Health Impacts of Exposure to Heavy Metals in Some Selected Lipstick Products Available in Mashhad, Iran

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Abstract
This study aimed to investigate the levels of heavy metals in the lipstick products. In general, 30 samples were randomly selected from Mashhad markets. Analysis of variance (ANOVA) and t test were used to compare color and price variables. Based on the results, the mean concentrations of studied elements were as follows: Cd (ND-0.52 µg/g), Cr (0.25-76.35 µg/g), Ni (ND-20.12 µg/g), Zn (ND-224.22 µg/g), Pb (ND-44.77 µg/g), and Hg (ND-0.32 µg/g). The presence of toxic metals in the samples, and on the other hand, continuous use and unintended ingestion of these metals can lead to their biological accumulation in the human body, thereby causing the development of different diseases. Hence, it deems necessary to apply suitable actions for the improvement of quality control over these products.

Keywords: Lipstick, Heavy metals, Contamination, Mashhad

1. Introduction
Many thousands of years ago, the people of primary societies used various materials in order to beautify their faces, as well as cosmetic products since the dawn of civilization (1). Cosmetics are used by all strata of society as a part of routine body care (2). Although the skin provides a protective barrier, ingredients penetrate through face, lips, and eyes, and can provide human systemic exposure (3). Lipstick products are mixtures of many materials such as pigments, oil, and organic matters (4). Facial cosmetics are used daily and applied to the thinnest area of the facial skin, such as the periorcular areas and lips, where absorption may be very high (2). Although lipstick as a product is intended for the topical use, it can be unconsciously ingested and therefore it provides an obvious oral route of exposure to metal contaminants in cosmetics (2,5). In general, cosmetic products enter the human body by three routes of ingestion, skin contact, and inhalation of spray (6). Although the existence of heavy metals in cosmetic products is not considered a major source of threat to human health, their presence in these products should not be neglected (7,8). Metals are of environmental and human health significance because these toxic elements can be accumulated in the human body organs and exhibit a wide range of toxic and chronic health effects, such as cancer; reproductive, developmental, and neurological disorders; cardiovascular, kidney, and renal problems; lung damage; contact dermatitis; brittle hair; and hair loss (2,9,10). Furthermore, exposure to lead (Pb), chromium (Cr), and cadmium (Cd) can increase the risk of cancers (11,12). Cosmetic products are found in different colors made by the addition of pigments, and the application of these elements by manufacturers has interdicted in most countries (13). Lipsticks are frequently applied by the women. In addition to skin absorption, lipsticks are ingested when consumer eats and drinks (14). Some heavy metals such as Pb, Cd, nickel (Ni), Cr, zink (Zn), and silver (Hg) are usually found in some lipstick products (15). Chronic exposure to Pb could cause neurological, teratogenic, and blood systemic effects. The heavy metal can also pass from the placenta of mothers and injure the fetus (7,16). Furthermore, Pb damages the production system and cellular processes, and decreases brain functions (17). Exposure of the skin and mucus to Pb for a long time also leads to cancer (18). Absorbed Pb (half-life greater than 20 years) can also accumulate in the bones of the human body (19). On the
other hand, prolonged exposure to Cd is closely linked with cardiovascular diseases, such as atherosclerosis and hypertension. Cd has also been reported as the most toxic metal even in low concentrations which can cause kidney dysfunction, bone degradation (Itai-itai disease), metabolic disturbance, and cancer by a direct effect on DNA (7,20,21). Additionally, long term exposure to Ni can cause kidney failure, skin reactions, and weight loss (6,22). For people who are extremely sensitive to Cr, allergic reactions including severe redness and swelling of the skin have been observed (7). Cr is considered as a metal that can increase the risk of some cancers such as kidney and lung, and can also damage the liver and stomach (23,24). Pollution of the environment by hexavalent Cr can result in phototoxic effects such as chlorosis and necrosis on plants, the inhibition of seed germination, depressed biomass, and the reduction of root growth (25). Some effects of Zn on human health are similar to Pb. This metal can cause stomach cramps, anemia, vomiting, tremor, and ataxia (17). Mercury can cause major problems in nervous system function, along with toxicity in reproductive, immune, and respiratory systems. It is also found in thiomersal, which is a mercury-based preservative and is used as a direct ingredient with impurities. However, the high toxicity of this metal implies that the presence of mercury in any cosmetic is a concern (26). The highest rate of cosmetic products consumption has been assigned to Iran after Saudi Arabia. Women annually pay more than 1 billion dollars for buying cosmetic products (27). Regarding the adverse effects of heavy metals on the human health and the overuse of cosmetic products, therefore this study aimed to investigate the presence of heavy metals (Pb, Cd, Cr, Ni, Zn, and Hg) in the most popular lipstick brands used by the women residing Mashhad city, Iran.

