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Analysis of Heavy Metals in Human Scalp Hair Using Energy Dispersive X-Ray Fluorescence Technique

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ABSTRACT

Analysis of six heavy metals (Cr, Mn, Ni, Cu, Zn and Mo) in human scalp hair was carried out among various occupational distributions to ascertain their heavy metal burden, using energy dispersive x-ray fluorescence technique (ED-XRF). The result of the analysis shows that mean concentrations (mg/kg) of heavy metals obtained were as follows: Cr = 17.1 ± 12.7; Mn = 3.11 ± 0.50; Ni = 11.3 ± 9.3; Zn = 451 ± 128; Cu = 83.3 ± 35.8 and Mo is 9.16 ± 9.1.While the mean concentrations of Cr, Cu, and Mo were higher in the females, that of Mn, Ni and Zn were more in the males. Statistical analysis of the results for both genders at 0.05 probably shows significant difference for Ni, Zn and Mo while Cr, Mn and Cu showed no significant difference. The relationships between age, body mass, height, and heavy metal concentrations were also investigated. Statistical analysis of the results indicates that there was no correlation between the body mass ($R^2 \leq 0.048$), height ($R^2 \leq 0.002$) and heavy metal concentration in hair. Zn showed the highest deviation among other elements in the individual samples for both genders which reflect the individual variation in the concentration of Zn.

Keywords: Human Hair; Heavy Metals; X-Ray Fluorescence; Risk Assessment

1. Introduction

Hair is a site of excretion for essential, nonessential and potentially toxic elements. The amount of an element that is irreversibly incorporated into growing hair is proportional to the level of the element in other body tissues [1]. Therefore, hair analysis may provide an indirect screening test for physiological excess and deficiency of elements in the body. Clinical research indicates that hair levels of specific elements, particularly potentially toxic elements are highly correlated with pathological disorders. For such elements, levels in hair may be more indicative of body stores than the levels in blood and urine. Comparing hair-analysis with blood- or urine-analysis with the same purpose, a couple of factors such as simplicity of matrix, relatively high concentration of trace elements, easier sample gathering, transfer and storage should be considered [2,3]. Previous investigation has shown that high concentration of heavy metal in the hair indicates that the person is contaminated by that heavy metal. Although, blood or urine analysis may exhibit normal concentration level even though sample is gathered in the same time as hair, the analytical results for hair analysis should be observed more carefully [4]. Hair is not only a good index of exposure to elements because of the partitioning, it also allows for a non-invasive biological sample collection [5].

Heavy metal contamination requires adequate attention because of sporadic outbreak of epidemics and other endemic illnesses.

Although, some heavy metals were required in trace amounts to maintain the metabolism of the human body, especially iron that is an important component of haemoglobin—the pigment that transports oxygen in the red blood cells. Others such as Mn, Ni, Cu, Zn etc are essential micronutrients for life processes in plants and micro-organisms. Deadly diseases like oedema of eyelids, tumour, and congestion of the nasal membrane, muscular, reproductive, neurological, and genetic malfunction were caused by some of these heavy metals [6]. Apart from natural sources, significant amount of heavy metals in the soil come from anthropogenic activities [7]. Research has shown that Cr concentration may be partly attributed to the Cr content in tobacco leaves, from the soil and this may be more in smokers [8]. Researchers have found many correlations of essential elements to diseases, metabolic disorders, nutritional status etc. [9]. Hence, monitoring of heavy metals from human hair has been of interest to researchers in the fields of environmental chem-

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istry and medical science because the amount of heavy metals of hair samples can reflect the nutritional state of the person or the environment where that person resides or works [4]. Many analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), x-ray fluorescence, neutron activation analysis (NAA) and proton-induced x-ray emission (PIXE) have been widely used for analysis of hair samples [4]. In this research, energy dispersive x-ray fluorescence technique (EDXRF) was used for analysis of six (6) heavy metals (Cr, Mn, Ni, Cu, Zn and Mo) in human scalp hair due to its high sensitivity, low detection limit and as well as minimal loss of analyte of interest during digestion processes in the conventional method of AAS.

