

Influence of Gender and Smoking Habit on the Minor Elements Levels of Washed Scalp Hair from Karbala, Iraq

تأثير الجنس والتدخين على مستويات العناصر الثانوية في عينات شعر فروة الرأس في كربلاء، العراق

Joda A. Baker

Faculty of Sciences, University of Kerbala, Karbala, Iraq

(E-mail of the corresponding author: phdhchemistry2008@googlemail.com)

Abstract

In recent years elemental analysis of human biological samples has become more advanced in terms of human health. This study has investigated the chemical analysis of Iraqi human hair samples in relation to differing forms of smoking (active and non-smokers) collected from Karbala, Iraq. Hair samples were obtained from 236 individuals (32 females, 204 males) aged between 3 to 70 years. Methods were developed and validated for the analysis of minor elements (namely Na, Ca, Mg and Fe) in human scalp hair samples. Different washing procedures and digestion methods were studied. The effect of gender and smoking habit on elemental levels was examined and the results compared with published values in the literature. Significant correlations were found between the gender and the levels of these elements in scalp hair samples at a probability level P of 0.05. In addition, significant differences were found between the Ca levels in smokers and non smokers at P of 0.05.

Key Words: Human hair; Minor elements; Karbala-Iraq; atomic absorption spectrometry.

الخلاصة:

أن تحليل العناصر بالبيولوجية أصبح متقدما جدا من ناحية صحة الإنسان. في هذه الدراسة تم تحليل نماذج لشعر فروة الرأس لعينات من المدخنين وغير المدخنين تم جمعها من مدينة كربلاء. أن عدد العينات هو 236 (32 نساء و 204 رجال) وبفئات عمرية تراوحت بين 3 الى 70 سنة. تم استخدام وتثبيت عدد من الطرق اللازمة لتحليل عناصر Na, Ca, Mg, Fe في عينات شعر فروة الرأس. حيث تمت دراسة طرائق غسل وهضم مختلفة لهذا الغرض. تمت دراسة تأثير التدخين والجنس على تراكيز العناصر ثم قورنت النتائج مع النتائج المنشورة في ادبيات الكيمياء. لقد وجد ان هناك علاقة مهمة بين جنس الفرد وتراكيز هذه العناصر في نماذج الشعر عند قيمة احتمالية تساوي 0.05. من جهة اخرى فان الاختلاف المهم بين الأفراد المدخنين وغير المدخنين وجد في تراكيز عنصر الكالسيوم عند نفس القيمة من الاحتمالية.

1. Introduction

The connection between inorganic elements and human health has long been recognized, particularly when in the 17th century it was discovered that iron (Fe) was essential for normal human health [1]. Human and other living organisms are exposed to essential, non-essential and toxic elements that are introduced into the environment from natural sources as well as a result of anthropogenic (or man-made) activities. Therefore, the levels of these elements in environmental samples (soil, sediment, water, etc) may be a potential risk factor in assessing the quality of human health. Eventually, these elements are transported from aquatic media, air, foods etc. to the human body and can then be stored in different tissues and fluids [2], as shown in Figure 1.

In the last century, human scalp hair has been used an alternative biological material for blood and urine as a biomarker in the assessment of exposure to various pollutants in an occupational and/or environmental setting [4,5]. This is due to that a blood test provides different results from a hair analysis as the former only reflects the composition at the time of sampling, there are difficulties in blood collection and the stress factor for the patient associated with taking the sample [6-8]. In addition, human scalp hair is a long-term growth material which may provide useful data

in determining health status of an individual for long periods and several trace elements being accumulated in hair as well [6,8].

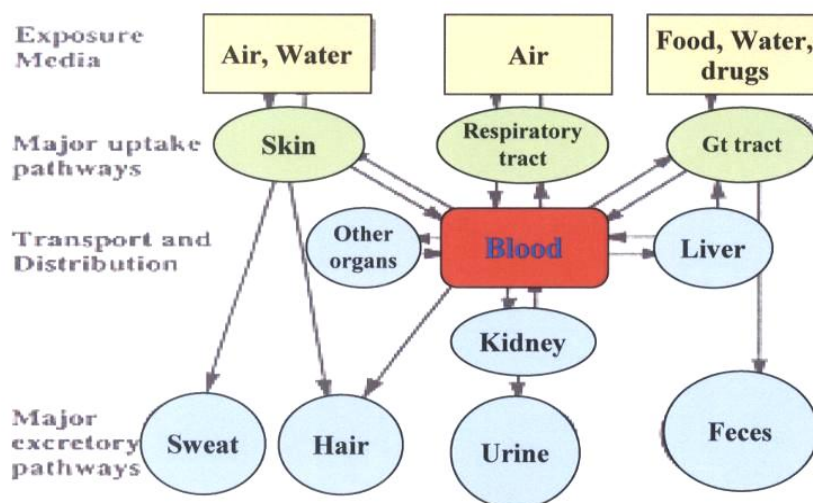


Figure: 1- Exposure and metabolic pathway for elements [3].

The role of minor elements in the human body is not yet fully understood. Previous study has reported that the minor elements maintain electrolyte balance processes [9,10]. Metallic and trace element analysis of biological samples has improved especially over the last forty-seven years providing more scientific evidence of the important role of these elements in the human body [11,12]. Therefore, any excess or deficiency of essential elements (Na, Mg, Ca and Fe) can relate to possible serious problems in terms of the physiology of the body [13]. On the other hand, the concentration of these elements can be used to investigate dietary exposure to chemical contaminants, especially toxic metals, including the effects of smoking and drug usage of an individual or populations [14]. Previous studies have reported that human scalp hair samples can be used to evaluate the effect of age, sex and healthy on element levels inside human body [15]. Furthermore, hair analysis may evidence of raised chemical levels in domestic and occupational exposure cases [4,16].

In Iraq, only blood, saliva and urine analysis are used to determine human minor element levels. However, no study has been published on the use of human hair material in Karbala, Iraq and therefore, this study will be valuable in establishing a database of 'control' levels for comparison with other body tissues and for comparative analysis for future environmental pollution or health disorders and disease studies. The aims of this study are: to determine the levels of Na, Mg, Ca and Fe in the human scalp hair for individuals living in Karbala, Iraq and to investigate the levels of these elements in relation to smoking activity (non and active), and gender of an individual under investigation.

2. Methods and Materials

2.1 Subjects and Methods:

Karbala is a city in Iraq located about 60 miles south west of Baghdad with approximately one million inhabitants. This town was subjected to military weapons during the two wars in 1991 and 2003[17]. Therefore, air, soil and water have been used to report levels of chemical contamination, especially trace and minor elements. In recent years, many individuals in Karbala have suffered from different health disorders such as cancer, sclerosis, diabetes, asthma, heart diseases, leukemia and various unknown diseases [18]. As part of this study, males (204) and females (32) were studied. The study population consisted of active (100), passive (32) and non-smokers (104), varying in age from three to seventy years. The subjects completed a brief

questionnaire to provide information about their age, body mass, health state, profession, diet program, life-style, smoking activity, drinking water and alcohol consumption.

2.2 Sample Collection and Treatment:

Human scalp hair samples were collected from the same region of the scalp for each individual, namely, from the back of head, less than 1 cm from the scalp. At least 0.5 g of scalp hair were collected and cut into pieces of about 1 cm in length using special scissors and plastic tweezers. The samples were then stored in polyethylene bags in a dry place at room temperature [19, 8, 20]. Previous studies have reported that an ideal washing procedure should remove only external contaminants and leave the endogenous elements in the sample for analysis [21, 3]. Therefore, the sequential washing procedure (method A: acetone-water-water-water-acetone) was adopted in this study [20,5, 22]. A wet digestion method using nitric acid in a Kjeldhal™ tube was used to digest the hair sample. In this method, concentrated nitric acid (1 ml) was added to the hair sample, heated at 160° C ± 10° C in an electric digester within fume until the solution became clear (about 30 min). Samples were then dissolved with deionised water in polypropylene volumetric flasks using constant dilution factor method [23,24].

2.3 Instrumentation

The concentrations of Na, Mg, Ca and Fe were determined by flame atomic absorption spectrometry (FAAS) Perkin Elmer model 400.

