease. HSH has been successfully utilised as a biomarker to both Pb and Cu.

With the above considerations in mind, the present study was undertaken with the following objectives:

a) To determine occupational and environmental exposure to Pb using scalp hair from exposed and unexposed individuals
b) To determine the Cu status among the exposed and unexposed individuals using scalp hair
c) To analyze the relationship between scalp hair Pb and Cu concentrations.
d) To determine the reliability of HSH as a biomarker.

MATERIAL AND METHODS

Scalp hair samples were collected from 355 adult male individuals residing in three different regions of the state of West Bengal, India. The individuals belong to two occupational groups potentially exposed to Pb (motor mechanics, leather tannery workers and printers), their controls and from individuals engaged in agriculture. The individuals belonging to the three occupational groups were the residents of Cooch Behar, Budge Budge and Rajabazar respectively. The town of Cooch Behar is situated about 600 km north of Calcutta and individuals working as motor mechanics (N=75) was categorized as the exposed group. Those individuals who were not engaged as motor mechanics (N=25) were categorized as the control group. The leather tannery workers were classified as exposed (N=75) and those in the packaging work as controls (N=25). The printers were sampled from the Rajabazar area of Calcutta and were further classified into those engaged as compositors (occupational group, N=35), machine men (occupational group, N=35) and binders (control group, N=35). All the individuals were working in the respective occupations for at least the last three years. The samples from the agricultural community (N=50), were collected from a village named Pipha, about 60 km northeast of Calcutta. The detailed breakup of the number of samples in the present study is reported in Table 1.

TABLE-1: BREAKUP OF THE NUMBER OF INDIVIDUALS IN THE PRESENT STUDY

Nature of group/Area studied	Number of individuals
Motor mechanics/Cooch Behar	75
Motor mechanic controls/Cooch Behar	25
Leather tannery workers/Batanagar	75
Leather tannery worker controls/Batanagar	25
Printer compositors/Rajabazar	35
Printer machinemen/Rajabazar	35
Printer binders/Rajabazar	35
Agriculturalists/Pipha	50
Total Number of individuals (Exposed and Control)	355
Total Number of individuals (Exposed)	220
Total Number of individuals (Control)	135

A number of variables have a strong influence on HSH elemental content. These are sex (Chlopicka et al. 1998; Senofonte et al. 2000; Dunicz-Sokolowska et al. 2007), age (Meng, 1998; Srogi, 2005), income (Sukumar and Subramanian, 2003), family size (Agte et al. 2005) and ethnic group (Barbosa et al. 2005). To eliminate the effects of these variables, all the sampled individuals in the present study belong to the same sex (male), a narrow age group of 25 years – 35 years, having an annual income <Rs.60, 000.00, a family size of <6 members and to the same ethnic group known as the Bengalee Hindu Caste Population (BHCP). The nature of the BHCP has been described earlier by Das Chaudhuri et al. (1993).

During the collection of the hair samples in the present study, individuals using hair dyes and bleaches were excluded, as these treatments are known to alter hair trace element concentrations to appreciable degrees (Maes and Pate, 1977; McKenzie, 1978). The hair samples were collected from the nape of the neck of the subjects. Following Gregar et al. (1978), the proximal 5 cm of the hair was sampled, as new growth hair is preferred for elemental analysis. Each hair sample weighed around 500 mg. The samples were cut with clean stainless-steel scissors and packed in small cellophane bags.

In the laboratory, the samples were washed and oven dried following the method of Schrauzer et al. (1988). The samples were washed in a 1% solution of a non-ionic detergent (Extran MA 01), distilled water and acetone. The dried hair samples were subsequently wet-digested using a 3:1 mixture nitric and perchloric acids at 300^{o}C following a standard method (Harrision et al., 1969). The estimations of Pb and Cu in the samples were done by flame atomic absorption spectrophotometry (Varian Techron: AAS-575-ABQ). Pure metal standards manufactured by Johnson Matthey Materials Technology have been used. Recovery studies have been done in the present study using standards obtained from the US National Institute of Standards and Technology (spinach #1570 and orchid #1571). For both Pb and Cu, the percentages of recoveries were over 90%. The detailed methodology has been described earlier (Sen and Das Chaudhuri, 2001).

