WORLD SCIENCE RESEARCH JOURNALS

World Journal of Engineering and Physical Sciences Vol. 3 (4), pp. 043-050, December 2015
Available online at http://wsrjournals.org/journal/wjeps
ISSN 2331-1878 ©2015 World Science Research Journals

Full Length Research

Comparative assessment of chromium level in human scalp hair using atomic absorption spectrophotometry and x-ray fluorescence techniques

Ishaq S. Eneji*, Peter O. Onuwa and Rufus Sha'Ato

Department of Chemistry and Centre for Agrochemical Technology, University of Agriculture, P. M. B. 2373, Makurdi, Benue State, Nigeria.

Accepted July 08 2015

This study was carried out to determine Chromium in human scalp hair samples using Atomic Absorption Spectrophotometry (AAS) and X-ray Fluorescence (XRF) techniques. The results of the analysis showed that the mean concentrations (mg/kg) of chromium in human scalp hair using AAS and XRF were; 2.82±3.11 and 16.90±26.7, respectively. The concentrations (mg/kg) in male samples were 3.28±3.43 for AAS and 16.34±28.6 for XRF. Similarly, female concentrations of Cr were 1.72±1.86 and 18.2±22.7 for AAS and XRF, respectively. Based on the comparison between the two gender in each method, no significant difference was found in Cr concentration between the male and female gender (p> 0.05) using the same method. However, significant difference was observed in Cr concentration between the same and different gender for AAS and XRF methods, respectively. The results suggest that XRF coupled with an appropriate quality controlled method would be more suitable for the analysis of Cr in human hair.

Key words: Atomic absorption spectrophotometry (AAS), ANOVA, chromium, concentration, human hair, x-ray fluorescence (XRF).

INTRODUCTION

The determination of poison in human hair was first published in the 1850s when the presence of Arsenic was reported in the hair of a body exhumed after 11 years. Since then, several literatures have been published on the analysis of heavy metals and drugs in hair (Cooper et al., 2012). It is important to note that human hair is an attractive biological material due to the simplicity of sampling, transport and handling, as well as providing information about the concentrations of some heavy metals that is considerably more concentrated in hair than in other biological materials, which makes analysis easier (Zhunk and Kist, 1995). Scalp hair is the metabolic end product that has recognized ability to reflect the body metal burden. The quantification of heavy metals in hair has been used for the assessment of long – term environmental and occupational exposure to heavy metal, as well as metabolic status (Perumal and Thangamani, 2011). Therefore, human hair analysis is a more reliable and convenient biological indicator of environmental pollution than blood or urine analysis. Various methods have been used in the determination of heavy metal concentrations in human hair, especially Cr. Among others, atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma atomic emission spectrometry (ICP–AES), spectrofluorimetric, differential pulse anodic stripping voltammetry (DPASV) and X – ray fluorescence (XRF) are widely used for heavy metal analysis. Other modern analytical methods could also be used to determine longitudinal profiles of the metal in human hair to assess the temporal variation

*Corresponding author. Email: eneji.ishaqs@uam.edu.ng.

(Normura and Oliveira, 2010).

Chromium exists primarily in two forms: Cr (III) and Cr (VI) and the sources of Cr in human body could be due to diffused sources such as metal ores, tannery, dumpsite exposure, food and water. The high levels of Cr in soils and vegetables could also be attributed to excessive usage of fertilizers and other agro-chemicals, as well as the use of waste water in irrigating the soils and other environmental factors in these areas (Uwah et al., 2011). Cr (III) participates in carbohydrate and lipid metabolism (Hati et al., 2005b). However, under certain environmental conditions and certain metabolic transformations, chromium (III) can also be toxic to human health (ATSDR, 2000a; Hati et al., 2009). Chromium (VI) is toxic and exists mainly as an anion, either as chromate ion (CrO_4^{-2}) or dichromate ion ($Cr_2O_7^{-2}$) (Velma and Tchounwou, 2011; Velma and Tchounwou, 2013). Breathing in high levels (that is, greater than 2 µg/m^3) of Cr (VI), such as in a compound of chromic acid or chromium (VI) trioxide, can cause irritation to the nose such as runny nose, sneezing, itching, nosebleeds, ulcers and holes in the nasal cavity (ATSDR, 2000a). Therefore, the effect and toxicity of chromium present in a particular environment depends more on the nature or form (oxidation state) in which the metal species exists. Meaning that the knowledge of inter-conversion processes between different Cr forms is necessary to understand its behaviour and role in the environment, in addition to enabling reliable Cr speciation analysis to be performed (Kotaś and Stasicka, 2000).

