Evaluation of the accumulation of heavy metals, including lead and cadmium, in hair samples dyed using both natural and artificial formulations- Aden, Yemen.

ABSTRACT:

Background: Heavy metal contamination has arisen as a major global public health concern, owing to its persistence, bioaccumulation, and toxicity even at low levels. Lead (Pb) and cadmium (Cd) are especially concerning due to their broad environmental dispersion and well-documented negative effects on human health.

Aim: The current study aims to measure the concentrations of Pb and Cd in natural hair, dyed hair, and selected hair coloring products to evaluate the health risks and clarify the extent of cosmetic-related heavy metal contamination within local consumer markets.

Methods: Hair samples were obtained from the back of the scalp of seven females aged 24 to 60 years defined and numbered as samples 1-7. Hair samples were cut into 0.3 cm pieces with ethanol-rinsed stainless-steel scissors. Three had natural hair, while four had dyed hair. Six hair dye brands were acquired in local marketplaces in Aden: three industrial dyes (MODA, Katrina, and Galaxy) and three natural colors (Black Henna, Hor, and Black Stone). All samples were analyzed using a Flame Atomic Absorption Spectrophotometer (FAAS) and the absorption was measured at 283.3 nm for Pb and 228.8 nm for Cd.

Results: Natural hair exhibited lower Pb and Cd levels than colored hair. Natural sample 1 had the lowest concentrations in hair (0.143µg/g Pb, 0.443µg/g Cd). The maximum concentration was found in dyed sample 7 (Pb: 0.292 µg/g, Cd: 0.537 µg/g). For dyes, Black Stone had the lowest metal content (Pb: 0.07833, Cd: 0.038), while Galaxy had the highest (Pb: 3.413, Cd: 1.238).

Conclusion: Hair dyed with synthetic dyes contains higher levels of the heavy metals Pb and Cd than hair dyed with natural dyes or undyed hair. The Galaxy dye had the greatest mean amounts of Pb (3.413 ppm) and Cd (1.238 ppm), whereas the Black stone dye and the Hor plant had the lowest Pb values. Sample 2 had the lowest Cd levels. Enhancing public health awareness and advocating botanical formulations are recommended.

Keywords: Cadmium (Cd); Lead (Pb); Hair dye; Aden

INTRODUCTION:

Heavy metal contamination in cosmetic products has emerged as a global public health concern, due to the widespread use of personal care products and the cumulative exposure to hazardous metals such as Pb and Cd. According to recent evaluations, these metals can enter cosmetic goods thru contaminated raw materials, pigments, manufacturing processes, or packaging, providing dangers even at trace levels (Abed et al., 2024).

Hair dyes are among the most commonly used beauty products globally, however numerous recent studies have found significant levels of hazardous metals in commercial formulas. Chronic exposure to Pb and Cd in cosmetic products has been linked global public health making their elimination from cosmetic items in concern (Anita et al., 2024; Sharma et al., 2024).

Previous epidemiological studies, including those published by the World Health Organization, have shown that even low blood Pb concentrations are linked to neurotoxicity, nephrotoxicity, reproductive issues, bone demineralization, cardiovascular complications in adults, and carcinogenic effects (Åkesson, et al. 2005).

Human hair is widely known as a viable, non-invasive biomarker for measuring long-term exposure to environmental pollutants because it represents the accumulation of trace elements over time. Simultaneous examination of hair samples and cosmetic goods gives useful information for assessing potential exposure pathways and health hazards (Kempson&Lombi, 2011).

Despite increased international interest in heavy metal pollution in cosmetics, data from Yemen are severely limited. No previous study has measured Pb and Cd levels in both human hair and commercially available natural and industrial hair dyes in impoverished nations, including Aden. As a result, the purpose of this study was to use Flame Atomic Absorption Spectroscopy (FAAS) to determine Pb and Cd concentrations and compare contamination levels between natural hair, dyed hair, natural dyes, and industrial dyes, thereby providing baseline data for cosmetic safety assessment and environmental health monitoring in Yemen.

MATERIALS AND METHODS:

Study design and area: A comprehensive experimental cross-sectional study design- Aden–Yemen.

