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Trace Metal Profiles of Human Hair and Nail Samples Collected from Urban and Rural Areas of Zonguldak, Turkey

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ABSTRACT

Hair and nail samples were collected during a period of October to December 2012 from 63 healthy humans who have been living in Zonguldak for at least ten consecutive years. The humans aged over 30 years and having no serious health problems were participated in the study. The methodology involved the collection, ultrasonic cleaning and microwave digestion of samples. The measurements of trace metals (As, Cu, Zn, Se, Cd, Pb, Cr, Fe and Ni) in samples were performed with ICP-MS. The predominant metals determined in the both hair and nail samples were the essential metals with the decreasing concentrations in the order of Zn>Fe>Cu. Other metals determined in the hair samples followed the concentration order of Pb>Se>Ni>Cr>As>Cd, whereas in the nail samples, the order was Se>Ni>Cr>Pb>As>Cd. Subgroups were formed according to location and gender. Se and Fe concentrations in hair samples obtained from urban residents were significantly higher. With regard to nail samples, the rural residents exhibited more elevated Fe levels significantly. Gender had a significant influence on the concentration of Zn and Cu in hair samples with females presenting higher values. However, the effect of gender on the trace metal concentrations in nail samples was negligible. The metal concentrations obtained for Zonguldak were within or below the values reported worldwide.

Keywords: *Hair, Nail, Trace Metals, ICP-MS, Zonguldak*

Zonguldak'ın Kentsel ve Kırsal Bölgelerinden Toplanan İnsan Saç ve Tırnak Örneklerinin İz Metal Profilleri

ÖZET

Ekim-Aralık 2012 döneminde Zonguldak'ta en az on yıldan beri yaşayan 63 sağlıklı insandan saç ve tırnak örnekleri toplanmıştır. Çalışmaya 30 yaşın üzerinde olan ve ciddi sağlık sorunu olmayan insanlar katılmıştır. Metodoloji, numunelerin toplanması, ultrasonik ile temizlenmesi ve mikrodalga ile parçalanması işlemlerini içermektedir. Örneklerdeki iz metallerin (As, Cu, Zn, Se, Cd, Pb, Cr, Fe ve Ni) ölçümleri indüktif eşleşmiş plazma-kütle spektrometresi ile yapılmıştır. Hem saç hem de tırnak numunelerinde belirlenen baskın metaller, Zn>Fe>Cu şeklinde azalan konsantrasyon sırasına sahip temel metallerdir. Saç numunelerinde belirlenen diğer metaller Pb> Se> Ni> Cr> As> Cd konsantrasyon sırasını takip ederken, tırnak numunelerinde sıra Se> Ni> Cr> Pb> As> Cd olarak bulunmuştur. Yerleşim yeri ve cinsiyete göre alt gruplar oluşturulmuştur. Kentsel bölgede yaşayanlardan elde edilen saç örneklerinde Se ve Fe konsantrasyonları kayda değer şekilde daha

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yüksek bulunmuştur. Tırnak örnekleri ile ilgili olarak, kırsal kesimde yaşayanlar önemli ölçüde daha yüksek Fe seviyeleri sergilemiştir. Saç örneklerindeki Zn ve Cu konsantrasyonları üzerine cinsiyetin önemli bir etkiye sahip olduğu ve kadınlarda bu metallerin daha yüksek seviyelerde olduğu tespit edilmiştir. Ancak, tırnaktaki metal konsantrasyonları üzerine cinsiyetin etkisi önemsizdir. Zonguldak için tespit edilen metal konsantrasyonları, dünya çapında rapor edilen değerlerin arasında veya altındadır.

Anahtar kelimeler: *Saç, Tırnak, İz Metaller, ICP-MS, Zonguldak*

I. INTRODUCTION

Trace metals are non-biodegradable environmental contaminants that may be released into the environment by various natural and anthropogenic sources such as crustal materials, road dust, construction activities, motor vehicles, coal and oil combustion, incineration and other industrial activities [1]. People get exposed to trace metals through inhalation, dietary intake and dermal contact pathways [2]. The excess of toxic metals as well as the absence of essential ones may cause serious problems in the human health. Therefore, the biomonitoring of trace metals in human body tissues is of great importance.