The data obtained have been statistically analyzed using Minitab 6.1 and SPSS 15.0. The normality of the elemental distributions has been determined by using normal scores. The relationship between Pb and Cu concentrations has been assessed using Pearson's correlation and linear regression analysis. The statistical differences in HSH Pb and Cu concentrations between the occupationally exposed and the respective control groups have been evaluated using one way analysis of variance (ANOVA). The statistical differences in the elemental concentrations between the control groups and the agricultural group have been observed by using ANOVA and the Tukey test (post-hoc test).

RESULTS

HSH Pb and Cu concentrations

From Table 2, it appears that there are differences in mean concentrations of Pb and Cu between occupationally exposed groups and their respective controls. With respect to Pb, the most affected occupational group is the printing group. However, the compositors have the higher levels of Pb (mean: 20.250 µg/g) in their scalp hair, compared with the machine men (mean: 8.289 µg/g). The motor mechanics and leather tannery workers have almost identical HSH Pb concentrations clustered around 3.000 µg/g. However, HSH Cu concentrations do not show differences between the means of the three occupational groups, clustering around 11.000 µg/g. Among the control groups (Table: 2), the highest mean hair Pb concentration is from Rajabazar (5.607 µg/g), followed by Batanagar (3.098 µg/g), Cooch Behar (3.008 µg/g) and finally Pipha (2.498 µg/g). However, such a decreasing trend is not shown among these respective control groups with regards to HSH Cu concentration as all the means are clustered around 11.000 µg/g, except the Cu from Batanagar (mean: 9.416 μg/g). The mean Pb and Cu concentrations among all the individuals, i.e., exposed and control (N=355), are 5.533 μg/g and 11.280 μg/g respectively, with the occupationally exposed individuals (N=220) expectedly showing higher means than their control counterparts (N=135). The data set also shows 4 outliers. The outliers have an abnormally high Cu content but low Pb content. The mean Pb and Cu concentrations of the outliers are 5.470 μg/g and 32.640 μg/g. The outliers have been removed from all subsequent analysis.

TABLE-2. LEAD (Pb) AND COPPER (Cu) CONCENTRATIONS IN SCALP HAIR FROM INDIVIDUALS OF OCCUPATIONAL, AGRICULTURALIST AND CONTROL GROUPS IN THE PRESENT STUDY (ACTUAL VALUES USED; VALUES IN Mg/g)

Group	N	Mean		Mean	
		Pb	Cu	Pb	Cu
Motor mechanics	75	3.330 (0.882)	11.956 (5.826)	2.500-7.760	5.000-38.980
Motor mechanic controls	25	3.008 (0.649)	11.384 (2.587)	2.500-5.400	7.700-16.500
Leather tannery workers	75	3.476 (0.729)	11.322 (2.945)	1.900-7.200	7.700-27.000
Leather tannery worker controls	25	3.098 (0.806)	9.416 (2.294)	2.100-4.900	6.500-15.100
Printer compositors	35	20.250 (4.510)	12.181 (1.730)	11.400-26.720	8.940-16.000
Printer machinemen	35	8.289 (3.570)	1.652 (1.826)	3.810-19.160	6.290-14.640
Printer controls	35	5.607 (1.881)	11.368 (1.508)	2.300-9.790	8.790-14.870
Agriculturalists	50	2.498 (0.544)	10.164 (2.291)	1.700-3.600	5.390-16.440
Total occupationally exposed	220	6.732 (6.608)	11.723 (3.930)	1.900-26.720	5.000-38.980
Total occupationally unexposed	135	3.580 (1.665)	10.564 (2.403)	1.700-9.769	5.390-16.500
Overall	355	5.533 (5.514)	11.280 (3.472)	1.700-26.720	5.000-38.980

(figures in parenthesis indicate standard deviation)

