INTRODUCTION

In most societies across the globe, fairness is considered as the label of beauty, grace, and high social standard in society (1). Due to this perception, skin whitening products have been extensively used especially among women (2). These whitening products are available in different forms such as skin whitening creams, solutions, ointments, and gels that are used by both women and men for beautification purposes (3). There are a variety of chemicals that are used as ingredients in skin care products and some of them are hazardous due to their adverse health impacts especially the presence of heavy metals in these products pose threat to human health (4, 5). These heavy metals are added unintentionally or intentionally in the skin whitening products and mostly the consumers are completely unaware of their presence and toxic effects (6). The exposure of humans to toxic metals present in skin whitening creams mainly occurs through the skin. Such products are either applied to specific skin areas or to the entire body surface and humans remain in contact with the creams for several hours or even days (1, 7). The excessive and continuous use of such whitening creams in daily routine may result in the accumulation of heavy metals in the body and are responsible for causing chronic health issues like reproductive, neurological, and developmental disorders, increase the risk of cancer, and may cause skin allergies (1, 8).

Several studies have been conducted in different regions of the world to examine the health effects that may occurred due to heavy metals exposure through different cosmetic products. Lead exposure through Surma (used for beautifying eyes) is being reported which is the potential source for causing lead toxicity in children that ultimately cause permanent damage to various organs (9–11). Another similar study conducted in Nigeria showed that the application of eye cosmetic ‘Trio’ on eyelids of infants contains 82.6% of lead which can cause lead poisoning in infants (12). In Pakistan, recent research was conducted to evaluate five heavy metals (Pb, Cr, Ni, Fe, and Cd) in different cosmetic products such as whitening creams, lotions, foundations, hair dyes, lipsticks, etc. and the results showed that the LCR values (Lifetime Cancer Risk) exceeded the acceptable limits in all beauty products except for lipsticks (13). A similar study, conducted in KPK showed that Pb, Cu, Zn, and Fe were higher in the cosmetic samples collected from local markets of Kohat District (14). Heavy metals analysis in cosmetic products sold in local markets of Quetta...
also disclosed that the concentrations of Cu, Cr, Mn, and Zn were found to be at elevated levels (15). Although these metals pose a serious threat to human health and the environment, very limited literature is available related to heavy metal analysis in local cosmetic products sold in various regions of Pakistan. Moreover, no such study is conducted till present in the densely populated city of Karachi to report the concentrations of heavy metals in cosmetic products sold in local markets. As skin whitening creams are one of the most demanding cosmetic products but the consumers are unaware of the presence of heavy metals in them which pose a serious threat to their health. Therefore, the present study is conducted for the first time in Karachi and it aims to analyze selected heavy metals (Pb, Ni, Cr, As, and Cu) in the unbranded skin whitening creams which are sold in local markets of the city and their health risk assessment.

MATERIALS AND METHODS

STUDY AREA

Karachi is Pakistan’s largest financial centre, most populous and industrialized metropolis, with a total size of 3,527 km². According to the 2017 official census, Karachi has a population of 16.21 million people; however unofficial figures suggest that the population is closer to 25 million. According to estimates, there are about 10,000 registered industrial units producing food, pharmaceutical, chemical, fabric, paints, oil, steel, and paper products (with a significant number in the informal sector).

SAMPLE COLLECTION

During the study, 25 samples of unbranded whitening creams (N = 25) were collected from three different local markets of Karachi i.e. (i) Sunday Market near Farooq e Azam, (ii) Paposh Market, and (iii) Boltan Market respectively. At these markets, the locally manufactured cosmetic products are sold by unauthorized local companies where quality control is largely ignored. Therefore, the consumers are mostly unaware of how they are exposed to various harmful heavy metals through the application of such cosmetic products. In this regard, 25 skin whitening creams samples were collected labeled properly with sample code, and then stored at room temperature for laboratory analysis.

SAMPLE DIGESTION

The collected skin whitening cream samples were subjected to the wet digestion method (16). Each sample was put into a china dish and then oven-dried at the temperature of 103°C in a hot air oven. After this, 1gm of the oven-dried sample was taken out and transferred into a 100ml digestion flask in which 10ml of concentrated HNO₃ (67%) was added. The mixture was then heated until it became pale yellow or colorless indicating the completion of the sample digestion and then the sample was cooled at room temperature. After this, the sample was filtered through Whatman filter paper no. 42 then 1ml of the filtrate was transferred into the measuring cylinder and diluted up to 50ml with deionized water. Lastly, the 50ml diluted sample was again filtered through Whatman filter paper no. 42 and then the filtrate was transferred into labeled disposable bottles for further heavy metals analysis.