3. Results and Discussion

3.1 Elemental levels of human scalp hair

The results were reported as mean, standard deviation (SD), and of Na, Mg, Ca and Fe in human scalp hair. Sodium, Mg, Ca levels were found to be 41 ± 42, 69 ± 84, 929 ± 563 mg/kg dry wt, respectively, as shown in Table 1. Similar results have been reported by other researches for hair samples [26,27,28,19,29]. In some cases, the value of Fe is higher than literature value [15,10].

Variables	Elemental concentrations (mg/kg)			
	Na	Mg	Ca	Fe
Mean	41	69	929	79
SD	42	84	563	104
Range	1 - 323	8 - 874	113 - 2849	26 - 414

: n is the number of samples

The confidence limits (95 %) of the mean value are given by the following formula:

$$95\% \text{ confidence limits} = x \pm zs / \sqrt{n}$$

Where: x is mean value, z is the degree of confidence required, s is standard deviation and n is the number of samples. The value of 95% confidence limits of sodium are (35.6 to 46.5 mg/kg), including the mean value of Na (41 mg/kg). This result has confirmed that there is not any systematic error in this analysis [30]. Similar results were also reported for Ca, Mg and Fe, as shown in Table 2. The Grubb's outlier test was used in order to check any normally distribution data for outliers. It was found that the lowest value of sodium (1 mg/kg Na) was not an outlier, but the highest value (323 mg/kg Na) was an outlier (low G_{calc} 0.95; high G_{calc} 6.7). The critical value (G_{crit}) ($P = 0.05$) is 3.62, therefore, the maximum measurement is rejected at the 5% significance level. The Grubb's results for Na, Mg, Ca and Fe are shown in Table 2.

Table 2: Grubb's test and 95% confidence limit for Na, Mg, Ca and Fe in washed scalp hair samples from Karbala, Iraq.

Element	95% confident limit (mg/kg)	Grubb's test			Test result
		G _{calc} (min)	G _{calc} (max)	G _{crit}	
Na	36 to 47	0.95	6.7	3.45	Maximum value is rejected
Mg	58 to 80	0.73	9.58	3.5	Maximum value is rejected
Ca	855 to 999	1.44	3.4	3.5	Significant result
Fe	66 to 92	0.73	3.2	3.4	Significant result

G_{calc}= Grubb's calculate, G_{crit}=Grubb's critical.

3.2 Influence of Gender

The effect of gender on the concentration of Na, Mg, Ca and Fe was investigated in this study. In fact, approximately, 14 % of the hair samples were taken from females and 86% from males. Table 3 reports the elemental levels as a function of gender. In female's hair, the levels of Na (146 ± 150 mg/kg), Mg (157 ± 89 mg/kg) and Ca (1708 ± 606 mg/kg) were higher when comparing with males hair. In contrast, male's hair samples have higher levels of Fe(44 ± 44 mg/kg). Surprisingly, the content of the majority of elements in hair of females was higher than in the hair of males from Karbala, Iraq. A possible explanation is that the majority of women living in the study area have long term exposure to exogenous contamination; namely permanent solutions, dyes and bleaches [15]. Similar findings have been previously reported for Na, Ca, Mg and Fe [24,28]. Previous study has found that the compositions of human scalp hair are varied in individual having a natural color hair comparison with those that were artificially colored. This study was found that colored hair contained more Ca (270%), Mg (260%), than naturally colored hair [15]. In the case of Fe, mean values were lower in females than in males. This is happening may be due to the menstruation which is a woman's monthly bleeding [31]. In order to assess whether the effect of age makes any significant contribution on the elemental levels of hair samples, the results were examined by an F-test and a two tailed t-test, as shown in Table 3. The findings show that there is a significant effect of gender on the levels of Na ($t_{(234)} = 3.87$, $t_{crit} = 1.97$, $P < 0.05$), Mg ($t_{(234)} = 7.09$, $t_{crit} = 1.97$, $P < 0.05$), Ca ($t_{(234)} = 10.08$, $t_{crit} = 1.97$, $P < 0.05$), and Fe ($t_{(234)} = 3.29$, $t_{crit} = 1.97$, $P < 0.05$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at the probability level of $P = 0.05$), as shown in Table 3. As a result, on the basis of our results, female hair is not an appropriate biomarker for most elements such as those evaluated in the present study. In that case, human toe nails could be considered as a useful material for this purpose since most women usually cover their feet when they leave the home.

Table 3: Elemental levels in washed human scalp hair for males and females.

Element	Mean ± SD (mg/Kg)		F – test		Two - tailed t-test			
	Male n ₁ = 204	Female n ₁ = 32	F _{calc}	Sig.	t _{calc}	df	Sig.	t _{crit}
Na	69 ± 91	146 ± 151	2.79	<0.05	3.87	234 ⁺	< 0.05	1.97
Mg	55 ± 74	157 ± 89	1.50	<0.05	7.09	234 ⁺	< 0.05	1.97
Ca	805 ± 447	1708 ± 606	1.84	<0.05	10.08	234 ⁺	< 0.05	1.97
Fe	44 ± 44	19 ± 14	9.24	<0.05	3.29	234 ⁺	< 0.05	1.97

SD is standard deviation, n₁, n₂ are the number of samples, df = degrees of freedom at n₁ - 1 and n₂ - 1 for F-test, ⁺ degrees of freedom for t-test (n₁ + n₂ - 2), F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at P = 0.05.

3.3 Influence of Smoking

The determination of the elemental levels in biological and environmental samples is very important due to they play an important role in physiological processes of human and other living organisms [32]. However, hair analysis recently becomes a powerful diagnostic tool to monitor the environmental exposure, human health and nutrition studies of individuals. Therefore, it is necessary to establish standard ranges of hair composition for variable groups of population with respect of age, gender and smoking habits. In general tobacco leaves have widely been used to manufacture smoking. Typically, tobacco plants are absorb many of an essential, non-essential and toxic elements from the soils, fertilizers, pesticide treatments, storage, processing, packing and other processes [32]. Previous study has been identified the concentrations of 21 elements in 16 cigarette brands that commonly used in Karbala, Iraq [33]. The obtained results were namely: 84 – 383 mk/kg Na dry wt, 7852 – 8768 mg/kg Mg dry wt, 12392 – 19600 mg/kg Ca dry wt, 143 – 301 mg/kg Fe dry wt, In this study the effect of smoking habits on the elemental composition of hair was investigated by using two subject groups, smokers and non-smokers. Therefore, 50 % of samples were taken from male smokers and 50 % from male non-smokers who were of the same age range. The findings in Table 4 show that a highly significant difference was found for calcium between smokers (981±488 mg/kg) and non-smokers (769±375). In the case of Mg, mean values were slightly higher in smokers than non-smokers, although the difference was not significant (at $P = 0.05$). Conversely, there are no significant differences for the other elements between smoker and non-smoker groups. A higher level of Ca and Mg and lower levels of Na and Fe in smokers was also found in other study [15,24]. One study has reported that the levels of Ca (16000 – 27800 mg/kg dry weight) and Mg (8950 – 16750 mg/kg dry weight) in commercial cigarette tobacco are higher than other elements [10]. The results were evaluated by using F-test and two tailed t-test, as shown in Table 4.

Table 4: Elemental levels in washed human scalp hair for smokers and non-smokers.

Element	Mean ± SD (mg/Kg)		F – test		Two - tailed t-test			
	Smokers (n ₁ = 100)	Non-smokers (n ₂ = 104)	F _{calc}	Sig.	t _{calc}	df	Sig.	t _{crit}
Na	93 ± 90	114 ± 114	1.63	< 0.05	0.99	202 ⁺	< 0.05	1.97
Mg	71 ± 116	50 ± 21	29.79	< 0.05	1.27	202 ⁺	< 0.05	1.97
Ca	981 ± 488	769 ± 375	1.66	< 0.05	2.47	202 ⁺	< 0.05	1.97
Fe	38 ± 26	47 ± 60	5.36	< 0.05	1.08	202 ⁺	< 0.05	1.97

SD is standard deviation, n₁, n₂ are the number of samples, df = degrees of freedom at n₁ - 1 and n₂ - 1 for F-test, ⁺ degrees of freedom for t-test (n₁ + n₂ - 2), F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at P = 0.05.

4. Conclusions

The optimum washing procedure was carried out by using acetone-water-water-acetone which produced the highest level of element removal. Wet digestion using Kjeldhal™ tubes was found to be the preferred digestion method for most elements under investigation resulting in acceptable percentage recoveries.

The highest mean values in the two gender groups are found for Ca followed by Mg, Na and iron for females,. The order of increasing trace element levels in the tear drops for females is: Fe < Na < Mg < Ca, whilst for males is Fe < Mg < Na < Ca. The findings show that there is a significant effect of gender on the levels of Ca, Mg, Na and Fe. In the case of smoking activity effect, no significant effect ($P = 0.05$) was found for Mg, Na and Fe. In contrast, there is a significant effect for Ca at $P = 0.05$.

5. Acknowledgment

The authors wish to thank the Iraqi government for its financial support. The authors also wish to acknowledge Al-Hussein hospital, Karbala, Iraq, University of Surrey, UK and the University of Kerbala, Iraq.

6. References

- 1- **Iyengar, G.V. (1989)**. Elemental Analysis of Biological System, Volume 1: Biomedical, Environmental, Compositional and Methodological Aspects of Trace Elements. CRC Press, Boca Raton, Florida. 11-12.
- 2- **Arain, M.B., Kazi, T.G., Jamali, M.K., Jalbani, N., Afridi, H.I. & Sahah, A. (2008)**. Total dissolved and bioavailable elements in water and sediment samples and their accumulation in *Oreochromis mossambicu* of polluted Manchar Lake. *Chemosphere*, **70**(10): 1845-1856.
- 3- **Apostoli, P. (2002)**. Elements in environmental and occupational medicine. *Journal of Chromatogram B*, **778**: 63-97.
- 4- **Amaral, A.F.S., Arruda, M., Cabral, S. & Rodrigues, A.S. (2008)**. Essential and non-essential trace metals in scalp hair of men chronically exposed to volcanogenic metals in the Azores. Portugal, *Environment International*, **34**: 1104-1108.
- 5- **Gault, A.G., Rowland, H.A.L., Charnock, J.M., Wogelius, R.A., Gomez-Morilla, I., Vong, S., Leng, M., Samreth, S., Sampson, M.L. & Polya, D.A. (2008)**. Arsenic in hair and nails of individuals exposed to arsenic-rich groundwater in Kandal province, Cambodia. *Science of the Total Environment*, **393**: 168-176.
- 6- **Wang, T., Fu, J., Wang, Y., Liao, Ch., Tao, Y. & Jiang, G. (2009)**. Use of scalp hair as indicator of human exposure to heavy metals in an electronic waste recycling area. *Environmental Pollution*, **157**(8-9): 2445-2451.
- 7- **Batista, B.L., Rodrigues, J.L., Nunes, J.A., Tormen, L., Curtius, A.J. & Barbosa, F. (2008)**. Simultaneous determination of Cd, Cu, Mn, Ni, Pb, and Zn in nail samples by inductively coupled plasma mass spectrometry (ICP-MS) after tetramethylammonium hydroxide solubilization at room temperature. *Talanta*, **76**(3): 575-579.
- 8- **Sukumar, A. & Subramanian, R. (2007)**. Relative element levels in the paired samples of scalp hair and fingernails of patients from New Delhi. *The Science of the Total Environment*, **372**: 474-479.
- 9- **Thomson, C.D. (2004)**. Assessment of requirements for selenium and adequacy of selenium status: a review. *European Journal of Clinical Nutrition*, **58**: 391-402.
- 10- **Ward, N.I. (2000)**. Trace Elements, In: Fifield, F.W. & Haines, P.J. (Editors), *Environmental analytical chemistry*. 2nd Edition, Blackwell Science Ltd, Oxford, UK, 360-392.
- 11- **Manso, M., Carvalho, M.L. & Nunes, M.L. (2007)**. Characterization of essential and toxic elements in cephalopod tissues by EDXRF and AAS. *X-ray Spectrometry*, **36**(6): 413-418.
- 12- **Villanueva, R. & Bustamante, P. (2006)**. Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae. *Aquaculture*, **261**(1): 225-240.
- 13- **Goldhaber, S.B. (2003)**. Trace element risk assessment: essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, **38**(2): 232-242.
- 14- **Esteban M, Castano A (2009)**. Non-invasive matrices in human biomonitoring: A review. *Environ Int* 35: 438-449.
- 15- **Chojnacka K, Gorecka H, Gorecki H (2006)**. The effect of age, sex, smoking habit and hair color on the composition of hair. *Environ Toxicol Pharmacol* 22: 52-57.
- 16- **Ashraf W, Jafaar M, Anwer K, Ehsan U (1995)**. Age and sex based comparative distribution of selected metals in the scalp hair of an urban population from two cities in Pakistan, *Environmental Pollution* 87: 61-4.
- 17- **Senofonte O, Violante N, Caroli S (2000)**. Assessment of reference values for elements in human hair of urban schoolboys. *J. Trace Elements Med. Biol* 14: 6 -13

- 18- **United Nations Environment Programme (2003)**. Desk study on the environment in Iraq, Geneva, UNEP.
- 19- **Senofonte O, Violante N, D'Ilio S, Caimi S, Peri A, Caroli S (2001)**. Hair analysis and the early detection of imbalances in trace elements for members of expeditions in Antarctica. *Microchem J* 69: 231-238.
- 20- **Rodrigues JL, Batista BL, Nunes JA, Passos CJS, Jr FB (2008)**. Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. *Sci Total Environ* 405: 370-376.
- 21- **Hawkins, D.P. & Ragnarsdóttir, K.V. (2009)**. The Cu, Mn and Zn concentration of sheep wool: influence of washing procedures, age and colour of matrix. *Science of the Total Environment*, **407**(13) 4140-4148.
- 22- **IAEA (International Atomic Energy Agency), (1978)**. Co-ordinated research programme on trace element pollutants, IAEA/RL/50, Vienna, Austria.
- 23- **Kazi, T.G., Afridi, H.I., Kazi, N., Jamali, M.K., Arain, M.B., Jalbani, N. & Kandhro, G.A. (2008)**. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. *Biological Trace Element Research*, **122**(1): 1-18.
- 24- **Forte, G., Alimón, A., Violante, N., Di Gregorio, M., Senofonte, O., Petrucci, F., Sancesario, G. & Bocca, B. (2005)**. Calcium, copper, iron, magnesium, Silicon and zinc content of hair in Parkinson's disease. *Journal of Trace Elements in Medicine & Biology*, **19**: 195-201.
- 25- **Bass, D.A., Hickok, D., Quig, D. & Urek, K. (2001)**. Trace Element Analysis in Hair: Factors Determining Accuracy, Precision, and Reliability. *Thorne Research, Inc*, **6**(5): 472-482.
- 26- **Skalnaya, M.G. & Demidov, V.A. (2007)**. Hair trace element contents in women with obesity and type 2 diabetes. *Journal of Trace Elements in Medicine and Biology*, **21**: 59-61.
- 27- **Chojnacka, K., Gorecka, H., Chojnacki, A. & Gorecki, H. (2005)**. Inter-element interactions in human hair. *Environmental Toxicology and Pharmacology*, **20**(2): 368-374.
- 28- **Rodushkin, I. & Axelsson, M.D. (2003)**. Application of double focusing sector field ICP-MS for Multielemental characterization of human hair and nails. Part III. Direct analysis by laser ablation. *The Science of the Total Environment*, **305**(1-3): 23-39.
- 29- **Miekeley, N., Dias Carneiro, M.T.W. & de Silvera, C.L.P. (1998)**. How reliable are human hair reference intervals for trace elements? *The Science of the Total Environment*, **218**: 9-17.
- 30- **Miller, J.N. & Miller, J.C. (2010)**. *Statistics and Chemometrics for Analytical Chemistry*. 4th Edition, Pearson Education Limited, England, 1-272.
- 31- **Jian, J., Yang, Q., Dai, D., Eckard, J., Axelrod, D., Smith, J. & Huang, X. (2011)**. Effects of iron deficiency and overload on angiogenesis and oxidative stress—a potential dual role for iron in breast cancer. *Free Radical Biology and Medicine*, **50**(7): 841-847.
- 32- **Martinez, T., Lartigue, J., Zarazua, G., Avila-Perez, P., Navarrete, M. & Tejeda, S. (2008)**. Application of the Total Reflection X-ray Fluorescence technique to trace elements determination in tobacco. *Spectrochimica Acta Part B*, **63**(12): 1469–1472.
- 33- **Joda, B. (2012)**. *Trace Element Levels of Human Fluids and Tissues for Iraqi Individuals*. Ph.D. Thesis, Department of Chemistry, University of Surrey, Guildford, Surrey, England.