Normality tests for distribution of HSH Pb and Cu concentrations

Non-significant r values (r_{Pb} = 0.778, d.f. 353, p>0.05 and r_{Cu} = 0.890, d.f. 353, p>0.05) based on the actual values of Pb and Cu with their respective normal scores reveal non-normality of the distributions of Pb and Cu. However, significant correlations (r_{Pb} = 0.913, d.f. 353, p<0.05 and r_{Cu} = 0.978, d.f. 353, p<0.05) based on log values of Pb and Cu with their normal scores indicate the distributions of HSH Pb and Cu concentrations in the present study to be log normal. In all subsequent statistical analysis, log values have been used. The tests also showed the 4 outliers having very high Cu concentrations (mean: 32.640 μg/g). These outliers have been rejected in all the subsequent analysis.

Relationship between HSH Pb and Cu concentrations

The correlation coefficient between Pb and Cu concentrations in HSH for both the exposed and control data in the present study is 0.307 which is statistically significant (d.f. 349, $p<0.05$). The regression equation for Pb on Cu is $Pb = -0.226 + 0.822\ Cu$. The regression coefficient is 0.822 which is statistically significant ($F = 36.35$, d.f. 1,349, $p<0.05$). When the data set is divided into unexposed ($n=135$) and exposed ($n=216$, excluding the outliers), the correlation coefficients between Pb and Cu are also statistically significant at $p<0.05$ ($r = 0.306$ and $r = 0.285$ respectively).

Occupational exposure

The occupational exposure is determined by examining the differences in scalp hair Pb concentrations between the occupationally exposed and the respective control groups using ANOVA. The results are presented in Table 3. The F-ratios show significant statistical differences ($p<0.05$) in HSH Pb levels between all the occupational groups and their corresponding controls, barring the motor mechanics group. The F-ratio is 3.33 (d.f. 1,97; $p>0.05$) for motor mechanics versus their controls. The F-ratio is 7.42 (d.f. 1,97; $p<0.05$) for leather tannery workers and their respective controls. For printer compositors and their controls, it is 311.01 (d.f. 1,68; $p<0.05$), while the F-ratio is 19.57 (d.f. 1,68; $p<0.05$) for printer machine men versus their controls.

TABLE-3. RESULTS OF ANOVA OF HAIR Pb CONCENTRATIONS BETWEEN INDIVIDUALS OF OCCUPATIONAL GROUPS AND CONTROL GROUPS

Occupational group versus control group	F-ratio	d.f.	Level of significance
Motor mechanics versus controls	$F_{Pb}= 3.33$	1,95	>0.05
Leather tannery workers versus controls	$F_{Pb}= 7.42$	1,97	<0.05
Printer compositors versus controls	$F_{Pb}=311.01$	1,68	<0.05
Printer machine men versus controls	$F_{Pb}= 19.57$	1,68	<0.05

Environmental exposure

The environmental exposure is estimated by assessing the differences between the concentrations of Pb in the scalp hair of the individuals forming the control groups and the agriculturalist group in the areas under study using ANOVA. The areas are Cooch Behar (motor mechanic control), Batanagar (leather tannery worker control), Rajabazar (printer control) and Pipha (agriculturalists). The results are shown in Table 4. The F-ratios are statistically significant ($p<0.05$) between all the four groups and also when two groups are taken at a time. The F-ratio between all the 4 areas is 58.61 (d.f. 3,131; $p<0.05$). The F-ratio for Cooch Behar and Batanagar is 5.26 (d.f. 1,48; $p<0.05$) while for Cooch Behar and Rajabazar it is 51.15 (d.f.

1,58; P,0.05). The F-ratio for Cooch Behar and Pipha is 13.39 (d.f. 1,73; $p<0,05$). The ratio is Batanagar and Rajabazar is 27.14 (d.f. 1,58; $p<0.05$). For Batanagar and Pipha, the F-ratio is 34.11 (d.f. 1,73; $p<0.05$). The F-ratio between Rajabazar and Pipha is 140.38 (d.f. 1,83; $p<0.05$). For a comprehensive understanding of the differences in hair Pb levels, the Tukey test which is a post-hoc test has been employed, whose results are shown in Table 5.

