

*Original Research*

# Trace Elements in Scalp-Hair of Students from Four Different Environmental Contexts

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## Abstract

Many individuals come into contact, at times unawares, with substances that due to concentrations can be classified as environmental pollutants. These substances can have marked adverse health effects on the individual when present in toxic concentration ranges. Excess accumulation of these elements can be determined in hair since hair does not have an excretion point. To investigate any such occurrences, scalp-hair samples were collected from male and female student volunteers (n = 183), aged between 20 and 23 years. These students were attending the Sudan University of Technology, but originated from four regions in Sudan. Elemental concentrations were quantitatively determined by X-ray emission. The study shows that the different inter-region and inter-gender results revealed alarming health effects of environmental pollution.

**Keywords:** mining, agriculture, effluent, environmental pollution, scalp-hair, X-ray emission

## Introduction

Global industrialization has been spurred on the rapid development of economic activities such as mining and agriculture [1, 2]. Despite this global initiative, there are still impoverished communities that face hazardous circumstances daily when plying their trade. Scalp hair specimens become the most preferable specimen for non-invasive sampling [3, 4]. This is due to the ease of sampling that can be effected at low cost,

which facilitates the storage and transport processes that offer several advantages for analysis including matrix stability in the human body.

An investigation of Pb poisoning was conducted [5] of individuals who were employed in an acid lead battery plant. To obtain a detailed concentration, the hair of only employees working for at least six consecutive months were analysed. In that instance, the workers were occupationally exposed to Pb fumes and dust from various activities. The hair analyses indicated significantly higher levels of Pb, Sb, As, and Cd. More so, a linear concentration dependence existed of Cr with Pb, Ni and Sb. For Mn concentration, there were linear correlations with the concentrations of Ni

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and Sb. The Ni concentration on the other hand, correlated positively with those of Mn and Cr, and negatively with the concentration of Cd. Similarly, the concentration of As correlated linearly with those of Sn, Sb and Hg, the concentration of Se correlated linearly positive with that of Ni; the Sn concentration with that of As, the Sb concentration with those of As, Mn, Sn and Se, and negatively with those of Ni and Hg. These metals are present in Pb ores and as such pose an additional toxicity threat to the individuals present in the occupational environment. Various other investigations have also been performed to assess the concentrations of other elements such as As and Mn [6-8]. It was found that these and other toxic elements can cause irreversible damage to the organs of the body.

The presence and effects of toxic elements are not limited to industrial activities. Fish samples from the northern part of the Persian Gulf [9]. Fish from five different species: grunt, flathead, greasy grouper, tigertooth croaker, and silver pomfret were analysed. It was found that these fish contained excessive amounts of Hg. Hence the contaminations was not limited to one species; and hence a large marine volume have been polluted. In addition, Hg and the compound methylmercury were analyzed in scalp hair of 19 fishermen. These men were living in the same coastal regions for several weeks and hence have been consuming fish from the marine area. Of concern was the Hg concentrations, which were higher than  $0.5 \mu\text{g.g}^{-1}$ . That concentration was also higher than the prescribed maximum concentration of the World Health Organization (WHO). A plethora of other investigations have been completed, of which [10, 11] are just a few examples. These investigations and subsequent findings however sketch a grim image of the extent of contamination of not only the natural environment but, important, also the health conditions of individuals present in these occupational environments. However, more importantly is the knowledge that those individuals were not aware of their physical contamination.

Sudan has also experienced an industrial revolution [12-15]. As would be expected, the contamination of individuals due to local pollution has also occurred. In the urban areas of Sudan are various micro industries. To investigate whether any contamination have happened, samples of the scalp-hairs were collected from different ages of worker of Sudanese origin, that were employed in an iron welding workshop and also in a perfume factory in Omdurman city [16]. The samples collected from different ages and genders in both these two sites. The resultant hair analyses of the two major groups of workers indicated that the concentrations of Ca, Fe and Sr in the workshop group and Mn, Cu, Zn, Pb and Hg in the perfume factory were significantly higher than those concentrations of the International Atomic Energy Agency (IAEA) data and of other countries [17]. It should be noted that those workers were not aware of the hazards that they faced in their occupational environment. More so, they were not

informed of these hazards. With industrial development in Sudan [18], such instances are a great cause for concern. In addition, Sudan only has a per capita income of US \$2898.15 per year; [19] which translates to approximately US\$ of 240 per month. This fact in turn places a burden on the university students when study, as an income of that nature do not ensure a successful study period. Thus students would be compelled to seek university vacation employment. Since the country economy is growing they would seek employment with companies such as mining.

It is of great importance whether these students would be alerted to the occupational hazards present in the work environment. It is therefore important that the areas of industrial development be considered so as to delineate the work environments to which the students might be exposed. Mineral resources present in Sudan are petroleum, natural gas, gold, silver, gold, silver, chrome, asbestos, manganese, gypsum, mica, zinc, iron, lead, uranium, copper, kaolin, cobalt, granite, nickel, tin and more [12-14, 20-22].

Gold ores occurs in nearly the entire Red Sea state, the central and northern parts of the River Nile state and the north-eastern part of the Northern state [12-44]. Ores are also present the states of Kassala, Gedaref, Northern and Southern Kordofan states and the southern part of the Southern Darfur state. Hence, gold occurs over approximately 40% of the surface area of Sudan. Mining of such large area has to be effected in the least costly manner. Cyanidation has been perfected over a long period of time and is therefore the least costly process [20]. Even though the resultant concentration of cyanide is less than 10 ppm, this concentration is still toxic to the environment.

Other process methodologies exist; these methodologies are extremely costly and considering the vast tonnage of gold that can still be mined would render the mining not feasible. Studies of heavy metal pollution in mining areas have mainly focused on the mined elements; the associated heavy metals, which are important environmental pollutants that lower the mining grade, have mostly been ignored. [12-14].

Among these problems, heavy metal pollution has attracted more attention because of their toxicity for human health, and their mobility from the polluted soil/ water/air to living beings.

The diseases with which the concentration imbalances of these elements have been associated are liver disease [22, 23] changes in micronutrient concentrations following anti-inflammatory treatment in patients with gastrointestinal cancer risk [23], uræmic toxicity [24], cardiovascular diseases [24] and also various other elements [24-26]. More so, a database of the concentration of elements in the human body was established by Versieck [25]. In addition to their own environmental pollution, the interactions among heavy metals and pesticides may also produce synergistic or antagonistic effects to form compound pollution.

The limit of detection of the converted toxic concentrations also determine the type of instrumental technique of analysis that should be used. The concentrations of Frieden (quantity-, essential-, promotive- and toxic-) elements [26] present in the human body, have been analyzed mostly after invasive clinical sampling of blood and preparation of the serum and plasma [27] in the blood, and at times whole blood, analysis [28]. Of late, there has been a trend towards non-invasive analysis. The use of fingernails [29] and hair [30] have shown promising results.

The hair ethnicity was classified as European, Asian and African [31, 32]. Since the different types of hair have significantly different concentrations, it is expected that other elements would also differ in concentrations. Hence, different normal concentration values (norms) for evaluating toxic concentrations are needed. Norms of elemental concentrations have already been established [33, 34]. Ethnicity in Sudan is widely spread and hence no clear limitation on hair composition could be achieved. The lower and upper limits from these studies were used to delineate toxic limits [35, 36].

The advantage of hair specimens is the simplicity of sampling and ease of handling and storing of the samples for extended periods. Since the elemental content of hair is high relative to other biological tissues and fluids, it can advantageously be analyzed and for this reason hair element analysis has attracted the interest of many disciplines including environmental, forensic and medical sciences [32, 37, 38]. As element levels in human hair may reflect the course of the corresponding blood plasma concentrations, hair analysis is of increasing interest both in nutrition research and in toxicology [39]. Thus, hair elemental concentration can be used to retrace the history of exposure to a pollutant or the evolution of a biomarker for an extended time period. In order to achieve such a past history tracing. Some of these elements are of endogenous origin, others of exogenous origin, reflecting the high propensity of hair for absorbing chemical elements. Because of its growth process, hair might in addition reveal the biomedical and environment history of the patient [39, 40]. Human hair consists mainly of  $\alpha$ -keratin [34]. It is thus of consequence that the concentrations of elements in hair be determined with the utmost accuracy.

Ion beam analysis techniques, especially X-ray emission induced by proton bombardment, PIXE [41] have increasingly been used in the biological and medical fields for elemental composition analysis of tissue [29, 42]. The principal application is the determination of the concentrations of trace and minor elements, and in some cases also major elements, in the matrices of various types of samples [29, 43]. Backscattering spectrometry (BS) [43] is performed simultaneously with X-ray emission.

Table 1. Regions, principal towns or cites of origin, the gender and number of students who participated in the study.

Region	Place	Gender	n	
Central	Bahri	Female	9	
Central	Khartoum	Female	10	
Central	Omdurman	Female	7	
Central	Bahri	Male	7	
Central	Khartoum	Male	9	
Central	Omdurman	Male	8	50
Eastern	Gedaref	Female	8	
Eastern	Halfa	Female	5	
Eastern	Kassala	Female	10	
Eastern	Gedaref	Male	9	
Eastern	Halfa	Male	9	
Eastern	Kassala	Male	5	46
Northern	Atbara	Female	11	
Northern	Dongala	Female	4	
Northern	Marawe	Female	7	
Northern	Atbara	Male	6	
Northern	Dongala	Male	7	
Northern	Marawe	Male	8	43
Western	Al Fashir	Female	9	
Western	Al Obied	Female	4	
Western	Nyala	Female	6	
Western	Al Fashir	Male	7	
Western	Al Obied	Male	12	
Western	Nyala	Male	6	44
Total				183

## Experimental

### Sample Preparation

Hair samples were collected from  $n = 183$  healthy appearing male and female volunteering students, attending the Sudan University of Science and Technology in Khartoum, Sudan. Their ages ranged from 20 to 23 years. Though the students had been living in the urban areas of Khartoum for the past 5 years, they spent university recess periods in their respective regions of permanent residence. The principal towns or cites of origin in Table 1 can be correlated with the map of Sudan, shown in Fig. 1.

