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**Determination of Some Heavy Metals in Human  
Hair by Using Atomic Absorption  
Spectrometry (AAS)**

A thesis submitted to the college of science for women  
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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

(وَالرَّاسِخُونَ فِي الْعِلْمِ يَقُولُونَ آمَنَّا بِهِ كُلٌّ مِنْ عِنْدِ

رَبِّنَا) سورة آل عمران الآية ٧

صدق الله العلي العظيم

## شكر وتقدير

ومن حق النعمة الذكر، وأقل جزاء للمعروف الشكر فبعد شكر المولى عز وجل،  
المتفضل بجليل النعم، وعظيم الجزاء.... يجدر بي أن أتقدم ببالغ الامتنان وجزيل  
العرفان إلى كل من وجهني و علمني، وأخذ بيدي في سبيل إنجاز هذا البحث .....

وأخص بذلك مشرفي

الدكتور : محمد عيدان

الذي قوم، وتابع، وصوب، بحسن إرشاده لي في كل مراحل البحث .  
وأقدم بخالص الشكر والتقدير إلى رئاسة قسم الكيمياء – كلية العلوم المتمثلة برئيس  
القسم والأساتذة في قسم الكيمياء على مساعدتهم ودعمهم لي في هذا  
البحث ....

وأحمل الشكر والعرفان إلى كل من أمدني بالعلم والمعرفة، وأسدى لي النصيح،  
والتوجيه، وإلى ذلك الصرح العلمي الشامخ متمثلاً في جامعة بابل، وأخص بالذكر  
كلية العلوم قسم الكيمياء.

## الإهداء

اهدي بحثي هذا الى الذي قال الله فيهما (وَإِخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُلْ  
رَبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا)

يا من احمل اسمك بكل فخر يا من أفقدتك منذ الصغر

يامن يرتعش قلبي لذكرك يامن أودعتني الله أهديك هذا البحث الماس الذي لا ينكسر  
نبع العطاء الذي زرع الاخلاق في داخلي و علمني طرق الارتقاء "الى ابي"

إلى .... الذي أتمنى أن تغيب عنه كل مظاهر العنف ويسوده الأمن والاستقرار بلدي  
الجريح العراق

الزهرة التي لا تذبل ... نبع الحنان التي ساندتني و وقفت الى جانبي حتى وصلت  
الى هذه المرحلة من التقدم والنجاح الى من تعجز الكلمات عن وصفها وتسكن امواج  
البحر لسماع اسمها. " إلى أمي

ملائكة الأرض .... شقائق النعمان الذين احتضنوني وزرعوا الورود في طريقي

الى اشقائي رفاق الدرب ... بناء المستقبل الى ارووع واصدق واقبل البشر الى  
اصدقائي المخلصين الذين رفعوا رايات العلم والتعليم واخمدوا رايات الجهل  
والتجهيل ... الى استاذتي الأفاضل لاسيما الدكتور محمد عيدان أهدي هذا الجهد  
المتواضع الى كل من قال " لا إله الا الله - محمد رسول الله - علي ولي الله " لكم  
جميعا اهدي سهري وتعبي وجهدي.

# Determination of Some Heavy Metals in Human Hair by Using Atomic Absorption Spectrometry (AAS)

## ABSTRACT

Hair samples of individual were analysed for heavy metals (Cd, Cr, Pb and As) across gender and various occupational distributions by Atomic Absorption Spectrophotometric technique (AAS). The results of replicate analysis shows the following mean concentrations (mg/kg): Cd =  $27.8 \pm 8.0$ , Cr =  $2.70 \pm 0.7$ , Pb =  $73.8 \pm 42.3$  and As =  $222 \pm 34.1$ . The coefficients of variation for the total distribution is; Cd = 28%, Cr = 26%, Pb = 57% and As = 15%. The distribution of the metals follows the series in decreasing order of As > Pb > Cd > Cr, while their coefficients of variation is in the order of Pb > Cd > Cr > As. The result shows the presence of all the metals in relatively large amounts with As having the highest concentration between the two genders. The difference between male and female concentration could be due to individual differences in exposure to heavy metal load as a result of habitual or environmental factors.