TABLE-4. RESULTS OF ANOVA OF HAIR Pb CONCENTRATIONS BETWEEN INDIVIDUALS OF THE CONTROL GROUPS AND THE AGRICULTURALIST GROUP

Control groups versus control/ agriculturalist groups	F-ratio	d.f.	Level of significance
All four groups	F_{Pb}= 58.61	3,131	<0.05
Motor mechanic control (Cooch Behar) versus leather tannery worker controls (Batanagar)	F_{Pb}= 5.26	1,48	<0.05
Motor mechanic control(Cooch Behar) versus printer binders (Rajabazar)	F_{Pb}= 51.15	1,58	<0.05
Motor mechanic control (Cooch Behar) versus agriculturalist group (Pipha)	F_{Pb}= 13.39	1,73	<0.05
Leather tannery worker controls (Batanagar) versus printer binders (Rajabazar)	F_{Pb}= 27.14	1,58	<0.05
Leather tannery worker controls (Batanagar) versus agriculturalist group (Pipha)	F_{Pb}= 34.11	1,73	<0.05
Printer binders (Rajabazar) versus agriculturalist group (Pipha)	F_{Pb}=140.38	1,83	<0.05

TABLE-5. RESULTS OF TUKEY TEST OF HAIR Pb CONCENTRATIONS BETWEEN INDIVIDUALS OF THE CONTROL GROUPS AND THE AGRICULTURALIST GROUP

Control groups versus control/agriculturalist groups	Standard error	Level of Significance
Motor mechanic controls versus leather tannery worker controls	0.033	0.275
Motor mechanic controls versus printer binders	0.030	0.000
Motor mechanic controls versus agriculturalist group	0.028	0.019
Leather tannery worker controls versus printer binders	0.030	0.000
Leather tannery worker controls versus agriculturalist group	0.028	0.000
Printer binders versus agriculturalist group	0.025	0.000

DISCUSSION

In the present study, samples were collected from individuals belonging to the same socio-economic status (based on income and family size), thereby controlling the variations due to nutrition, diet and living conditions as far as practicable. To overcome the possible effects of ethnic influences on the elemental composition in HSH, the individuals sampled in the present study belonged to the BHCP. Individuals using hair dyes and hair treatments were also excluded.

Previous studies have indicated that the distributions of hair trace element concentrations were log normal (Baumslag et al. 1974; Boiteau et al. 1983; Ahmed and El-Mubarak, 1990a; Samanta et al. 2004). The results of the present study are in agreement with these earlier studies.

The present study has observed statistically significant ($p<0.05$) correlations between HSH Pb and Cu concentrations. The regression coefficients of Pb on Cu are also statistically significant. The overall correlation coefficient value of 0.307 obtained between hair Pb and Cu concentrations in course of the present study is not very dissimilar with those reported by Ahmed and El-Mubarak (1990b) and Baumslag et al. (1974) who observed it to be 0.240 and 0.284 respectively. However, Dunicz-Sokolowska et al. (2007) and Wilhelm and Ohnesorge (1990) have reported found a low correlation between these two variables ($r = 0.090$ and 0.041 respectively) between HSH Pb and Cu levels. Positive correlations have also been reported by Zaborowska and Wierciński (1997) and Berzini and Ziakotiuk (1994). Hence, there is an evidence of a synergistic effect between Pb and Cu. Many variables such as duration of exposure and life style factors are responsible for this interaction between essential and toxic trace elements (Telisman, 1995).

Although the overall mean Pb concentration of the unexposed individuals in the present study (3.580 µg/g) is well within the range of values shown in Table 6 (0.200 µg/g - 44.900 µg/g), the documented high mean HSH Pb concentrations reported by Burguera et al. (1987), Baumslag et al. (1974) and Petering et al. (1973) are difficult to explain. The mean HSH Pb concentrations reported in the present study are lower than the values obtained by Sukumar and Subramanian (1992a) on Indian farmers and labourers as far as the unexposed group is concerned (Table 7). Probably the various confounding factors of age, sex, other attributes of the samples and the methods of analysis have lead to the high Pb values in the above-mentioned studies.