In recent years, human hair and nail have been regarded as suitable tissues for monitoring human exposure to many toxic and essential metals over a period of time [3]. Hair and nail samples can be collected easily in sufficient quantity without causing pain and health risk for the individual during sampling [4]. The significantly different levels of trace metals in hair and nail between exposed and non-exposed populations have been reported in previous studies, indicating that environmental exposure can partly affect the elemental content of these tissues [5,6]. In addition, some certain associations have been found between trace metals and several diseases with the analysis of hair and nail samples collected from patients and healthy controls [7,8].

To date, some researchers have investigated trace metal concentrations in environmental samples in Zonguldak province [9,10]. However, there has not been any research data reported on the biological monitoring of trace metal levels in hair and nail samples. Therefore, the main objective of this study was to investigate possible human exposure to trace metals in Zonguldak province, where coal is mined and burned for domestic heating and electricity generation in power plants.

II. MATERIALS AND METHODS

A. STUDY AREA AND COLLECTION OF THE SAMPLES

The study was performed in the cities of Zonguldak and Devrek, located in the Western Black Sea Region in Turkey. Zonguldak has a population of about 125,000 inhabitants. The city is known as a center for coal mining and energy production, thus characterized as urban area. Devrek is located 50 km away from Zonguldak with a population of more than 57,000 inhabitants. This area is mostly covered by agricultural fields and grasslands, so considered as a rural area. The map of the sampling sites is shown in Figure 1.

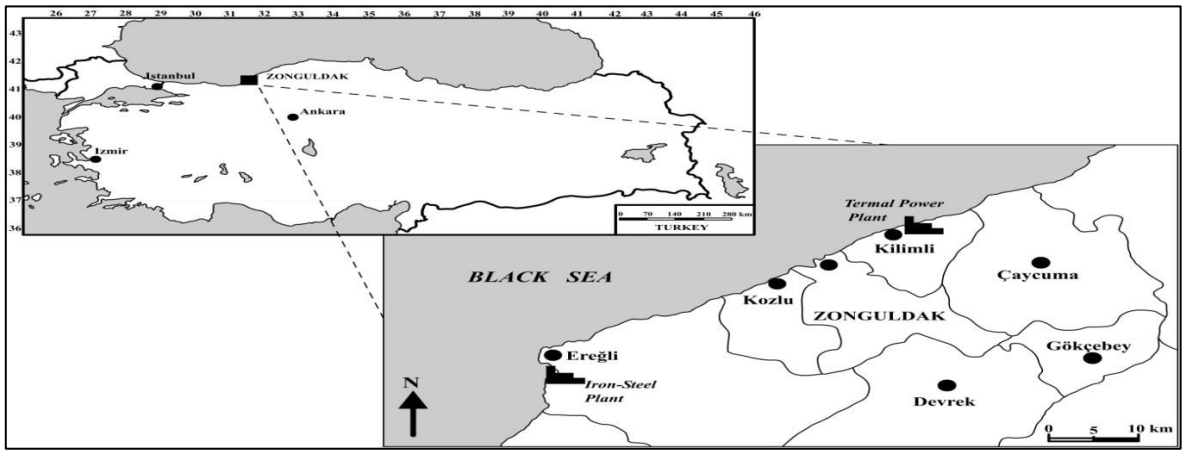

Figure 1. *Location map of the sampling sites.*

Hair and fingernail samples were collected during a period of October to December 2012 from 63 healthy humans who have been living in Zonguldak (n=53) and Devrek (n=10) for at least ten consecutive years. The humans aged over 30 years and having no serious health problems were participated in the study. The humans were initially briefed about the purpose and objectives of the study and then a written consent was obtained. The study was approved by the Ethics Committee of Abant Izzet Baysal University (B.30.2.ABÜ.0.20.05.04-050.01.04-21). Hair samples 1–3 cm long were cut with stainless steel scissors from the occipital position of the head. All the fingernails of the humans were clipped with a stainless steel clipper. The samples were packed into the polyethylene bags, labeled with identifying numbers and transferred to the laboratory for the trace metal analysis.

B. DETERMINATION OF TRACE METALS

Prior to chemical analysis, a standardized washing protocol, established by International Atomic Energy Agency (IAEA), was used to wash the hair and nail samples to avoid the exogenous contamination [11]. The hair and nail samples were firstly washed with distilled water in the ultrasonic bath for 15 minutes. The samples were subsequently washed again with acetone-water-water-water-acetone, then placed in glass containers and dried in the oven overnight at 50 °C. After drying, the samples were placed in sealed polyethylene bags and labeled.

For metal analysis, 50 mg of the samples was digested with 0.5 mL of HNO_3 and 0.5 mL of H_2O_2 in a microwave digestion system (Berghof Speedwave, Germany). After digestion, the solutions were diluted by the addition of deionized water to reach a volume of 10 mL. Deionized water was obtained from Zeener Power I Scholar-UV (18.2 MΩ) instrument.

The measurements of trace metals, namely As, Cu, Zn, Se, Cd, Pb, Cr, Fe and Ni were performed with ICP-MS using a Perkin-Elmer NexION 300D ICP-MS device. Calibration of the instrument for the determination of As, Cu, Zn, Se, Cd, Pb, Cr, Fe and Ni was carried out using NexION SETUP solution (Perkin Elmer, Shelton, USA) and VHG Labs standard solutions (Manchester, USA). Blank samples were also analyzed and the operational detection limit for each metal was calculated as three times the standard deviation of the analyte concentration in blank samples (n=12). Detection limits were calculated to be 0.018 µg/g for As, 0.214 µg/g for Cu, 1.260 µg/g for Zn, 0.092 µg/g for Se, 0.011 µg/g for Cd, 0.009 µg/g for Pb, 0.120 µg/g for Cr, 1.541 µg/g for Fe and 0.110 µg/g for Ni. The validity of the analytical procedure was checked by the hair standard reference material (NCS DC 73347). The recovery rates of certified metals in the reference material ranged between 95% for Pb and 104% for Zn.

C. STATISTICAL ANALYSIS

The distribution of the data was evaluated using the Shapiro–Wilk test. Due to the non-normal distribution of most variables, the differences between two groups for continuous dependent and independent variables were tested by Wilcoxon and Mann Whitney U tests, respectively. The relationships among different metals in hair and nail samples were determined by Spearman's rank correlation test. For all statistical analyses, the IBM SPSS 22 statistical software was used.

III. RESULTS AND DISCUSSION

The normality of results was checked by the Shapiro-Wilk normality test. Almost all variables exhibited significant (with almost all at 99.9% level) deviance from normality, except for Zn content in hair and Cu content in nail samples. The same observation was reported in the previous surveys for Zn in the hair [2,3] and Cu in the nail samples [12]. A possible reason for this observation may be that those elements are essential in body metabolism, so their concentrations are internally controlled, leading to a normal distribution of measurements. The levels of non-essential elements are externally controlled via occupational, dietary or environmental exposure, which may be the cause of the non-symmetrical distribution of data.

Table 1 presents maximum, minimum, median and mean concentration with standard deviation (SD) values for trace metals determined in both hair and nail samples. As the majority of the data were non-normally distributed, median concentrations for trace metals were used throughout the ongoing discussions. The predominant metals determined in the both hair and nail samples were the essential metals with the decreasing concentrations in the order of Zn>Fe>Cu. Other metals determined in the hair samples followed an order Pb>Se>Ni>Cr>As>Cd, whereas in the nail samples, the order was Se>Ni>Cr>Pb>As>Cd. Generally, the concentrations of metals (Zn, Cu, and Fe) associated with enzymes are present at the higher levels in tissues. Since hair and nails are chemically related, it might be expected that they would incorporate trace elements in a similar manner [13]. The Wilcoxon test showed that Cu, Zn, Cd and Pb concentrations in hair samples were significantly higher than those in nail samples (P<0.05 for Cu and P<0.01 for the others), while the opposite was the case for Se, Cr, Fe and Ni (P<0.01). No significant difference was observed between hair and nail As levels. The distribution profiles of Zn, Cu, and Fe between hair and nails were in agreement with the previous observations [13,14]. Among the other metals, Ni and Cd also exhibited consistent distributions [15].