This research is therefore, aimed at analysing and comparing the average cumulative concentration of chromium in human scalp hair using two different methods (AAS and XRF). Statistical tools were used to see if there is a meaningful variation in Cr concentration between the two techniques.

MATERIALS AND METHODS

a) Laboratory materials: oven, hot plate (model: SV3, voltage: 220V, current: 2A, power rating: 450W and a maximum temperature of 425^{o}C), atomic absorption spectrometer (Younglin AAS 8010) from Younglin Instrument Ltd South Korea, X-ray fluorescence machine (Minipal 4, 4030 X-ray spectrometer with Si-Li diode detector), analytical balance.

b) Reagents: Ethanol, deionised water, ultra pure 70%HNO_3, 30% w/w H_2O_2.

All reagents 70%HNO_3, 30% (w/w) H_2O_2 and 99% CH_3CH_2OH) used in this research were of high purity procured from Sigma Chemical Company St Loius, USA. Certified reference material [(CRM) (GBW 09101)] of hair (control) was obtained from Shanghai Institute of Nuclear Research Academia, China. Atomic absorption spectrometer (Younglin AAS 8010) and X – ray fluorescence machine 44030 X – ray spectrometer used was obtained from Younglin Instrument Limited, South Korea.

Collection and treatment of samples

Hair samples from human head were collected in sealed plastic bags from 50 persons across genders (35 males and 15 females) and were analysed. The samples were collected from people with diverse geographical, ethnic and occupational backgrounds residing within Makurdi metropolis (bearing of latitude 7^0 44^1 and longitude 8^0 31^1) of Benue State, Nigeria. The personal bio-data of the individuals alongside relevant details (such as sex, age, occupation and habit) were obtained through a questionnaire based on World Health Organization (WHO) guideline. The hair samples were separated according to gender (male and female), age group, coded alphabetically (A, B, C,.... AY) and finally cut into uniform length of 1 cm. Each of the sub samples were repeatedly washed with water, cleaned with analytical grade ethanol (99%) and deionised water following the procedure developed by International Atomic Energy Agency (IAEA) (Jauharah et al., 2011). The hair samples were first washed once with ethanol, then thrice with distilled water, the samples were then washed again with ethanol and finally with distilled water, accordingly. They were placed in crucibles and were oven dried at 105°C for an hour and kept for further sample preparation and analysis. A hair washing step is required to remove exogenous contamination of heavy metals from the hair surface. The hair samples before pre-treatment are shown in Figure 3.

Preparation of chromium standards

A stock solution of Cr metal was prepared from the soluble compound of the salt ($K_2Cr_2O_7$), that is, 2.8285 g of anhydrous $K_2Cr_2O_7$ was dissolved in deionised water and made up to 1 L mark of solution in 1000 mL volumetric flask (1 mL = 1 mg Cr).

Preparation of blanks

Two blanks were prepared to check the effects of matrix:

(a) Sample or method blank – This involves digested blank, that is, distilled H_2O (everything except hair) were allowed to goes through the digestion process.
(b) Instrument blank- only deionised water used in the instrument.

Calculation of actual concentration of the metal (µg/g):

$$(Cr) = \frac{\text{Conc. of metals in sample solution (µg/L)} \times \text{mL of sample}}{\text{Sample weight (g)} \times 1000}$$