TABLE-6. MEAN SCALP HAIR Pb AND Cu CONCENTRATIONS IN DIFFERENT NON-INDIA RURAL/UNEXPOSED/CONTROL POPULATIONS (VALUES IN MG/G; A.M. OR AS STATED)

Authors	Pb	Cu	Nature of sample
Bate and Dyer (1965)	-	15.300	New Zealand
Harrison et al. (1969)	-	14.5	US adults
Petering et al. (1973)	24.400	29.600	US adults

Baumslag et al. (1974)	31.500	17.900	US mothers
Creason et al. (1975)	12.200 (G.M.)	-	US adults
Rendic et al. (1976)	1.400	4.700	Yugoslav adults
Chattopadhyay et al. (1977)	10.100	-	Canadian adults
Gregar et al. (1978)	-	31.000	Adolescent girls
Bergomi et al. (1985)	15.310	-	Rural
Moon et al. (1986)	4.900	-	Amerindian controls
Piccinini et al. 1986	10.700	-	Italian children
Burguera et al. (1987)	17.200	-	Venezuelan controls
Jamall and Jaffer (1987)	-	11.300	Pakistani controls
Jones et al. (1987)	3.000	23.200	Papuan controls
Bergomi et al. (1989)	7.110 (G.M)	-	Italian children
Zaborowska et al. (1989)	4.200	-	Polish
Ahmed and El-Mubarak (1990a)	9.200; 6.300; 14.100; 7.600	-	Students
Ahmed and El-Mubarak (1990b)	6.300	14.400	Saudi Arabian males
Attar et al. (1990)	-	21.400	Saudi Arabian adults
Jamall and Allen (1990)	5.000 (G.M.)	30.700	Bangladeshi women
Leotsinidis and Kondakis (1990)	4.400	10.480	Greek males
Wilhelm and Ohnesorge (1990)	1.720	17.700	German adults
Bache et al. (1991)	1.620; 4.620	-	US controls
Gonzalez-Reimers et al. (1991)	4.600	19.800	Gran Caneri adults
Schuhmacher et al. (1991)	7.800	-	Spanish adults
Donma et al. (1993)	-	8.400	Turkish children
Kozielec and Drybanska (1994)	0.200-9.500 (Range)	-	Polish children
Sturaro et al. (1994)	-	22.000	Italian adults
Wolfsperger et al. (1994)	3.420	-	Italian adults
Esteban et al. (1999)	7.200	-	Russian children
Wilhelm et al. (2002)	0.870 (G.M.)	-	German
Sanna et al. (2003)	4.030	-	Italian boys rural
Hasan et al. (2004)	0.790	-	UAE children rural
Strumylaite et al. (2004)	3.200	-	Polish controls
Souad et al. (2006)	6.600	-	Moroccan infants
Zaida et al. (2007)	6.600	-	Moroccan infants

TABLE-7. MEAN SCALP HAIR Pb AND Cu CONCENTRATIONS IN DIFFERENT INDIA POPULATIONS (VALUES IN MG/G; A.M.)

Authors	Pb	Cu	Nature of sample
Sharda and Bhandari (1984)	-	27.200	ICC controls
Misra et al. (1985)	-	24.600	ICC controls
Quereshi et al. (1985)	-	15.300	Adults
Sukumar and Subramanian (1992a)	13.700	54.500	New Delhi controls
Sukumar and Subramanian (1992b)	13.000; 13.900	6.500; 7.900	Farmers, workers
Sukumar and Subramanian (1992c)	6.800; 4.600 12.600; 4.700	13.100; 14.700; 14.200; 10.400	Farmers, laborers, businessmen, officers
Sen and Chaudhuri (1996)	4.520; 6.910; 3.480	8.040; 11.340; 9.380	BHCP adult males unexposed

Studies have reported occupational exposure to different elements using HSH (Stephen et al. 2000; Gerhardsson et al. 2002; Strumylaite et al. 2004; Martin et al. 2005; Afridi et al. 2006; Cespon-Romero and Yebra-Biurrun, 2007).