HEAVY METAL ANALYSIS

The selected heavy metals (Pb, Ni, Cr, As, and Cu) were analyzed in all the 25 digested samples of skin whitening creams by using Merck Super Nova60 kits. The experiment for each sample was repeated 3 times and then the results were obtained in the form of mean values and standard deviation.

RESULTS AND DISCUSSION

The present study was conducted to assess the concentrations of five heavy metals i.e. Lead (Pb), Nickel (Ni), Chromium (Cr), Arsenic (As), and Copper (Cu) in unbranded whitening creams sold in local markets of Karachi. The standards given by the Federal food drug and cosmetic act given by FDA Canada were followed in this study (17). The concentrations of heavy metals found in all the 25 whitening creams
samples are presented in Table I. The mean concentrations of heavy metals were found in the order Pb > Cr > Ni > Cu > respectively.

**LEAD**

Overall, the mean concentration of lead was found highest among all the heavy metals. The minimum and maximum levels of lead were detected in the range of 2.11-9.12 ppm with the highest value observed in sample P-1 and the lowest was detected in sample P-23 as given in (table I). It was found that in all the samples, the concentration of lead was crossing the permissible limit defined by FDA Canada i.e. 1 ppm. There are some studies conducted in different regions of Pakistan to report lead concentration in various cosmetic products which can pose a serious health threat to humans. A similar study was conducted in Quetta to assess heavy metals in locally available cosmetic products showed that the concentration of lead was much lower as compared to the present study (15). Another study conducted in KPK revealed that the mean concentration of Pb was much higher in cosmetic products i.e. 141.6 ± 0.016 ppm (14). It was also observed that the use of eye cosmetics such as Surma and Kohl containing lead has been linked with the higher levels of lead in the blood of women and children (9). Children and pregnant women are more vulnerable to lead toxicity because it can enter the placenta and damage the brain of the fetus or it can also transfer through lactating mothers and stored in the bones (18, 19). Exposure to lead was also associated with hormonal imbalances; miscarriages, infertility issues. Additionally lead has been also classified as a suspected carcinogen to humans (20).

**NICKEL**

Humans are exposed to a small amount of nickel mainly through the air, water, food, dust, and also through dermal contact with various products used for skin especially cosmetics (21, 22). Exposure to nickel can also cause health issues depending upon the route and level of exposure (23). Nickel can also be responsible for allergic reactions especially associated with severe dermatitis (21). In the present study, the minimum concentration of Ni was observed in sample P-22 (0.12ppm) while the maximum was observed in sample P-9 (1.21ppm). Overall the mean concentration and standard deviation of Ni in all 25 samples of local whitening creams were found to be 0.546±0.274 ppm. Research conducted in the Malaysian market to assess selected heavy metals concentration in skin lightening products showed that the mean concentration of Ni was found slightly higher in non-local products (0.207±0.15 mg/kg) as compared to the local skin lightning products 0.180±0.17 mg/kg (24). Some studies are also conducted in Pakistan to assess heavy metals in cosmetic products and scientific literature revealed that in KPK, the mean level of nickel in different cosmetic products was 0.674±0.002 μg/g (14). Another study conducted in local markets of Lahore disclosed that Ni was detected in higher concentrations i.e. 55.6821 μg/g in only one locally manufactured lipstick sample which was crossing the permissible limits defined by US-FDA and poses threat to human health (25). According to scientific evidence, a concentration of nickel less than 5mg/kg is considered to be a safe manufacturing practice (26). Therefore, in the present study, nickel concentration remained within safe limits.

**CHROMIUM**

Among all the selected heavy metals represented in (table I), the results revealed that the highest and lowest concentrations of chromium were 2.68 ppm in sample P-14 and 1.13 ppm in sample P-23 respectively. Overall, the mean concentration of Cr in all skin whitening cream samples collected from local markets of Karachi was found to be 2.013±0.421 ppm. A study reported that the lowest and highest concentrations of chromium observed in locally available lipsticks in markets of Lahore were found 0.1527 – 42.574 μg/g (25). Another study conducted for assessing heavy metals in beauty creams sold in local markets of Bangladesh chromium was detected in only one sample in the concentration of 2.82 μg/g (27). The acceptable limit for chromium in cosmetic products according to EU standards is 1 μg/g (28). Consequently, in the present study, the levels of chromium were slightly higher than the permissible limits.
Table I. Concentration of selected heavy metals (ppm) in unbranded whitening creams sold in Karachi