Study population: 7 females residing in Aden aged 24-60 years participated in this study; three of them used natural dyes and were enrolled in group 1, whereas four used synthetic dye and were enrolled in group 2. Those groups were selected to provide a representation of different age brackets and hair care habits among local residents. The study categorized participants strictly by their hair status (natural vs. dyed) and focused on a specific age range to maintain demographic consistency.

Sampling:

Hair Sample Collection: Human Hair Sampling: Seven freshly hair samples were collected from the back of the scalp (near the neck) using stainless-steel scissors that were thoroughly rinsed with ethanol to prevent cross-contamination. Participants were categorized into two groups: those with natural hair Group 1 (n = 3) and those with dyed hair Group 2 (n = 4).

Hair Dye Sample Collection: Hair Dye Sampling: Six distinct brands were purchased from local markets in Aden. These included three industrial dyes (MODA, Katrina, Galaxy) and three natural/herbal dyes (Black Henna, Hor, Black stone).

Hair Sample Digestion:

Procedure: Hair samples were cut into 0.3 cm pieces using ethanol-rinsed stainless-steel scissors. A 0.1063 g portion of each sample was weighed into a 50 mL beaker. Then, 8 mL of concentrated

HNO_3 (69–70.5%) was added. The mixture was heated on a hot plate at 70–85°C for 25 minutes until complete digestion. After cooling, 1 mL of 30% H_2O_2 was added and gently heated at 42°C until bubbling stopped, then heated to 80°C until the volume reduced to 2.5mL. The solution was transferred to a 100 mL volumetric flask and diluted to volume with deionized water. If cloudy, it was filtered using Whatman No. 1 and No. 40 filter paper. Solutions were stored in a refrigerator until analysis.

Hair Dye Sample Preparation: Hair samples were digested using a 4:1 mixture of concentrated HNO_3 and HCl (37%) on a hot plate under a fume hood for 2–3 hours until near dryness. If a brown/black color appeared, the digestion was repeated. After cooling, solutions were filtered using Whatman No. 42 filter paper into a 20 mL volumetric flask and diluted to volume with distilled water.

Atomic Absorption Spectroscopy (AAS): FAAS and the Buck Scientific model ACCUSYS 211 were used to analyses every sample. Using the same acid matrix as the samples, fresh calibration standards (1–20 ppb) were made from stock solutions. The absorbance was measured at wavelengths of 283.3 nm for Pb and 228.8 nm for Cd using a blank and at least four calibration standards.

Ethical Approval: Research activities were conducted following the principles of the Helsinki Declaration. All participants provided informed consent before sample collection, and data gathered through questionnaires were handled with confidentiality.

Statistical analysis: All data were processed and analyzed using SPSS software. A descriptive statistic for the trace elements (Pb and Cd) in the hair sample was done and represented in numerical and graphic format in addition of comparison of the concentration of Pb and Cd in group 1 and 2 of the sample. Calculation of the Mean, Standard Deviation (SD), and range (Minimum/Maximum) for Pb and Cd concentrations. Pearson's correlation was performed to find the association between Pb and Cd concentrations.

Methodology for Hazard Quotient HQ Calculation

Based on the equations and reference values provided in the sources (e.g., Mohammed et al., 2023 and Buzid& Burke, 2026), here is a Table (1) illustrating the calculation methodology and the assessment of your samples from Aden.

The non-carcinogenic risk is determined by calculating the Average Daily Dose ADD or Chronic Daily Intake (CDI), which is then compared against the Reference Dose RfD.

Table 1. Exposure Parameters and HQ Formulas

Parameter	Symbol	Unit	Value / Source
Metal Concentration	*C*	mg/kg	Sample results from FAAS
Ingestion Rate	*IR*	mg/day	24 (for lipsticks/dyes)
Exposure Frequency	*EF*	days/year	365
Exposure Duration	*ED*	years	30
Body Weight	*BW*	kg	60

Averaging Time	*AT*	days	*ED* x 365
RfD for Pb	RFD_{Pb}	mg/kg/day	0.0035 – 0.004
RfD for Cd	RFD_{Cd}	mg/kg/day	0.001