Keywords: Heavy Metals; Human Scalp Hair; AAS; Concentration

## 1 . Introduction

Research has shown 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 [1]. Lemos et al [2] have also reported that individual's deviation of elemental concentrations reflects the degree of environmental pollutants exposure to the human body, intakes of food and metabolism. Heavy metals, such as chromium, lead, mercury, cadmium, arsenic are extremely toxic even in very small amounts. When any of these elements is present in the environment at high concentrations, living organisms are subjected to strong natural selection for tolerance. Environmental contamination by metals

exerts physiological pressures that are clearly too severe for survival of most species by means of phenotypic plasticity or physiological acclimation, rather than genetic adaptation [3]. Heavy metals are poisonous to living organisms including humans due to their biotoxic effects, which could be acute, chronic or subchronic, neurotoxic, carcinogenic, mutagenic or teratogenic [4]. Cadmium is toxic at extremely low level, it is also associated with bone defects like osteomalacia, increased blood pressure and myocardic dysfunctions. McCluggage [5] reported that severe exposure to cadmium may result in pulmonary oedema and death. Smoking has also been reported to be a contributing factor to higher bioaccumulation of cadmium [6]. Chromium is an essential nutrient in our diet that helps insulin to maintain normal glucose level. Chromium toxicity can cause stomach upsets, ulcer, kidney-, liver-damage and even death. Femer [7] reported that lead is the most significant of the toxic heavy metals and the inorganic forms are absorbed through ingestion of food and water as well as inhalation. Apart from the teratogenic effects of lead, its poisoning also causes inhibition of the synthesis of haemoglobin, dysfunctions in the kidneys, joints and reproductive systems, acute and chronic damage to the central nervous system, etc. [8]. Workers with chronic headache and dizziness have higher levels of Cr and Pb in the scalp hair samples, such as in those working in a fireworks factory [9]. Arsenic toxicity symptoms depends on the chemical form ingested [10]. Arsenic acts to coagulate protein, forms complexes with coenzymes and inhibits the production of adenosine triphosphate during respiration [11].

Hair analysis is inexpensive and fast; it also detects and measures the content of heavy metals and minerals of the hair. The Global Environmental Monitoring System (GEMS) of the United Nations Environment Program selected human hair as one of the important monitoring materials for worldwide biological monitoring of pollution [12]. Therefore analyses of heavy metals (Cr, Pb, Cd and As) in human scalp hair serves as an assessment for environmental

contamination and can be used to sensitise individuals towards maintaining a healthier life style in their environments.

## 2. Materials and Methods

All reagents (absolute ethanol, HNO<sub>3</sub>, 20% H<sub>2</sub>O<sub>2</sub>) used were analytical grade reagents obtained from Sigma Chemical Company, St Louis, USA. Atomic absorption spectrometer (Younglin AAS 8010) used was obtained from Shimadzu Japan.

### 2.1. Sample Collection

Freshly cut human hair samples were collected from the head of 51 individuals between the ages of 7 - 55 years (35 males and 16 females) across several occupational distributions within Babil in Iraq. The samples were quickly transferred into coded polythene bags, sealed tightly and kept for pre-treatment.

### 2.2. 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 0.125 in (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) [13]. Exactly 0.1065 g of hair sample was weighed accurately into a 50 mL crucible. The sample was covered with 8 mL conc. HNO<sub>3</sub>, 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<sub>2</sub>O<sub>2</sub> 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 [14]. The contents of each crucible were quantitatively transferred to a cleaned and dried 100 mL volumetric flask. The digestion vessel was rinsed three times with 1.5 mL each with deionised water and added to the volumetric flask and made up to volume with deionised

water. (It could be filtered using Whatman paper no. 1 and no. 40 if the solution looks cloudy to prevent clogging the nebulizer). It was then transferred to a cleaned sample bottle, corked, labelled well and stored in the refrigerator until ready to be analysed. Standard solutions of all the metals investigated were prepared from concentration levels of 1 - 20 ppb.

### 3. Results and Discussion

The results of AAS analysis shows, the ranges in concentrations of heavy metals (mg/kg) as follows: Cr varies from 0.33 - 16.16; Pb varies from 5.80 - 347.19; Cd ranges from 2.46 - 130.17 while As ranges from 21.29 - 447.04. Table 1 shows the distribution of heavy metals among male samples by age with As having the highest concentration ( $243 \pm 125$  mg/kg) within 21 - 31 years of age interval.