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Lead (Pb)</th>
<th>Nickel (Ni)</th>
<th>Chromium (Cr)</th>
<th>Arsenic (As)</th>
<th>Copper (Cu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-1</td>
<td>9.12</td>
<td>0.78</td>
<td>2.31</td>
<td>0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>P-2</td>
<td>8.67</td>
<td>0.65</td>
<td>2.14</td>
<td>0.02</td>
<td>0.72</td>
</tr>
<tr>
<td>P-3</td>
<td>5.34</td>
<td>0.85</td>
<td>2.18</td>
<td>0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>P-4</td>
<td>6.13</td>
<td>0.76</td>
<td>2.22</td>
<td>0.008</td>
<td>0.43</td>
</tr>
<tr>
<td>P-5</td>
<td>4.31</td>
<td>0.54</td>
<td>2.34</td>
<td>0.005</td>
<td>0.54</td>
</tr>
<tr>
<td>P-6</td>
<td>3.98</td>
<td>0.67</td>
<td>2.52</td>
<td>0.007</td>
<td>0.52</td>
</tr>
<tr>
<td>P-7</td>
<td>5.12</td>
<td>0.43</td>
<td>2.34</td>
<td>0.006</td>
<td>0.62</td>
</tr>
<tr>
<td>P-8</td>
<td>3.42</td>
<td>0.83</td>
<td>2.18</td>
<td>0.027</td>
<td>0.42</td>
</tr>
<tr>
<td>P-9</td>
<td>3.69</td>
<td>1.21</td>
<td>2.17</td>
<td>0.022</td>
<td>0.46</td>
</tr>
<tr>
<td>P-10</td>
<td>3.48</td>
<td>0.96</td>
<td>2.42</td>
<td>0.028</td>
<td>0.43</td>
</tr>
<tr>
<td>P-11</td>
<td>4.19</td>
<td>0.88</td>
<td>2.19</td>
<td>0.029</td>
<td>0.42</td>
</tr>
<tr>
<td>P-12</td>
<td>4.75</td>
<td>0.41</td>
<td>2.34</td>
<td>0.023</td>
<td>0.44</td>
</tr>
<tr>
<td>P-13</td>
<td>3.24</td>
<td>0.67</td>
<td>2.54</td>
<td>0.01</td>
<td>0.53</td>
</tr>
<tr>
<td>P-14</td>
<td>4.35</td>
<td>0.54</td>
<td>2.68</td>
<td>0.02</td>
<td>0.48</td>
</tr>
<tr>
<td>P-15</td>
<td>4.45</td>
<td>0.36</td>
<td>1.98</td>
<td>0.01</td>
<td>0.63</td>
</tr>
<tr>
<td>P-16</td>
<td>3.72</td>
<td>0.43</td>
<td>1.88</td>
<td>0.008</td>
<td>0.43</td>
</tr>
<tr>
<td>P-17</td>
<td>3.85</td>
<td>0.27</td>
<td>1.78</td>
<td>0.005</td>
<td>0.58</td>
</tr>
<tr>
<td>P-18</td>
<td>3.54</td>
<td>0.49</td>
<td>1.67</td>
<td>0.007</td>
<td>0.36</td>
</tr>
<tr>
<td>P-19</td>
<td>3.12</td>
<td>0.18</td>
<td>1.54</td>
<td>0.006</td>
<td>0.37</td>
</tr>
<tr>
<td>P-20</td>
<td>3.28</td>
<td>0.24</td>
<td>1.82</td>
<td>0.027</td>
<td>0.38</td>
</tr>
<tr>
<td>P-21</td>
<td>2.87</td>
<td>0.21</td>
<td>1.35</td>
<td>0.022</td>
<td>0.28</td>
</tr>
<tr>
<td>P-22</td>
<td>2.65</td>
<td>0.12</td>
<td>1.28</td>
<td>0.028</td>
<td>0.22</td>
</tr>
<tr>
<td>P-23</td>
<td>2.11</td>
<td>0.43</td>
<td>1.13</td>
<td>0.066</td>
<td>BDL</td>
</tr>
<tr>
<td>P-24</td>
<td>7.91</td>
<td>0.33</td>
<td>1.67</td>
<td>0.091</td>
<td>BDL</td>
</tr>
<tr>
<td>P-25</td>
<td>6.91</td>
<td>0.41</td>
<td>1.81</td>
<td>0.055</td>
<td>BDL</td>
</tr>
<tr>
<td>Mean</td>
<td>4.568</td>
<td>0.546</td>
<td>2.013</td>
<td>0.022</td>
<td>0.489</td>
</tr>
</tbody>
</table>

During the manufacturing process of cosmetic products, hexavalent chromium which is a known human carcinogen can be added as an impurity and may cause allergic dermatitis due to exposure through skin contact from such products (29). Moreover, it is also reported that the elevated levels of chromium in urinary samples of pregnant women may be associated with chromium exposure through the use of cosmetics, passive smoking, and other sources (30). Exposure to hexavalent chromium may also lead to lung cancer through inhalation and can cause an increased risk of stomach tumors due to exposure through consuming chromium contaminated drinking water (31).