(a) AAS Digestion/analysis techniques: Exactly 0.1065 g of the cleaned hair sub-samples was weighed using analytical balance (AB 54-S METTLER TOLEDO) into a 50 mL crucible for digestion. Precisely 8.0 mL of conc. HNO_3 was added, covered with the lid, placed on a hot plate and heated to a gentle boil. Hair was digested at 85°C for about 30 min until the solution becomes clear (yellowish). The crucible was not allowed to go dry as exactly 1.0 mL of 30% H_2O_2 was added to each sub-sample and heating resumed at the lowest setting until bubbling stopped. The solution was allowed to cool, the crucible was rinsed twice with about 3 cm^3 deionised water and was then transferred into a 100 mL sample bottle, made up to volume and run using AAS at the Federal Polytechnic, Zaria, Kaduna State, Nigeria. A wavelength scan for Cr was performed in which the wavelength of maximum absorption was selected. Four serial dilutions of each stock solution (Standard solutions of Cr) were prepared and injected into the instrument (AAS), run at a wavelength of 357.9 nm using air-acetylene flame for which a plot of absorbance or peak area versus concentration of the metal in the standard solution was made. A linear correlation was obtained, which was used as calibration curve (Figure 4) and serves as standard for the measurement of unknown samples.

(b) **XRF analysis technique:** Exactly 0.1065 g of pre-treated hair sample in the same way as AAS analysis was weighed using analytical balance and stored in an inert plastic container of about 10 cm^3 capacities, corked tightly and ground in an agate mortar. 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 into the sample chamber of the spectrometer and voltage (30 KV maximum) and a current (1 mA maximum) was applied to produce the x-rays, which excited the sample for a preset time of 10 min. The spectrum from the sample was then analysed to determine the concentration of the element (Cr). Readings were printed out immediately by the interfaced PC printer for both the standardless and the individual elemental concentrations in the appropriate units. Certified reference material [(CRM) (GBW 09101)] obtained from Shanghai Institute of Nuclear Research Academia, Sinica, Shanghai, China was also processed in the same way as sample. Eleven beam filters were filled with hair sample, while the twelfth position the certified reference material (CRM), that is, hair which was run concurrently with each running of the hair sample in both the standardless run and the run for individual elemental concentration. Each CRM run was calculated as a common factor employed for the elements determined within each filter position to correct for deviations. A good correlation with the CRM value was obtained (4.77 ± 0.15 mg/L) within an acceptable standard error limit (3.14%), which serves as quality control parameter. All XRF analysis was carried out at the Centre for Energy Research and Training, Zaria, Kaduna State, Nigeria.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 16.0, for some descriptive statistics, paired sample correlation, paired sample t – test and one-way analysis of variance (ANOVA). The mean and standard deviation were calculated for each range of age group and gender for the two instrumental methods. All comparisons were made at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Chromium was determined in human scalp hair in various occupational and cultural diversities between ages 7-55 years using AAS and XRF methods of analyses. The individual concentration of Cr in all the samples with respect to sample codes for XRF and AAS is presented in Figure 1. In terms of XRF, point F has much higher concentration of 151 mg/kg whose age ranged the highest as well. This could be due to the cumulative effect of occupational exposure over the years in the work place, because the subject has been working in the Chemistry laboratory and had been involved in reagents preparations for more than twenty five (25) years. This is followed by points AK and AM with values of 97.2 and 93.2 mg/kg respectively, where as the lowest value was observed at point AS (2.02 mg/kg). Point P has the highest value of 16.2 mg/kg followed by point L (10.4 mg/kg) for AAS, while lowest concentration was observed at point AK (0.36 mg/kg) followed closely by AB, AE, AI, AO, AS and Y, all having a common value of 0.36 mg/kg. The mean Cr concentration (mg/kg) in all the 50 hair samples using AAS and XRF techniques were 2.83 and 16.9, respectively as shown in Figure 2. The concentration of Cr was found to be much higher using XRF than AAS method, invariably due to non-destructive nature of sample matrix and elimination of solvent interference during sample preparation among other factors (Onuwa et al., 2012a). The standard deviations were generally high in both methods and this is an indication of low precision as expected since these samples were not replicate, but obtained from different persons, sex, occupational variations, habitual practice and age (Onuwa et al., 2012b). This trend was also observed in the average Cr concentration across male and female genders using AAS (3.28 and 1.72 mg/kg, respectively) and XRF (16.3 and 18.2 mg/kg, respectively) methods as shown in Figure 2. Table 1