The distribution of the metals among female samples by age are shown in Table 2, where all the metals analysed were more distributed around age bracket of 21 - 31 years except Cd where the highest was between 7 - 20 years. The distribution and the average concentration of heavy metals by age in the entire samples is shown in Table 3 in which Cr has the least concentration but has a high value between the ages 21 - 31 and  $\geq 43$  and As has the highest concentration followed by Pb and Cd. The total mean concentrations of the metals (mg/kg) were Cr =  $2.70 \pm 0.7$ , Cd =  $27.8 \pm 8.0$ , Pb =  $73.8 \pm 42.3$  and As =  $222 \pm 34.1$  as also shown in Figure 1. This shows that As has the highest and Cr the least concentration. The total coefficient of variation were; Cr = 26%, Cd = 29%, Pb = 57%, and As = 15%. This indicates that

**Table 1. Distribution and mean concentration (mg/kg) of heavy metal in male samples by age.**

Range (years)	Number of male	Heavy metals			
		Cr	Cd	Pb	As
7-20	4	$4.31 \pm 4.10$	$40.5 \pm 5.90$	$104 \pm 73.7$	$241 \pm 210$
21-31	19	$3.57 \pm 4.00$	$28.7 \pm 22.2$	$42.4 \pm 30.3$	$243 \pm 125$
32-42	7	$1.64 \pm 0.90$	$20.1 \pm 12.0$	$24.6 \pm 30.0$	$181 \pm 114$
$\geq 43$	5	$3.66 \pm 2.60$	$28.5 \pm 21.9$	$60.8 \pm 4.10$	$211 \pm 133$



**Table 2. Distribution and mean concentration (mg/kg) of heavy metal in female samples by age.**

Range (years)	Number of female	Heavy metals			
		Cr	Cd	Pb	As
7-20	5	1.08 ± 0.800	31.9 ± 0	127 ± 78.3	298±0
21-31	10	2.60 ± 2.10	27.0 ±17	194 ± 158	223 ± 200
32-42	-	-	-	-	-
≥43	1	0.72 ± 0.00	2.46 ± 0.00	40.51 ± 0.00	Nil

**Table 3. Summary of distribution and average concentration (mg/kg) of heavy metal in the 51 samples by age.**

Range (years)	Heavy metals			
	Cr	Cd	Pb	As
7-20	2.70 ± 3.23	38.8 ± 6.37	118 ± 67.7	260 ± 153
21-31	3.28 ± 3.56	28.2 ± 20.3	98.4 ± 121	233 ± 144
32-42	1.64 ± 0.94	20.1 ± 12.0	24.6 ± 30.0	181 ± 114
≥43	3.17 ± 2.58	24.2 ± 22.3	54.0 ± 12.0	211 ± 133

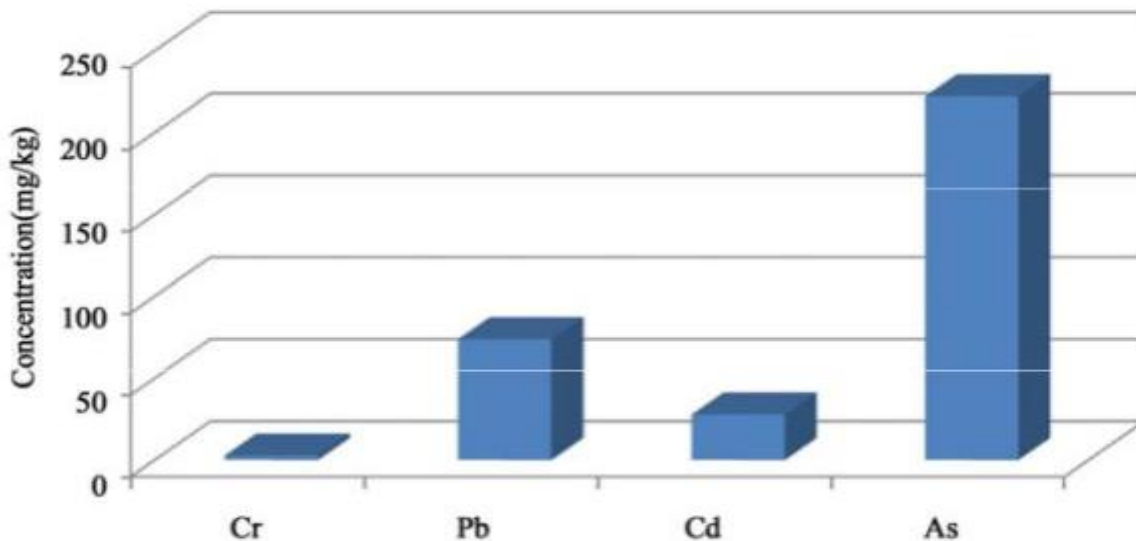


Figure 1. Total mean distribution of individual heavy metals using AAS technique.