**ARSENIC**

In the present study, the concentration of arsenic in locally manufactured skin whitening creams was found in the range of 0.005-0.091 ppm with the lowest concentration observed in sample P-5 and the highest recorded in sample P-24 as shown in Table 1. The mean concentration of arsenic in all 25 samples of
whitening creams was found at 0.022±0.021 ppm which showed that the levels of As in the samples were found within the safe limits i.e. <3ppm according to FDA Canada guidelines. Comparison of the results obtained with the previous studies showed that the levels of arsenic in cosmetic products sold in local markets of Iraq were found much higher i.e. in the range of 4.83-5.73 mg/kg (or ppm) compared to the level found in the present study (32). A study conducted to assess the concentration of heavy metals in various cosmetic products sold in Iran showed that arsenic levels were found highest (0.359–3.853 μg/g) compared to other heavy metals found in the samples (4). Moreover, a recent study conducted in Pakistan to assess heavy metals in colored cosmetic products also revealed that higher levels of arsenic were detected in eye shadow and lipsticks samples (33).

Human exposure to arsenic may result in adverse health issues such as high blood pressure, skin lesions, gangrene, cancer, peripheral vascular disease, and melanosis (34). Long-term exposure to arsenic can also cause chronic diseases such as macrophage dysfunction due to deterioration of immune systems and also affecting lymphocytes (35). It was also reported that exposure to arsenic and UV light are the important risk factors that are associated with squamous and basal cell carcinoma (36).

COPPER

Exposure to copper through dermal contact may result in skin discoloration, dermatitis and can also cause throat or nose irritation (37). Furthermore, long term human exposure to copper can also cause behavioral and physiological disturbances (such as progressive demyelination and brain damage), psychiatric issues (including aggressive behavior, depression, and suicidal tendencies), and other health issues including motor dysfunction, liver cirrhosis and hemolytic anemia (37–39). In the present study, the results revealed that the minimum and maximum concentrations of copper in 25 local skin whitening creams sample were found to be 0.22 ppm in sample P-22 and 0.85 ppm in sample P-1. Only scanty literature is available related to copper analysis in cosmetic products and their related health effects due to exposure. A study conducted in Nigeria to assess heavy metals in personal care products reported that the highest concentration of copper was observed in hair cream i.e. 0.783 ppm (37). Similarly, copper concentrations in traditional cosmetics (henna and kohl) sold in local markets of Tunisia were found in the range of 3.7-90.0 μg/g in henna products and 2.5-162.5 μg/g in kohl products (40). However, in the present study, the mean concentration of Cu was found to be much lower compared to previous studies i.e. 0.489±0.145 ppm which indicates that the samples are not associated with copper toxicity to the consumers.

CONCLUSION

The present study investigated for the first time to assess the concentration of selected heavy metals (Pb, Ni, Cr, As, and Cu) in unbranded skin whitening creams sold in local markets of Karachi, Pakistan. The results revealed that lead was found to be in the highest concentration in all the samples as compared to other heavy metals which are found in order of Pb>Cr>Ni>Cu>As respectively. It shows that the high level of heavy metals contamination in the samples is mainly due to the mismanaged manufacturing of such products without any proper monitoring of toxic metals during the production process. Quality control methods are not applied. Therefore, it is necessary to monitor and control the fate of heavy metals in cosmetic products, especially the unbranded ones to avoid human exposure to such toxic heavy metals. There are no safety regulation guidelines defined in the country to assess the heavy metals in cosmetic products which is another major drawback that needs attention. Moreover, further studies are need to be performed related to the daily use of cosmetic products contaminated with heavy metals to assess the exposure risk of humans to different toxic heavy metals. There is also an urgent need to define acceptable limits of potential pollutants in cosmetic products that must be enforced at local levels. Keeping in view that it is not possible to remove heavy metals from such demanding products after their manufacturing; therefore, safe manufacturing practices must be implemented during the production of cosmetic products so that the quality of products can be improved without compromising human health.
Acknowledgments:
Authors are thankful to the Director of the institute for providing laboratory facility to conduct this study

Conflict of Interest:
The authors declare that they have no conflict of interest.

References:


