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## **Determination of the Trace Element Levels in Hair of Smokers and Non-Smokers by ICP-MS**

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**Abstract:** For at least 50 years, determination of the trace element levels in human hair has been used to assess environmental and vocational exposure to toxic elements. As compared to other biological matrices (e.g. blood, urine), human hair is stable and therefore useful as a matrix. In this study, analyses of toxic and essential trace elements, such as Cd, Pb, Cu, and Fe were done in hair samples which we collected from male smokers (10 people) and non-smokers (10 people) who live in Diyarbakir, Turkey and concentrations in hair samples were compared. Hair samples were washed by a standard procedure proposed by the International Atomic Energy Agency. Then the samples were dried for 16 h at 110°C in an oven. Solubilization procedure was carried out by nitric acid-hydrogen peroxide mixture (3:1) in closed vessels in a microwave oven. Trace element analyses were carried out by using inductively coupled plasma-mass spectrometry (ICP-MS) technique. In this study, while concentrations of Cd, Pb, and Fe elements were found to be considerably higher in smokers than non-smokers, similar results were observed in Cu concentrations. The accuracy of the method was evaluated by applying spike method to samples. There was a good agreement between added and found analyte content. Analytical recovery values were determined between 91.2% and 104.6%. The values of R were found to be higher than 0.99.

**Keywords:** Human Hair, Smokers, Non-smokers, Trace Element, ICP-MS.

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## INTRODUCTION

In recent years, the increasing environmental and vocational awareness of toxic elements has been seen. Being exposed to heavy metals can cause to poisoning and this fact can reveal significant threats to the human health and all living organisms (1). The heavy metals are accumulated inside cells that can cause several pathological changes, affection or death in the case of excessive poisoning (1).

Effect of environmental pollution on human health can be explained with some biological markers such as tooth, hair, and fingernail. Analyzing the hair samples taken from human ensured us the helpful information in order to analyze the changes in our bodies and it became successful in various applications. Analyzing the trace metals on hair samples has some advantages in comparison with the analyzing carried out by using blood or urine samples. The simplicity of matrix, presence of high concentration of trace elements, being collected, stored, and treated easily can be counted in the scope of mentioned advances of analyzing the trace metals on hair samples (2). Furthermore, precious information regarding our medical condition, the implementation of certain drugs and diagnosis about various diseases can be provided by the hair analysis. Thus, human hair analyzing became a significant method for understanding any of quantitative alterations about the certain elements within the human body (2).

The heavy metals are absorbed by tobacco supposedly from the soil as the natural environment of it and from the fertilizers, or pesticides which are used to protect the plant. The alternative environmental effects that can create an impact on the removal of toxic elements by using the plant of tobacco that contains some substances as pH of the soil, and contaminated irrigated water and sewage sludge that is used as fertilizer. Smoking the tobacco takes 87 kinds of organic carcinogens to the lungs as well as the toxic elements that can be parted during the smoke phase of combustion. Some metals [cadmium (Cd), nickel (Ni), and lead (Pb)] easily mix with blood stream and can accumulate within the body of particular organs as the kidney and liver (3). There can be mentioned some studies which were notified on the great diversities about the toxic elements in the compositions of commercial tobacco products where smoking-related troubles have been tried to be connected with toxic elements arisen from the burning of tobacco (3, 4).

Many different techniques such as graphite furnace atomic absorption spectrometry (GF-AAS) (5), flame atomic absorption spectrometry (F-AAS) (6), inductively coupled plasma optical emission spectrometry (ICP-OES) (7, 8), inductively coupled plasma mass spectrometry ICP-MS (9, 10) and X-ray fluorescence spectrometry (XRF) (11) are used in determining the levels of trace elements. ICP-MS, being one of these techniques, is used effectively and widely because of its high sensitivity, accuracy, wide dynamic range in determining of multiple elements at trace levels.

The aim of this study was evaluating the trace toxic and nutrient elements concentrations in the hair of human groups consist of smokers and non-smokers living in urban parts of the city of Diyarbakır. Ten (10) smoker and ten (10) non-smoker male individuals were chosen as referents in order to perform a comparative study. Trace elements levels were determined by ICP-MS, prior to microwave acid digestion. The precision and accuracy of the method was evaluated by applying spike method to solutions.

## **MATERIALS and METHODS**

### **Instrument**

Agilent 7700X model ICP-MS was used for finding the trace elements within the hair samples. The operating conditions for this device are shown in Table 1. The solubilization procedure of hair samples before the analysis was carried out in Milestone Start D brand microwave oven which has the PTFE vessels.

