



A Comprehensive Artificial Sweat Study for Quantitation of Nickel and Other Inorganic Elements Released from Imitation Earrings Purchased in Istanbul Market

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Received: 6 December 2019 / Accepted: 7 January 2020
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Abstract

Element sensitization is frequent cause of allergic contact dermatitis, and Ni is the main reason for this condition owing to daily use of different Ni-containing items. European Union established a limit of $0.2 \mu\text{g}/\text{cm}^2/\text{week}$ Ni release for the items by the Ni Directive in 2004. The aim of this study was to determine the amount of element release of imitation earrings sold in different points of Istanbul with different prices and consider the element release levels including 29 trace and toxic elements. After calculation of released nickel amounts, 31.80% of earrings (71 of 223) were observed over the limit of European Commission Directive's migration limit. Minimum and maximum nickel release amounts were found as 0.21 and $30.11 \mu\text{g}/\text{cm}^2/\text{week}$, respectively, and mean level was found $3.24 \mu\text{g}/\text{cm}^2/\text{week}$ among 71 positive earrings. Results showed that essential part of imitation earrings used especially by women release nickel particles over the European Commission Directive's migration limit. In addition, other elements such as Cu, Cr, and Co were found to be in the structure of the imitation earrings, as well as high amounts of toxic element Pb.

Keywords Artificial sweat · Earrings · Nickel allergy · Nickel release · Toxic elements

Introduction

Allergic contact dermatitis (ACD) is a pruritic inflammation of the skin with an increasing incidence worldwide, especially as a result of industrialization. The most common cause of ACD is the contact to various allergens, especially element-containing sources [1].

In a recent meta-analysis, the prevalence of element hypersensitivity has been reported to be at least 20% in the general population [2]. Among the elements, Ni is considered the most prevalent cause of ACD worldwide. Ni is also present in a wide variety of daily objects, including coins, hand tools, cosmetics, clothing accessories, cell phones, and imitation jewelry, as well as the occupational sources [3].

Human sweat has a corrosive nature, causing the release of and dermal absorption of element ions bound onto the object containing the allergen. Although Ni is the most common cause of element-induced ACD, other elements such as Co,

Cr, Cu, Zn, Al, as well as more valuable elements Ag, Au, and the platinum group elements has been shown [2, 4]. Among these, Co is accepted as a potent allergen, and simultaneous reactions to Co and Ni are frequent [5]. Although Co and Cr are mainly used in industrial sites, and occupational exposure to these elements has been reported in different case series, there are studies addressing these elements in the etiology of ACD caused by the use of imitation jewelry [6, 7]. Cr hypersensitivity was reported following skin contact with hexavalent and trivalent compounds of the element [8]. Oral ingestion of nutritional multielement supplementations and placement of joint prostheses and other implants were reported to cause adverse reactions to Cr [9]. Cr is a common known cause of ACD, both in adults and children due to the use of tanned leather shoes, paints, and artificial tattoos [3]. Although contact dermatitis to Cu and Zn are uncommon, the former is the primary element used in the alloys in the production of jewelry, and the latter was the main reason in several ACD cases treated with element-containing dental fillings [5, 10]. Al is also an element to which everyday contact is inevitable as a result of its abundance in the nature and wide use in various objects in daily use, such as antiperspirants, cookware, packaging and preserving materials, and vaccines. It was reported that the probability of allergic reactions to Al depends on the exposure dose and duration [11].

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The ACD cases due to the use of element-containing imitation jewelry products show female predominance related to the daily use of these items by women of different age groups [12, 13].

As a result of a high frequency of Ni-related ACD cases, European Union (EU) adopted a directive on the amount of Ni release from element-containing sources in an attempt to regulate the manufacturing processes of Ni containing objects in daily use. According to this legislation, the migration limit of the element in the penetrating products was set below the limit of $0.2 \mu\text{g}/\text{cm}^2/\text{week}$ [14]. However, regulations limiting the exposure of consumers to other elements have not been implemented long after the release of the EU Commission Directive in 2004 for Ni. Furthermore, ACD is a frequent dermatological condition reported worldwide; studies on the comprehensive element content of jewelry products are scarce, and there is no study in the literature on the simultaneous quantification of 29 elements per sample using a highly specific and validated ICP-MS method. ICP-MS is an analytical technique that depends on the atomization and ionization of the samples under high-temperature argon plasma and then mass detection of the ions in the content of the sample according to their mass-charge ratio (m/z) by an electron multiplier. The reason to choose ICP-MS-based analytical approach in the current study is due to its multielement capability, less complex pre-analytical sample preparation process, and lower detection limit compared to the other analytical techniques for element detection.