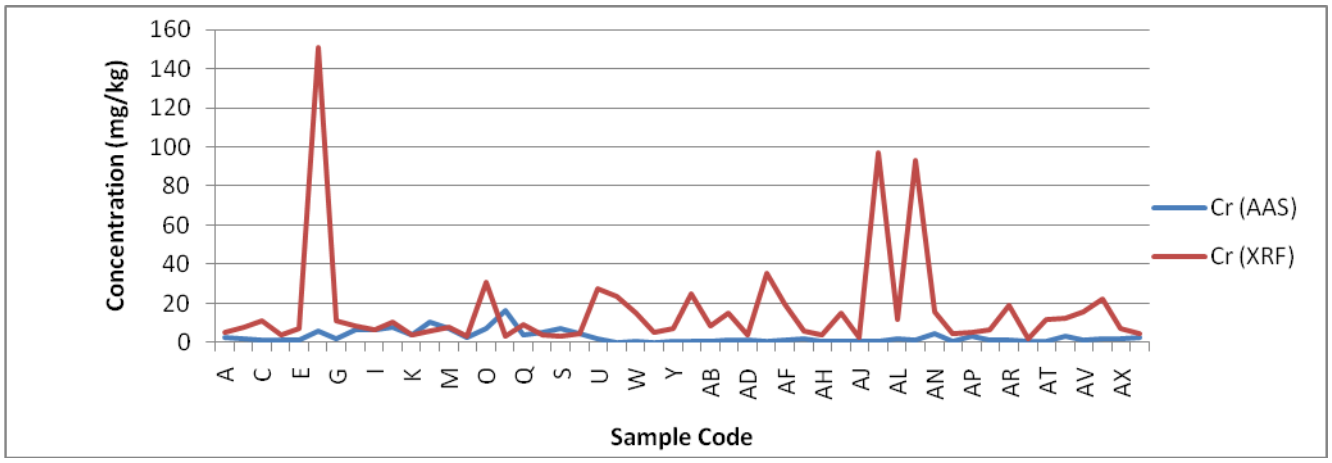

Figure 1. Comparative representation of Cr concentration (mg/kg) in AAS & XRF techniques.

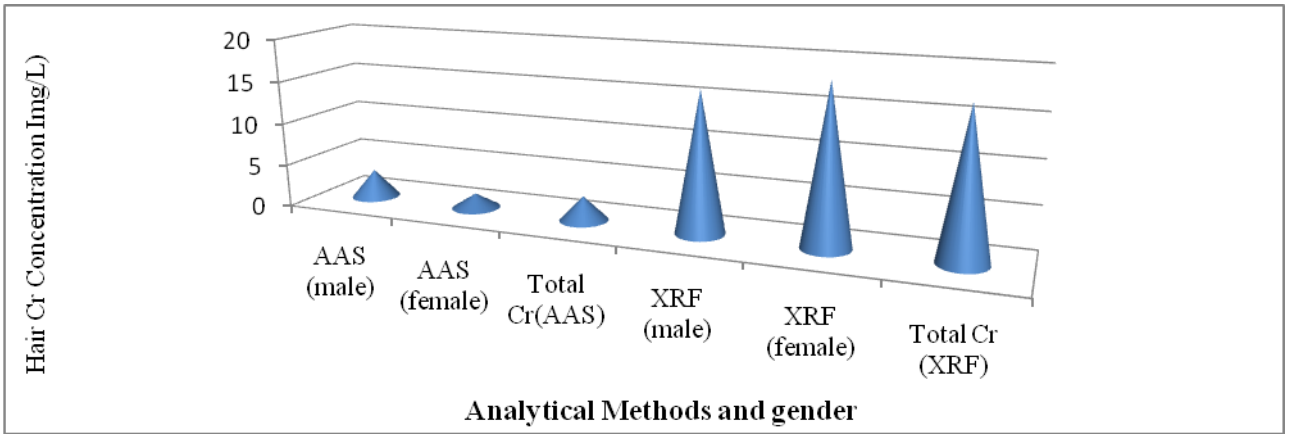

Figure 2. Chromium distribution (mg/kg) in 50 hair samples using AAS and XRF techniques.

Figure 3. Hair samples before pre-treatment.

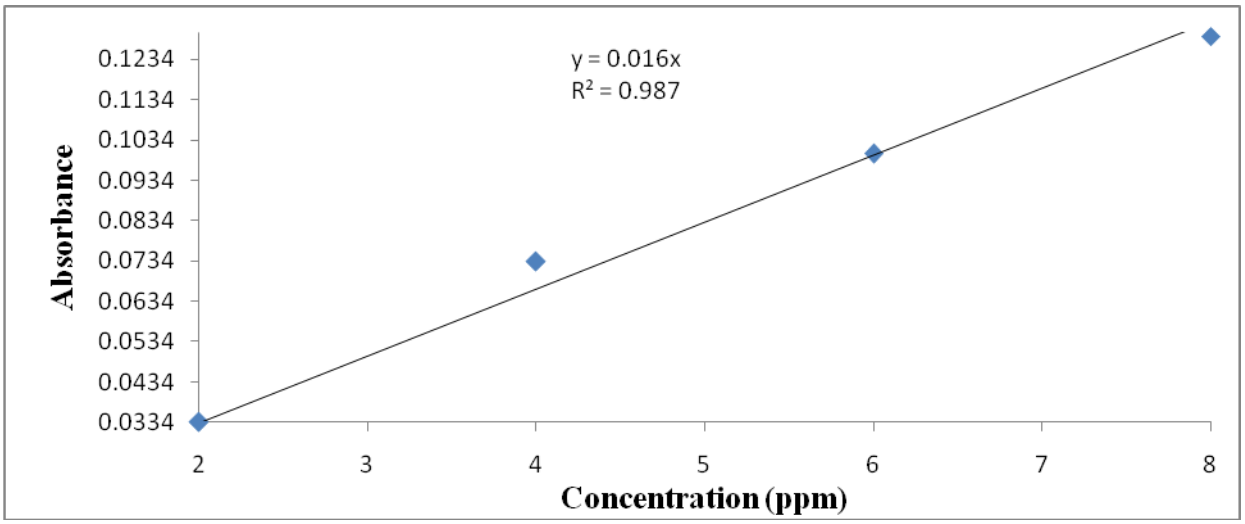

Figure 4. Calibration curve for Cr.

Table 1. Age distribution and mean concentration (mg/kg) of Cr in hair samples.

Range (years)	Number of person	AAS	XRF
0 – 20	8	2.69 ± 2.29	5.75 ± 0.023
21 – 40	36	2.83 ± 0.317	19.1 ± 6.75
≥ 41	6	2.19 ± 2.08	25.5 ± 19.3

Table 2. Descriptive Statistics of Cr concentration (mg/kg) in human hair samples and correlation for the AAS and XRF methods.

	N	Mean	Std. deviation	Std. error mean	Correlation	Sig.
Pair1:AAS Method	50	2.82	3.11	0.40	- 0.21	0.884
XRF Method	50	16.9	26.7	3.78		

shows the age-based concentration of Cr among the sampled population using the AAS and XRF methods. The samples were widely distributed between ages 21–40 years, with the highest total concentration obtained in the range of age bracket above forty years, which could be due to accumulation with time alongside other factors. Better precision was obtained within ages 0 – 20 years probably due to similarity in behavioural pattern.

Table 2 shows the descriptive statistics of Cr concentration (mg/kg) in human hair samples for the two methods, where mean, standard deviation and standard error of the mean were higher in XRF method when compared with AAS. The Paired sample correlation of Cr concentration between AAS and XRF methods was – 0.021. This indicates a low negative correlation value between the two methods, reflecting a method difference in this regard. Paired sample t – test for comparing the mean concentration of Cr level in human hair using the AAS and XRF instrumental methods is shown in Table 3. The two-tailed Paired sample t – test shows p value of 0.001 indicating that the difference between the methods is significant ($p < 0.05$).

Table 4 shows the descriptive statistics, giving a summary of individual method with their gender-based concentrations, where the highest concentration was found in female using XRF method. One-way analysis of variance (ANOVA) was used to compare the mean Cr concentration with regaed to the two methods used in conjunction with gender as shown in Table 5. The result of the analysis also shows p – value of 0.005 and F – value of 4.53, indicating a significant difference between the AAS and XRF techniques. Table 6 shows the Levene Statistics for test of homogeneity of variance to assess if there is difference or similarity in variance across samples so as to predetermine the suitability of ANOVA. Welch Robust tests of equality of means for confirmation

Table 3. Paired sample test showing 2-tailed level of significance of Cr in human hair samples between AAS & XRF methods. This is used for comparing between AAS and XRF methods as paired in which p value is 0.001 and shows that the difference between the two methods is significant ($p < 0.05$).

	Paired differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. error mean	95% confidence interval of the difference				
				Lower	Upper			
Pair 1 AAS Method XRF Method	-14.08300	26.99011	3.81698	-21.75351	-6.41249	-3.690	49	0.001

Table 4. Gender- based descriptive statistics of Cr distribution for the AAS and XRF methods.

Statistics								
Group	N	Mean	Std. deviation	Std. error	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
AAS_Male	35	3.28	3.43	0.579	2.11	4.46	0.33	16.2
AAS_Female	15	1.72	1.86	0.480	0.693	2.75	0.00	6.46
XRF_Male	35	16.3	28.6	4.83	6.52	26.2	2.02	151
XRF_Female	15	18.2	22.7	5.87	5.62	30.8	3.97	93.2
Total	100	9.86	20.2	2.02	5.84	13.9	0.00	151

Table 5. Analysis of variance (ANOVA) for Cr distribution in human hair samples.

Variable	Sum of squares	Df	Mean square	F	Sig.
Between groups	5020.428	3	1673.476	4.531	0.005
Within groups	35460.146	96	369.377		
Total	40480.574	99			

Table 6. Test of homogeneity of variances and Rubust tests of equality of means for the two methods.

Test		df1	df2	Sig.
Levene statistic:	4.72	3	96	0.004
Welch statistic (a):	6.39	3	40.8	0.001

a, Asymptotically F distributed.

of ANOVA was used and this actually confirmed the significant difference that was also observed using the one – way ANOVA.

Table 7 shows the Post Hoc Test of multiple comparisons in which the difference in means were significant between the following pairs: [AAS male-XRF male, AAS male- XRF female, AAS female-XRF female, XRF male-AAS female], whereas the following pairs show no significant difference ($p>0.05$): [AAS male-AAS female, XRF male-XRF female].

The mean concentration of Cr (2.83 ± 3.11 mg/kg) reported in this study using AAS is within the range of literature values reported in other areas. Abdulrahaman et al. (2012) investigated the level of heavy metals in human hair and nail samples from Maiduguri metropolis, Borno State, Nigeria. In their study, they reported Cr concentration (µg/g) range of 0.11 to 0.60 for liquor users, 0.267 to 4.06 for workers in iron welding workshop and 0.11 to 1.77 for liquor – and non – liquor – users. In this study, irrespective of age, gender and habit, the range of Cr level in human scalp hair in Makurdi metropolis was 1.72 to 3.28 mg/kg. Buchancova et al. (1993) and Halasova et al. (2010) reported Cr concentration in human hair to be between 0.03 to 19.7

Table 7. Post hoc tests of multiple comparisons for the AAS and XRF methods with regards to gender.

(I) Method_Sex	(J)Method_Sex	Mean difference (I-J)	Std. error	Sig.	95% confidence Interval	
					Lower bound	Upper bound
AAS_Male	AAS_Female	1.56	5.93	0.793	-10.2	13.3
XRF_Male	AAS_Male	13.1(*)	4.59	0.005	3.94	22.2
	AAS_Female	14.6(*)	5.93	0.016	2.84	26.4
XRF_Female	AAS_Male	14.9(*)	5.93	0.014	3.15	26.7
	AAS_Female	16.5(*)	7.02	0.021	2.55	30.4
	XRF_Male	1.87	5.93	0.754	-9.91	13.6

* The mean difference is significant at the 0.05 level.