### **Reagents and Solution**

18.2 MΩ deionized water was used in all experiments and also nitric acid (Merck) and hydrogen peroxide (Merck) were used in the procedure of solubilization. All used reagents were analytical grade.  $^6\text{Li}$ ,  $^{45}\text{Sc}$ ,  $^{72}\text{Ge}$ ,  $^{115}\text{In}$ , and  $^{209}\text{Bi}$  in the concentration of 100 mg/L were used as mix internal standard (Matrix: %10  $\text{HNO}_3$ , Agilent technologies) in ICP-MS measurements. The calibration graphs were prepared by diluting in the concentration range of 0-100  $\mu\text{g/L}$  from the standard of mix about 10 mg/L for the metals.

### Sample Collection

The hair samples were collected from the male individuals live initially in the city center of Diyarbakır. Totally 20 healthy individuals consist of 10 smoker individuals and 10 non-smoker individuals from the volunteers informed about the research were added to the research randomly. The hair samples (approximately 1 g) were taken from the points in nape which are near scalp area by cutting with steel scissors. Hair samples were washed with acetone/water (1:3) according to the International Atomic Energy Agency to remove external contamination (12). The washed samples were dried in an oven at 110 °C about 16 hours.

**Table 1:** Optimum operating conditions of ICP-MS for analyzing the samples.

Instrument parameter	Condition
RF power	1550 W
RF frequency	27.12 MHz
RF Matching	1.80 V
Carrier gas (inner)	1.1 L/min
Makeup Gas	0.9 L/min
Plasma gas	Ar X50S 5.0
Plasma gas flow (Ar)	15 L/min
Nebulizer pump	0.1 rps
Sample intake	0.5 mL/min
Spray chamber temperature	2 °C
Resolution m/z	244 amu
Background	<5 cps (9 amu)
Short-term stability	<3% RSD
Long-term stability	<4% RSD/2 h
Isotopes measured	<sup>111</sup> Cd, <sup>208</sup> Pb, <sup>56</sup> Fe, <sup>63</sup> Cu

### Analytical Methods

The amount about 0.2 g from each of dried hair samples was weighed and concentrated nitric acid (6 mL) and hydrogen peroxide (2 mL) at analytical purity were added (3:1 ratio) and it was made soluble in the microwave oven system. Afterwards, it was taken to the volumetric containers and last volumes of it were completed until 15 mL with deionized water. The samples were stored in refrigerator until the measurement was carried out. The trace elements measurements were carried out with the method of ICP-MS.

### Method Validation

Known amounts of the analytes were spiked to the solutions in order to evaluate the accuracy of the method. The results are given in Table 2. Good agreement was obtained between added and found analyte contents using the recommended procedure and recovery values were between 91.2 and 104.6 percent, which is within expected ICP-MS performance.

**Table 2:** Spike recovery values for Cd, Pb, Fe and Cu

Elements	Added ( $\mu\text{g/L}$ )	Found ( $\mu\text{g/L}$ )	Recovery (%)
Cd	50.0	45.6 $\pm$ 1.37	91.2
Pb	50.0	47.5 $\pm$ 1.42	95.0
Fe	50.0	51.3 $\pm$ 2.05	102.6
Cu	50.0	52.3 $\pm$ 2.61	104.6

[mean  $\pm$  SD, n=3]

### Analytical Figures of Merit

The quantitation of the analytes was performed by using the calibration curve method for each analyte, the calibration curves were prepared from the standard solutions of the analytes. The correlation coefficients were obtained at least 0.9993. The limits of detection ( $\text{LOD}=3s$ ) and limits of quantification ( $\text{LOQ}=10s$ ) for four metals were also calculated by analyzing 3 blank solutions. The linear range of calibration graph drawn under the optimized conditions functioning for four elements and values of R, LOQ and LOD are shown in Table 3.

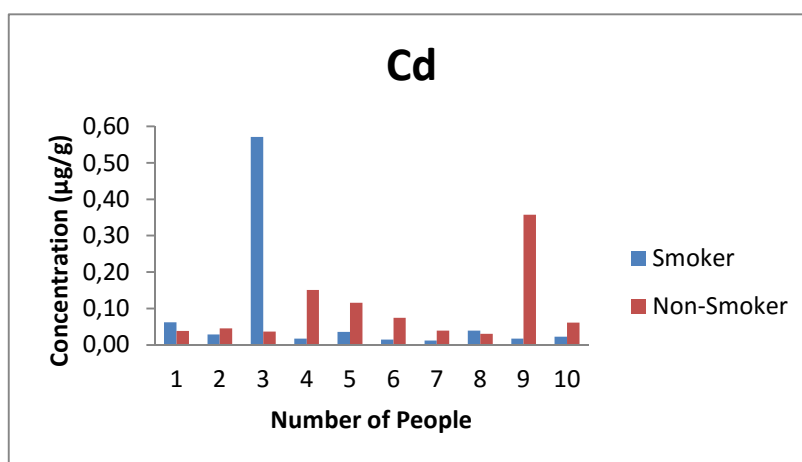
**Table 3:** Parameters of merit for ICP-MS.