2. Materials and Method

2.1 Sampling

Freshly cut human scalp hair samples were collected from 50 individuals between the ages of 7 - 55 years (male and female) and across several occupational distributions within Makurdi town in Central Nigeria (latitude 7˚44' N and longitude 8˚31' E). The samples were quickly put in a pre-coded polythene bag, sealed tightly and kept for pre-treatment. A questionnaire was given to each respondent which contained a highlight of information on gender, age, occupation, population density of residential area, type of food consumed, water source, presence of refuse dump, behavioural pattern etc. prior to sample collection. Height and body mass of respondent were also measured and recorded alongside sample hair code at the time of collection.

2.2 Sample Cleaning

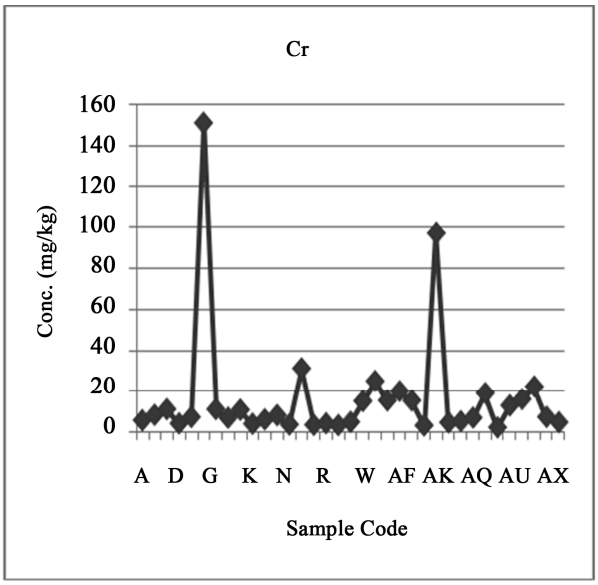
The hair samples collected were cut to about 200 - 250 mg by using stainless steel scissors rinsed in ethanol, then coded and stored. The stored samples were further cut into approximately 0.3 cm pieces and mixed to allow a representative sub sampling. These were washed according to the recommendation of International Atomic Energy Agency (IAEA) [10]: first in ethanol once, then three times in distilled water, once again in ethanol and followed finally in distilled water, accordingly. They were placed in crucibles and dried in the oven at 75˚C ± 5˚C for 15 - 25 minutes. About 0.1065 g of pre-treated hair sample was weighed using analytical balance (AB 54-S METTLER TOLEDO Model from Switzerland) and stored in an inert plastic container of about 10 cm^3 capacity, corked tightly and kept for EDXRF analysis.

3. Instrumentation and Procedure

The analysis was carried out in the XRF laboratory at the Centre for Energy Research and Training (CERT), Zaria, Kaduna State, Nigeria, using a Minipal 4 PW 4030/42B x-ray fluorescence with molybdenum tube and spinner. Minipal is a compact energy dispersive x-ray spectrometer designed for the elemental analysis of a wide range of samples. The system is controlled by a PC running the dedicated Minipal analytical soft ware. The Minipal 4 version in use is an energy dispersive micro processor controlled analytical instrument designed for the detection and measurement of elements in a sample (solids, powders and liquids) from sodium to uranium. The weighed samples for EDXRF analysis were ground in an agate mortar and a binder (PVC dissolved in toluene) was added to the sample, carefully mixed and pressed in a hydraulic press into a pellet. The pellet was loaded in the sample chamber of the spectrometer and voltage (30 kV) and a current (1 mA) applied to produce the x-rays to excite the sample for a preset time of 10 minutes. The spectrum from the sample was then analysed to determine the concentration of the elements in the sample. The Minipal 4 x-ray had an excitation system of 30 KV maximum, a minimum and maximum current of 1 μA, 1 mA respectively with a power rating of 9 W. It is fitted with thin film circle sample support (cup) with diameter of 63.5 mm where the pelletised hair sample was placed. The primary beam filters were arranged in twelve positions for optimum function across the periodic table. Eleven beam filters were filled with hair sample while the twelfth position the certified reference material (CRM) hair. The XRF had silicon-lithium (Si-Li) diode detector. The collimator, the target and the source of radiation had close coupling to the detector which increases the versatility, and high x-ray output with lower power tube [10]. Quality assurance energy calibration was first performed. A quality control system was employed to ensure data quality by using a certified reference human hair (GBW 09101) obtained from Shanghai Institute of Nuclear Research, Academia, Sinica, Shanghai 201849, China. The CRM was placed in the twelfth position and run concurrently with each running of the hair sample for individual elemental concentration.