Burguera et al. (1987) found a very high scalp hair Pb mean concentration of 48.700 µg/g among individuals working in petrol stations. Mean HSH Pb concentrations among three different occupational groups exposed to Pb in Poland were 17.480 µg/g, 29.030 µg/g and 25.740 µg/g (Zaborowska et al. 1989). Man et al. (1996) reported significantly higher Pb concentrations ($p<0.05$) among professional drivers when compared with those in the teaching profession. In a study done in India, Viswanathan et al. (2002) reported very high Pb levels in the hair of autodrivers and further observed that such drivers are at risk. Babalola et al. (2005) obtained very high hair concentrations of Pb (17.750 µg/g) among auto mechanics in Nigeria as compared with their controls (14.300 µg/g). Traffic policemen have recorded elevated concentrations of Pb in hair due to exposure to automobile exhaust and the levels bear significant positive correlations with duration (Mortada et al., 2001). In just one documented study among leather tannery workers, Randall and Gibson (1989) reported elevated scalp hair chromium levels. No such studies are available in the literature among printers. The present study yielded elevated HSH Pb concentrations among individuals working as motor mechanics, leather tannery workers and printers, as compared with their controls (Table 2). It is a high possibility that exposure to Pb among the motor mechanics can be due to the fact that they use leaded petrol, solvents, metal cleaners and rust prevention paints in the course of repairing and cleaning vehicles. However, since this study, the use of leaded petrol has been phased out in India. Leather tannery workers use a number of solvents, adhesives and thinners, and this can be a possible source of exposure through direct contact and inhalation. The printer compositor group is the most exposed as the printing blocks they use are made of Pb. The printing blocks are manually set up for composition and there is exposure due to direct contact with the skin. The printer machine men are less exposed primarily because they are not directly involved in the composing of the blocks. There is also a widespread habit of Indian workers rubbing their fingers on their clothes and their head. Often while working, the individuals also rub their fingers on their lips and tongue. Doing this with a contaminated finger at the place of work is a significant route of exposure. The results, thus, agree these above studies as elevated hair Pb levels are obtained from individuals engaged in Pb exposed occupations.

As this study indicated higher concentrations of Pb in the scalp hair of individuals engaged as motor mechanics, leather tannery workers and printers, compared with their respective controls, ANOVA was employed to find out the statistical differences, if any, in Pb concentrations between these groups and their respective controls. The results are shown in Table 3.

In the case of motor mechanics with their controls, no significant statistical differences were found for Pb ($p>0.05$), although the concentrations of this element was elevated among the exposed group. On the other hand, a statistically significant F-ratio ($p<0.05$) is obtained for Pb when the leather tannery workers and their control group are analyzed. A statistically significant F-ratio is also obtained for Pb concentrations in both the printer groups and their controls.

Among the control groups, the highest Pb concentration (19.160 μg/g) was obtained from Rajabazar, as this area is more towards the heart of the city of Calcutta. Studies have indicated that the concentrations of Pb vary from area to area in Calcutta, and increases as one move into the city (Chakraborti et al., 1992; Das et al., 1993). Individuals residing in Rajabazar are more exposed to Pb, compared to the other groups of individuals from Cooch Behar, Batanagar and Pipha as Rajabazar is more towards the heart of the city. The differences in hair Pb concentrations between these four areas mentioned can be attributed to environmental exposure to Pb. A major source of this Pb is due to use of leaded petrol where Pb is emitted as particulates of oxides, halides and organo-Pb. This environmental Pb is a major concern in many developing countries, and it is pertinent to mention here that it is after the completion of the present study, India has recently completely phased out the production, sale and use of leaded petrol. A conclusion that is possible here is also that the agriculturalists represent a largely unexposed population to Pb.