The samples were washed with copious amounts of distilled water, rinsed with ethanol, and allowed to dry to room temperature in a dust-free environment. Samples were then embedded in resin. Cross sections

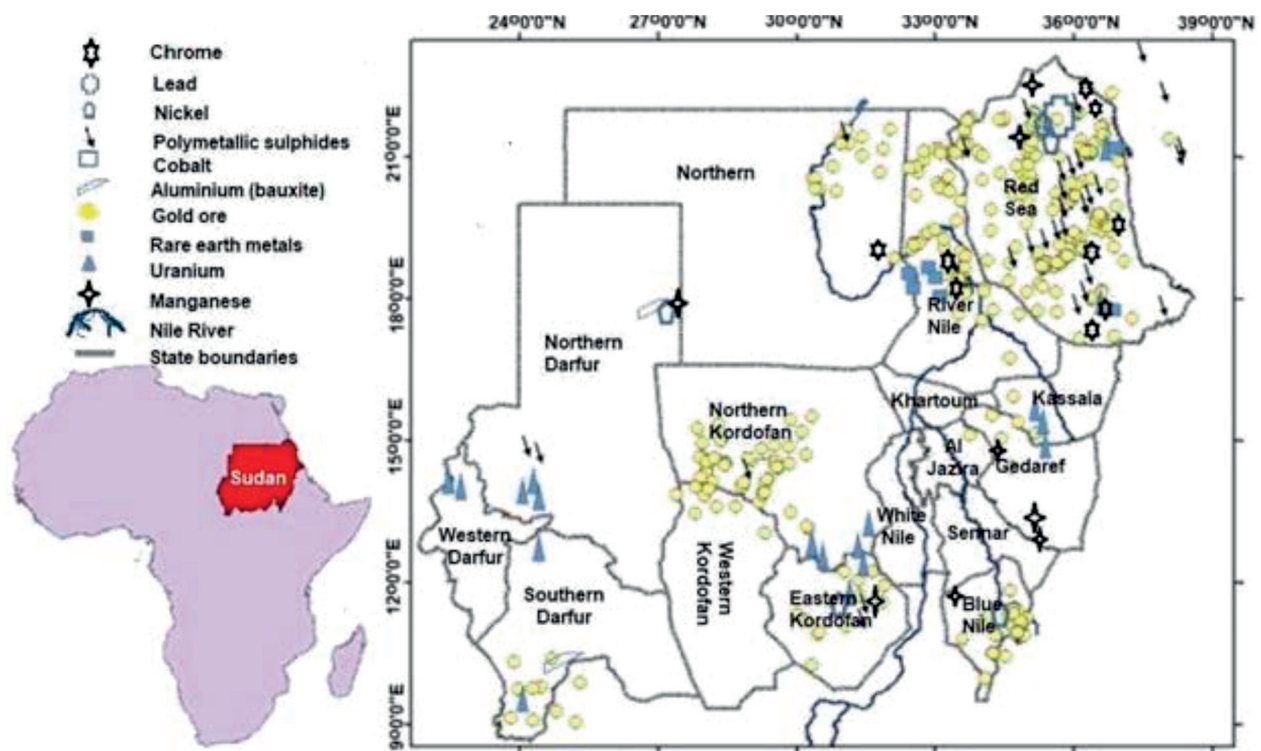


Fig. 1. Map of Sudan, indicating the mining locations and types of mining. Data were obtained from [4, 6]. Map outline obtained with courtesy from googlemaps (freely available).

of thicknesses varying from 5 to 6  $\mu\text{m}$ , were cut with a microtome (UNILAB-102FAM, Haryana, India). The sections were coated in a Quorum coater (Quorum Technologies Ltd, London, UK) with an approximately 10 nanometers thick layer of carbon. The coating prevents charging during irradiation of the sections with a beam of protons. The increase in the yield of the characteristic X-rays from an element in a hair

with the energy of the incident proton beam depends significantly on the distribution of the element over the transverse cross section of the hair.

#### Instrumentation

The samples were irradiated at the 6 MeV Van de Graff Accelerator Nuclear Microprobe (NMP) facility

Table 2. Evaluation of the certified standard IAEA and NIST 1515 used in this study. It should be noted that data supplied were only the element analysed and the concentration average, the standard deviation and the ranges of the concentrations.

Sym	NIST1515					IAEA 085				
	$c_i$	$\sigma_{ci}$	R	$x_i$	% $\epsilon$	$c_i$	$\sigma_{ci}$	R	$x_i$	% $\epsilon$
Ca	15250	1000	NA	15312	-0.41	929		847-1010	922	0.3
Cl	582	15	NA	594	2.06					
Cu	5.69	0.13	NA	5.71	3.51	16.8		15.7-17.8	17.3	0.3
Fe	82.7	2.6	NA	81.4	1.33	79.3		71.0-87.8	78.4	0.9
Mn	54.1	1.1	NA	55.3	2.40	8.8		8.4-9.2	9.2	2.27
Ni	0.936	0.094	NA	0.9	3.22					
P	1593	68	NA	1614	1.06					
K	16080	210	NA	15897	0.52					
V	0.25	0.027	NA	0.3	16.0					
Zn	12.45	0.43	NA	12.18	2.17	163		156-170	168	2.1
Se	NA	NA	NA	NA	NA	1.07		0.96-1.17	1.1	0.8



Table 3. Average concentrations and standard deviations of the quantity elements in the hair specimens per gender per place. The concentration of S is given in percentage, and those of the other elements are given in ppm. ND indicates that the concentration of the element is below the minimum detection limits (MDLs) and therefore not determinable. The MDLs, and therefore not determinable. The MDLs, and the normal values (Norms) are shown.

Place	Gender	P	S	Cl	K	Ca
Bahri	Female	ND	4.11±0.25	915±55	364±31	484±38
Khartoum	Female	ND	4.18±0.25	339±43	165±20	198±15
Omdurman	Female	140±18	4.59±0.14	12±5	145±31	422±55
Bahri	Male	3.9±0.8	3.91±0.16	92±9	167±18	108±47
Khartoum	Male	ND	5.13±0.36	1376±62	344±15	592±77
Omdurman	Male	ND	4.36±0.44	ND	119±26	378±39
Gedaref	Female	1389±44	4.90±0.10	128±29	647±59	1054±44
Halfa	Female	903±41	4.69±0.19	164±43	1347±47	914±64
Kassala	Female	1241±44	4.88±0.34	845±33	2401±37	833±46
Gedaref	Male	886±58	4.65±0.42	1769±70	1047±63	645±35
Halfa	Male	1021±89	4.85±0.39	1223±58	2788±82	1346±22
Kassala	Male	2185±152	5.04±0.05	280±18	1945±15	1073±30
Atbara	Female	2103±154	4.95±0.20	812±74	ND	598±27
Dongala	Female	1123±79	4.79±0.43	762±55	455±36	646±52
Marawe	Female	ND	4.86±0.10	877±36	528±41	712±66
Atbara	Male	752±45	4.79±0.19	1894±72	1703±47	645±44
Dongala	Male	1848±89	4.94±0.25	280±34	1679±35	1184±67
Marawe	Male	936±38	4.84±0.34	1223±150	2113±51	1247±73
Al Fashir	Female	ND	4.39±0.13	786±40	209±17	801±33
Al Obied	Female	ND	4.30±0.26	900±32	221±26	768±26
Nyala	Female	ND	4.19±0.25	708±46	382±13	568±38
Al Fashir	Male	5.2±0.4	4.67±0.09	135±17	168±28	691±37
Al Obied	Male	ND	4.43±0.13	612±20	335±47	541±40
Nyala	Male	ND	4.02±0.36	ND	230±60	411±18
	Norms	132–300	4.53–5.10	50–250	9–77	192–665
	MDLs	2.0	1.6	2.8	1.8	4.5

[45] at iThemba LABS, Somerset West, Rep. of South Africa, analysis were selected at particular micro-regions of hair-shaft cross sections using. Beam energy was varied from 1.50 to 3.00 MeV and the current was maintained at approximately 200 pico-amperes. The beam spot size was  $2 \times 3 \mu\text{m}^2$  and the dwell time of the beam per spot size area was 10 milliseconds. The emitted X-rays were detected with a PGT detector (Princeton Gamma Tech, Princeton, USA) of 100 mm<sup>2</sup> surface area and a resolution of 125eV at Mn K<sub>α</sub> X-ray line. The detector was mounted at 135° to the incoming beam. Back-scattered particles were measured with a silicon surface barrier detector (Ortec, Houston, USA), mounted at 176° to the incoming beam. Pile-up rejection was controlled by a beam-on-demand system.

Organic tissue consists mostly of the major elements C, H<sup>4</sup>, O and N and S, to a smaller percentage mass per mass [46]. Small changes in the Frieden elements [26] compositions would there not affect the concentration of the major elements significantly [46] and therefore the concentrations of these elements were not determined. The concentrations of the elements S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Br and Sr were however determined.

#### Quantification Qualification of Data

The PIXE data obtained were quantified using the Geo-PIXE II software program with dynamic analysis [47]. The back-scattered data were quantified with

Table 4. The average concentrations and standard deviations, in ppm, of the essential elements in the hair specimens. per gender per place. ND indicates that the concentration of the element is below the minimum detection limits (MDLs). The MDLs, and the normal values (Norms) are shown. Concentrations less than 10 ppm is expressed to the first decimal, and those of 10 ppm or higher concentration to whole numbers.

