Comparison of Trace Elements between Dyed and Non-Dyed Head Hair Samples among Females from Different Ethnic Groups in Malaysia using ICP-OES

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Hair dye is a commonly used cosmetic product applied directly on the scalp which exposes the users to possible harmful elemental dye components, via absorption especially through skin pores to body organs. Essential elements such as Zn, Cu and Fe play significant roles in proper human development while non - essential elements for example Cd and Pb are harmful even at low concentrations. The objective of this study is to investigate the presence and the concentration of heavy metals such as iron (Fe), zinc (Zn), cadmium (Cd), copper (Cu) and lead (Pb) in dyed and non-dyed hair samples among females from different ethnic groups (Malay, Chinese and Indian) in Malaysia using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The sample group used for this research compromise of 90 female individuals (45 with dyed hair and 45 with non-dyed hair). The identification of heavy metals in the hair samples was analysed by using microwave digestion method with ICP-OES. The analysis of calibration curve for each heavy metal showed a good linear regression with coefficient of determination (R^2) of 0.999 with a low limits of detection (LOD) and quantification (LOQ). The results indicate that the raised levels of heavy metals in dyed hair may be attributed to hair dye use while high levels in non-dyed hair highlights environmental exposure, largely caused by industrialization and urbanization. The statistical findings also indicate that both hair dyeing and ethnic background have significant effects on the buildup of heavy metals in hair ($p \leq 0.05$). There is no globally standardised "normal" range for heavy metals in hair due to large variability influenced by geography, age, sex, ethnicity, hair treatments and analytical methods. Therefore, the comparisons should ideally be with population data of similar geographical location, ethnicity, gender, lifestyle and working environment to get more comprehensive results on the presence of heavy metals in hair samples.

Keywords: Dyed hair; non-dyed hair; female; heavy metals; ICP-OES

INTRODUCTION

The use of hair dyes is common in Asian counties, as it allows consumers to either change hair color or cover gray hair in aging population. Hair dye products also serve as an external source of heavy metal which are mainly introduced as mordanting agents [1]. Hair dyes are common cosmetic products, and their ingredients should not be harmful to human health [2]. Most commercial hair dyes products have dozens of ingredients, which vary considerably from one brand to another. In general, hair dyes contain colorants, modifiers, antioxidants, alkalizers, soaps, ammonia, wetting agents, scents, and a mix of other chemicals in small quantities to pass special qualities to the hair such as softening texture and most of these substances contain heavy metals [3].

Metals such as iron (Fe), copper (Cu), and zinc (Zn) are essential elements and play an important role in biological systems. The needful amounts of essential elements are needed for proper human health, but these elements can be harmful in extravagant [4]. Cu is necessary for normal metabolic functions at optimal concentrations, but it can cause oxidative stress in immoderate [5]. Metals such as lead (Pb), cadmium (Cd) and arsenic (As) are not needed for the body's metabolism and even small amounts are harmful to humans [6]. Lead is a highly hazardous metal which can cause headaches, abdominal pain, memory loss, kidney failure, male reproductive problems, and weakness, pain, or tingling in the extremities [7]. Cadmium has the ability to accumulate and damage liver and kidneys [8] and exposure to the cadmium also can affect zinc (Zn), iron (Fe), and copper metabolism [9].

Human biomonitoring (HBM), used for the measurement of specific chemicals or their metabolites in human tissues, has received a great deal of attention over the last few decades [10]. In addition, heavy metal concentrations in hair depend on several factors such as gender, hair colour, eating habits, age and lifestyle [11]. The United States Environmental Protection Agency (USEPA) identified human hair as one of the tissues used for biological monitoring of the toxic metals and for determining toxic metal exposure [12]. The main objectives of this research are to determine the levels of selected elements in human hair samples collected from females of different ethnic groups in the state of Selangor, Malaysia using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), and to evaluate the effects of hair dyeing on the levels of elements.

MATERIALS AND METHODS

Chemicals and Materials

Chemicals and materials are essential for the precise and efficient preparation of hair samples for trace element analysis. Ethanol and deionised water were employed for washing procedures. Concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were utilised for the digestion of hair samples, while deionised water was employed to maintain the purity of solutions for dilution and equipment rinses. After the digestion process, the solution was filtered using filter paper to remove any residual particulate matter from the sample to enhanced accuracy and reliability in the measurement of trace elements. A 5 mL micropipette was employed for precise liquid measurements, and all hair sample solutions were stored in 30 mL medication bottles. These processes utilised various sterilised flasks and tubes, including conical flasks, 100 mL graded volumetric flasks, and 50 mL and 15 mL centrifuge tubes.

Collection of hair samples and storage

Hair samples were collected from 90 females (45 with dyed hair and 45 with non-dyed hair) from different ethnic groups in the Selangor state of Malaysia. Hair samples were taken by normal cutting and sealed immediately in clean and numbered plastic bags after collection and kept in a clean dry location to avoid any external contamination. Then they were kept in the laboratory until further analysis.

Washing procedure

After the collection, the hair was washed to remove any contamination and external pollutants from the surface, ensuring a true level of endogenous metals. The hair was washed three times with acetone ($(CH_3)_2CO$), then rinsed three times with deionised water to obtain a clean sample. Although several types of washing procedures exist, this method has been proven successful in removing external trace elements while preserving internal trace elements. This method was accepted by the International Atomic Energy Agency. After rinsing, the hair was left to dry to a stable weight [13].

Acid digestion procedure

0.5 g of dried hair samples were placed in a digestion vessel. Subsequently, 8 mL of nitric acid (HNO_3) was combined with 3 mL of hydrogen peroxide (H_2O_2) to produce a clear solution. The vessels were subsequently subjected to ETHOS EASY microwave digestion using this clear mixture and the process lasted approximately 47 minutes including cooling. After digestion, the solutions were filtered and diluted to the mark with deionized water in a 100 mL measuring flask. The hair solutions were preserved in the labelled bottles [14].

Instrument

The analysis of hair was performed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), PerkinElmer, Optima 2100 DV. The plasma gas flow rate used was tuned to 15 L/min, giving sufficient energy for the plasma to ionize the sample elements. Plasma stabilization was supported by adjusting the

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auxiliary gas flow rate to 0.2 L/min. To ensure effective aerosolization of the sample solution, the nebulizer gas flow rate was set at 0.80 L/min. In order to provide sufficient energy for the excitation and emission of the elements, the radiofrequency power was kept at 1300 watts. The sample's introduction into the plasma was regulated by setting the pump flow rate to 1.50 mL/min for more reliable analysis and to ensure the precise and reliable identification of trace components in both dyed and undyed hair samples, these parameters were optimised. Each of the samples and five standard trace elements were replicated three times. The intensity of light emitted were plotted against the known concentrations of the standards to create calibration curve of each of the trace elements.

Standard Preparation

The calibration curve is established by quantifying the intensity of the standard metal solution for calibration purposes. The standard metal solution is utilised for calibration purposes. It is crucial to verify the detection limit before establishing the standard curve. This research developed five standards, specifically zinc (Zn), iron (Fe), cadmium (Cd), copper (Cu), and lead (Pb). The starting stock solution of 1000 ppm (equivalent to 1000 mg/L) underwent dilution to yield five distinct concentrations; 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. To prepare the standard solution, 5 mL was extracted from each of the 1000 ppm stock solutions of the elements and transferred into a 50 mL volumetric flask. Subsequently, 45 ml of deionised water was added to achieve a concentration of 100 ppm for the standard solution. 5 mL of the 100-ppm solution was measured and transferred into a 50 mL volumetric flask, then diluted with deionised water up to the mark to achieve a 10-ppm standard solution. The dilution process were proceeded from the 10-ppm stock to achieve concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. Finally, a calibration curve was plotted to quantify the amount of heavy metal elements present in dyed and non-dyed hair samples.