This study aimed to determine the element release amount of 223 different cheap imitation earrings available on the Istanbul market and determine the distribution rates of the earrings with Ni content higher than the directive's limit in terms of purchasing places alongside 28 other toxic and allergic elements potentially found in the content of the element alloys used in the production of imitation jewels. In an attempt to measure the levels of inorganic elements found in the compositions of these earrings, an inductively coupled plasma mass spectrometry (ICP-MS)-based quantification study was performed on the levels of 29 elements including Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Mn, Mo, Ni, P, Pb, Pd, Sb, Se, Sn, Sr, Tl, U, Zn, and Zr. In addition, a correlation study was performed on the quantity of these elements in the study materials and analyzed the relationship between the elemental profile and purchase site and price range as well.

Materials and Methods

Reagents, Equipment and Calibrations

In the presented method, nitric acid (HNO_3) 65% (v/v) solutions were supplied from Merck Suprapur® (Merck, Darmstadt, Germany). Also sodium dodecyl sulfate (SDS) used for cleaning process was supplied from Merck

(Darmstadt, Germany). Certified tune solutions (including 10 mg mL^{-1} of Ba, Be, Bi, Ce, Co, In, Pb, Li, Ni, and U in 2% HNO_3) for short-term stability tests and calibration solutions for quantitative analysis (10 mg mL^{-1}) were purchased from High-Purity Standards (Charleston, SC, USA); Indium (In) and Gallium (Ga) (1000 mg mL^{-1}) as internal standards (ISs) were supplied from Absolute Standards Inc. (Hamden, CT, USA). All measurements were carried out using high purity argon gas (> 99.999% purity, Okser, Turkey) to obtain Ar plasma. For artificial sweat test, Suprapur® NaCl, lactic acid, and urea with a purity of 98% (Merck, Darmstadt, Germany) were used. Ultrapure water (Millipore Direct-Q UV 3, Molsheim, France) ($18.2 \text{ M}\Omega \text{ cm}$) was used for dilution of samples and standards as well as cleaning. A Thermo Scientific X Series II ICP-MS (Thermo Fisher Scientific, Bremen, Germany) coupled with a CETAC (Omaha, Neb., USA) autosampler unit (ASX 520) was used for elemental determinations.

The calibration solutions were prepared daily in 2% HNO_3 by incremental amount as 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, 100, and 150 ng mL^{-1} for Li, Be, Al, Cr, Mn, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Sb, Ba, Tl, Pb, Bi, and U; 1, 2, 5, 10, 15, 25, and 35 ng mL^{-1} for Hg; 5, 10, 20, 50, 100, 200, 250, 300, and 400 ng mL^{-1} for B, Mg, P, and Zn. Additionally, K, Ca, Zr, Pd, and Sn were analyzed with semiquantitative method since the lack of certified reference standards. All calibration solutions and samples were added internal standards (20 ng mL^{-1} of Ga and In) and prepared in triplicate for each experiments. All polypropylene materials and vials with flat-top screw caps (Falcon, Fisher Scientific, UK) were cleaned by soaking them in 10% (v/v) HNO_3 solution, thoroughly rinsed with ultrapure water and dried at room temperature before use. Nitric acid solutions for dilution (2%) and cleaning (10%) were daily prepared.

Sample Collection and Preparation

In this study, a total of 223 inexpensive imitation earrings including different penetration types (Fig. 1) were randomly purchased from 21 different stores in Istanbul city such as street bazaars ($n = 3$), street markets ($n = 9$), and accessory stores ($n = 9$). Every kinds of item were bought once. The price categories were determined as follows: Category 1: ≤ 1 Turkish Lira (TL)-0.5 USD; Category 2: 1.01–4.99 TL/0.5–2.49 USD; Category 3: 5–9.99 TL/2.50–4.99 USD; Category 4: 10–19.99 TL/5.00–9.99 USD; and Category 5: ≥ 20 TL/10 USD. Imitation earrings were listed and photographed firstly and then were cut to calculate the penetration surface area as can be seen in Fig. 2. Penetrating parts of earrings were measured via a caliper (Mitutoyo, CD-15-CPX, Japan), and measurements were recorded. To remove external contamination, all parts were washed in 0.5% SDS solution in ultrasonic bath



Fig. 1 An illustration of cheap imitation earrings sample types purchased from Istanbul bazaar

for 5 min and then thoroughly rinsed with ultrapure water prior to incubation process.

Artificial sweat solution was prepared by adding urea (0.1%), NaCl (0.5%), and lactic acid (0.1%) (w/v) according to EN 1811:2011 standard. Ammonia solution (1%) was used to adjust the pH solution at the value of 6–6.50. Sweat solutions were freshly prepared before 3 h than incubation in each batch. Each sample was soaked in the artificial sweat in the ratio 1:1 (1 mL of solution for each 1 cm² of the item's surface) and placed in a water bath at 30 ± 1 °C for 1 week according to the regulation UNI EN 1811:2011 [15].

Following proper dilution of 1-week incubated artificial sweat solution with 2% HNO₃ involved ISs (Ga and In), element release levels of earrings were determined by ICP-MS.

Method Development

To check out the recovery of sample preparation and analysis steps, blank artificial sweat samples were spiked with two different calibration concentrations (10 and 100 ng mL⁻¹) in six replicates, and each spiked samples were incubated with real samples simultaneously. Satisfaction range was assumed as 70–120% for recovery and < 20% RSD for precision as defined by SANCO/12571/2013 [16].

In every batch of analysis, blank samples with ISs were analysis to monitor possible carryovers after each sample. In order to maintain quality control and monitor matrix effect, after every 10 samples, blank solution, one of calibration solutions (20 ng mL⁻¹) and spiked sample (10 ng mL⁻¹ spiked to artificial sweat solution) were analyzed to the batch as well. In



Fig. 2 Preparation steps of samples: after labelling and cutting the penetrating part and calculation of surface area by a caliper

addition, all calibration points were performed using the same matrix, and a blank sweat solution was analyzed in replicates in order to check out matrix effect. Recovery analyses were done using the spiked concentrations of the elements in the blank sweat matrix.

The limit of detection (LOD) was calculated as three times the standard deviations of the blank artificial sweat solutions, which were determined by measuring 10 times a sample. Limit of quantitation (LOQ) was also calculated as 3.3 times of LOD values for each quantitatively determined analytes.

Experimental conditions of ICP-MS system were as follows: radio frequency power, 1400 W; plasma gas flow, 13 L min⁻¹; auxiliary gas flow, 0.8 L min⁻¹; dwell time, 0.01 s; concentric nebulizer gas flow, 0.87 L min⁻¹; sweeps/replicate, 100; replicates/sample, 3; spray chamber temperature, 3 °C; sample uptake, 60 s; washing, 45 s.

Evaluation of Results

After the analysis of samples via ICP-MS, released elemental concentrations of each earring item were calculated according to the $d = V(C_t - C_b)/1000 A$ formula presented in the study of Junping Y et al. where d is the nickel release rate, $\mu\text{g} \cdot (\text{cm}^2 \text{ week})^{-1}$; V is the dilution volume of the test solution in mL; C_t is the nickel concentration in the diluted test solution after 1 week, $\mu\text{g L}^{-1}$; C_b is the nickel concentration in the blank solution after 1 week, $\mu\text{g L}^{-1}$; A is the test surface area of the sample in cm^2 [17].

Release ratios were presented as $\mu\text{g}/\text{cm}^2/\text{week}$. All results were evaluated by statistical analysis, the normality of distribution was checked using the Kolmogorov-Smirnov test, and Spearman's correlation test was applied to evaluate the degree of the relationship between concentrations of elements in earring samples. Additionally, Kruskal-Wallis test was applied to compare means of released elemental concentrations according to price of earrings. Also mean concentrations were compared according to the purchasing point (i.e., street bazaar, street markets, and accessory stores). All statistical analyses were carried out using the SPSS statistics program (v.16.0, SPSS Inc.). The p values less than 0.05 were considered as statistically significant.

Results and Discussion

Analytical Performance Results

After analysis of calibration solutions three times including 2% HNO₃ blank solution, correlation coefficients of all quantitatively determined 24 elements were satisfactorily found $R \geq 0.999$, and calibration curves were obtained according to linear regression analysis. Linear ranges were obtained as follows: 0.1–150 ng mL⁻¹ for Li, Be, Mn, Co,

Ni, Cu, Sr, Mo, Cd, Ba, and Tl; 0.1–20 ng mL⁻¹ for U; 0.5–100 ng mL⁻¹ for Al, Cr, As, Se, and Pb; 1–35 ng mL⁻¹ for Hg; 2–50 ng mL⁻¹ for Sb and Bi; 5–400 ng mL⁻¹ for B, Mg, and Zn; and 50–400 ng mL⁻¹ for P. Also K, Ca, Zr, Pd, and Sn were determined semiquantitatively. According to the analytical performance results, LODs were obtained between 2.28–4.23 ng mL⁻¹ for quantitatively determined major elements (Mg, P, and Zn), 0.01–0.37 ng mL⁻¹ for trace elements (Li, B, Cr, Mn, Co, Cu, Se, Sr, Mo, and Ba), and 0.01–1.41 ng mL⁻¹ for toxic elements (Be, Al, Ni, As, Cd, Sb, Hg, Tl, Pb, Bi, and U). Table 1 shows linear ranges, LOD values, mean recovery values obtained from two concentrations spiked to artificial sweat solutions (10 and 100 ng mL⁻¹), and RSD% of spiked samples ($n = 6$) detailed for each analyte.

Since no certified or standard reference material (CRM) is available for sweat matrix including inorganic elements, the accuracy of the proposed sample preparation method was assessed by the use of recovery experiments performed at two concentrations using blank artificial sweat solution. For method performance studies, 10 and 100 ng mL⁻¹ were used to check out the precision, accuracy, and recovery of the incubation method. All concentrations were spiked into the blank sweat solutions in six replicates and incubated according to the procedure as mentioned above. Satisfactory recovery results of the elements varied from 79.2% (Mn) to 116.4% (Mg) for 10 ng mL⁻¹ except B and Bi with moderate recovery result by 65.3% mean value and 78.2% (Cu) to 94.7% (Sb) for 100 ng mL⁻¹. Relative standard deviations (RSD%) of recovery samples were below 14.5% for 10 ng mL⁻¹ and 12.3% for 100 ng mL⁻¹. All the recovery results are also presented in details in Table 1. Since any interference was not observed in blank sweat solution and the recovery results were within the acceptable interval for the analysis of these elements, the current method was evaluated satisfactory in terms of matrix interference.

Study Findings

This is the first study to quantify the amount of 29 different elements simultaneously in a high sample size of cheap imitation earrings and their release pattern in the artificial sweat. Released element concentrations obtained from ICP-MS analysis of incubated artificial sweat solutions were calculated according to the surface area of items as $\mu\text{g}/\text{cm}^2/\text{week}$. A number of 223 penetrating earrings were purchased from 21 different accessory markets.

Considering the results of earrings, concentrations of Li, Be, Se, Zr, Pd, and U among 29 analyzed elements in earring samples were all below the LOD in all items, as listed in Table 1. Other 23 elements were evaluated according to Spearman's correlation test results, and three

Table 1 Analytical performance of the ICP-MS method and results of elemental release in earrings

Element	Calibration ranges	LOD	Recovery % (RSD)		Mean $\pm$ Std ($\mu\text{g}/\text{cm}^2/\text{week}$) ($n = 223$)	Min.-Max.
	ng mL^{-1}		10 ng mL^{-1} ($n = 6$)	100 ng mL^{-1} ($n = 6$)		
Li	0.1–150	0.01	91.5 (0.93)	85.9 (1.24)	< LOD	< LOD
Be	0.1–150	0.01	84.4 (0.81)	84.1 (1.39)	< LOD	< LOD
B	5–400	0.37	63.8 (8.6)	81.1 (1.21)	0.001 ± 0.01	< LOD-0.08
Mg	5–400	4.23	116.4 (10.79)	88.0 (3.23)	0.02 ± 0.04	< LOD-0.29
Al	0.5–100	1.41	112.2 (7.21)	91.1 (7.78)	0.06 ± 0.40	< LOD-3.99
P	50–400	2.28	ND		0.01 ± 0.03	< LOD-0.19
K			SQ		0.05 ± 0.08	< LOD-0.38
Ca			SQ		0.35 ± 1.24	< LOD-15.7
Cr	0.5–100	0.04	107.6 (1.52)	84.5 (1.72)	2.04 ± 4.68	< LOD-26.7
Mn	0.1–150	0.17	79.2 (1.17)	79.3 (1.22)	1.81 ± 5.78	< LOD-50.9
Co	0.1–150	0.01	80.1 (0.72)	79.6 (1.31)	0.20 ± 2.73	< LOD-40.6
Ni	0.1–150	0.16	83.3 (1.05)	79.4 (0.65)	1.03 ± 3.27	< LOD-30.3
Cu	0.1–150	0.10	83.4 (8.12)	78.2 (0.69)	5.31 ± 14.6	< LOD-91.0
Zn	5–400	2.99	89.8 (6.52)	81.7 (6.94)	6.09 ± 30.6	< LOD-275.8
As	0.5–100	0.03	83.9 (2.59)	84.3 (0.94)	< LOD	< LOD-0.01
Se	0.5–100	0.07	81.8 (5.15)	81.5 (1.48)	< LOD	< LOD
Sr	0.1–150	0.20	87.8 (1.61)	86.6 (1.58)	< LOD	< LOD-0.01
Zr			SQ		< LOD	< LOD
Mo	0.1–150	0.09	90.8 (0.85)	89.3 (1.73)	0.001 ± 0.01	< LOD-0.05
Pd			SQ		< LOD	< LOD
Cd	0.1–150	0.01	80.5 (0.57)	81.4 (1.34)	0.23 ± 2.47	< LOD-36.3
Sn			SQ		0.22 ± 2.31	< LOD-34.2
Sb	2–50	0.01	90.2 (6.45)	94.7 (5.69)	0.01 ± 0.07	< LOD-0.70
Ba	0.1–150	0.06	87.3 (1.90)	89.3 (1.95)	< LOD	< LOD-0.02
Hg	1–35	0.09	79.5 (14.48)	90.3 (12.28)	< LOD	< LOD-0.01
Tl	0.1–150	0.06	83.5 (0.56)	85.7 (1.67)	< LOD	< LOD-0.02
Pb	0.1–100	0.04	86.0 (4.52)	84.4 (2.72)	0.92 ± 8.41	< LOD-122.3
Bi	1–50	0.28	66.8 (0.96)	79.2 (2.92)	< LOD	< LOD-0.01
U	0.1–20	0.01	88.6 (0.72)	90.9 (1.74)	< LOD	< LOD