Elements	Linear range (µg/kg)	Regression	R <sup>2</sup>	LOD (µg/kg)	LOQ (µg/kg)
Cd	0-100	y= 0.0344 x + 0.0280	0.9993	0.0103	0.0310
Cu	0-100	y= 0.0662 x + 0.1028	0.9999	1.0200	3.0600
Fe	0-100	y= 0.3359 x + 4.6289	0.9999	2.3108	6.9324
Pb	0-100	y= 0.1861 x + 0.2314	0.9996	0.0832	0.2777

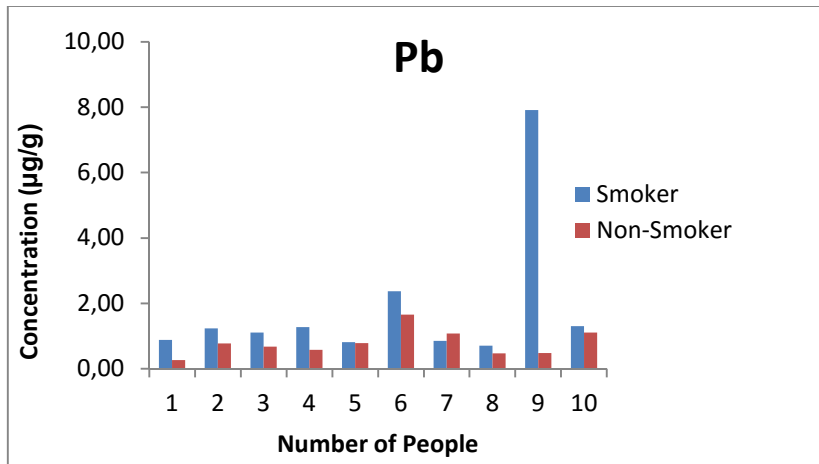
R=Correlation coefficient , LOD= Limit of detection, LOQ=Limit of quantification

### RESULTS and DISCUSSION

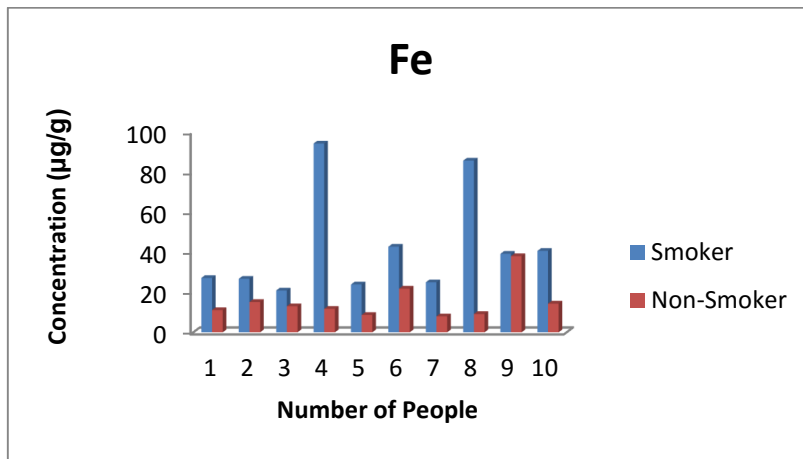
The metal analysis results of smokers and non-smokers hair samples are given in Figure 1-4.



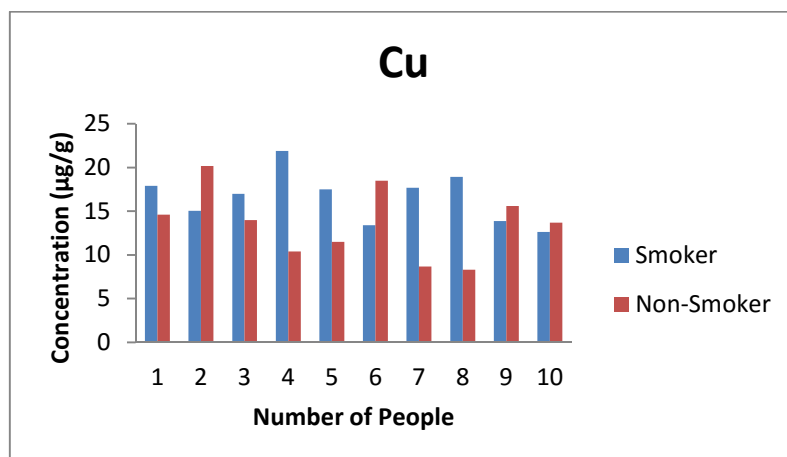
**Figure 1:** Cd concentrations of smokers and non-smokers.