lead has the highest coefficient of variation while arsenic has the least. Since coefficient of variation is a measure of dispersion, the implication is that while lead is widely dispersed among the sampled population, arsenic is more closely dispersed within same sample population. Based on AAS analysis, the average concentration of Cr, and Cd are higher in males while that of Pb and As are higher in females as shown in Figures 2 and 3. According to Lee et al [15] this could be attributed to use of cosmetic products by females among other sources.

The standard deviation is higher in females for Pb and Cd while it is higher for Cr in males. This indicates that the deviation in the individual content of heavy metals is more pronounced in females compared to males. The coefficient of variation (CV) for Cr, Cd and As are higher in females except Pb which is higher in males.

The mean concentration of heavy metals in both males and females were compared statistically at 0.05 probability which showed significant difference in all the elemental content. Similar samples analysed by x-ray fluorescence analysis showed that the average concentrations of Cr in XRF method for both genders were relatively higher than that in AAS method [16]. This is likely due to the non-destructive nature of sample matrix used in XRF analysis as the samples were not digested before analysis [17], where as, the samples for AAS were completely digested before analysis. This could lead to loss of volatile proportion of analytes.

Also, a difference was observed between the concentration of Cr in XRF technique and AAS method when compared statistically [16].

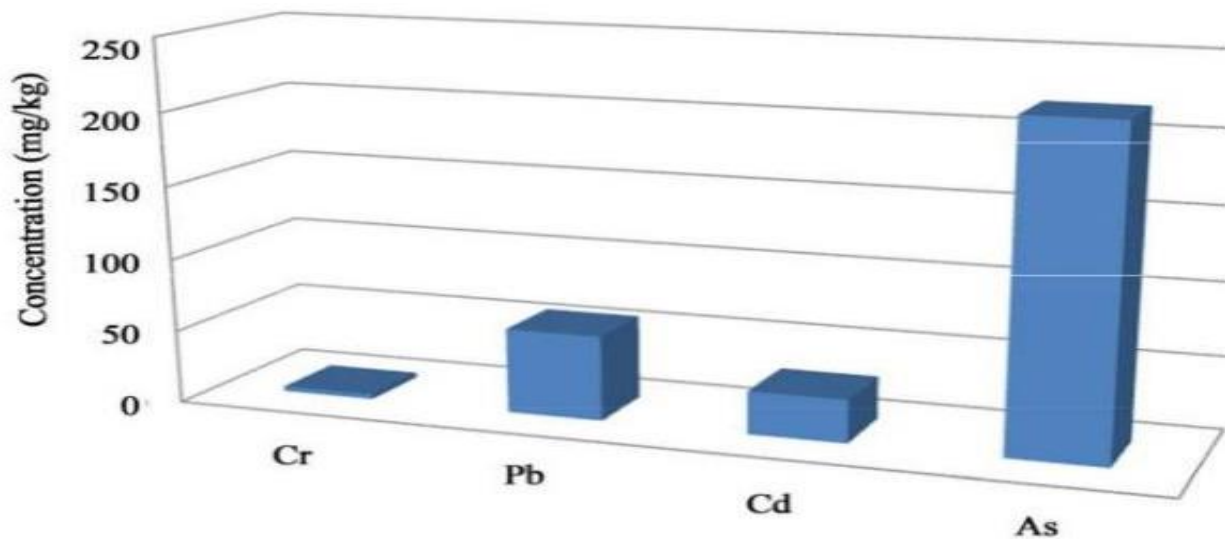


Figure 2. Average concentration of heavy metals in males using AAS technique.