ND not determined, SQ semiquantitative determination

correlation clusters were considered as low correlation ($0.5 < r < 0.3$), moderate correlation ($0.7 < r < 0.5$), and high correlation ($0.9 < r < 0.7$). All three clusters were also classified in terms of positive and negative correlation. The relationship between released elements in sweat samples was presented in Table 2. Significant relationships ($p < 0.01$, $p < 0.05$) were detected between studied elements. According to Spearman's correlation test results, a high positive correlation was detected between Co and Cr. Being a major content of element alloys alongside the Ni, simultaneous use of Co and Cr in the structure of the earrings, and their high correlation ratio is an expected finding [18–20].

Moderate positive correlations were detected between the following pairs of elements: Ca-K, Mn-Cr, Co-Mn, Ba-Sr, and

Pb-Sb. The positive correlation of toxic elements and their presence in the structure of imitation earrings might be consequence of the manufacturer's attempt to reduce the production costs. Furthermore, although Ni is reported to be the main allergen in inexpensive jewels, the presence of the toxic elements in the content of these items and their possible relation to the hypersensitivity reactions should not be overlooked. Most of the element pairs showed significant positive low correlation such as Al-Mg, P-Mg, Zn-Cu, Sr-Hg, Mo-Cr, Sn-Cr, Sn-Cu, Sn-Cd, Sb-Cu, Sb-Sn, Pb-Cu, Pb-Sn, Ni-Cr, Ni-Co, Ni-Cu, and Ni-Pb. Only Zn-Mn pairs showed a low negative correlation.

Tables 3 and 4 report the Ni release amount of the earrings according to purchasing places and prices, respectively. Also Table 5 shows the number of the samples detected below and

Table 2 Spearman's correlation coefficients (r) of elements (significant results were highlighted)