Place	Gender	Cu	Fe	Mn	Zn	Co	Cr	Se
Bahri	Female	5.8±1.6	74±5	1.8±0.2	183±5	1.5±0.3	5.6±0.6	12±3
Khartoum	Female	8.3±1.1	66±7	2.8±1.2	124±18	39±9	3.6±0.4	1.5±0.2
Omdurman	Female	9.7±1.1	75±7	0.8±0.2	28±3	0.8±0.2	0.5±0.2	0.8±0.1
Bahri	Male	3.5±0.8	ND	1.7±0.9	191±12	43±9	ND	12±2
Khartoum	Male	6.8±1.4	14±2	8.0±1.7	31±5	21±2	5.0±0.1	8.5±0.3
Omdurman	Male	12±2	66±5	6.8±1.4	214±32	39±6	4.6±0.1	9.0±0.2
Gedaref	Female	14±4	32±2	ND	12±3	43±3	2.2±0.5	1.3±0.2
Halfa	Female	7.2±1.2	14±2	ND	116±4	33±3	2.9±0.3	2.8±0.3
Kassala	Female	4.0±0.4	7.7±1.1	ND	0.4±0.1	44±4	1.1±0.2	6.0±0.1
Gedaref	Male	11±3	48±4	2.8±1.8	15±2	3.8±0.2	3.6±0.1	2.9±0.2
Halfa	Male	13±3	94±5	3.3±2.4	17±3	0.9±0.3	ND	2.9±0.1
Kassala	Male	16±4	120±10	ND	114±8	19±5	2.8±0.2	2.9±0.3
Atbara	Female	9.2±2.3	65±5	ND	130±35	55±4	19±4	2.3±0.1
Dongala	Female	10±3	74±6	ND	262±58	61±7	26±3	2.3±0.2
Marawe	Female	22±3	101±7	ND	108±14	43±4	13±3	2.3±0.3
Atbara	Male	11±2	56±2	ND	25±2	263±16	12±2	2.9±0.1
Dongala	Male	19±4	10±2	1.3±0.4	37±6	41±5	5.5±0.5	3.0±0.3
Marawe	Male	17±2	37±3	ND	234±26	112±9	10±2	2.3±0.2
Al Fashir	Female	9.8±1.8	14±2	1.1±0.5	30±6	83±4	4.9±0.1	ND
Al Obied	Female	12±3	21±3	1.4±0.6	29±4	39±4	2.3±0.2	1.9±0.3
Nyala	Female	19±4	9.4±2.1	ND	221±23	46±3	3.6±0.2	ND
Al Fashir	Male	5.1±1.5	24±3	2.8±0.3	28±7	38±4	4.3±0.1	2.5±0.2
Al Obied	Male	8.0±2.3	19±2	1.9±0.2	58±7	62±5	4.7±0.1	1.7±0.2
Nyala	Male	8.7±0.9	14±2	1.1±0.5	107±12	43±3	3.3±0.3	5.0±0.2
	Norms	8-27	9-75	0.3-0.9	6-21	0.3-1.3	0.2-0.6	0.1-1.9
	MDLs	1.4	0.2	0.2	0.9	0.2	1.8	1.3

the RUMP [44] and the SimNRA [48] software programs.

The R statistical software [49] was used in the statistical evaluation of the data. A probability value of  $p \leq 0.05$  at the 95% confidence level [50], indicated that there is no significant difference in the resultant concentrations. The R software was also used to determine the linear adjusted regression coefficients [50]. Certified standards obtained from IAEA and NIST, standards IAEA 085 and NIST 1515.

It is of essence to describe variability among observed variables, that is, the concentrations of the elements in the hair, that could be correlated in terms of a potentially lower number of unobserved variables [51]. This variability can be determined by factor analysis

(FA) or principal component analysis (PCA) [51, 52]. In factor analysis an assumption that an underlying causal model exists, was made. Although many hair analyses have been performed, there still exists an inadequate understanding of the hair system and the elemental distribution in the hair. PCA is therefore more applicable since it simply entails the reduction of variables, without the need of any knowledge of correlations that might exist. For completion, both factor analysis and PCA were performed.

Normal values (Norms) for the elements were obtained from [32-36, 53]. The minimum detection limits (MDLs) were obtained from the X-ray emission analysis.

Table 5. Average concentrations and the standard deviations, in ppm, of the promotive and toxic elements in the hair specimens. ND indicates that the concentration of the element is below the minimum detection limits (MDLs, also shown) as performed with X-ray emission. Concentrations less than 10 ppm is expressed to the first decimal, and those of 10 ppm or higher concentration to whole numbers.

Place	Gender	Br	V	Ti	Ni	Sc	Pb	As
Bahri	Female	ND	1.8±0.3	ND	14±3	39±9	ND	6.0±0.2
Khartoum	Female	ND	2.6±0.1	16±3	35±7	16±1	ND	2.1±0.3
Omdurman	Female	ND	4.2±0.1	1.6±0.4	3.5±0.3	3.6±0.1	0.2±0.1	ND
Bahri	Male	ND	ND	16±4	9.0±0.2	3.5±0.1	12±3	6.0±0.2
Khartoum	Male	ND	ND	ND	50±13	47±6	13±2	2.1±0.5
Omdurman	Male	4.0±0.1	0.7±0.4	ND	35±7	26±2	32±4	10±2
Gedaref	Female	1.9±0.4	ND	ND	7.0±0.1	33±6	12±2	ND
Halfa	Female	ND	11±2	1.9±0.7	0.9±0.2	19±4	ND	ND
Kassala	Female	ND	7.0±0.1	ND	5.0±0.9	9.0±0.1	12±3	ND
Gedaref	Male	ND	ND	3.3±0.2	2.4±0.1	4.7±0.1	ND	2.5±0.4
Halfa	Male	6.7±0.2	ND	ND	8.1±0.2	10±2	12±1	0.7±0.4
Kassala	Male	ND	ND	2.2±0.1	3.7±0.1	4.7±0.1	ND	2.5±0.5
Atbara	Female	ND	27±5	1.8±0.1	58±13	68±8	43±6	ND
Dongala	Female	ND	9.7±0.3	ND	29±2	72±9	36±2	0.8±0.2
Marawe	Female	ND	6.2±0.5	0.9±0.2	18±1	89±14	ND	0.8±0.6
Atbara	Male	6.4±0.1	15±2	19±4	140±17	140±8	12±2	2.5±0.1
Dongala	Male	ND	17±4	ND	88±11	35±3	15±3	ND
Marawe	Male	ND	22±6	ND	49±13	7.0±0.1	7.8±0.2	0.8±0.3
Al Fashir	Female	ND	ND	ND	ND	34±5	ND	ND
Al Obied	Female	1.5±0.4	4.8±0.1	ND	ND	36±4	ND	ND
Nyala	Female	ND	ND	ND	12±2	21±5	ND	ND
Al Fashir	Male	ND	ND	12±1	3.9±0.1	37±2	ND	1.2±0.2
Al Obied	Male	ND	ND	ND	5.8±0.1	28±6	ND	1.5±0.2
Nyala	Male	3.3±0.1	ND	ND	3.0±0.1	12±3	11±2	9.2±0.3
	Norms	≤10	0.5-2.8	≤4.5	2.0-5.3	0.5-2.9	≤4	≤0.9
	MDL's	0.7	0.5	2.5	1.5	2.4	3.9	0.2

## Results and Discussions

The concentrations and standard deviations of the quantity elements, P, S, Cl, K and Ca in the hair specimens are shown in Table 3. Phosphorus (P) was only found the hair of men residing in the eastern (1190±633) ppm and northern (940±892) ppm regions. More so, only n = 7 (3.8%) of the students had hair P concentrations that were within the normal values and n = 84 (45.9%) had concentrations less than the MDL of 1.6 ppm. These high average values indicate that the students originating from the eastern and northern regions were in contact with a P contaminating environment for long periods of time. It is also tentative

to compare the inter-gender and inter-location data since the standard deviations is at times approximately 50%.

The S concentrations of the Eastern and Northern regions are within the normal concentration levels. Those of the central and western regions are lower than the normal concentration values. Since the concentrations are not in excess, it can be concluded at this stage that these values are physiological (or ethical) in nature, and no S contamination occurred. Of the Cl concentrations values 92% were determinable. The average and standard deviation values were however 456±567, 735±667, 975±542 and 524±368 in ppm for the respective regions central, eastern, northern and western. These standard deviations in concentrations

Table 6. Linear adjusted regression correlation coefficients,  $-0.200r_{ad}0.200$ , of the elements found in the scalp-hair specimens. NA indicates that the correlation is not applicable.

	P	S	Cl	K	Ca	Cu	Fe	Mn	Zn	Co	Cr	Se	Br	V	Ti	Ni	Sc	As	Pb
P	NA	0.639	0.779	0.589	0.633	NA	0.276	0.223	-0.201	-0.366	NA	NA	NA	0.499	NA	0.275	NA	0.296	-0.326
S	0.639	NA	0.792	0.523	0.667	0.282	0.295	0.286	-0.246	NA	NA	-0.429	0.321	0.416	-0.239	0.327	NA	0.338	-0.544
Cl	0.779	0.792	NA	0.565	0.639	0.266	0.284	NA	-0.262	-0.274	NA	-0.347	0.300	0.431	NA	0.362	NA	0.246	-0.547
K	0.589	0.523	0.565	NA	0.763	NA	0.209	NA	NA	NA	NA	NA	NA	0.252	NA	0.205	0.339	NA	-0.284
Ca	0.633	0.667	0.639	0.763	NA	NA	0.471	NA	NA	-0.277	NA	-0.437	NA	0.298	-0.419	NA	NA	NA	-0.517
Cu	NA	0.282	0.266	NA	NA	NA	NA	NA	NA	-0.207	0.679	-0.231	0.663	0.392	0.276	0.679	0.295	0.308	NA
Fe	0.276	0.295	0.284	0.209	0.471	NA	NA	0.300	0.231	-0.348	NA	-0.415	NA	0.220	-0.320	0.210	NA	NA	-0.253
Mn	0.223	0.286	NA	NA	NA	NA	0.300	NA	0.286	NA	NA	NA	0.306	NA	NA	NA	0.214	0.202	NA
Zn	-0.201	-0.246	-0.262	-0.230	NA	NA	0.231	0.286	NA	-0.239	NA	0.213	0.458	0.225	NA	NA	-0.204	0.313	0.275
Co	-0.366	NA	-0.274	-0.239	-0.277	-0.207	-0.348	NA	-0.239	NA	-0.369	NA	-0.323	-0.413	NA	NA	NA	NA	NA
Cr	NA	NA	NA	NA	NA	0.679	NA	NA	NA	-0.369	NA	-0.221	0.368	0.444	0.473	0.727	0.397	NA	NA
Se	NA	-0.429	-0.347	NA	-0.437	-0.231	-0.415	NA	0.213	NA	-0.221	NA	-0.232	NA	NA	NA	NA	NA	0.646
Br	NA	0.321	0.300	NA	NA	0.663	NA	0.306	0.458	-0.323	0.368	-0.232	NA	0.605	NA	0.433	NA	0.612	NA
V	0.499	0.416	0.431	0.252	0.298	0.392	0.220	NA	0.225	-0.413	0.444	NA	0.605	NA	NA	0.620	NA	0.501	-0.336
Ti	NA	-0.239	NA	NA	-0.419	0.276	-0.320	NA	NA	NA	0.473	NA	NA	NA	NA	0.382	NA	-0.210	NA
Ni	0.275	0.327	0.362	0.205	NA	0.679	0.210	NA	NA	NA	0.727	NA	0.433	0.620	0.382	NA	0.350	0.394	NA
Sc	NA	NA	NA	0.339	NA	0.295	NA	0.214	-0.204	NA	0.397	NA	NA	NA	NA	0.350	NA	0.223	0.298
As	0.296	0.338	0.246	NA	NA	0.308	NA	0.202	0.313	NA	NA	NA	0.612	0.501	-0.210	0.394	0.223	NA	NA
Pb	-0.326	-0.544	-0.547	-0.284	-0.517	NA	-0.253	NA	0.275	NA	NA	0.646	NA	-0.336	NA	NA	0.298	NA	NA



Table 7. Correlation of the principal components, *pca*, and the elements for limitation (of 10% dependence)  $-0.100 \geq pca \geq 0.100$ . NA indicates that the dependency is not within the limitation.

Elem	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5	PCA 6
P	0.310	0.174	NA	0.222	NA	0.307
S	0.344	0.142	NA	NA	0.324	-0.149
Cl	0.356	0.141	NA	NA	0.180	NA
K	0.248	0.227	0.211	0.291	-0.194	0.141
Ca	0.300	0.316	NA	NA	-0.161	NA
Cu	0.211	NA	-0.188	NA	-0.434	-0.348
Fe	0.108	NA	-0.219	0.379	NA	-0.392
Mn	-0.183	NA	0.118	0.127	0.535	-0.342
Zn	NA	-0.213	-0.502	NA	-0.270	NA
Co	0.188	-0.342	0.310	-0.125	-0.226	NA
Cr	0.221	-0.326	-0.313	-0.144	0.121	NA
Se	-0.213	-0.117	NA	0.435	NA	0.351
Br	NA	-0.140	0.325	0.431	-0.173	-0.299
V	NA	-0.271	0.400	NA	NA	0.137
Ti	0.245	-0.338	0.207	NA	NA	NA
Ni	0.302	-0.188	-0.122	NA	NA	0.373
Sc	0.205	-0.376	0.113	-0.139	NA	-0.275
As	-0.243	-0.212	NA	0.423	-0.110	NA
Pb	0.165	-0.240	-0.267	0.256	0.352	NA

Table 8. Factor analysis in terms of the loadings (*l*) and communalities (Comm), *c*, after varimax rotation. The communality was limited to  $c \geq 1000$ .

El	FA 1	FA 2	FA 3	FA 4	FA 5	FA 6	Comm
P	386	191	-605	62	7	-51	557662
S	0	0	0	0	0	0	0
Cl	642	130	-222	8	133	-90	504382
K	302	759	-168	95	41	-12	706337
Ca	179	167	-71	206	-25	15	108318
Zn	-30	-11	5	17	2	-70	6240
Co	-2	7	-1	4	50	4	2582
$\sigma$	678593	665951	443212	54835	18052	14878	1885521
% $\sigma$	0	0	0.237	0	0	0	1

are relatively high and it is inferred at this stage that Cl contamination occurred in all four regions.

All the K concentrations are in excess of the normal values. A similar trend as with S and Cl is present in the K concentrations, that is, the concentration values for the central and western regions are lower than those of the eastern and northern regions.

The concentrations of Ca in the central ( $364 \pm 181$ ) ppm and western regions ( $630 \pm 149$ ) ppm are within the normal concentration range. As with the other element concentrations, the Ca concentrations of the eastern and northern regions were in excess of the normal values. Ca contamination might therefore have occurred in these regions.

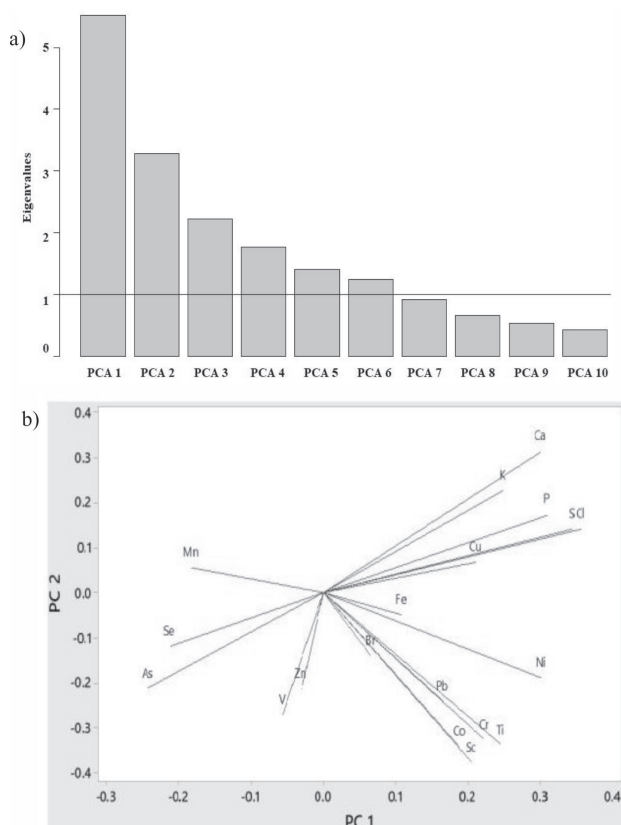


Fig. 2. Principal component analysis of the elemental concentrations: a) bar scree plot of the eigenvalues (variances), b) Plot of the eigen-values (variances) on PC1 and PC2.

Of the quantity elements, S was not in excess and the lower than normal concentrations are due to ethnic origins. The high P, Cl, K and Ca concentrations could only be due to contamination by compounds of  $\text{PO}_4^{3-}$ ,  $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , organic P and  $\text{POCl}_x$ .

The average concentrations and standard deviations of essential elements in the hair specimens, are shown in Table 4. Of the Cu concentrations, 84% was within the normal concentration range. Hair of students from the eastern and northern regions contained more Cu than those of the central and western regions, though these concentrations were within the normal values. Even though the average concentration for the eastern region ( $10.9 \pm 4.5$ ) ppm is marginally greater than that of western region ( $10.4 \pm 4.8$ ) ppm, the trend is similar to the trend of the quantity elements.

Similarly, 85% of the hair specimens contained Fe concentrations was within the normal values. Only hair from male students of the Bahri area had an Fe content of less than 0.2 ppm. In addition, hair of students from the eastern and northern regions contained more Fe than those of the central and western region, although these concentrations were within the normal values.

Mn was only found in  $n = 121$  (66.1%) of the students; and was not found in hair of most eastern and northern

regions students ( $n = 62$ ). Hence, concentrations of Mn in the hair of these students cannot be used in correlation. The concentrations of Zn, Co and Cr were relatively high; more than an order of magnitude in some instances. These high concentrations are of great concern as underlying metallic-caused diseases might be present. Se was only present in normal concentration ranges in hair of students from the western region. Br was only present in  $n = 34$  (19.6%) of the hair samples of the students.

Similarly, the concentrations of V, Ni, As and Pb, given in Table 5, were also found in low percentages of the hair samples. Ti concentrations were relatively high. Of grave concern is the relatively high concentrations of As and Pb in the hair samples.

The linear adjusted regression coefficients,  $-0.200 \geq r_{ad} \geq 0.200$ , inter-element correlation are given in Table 6. Concentration of P correlated positively with Cl (0.779), S (0.639), K (0.589), Ca (0.633) and Ni (0.499). There was high inter-element correlation (0.659) among the quantity elements P, S, Cl, K and Ca. Importantly, the high correlations of toxic elements As and Pb. As correlates positively with Se (0.646). However, the element concentration correlated negatively with those of S (-0.544), Cl (-0.547) and Ca (-0.517). Hence As is present with Se. The Co concentration was the most negatively correlated, which is evident from the abnormally high concentration values, whereas Mn was only positively correlated.

Interest was also focused on determining levels of variability in elemental concentrations of the elements such as P, S, Cl, K and Ca. In addition, the objective of this study to assess the concentration profiles of trace and toxic elements. The scree plot of the principle components (PCA) and the eigenvalues is shown in Fig. 2a). The eigenvalues were set to limit of unity. Consequently, six components were identified, which was indicative the wide variety of ores found in most of the regions. The dependence of the element concentrations on the first and second principal components is shown in Fig. 2b). The Mn concentration was in principle only negatively dependent on PCA 2. The quantity elements are equally dependent on PCA 1 and PCA 2. The promotive elements were positively correlated with PCA 1 and negatively correlated with PCA 2. K was the only element that was dependent on all six components. The Zn concentration correlated only negatively on the PCAs.

Factor analysis, in terms of the loadings ( $l$ ) and communalities ( $c$ ), is shown in Table 7. The S concentration, although  $l = 0$  and  $c = 0$ , was included to illustrate the element concentration had no communality; confirming the inference that the deviations in S concentration could be ascribed to ethnical differences. All the concentrations of the other quantity elements exhibited high communalities. Of the remaining elements only Zn and Co concentrations showed communalities of more than 1000.

## Conclusions

Although the students have been residing for a long period in the urban area of the capital city, they spent working holidays the rural regions. As there is little mining and agricultural activities *per se* in the urban areas, these students have thus been polluted during their holiday periods. The high concentrations of the toxic elements are of great concern. More so, even though the students after visual inspection appeared normal, such high toxic concentrations would have a detrimental effect on the organs in the body. Should such detrimental effects be found, then forensic investigations should be followed. It is recommended that further clinical evaluations should be performed to assess direct and indirect organ malfunctions.

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## Conflict of Interest

The authors declare no conflict of interest.

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