It has been observed that the most important factor influencing Pb concentrations in hair is the location (Wibowo et al., 1986). During recent times, a series of studies have pointed out the use and importance of HSH in documenting environmental exposure to Pb (Seifert et al., 2000; Mortada et al., 2002). It has been reported by various authors that there are significant differences in hair Pb concentrations between individuals residing in the urban areas and those from the rural areas. Hammer et al. (1971) reported HSH Pb concentrations to be 80.200 μg/g when individuals from Pb and zinc mining areas were studied, as compared to 8.200 µg/g when rural based individuals were sampled. A mean hair Pb concentration of 10.100 µg/g for individuals residing in a rural area, in comparison to a mean of 16.900 µg/g for those in an urban area in Canada has been reported (Chattopadhyay et al., 1977). Bergomi et al. (1985) also showed that hair Pb concentrations were significantly higher in the industrial area than the rural area in Italy, mean Pb concentration being 25.110 µg/g and 15.310 µg/g respectively. Bertillo and Bonard (1989) observed that in Argentina, the maximum hair Pb concentration in the rural area is less than 10.000 µg/g, while it is less than 20.000 µg/g in the urban area. Jamall and Allen (1990) also found elevated hair Pb concentrations among individuals from the city of Karachi, as compared to those from Bangladesh. Similarly, Schuhmacher et al. (1991) also reported high hair Pb concentrations among children from an industrial area (mean: 9.380 µg/g) compared to that from those from a rural area (mean: 7.800 µg/g). It has been shown by Sukumar and Subramanian (1992b) that persons employed in Delhi as businessmen have elevated hair Pb concentrations (means: 12.600 µg/g) as compared to those residing in the same village, but engaged in agriculture (means: 7.900 µg/g). Diaz-Barriga et al. (1993) reported high HSH Pb concentrations among individuals near a hazardous waste disposal site. Differences in scalp hair Pb concentrations have also been found among three populations occupationally unexposed to Pb in Calcutta by the present authors (Sen and Chaudhuri, 1996). Elevated Pb concentrations were also reported from children residing in an industrial area by Chlopicka et al. (1998) (mean: 8.210 µg/g). In a significant study done in Istanbul, Furman and Laleli (2000) reported that children exposed to traffic had elevated levels of hair Pb (11.820 µg/g) as compared with their controls (2.700 µg/g). The same study has observed that street vendors in areas of higher traffic density had higher hair Pb concentrations (14.180 µg/g) than their counterparts from the low-density areas (9.860 µg/g). In an Italian study, Sanna et al. (2003) observed hair Pb levels among children from two towns, one being exposed to Pb and the other unexposed to Pb. Boys and girls from the exposed town had significantly more hair Pb concentrations (15.510 µg/g and 8.820 µg/g) than their unexposed counterparts (4.030 µg/g and 2.830 µg/g). In yet another study, Hasan et al. (2004) reported that children from rural areas had mean hair Pb levels (0.790 µg/g) whereas children from urban area had higher hair Pb levels (3.470 µg/g). Recently, it has been observed that children living in central area of Lahore city had higher hair Pb concentrations than those living outside Lahore. This is suggestive of the fact that dust containing this heavy metal was attached to hair samples due to a typical urban environment with heavy traffic load, congested population and industrial activities (Anwar, 2005).

Using ANOVA, it is seen that statistically significant F-ratios are obtained for Pb levels among the individuals comprising the control groups and the agriculturalist group. Statistically the ANOVA results further indicate that significant differences exist with respect to Pb in all the cases when the areas are analyzed two at a time. Hence, there are differences due to exposure to Pb in the environment. The results are shown in Table 4. The Tukey test was also utilized for comparisons with respect to hair Pb concentrations (Table 5). In a single pair (motor mechanic controls versus leather tannery controls) ANOVA detected significant differences with respect to hair Pb concentrations, while the Tukey test failed to do so. Such an occurrence is usually not encountered, but it reflects the fact that the analysis of variance is a more powerful test than the multiple comparison tests such as Tukey. This anomaly can be rectified by using a larger sample size that would result in a multiple comparison analysis more capable of locating differences among means. It needs to be recalled here that HSH Pb levels from these two areas are almost identical (3.008 μg/g and 3.098 μg/g).

