# Concentration of Heavy Metals, Namely Lead, Cadmium, and Chromium, in Hair Color Available in the Markets of Qom, Iran

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**Background & Aims of the Study:** Heavy metals, such as cadmium, lead, and chromium, are common environmental pollutants; however, they are used in various cosmetics. Regarding this, the aim of the present study was to measure the concentrations of lead, cadmium, and chromium in the hair colors available in the markets of Qom, Iran.
**Materials and Methods:** This cross-sectional descriptive study was performed on 12 hair color brands (i.e., 7 domestic and 5 foreign brands). Three popular colors were chosen from each brand and purchased from randomly selected famous stores located in four districts of the city. After preparing the samples, the levels of lead, cadmium, and chromium were measured by graphite furnace atomic absorption spectroscopy (µg/kg). Collected data were analyzed in SPSS software (version 22) using independent t-test to compare the amount of heavy metals in different brands at a p-value of 0.05.
**Results:** The level of lead in the studied hair colors was higher than those of cadmium and chromium. In this regard, cadmium level was lower than the detection limit of the device and was about zero. In addition, the lead and chromium levels in hair colors were estimated at 552.37-11.5 and 35.12167-62.97, respectively. Furthermore, the mean weight of chromium and lead in Iranian hair color was significantly higher than that in the foreign ones.
**Conclusion:** Regarding the potential impacts of heavy metals on health and their accumulation property, it is required to perform comprehensive interventions to raise the awareness of the society in this regard.

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## Background

In recent years, cosmetic industry has experienced a significant progress, including the production of various types of cosmetics, such as soaps, creams, face powder, tanning lotions, toothpaste, and lipsticks, for the skin, hair, teeth, body, and nails care (1). Based on previous research, the consumption of cosmetic products is very common in Iran. According to the statistics, Iran and Saudi Arabia have the highest cosmetic consumption rate in the Middle East; in this regard, about $ 2.1 million is spent on cosmetic products in Iran (2). Hair color is one of the best-selling products since about 25% of women aged above 18 years and 10% of men aged over 40 years use hair color in Europe, North America, and Japan (3).

Hair colors include ammonium compounds (e.g., 4-amino2-hydroxytoluene and amino phenol), antioxidants, ammonia, soap, aroma compounds, moisturizers, forming creams, alkalisers, and metal oxides (e.g., titanium dioxide and iron oxide) (4) Some of the substances like heavy metals in cosmetics are unwanted or produced during the production process (5). Heavy metals are among the most important environmental pollutants, many of which are toxic to humans even in small quantities (6). These kinds of metals are capable of producing free radicals and active oxygen species and damaging the cell cycle, cellular organelles, and their activities (7). The persistent usage of polluted products results in the accumulation of non-metabolized toxins in the target tissue, thereby leading to the incidence of several diseases and complications (8).

With regard to the high prevalence of using cosmetics in Iran, the present study was conducted to determine the concentration of three heavy metals, namely lead, chromium, and cadmium, in the most popular chemical hair color brands available in the market of Qom, Iran.

## Materials & Methods

### *Sampling Method*

This cross-sectional descriptive-analytical study was conducted in Qom in 2017. Information about the most commonly used hair color brands was gathered by interviewing and distributing questionnaires among famous makeup sale representatives, hairdressers, and women aged above 40 years. The last-mentioned group was selected because of their persistent and frequent use of hair colors as a result of white hair and lack of natural pigments. After reviewing 12 brands (i.e., 7 and 5 domestic and foreign brands, respectively), 3 samples from each brand were randomly purchased from the most popular stores located in four areas of Qom. The samples were prepared in the laboratory using digestion method and then the levels of lead, cadmium, and chromium were measured by means of the atomic absorption spectrometer (AAS).