2. Materials and Methods

2.1. Sample Collection

Samples included 15 different brands of lipstick in 2 different colors (pink, red, n = 30) commonly used by women and purchased from different markets in Mashhad city in 2018 (Table 1). The most popular brands available in orange, creamy, brown, and violet colors were analyzed separately. All glassware and plastic containers were soaked in 10% HNO₃ (Merck, Germany, 67%) for 24 hours and rinsed with deionized water. All chemical reagents used were of high purity. The samples were analyzed using the wet digestion method. In this method, HNO₃ and H₂O₂ (Merck, Germany, 33%) were used for digestion. One gram from each sample was weighted by a digital balance (model: Adam, England) and placed into a glass beaker. Then, 5 mL of HNO₃ was used for digestion of each sample in the beaker, heated at 80 °C (model: Labinco, Italy). Over the time, the brown fumes converted to white fumes. Afterward, 1 mL of H₂O₂ was added to the samples for the complete oxidation of organic matter and then the indigested samples were filtered using the Whatman filter No. 42 (Merck, Germany). Finally, the samples were diluted to 50 mL using the deionized water. All samples were transferred into polyethylene containers and placed in the refrigerator until their analysis. The samples were tested in duplicate. Moreover, the concentrations of Pb, Cd, Cr, Ni, Zn, and Hg in the samples were determined using the ICP-OES technique (model: Spectro 76004555, America). Three blank samples were analyzed in a similar way as the control samples (1, 28).

2.2. Statistical Analysis

The data were analyzed using the statistical software package for Stata. Analysis of variance (ANOVA) and t test were done to determine the statistical correlation between selected heavy metal concentrations with different colors and prices of lipstick samples at a significance level of P < 0.05. For each sample, the measured concentration was analyzed twice.

2.3. Quality Control

The accuracy of the method was evaluated by calculating the recoveries of Pb, Ni, Cr, Cd, Hg, and Zn added to the matrix because of unavailability of certified materials for

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**Table 1. Thirty Lipstick Samples Collected From Mashhad Local Markets**

| Number | Color | Manufacturing Country |
|--------|-------|-----------------------|
| 1      | Red Pink | Iran                  |
| 2      | Red Pink | Canada                |
| 3      | Red Pink | Turkey                |
| 4      | Red Pink | Turkey                |
| 5      | Red Pink | Italy                 |
| 6      | Red Pink | Iran                  |
| 7      | Red Pink | Germany               |
| 8      | Red Pink | China                 |
| 9      | Red Pink | Italy                 |
| 10     | Red Pink | Korea                 |
| 11     | Red Pink | America               |
| 12     | Red Pink | China                 |
| 13     | Red Pink | Spain                 |
| 14     | Red Pink | China                 |
| 15     | Red Pink | France                |
lipstick analysis. Hence, the extraction recoveries of Pb, Cd, Cr, Ni, Zn, and Hg were 99.82%, 90.22%, 100.62%, 98.75%, 102.45%, and 100.62%, respectively, for two spiked lipstick samples. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the mean plus 3*standard deviation of the blank solution. The LOD for studied elements were as follows: Pb (0.05 µg/g), Cd (0.025 µg/g), Ni (0.025 µg/g), Zn (0.075 µg/g), Cr (0.1 µg/g), and Hg (0.025 µg/g), while the LOD for Pb, Cd, Ni, Zn, Cr, and Hg were as 0.075, 0.025, 0.23, 0.3, 0.16, and 0.085 µg/g, respectively. The precision of the method was determined by relative standard deviations. Relative standard deviations for repeated analyses were calculated to be <15% for all studied elements.