The critical level for indicating high exposure to Pb is 20.000 μg/g for HSH Pb concentrations (Krause and Chutsch, 1987). The present study has shown that most of the printer compositors and some of the printer machine men have hair Pb concentrations higher than this critical value. These individuals should be put on a chelation therapy which is the conventional recommendation in such cases, using 2, 3-dimercaprol (Kalia and Flora, 2005) or intravenous CaEDTA (Ogawa et al., 2008).

Mean HSH Cu levels are more difficult to assess and comprehend than Pb, primarily because the former is an essential element and the latter a toxic element. From Tables 6 and 7, it can be observed that there is a wide variation in HSH Cu levels. Sukumar and Subramanian (1992b, 1992c) reported appreciably lower mean HSH Cu concentrations for farmers, workers and labourers from a village near Delhi and very high mean HSH Cu concentration among healthy Indian individuals from Delhi respectively. Jamall and Allen (1990) reported very high mean HSH Cu levels among women from Bangladesh that is in sharp contrast to the mean HSH Cu concentration obtained by Khan and Biswas (1985) on Bangladeshi adults. The mean HSH Cu concentrations obtained in the present study are 10.564 μg/g and 11.723 μg/g for the unexposed and exposed populations respectively. Wide variations in HSH Cu concentrations have also been reported in other studies. Given such wide variations in HSH Cu levels, the nature of the diet of the individuals needs to be explored. So, hair Cu levels do not appear to be the prime biomarker to screen populations for Cu exposure.

Since it is widely recognized that the presence of essential elements such as Cu may contribute to the protection of individuals from the effects of toxic metal exposure, appropriate dietary manipulation may be valuable in the prevention and treatment of such metal toxicity. However, there are large variations in HSH Cu levels as reported in the literature. So the primary objective would be to select cut-off values for low, normal and high nutritional status based on HSH Cu concentrations. It needs to be mentioned here that Petering et al. (1978) had proposed that the toxicological investigations of Pb must be based on a definite consideration of the nutritional status of the individuals.

The present study has thus, been successful in screening individuals who are highly exposed to Pb using HSH as the biomarker. The influence of nutritional status on susceptibility to the toxicity of lead bears importance as nutrition can be utilized as a component of intervention. It has been reported that the role of nutrition should be considered to be an adjunct to reduction of environmental Pb exposure, which is the primary means of reducing adverse health effects of Pb (Mahaffey, 1990; Ahamed and Siddiqui, 2007). Most food patterns that reduce susceptibility to Pb toxicity are consistent with recommendations for a healthy diet (Ros and Mwanri, 2003). The relationship between nutritional status and Pb uptake and toxicity is most clearly established for irregular food intake. These issues need to be taken up in subsequent studies.

CONCLUSION

All the 3 occupational groups (motor mechanics, leather tannery workers and printers) are exposed to Pb. The agriculturalists represent a largely unexposed population to Pb. The present study has successfully screened different occupational groups for Pb exposure using HSH analysis and individuals tending to high risk have been identified. As far as HSH Cu is concerned, the diet and nutrition assumes great importance. The results of the present study support the hypothesis that hair Pb levels can be considered an indicator of exposure of populations to Pb. Measuring elemental concentration in HSH could be a useful method for studying exposure and assessing environmental pollution. The technique has the potential of being an effective tool for evaluating extent of pollution and identifying potentially toxic elements.

Interpretation of data on elemental content of hair analysis will still remain the subject of some controversy because of some areas of concern like endogenous and exogenous sources of an element in hair, washing or other pretreatment of hair, choice of statistical methods etc. In spite of these, conclusion may be drawn that evaluation of trace element levels in hair as a measure of occupational and environmental exposures of a population notably to the toxic element Pb have been successfully accomplished in the present study.

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