### *Sample preparation method*

The extraction of the heavy metals was accomplished using the dry and wet methods, as well as microwave digestion system. In the dry method, 10 g of the sample was weighed in a crucible and then placed in the oven at 100ºC until reaching a constant weight. In the next stage, for the ash preparation procedure, the crucible was first placed in a furnace at 200ºC. Subsequently, the furnace was heated to 400ºC, and its temperature was added 50ºC per hour. The solution was kept at this temperature for 8 h. Afterward, 5 ml of 6 M hydrochloric acid was added to the crucible so that all the ashes could be exposed to acid.

The solution was heated until evaporation, and the resultant sediments were dissolved in 10-30 ml nitric acid 0.10N-standardized. The crucibles were carefully centrifuged so that all the ashes would be in contact with the acid. Then, the solution was covered by a plastic cover and left intact for 1-2 h. In the next stage, deionized water was added to the solution until its volume reached 25 cc (9).

To prepare the samples through the wet method, 5 ml of nitric acid 0.10N-standardized was added to 1 g of the sample. Then, the samples were placed on a heater at 85º C for 4 h to extract the yellow vapors. After reaching the room temperature, 1 ml of 0.0030 oxygenated water was added to the solution and then passed through Whatman filter paper. Subsequently, deionized water was added to the solution until obtaining a volume of 25 cc. Finally, the absorption rate was measured by graphite furnace AAS (GFAAS) (10).

In order to investigate the influence of the acid types, used in sample preparation stages, on the adsorption rate, measured by GFAAS, one of the samples that had the highest concentration of heavy metals (i.e., lead, cadmium, and chromium) was chosen to be subjected to digestion and preparation procedures using different acids. The methods are explained below.

To this end, five crucibles containing 1 g of the sample were dried in the oven at 105°C for 12 h in order to obtain a constant weight. Subsequently, 5 ml nitric acid was added to the samples so that all the samples were exposed to the acid. Subsequently, each sample was put on heater at 85ºC for 4 h until extracting the yellow smoke produced as a result of the reaction of the sample with acid. After the samples were cooled, 15 ml hydrochloric acid, 10 ml sulfuric acid, 5 ml perchloric acid, 2.5 ml trichloroacetic acid, and 1 ml oxygenated water were added to the first, second, third, fourth, and fifth samples, respectively. After passing all samples through the Whatman filter paper, they increased to a volume of 25 ml by adding double distilled water.

In the digestion method accomplished through the digestion system, 0.2 g hair color was placed on the special digestion plates. Subsequently, each sample was added with approximately 5 ml of high concentration nitric acid and 1 ml oxygenated water, according to the microwave instruction. Afterwards, the plates containing samples and acids were carefully placed in the vessels. Then, the vessels were put in the microwave, the lid was closed, and the special pressure and temperature schedule of the machine was set for the samples.

After the completion of the digestion and cooling of the device, the digested samples were extracted and added with deionized water until reaching a volume of 25 cc. Finally, it was read by the GFAAS; moreover, acetate buffer and NaHO were used to prepare samples with various pH values.

***Preparation of samples with various pHs***

In order to investigate the effect of pH applied in the sample preparation stages on the measured absorption rate by the GFAAS, nitric acid, buffer acetate ($C_2H_3O_2NH_4$), buffer potassium dihydrogen phosphate ($KH_2PO_4$), and borate buffer (Na2B4O7) were respectively used for the pH values of 2, 4, 6-8, and 10, at a volume of 1 ml. These substances were added to 5 ml of the standard levels of lead (500 ppb), cadmium (5 ppb), and chromium (200 ppb). Finally, the absorption rate was read by the GFAAS (11-12).

***Heavy Metals Measurement Method***

Initially, standard curves were drawn for the metals under investigation. The stock solutions of metal analytes were used (lead, cadmium, and chromium) for drawing standard curves (Figure1. Chart 1,2and3).