3. Results and Discussion

The presence of heavy metals was evaluated in 30 lipstick samples from 15 different brands in 2 different colors (red, pink) which were frequently used by the females residing Mashhad city, Iran. Table 2 represents the mean concentrations of Zn, Cr, Cd, Ni, Pb, and Hg in lipstick samples and their comparison with acceptable limits set by the Food and Drug Administration (FDA) and Health Canada. In this study, the Pb content in the red color of the brand numbers of 4, 5, 15 and 14 was higher than the LOD of the FDA (20 µg/g). In addition, compared to the acceptable limit of Health Canada (10 µg/g), its concentration in the brand number 12 was higher than 10 µg/g. The Pb levels in marketed lipsticks in India was observed to be in the range of ND- 42.03 ppm (27); this result was in line with our result (ND - 44.77 µg/g). The Ni concentration in all of the studied lipstick samples was within acceptable limits provided by the FDA. While the suggested limit for Ni under the Health Canada is 5 µg/g, the brands of 4, 5, and 13 had the Ni concentrations higher than this limit. The average concentrations of Cr and Ni (24.7-36.9 and 7.4-25.8) in lipstick samples of the study conducted by Iwegbue et al were found to be lower than the acceptable limits defined by the FDA (170 µg/g) and higher than the suggested limits by the Health Canada (2.5 µg/g) (2). Cd content in all brands was within the permissible limits determined by the Health Canada (3 µg/g), but in comparison with the FDA, all the tested brands were out of the safe range (1). Cd concentration in lipstick samples was 20.84 µg/g which was in the allowed range of 4.08-60.2 µg/g. The results of this study showed that the amounts of Cd were higher. Mayiludurai et al reported that the Zn contents in lipstick samples were in the range of 0.489-1.51 ppm (29). Moreover, the Hg concentration in all of the studied lipstick samples was within the acceptable limits.

ANOVA test was used to evaluate the effect of colors on heavy metals contents in lipstick samples. To do the test, 4 different colors (violet, brown, orange, creamy) from the most common brands were chosen (the results are summarized in Table 3). According to the results, in the case of Pb and Ni, a significant difference was observed ($P<0.05$). It can be concluded that the type of pigment used in lipstick samples affects its heavy metal content. Regarding Zn, Cd, Hg, and Cr contents, a significant relationship was also observed among different colors of the most popular brands ($P>0.05$). However, our results for two colors of red and pink displayed that the type of pigment used in lipsticks has no relationship with its heavy metal content. Nourmoradi et al reported similar results in their study (1). In another report from these authors, the statistical analysis confirmed the presence of a meaningful difference between the Pb and Cd contents in lipstick samples of Iranian brands. Moreover, there was a clear relationship between Pb contents in foreign brands. The results of a study by Ziarati el al indicated that the type of pigments used in lipsticks might have a significant relationship with the contents of selected metals (30).

Table 4 shows the concentrations of heavy metals in lipstick samples with different price categories in 2 colors (red and pink). Determination of the relationship between heavy metal contents in price categories and colors were accomplished by the ANOVA test. The results depicted that there was no significant difference between Ni, Pb, Cd, Cr, Hg, and Zn contents among the lipsticks regarding the price categories of cheap, intermediate, and expensive, where $P$ value was more than 0.05. According to the results, the highest concentrations of Cr and Ni were found in expensive lipsticks. The highest Cd, Hg, and Zn contents were seen in cheap lipsticks, while the highest Pb content in red color was found in the lipsticks with intermediate price and the highest Pb content in pink color was found in expensive lipsticks. In all the samples, heavy metals contents were higher in dark colors (red). Al-Qahtani et al studied the relationship of heavy metal contents in the cosmetic products with different prices and found that there was no significant difference between the amounts of heavy metals and lipstick prices (9). In another study conducted in Malaysia by Zakaria and Ho, a remarkable relationship was reported between the Pb contents and the prices of sample lipsticks, while such result was not observed for Cr and Cd (7). In general, it can be concluded that the prices of lipstick brands are not a reliable indicator of the quality of cosmetic products regarding the concentration of heavy metals.

In this study, the correlation between heavy metal contents in 2 colors (pink, red) was assessed using ANOVA (the results are summarized in Table 5). There was no significant difference between Zn, Cr, Pb, Hg, and Ni ($P>0.05$), however $P<0.05$ was obtained for Cd. The results represented that there were greater concentrations of Cd, Pb, Ni, Zn, Hg, and Cr in dark-colored samples (dark red) compared to the light-colored ones (pink). Nourmoradi et al and Al-Saleh et al also reported higher amounts of heavy metals in dark-colored samples compared to the light-colored ones (1,31).

Lipsticks are produced using natural colors obtained...
from plants or artificial colors from minerals. Artificial colors contain different amounts of heavy metals and the concentration of these elements in dark-colored lipsticks is more than their concentration in light-colored ones.

4. Conclusion

The present study revealed that in some brands and colors of lipsticks, the amounts of heavy metals were higher in comparison with the acceptable limits determined by the FDA and Health Canada. According to our results, no significant difference was observed between the contents of Cr, Cd, Zn, and Hg and different colors of the studied brands. However, a significant difference was observed between the Pb and Ni contents regarding different colors of a brand. Moreover, no significant relationship was found between the concentrations of Hg, Ni, Cr, Cd, and Zn, and different price categories in lipstick samples, while there was a significant relationship between the concentrations of Pb in lipstick samples with different prices. Furthermore, no meaningful difference was observed between the Ni, Cr, Pb, Zn, and Hg contents and light and dark colors, though this relationship was significant for Cd. Since the toxic metals are accumulated in the body organs and the consumption of the lipstick

| Lipstick Samples | Color | Zn   | Pb   | Ni   | Cr   | Cd   | Hg   |
|------------------|-------|------|------|------|------|------|------|
| 1                | Red   | 3.35 | 0.52 | ND   | 0.25 | ND   | 0.16 |
|                  | Pink  | 3.85 | 0.67 | 0.15 | 0.32 | ND   | 0.19 |
| 2                | Red   | 1.5  | 0.3  | 0.4  | 0.92 | ND   | 0.22 |
|                  | Pink  | 0.87 | 0.5  | 0.67 | 2.37 | ND   | 0.18 |
| 3                | Red   | ND   | 0.22 | 0.27 | 0.52 | 0.1  | 0.26 |
|                  | Pink  | ND   | 0.27 | 0.32 | 0.3  | ND   | 0.23 |
| 4                | Red   | 23.85| 39.82| 20.12| 74.02| 0.52 | 0.18 |
|                  | Pink  | 24.17| 37.07| 17.67| 69.2 | ND   | 0.21 |
| 5                | Red   | 214.57| 44.77| 19.1 | 73.2 | 0.42 | 0.19 |
|                  | Pink  | 224.22| 34.65| 17.02| 75.77| 0.35 | 0.22 |
| 6                | Red   | 0.8  | 0.57 | 0.1  | 0.52 | ND   | ND   |
|                  | Pink  | 0.27 | 0.4  | 0.37 | 0.5  | ND   | 0.31 |
| 7                | Red   | 29.7 | 0.9  | 0.32 | 4.7  | 0.07 | 0.3  |
|                  | Pink  | 27.2 | 0.85 | 0.17 | 2.7  | 0.02 | 0.25 |
| 8                | Red   | 15.4 | 0.9  | 0.1  | 0.82 | 0.06 | 0.12 |
|                  | Pink  | 6.65 | 0.45 | 0.2  | 1.27 | 0.02 | 0.19 |
| 9                | Red   | 5.12 | 0.27 | 0.35 | 1.87 | 0.02 | 0.18 |
|                  | Pink  | 3.92 | 0.52 | 0.27 | 0.7  | 0.02 | 0.23 |
| 10               | Red   | 0.27 | 0.95 | 2.42 | 8.95 | 0.1  | 0.15 |
|                  | Pink  | ND   | 0.45 | 3.22 | 6.65 | ND   | 0.21 |
| 11               | Pink  | 3.15 | ND   | ND   | 0.42 | ND   | 0.23 |
| 12               | Red   | 4.97 | ND   | 0.35 | 1.42 | ND   | 0.15 |
|                  | Pink  | 17.07| 18.02| 4.72 | 8.37 | 0.02 | 0.18 |
| 13               | Red   | 16.37| 10.37| 3.92 | 8.57 | ND   | 0.22 |
|                  | Pink  | 10.27| 7    | 7.02 | 31.25| 0.108| 0.23 |
| 14               | Red   | 7.82 | 25.82| 1.45 | 9.32 | 0.16 | 0.32 |
|                  | Pink  | 13.72| 16.5 | 3.5  | 10.67| 0.1  | 0.18 |
| 15               | Pink  | 4.95 | 40.9 | 4.87 | 21.92| 0.09 | 0.2  |

| Permissible Limits | Zn | Pb | Ni | Cr | Cd | Hg |
|--------------------|----|----|----|----|----|----|
| FDA                | <170| <170| <170| 0  | 1  |    |
| Health Canada      | 10 | 5  | 2  | 3  | 3  |    |
| Germany            | 20 | -  | -  | -  | -  |    |

ND: Not Detected
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products is increasing, monitoring and quality control of these products are necessary to decrease potential hazards due to the presence of these toxic metals in cosmetic products.

Conflict of Interests
Authors have no conflict of Interests.

Acknowledgment
The authors would like to express their gratitude for the assistance and financial and spiritual support of Mashhad University of Medical Sciences.

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