Limit of detection (LOD) is the lowest concentration of the analyte that can be distinguished from the background noise. In this study, the method was validated by using Signal-to-Noise (S/N) ratio technique. In chromatography, the LOD is the injected amount that results in a peak with a height at least three times higher than the baseline noise level (S/N: 3/1). Limit of quantification (LOQ) is the lowest concentration of an analyte in the sample that can be determined with acceptable precision and accuracy under stated conditions of test. Both LOD and LOQ are two basic elements of method validation that define the limitations of an analytical method. LOQ was determined based on the standard concentration of heavy metals with a peak of ten times higher than the baseline noise level (S/N: 10/1).

Statistical Analysis

Data were statistically analysed using a multivariate analysis of variance (MANOVA) to evaluate the significant differences of hair type (dyed and non-dyed) and ethnicity (Malay, Chinese, Indian) on the levels of Zn, Fe, Cd, Cu and Pb in female hair samples. The significance threshold was set at $p < 0.05$. Mean values and standard deviations (±SD) were calculated for each trace element. Statistical analyses were performed using XLSTAT 2025 and findings were presented in graphical formats for clarity.

RESULTS AND DISCUSSION

ICP-OES Analysis of Standard Trace Elements: Zinc (Zn), Iron (Fe), Cadmium (Cd), Copper (Cu) and Lead (Pb)

The calibration curve for Zn element represented by the equation $y = 43549x - 822.6$. The calibration curve show strong linearity with a coefficient of determination (R^2) of 0.9997, as illustrated in Figure 1.1. For the Fe element, the linear regression equation was $y = 87779x - 30924$, with a R^2 value of 0.9951, as depicted in Figure 1.2. The calibration curve for the Cu element, illustrated in Figure 1.3, produced a linear regression equation of $y = 126965x – 732.98$, accompanied by a R^2 value of 0.9987. Figure 1.4 indicates that the linear regression for the Cd element represented by the equation $y = 81903x – 3613.3$, exhibiting a R^2 value of 0.9996. In contrast, the linear regression for the Pb element, as shown in Figure 1.5, is given by $y = 8976.9x + 544.8$, with a R^2 value of 0.9985. Overall, the calibration curves for each element achieved good linearity with R^2 values close to 1 to

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ensure accurate quantification of all five elements, together with their respective Limit of Detection (LOD) and Limit of Quantification (LOQ) as shown in Table 1.

Table 1: Calibration curve details, LOD, and LOQ for hair analysis

Trace elements	Slope	Intercept	Coefficient of Determination (R^2)	LOD (ppm)	LOQ (ppm)
Zn	43580	-1028.0	0.9997	0.00136	0.00412
Fe	87780	-30939.7	0.9951	0.00049	0.00148
Cd	81860	-3309.0	0.9987	0.00132	0.00400
Cu	128400	-10910.2	0.9996	0.01523	0.04616
Pb	9145	-690.1	0.9985	0.01023	0.03101