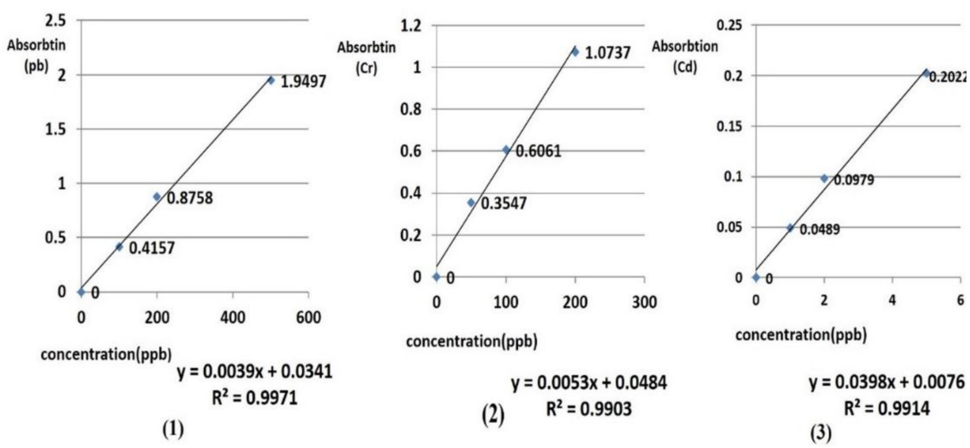



**Figure 1) Standard curves of lead (1), chromium (2), and cadmium**

After reading the standard solution absorption by the GFAAS, the original samples were subjected to the device and their absorption rates were read (µg/kg).

Concentrations of the elements in the digested samples were calculated based on the standard curve equation of each metal (which was already drawn in excel). The linearity of the graph and validity of the equation were obtained by calculating $R^2$ rendering the values of 0.997, 0.991, and 0.990 for lead, cadmium, and chromium, respectively.

In order to validate and evaluate the efficiency of the digestion method and determine the reliability of the test applied for estimating the levels of heavy metals, the percent recovery of lead in hair color samples, limit of detection and limit of quantification, and intraday and interday variation coefficients were calculated. To this end, the digestion of a certain amount of metal was added to a specific sample; however, this amount was not added to the other samples. Afterward, the absorption of the two samples and the absorption of the added amount were calculated according to the following recovery formula:

R=100×(A2-A1)/ As

where $R$ is the percent recovery, $A_s$ is the standard adsorption, $A_2$ is the absorption rate of the sample containing the standard, and $A_1$ is the absorption of the sample without standard. In this study, to determine the percent recovery, out of the 12 hair color brands, one brand of domestic (Dopin) and one brand of foreign samples (Beauty) (showing the highest absorption rate of heavy metals) were selected to determine the percent recovery by adding 100 µl of each of 1 ppb and 5 ppb lead concentrations and brought to a volume of 10 ml (13).

After drawing the standard curve based on the standard deviation (SD) and estimated line slope, the "limit of detection" and "limit of quantitation" for both lead and chromium metals were calculated according to the following formula:

$$LOD=3\ \delta_b/m \qquad LOQ=3\ LOD$$

where $\delta_b$ is the SD of six consecutive measurements of the blank solution, and $m$ is the slope of the calculated line in the standard curve.

To evaluate the intraday variation coefficient, three lead concentrations (i.e., 50, 250, and 1250 ppb) were prepared, and their absorbance was measured six times a day by the AAS.

Furthermore, the evaluation of inter-day variation coefficient was accomplished by measuring the absorbance of three lead concentrations of 50, 250, and 1250 ppb by AAS for six consecutive days (14).

### *Statistical Analysis*

Data analysis was performed in SPSS software (version 22). To compare the mean levels of lead, cadmium, and chromium between the domestic and foreign brands, independent sample t-test was employed. A p-value less than 0.05 was considered statistically significant.