	Hg	B	Mg	Al	P	K	Ca	Cr	Mn	Co	Cu	Zn	As	Sr	Mo	Cd	Sn	Sb	Ba	Tl	Pb	Bi	Ni
Hg	1																						
B	.289**	1																					
Mg	.092	.040	1																				
Al	-.005	-.003	.419**	1																			
P	.069	.188**	.390**	.200**	1																		
K	-.111	.053	.150*	.221**	.239**	1																	
Ca	.010	.017	.269**	.240**	-.090	.642**	1																
Cr	.114	.120	.182**	.214**	.224**	-.131	-.101	1															
Mn	.126	.141*	.203**	-.018	.117	-.001	.116	.681**	1														
Co	.150*	.127	.152*	.003	.140*	-.100	-.006	.712**	.662**	1													
Cu	.172*	.009	.123	.134	.269**	-.052	-.106	.295**	-.114	.209**	1												
Zn	.032	-.042	.072	.115	.007	-.027	.017	-.108	-.367**	-.123	.337**	1											
As	-.010	-.016	-.069	-.046	-.058	-.055	-.043	.031	.014	-.049	.079	.070	1										
Sr	.345**	-.023	.169*	.133	.041	-.078	.184*	.146*	.157*	.183**	.091	.056	-.007	1									
Mo	-.034	-.058	.087	.119	.082	.003	-.018	.320**	.208**	.281**	.250**	.057	-.017	-.024	1								
Cd	.233**	.021	.053	.009	.019	.054	.075	.148*	.199**	.151*	.156*	.259**	.176*	.105	.193**	1							
Sn	.026	-.027	.293**	.227**	.138*	.165*	.106	.309**	.234**	.252**	.366**	.071	.123	.066	.198**	.326**	1						
Sb	.174*	.054	.171*	.141*	.178**	.103	-.005	.079	.000	-.022	.325**	.086	.210**	.129	.162*	.075	.339**	1					
Ba	.169*	-.044	.272**	.176*	.162*	.047	.225**	.130	.077	.073	.104	.065	-.013	.538**	-.046	.002	.052	.182**	1				
Tl	-.010	-.016	.071	.102	.103	.127	-.043	.052	.002	.128	.115	.025	-.005	-.007	.279**	-.030	.063	-.025	-.013	1			
Pb	.072	-.025	.281**	.106	.202**	-.077	-.172*	.195**	-.059	.091	.479**	.215**	.134	.050	.138*	.057	.445**	.532**	.187**	.067	1		
Bi	-.010	-.016	-.069	.103	-.058	-.055	-.043	.012	-.017	-.049	.054	.005	-.005	-.007	-.017	-.030	.124	.212**	-.013	-.005	.136*	1	
Ni	.128	.062	.066	.042	.191**	-.082	-.058	.484**	.225**	.493**	.462**	.168*	.110	.109	.211**	.130	.148*	.188**	.142**	.098	.318**	.066	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 3 Distribution of earrings by purchasing places and nickel release rates of each group ($\mu\text{g}/\text{cm}^2/\text{week}$)

Purchasing places	Number of samples (%)	Mean nickel release	Median nickel release	Range of nickel release	Std deviation
Street bazaar ($n = 3$)	39 (17.5)	1.21	0.11	< LOD-16.2	3.330
Street market ($n = 9$)	139 (62.3)	1.34	0.06	< LOD-30.3	3.927
Accessory stores ($n = 9$)	45 (20.2)	0.30	0.01	< LOD-6.36	1.012
Total	223 (100)				

above the EU limit. The mean Ni release of items $1.03 \pm 3.27 \mu\text{g}/\text{cm}^2/\text{week}$ was found to be over the EU Commission directive. A total of 71 earrings exceeded the Ni release limit of $0.20 \mu\text{g}/\text{cm}^2/\text{week}$. Among these, 49 (69%) were purchased from the street markets. The range of Ni release in the earrings obtained from the street markets was also higher than the samples purchased from the street bazaar and accessory stores and varied between below the LOD and $30.3 \mu\text{g}/\text{cm}^2/\text{week}$. The amount of Ni release was less than the EU limit in 152 of the earrings. In all purchasing place groups, median Ni release levels were below the safety limits, revealing that at least half of the earrings purchased from different places were within the determined limit (Table 5). Kruskal-Wallis analyses revealed that the purchasing place and price was correlated with the Ni content ($p = 0.032$; $p = 0.029$). Intergroups Mann-Whitney U test did not yield a difference between the street bazaar and street market ($p = 0.663$), whereas the relationship between street bazaar and accessory stores ($p = 0.019$) and street market and accessory stores ($p = 0.017$) were significantly different. There were also significant relationship between the price groups 1–5 ($p = 0.003$), 2–5 ($p = 0.006$), 3–5 ($p = 0.003$), and 4–5 ($p = 0.020$) in the Mann-Whitney U analyses.

Bocca et al. observed that the frequency of ACD due to earrings did not decrease after the introduction of the Ni limit, and half of the earrings purchased from the Italian market exceeded the defined limits for Ni release [21]. Although this ratio is higher than the findings of this study, there are several points that need to be mentioned: Firstly, the number of earring samples in their analysis was lower than the number evaluated in this presented study (10 vs. 223). Secondly, the limit

they used in their analysis was according to the previous EU directive's, which is $0.5 \mu\text{g}/\text{cm}^2/\text{week}$. Thus, if they conducted their study with the recent limit, the ratio of the earrings above the permitted limit would be higher. The relatively lower ratio in this presented study might be a result of the introduction of the current Ni limit and possible controlling of the manufacturing processes by the authorities in order to provide a higher standard in compliance with the current directive. Meijer et al. and Warshaw reported that the rate of Ni sensitivity is higher in the pierced individuals, and the jewelry use is the most common cause of element-related allergic reactions [13, 22]. It is a fact that the sources of element sensitivity may also be other sources than earrings, including other types of inexpensive jewelry, mobile phone accessories, jean buttons, and zippers.

It is observed that, together with Ni, considerable amounts of Cr, Cu, and Zn were also present in the structure of the samples. The greatest release of Cr was $26.7 \mu\text{g}/\text{cm}^2/\text{week}$, Co was $40.6 \mu\text{g}/\text{cm}^2/\text{week}$, and Zn was $275.8 \mu\text{g}/\text{cm}^2/\text{week}$. Stainless steel is constituted from a percentage of 8% of Ni and 18% of Cr, and Cu and Zn are widely used in the manufacturing process of jewels in order to provide element alloys; thus the simultaneous presence of Cr, Cu, Zn and their high positive correlation with Ni is an expected finding [23]. In their study, Duarte I et al. reported the ratio of sensitivity to Ni as 77%, to Co as 32%, and to Cr as 29% [19]. In a study from Turkey, the frequency of sensitivity to Ni was 17.6%, whereas it was 5.3% to Co [24].

Although the release of hexavalent and trivalent Cr has been shown in the tanned leather shoes, our study adds the presence of a strong correlation between the Cr and Ni content

Table 4 Distribution of earrings by price groups and nickel release rates of each group ($\mu\text{g}/\text{cm}^2/\text{week}$)

Price	Number of samples (%)	Mean nickel release	Median nickel release	Range of nickel release	Std deviation
1	29 (13.0)	1.11	0.09	0.01–16.2	3.41
2	64 (28.7)	1.39	0.09	< LOD-30.3	4.59
3	95 (42.6)	1.20	0.06	< LOD-16.6	3.14
4	17 (7.62)	0.66	0.07	< LOD-6.36	1.63
5	18 (8.07)	0.05	0.01	< LOD-0.37	0.11
Total	223 (100)				

Table 5 Number of samples over the EU limit and their distribution rates by purchasing places

EU Limit (0.2 µg/cm ² /week)	Street bazaar	Street market	Accessory stores
Over the limit	11 (28.2%)	49 (35.3%)	11 (24.4%)
Below the limit	28 (71.8%)	90 (64.7%)	34 (75.5%)
Total	39 (100%)	139 (100%)	45 (100%)

of the earrings in the literature. Although Cr is a known occupational allergen, it should be noted that some of the cases with ACD might be a consequence of an adverse reaction to the elements other than or beneath Ni.

Releases of Ca, Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, and Pb were highly variable among the earrings in the present study. A common pattern of alloying practice was not present among the items, suggesting that different production sites have their own formula of various types of element alloys. Also, the variations in the release rate of these elements might be a result of the different covering practices. For instance, a superficial presence of Ni might be providing a less stable structure and resulting in an increased migration pattern in artificial sweat.

As another remarkable finding, Pb was released from the objects with a mean concentration of 0.92 ± 8.41 µg/cm²/week, with the highest concentration of 122.3 µg/cm²/week for one sample. Bocca et al. observed that the maximum concentrations of Pb and Cr were detected in the stud part of the earrings in their sampling group [21]. In a study by Hamann CR et al., Pb was present in minor amounts in the 27% of the earrings in their sampling group [25]. Together with the data presented herein, these findings suggest that Pb is an overlooked common component of element alloys in the structure of imitation earrings. Being a highly toxic element, wide use of Pb in the structure of these items needs certain restriction and regulations worldwide both for the consumers and the individuals working in the manufacturing of these products.

Although there was a correlation between the rate of Ni release and the purchase price, there were still earring samples with high Ni content in the highest price group. Therefore, customers purchasing earrings in relatively higher prices are still potentially at risk of Ni exposure and ACD. Furthermore, the maximum mean amount of Ni release was found in the samples of Price Group 2, which is between the 1.01–4.99 TL (0.5–2.49 USD) range. This might be possibly due to relatively larger number of samples in this group compared to the Price Group 1 (61 vs. 26). Following the release of the EU directive on Ni amount in the element products, a Danish study found an excessive amount of Ni in earrings using the dimethylglyoxime (DMG) test, irrespective of the price or purchase site. They also stated that excessive nickel release was not present in any of the earrings they purchased from the supermarket or department stores [26]. This is in concordance with the findings of the presented study that the ratio of the earrings above the EU limit is lower in the earrings purchased

from accessory stores, possibly due to more strict regulations in the batch order process of these items by the purchasing departments of corporate companies. Ahlström MG et al. also stated that although there was a consistent pattern of decreasing prevalence of Ni allergy in some EU countries, the prevalence among young women aged between 18 and 35 years still remains high [27].

Similar to the ratio of 30.7% in their 2008 study by Thyssen and Maibach, the 31.8% ratio of earrings that released Ni above the permitted limits in this study reveals that, although there are reports indicating a significant decrease in the ratio of excessive Ni containing earrings, more than a decade after the implementation of the EU Directive, earrings with excessive Ni content are still in the markets [28].

There is not a regulation on the inspection and reporting of the violation of EU Directive on Ni content of jewels in Turkey. For the imported materials, the element content analysis of the items in the customs or clearance processes is not a routine procedure. Besides, there is not a regulation on the random sampling and control on these objects while they are on the markets for sale.

All the purchases and analyses were performed by the same investigator, and the experiments have been repeated in order to confirm the findings. The equal surface measures had taken in order to prevent a bias between the samples and analyses. The environmental settings in terms of temperature and humidity (20 ± 2 °C; 35–50%, respectively) were carefully controlled, and all the experiments were performed using the same instruments. An artificial sweat corrosion step was also performed in order to imitate the personal use conditions of the earrings.

It is a well-known fact that epidermal thickness, the content of the sweat, and body temperature vary between individuals, and subjects with fair skin color have more tendency to allergic reactions. Since the setup of this research is an in vitro experimental study, it is not possible to assess the influence of these variables on the release of element contents from the earrings. Also, there is no data available on the countries of origin for the earrings randomly purchased and tested in the study.

It should be noted that the city, Istanbul, which the earring samples have been purchased is a touristic location, and pricing practices differ between different parts of the city, irrespective of the quality or selling places of the earrings. Thus, the pricing might not be reflecting the element status of the items for this particular reason.

The most important strength of the study is its analytical quality with highly standardized ICP-MS method. The method used in the analysis of the samples has a high accuracy and precision, with high sensitivity for all 29 elements tested. Although there are studies implementing the use of rapid color development-based spot tests such as DMG in determination of Ni content, and 1,5-diphenylcarbazine (DPC) in determination of Cr content, the findings presented here brings a quantitative approach to the current data on the content of these products by analyzing wide-ranged inorganic profile simultaneously [29].

Development of a new ICP-MS method with low detection and quantitation limits for a wide range of elements is a valuable contribution to the literature on the detection of element content of these products. The method and data presented here bring new insights with higher analytical grade to the future analyses of element content of these materials.

Conclusion

The study employed ICP-MS quantification of alloying and elemental profile in 223 earring samples purchased from Istanbul market. Taken together, this study showed that one third of the imitation earrings in the market have excessive Ni release among other allergen elements. Analysis results also showed that Cu, Cr, and Zn were the most predominant elements after Ni. It is worth noting that Pb was also present in some of the samples within a range of $0.92 \pm 8.41 \mu\text{g}/\text{cm}^2/\text{week}$, with the highest concentration of $122.3 \mu\text{g}/\text{cm}^2/\text{week}$.

We also observed that the Ni content was negatively correlated with the price of the items, and the Ni contents of the imitation earrings were lower in the samples obtained from brand stores.

There is currently no regulation of topical element exposure except for Ni in Europe. Not only earrings but also other types of jewelry and hair accessories are the potential causes of ACD in the individuals subjected to these materials. Although Ni is the predominant element in the structure of the earrings analyzed, potential sensitivity to other elements should be considered and limiting actions need to be undertaken. The high amounts of Pb in some of the samples we analyzed might be an alarming call to the authorities for settlement of new limits, including a wide range of elements besides Ni.

Acknowledgements Author would like to thank The Scientific And Technological Research Council of Turkey (TUBITAK) and Scientific Research Fund of Istanbul University-Cerrahpasa, Turkey, for support of this work with the Project numbers 213S098 and BYP-54488, respectively. The author also grateful to Assoc. Prof. Hayriye Vehid for her valuable contribution to the statistical evaluation of the study's findings and to Murat Yayla and Sevcin Şemen for their technical assistant.

Compliance with Ethical Standards

Conflict of Interest The author declares that she has no conflict of interest.

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