Table 1. *Descriptive statistical parameters for the trace metals (μg/g dry-weight basis) in hair and nail samples.*

	Hair (μg/g)					Nail (μg/g)					
Metals	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max	P^a
As	63	0.12 ± 0.07	0.11	0.03	0.32	63	0.14 ± 0.09	0.11	0.05	0.56	0.187
Cu	63	12.72 ± 3.59	11.70	5.00	24.85	63	11.31 ± 3.04	10.98	3.25	18.57	0.043
Zn	63	141.9 ± 39.0	152.0	52.2	220.0	63	108.0 ± 25.9	105.0	38.56	198.5	0.000
Se	63	0.61 ± 0.15	0.58	0.43	1.49	63	0.83 ± 0.19	0.82	0.30	1.58	0.000
Cd	63	0.04 ± 0.01	0.03	0.01	0.30	63	0.02 ± 0.01	0.02	0.01	0.07	0.005
Pb	63	1.46 ± 1.16	1.09	0.24	5.03	63	0.45 ± 0.32	0.33	0.18	1.75	0.000
Cr	63	0.17 ± 0.08	0.15	0.08	0.39	63	0.91 ± 0.72	0.63	0.24	3.01	0.000
Fe	63	13.83 ± 5.89	14.65	3.84	24.90	63	35.98 ± 28.58	30.33	8.00	142.6	0.000
Ni	63	0.49 ± 0.34	0.36	0.11	1.46	63	1.07 ± 0.92	0.71	0.24	4.55	0.000

[a] P values that resulted from the Wilcoxon test.

A. COMPARISON OF THE METALS ACCORDING TO LOCATION AND GENDER

Table 2 and Table 3 present the metal concentrations with respect to location and gender in hair and nail samples, respectively. The humans living in urban area (n=53) had higher Se concentration in hair (P<0.05) compared to those living in rural area (n=10). This difference is most likely due to the higher consumption of fish and other seafood within the urban population residing near the coastal area. Beside fish, other food items including meat, nuts, cereals and bread can also be the potential sources of exposure to Se [16]. In addition, Fe concentration in hair was found to be statistically higher (P<0.05) in urban residents which might be due to the external air exposure. The dust containing metals can attach to human hair in a typical urban environment and this external contamination cannot be totally removed by the decontamination procedure [17].

Table 2. *Metal concentrations with respect to location and gender in hair samples.*

Metals (μg/g)	Hair urban		Hair rural			Hair male		Hair female		
	N	Median	N	Median	P^a	N	Median	N	Median	P^a
As	53	0.11	10	0.11	0.807	31	0.12	32	0.11	0.280
Cu	53	11.92	10	11.65	0.807	31	11.55	32	11.81	0.650
Zn	53	141.8	10	168.6	0.158	31	130.4	32	162.7	0.005
Se	53	0.59	10	0.53	0.020	31	0.57	32	0.58	0.826
Cd	53	0.03	10	0.02	0.284	31	0.03	32	0.03	0.224
Pb	53	1.09	10	1.01	0.778	31	1.14	32	0.96	0.372
Cr	53	0.16	10	0.12	0.102	31	0.15	32	0.15	0.826
Fe	53	14.90	10	7.70	0.034	31	13.50	32	15.02	0.290
Ni	53	0.33	10	0.56	0.244	31	0.24	32	0.57	0.000

[a]*P* values that resulted from the Mann-Whitney U test

Table 3. *Metal concentrations with respect to location and gender in nail samples.*

Metals (μg/g)	Nail urban		Nail rural			Nail male		Nail female		
	N	Median	N	Median	*P*	N	Median	N	Median	*P*
As	53	0.12	10	0.10	0.071	31	0.13	32	0.11	0.042
Cu	53	11.22	10	10.61	0.638	31	11.74	32	10.41	0.099
Zn	53	106.7	10	97.43	0.176	31	106.4	32	104.8	0.417
Se	53	0.82	10	0.76	0.259	31	0.82	32	0.81	0.564
Cd	53	0.02	10	0.03	0.071	31	0.02	32	0.02	0.072
Pb	53	0.34	10	0.31	0.529	31	0.33	32	0.31	0.902
Cr	53	0.62	10	0.65	0.821	31	0.73	32	0.61	0.316
Fe	53	26.31	10	66.21	0.001	31	26.31	32	33.99	0.136
Ni	53	0.74	10	0.65	0.377	31	0.79	32	0.71	0.573

[a]*P* values that resulted from the Mann-Whitney U test.

With regard to nail samples, the rural group (n=10) exhibited more elevated Fe levels significantly (P<0.01) than the urban group (n=53). The rural residents are generally in contact with soil particles more during the farming activity, one may then think that the result found here is attributable to external contamination of nails by soil containing Fe. However, any external contamination in most nail specimens can be effectively removed using ultrasonic cleaning protocols with both polar and nonpolar solvents [18]. In addition, it has been found that the adsorption of external heavy metal on nail surface is negligible compared to hair [19,20]. Similar results were noted in the previous surveys

with nail Fe levels significantly higher in those living in the rural areas and the difference was ascribed to the nutritional factors [3,21].

Hair trace metals analysis in men and women indicated that the hair levels of Zn and Ni were gender-related. In detail, women were characterized by significantly higher Zn (P= 0.005) and Ni (P<0.001) content in comparison to men. No significant difference was found for the remaining metals between men and women in hair. The obtained data are in agreement with earlier studies indicating gender-related difference of Zn [22-25] and Ni [26-28] in hair. Different metabolism and physiological roles of the elements have been referred as possible explanations for different hair metal content between the two genders [28]. With regard to nail samples, no significant differences were found for trace metals in relation to gender except As (P =0.042), indicating that the effect of gender on the trace metal concentrations in nail was negligible.

B. CORRELATIONS BETWEEN THE METALS

The relationships between individual metals were investigated by Spearman correlation analysis. The correlation coefficients (r) obtained for both hair and nail metals are shown in Table 4. The correlation coefficients were marked in the table to indicate the significance levels (P<0.01 or <0.05). In case of hair of the humans, strong positive correlations were observed among Pb/Cd, Cr/Cd, Fe/Cr, Ni/Zn and Ni/Cd. Some significant but weak correlations were also found among Pb/Se, Cr/Se, Cr/Pb and Ni/Cu. For nails of the humans, strong positive correlations were found among Zn/Cu, Cd/Cu, Pb/Cd, Cr/Cu, Fe/Cr, Ni/Cu and Ni/Cd. Positive correlations were also observed among Se/Zn, Pb/Cu, Cr/As and Cr/Pb but these correlations remained weak. A negative correlation was only observed between Pb and Se but this correlation was also weak. All other metal pairs in both hair and nail exhibited insignificant positive or negative relationships. The comparative literature survey exhibited common significant correlations among Pb/Cr, Fe/Cr, Pb/Cd and Zn/Cu, nevertheless the reason underlying these correlations has not been clearly explained [29-32].

Table 4. *Spearman correlation coefficients for trace metals in hair and nail samples (n=63).*

Hair	As	Cu	Zn	Se	Cd	Pb	Cr	Fe	Ni
As	1.000								
Cu	0.035	1.000							
Zn	-0.161	0.214	1.000						
Se	-0.048	0.089	-0.070	1.000					
Cd	-0.174	0.180	0.130	0.218	1.000				
Pb	0.214	0.069	-0.094	0.272*	0.511**	1.000			
Cr	0.010	-0.012	0.183	0.305*	0.374**	0.287*	1.000		
Fe	0.040	-0.007	0.090	0.097	0.141	-0.007	0.413**	1.000	
Ni	-0.214	0.256*	0.325**	0.086	0.347**	0.007	0.234	0.197	1.000
Nail	As	Cu	Zn	Se	Cd	Pb	Cr	Fe	Ni
As	1.000								
Cu	0.138	1.000							
Zn	0.137	0.587**	1.000						
Se	0.089	0.131	0.253*	1.000					
Cd	-0.189	0.383**	0.166	0.030	1.000				
Pb	0.037	0.314*	0.161	-0,312*	0.357**	1.000			
Cr	0.305*	0.498**	0.207	0.040	0.186	0.279*	1.000		
Fe	0.174	0.198	-0.027	-0.059	0.158	0.166	0.344**	1.000	
Ni	-0.017	0.401**	0.186	0.046	0.430**	0.244	0.084	-0.071	1.000

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

The measured concentrations of As, Cu, Zn, Se, Cd, Pb, Cr, Fe and Ni in hair and nail were compared with data from the literature. The results were found to be within the range of concentrations published in the literature (Table 5). The reference range and possible health consequences have been discussed in previous papers [4,33]. Based on the comparison results, it may be concluded that the people residing in Zonguldak area were not exposed to related metals via environmental factors.

Table 5. *Comparison of trace metal concentrations (µg/g) reported worldwide.*

Hair samples										
Location	As	Cu	Zn	Se	Cd	Pb	Cr	Fe	Ni	Reference
Wroclaw, Poland	0.73	-	-	-	0.08	2.29	-	-	0.99	[34]
Madrid, Spain	0.12	50.8	131.0	1.0	0.03	1.23	0.5	17.3	0.8	[32]
Norway	0.01	76.5	190	0.39	0.04	1.00	-	13.3	-	[35]
Palermo, Italy	-	22.87	189.2	0.46	0.04	1.01	0.11	-	0.55	[22]
Guangzhou, China	0.83	16.66	71.17	-	0.11	4.24	2.19	-	-	[11]
Lahore, Pakistan	-	11.64	255.28	-	0.13	8.08	1.02	-	7.74	[2]
Kayseri, Turkey[a]	0.24	11.93	63.7	5.06	0.17	6.2	0.99	28.19	0.69	[36]
Ankara, Turkey[a]	-	10.3	98	0.6	-	-	-	24.3	-	[37]
Zonguldak, Turkey[b]	0.11	11.70	152.0	0.58	0.03	1.09	0.15	14.65	0.36	This work
Nail samples										
Location	As	Cu	Zn	Se	Cd	Pb	Cr	Fe	Ni	Reference
Lublin, Poland[a]	-	18.23	129.8	-	-	0.1	-	78.0	-	[12]
Tainan, Taiwan[a]	-	17.93	131.8	-	-	1.0	-	75.32	-	[12]
Aswan, Egypt	-	11.3	171	-	0.74	13.3	-	-	-	[14]
Nairobi, Kenya	-	-	94.8	-	0.73	27.5	-	66.9	-	[3]
Lahore, Pakistan	-	20.74	251.2	-	0.14	10.57	3.0	-	10.68	[2]
France[b]	0.07	6.5	108	0.74	0.03	0.72	0.42	-	0.91	[17]
Zonguldak, Turkey[b]	0.11	10.98	105.0	0.82	0.02	0.33	0.63	30.33	0.71	This work

[a]Healthy controls

[b]Median concentrations

IV. CONCLUSION

In the present study, hair and nail samples collected from the humans residing in Zonguldak area were analyzed with ICP-MS for nine selected metals (As, Cu, Zn, Se, Cd, Pb, Cr, Fe and Ni). The obtained data were evaluated in terms of location and gender. Hair concentrations of Se and Fe were significantly higher in urban residents. With regard to nail samples, the rural residents exhibited more elevated Fe levels significantly. Gender had a significant influence on the concentration of Zn and Cu in hair with higher concentrations found in females, whereas the effect of gender on the trace metal concentrations in nail was found to be negligible. The metal concentrations obtained for Zonguldak were within or below the values presented in the literature, indicating that the people residing in Zonguldak were not exposed to related metals via environmental factors. Nevertheless, a wider study with more number of samples and collecting information on food intake/individual habits should be carried out in order to confirm whether people are exposed to trace metals or not.

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