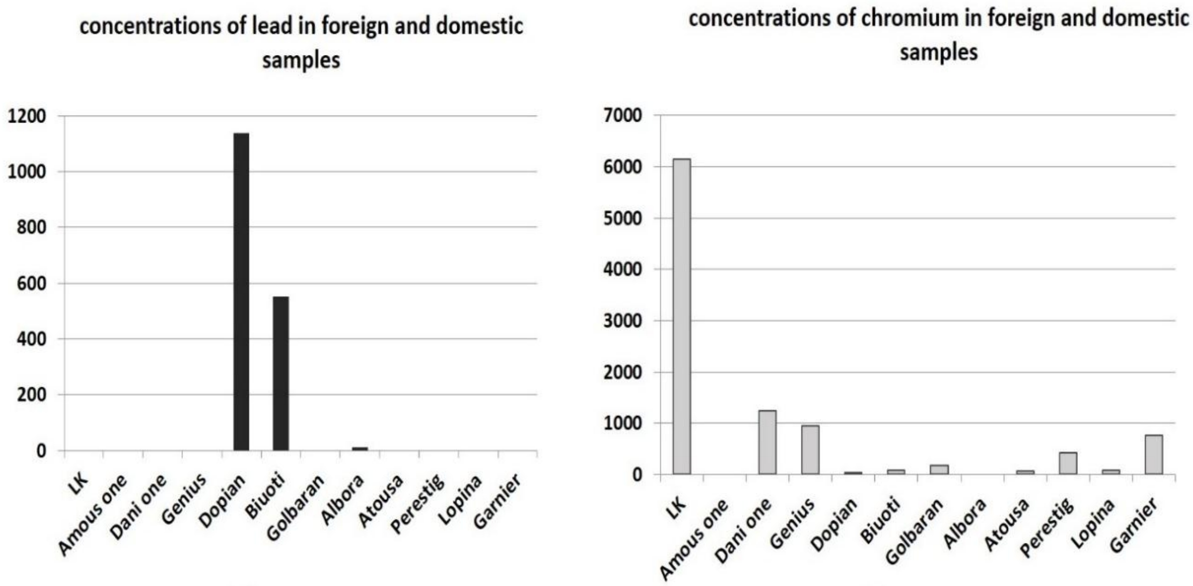
## Results

According to the results of the study, the concentration of cadmium, which was almost equal to zero, was lower than the device limit of detection (Table 1). Moreover, the concentration of lead was reported to be higher than that of chromium in all samples (Figure2. Chart4and5).

Regarding the results presented in Table 2, there was a significant difference between the . domestic and foreign hair colors regarding the mean weight of lead and chromium concentrations. In this regard, the mean weight of lead and chromium concentrations in the domestic hair color was significantly higher

**Table 1) Concentrations of heavy metals (µg/kg) in domestic and foreign hair color samples**

| Hair Color Number | Manufacturer Country | Cadmium | Lead | Chromium |
|---|---|---|---|---|
| **Lk** | Foreign | -* | -* | 6148.6 |
| **Amousone** | Domestic | -* | -* | -* |
| **Danione** | Foreign | -* | -* | 1243 |
| **Genius** | Foreign | -* | -* | 944.37 |
| **Dopian** | Domestic | -* | 1137.5 | 35.12167 |
| **Biuoti** | Foreign | -* | 552.37 | 88.75 |
| **Golbaran** | Domestic | -* | -* | 172 |
| **Albora** | Domestic | -* | 11.5 | -* |
| **Atousa** | Domestic | -* | -* | 62.97 |
| **Perestig** | Domestic | -* | -* | 417.75 |
| **Lopina** | Domestic | -* | -* | 88 |
| **Garnier** | Foreign | -* | -* | 771.5 |




(4) (5)

**Figure 2) Mean concentrations of lead (4) and chromium (5) in foreign and domestic samples**

than that in the foreign hair colors.

***Percent recovery of lead in hair color***

As mentioned earlier, percent recovery is used to measure the efficiency of the digestion method and the method used to determine the level of lead in hair color samples.

(Table 3) presents the results regarding the percent recovery of lead.