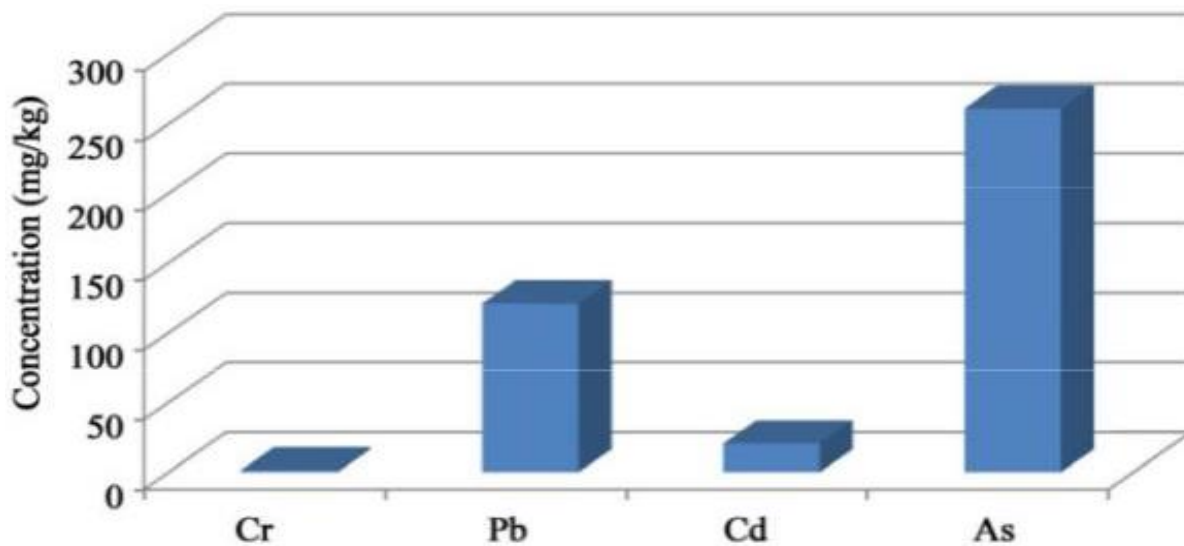


Figure 3. Average concentration of heavy metals in females using AAS technique.

The difference between the concentration of the same element (Cr) in same gender under two methods where as, it showed no difference under same method could have resulted from method variability such as sensitivity, detection limit, analytical conditions of instrument, pretreatment methods, possibility of the sample matrix being contaminated by exogenous material and the efficiency

of the analyst [12,18]. The difference between male and female concentration could be due to individual differences in heavy metal load as a result of habitual or environmental factors.

The distribution of the metals follows the series:  $As > Pb > Cd > Cr$ , while their general coefficients of variation is of the order:  $Pb > Cd > Cr > As$ . This showed that Pb recorded the highest variation in the distribution among individual respondents as compared to As which has the least. Lead has diffuse possible sources which could be from drinking water, which for most respondents were tap water (boilers ring in the pipes) and bore holes. Others are packaging materials, contaminated food grown in lead-deposited soils either from point or non-point sources, use of agrochemicals during cropping season, use of leaded fuels, lead plate accumulators, use of alloys like solder, bearing metals, type metals etc. The high accumulation of As could have resulted from the use of insecticides, doping agents in semi-conductors, use of some lead-based alloys to promote hardening etc.

#### 4. Conclusion

Analysis carried out by AAS technique for As, Cd, Cr and Pb indicates the presence of all the metals in relatively large amounts with As having the highest concentration across age distribution and between the two genders. The presence of all the heavy metals under investigation is a clear indication of the environmental content as well as the behavioural pattern of the respondents who are randomly selected from the general society. As and Pb were much higher in females than males probably due to use of cosmetics formulations and hair treatment among them. For all the four different heavy metals determined, the coefficients of variation were higher for three elements (Cr, Cd and Pb) among the females while only As has higher coefficient of variation among the males. This reflects 75% of the total population which indicates that these metals have greater degree of dispersion among the female population than the males.