RfD= Reference Dose

The potential non-carcinogenic health risks associated with heavy metal exposure were quantified using the standard risk assessment framework established by the U.S. Environmental Protection Agency (USEPA, 1989). The ADD for ingestion and dermal contact was calculated using Equation (1), while the HQ, representing the ratio of exposure to the oral reference dose (RfD), was determined following the methodology described by Alam et al. (2019) and Buzid and Burke (2026)

Fundamental Formulas:

$$\text{Average Daily Dose} = (C \times IR \times EF \times ED) / (BW \times AT) \;\ldots\ldots (1)$$

$$HQ = \boldsymbol{ADD / RFD}$$

RESULTS:

Data analysis was performed for the two elements Pb and Cd to different types of samples.

1. **Descriptive Statistics:**

Table 2 represents the grouping of hair sample according to the type of dye in addition the age of the participants. The overall mean concentrations of Pb and Cd are relatively low, but the high standard deviations indicate significant variability across samples.

Table 2**: Descriptive statistics of the study sample**

Hair Sample	Age	Type of dye
1	25	**Natural**
2	32	
3	58	
4	55	
5	48	**Synthetic**
6	34	
7	24	

The mean concentration of Pb and Cd was relatively low Table 3.

Table 3: Descriptive Statistics of Pb and Cd

Variable	Mean	Std. Deviation
Pb µg/g	0.681	1.003
Cd µg/g	0.272	0.422

Pb lead; cadmium **Cd; µg/g=**microgram per/gram

Means Plots:

The means plots visually represent the mean concentrations of Pb with each sample type.

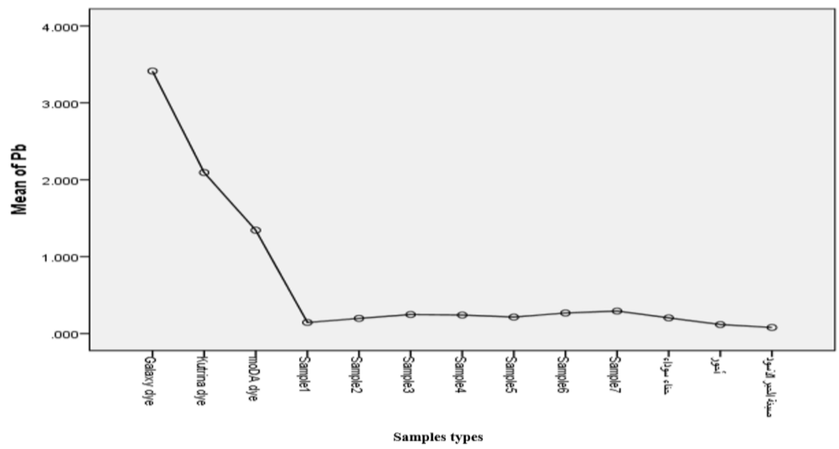

Figure 1: Pb Means Plot

Pb Means Plot: The plot shows a clear trend, with Galaxy dye having the highest mean Pb concentration, followed by Kutrina dye and moDA dye. Cd Means Plot: Similarly, Galaxydye has the highest mean Cd concentration, while other samples have much lower concentrations.