The best percent recovery is 100% but for various reasons, such as mistakes made by the researcher or the device and disturbances caused by other metals, this percentage is not usually achieved. However, the statistical results and percent recovery for the selected hair color showed that the difference of percent recovery for was not significant for lead the two added values of 1 and 5 ppb ($P<0.05$). Moreover, the results showed that the method used for digesting and measuring

**Table 2) Independent sample t-test for comparing the mean logarithm of lead concentration in foreign and domestic hair colors**

| Heavy Metal | Manufacturer country | Descriptive statistics | | Analysis of variance | | Compare means | |
|---|---|---|---|---|---|---|---|
| | | Mean | Standard Deviation | F | P-value | t | P-value |
| **Lead** | Domestic | 1.49 | 2.83 | 16.963 | 0.001 | 2.122 | 0.048 |
| | Foreign | 0.25 | 1.26 | | | | |
| **chrome** | Domestic | 2.83 | 2.56 | 0.853 | 0.361 | 2.104 | 0.042 |
| | Foreign | 1.07 | 2.57 | | | | |

*Considering the difference in the variances of the two groups

**Table 3) Mean percent recovery in the method used for extracting and measuring domestic and foreign hair color**

| Hair Color | Percent Recovery (Mean±SD) | Added Amount of Lead (ppb) |
|---|---|---|
| **Dopian** | 98.66±1.5[a] | 1 |
| **Dopian** | 97±1[a] | 5 |
| **Biuoti** | 100±2[a] | 1 |

the amount of lead in the hair color was appropriate.

***Limit of detection, limit of quantitation, and intraday and inter-day lead variation coefficients***

In order to determine the limit of detection and limit of quantitation, first, the SD and line slope were determined at a wavelength of 283.3 nm for averagely six-time test repetitions at the concentrations of 500, 1000, 1500, 2000, 2500, and 3000. After drawing a standard curve, the limit of detection and limit of quantitation for the lead were calculated as 1.5 and 4.5 ppb, respectively.

The accuracy and reproducibility of the method by using intraday and inter-day variation coefficients of lead are shown in( tables 4 and 5), respectively. According to the obtained variation coefficient, the method had a proper intraday and inter-day accuracy for lead measurement.

**Table 4) Intraday evaluation for lead**

| Level (ppb) | Mean | SD | cv% |
|---|---|---|---|
| **50** | 49.5 | 2 | 4.03 |
| **250** | 249 | 3 | 1.2 |
| **1250** | 1252 | 4 | 0.32 |

**Table 5) Inter-day evaluation for lead**

| Level (ppb) | Mean | SD | cv% |
|---|---|---|---|
| **50** | 49 | 2 | 4.07 |
| **250** | 251 | 3 | 1.19 |
| **1250** | 1252 | 4 | 0.31 |

***Effect of acid type on the absorption of heavy metals***

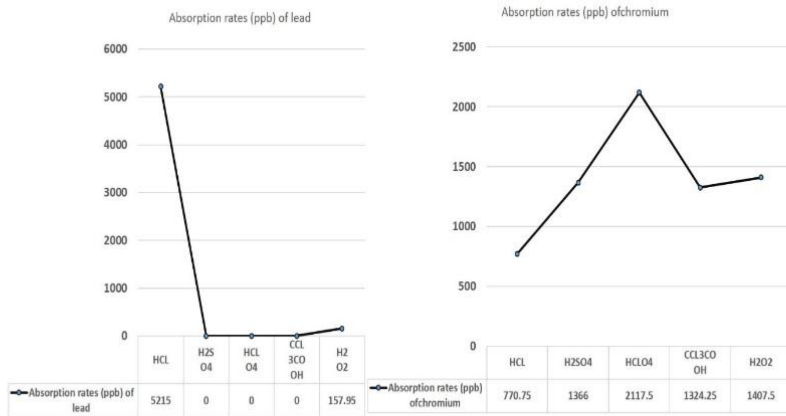
According to (figures3. Chart 7 and 6) the absorption rate of lead when using hydrochloric acid for the digestion of the sample was higher than that for other acids. Furthermore, the absorption rate of chromium when using perchloric acid to digest the sample was higher than that of other acids.

***Effect of Different Sample Preparation Methods on Heavy Metal Absorption***

According to Figure 4, the absorption rates of lead and chromium using wet method were higher than those estimated by the dry method.

***Effect of Sample pH Values on Absorption rate of Heavy Metal***

As depicted in (Figure5.Chart 8, 9, and 10)




(6) (7)

**Figure 3) Absorption rates (ppb) of lead (6) and chromium (7) using different acids in Dopin hair color**

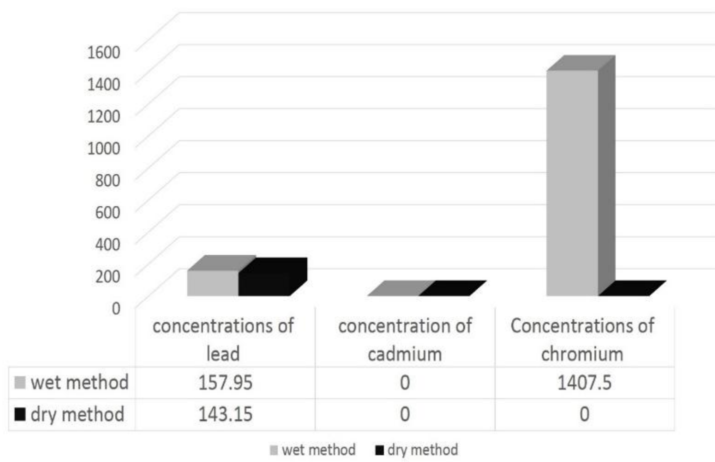
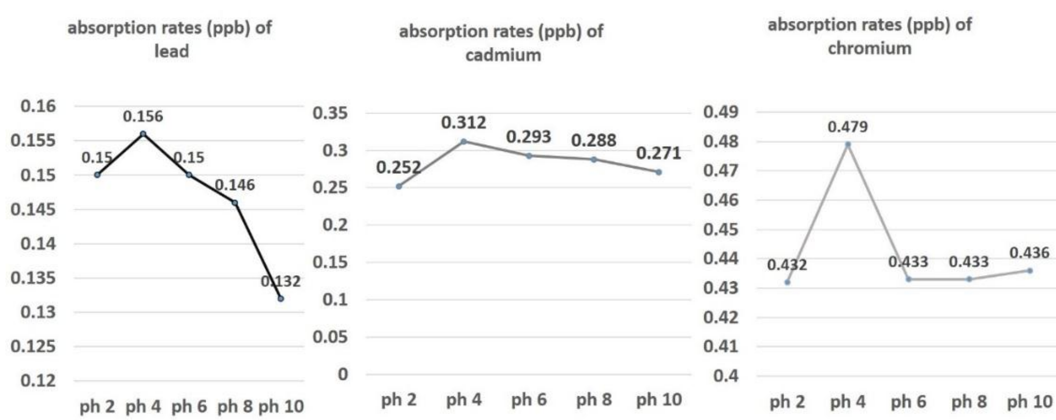



**Figure 4) Comparison of metal absorption rate in Dopin hair color using different preparation methods**




(8) (9) (10)

**Figure 5) Investigation of absorption rates (ppb) of lead (8), cadmium (9), and chromium (10) at different pH values**

a pH value of 4 resulted in the highest absorptionrate in lead, chromium, and cadmium.

## Discussion

Heavy metals are among the most important environmental pollutants the presence of which in cosmetics can pose consumers to different health-related issues. The current study was conducted to determine the concentrations of lead, cadmium, and chromium in 12 hair color brands available in the market of Qom.

Cadmium is a heavy metal the exposure to which, even in small amounts, affects the cardiovascular system of the exposed person. Previous studies have shown that exposure to high concentrations of this metal is associated with the development of diabetes and hypertension (15). Bone destruction is another adverse effects of this metal (16). In addition, cadmium is a potential toxin to cells by causing cell death or multiplication (17). Moreover, cadmium is a mutagen which is toxic to the kidneys (19, 18). This heavy metal exists in many cosmetic products and is used as yellow and orange pigments. In the current study, the level of cadmium in the hair color samples was lower compared to other reports to the extent that it was below the device limit of detection. Moreover, according to the developed standards, the use of any amount of cadmium in cosmetics is forbidden (20).

Lead is an unnecessary element for the human body which can cause loss of appetite, weakness, anemia, vomiting, seizures, and autoimmune diseases (21). It can also lead to mutation and cancer (18). In addition, this metal damages the central nervous system and reduces intelligence in children (19). Studies have shown that lead can cross the placenta during pregnancy and lead to fetal death, preterm labor, and low weight (23, 22).

In the present study, the concentration of lead in the hair color samples was obtained as 11.5-1137.5 ppb, which is lower than the mean value determined in a study conducted by Amartey et al., reporting a mean lead concentration of 8.269 ppm in 34 hair ointments (5). Moreover, in another study conducted by Sukender et al., the amount of this metal in natural hair color (henna) was estimated at 1.47-33 ppm (24). The level of lead obtained in the present study is lower than the value reported by Khalili Naji for hair color (25). In addition, the lead concentration values in the products under study were significantly lower than the standard set by the Food and Drug Administration (20 ppm) (27, 26).

Chromium is one of the metals that can cause severe skin allergies (28). Exposure to large amounts of chromium can also lead to severe corneal damage, deep skin burns, and oral and esophageal burns (29). Mansouri et al., investigating hair color in the markets of Sanandaj, Iran, reported a chromium concentration of 0.865-0.034 μg/g in these products (29). Amount of chromium present in the hair colors of the present study was 88-771.5 ppb, which is higher than the permissible level of chromium in cosmetics forbidding the use of chromium at any amount (20). In addition, the concentrations of lead, cadmium, and chromium in Iranian hair colors were higher than that of foreign hair colors, which is in accordance with the results of a study conducted by Zafar Zadeh in 2018 (30).

In the present study, wet, dry, and digestive methods were used for the preparation and extraction of the samples. In addition, different acids were used in the wet method. As the results indicated, percent recovery was higher in the digestive method performed under controlled temperature and pressure in a closed system. However, the disadvantage of this method is that according to the system instruction, the base acid for digestion is nitric acid, which is not suitable for the digestion of the samples containing metals that are better digested with other acids. Digestion with hydrochloric acid+nitric acid (aqua regia) and nitric acid+oxygenated water in wet method showed higher absorption in the solid samples containing tin, lead, cadmium, and chromium (11).

Furthermore, the samples prepared using hydrochloric acid showed a higher lead absorption rate. Moreover, in the samples prepared by means of perchloric acid, a higher chromium absorption rate was detected. In addition, cadmium absorption rate was lower than the device limit of detection in all samples. These results are indicative of the effect of the applied acid on sample preparation processes. Moreover, the samples obtained at different pH values had the highest absorption at the pH of 4. This finding is inconsistent with the results of a study conducted by Altunay (2016) in Turkey reporting a pH of 8.5 as the most suitable value for sample preparation (12).

According to the results of the present study the following conclusions can be drawn:

1. Use of hair colors, especially at an early age, can be harmful and should be restricted.
2. Despite the low concentration (according to the aforementioned standards) of heavy metals in the hair colors sold in Qom, it is necessary to be careful about the potential harmful effects of heavy metals on health status and also their accumulation properties.
3. Due to the lack of regular industrial testing in the manufacturing process of cosmetic products, companies are probably unaware of the contamination of their products. Therefore, the existence of quality control systems for contaminated products is expected in the manufacturing companies.
4. It is essential to provide necessary training and raise the awareness of the society about the dangers of using non-standard cosmetics and reducing their use in order to prevent contact with these substances.

## Conclusion

Regarding the potential impacts of heavy metals on health and their accumulation property, it is required to perform comprehensive interventions to raise the awareness of the society in this regard.

## Footnotes

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**Conflict of Interest**

The authors declare that there is no conflict of interest.

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