4. Results and Discussion

Variation of concentration of heavy metals in human hair can be attributed to human history [11]. Hence, individual's burden of heavy metal concentrations reflects the extent of the person's exposure to atmospheric pollutants, intakes of food and metabolism. The distribution pattern of various heavy metals among the males hair samples investigated is shown in **Figure 1** while that of female is presented in **Figure 2**. The total mean concentration of individual heavy metals in males and females hair samples is presented in **Table 4**, which showed that the concentration of Zn is comparably higher than all the other



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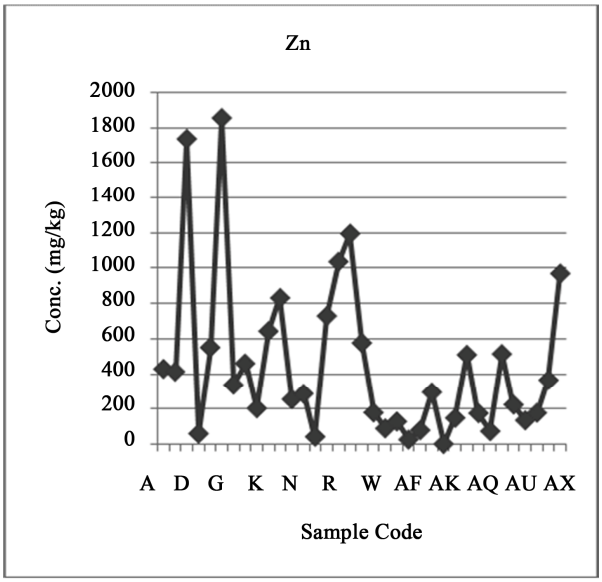

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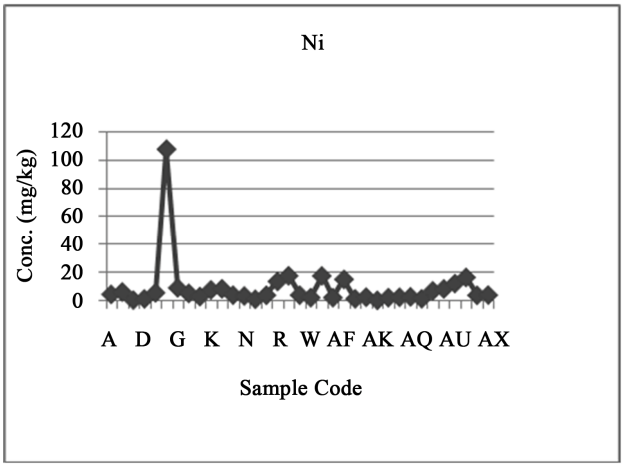

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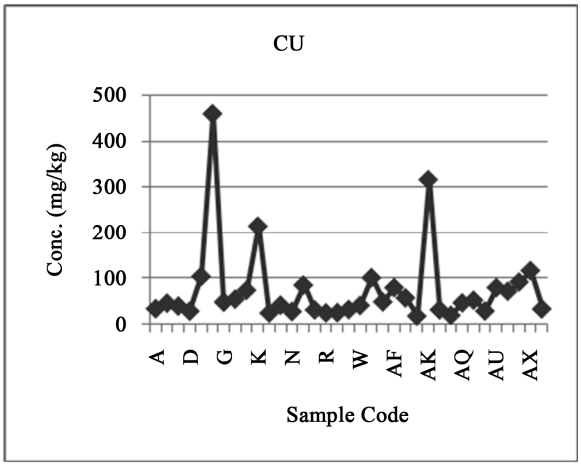

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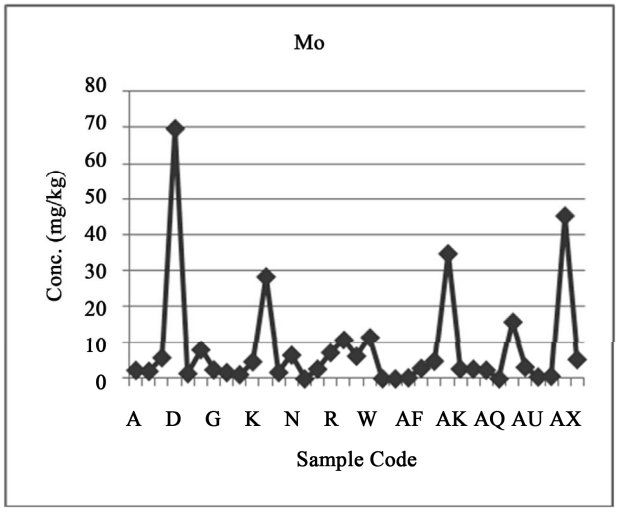

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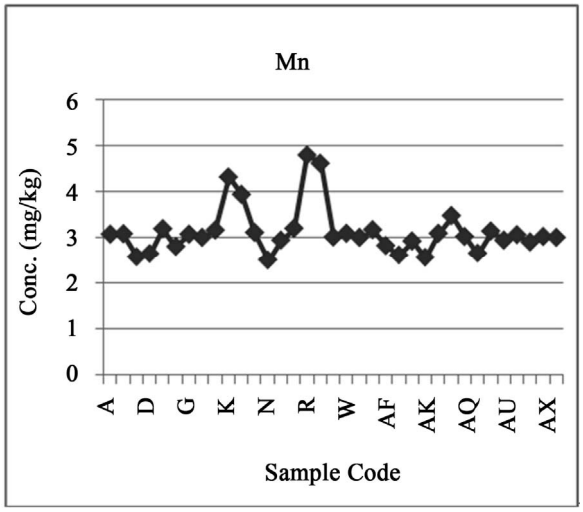

(f)

Figure 1. Mean concentration of individual heavy metals in hair samples from males.

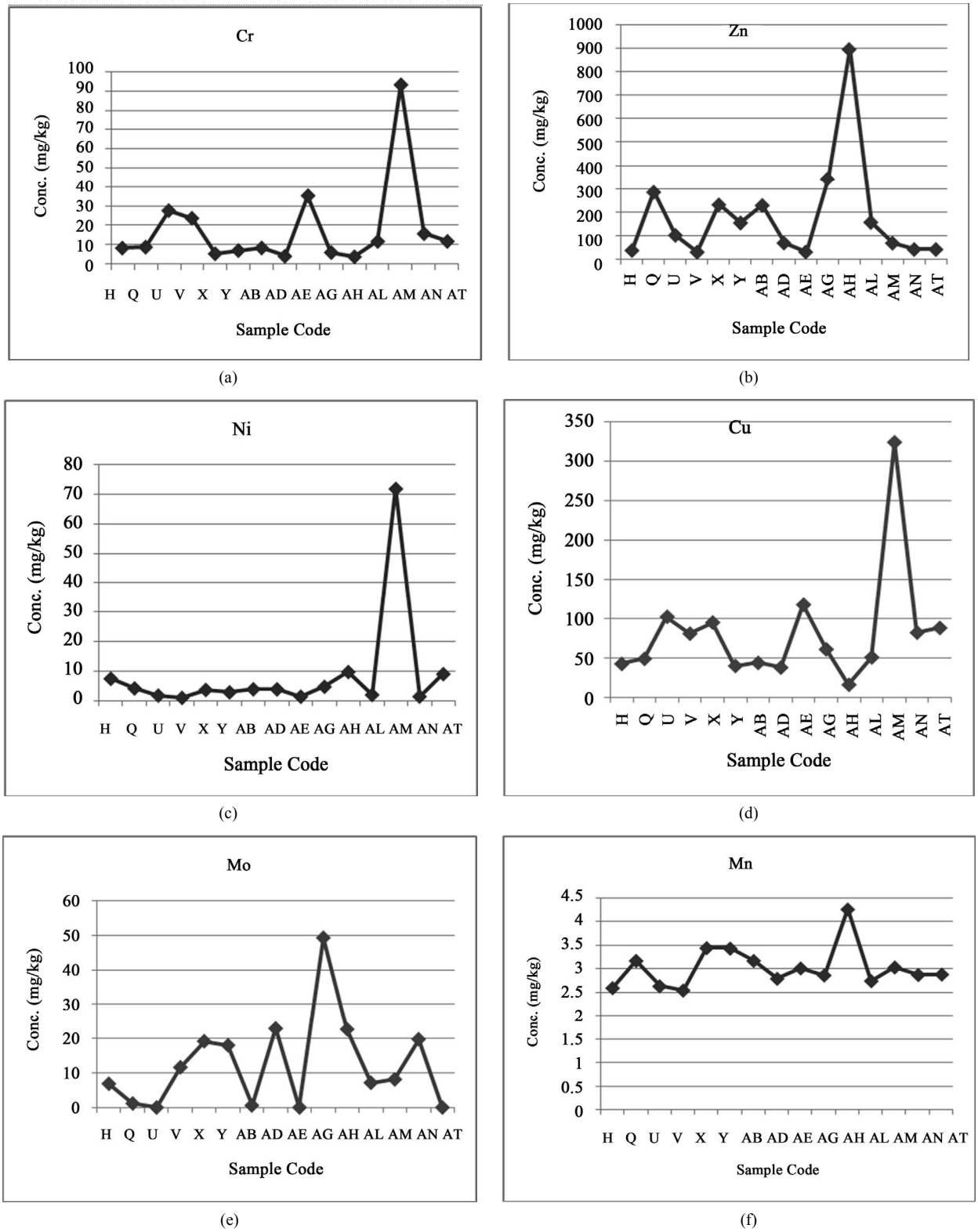

Figure 2. Mean concentration of individual heavy metals in hair samples from females.

elements while Mn has the least. There was however an outlier in the heavy metal concentration in sample code F in the males' hair sample for Cr, Ni, Zn and Cu which were 151.2, 107.9, 1,850.1 and 460.1 (mg/kg) respectively as shown in **Figure 1**. This might be related to the nature of the individual's work, which also has the long-

est exposure time based on the completed questionnaire. Sample AM showed higher peaks for Cr, Ni, and Cu; samples AH was higher in concentration of Mn and Zn while AG was higher for Mo concentration in females' hair samples as shown in **Figure 2**.

The analytical process for the analysis of heavy metals in human hair was carried out successive to the analysis of the certified sample with each cycle of beam filter arrangement. The adjusted value was used as a factor for converting the concentrations of other samples analysed. The relationships between age, body mass, height and heavy metal concentrations were investigated. Statistical analysis of the results indicates that there was no correlation between the body mass ($R^2 \leq 0.048$), height ($R^2 \leq 0.002$) and heavy metal concentration in hair. However, it is generally believed that the amount of heavy metals in human body should correlate with age as a result of bioaccumulation [11,12]. These toxic metals have relationship with duration of exposure (age) since their effects are cumulative [13]. **Tables 1 and 2** indicate the age distribution for concentration of heavy metals under investigation between male and female genders. The mean concentrations of heavy metals for the fifty samples are presented in **Table 3**.

Zn showed the highest concentrations (mg/kg) in both genders between ages 7 - 20 with values of 646 ± 297 (mg/kg) and 384 ± 361 for males and females respectively. While Mo has the lowest value of 2.47 ± 2.1 (mg/kg) for males and Mn was 2.88 mg/kg for females. **Table 4** shows their total distribution in which Zn has the highest over all concentration of 451 ± 128 mg/kg while Mn has the over all lowest value of 3.11 mg/kg for this analysis method. Afridi *et al.* [14] reported values of 224.0 ± 7.07 and 214.14 ± 8.2 mg/kg for Zn between the ages 30 - 45 and 46 - 60 years respectively using flame atomic absorption spectrometer (FAAS) to analysed scalp hair of non hypertensive group. The value of Ni within age range of 32 - 42 years for this work is 7.54 mg/kg. Ni was also reported to be 6.79 mg/kg for ages 30 - 45 years in a similar biological matrix *i.e.* human hair [14]. **Table 4** shows heavy metal distribution (mg/kg) in 50 human hair samples. Some elements such as Zn, Cr, Mn, Cu and Mo are important in human nutrition. These elements are also present in pharmaceutical products. This is because a wide variety of metals mostly transition elements are employed as complexes or salts to act as catalysts, therefore, any pharmaceutical excipient whose synthesis involves the use of one or more metal catalysts may

Table 1. Age distribution and level of heavy metals in male hair samples.

Range (years)	Number of male	Heavy Metals (mg/kg)					
		Cr	**Mn**	**Ni**	**Cu**	**Zn**	**Mo**
7 - 20	4	5.77 ± 1.1	3.27 ± 0.5	5.59 ± 2.2	50.9 ± 43.4	646 ± 297	20.3 ± 20.3
21 - 31	19	14.8 ± 21.5	3.16 ± 0.6	5.55 ± 5.4	57.6 ± 66.4	356 ± 341	8.9 ± 16.8
32 - 42	7	10.3 ± 5.7	3.16 ± 0.6	7.54 ± 5.1	85.0 ± 61.4	523 ± 579	2.47 ± 2.1
≥43	5	39.2 ± 63.0	2.92 ± 0.1	7.54 ± 5.1	140 ± 181	600 ± 707	3.71 ± 2.9

Table 2. Age distribution and level of heavy metals in female hair samples.

Range (years)	Number of female	Heavy Metals (mg/kg)					
		Cr	**Mn**	**Ni**	**Cu**	**Zn**	**Mo**
7 - 20	4	5.74 ± 2.1	3.27 ± 0.7	5.52 ± 2.8	40.2 ± 18.5	384 ± 361	23.8 ± 19.9
21 - 31	10	23.8 ± 26.3	2.95 ± 0.3	9.72 ± 21.9	98.8 ± 83.7	113 ± 91	9.17 ± 7.7
32 - 42	0	-	-	-	-	-	-
≥43	1	11.0	2.88	8.97	88.4	40.7	-

Table 3. Age distribution and level of heavy metals in the 50 hair samples.

Range (years)	Heavy Metals (mg/kg)					
	Cr	**Mn**	**Ni**	**Cu**	**Zn**	**Mo**
7 - 20	5.75	3.27	5.55	45.5	515	22.1
21 - 31	17.9	3.09	6.99	71.8	269	8.99
32 - 42	10.3	3.16	7.54	85.0	523	2.46
≥43	34.6	2.92	25.2	131	506	3.09

Table 4. Heavy metal distribution in 50 hair samples.

Heavy metals (mg/kg)	Male	Female	Total average
Cr	17.52 ± 14.9	13.81 ± 9.18	17.1 ± 12.7
Mn	3.13 ± 0.15	3.03 ± 0.21	3.11 ± 0.5
Ni	6.56 ± 19.14	8.07 ± 2.24	11.3 ± 9.3
Cu	83.4 ± 40.5	75.8 ± 31.3	83.3 ± 35.8
Zn	531 ± 127	179 ± 181	451 ± 128
Mo	9.26 ± 8.18	17.6 ± 8.18	9.16 ± 9.1

contain residual metal(s) in form of the original catalyst(s) or as derivative [13]. This may accumulate in human body when taken which may add to this metal content in the body.

Zinc showed the highest deviation among other elements in the individual samples for both genders which reflects the individual variation in the concentration of Zn. Mo showed the highest coefficient of variation while Mn indicated the least. This implies that Mn is more closely distributed among the individual samples than Ni and other elements discussed. The mean concentration of heavy metals in both males and females were compared statistically at 0.05 probability for the method used. Significant difference was found between both genders ($P > 0.05$) for Ni, Zn and Mo while Cr, Mn and Cu showed no significant difference.

There was observed variations in the concentration of individual elements in both genders with the highest found among the females for Mo and Ni but Cr, Mn, Cu and Zn were more distributed in the male population. The exposure of hair to the environment and the chemical treatment of hair for cosmetic purposes, which leads to increase or decrease in metal concentration (external contamination) could account for this observation [15,16]. It was also asserted that there is personal difference in concentrations of heavy metals in the human hair according to human life or history such as occupation, sex, age, food, habit, social condition [11]. Individual's deviation of heavy metal concentrations reflects the degree of environmental pollutants exposure to the human body and intakes of food. Meaning that, there is potential for human exposure to heavy metals from drinking contaminated water or eating fish from contaminated water bodies [17-19]. They are finally transferred to other animals including humans through the food chain.

5. Conclusion

Analysis of Cr, Mn, Ni, Cu, Zn and Mo in human hair has been carried out to ascertain the accumulation of heavy metals. The mean concentrations of heavy metals showed that all the 50 hair samples were contaminated with the elements investigated. The mean concentrations of Ni and Mo were determined to be higher in the females' samples while that of Cr, Mn, Cu, and Zn were more in the males. Samples F showed higher values for Cr, Ni, Zn, Cu and D for Mo respectively among male gender while samples AM, AH and AG also showed higher values for (Cr, Ni, Cu), (Mn, Zn) and Mo respectively among female gender. These samples use the same means of cooking *i.e.* fire wood and similar sources of drinking water (well, bore hole, tap) and consume the same class of food, which actually reflect the life history, nutritional pattern as well as the environmental content of the individuals. The pattern of concentration of the heavy metals in the hair samples were in decreasing order of Zn > Cu > Cr > Ni >Mo > Mn.

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