Analysis of some trace metals in human hair using atomic absorption spectrophotometry for forensic applications.

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Abstract

Hair samples collected from thirty-four (34) individuals in Wudil Local Government Area of Kano State Nigeria with respected to age, sex and smoking habits were analyzed for trace metals (Ca, Zn, Cu, Cd, Cr, Mg and Pb) using Atomic Absorption Spectrophotometric (AAS) technique. The result obtained indicated the mean concentrations of trace metals in the human hair of both male and female respondent follow similar trend as Ca>Zn>Cu>Pb>Cr>Mg>Cd. The levels of Ca and Mg were found higher in female respondents compared to the men. Also, the standard deviation obtained for the level of Pb and Cd were higher in males while Cr was found to be higher in females. Cd and Ca shows a gradual increase as it rises thorough the studied age groups, and the abruptly drops at the last age group (≥ 41). Cu was also found to have the highest concentration in individuals of the highest age group (≥ 41). Age and smoking habits also had great influence on trace metal hair concentrations among the Wudil inhabitants. The results of the study may provide information regarding hair sex, age, and habit of the individuals during forensic investigations.

Keywords: Atomic absorption spectroscopy, Trace metals, Human hair, Forensic application

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Introduction

Human hair is one of the most frequently found pieces of evidence at the scene of a violent crime. It may also be may be transferred from the suspect or the suspect's clothes to the victims' and vice versa. It can provide a link between the criminal and the act. Hair have gained a wide interest in its examination for various fields counting taxonomy, zoology, wildlife, clinical biology, dermatology and forensic investigation due to its non-invasive and quick analysis along with précised and accurate results [1]. The forensic evaluation of hair can be very important in the physical evidence examination and assessment by representing the association between the suspect flanked due to a crime scene or a victim and a suspect or representing that no evidence exists for an involvement connecting a criminal with a crime scene or a culprit with a victim [2].

Various techniques have been employed in the detection of trace elements present in human hair. Among others, atomic fluorescence spectrometry inductively coupled plasma atomic emission spectrometry (ICP-AES) spectrofluorimetric and differential pulse anodic stripping voltammetry (DPASV), Particle – induced X-ray emission (PIXE), Energy dispersive spectrum (EDS), energy dispersive X-Ray fluorescence, (EDXFA), Electro Thermal Atomic Absorption Spectroscopy (ETAAS), neutron activation analysis (NAA), microscopy and DNA analysis have been used widely used for forensic hair analysis [2,3-15]. It has been established that there is personal difference in concentrations of trace elements in the human hair according to human life or history such as occupation, sex, age, food, habit, social condition and so on [4]. Literature on the use of atomic absorption spectrometry technique in the determination of trace elements in human hair for forensic investigation is scanty. The use of AAS technique to detects and measures the content of heavy metals and minerals of the hair samples recovered from crime scene may offer inexpensive and fast way of getting information about the questioned hair samples. It will also give the actual nutritional status, as well as the quantity of stored and accumulated toxins stored in the organism.

The study is aimed at evaluating the concentration of some trace metals (Ca, Zn, Cu, Cd, Cr, Mg and Pb) in scalp hair samples of selected groups of people in Wudil Local Government Area (Lat. 11° 49'N, Log. 8° 51'E) of Kano State of Nigeria using Atomic Absorption Spectrophotometric(AAS) technique. The relationship between age sex and smoking habits with trace metals among the selected population was evaluated with a view of providing information regarding hair sex, age, and habit of individuals during forensic investigations.

Materials and Methods

Materials

All reagents (absolute ethanol, HNO_3 and 20% H_2O_2) used were analytical grade reagents obtained Sigma Aldrich company U.S.A. Double distilled water was used throughout for the preparation of samples and dilution of the stock standard solutions. The glasswares used in the study were decontaminated by overnight soaking in 5% HNO_3. Atomic Absorption Spectrophotometer (AAS Unicam 969) was used for the determination of the trace metals.