**Figure 2:** Pb concentrations of smokers and non-smokers.



**Figure 3:** Fe concentrations of smokers and non-smokers.



**Figure 4:** Cu concentrations of smokers and non-smokers.

Mean values of the metal analysis of smokers and non-smokers hair samples are given in Table 4.

**Table 4:** Comparisons between smokers and non-smokers in relation to levels Cd, Pb, Fe and Cu in hair samples.

Individuals	n	Cd ( $\mu\text{g/g}$ )	Pb ( $\mu\text{g/g}$ )	Fe ( $\mu\text{g/g}$ )	Cu ( $\mu\text{g/g}$ )
Smokers	10	0.140 $\pm$ 0.04	1.85 $\pm$ 0.05	42.59 $\pm$ 1.28	16.59 $\pm$ 0.49
Non-Smokers	10	0.037 $\pm$ 0.001	0.79 $\pm$ 0.01	15.07 $\pm$ 0.45	13.55 $\pm$ 0.41

Analyzed metals content [mean  $\pm$  SD, n=3]

Cadmium is one of very scarce metals within the soil as in the atmosphere. Cadmium enters the body through respiration and digestion. 15-30% of cadmium taken through respiration is absorbed. One of the most important cadmium sources is cigarette, in a single of which there is 1-2  $\mu\text{g}$  of cadmium. 10% of this amount (0.1-0.2  $\mu\text{g}$ ) is taken through inhalation. Besides, cadmium is also present in the inhaled environmental atmosphere (13). Cadmium is able to affect kidney, lung, and gastrointestinal system. Long-term low-dose Cd exposure leads to bone loss. Low birth weight, skeletal abnormalities, behavioral and learning problems were observed in animals exposed to Cd during the prenatal period. It was observed in animal testing that Cd exposure during pregnancy period affected mostly the nervous system (14, 15). Hair is one of the tissues, where heavy metals such as cadmium are removed out of the body (16, 17). The differences were found significant while the cadmium levels in collected hair samples were compared between smokers and non-smokers in this study. Mean Cd levels of male smokers (0.140  $\mu\text{g/g}$ ) were found to be nearly 4 times higher than those of non-smokers (0.037  $\mu\text{g/g}$ ) as seen in Table 4. Sukumar and Subramanian found that the amount of Cd (1.7  $\mu\text{g/g}$ ) in the hair samples of smokers was higher than that of non-smokers (0.5  $\mu\text{g/g}$ ) (18). Özden *et al.* found Cd level of hair high in children whose schools are near the main street and live in centrally heated houses. Furthermore, as the number of smokers in the house increases, they stated that the rate of determination of Cd level in hair increased, too (19). It was emphasized that the external environment activities, the season and the Cd amount in potable water are important exogenic sources determining the Cd level of hair (14, 20).

Lead is an element existing naturally in nature. An important part of lead in the body is taken by means of foods. Potable water, old houses, and smoking may lead to exposure



to toxic lead. While the lead enters into the body, it mixes with blood stream and starts entering into bone tissues, soft tissues, and the brain (21, 22). Exposure to excessive lead reveals itself with lead poisoning. As symptoms, diagnosis is established with weakness, tiredness, stomachache, anaemia, and sensitiveness. Lead poisoning affects functions of central nervous system in children more (22). It was found in this study that the mean level of Pb (1.85  $\mu\text{g/g}$ ) accumulating in hair of smokers was 2.5 times more than mean level of Pb (0.79  $\mu\text{g/g}$ ) in hair of non-smokers (Table 4). Many studies, by supporting our study, show that Pb amount in hair samples of smokers is higher than that of non-smokers (19, 23, 24). In a study done on smoker mothers by Serdar *et al.* they expressed that there was a significant connection between smoking levels of mothers and concentrations of toxic element (especially Pb) in hair. Furthermore, it is found in the same study that although mothers said they did not smoke around their children, Pb amount in hair of their children was in high concentrations (23). In another study done on school children between the ages 11-13 by Özden *et al.* They determined higher levels of Pb in hair as the number of smokers in the house increased (25). When Mortada *et al.* compared levels of Pb in hair of smokers and non-smokers between the ages 25-35 in Egypt; they found the level of Pb in the hair of smokers significantly high (26).

Iron is an essential trace element which exists in especially the structure of red blood cells in the human organism and is important in terms of being a functional part of hemoglobin. Besides, iron is a vital mineral present in the myoglobin of muscles, the cytochrome, peroxidase, and catalase systems. Iron has important tasks in terms of biochemical reactions especially in terms of respiratory system (22).

Copper is an important element nutritionally and in terms of toxicity (23). The copper is the basic component of hair, skin flexible parts, bone and some internal organs as well as being important in terms of bodily functions. Copper, which is present by average 50-120 mg in adult people, is essential element of reactions of amino acids, fatty acids and vitamins in metabolism under normal conditions. Copper, which exists in the structure of many enzymes and proteins, takes on a task of activator for iron to carry out its functions. In the event of copper deficiency, abnormalities, anemia, bone problems, and nervous system disorders were detected in animals (27).

Metals tied to enzymes such as Cu and Fe exist in tissues with high rates (28). In this study, these elements were also found in high concentrations in hair samples as seen in

Table 4. When Fe and Cu levels were compared while the mean Fe level (42.59 µg/g) of smokers was found to be approximately 3 times higher than non-smokers (15.07 µg/g), the mean Cu level (16.59 µg/g) of smokers was found to be similar to that (13.55 µg/g) of non-smokers. In the study of Serdar *et al.*, by supporting our study, Fe amount was found high in hair of children who live in smoking houses (23). Samanta *et al.* found Fe amount in hair samples as 69.50 µg/g and Cu amount as 14.76 µg/g in their study (28). Szykowska *et al.* found Cu level as 10.23 µg/g in hair samples of smokers and as 9.40 µg/g in those of non-smokers in the study in 2015 (1); Sukumar and Subramanian found Cu level as 13.8 µg/g in hair samples of smokers and as 14.8 µg/g in those of non-smokers in the study in 2007 (18).

## CONCLUSIONS

In this study, while the concentrations of toxic trace elements (Cd, Pb) and essential element (Fe) were found to be considerably higher in smokers than non-smokers, Cu which is nutritionally and toxicologically important element concentration was found similar. Applying spike method to samples assured the accuracy of the method. Good agreement was obtained between added and found analyte contents using the recommended procedure. Recovery values were between 91.2% and 104.6%, thus it is possible to conclude that no analyte loss and no contamination were occurred during the whole procedure. The value of R to be bigger than 0.99 was evaluated as "the linearity of acceptability". The analyte contents in the hair samples were clearly higher than the detection limits observed.

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**Türkçe Öz ve Anahtar Kelimeler**  
**Sigara İçen ve İçmeyen Kişilerde Eser Element Seviyelerinin ICP-MS ile Belirlenmesi**

**Öz:** En az 50 yıldır, insan saçındaki eser element seviyelerinin belirlenmesi çevresel ve mesleki anlamda zehirli elementlere maruziyetin değerlendirilmesi için kullanılmaktadır. Diğer biyolojik matrislere (örneğin kan, idrar) nazaran, insan saçı kararlıdır ve bu nedenle de bir matris olarak faydalıdır. Bu çalışmada Diyarbakır ilinde yaşayan, sigara içen (10 kişi) ve içmeyen (10 kişi) erkeklerden topladığımız saç örneklerinde Cd, Pb, Cu ve Fe gibi toksik ve besleyici eser elementlerin analizleri yapıldı ve saç örneklerindeki konsantrasyonları karşılaştırıldı. Saç örnekleri, Uluslararası Atom Enerjisi Kurumu tarafından önerilen standart prosedüre göre yıkandı ve 16 saat 110 °C' de etüvde kurutuldu. Çözünürleştirme işlemi, nitrik asit-hidrojen peroksit karışımı (3:1) ile kapalı tüplerde mikrodalga fırında gerçekleştirildi. Eser element analizleri ICP-MS tayin tekniği ile gerçekleştirildi. Çalışmada Cd, Pb, Fe elementlerinin konsantrasyonları sigara içen kişilerde içmeyenlere oranla önemli derecede yüksek bulunurken, Cu konsantrasyonunda benzer sonuçlar gözlemlendi. Örnek numunelere spike yöntemi uygulanarak metodun doğruluğu ve kesinliği kontrol edildi. Eklenen ve bulunan analit içerikleri arasında iyi bir ilişki bulundu. Analitik geri kazanım sonuçları % 91.2 ile % 104.6 arasında belirlendi. R değerleri 0.99'dan büyük bulundu.

**Anahtar Kelimeler:** İnsan Saçı , Sigara İçen Kişiler, Sigara İçmeyen Kişiler, Eser Element, ICP-MS.

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