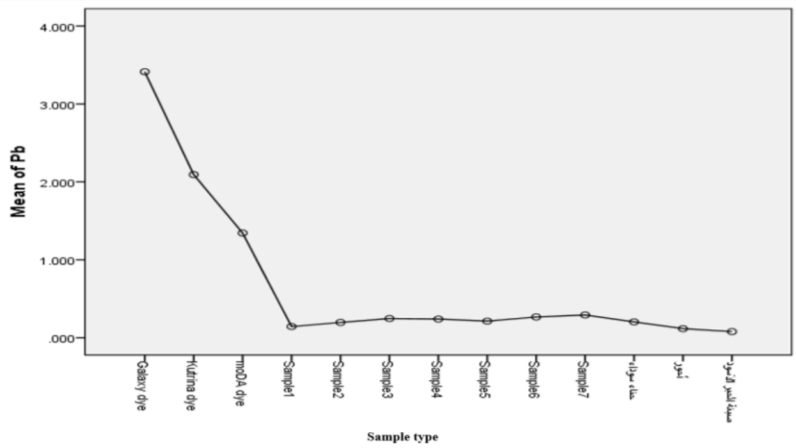

Figure 2: Cd Means Plot

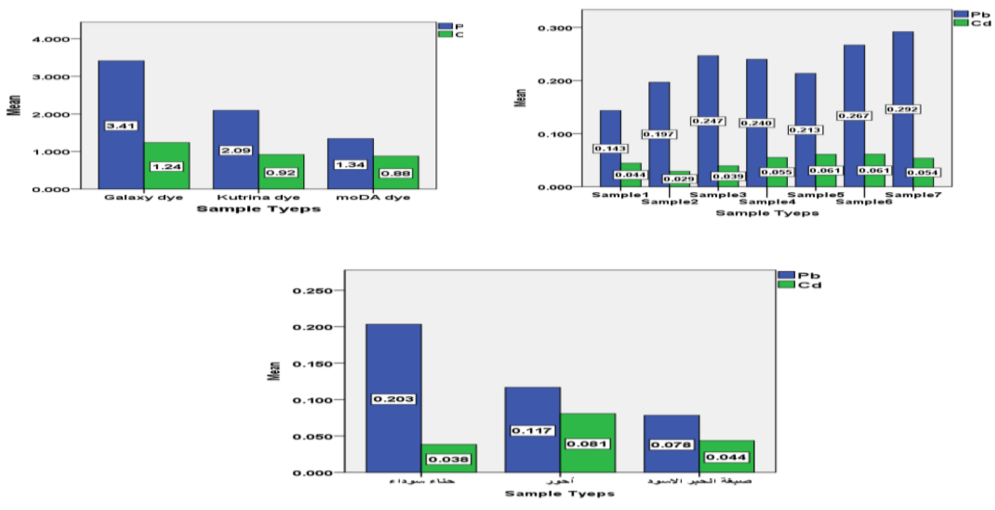

Figure 3: Distribution of Pb and Cd concentrations across sample types

The Galaxy dye is an outlier in terms of Pb and Cd concentrations. The plots also highlight the variability between sample types, with some samples having very low concentrations of Pb and Cd. Table 4, 5 and 6 provide descriptive statistics for different types of dyes and synthetic and natural dyes. Galaxy dye has a mean Pb concentration of 3.413 and Cd concentration of 1.238. The Galaxy dye has the highest mean concentrations of both Pb and Cd, indicating it may contain higher levels of these heavy metals compared to other samples. While the black stone dye sample has the lowest mean Pb concentration (0.078). Sample 2 has the lowest mean Cd concentration (0.029).

Table 4: Synthetic dyes

Dye commercial name	Characteristics	**Pb**µg/g	P. value	**Cd** µg/g	P. value.
1. Galaxy dye	Mean	3.413	**0.720**	1.238	**0.675**
	Std. Deviation	0.217		0.246	
	Minimum	3.180		1.020	
	Maximum	3.610		1.504	
2. Kutrina dye	Mean	2.093	**0.778**	0.919	**0.474**
	Std. Deviation	0.795		0.098	
	Minimum	1.250		0.810	
	Maximum	2.830		0.998	
3. moDA dye	Mean	1.343	**0.510**	0.878	**0.619**
	Std. Deviation	0.227		0.020	
	Minimum	1.090		0.860	
	Maximum	1.530		0.900	

Pb lead; cadmium **Cd; µg/g=**microgram per/gram

Natural dyes generally showed substantially lower heavy metal levels compared to synthetic alternatives Table 5.

Table 5: Natural dyes

Dye commercial name	Characteristics	**Pb** µg/g	P. value	**Cd** µg/g	P. value
1. Black Henna	Mean	0.203	**0.637**	0.038	**0.027**
	Std. Deviation	0.015		0.028	
	Minimum	0.190		0.006	
	Maximum	0.220		0.055	
2. Hour	Mean	0.117	**0.029**	0.081	**0.339**
	Std. Deviation	0.033		0.009	
	Minimum	0.097		0.071	
	Maximum	0.155		0.087	
3. Black stone dye	Mean	0.078	**0.637**	0.044	**0.510**
	Std. Deviation	0.008		0.006	
	Minimum	0.070		0.039	
	Maximum	0.085		0.050	

Pb lead; cadmium **Cd; µg/g=**microgram per/gram

Participant hair samples (Samples 1-7) displayed consistent baseline levels of heavy metals Table 6.