Sample collection

Freshly cut human hair samples were collected from the head of 34 individuals (23 males and 11 females) between the ages

of 7 to ≥ 41 years residing Sabon Gari Wudil in Wudil Local Government Area of Kano State. All individuals used in the study agreed to participate in this study. A questionnaire given to all participants provided the following information: sex, age, smoking, nutritional habits, water source and occupational exposure to heavy metals.

Sample cleaning/digestion

The hair samples collected were cut to about 200-250 mg using stainless steel scissors rinsed in ethanol, then coded and stored. The stored samples were further cut into approximately 0.125inch (0.3 cm) pieces and mixed to allow a representative subsampling of the hair specimens and were washed according to the recommendation of International Atomic Energy Agency (IAEA) [16]. Exactly 0.1065 g of hair sample was weighed accurately into a 50 ml crucible. The sample was covered with 8 ml conc. HNO_3, after which the crucible was covered with the crucible lid and placed on a hot plate. Hair was digested at 70°C -85°C for about 25 minutes or until the hair is completely digested and the solution becomes clear. The crucible was not allowed to go dry until the digestion was complete. After cooling to room temperature inside the fume hood, 1 ml of 30% H_2O_2 was added to each sample, and heated again on hot plate at the lowest setting (first setting i.e., 42°C) just until bubbling stops. After this, heat was increased to about 80°C or as needed until the volume is reduced to about 2.5 ml [17]. The contents of each crucible were quantitatively transferred to a cleaned and dried 100 m volumetric flask. The digestion vessel was rinsed three times with 1.5 ml each with deionized water and added to the volumetric flask and made up to volume with deionized water. It was then transferred to a cleaned sample bottle, corked, labelled well and stored in the refrigerator until ready to be analyzed. Standard solutions of all the metals investigated were prepared from concentration levels of 1-20 ppm.

Determination of metal contents in hair samples

The contents of the trace metals were determined using the method of atomic absorption spectrometry - (AAS, Unicam, 969). The instrument was previously calibrated with standard solutions (1000 mg/l).

Results

The results of trace metal measurements were analyzed for a total of 35 healthy individuals who met the selection criteria set by the researchers. The characteristics of the participants, Including gender and age, are presented in Table 1. Table 2 gives the mean concentration of trace metals in the hair samples collected from Wudil with respect to the studied age groups (7-20, 21-30, 31-40 and ≥ 41). The distributions of the variation of the trace metal with age are presented pictorially in Figure 1. The results obtained indicated a regular change in the concentration of the studied trace metals with age.

The mean results from AAS measurements to study the effect of sex on the trace metal concentration in hair samples collected from a total of 11 women and 24 men are presented in Table 3 while Figure 2 gives the graphical variation of the mean concentration of trace elements in hair samples from Wudil. From the results presented, it can be seen that the variation of the mean concentration for the studied trace metals of both male and female respondent follow similar trend as Ca>Zn>Cu>Pb>Cr>Mg>Cd.

The mean concentrations of heavy metals in the human hair samples from smokers and non-smokers are as presented in Table 4 and graphically in Figure 3. It can be seen from the results obtained that the mean concentration of both smokers and non-smokers follow the pattern Ca>Zn>Cu>Pb>Cr>Mg>Cd.

Discussion

Influence of age on mean metal levels in human hair

Table 2 gives the mean concentration of trace metals in the hair samples collected from Wudil with respect to the studied age groups (7-20, 21-30, 31-40 and ≥ 41). The distribution of the variation of the trace metal with age are presented pictorially in Figure 1. The results obtained indicated a regular change in the concentration of the studied trace metals with age. Age group 7-20 years was the one with the lowest calcium concentration. This may be due to the fact that human bones need a lot of calcium during childhood and at young age. A lot of calcium are used up by the organism for bone formation in this new age group hence accounting for the low values obtained by the research [18].

Zn, Mg and Cr have their highest concentration at age group 21-30. Zinc plays an important role in immunity, would healing, reproduction, neurotransmission and metabolism of proteins and carbohydrates and hence, as a higher concentration in middle aged individuals [13]. Mg and Cr are used in electroplating and are more abundant in individuals of working class age range [19].

Cd and Ca shows a gradual increase as it rises thorough the age groups, and the abruptly drops at the last age group (≥ 41).

Table 1. *Age and gender of people who participated in this study.*

Age	Number of Subjects			
	Male	Female	Number of Smokers	Total
7-20	5	3	0	8
21 – 30	6	4	3	10
31 – 40	7	3	3	10
≥ 41	5	1	1	6
Total	23	11	7	34

Table 2. *Mean concentration of heavy metals in hair samples from Wudil with respect to age groups.*

Range	Cd	Ca	Zn	Cu	Mg	Pb	Cr
7 – 20	1.15 ± 0.077	403.50 ± 17.18	164.95 ± 10.94	10.48 ± 1.50	3.03 ± 0.29	8.30 ± 1.09	2.64 ± 0.13
21 – 30	1.59 ± 0.12	444.78 ± 21.45	205.55 ± 16.77	13.16 ± 2.62	4.99 ± 0.89	7.801 ± 2.61	4.312 ± 0.12
31 – 40	2.132 ± 0.12	515.71 ± 30.72	175.18 ± 13.26	12.48 ± 3.37	1.40 ± 0.28	6.71 ± 1.68	3.91 ± 0.18
≥ 41	1.86 ± 0.07	440.52 ± 19.17	178.65 ± 11.79	16.46 ± 3.59	2.92 ± 0.66	7.69 ± 0.41	3.14 ± 0.21

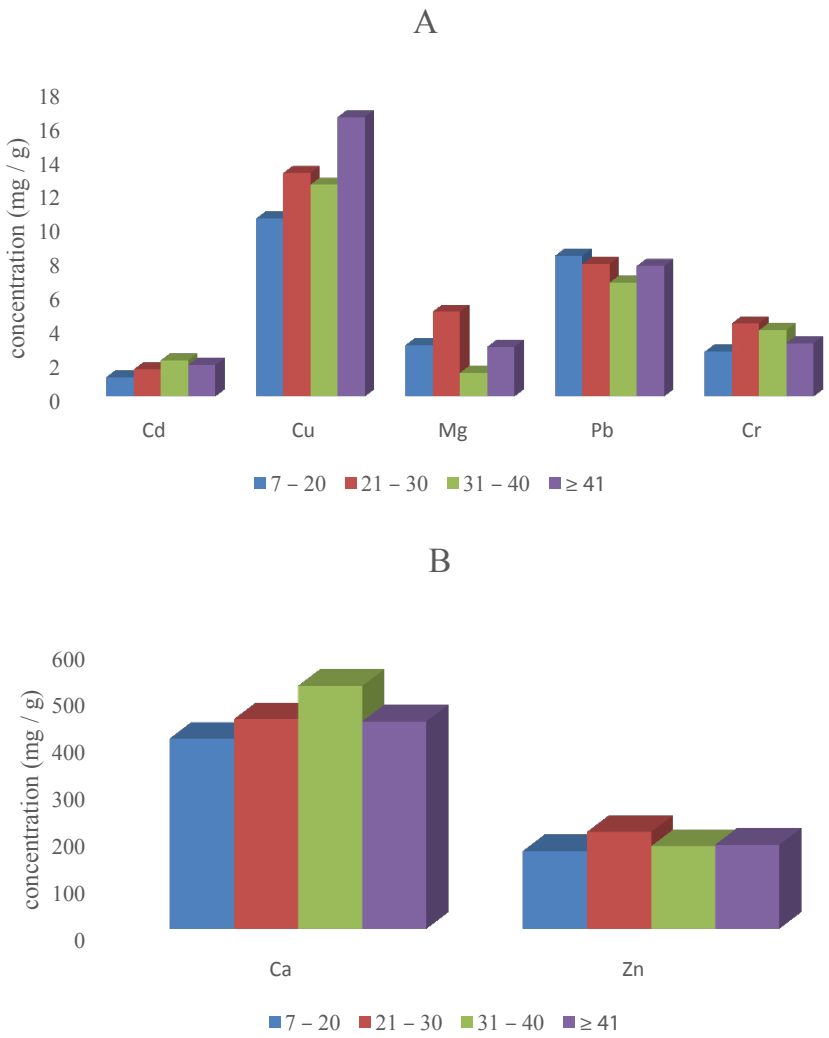

Figure 1. *Relationship between age and mean metal values in hair samples from Wudil.*

This increase could be as a result of environmental exposure (as children and old persons are rarely exposed to environmental conditions compared to middle aged individuals). Pb is has the highest mean concentration in individuals in the 7-20 age bracket. It then reduces gradually as the age group increases but increases sharply at ≥ 41. Pb gets into the body through air, food and water [20]. Contaminated food is the easiest source of lead intake. Children and old persons are more disposed to consuming food contaminated with lead without concern as shown in the result. Cu has the highest concentration in individuals of the highest age group (≥ 41). Cu is the most common environmental metal and is essential in cellular metabolism, but its critical doses can cause anemia, hair loss, arthritis, cancer, depression, elevated cholesterol, heart attack, hypertension and some other ailments known to individuals of old age, hence its elevated levels in individuals of the highest age range shows that they are prone to these ailments characteristic of advanced age [21].

Influence of sex on mean metal levels in human hair

The mean results from AAS measurements to study the effect of sex on the trace metal concentration in hair samples collected from a total of 11 women and 24 men are presented in Table 3. Figure 2 gives the graphical variation of the mean concentration of trace elements in hair samples from Wudil. From the results presented, it can be seen that the variation of the mean concentration for the studied trace metals of both male and female respondent follow similar trend as Ca>Zn>Cu>Pb>Cr>Mg>Cd. However, the mean concentration of Cd, Zn, Cu, and Pb were found to be higher in the male respondents. It has been reported that Pb accumulation in organ tissues (hair, liver, blood, etc.) is higher in male than in female individuals [22]. These differences in Pb and Cd levels between male and female individuals could be associated with differences in lifestyle and occupation-related Pb exposure.

It was also found that the levels of Ca and Mg were in female respondents compared to the men. Females have a higher risk of having osteoporosis (bone disease that makes the bone become fragile), hence a higher level of Calcium and Magnesium is needed by them since these metals are essential to the formation of strong bones [23]. The higher level of magnesium in the female respondents could also be as a result of the use of hair dye. Other metals having higher mean concentrations in the male respondents could be as a result of environmental exposure. The standard deviation is higher in males for Pb and Cd while it is higher for Cr in females. This indicates that the deviation in the individual content of heavy metals is more pronounced in females compared to males.

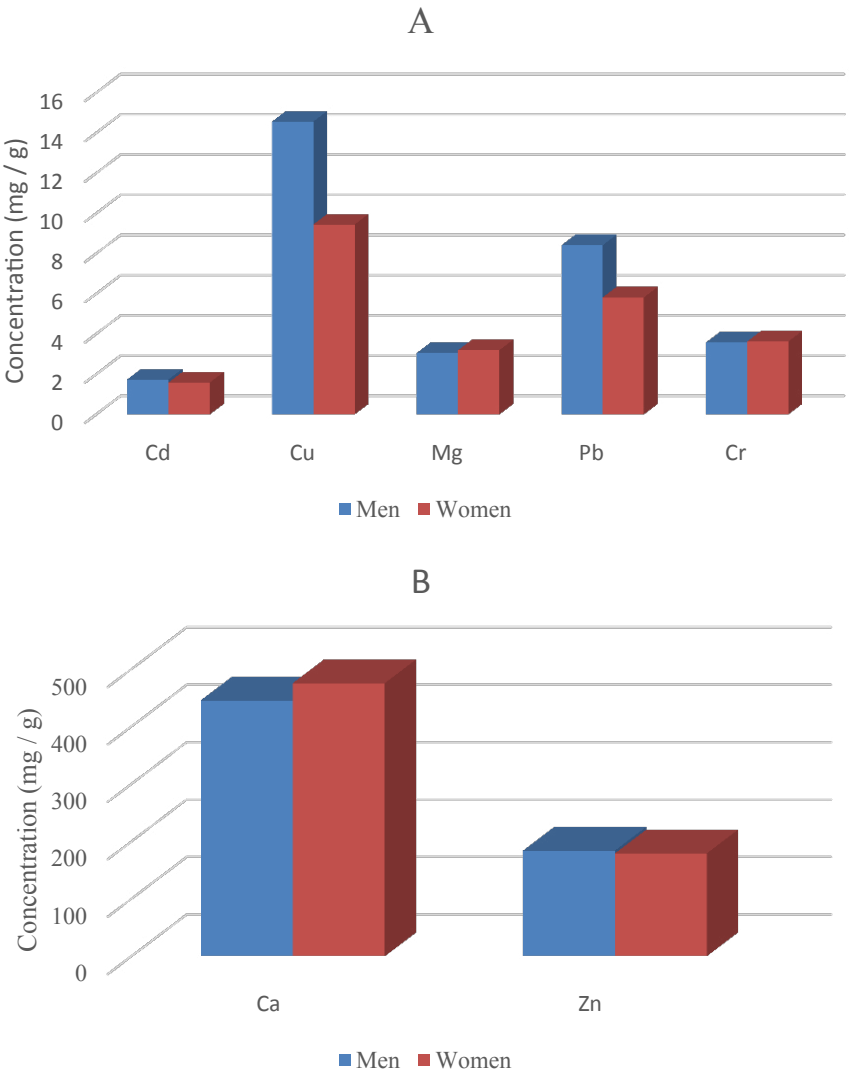

Figure 2. *Relationship between sex and mean metal values in hair samples from Wudil.*

Table 3. *Mean concentration of heavy metals in hair samples from Wudil with respect to sex groups.*

Sex	Cd	Ca	Zn	Cu	Mg	Pb	Cr
Male	1.74 ± 0.40	445.41 ± 44.53	183.89 ± 21.34	14.58 ± 2.67	3.06 ± 1.47	8.42 ± 1.35	3.58 ± 0.64
Female	1.59 ± 0.36	475.60 ± 51.48	179.02 ± 19.63	9.45 ± 1.33	3.20 ± 1.67	5.82± 1.34	3.63 ± 0.81

Table 4. *Mean concentration of heavy metals in hair samples from Wudil with respect to smokers and non-smokers groups.*

Habits	Cd	Ca	Zn	Cu	Mg	Pb	Cr
Non – Smokers	1.64 ± 0.39	448.30 ± 48.66	175.93 ± 16.07	12.03 ± 3.14	3.02 ± 1.40	7.21 ± 1.62	3.48 ± 0.71
Smokers	1.88 ± 0.35	481.71 ± 39.05	206.94 ± 18.20	16.30 ± 1.41	3.44 ± 2.00	9.01 ± 1.91	4.02 ± 0.36

Sharma et al. [18] in their research to compare the effect of sex on heavy metal concentration in hair observed that male hairs contain more amount of Pb and Cu while the females have highest amount of Ca. These results have been corroborated in our study.

Influence of smoking on mean metal levels in human hair effect of smoking

The mean concentrations of heavy metals in the human hair samples from smokers and non-smokers are as presented in Table 4 and graphically in Figure 3. It can be seen from the results obtained that the mean concentration of both smokers and non-smokers follow the pattern Ca>Zn>Cu>Pb>Cr>Mg>Cd. All the trace metals determined however have higher mean concentrations in smokers as compared to non-smokers which is as expected.

The observation on Cu is in a good agreement with literature reports [23,24]. A higher Cu in smokers could be due to an increased release of corticosteroids and catecholamine, which are known to influence the body Cu status [25]. For Zn, we observed only a slight, yet significant increase of Zn among the smokers.

Cd is known to be higher in smokers, because tobacco leaves naturally accumulate Cd and are the known source of Cd exposure [26]. Other reports in literature have shown the similar outcomes [27-30].

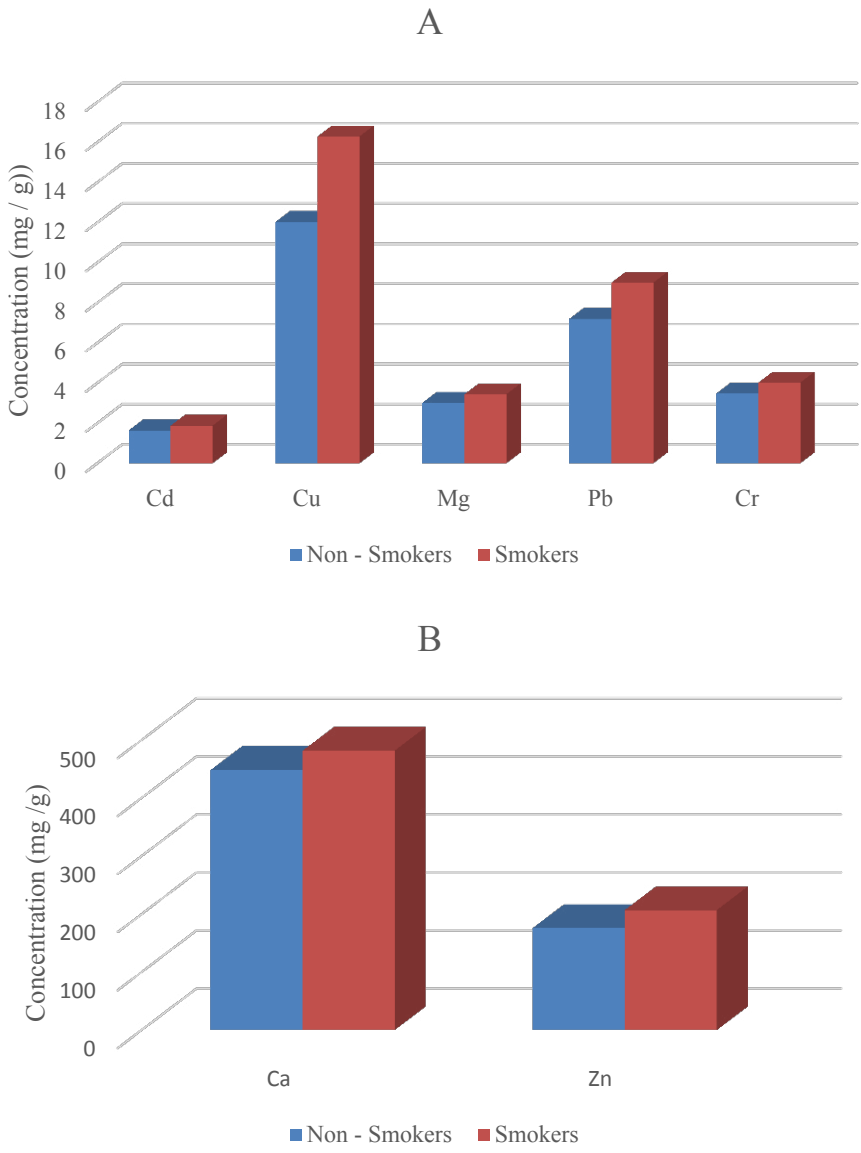

***Figure 3**. Relationship between smoking habits and mean metal values in hair samples from Wudil.*

The higher Mg concentration found in hair samples taken from smokers can be explained by the fact that tobacco contains magnesium which participates in photosynthesis as an integral part of chlorophyll to a lower degree, and to a higher degree was found in the form of other compounds [31].

Conclusion

From the current study, the following conclusion can be drawn:

1. Age, sex and smoking habits have strong dependence on the trace metal concentrations in Wudil, Kano State Nigeria.
2. The mean concentration of trace metals of both male and female respondent follow similar trend as Ca>Zn>Cu>Pb>Cr>Mg>Cd.
3. Zn, Mg and Cr have their highest concentration in the hair of individual between the age group of 21- 30 while Pb is has the highest mean concentration at the lowest age group (7-20).
4. Cu has the highest concentration in individuals of the highest age group (≥ 41).
5. All the trace metals studied had a higher mean concentration in smokers compared to non-smokers.
6. The analysis of trace metals on human hair recovered from crime scene may give forensic investigators clues about the source individual.

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