mg/kg in Italy, where sixty seven (67) workers were exposed to ferrochromium alloys. Randall and Gibson (1989) investigated health problems related to hair Cr as an index of chromium exposure of tannery workers from four Ontario tanneries in Canada using flameless atomic absorption spectrometry (FAAS). It was found from their research that Cr(III) compounds used in the leather tanning industry was absorbed because the concentrations of Cr in serum and urine of tannery workers were significantly increased when compared with concentrations for unexposed control. The results of the FAAS analysis showed that the median hair Cr concentrations for the tannery workers (551 ng/g) was significantly higher (p=0-0001) than for the controls (123 ng/g). For the tannery workers, hair Cr concentrations were positively and significantly correlated with serum Cr (r = 052, p < 0-01) and with the preshift and postshift urinary Cr/creatinine ratios (r = 043, p < 0-01; r = 0-64, p < 0-01, respectively). These data indicate that trivalent Cr absorbed from leather tanning compounds results in raised concentrations of Cr in hair and that hair Cr concentrations may be used as an index of industrial Cr exposure. AAS has also been used to determine Pb and Cr levels in human hair of people living in Katpadi and Yelagiri hills of Vellore District, India (Perumal and Thangamani, 2011). They reported mean concentration Cr level to be 1.27±2.58 and 1.06±0.456 for Katpadi and Yelagiri hills, respectively. In England, Cr concentration range of 0.03 to 1.88 mg/kg in human hair using AAS has also been documented (International Occupation` Safety and Health Information Centre, 1999). Mehra et al. (2010) reported mean concentration of Cr in human hair of the people residing near heavy traffic and less traffic areas of Rajasthan in India to be 5.79 ± 7.22 and 4.540 ± 4.542 µg/g, respectively.

Polonnikova et al. (2000) determined the trace elements in hair of pregnant women using XRF spectrometry and reported Cr concentration range of 2.6 to 15.3 mg/kg. Baranowska et al. (2004) reported XRF spectrometry in Multi-elemental analysis of hair and teeth from the inhabitants of Katowice, Gliwice, Pyskowice and Tychy (Silesia, Poland). The results of their analysis shows that among the twelve (12) elements analysed, Cd, Cr and Ni concentrations were below the detection limit of X – ray method. The reliability of the results obtained by XRF analysis were further verified by means of the ICP – OES method and reported the mean concentration (mg/kg) of Cr to be 4.48 ± 0.22 and 3.78 ± 0.20 for female and males subject, respectively. The high level of Cr in the hair samples investigated above the permissible limit of International Occupation Safety and Health Information Centre shows the bioaccumulation of Cr in the body of the subjects as a result of long term exposure. Since hair is an indicator of the history of individual's metabolism and environmental exposure, the results shows that Cr content of human scalp hair irrespective of the methods used for determination, strongly depends on age, sex, dietary habits and exposure (Komaromy-Hiller et al., 2000).

Conclusion

The comparison of the two methods of chromium analyses (AAS and XRF) showed that the average concentration of the metal in hair using XRF was generally higher than that of AAS method. This could be attributed to the fact that this instrumental method employs a non-destructive sample matrix means of analysis, whereas there could be solvent effect and other interferences during digestion process in terms of AAS method. Statistical comparison showed no significant difference ($p > 0.05$) between male and female hair samples using AAS, as well as male and female hair samples using XRF method of analyses, respectively. However, significant differences were observed ($p < 0.05$) between same and different genders under the two different methods used. This indicates variation in the methodological or instrumental procedure for the determination of Cr concentration in biological samples (human scalp hair) used in this research. From the two methods considered in the course of this research, XRF method deserved more credit due to some advance aforementioned reasons.

ACKNOWLEDGEMENTS

The authors wish to appreciate the assistance of the technical staff of Centre for Energy Research and Training, Zaria, Kaduna State, Nigeria, in using their XRF machine. The authors also acknowledge Dr. P. O. Agada, Department of Mathematics/Statistics/Computer Science, University of Agriculture, Makurdi, for his assistance in the statistical analyses of samples.

REFERENCES

Abdulrahman FI, Akan JC, Chellube ZM, Waziri M (2012). Levels of Heavy Metals in Human Hair and Nail Samples from Maiduguri Metropolis, Borno State, Nigeria, World Environment, © 2012 Scientific & Academic Publishing, 2(4): 81-89.

ATSDR: Agency for Toxic Substances and Disease Registry. (2000a).''Toxicological Profile for Chromium'' U.S. Department of Health and Human Services Public Health Service. Atlanta, GA.

Baranowska I, Barchanski L, Bak M, Smolec B, Mzyk Z (2004). X – ray fluorescence spectrometry in multi-elemental analysis of hair and teeth. Polish J. Environ. Stud., 13(6): 639-646.

Buchancova J, Vrlik M, Knizkova M, Mesco D, Holko L (1993). Levels of selected element (Fe, As, Cd, Pb, Zn, Mn) in biological samples from ferrochromium workers. Bratisl Lek. Listy., 94: 373-387.

Cooper GAA, Kronstrand R, Kintz P (2012). Society of Hair Testing guidelines for drug testing in hair. Forensic Sci. Int., 2018: 20-24.

Hai SS, Joseph CA, Ogugbuaja VO (20005b). ''Comparative Assessment of Chromium Discharge in Tannery Wastewaters in Kano State, Northern Nigeria'' Proceed of the 28th Ann. Int. Confer. CSN, 2(1): 137-139.

Halasova E, Adamkov M, Matakova T, Kavcova E, Poliacek I, Singliar A (2010). Lung cancer incidence and survival in chromium exposed individuals with respect to expression of anti-apoptotic protein survivin and tumor suppressor P53 protein, Eur. J. Med. Res., 15(Suppl 2): 55-59.

Hati SS, Dimari GA, Ogugbuaja VO (2009). Levels of Potentially Toxic Metals in Synthetic Industrial Essential Oils Utilized in Northern Nigeria. Pac. J. Sci. Technol., 10(1): 395-396.

International Occupation Safety and Health Information Centre (1999). Prevention today, 5(3): 1-12.

Jauharah K, Husin W, Imam H, Norddin I, Mohd AA (2011). Heavy metals Mn, Fe, Ni, Cu and Zn in human hair samples using energy dispersive X – ray fluorescence analysis. Int. J. Phys. Sci., 6(8): 2090-2094.

Komaromy-Hiller G, Owen Ash K, Coasta R, Howerton K (2000). Comparison of representative ranges based on U.S patient population and literature reference intervals for urinary trace elements. Cline Chim. Acta, 296: 71-90.

Mehra R, Thakur AS, Bhalla S (2010). Trace level analysis of chromium, manganese, cobalt and iron in human hair of people residing near heavy traffic area by bio-monitoring. Int. J. Pharma and Bio Sci., 1(4): 57-61.

Nomura CS, Oliveira PV (2010). Method for cadmium and lead longitudinal profiles determination in hair by solid sampling graphite furnace atomic absorption spectrometry. Anal. Methods, **2**: 49-53.

Onuwa PO, Eneji IS, Sha'Ato R (2012). Analysis of Heavy Metals in Human Hair Using Atomic Absorption Spectrometry (AAS). Am. J. Anal. Chem., 3: 770-773.

Onuwa PO, Nnamonu LA, Eneji IS, Sha'Ato R (2012). Analysis of Heavy Metals in Human Scalp Hair Using Energy Dispersive X-Ray Fluorescence Technique. J. Anal. Sci. Methods Instr., 2(4): 190-196

Perumal AS, Thangamani A (2011). Atomic Absorption Spectrophotometric Determination of heavy metals lead and Chromium in Human Hair of people living in Katpadi and Yelagiri Hills of Vellore Didtrict. Int. J. Res. Anal. Pharmacy (IJRAP), 2(5): 1568-1570.

Polonnikova VV, Karpukova OM, Smagunova AN, Ivanova EI, Proidakova OA (2000). Mutual interferences of elements in the atomic-absorption analysis of human hairs. J. Anal. Chem., 55: 1-29.

Randall JA, Gibson RS (1989). Hair Chromium as an index of chromium exposure of tannery workers. Br. J. Ind. Med., 46: 171-175

Uwah EI, Ndahi NP, Abdulrahman FI, Ogugbuaja VO (2011). Distribution of Some Trace Metals in Soils, and Fruit Vegetables Grown in Maiduguri, Nigeria. Continental J. Appl. Sci., 6(1): 31-41.

Velma V, Tchounwou PB (2011). Hexavalent chromium-induced multiple biomarker responses in liver and kidney of Goldfish, *Carassius auratus*. Environ. Toxicol., 26(6): 649-56.

Velma V, Tchounwou PB (2013). Oxidative Stress and DNA Damage Induced by Chromium in Liver and Kidney of Goldfish, *Carassius auratus*. Biomark Insights, 8: 43-51.

Zhunk LI, Kist AA (1995). Human hair instrumental neutron activation analysis and medicine. J. Radioanal. Nuclear Chem., 195: 75-81.