Table 6: Samples of participants hair

Hair Sample	Characteristics	**Pb** µg/g	P. value	**Cd** µg/g	P. value
1	Mean	0.143	**0.253**	0.044	**0.328**
	Std. Deviation	0.038		0.012	
	Minimum	0.100		0.031	
	Maximum	0.170		0.053	
2	Mean	0.197	**0.637**	0.029	**1.000**

	Std. Deviation	0.031		0.001	
	Minimum	0.170		0.028	
	Maximum	0.230		0.030	
3	Mean	0.247	**0.900**	0.039	**0.780**
	Std. Deviation	0.055		0.010	
	Minimum	0.190		0.030	
	Maximum	0.300		0.050	
4	Mean	0.240	**1.000**	0.055	**1.000**
	Std. Deviation	0.010		0.011	
	Minimum	0.230		0.044	
	Maximum	0.250		0.066	
5	Mean	0.213	**0.194**	0.061	**1.000**
	Std. Deviation	0.049		0.001	
	Minimum	0.180		0.060	
	Maximum	0.270		0.062	
6	Mean	0.267	**0.328**	0.061	**0.328**
	Std. Deviation	0.059		0.006	
	Minimum	0.200		0.057	
	Maximum	0.310		0.068	
7	Mean	0.292	**0.780**	0.054	**0.089**
	Std. Deviation	0.013		0.011	
	Minimum	0.280		0.047	
	Maximum	0.305		0.066	

Pb lead; cadmium **Cd; µg/g=**microgram per/gram

2. Association analysis:

Pearson's correlation analysis was used to measure the extent of the association between each of Pb, Cd and sample type which represented in Table 7.

There was a negative correlation between Pb, Cd and Sample Types (-0.704, Sig. = <0.0001) and (-0.720, Sig. = <0.0001) respectively suggesting that certain sample types are associated with lower Pb and Cd concentrations. The strong positive correlation between Pb and Cd suggests that these heavy metals may co-occur in the samples and the negative correlations with sample types indicate that some samples are consistently lower in Pb and Cd.

Table 7: correlation analysis between Pb, Cd and sample type

Variable		P. value	Sample Type
Pb µg/g	**Pearson Correlation**	0.949**	-0.704-**
	Sig. (2-tailed)	<0.0001	<0.0001
Cd µg/g	**Pearson Correlation**	0.949**	-0.720-**
	Sig. (2-tailed)	<0.0001	<0.0001

Correlation is significant at the 0.01 level (2-tailed). **Pb lead; cadmium **Cd; µg/g=**microgram per/gram

3. Analysis of variance (ANOVA):

Analysis of variance ANOVA Table 8 was used to compare the differences between the means concentration of Pb and Cd. There is statistically significant increase **P. value=** <0.0001 in Pb and Cd concentrations across the sample types. This suggests that the type of sample significantly affects the concentration of Pb and Cd.

Table 8: Comparison between mean concentration of Pb and Cd

Variable		Sum of Squares		df	Mean Square	F	P. value
		Between Groups	Within Groups				
Pb µg/g		36.773	1.489	12	3.064	53.507	<0.0001
	Total	38.262		26	0.057		
Cd µg/g		6.633	0.143	12	0.553	100.280	<0.0001
	Total	6.776		26	0.006		

Pb lead; cadmium **Cd; µg/g=**microgram per/gram

DISCUSSION:

Analytical Quantification of Metal Burdens identified synthetic formulations specifically Gaسlaxy dye as critical toxicological outliers, recording maximal mean concentrations for Pb and Cd respectively compared to the natural herbal alternatives such as Black Stone confirming a distinct safety dichotomy between industrial synthetics and natural products. However, samples 4-7 of human hair exhibited elevated metal burdens, with Pb in dyed samples compared to untreated controls. Pearson correlation between Pb and Cd concentrations indicates simultaneous co-occurrence, likely stemming from shared pigment precursors or contaminated raw materials during manufacturing. Furthermore, One-way ANOVA validation confirmed that sample matrix typology (natural versus synthetic) acts as a primary statistical determinant of heavy metal concentration. The findings highlight a deteriorating regional profile. While observed Pb spikes are lower than extreme anomalies documented in Moroccan henna (Benabbes et al., 2021), they align with Nigerian market metrics (Ojezele et al., 2026) and mirror prior local contamination trends identified in Aden lipsticks (Fadhel et al., 2025), skin-whitening cosmetics (Saleh et al., 2016; Saleh et al., 2020), and shampoos (Taher et al., 2026). Additionally, the utilization of human hair as a stable retrospective biomarker corroborates international frameworks (Liang et al., 2017), whereas the proliferation of substandard formulations aligns with conflict-zone dynamics observed in Iraq (Mohammed et al., 2023) and contrasts with controlled baselines in Saudi Arabia (Alqahtani et al., 2024; Buzid& Burke, 2026).

Health Risk Characterization: CalculatedHQ values across all tested commercial products remained below the unity threshold, indicating a negligible immediate non-carcinogenic health hazard under standard exposure scenarios (Table 9). Nevertheless, the high bio accumulative capacity of human hair supports concerns regarding chronic, low-level internal metal deposition (Taher et al. 2026). These findings indicate considerable product-to-product and geographical variability and emphasize the importance of local surveillance rather than assuming equivalent contamination levels across cosmetic markets.

Table 9. Hazard Quotient (HQ) Assessment for Lead (Pb) and Cadmium (Cd)

Sample ID	Type	Mean Pb (µg/g)	HQ (Pb)	Mean Cd (µg/g)	HQ (Cd)
Galaxy Dye	Synthetic	3.413	~0.00034	1.23800	~0.00049
Kutrina Dye	Synthetic	2.093	~0.00021	0.91933	~0.00037
moDA Dye	Synthetic	1.343	~0.00013	0.87767	~0.00035
Black Henna	Natural	0.203	~0.00002	0.03833	~0.00002
Black Stone	Natural	0.078	~0.00001	0.04367	~0.00002
Sample 7	Dyed Hair	0.292	~0.00003	0.05367	~0.00002
Sample 2	Natural Hair	0.197	~0.00002	0.02900	~0.00001

Pb lead; cadmium **Cd; µg/g=**microgram per/gram; **HQ=**Hazard Quotient< **1** Consider safe

CONCLUSION:

The findings showed distinct variations in Pb and Cd concentrations between the examined samples, regardless of whether the colors were synthetic or natural. The results showed that compared to hair dyed with natural dyes or left undyed, hair dyed with synthetic dyes had higher concentrations of heavy metals like Pb and Cd. In comparison to other samples, the Galaxy dye had the greatest mean values of Pb (3.413ppm) and Cd (1.238pm), indicating higher amounts of heavy metals. On the other hand, Sample 2 had the lowest Cd concentration, while Black stone dye and the Hor plant had the lowest Pb concentrations.

RECOMMENDATIONS:

- Enhancing Public Health Awareness: Educating consumers on the toxicological hazards associated with heavy-metal-containing hair dyes, specifically concerning potential nephrotoxic and neurotoxic impacts.
- Advocating Botanical Formulations: Encouraging the utilization of plant- and henna-based natural hair colouring alternatives to minimize chemical exposure.
- Occupational Biomonitoring: Implementing routine hair analysis protocols for high-risk cohorts, including salon professionals and chemical industry workers, to track heavy metal bioaccumulation.
- Longitudinal Investigation: Advancing comprehensive research initiatives to elucidate the long-term systemic health consequences of chronic heavy metal retention in hair matrices.

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Research Highlights

1. Precise FAAS quantification of Lead and Cadmium levels in Aden’s hair and dye market
2. Identification of Galaxy dye as a toxicological outlier with peak heavy metal concentrations
3. Validation of natural alternatives (Black Stone, Hor) as safer, low-metal options
4. Strong correlation (r = 0.949) confirms Lead and Cadmium coexist as simultaneous manufacturing impurities
5. Establishment of human hair as an effective non-invasive biomarker for long-term toxicological surveillance