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HUMAN HAIR AND NAILS AS BIO-INDICATOR OF HEAVY METALS CONTAMINATION BY HAIR DYE EXPOSURE AMONG POPULATION IN SAUDI ARABIA

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ABSTRACT

Objectives: The aim of this study is to assess hair and nails as bioindicators of heavy metals (arsenic, cadmium and lead) contamination by hair dye exposure, estimate levels of heavy metals in different hair dyes and then monitor their possible harmful effects. Additionally, the present work extends to study the correlations between the heavy metals levels in the hair and nails samples. Finally, the present results were compared to other studies.
Design and methods: Human hair and nail samples of one hundred participants were collected. They were classified into two groups; group (I) included ten participants served as control group and group (II) included ninety participants used different hair dyes and the amount of heavy metals, i.e. arsenic (As), cadmium (Cd) and lead (Pb) was meaured by using graphite furnace atomic absorption spectrophotometer (GF-AAS). **Result:** The concentrations of the heavy metals in the hair samples ranged from (0.53 to 3.38µg/g) for arsenic, (0.14 to1.57 µg/g) for cadmium and (1.85 to 279.9 µg/g) for lead. In nail samples, heavy metals ranged from (2.60 to10.04 µg/g) for arsenic, (0.18 to 0.89 µg/g) for cadmium and (2.69 to 17.77 µg/g) for lead. The measured heavy metals (As, Cd and Pb) levels in hair and nail were significantly increased in participants used Henna- black , Rani-black, Bigen, Vary hair dye, Garnier and Lakme hair dye groups compared to control group. Positive correlations were monitored between hair and nails samples content of As, Cd and Pb in participants used hair dyes group. **Conclusion**: Lead is one of the highest heavy metals used in hair dye products and it accumulates in hair and nails by continuous usage and produces serious damage effects.

KEYWORDS: Hair dyes, Heavy metals, Bioindicators, Arsenic, Cadmium, Lead, Henna.

1. INTRODUCTION

Hair coloring is the practice of changing the color of hair.[1] In developed countries, 70% of women dye their hair at least once, and many do so regularly.[2] Some hair colors contain heavy metal impurities, including lead, arsenic and others causing their adverse effects including allergic contact dermatitis, cancer and other systemic diseases.[3]

Heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair. Additionally, metal ions interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis. Several studies have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic, cadmium, chromium, lead and mercury. Because of their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance. According to the United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as either “known” or “probable” human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals.[4]

Lead has been described as the most harmful environmental contaminant to arise in human civilization and has been shown to impair renal, homopoietic and the nervous system with different reports linking it to deficiency in cognitive functioning.[5]

Arsenic is regarded as human carcinogen from extremely low levels of exposure, having no possible beneficial metabolic functions for humans. Its low level exposure causes nausea, vomiting, decreased production of RBCs and WBCs, abdominal pain and its long term exposure causes darkening of skin and appearance of small corns on palm soles.[6] Additionally, arsenic exposure affects virtually all organ systems including the cardiovascular, dermatologic, nervous, hepatobilliary, renal, gastro-intestinal, and respiratory systems.[4]

Hair and nails are biomaterials primarily composed of fibrous protein structure, notably keratin. The changes in their appearance and composition are visible signs of deficiency or presence of stored substances in the body. Therefore, they have been recognized as valuable tissues for monitoring human environmental exposure. The amount of elements in human hair and nails is a good alternative indicator of public and occupational exposure to natural radionuclides and other metals that can reflect the actual exposure over a long period of months, or even years.[7] Therefore, nail tissue is more attractive diagnostic tool in assessing heavy metals, as it is an economical method and not susceptible to infections and contamination.[8] Also, Hair analysis is inexpensive and fast; it also detects and measures the content of heavy metals and minerals of the hair. The Global Environmental Monitoring System (GEMS) of the United Nations Environment Program selected human hair as one of the important monitoring materials for worldwide biological monitoring of pollution.[9]

The main goal of the present work is to assess hair and nails as bioindicators of heavy metals contamination by hair dye exposure and estimate the levels of heavy metals (arsenic, cadmium and lead) in different hair dyes. Additionally, the present work extends to study the correlations between these heavy metals levels in the nails and hair, compare the results of the study with other studies and monitor the possible harmful effects of hair dyes.

2. SUBJECTS AND METHODS

2.1. Subjects

Human hair and nail samples of one hundred participants were collected. They were classified into the following two groups.
Group I: Ten participants served as control group (I).
Group II: Ninety participants used hair dyes. This group was subclassified into following nine groups:
Group IIa: Ten participants used Henna-black hair dye.
Group IIb: Ten participants used Rani-black hair dye.
Group IIc: Ten participants used Rani-red hair dye.
Group IId: Ten participants used Loreal hair dye.
Group IIe: Ten participants used Bigen hair dye.
Group IIf: Ten participants used Kolestone hair dye.
Group IIg: Ten participants used Vary hair dye.
Group IIh: Ten participants used Garnier hair dye.
Group IIi: Ten participants used Lakme hair dye.

Informed consents were collected from all participants in this study. Age, gender, hair dye brands, manufacturing country and duration of hair dye usage were also collected.

- Inclusion criteria.
1. Both genders from Saudi Arabia.
2. Age (20-60 years).
- Exclusion criteria:

Exposure to other sources of heavy metals.

2.2. METHODS

Organic matters in sample was digested by high pressure microwave digestion and determination of the amount of heavy metals, i.e. arsenic (As), cadmium (Cd) and lead (Pb) was done by using graphite furnace atomic absorption spectrophotometer (GF-AAS).

Accurately weighing, to the nearest mg in duplicate, 0.15 – 0.20 gm of sample into a high pressure resistance 50 mL quartz or TFM vessel was done. The contact with the side of the vessel was avoided. The 3 mL conc. nitric acid and 30 % hydrogen peroxide 1 mL by using graduated pipette were added. If sample contain talcum or pigment, conc. hydrochloric acid 1 mL was added. The vessel lid was closed. Leave for about 15 minutes to ensure complete reaction.
Digestion in microwave digestion system at the specified program was applied. After cooling to room temperature, 20 mL deionized water was added to the digested solution, the inner wall and lid were thoroughly rinsed. Filtration through Whatman paper no.1 into 50 mL volumetric flask and dilution to volume with deionized water were applied. Nail samples were diluted up to 25 mL with deionized water. Hair samples were diluted up to 50 mL with deionized water.

Standard calibration solutions were injected into the GF-AAS. The response (absorbance or peak height or area) versus concentration of each standard solution for As, Cd and Pb were plotted. Sample solutions were injected into GF-AAS. The response and concentrations (µg/L) of As, Cd and Pb in sample solution were recorded.

2.3. Statistical analysis

The SPSS Version 21.0 (SPSS, Inc., Chicago) package was used for all statistical analyses. Results were expressed as mean ± SD. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by a Tukey-Kramer post-hoc test. Moreover, correlations between different parameters were evaluated by Pearson correlation (r). The level of significance was set at $p \leq 0.05$.

3. RESULTS

The demographic characteristics of the studied groups are presented in Table (1). The Studied groups were not different with respect to the age and sex distribution. The participants used hair dyes group (II) showed a significant increase in age compared to control group (I) by 1.4 fold.

Table (1): Demographic characteristics of control group (I) and participants used hair dyes group (II).

Clinical parameters	Groups	
	Group (I)	Group (II)
N	10	90
Gender		
Male	3 (30%)	21 (23%)
Female	7 (70%)	69 (77%)
Age (years)	26.84 ± 6.49	38.68 ± 9.33*
Duration of hair dye usage (Years)	Nil	8.05 ± 2

Results are expressed as number (percentage) and mean ± SD.
N: Number of participants.
(*): Significance difference from control group (I). P values ≤ 0.05 were considered significant.

The data presented in Table (2) revealed that the measured heavy metals (As, Cd and Pb) levels were statistically significantly increased in participants used Garnier hair dye group (IIh) compared to control group (I) by about 3, 9 and 0.5 fold respectively. The calculated means ± SD of arsenic in participants used Rani-black hair dye group (IIb), Vary hair dye (IIg) and Lakme hair dye group (IIi) showed a significant increase compared to control group (I) by 3, 1.8 and 1.7 fold respectively. In addition, there were significant elevations in lead levels in participants used Henna- black hair dye group (IIa) and Rani-black hair dye group (IIb) compared to control group (I) by 85 and 3.5 fold respectively.

Table (2): Heavy metals of control group (I) and participant used Henna-black hair dye group (IIa), Rani-black hair dye group (IIb), Rani-red hair dye group (IIc), Loreal hair dye group (IId), Bigen hair dye group (IIe), Kolestone hair dye group (IIf), Vary hair dye group (IIg), Garnier hair dye group (IIh) and Lakme hair dye group (IIi) in human hair samples.

Heavy metals	Groups (Manufacturing country)									
	Group (I)	Group (IIa) (Saudi)	Group (IIb) (Saudi)	Group (IIc) (Saudi)	Group (IId) (Belgium)	Group (IIe) (Japan)	Group (IIf) (Germany)	Group (IIg) (Germany)	Group (IIh) (Egypt)	Group (IIi) (Spain)
As (µg/g)	1.05± 0.48	1.2± 0.36	2.94± 0.64*	0.53± 0.08*	0.60± 0.10*	1.11± 0.77	1.36± 0.22	1.79± 0.218*	3.38± 0.18*	1.69± 0.91*
Cd (µg/g)	0.16± 0.05	0.23± 0.12	0.2± 0.06	0.14± 0.04	0.16± 0.04	0.15± 0.02	0.33± 0.07*	0.48± 0.71	1.57± 0.73*	0.16± 0.02
Pb (µg/g)	3.30± 0.91	279.9± 115.89*	11.55± 0.99*	2.84± 0.18	1.85± 0.32*	3.57± 1.12	2.3± 0.15*	3.12± 0.64	4.16± 0.23*	3.47± 0.16

Results are expressed as mean ± SD.
(*): Significance difference from control group (I). P values ≤ 0.05 were considered significant.

The data presented in Table (3) revealed that the measured heavy metals (As, Cd and Pb) levels were statistically significantly increased in participants used Bigen hair dye group (IIe) compared to control group (I) by about 2, 3 and 1.4 fold respectively. The calculated means ± SD of lead in participants used Henna-black hair dye group (IIa) and Garnier hair dye group (IIh) showed a significant increase compared to control group (I) by 3 and 1.4 folds respectively. In addition, there were significant elevations in arsenic levels in participants used Garnier hair dye group (IIh) and Lakme hair dye group (IIi) compared to control group (I) by 1.6 and 1.3 fold respectively.

Table (3): Heavy metals of control group (I) and participants used Henna-black hair dye group (IIa), Rani-black hair dye group (IIb), Rani-red hair dye group (IIc), Loreal hair dye group (IId), Bigen hair dye group (IIe), Kolestone hair dye group (IIf), Vary hair dye group (IIg), Garnier hair dye group (IIh) and Lakme hair dye group (IIi) in human nails samples.

Heavy metals	Groups (Manufacturing country)									
	Group (I)	Group (IIa) (Saudi)	Group (IIb) (Saudi)	Group (IIc) (Saudi)	Group (IId) (Belgium)	Group (IIe) (Japan)	Group (IIf) (Germany)	Group (IIg) (Germany)	Group (IIh) (Egypt)	Group (IIi) (Spain)
As (µg/g)	4.67± 0.90	4.70± 1.06	3.93± 0.88*	5.13± 1.15	2.60± 0.42*	10.04± 2.5*	4.75± 1.1	2.80± 0.37*	7.38± 1.5*	5.95± 1.12*
Cd (µg/g)	0.32± 0.11	0.23± 0.03	0.18± 0.03*	0.27± 0.04	0.22± 0.06*	0.89± 0.09*	0.21± 0.06*	0.18± 0.04*	0.28± 0.05	0.25± 0.03
Pb (µg/g)	5.78± 1.12	17.77± 4.2*	3.42± 0.50*	3.72± 0.50*	3.11± 0.56*	7.82± 1.8*	2.88± 0.41*	2.69± 0.42*	7.93± 1.40*	6.27± 1.29

Results are expressed as mean ± SD.
(*): Significance difference from control group (I). P values ≤ 0.05 were considered significant.

Figure (1) shows the comparison between the levels of measured heavy metals (As, Cd and Pb) in hair and nails samples of participants used natural hair dyes (Henna-black, Rani-black and Rani-red hair dyes). The lead concentration was higher in the hair samples than nails samples for participants used Henna-black hair dye group (IIa) and Rani-black hair dye group (IIb).

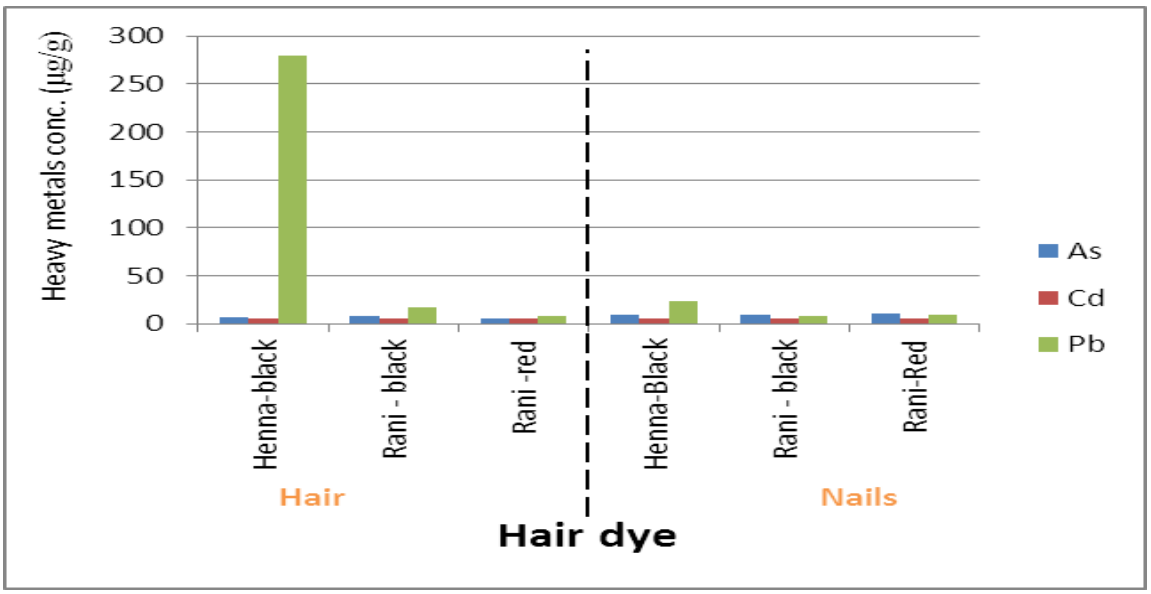

Figure (1): Arsenic, cadmium and lead concentrations in hair and nails samples of participants used natural hair dye.

Figure (2) shows the comparison between the levels of measured heavy metals (As, Cd and Pb) in hair and nails samples of participants used synthetic hair dyes. The arsenic and lead concentrations tended to be higher in the nails samples than hair samples for participants used Loreal hair dye group (IId), Bigen hair dye group (IIe), Kolestone hair dye group (IIf), Garnier hair dye group (IIh) and Lakme hair dye group (IIi).

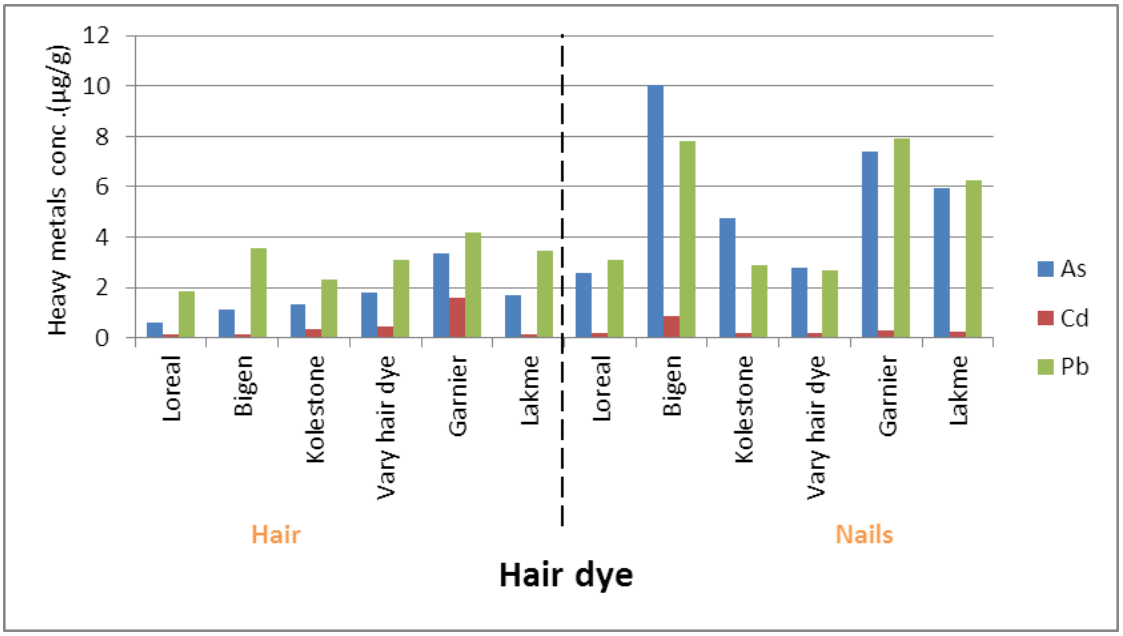

Figure (2): Arsenic, cadmium, and lead concentrations in hair and nails samples of participants used synthetic hair dye.

Table (4): Meta-analysis of heavy metals (As, Cd and Pb) levels in human hair samples.

Heavy metals	Other Studies					
	Our study (2016)	Malaysia (saat et al., 2013)[19]	Nigeria (Abdulrahman et al., 2012)[14]	India (Samanta et al ., 2003) [20]	Indonesia (Ponzetta et al., 1998)[21]	Nigeria (Oluwole et al., 1994)[22]
As (µg/g)	1.6	0.003	1.36	3.43	NA	0.09
Cd (µg/g)	0.36	0.009	0.28	0.40	0.6	NA
Pb (µg/g)	31.61	0.473	31.22	8.03	15.7	NA

Results are expressed as mean.
NA: Not available.

Table (5): Meta-analysis of heavy metals (As, Cd and Pb) levels in human nails samples.

Heavy metals	Other Studies					
	Our study (2016)	Malaysia (saat et al., 2013)[19]	Nigeria (Abdulrahman et al., 2012)[14]	India (Samanta et al ., 2003)[20]	Poland (Nowak and Chmielnicka, 2000)[23]	Japan (Hayashi et al., 1993)[24]
As (µg/g)	5.25	0.012	1.7	7.24	NA	NA
Cd (µg/g)	0.30	0.009	6.1	0.32	2.01	0.13
Pb (µg/g)	6.16	0.403	26.27	10.99	8.7	12.1

Results are expressed as mean.
NA: Not available

Positive and significant correlations were monitored between hair and nails samples content of lead in participants used hair dyes group (II) as shown in Table (6). On the other hand, there were positive correlations between hair and nails samples content of arsenic and cadmium in participants used hair dyes group (II).

Table (6): Correlations between hair and nails samples content of As, Cd and Pb in participants used hair dyes group (II).

Heavy metals in nails samples	Heavy metals in hair samples		
	As As (µg/g)	Cd (µg/g)	Pb (µg/g)
As (µg/g)	0.16	Nil	Nil
Cd (µg/g)	Nil	0.11	Nil
Pb(µg/g)	Nil	Nil	0.83**

Results are expressed as correlation coefficients (r).
(**):P < 0.01.

4. DISCUSSION

The aim of this study is to assess hair and nails as bioindicators of heavy metals (arsenic, cadmium and lead) contamination by hair dye exposure, estimate levels of heavy metals in different hair dyes and then monitor their possible harmful effects. Also, the present work extends to study the correlations between the heavy metals levels in the hair and nails samples. At the end, the present results were compared to other studies.

The use of cosmetics to change hair color occurs with high frequency and mostly among the females (to cover gray hair, or change to more fashionable color). Hair dyeing involves treatment of the hair with different natural products or chemicals. Cosmetics are seen as one of the most important sources of releasing heavy metals into the environment and the human biological system. Heavy metals have ability for accumulation in the body causing serious side effects by the time. Reaction caused by the use of hair dye in mild cases only involves dermatitis to the upper eyelids or the rims of the ears. In more severe cases, it often extends beyond the scalp to include the forehead, neck, eyelids and face. It manifests as pruritic, oedematous, erythematous scaly patches and plaques and vesicular lesions in some cases [10].

In addition, high doses of arsenic can be deadly and that even long-term exposure to low levels of arsenic can cause certain cancers. There is also a growing concern about the physiological and behavioral effects of toxic metals on human population in general.[5] Following such observation, there is an increasing need to investigate the concentration of toxic metals in some commonly used cosmetic products.

In the present study, arsenic (As) levels in hair samples of participants used Rani-black, Vary and Lakme hair dyes showed a significant increase compared to control group by 3, 1.8 and 1.7 fold respectively. In nail samples, there was statistically significantly increased in participants used Bigen hair dye group (IIe) compared to control group (I) by about 2 folds. Arsenic is regarded as human carcinogen from extremely low levels of exposure, having no possible beneficial metabolic functions for humans. Its low level exposure causes nausea, vomiting, decreased production of RBCs and WBCs, abdominal pain and its long term exposure causes darkening of skin and appearance of small corns on palm soles. Other effects include abnormal ECG, anorexia, fever, fluid loss, goiter, hair loss, headache, herpes, impaired healing, jaundice, keratosis, kidney and liver damage, muscle spasms, pallor, peripheral neuritis, sore throat, weakness and it also interferes with the uptake of folic acid.[6]

Assessing the amount of dermal absorption from a single component in a cosmetic product is complex and depends on many factors such as the concentration of arsenic in the product, the amount of product applied, the length of time left on the skin and the presence of emollients and penetration enhancers in the cosmetic product.[11] Meanwhile, the present study coincides with the work of Beatrice et al., 2014 who provided evidence that arsenic level in cosmetic products for hair (shampoo, hair conditioner and hair gel) was found at concentrations between 0.087- 0.252 (µg/g).[12] It is important to mention that, the lowest level of arsenic was observed in Loreal hair dye group (IId) among other hair dyes.

Cadmium (Cd) levels in hair samples of participants used Garnier hair dye group (IIh) and in nails samples of participants used Bigen hair dye group (IIe) showed a significant increase compared to control group by about 9.8 and 2.7 folds respectively. However, the lowest level of cadmium was observed in Rani-black, Kolestone and Loreal hair dye groups compared to other hair dyes. In both hair and nail samples, cadmium levels were the lowest concentrations compared to other heavy metals concentrations but even that very low concentration doesn't demonstrate its safety as it was mentioned by Ayenimo et al., 2009 who reported that any amount of Cd is forbidden in all types of cosmetics due to its threat to human health because significant dermal exposure can cause irritant dermatitis.[13]

Slow release of cadmium with low amount may also cause harmful effects to the human body, results from tests showed that it was minor exposure that caused high blood pressure. It targets blood vessel and heart tissue as well as, the kidneys, lungs and brain, and results in heart disease, liver damage.[14] The present results come in agreement with the observation of Huda, 2013 who stated that cadmium was detected in small amount at all samples of variety of hair dyes.[15]

Some Henna products claim to be pure natural product. The Henna powder used by mixing it with hot water stirred to a paste. After a cooling, the hot paste is massaged into the dry hair and skull of the head and left for 45 to 90 minutes before washing. The hair samples in participants used Henna- black hair dye (IIa) and Rani-black hair dye (IIb) and nail samples of participants used Henna-black hair dye (IIa) showed a significant increase in lead levels compared to control group (I) by 85, 3.5 and 3 fold respectively. Jallad et al., 2008 reported that highest lead concentration was detected in Henna paste with added materials[16] and Marzulli et al., 1978 conducted study on nine adult males who applied hair dye containing Pb acetate for ninety days; it was found that seven out of nine had elevated Pb levels in hair[17]; both studies come in agreement with the present results. Florence et al., 1988 showed that inorganic lead was absorbed through the skin and was rapidly distributed through the body leading to abnormal blood lead levels.[18] It is a serious cumulative body poison, which can affect every organ and system in the body. Lead at higher levels is well known to cause several health related abnormalities affecting the central nervous system, kidney, liver and reproductive system, etc.

Chronic exposure to Pb may result in birth defects, mental retardation, autism, psychosis, allergies, dyslexia, hyperactivity, weight loss, shaky hands, muscular weakness and paralysis.[19] Acute allergic contact dermatitis, eczema, chemical burn, acute renal failure, acute and severe angioneurotic edema, abdominal pain and vomiting as adverse health effects associated with the use of black Henna are well documented in the literature.[10]

Rani-black hair dye group has the second highest lead level in hair samples of participants used natural hair dye while Rani-red hair dye group has lowest level compared to other hair dyes. Red Henna made up only of Henna leaves which have active substance naphthoquinone relatively harmless in nature while the black Henna has some additives as para-phenylenediamine (PPD) which were responsible for most serious side effects of Henna. Kalicanin et al., 2015 found that Henna hair dye samples had lead range from 8.51 to 19.61 µg/kg with the highest level in Red Henna dye, this variation in the lead levels in Henna hair dye can be the result of the additives that used to make different colors.[20] Regarding to synthetic hair dyes, the lowest level of lead was observed in Kolestone and Loreal hair dye groups among other hair dyes.

Notably arsenic and lead concentrations tend to be higher in the nails samples than hair samples. The present work comes in agreement with Sahoo et al., 2015 who stated that the nails samples were observed to accumulate higher concentrations of heavy metals when compared to hair samples such differences might be attributed to the incorporation of elements into the keratin structure of hair which takes place by binding to the sulfhydryl groups that are present in the follicular protein. In this regard, the detergents such as soap, and shampoos, hair pomades, lotions, hair bleaches and dyes actually compete with the complexing ability of these reactive sites, thus leading to a significant leaching of elements from the shaft bulk.[7]

In addition, meta-analysis showed comparison of As, Cd and Pb levels in hair and nails samples to other studies. Arsenic concentrations in hair and nails samples of our study groups were higher than that of Saatm et al., 2013 in Malaysia[21] but lower than that of Samanta et al., 2003 in India, also cadmium levels were higher in Samanta et al., 2003 in India than our result with close variation. Comparison of Pb level in hair and nails samples in our work to other studies revealed that lead level in the hair samples was higher than that Samanta et al., 2003 in India[22] but the opposite in nails, and the lowest level was in Saatm et al., 2013 in Malaysia for both nails and hair samples, also it was observed that the highest lead concentration was in working people at iron welder workshop in Borno State Nigeria compared to other studies. On the other hand, the lowest level of heavy metals was found in Saatm, et al., 2013 among farmers exposed to pollution in Malaysia compared to other studies.[21]

Positive and significant correlations were monitored between hair and nails samples content of lead in participants used hair dyes group (II). On the other hand, there were positive correlations between hair and nails samples content of arsenic and cadmium in participants used hair dyes group (II). The present work comes in agreement with the observation of Mehra et al., 2004 who concluded that hair and nail lead concentrations were correlated to each other and hair concentrations of Pb are considered as useful biological indicator for monitoring exposure to Pb in the general or occupational environment.[23]

In this study; arsenic, cadmium and lead levels in various hair dyes of different types and brands were determined in hair and nails samples. According to the present results, it is revealed that the highest concentration of heavy metals in both hair and nails samples is lead but cadmium is the lowest concentration. Black Henna is one of the products that has high levels of all the three heavy metals and thus it has the highest risk on the users. Also, the present study indicates that the Bigen contains the highest level of arsenic in nails samples. It is worth mentioning that the most safe hair dyes based on the concentration of heavy metals are Kolestone and Loreal hair dyes.

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