## REFERENCES

- [1] S. J. Steindel and P. J. Howanitz, "Uncertainty of Hair Analysis for Trace Metals," *Journal of American Medical Association*, Vol. 285, No. 1, 2001, pp. 83-85. doi:10.1001/jama.285.1.83
- [2] P. C. D. Lemos, H. M. Dung, Vu C. Dong, Sy, N Thi and N. M. Sinh, "Analysis of Angolan Human Hair Samples by the Ko- NAA Technique on the Dalat Research Reactor: The Study and Application on the Ko-Standardization Method of Neutron Activation Analysis at Dalat Nuclear Research Institute(NRI), Vietnam," *Proceedings of the 5th Asian Workshop on Utilization Research Reactors*, Jakarta, 2005.
- [3] R. V. Bharathi, A. J. Suresh, M. Thirumal and B. Kumudhaveni, "Analysis of Heavy Metal and Inorganic Element Content in *Barringtonia acutangula* Leaf," *Ethnobotanical Leaflets*, Vol. 14, 2010, pp. 856-863.
- [4] J. O. Duruibe, M. O. C. Ogwuegbu and J. N. Egwurugwu, "Heavy Metal Pollution and Human Biotoxic Effects," *International Journal of Physical Sciences*, Vol. 2, No. 5, 2007, pp. 112-118.
- [5] D. McCluggage, "Heavy Metal Poisoning," *NCS Magazine*, 1991. [www.cocktatiels.org/articles/Diseases/Metals.html](http://www.cocktatiels.org/articles/Diseases/Metals.html).
- [6] P. Chattopadhyay, H. Joshi and K. Samaddar, "Hair Cadmium Level of Smoker and Non-Smoker Human Volunteers in and around Calcutta City," *Bulletin of Environmental Contamination and Toxicology*, Vol. 45, No. 2, 1990, pp. 177-180. doi:10.1007/BF01700180
- [7] D. J. Ferner, "Toxicity, Heavy Metals," *eMed.J.* Vol. 2, No. 5, 2001, p. 1.
- [8] M. O. C. Ogwuegbu and W. Muhanga, "Investigation of Lead Concentration in the Blood of People in the Copperbelt Province of Zambia," *Journal of Environment*, Vol. 1, 2005, pp. 66-75.
- [9] A. Sukumar and R. Subramanian, "Elements in Hair and Nails of Urban Residents of New Delhi, CHD Hypertensive and Diabetic Cases," *Biological Trace Element Research*, Vol. 34, No. 1, 1992, pp. 89-97. doi:10.1007/BF02783901
- [10] J. R. Holum, "Elements of General and Biological Chemistry," 6th Edition, John Wiley and Sons, New York, 1983.

- [11] Institute of Environmental Conservation and Research (INECAR), "Position Paper against Mining in RapuRapu," INECAR, Ateneo de Naga University, 2000.  
[www.adnu.edu.ph/institutes/Inecar/pospaper1.asp](http://www.adnu.edu.ph/institutes/Inecar/pospaper1.asp)
- [12] G. Dodd, "Heavy Metal Poisoning and Hair Analysis," 2010.  
[http://holisticvetpetcare.com/pdf/HeavyMetalpoisonhair Analysis-2.pdf](http://holisticvetpetcare.com/pdf/HeavyMetalpoisonhair%20Analysis-2.pdf)
- [13] J. Md Khudzari, H. Wagiran, Md. I. Hossain, N. Ibrahim and M. A. Agam, "Heavy Metals Mn, Fe, Ni, Cu and Zn in Human Hair Sample Using Energy Dispersion X-Ray Fluorescence Analysis," International Journal of Physical Science, Vol. 6, No. 8, 2011, pp. 2090-2094.
- [14] A. Johansen, "Environmental Health: Science, Policy and Social Justice Winter Quarter," Central Washington University, Ellensburg, 2010.
- [15] S. M. Lee, H. J. Jeong and I. S. Chang, "Simultaneous Determination of Heavy Metals in Cosmetic Products," Journal of Cosmetic Science, Vol. 59, No. 5, 2008, pp. 441-448.
- [16] O. P. Onuwa, "Determination of Heavy Metals in Human Hair," M.Sc. Thesis, Department of Chemistry, University of Agriculture, Makurdi, 2012.
- [17] D. A. Bass, D. Hickok, D. Quig and K. Urek, "Trace Element Analysis in Hair: Factor Determining Accuracy, Precision and Reliability," Alternative Medicine Review, Vol. 6, No. 5, 2001, pp. 472-481.
- [18] S. M. DeAntonio, S. A. Katz, D. M. Schiner and J. D. Wood, "Anatomically Related Variations in Trace-Metal Concentrations in Hair," Clinical Chemistry, Vol. 28, No. 12, 1982, pp. 2411-2413.
- [19] "Oxford Dictionary of Chemistry," 6th Edition, Oxford University Press, New York, 2008.