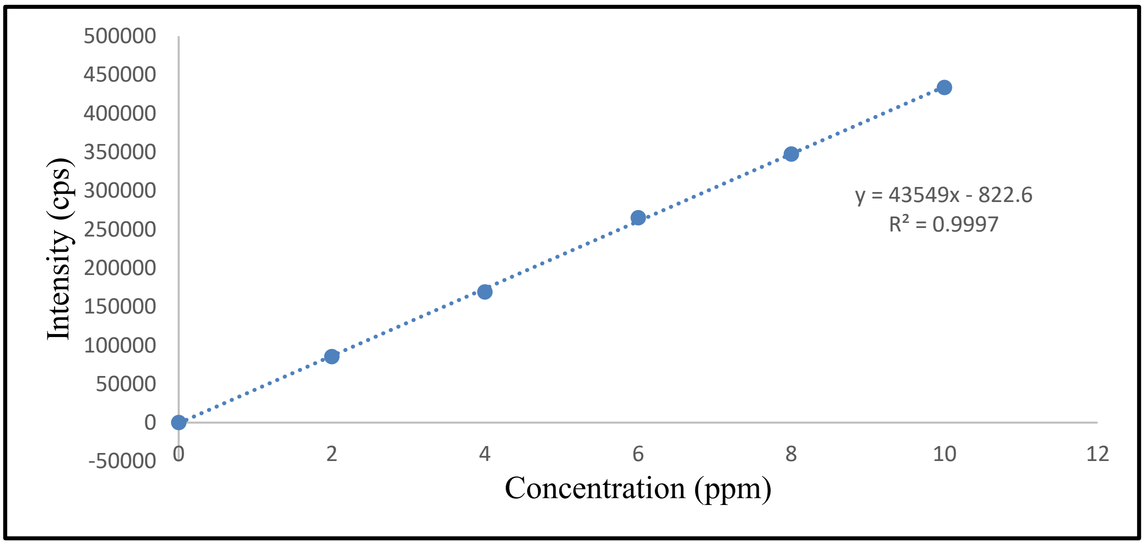

Figure 1.1: Calibration Curve of Zn standard

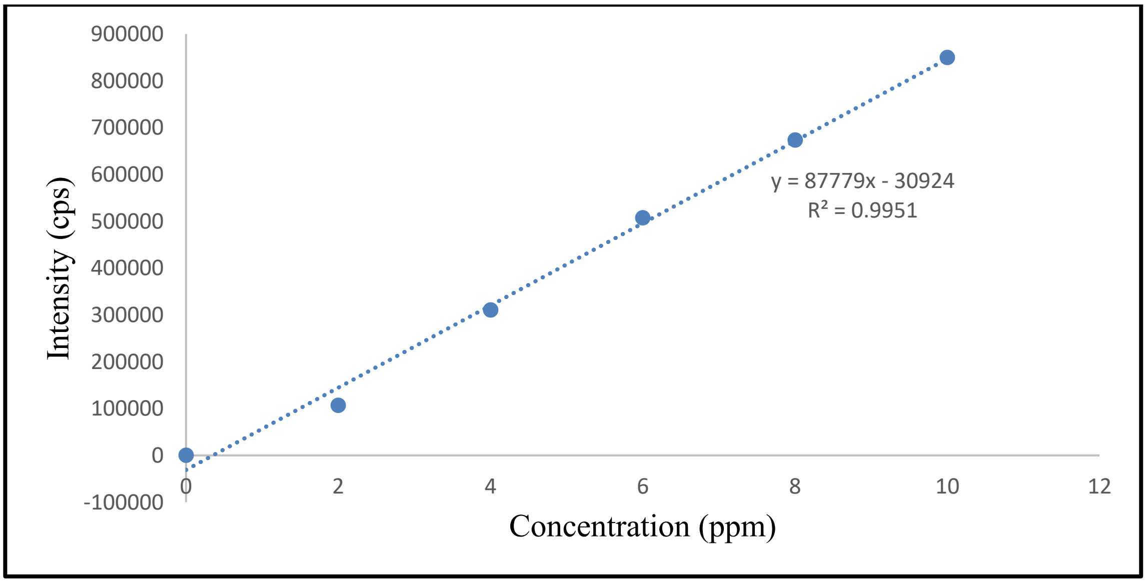

Figure 1.2: Calibration Curve of Fe standard

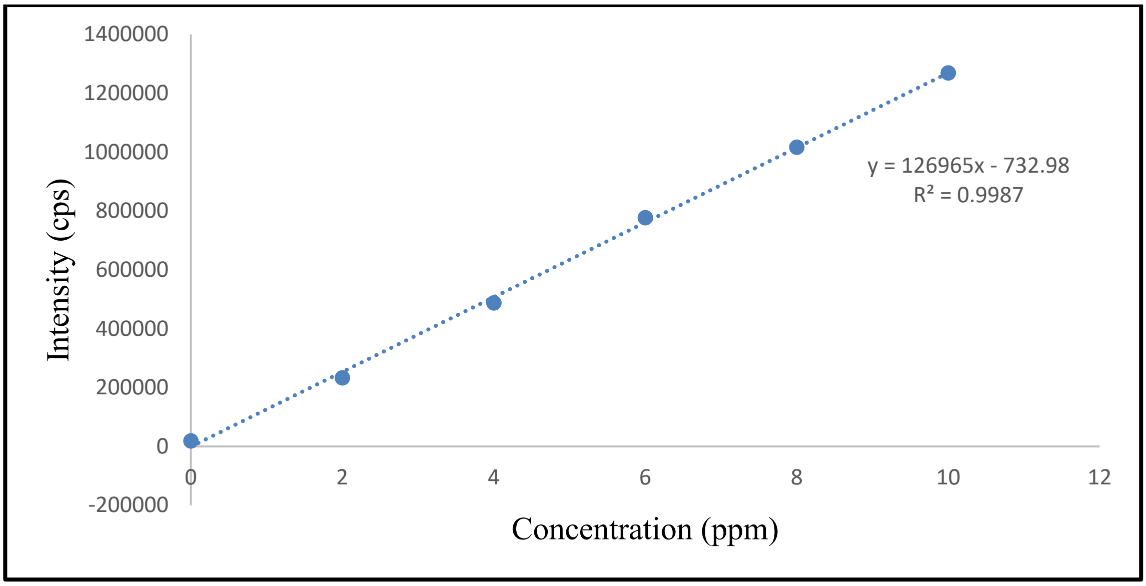

Figure 1.3: Calibration Curve of Cu standard

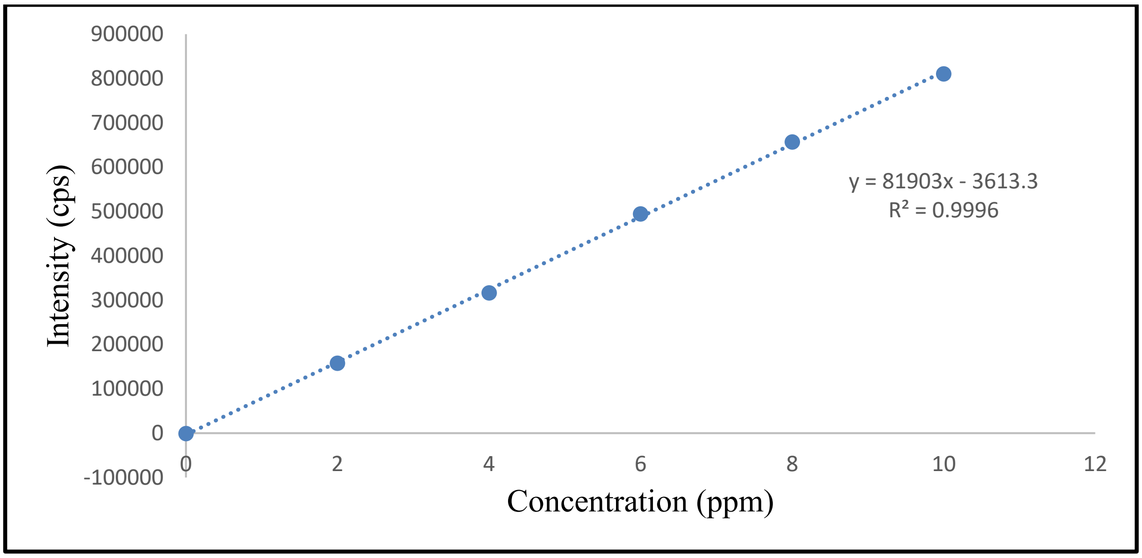

Figure 1.4: Calibration Curve of Cd standard

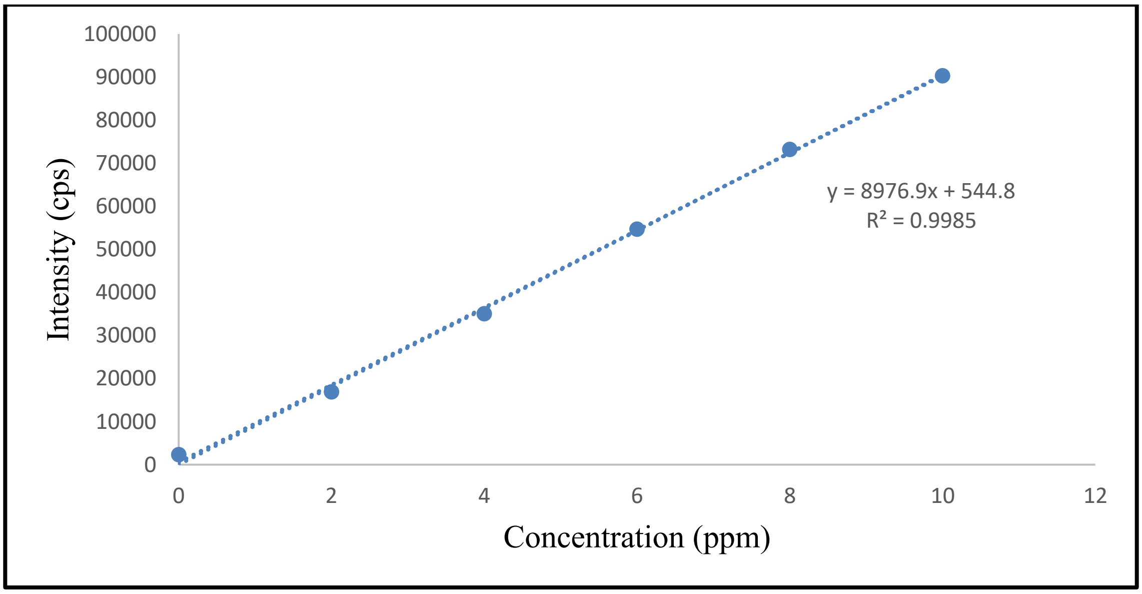

Figure 1.5: Calibration Curve of Pb standard

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Heavy metal presence in hair samples (dyed and non-dyed) among different ethnic female populations (Malay, Chinese, Indian)

Table 2 shows that Zn had the widest range of concentrations (0.335 - 6.174 ppm) with highest mean concentration (1.777 ppm) and highest variability (SD = 1.153) among all five trace elements. Fe concentrations with a narrower range (0.523 to 1.576) showed more consistent Fe level in hair samples among all females suggesting less inter-individual variation compared to Zn. In contrast, Cd concentrations were tightly clustered (mean = 0.067 ppm, SD = 0.016), very low concentration and consistent among all females from different ethnic group. Standard deviation (0.112) value of Cu suggests low to moderate variability. The negative minimum value for Cu (-0.038) suggests possible noise measurement or instrument calibration drift. Pb concentrations were found to be elevated in both hair samples, with values ranging from 0.157 to 0.853 ppm. SD value of 0.171, reveals moderate variability between individuals and could be influenced by hair dye ingredients and environmental exposure.

Table 2: Heavy Metal Concentrations in Hair Samples (n = 90)

Element	Minimum Concentration (ppm)	Maximum Concentration (ppm)	Mean Concentration (ppm)	Standard Deviation (SD)
Zn	0.335	6.174	1.777	1.153
Fe	0.523	1.576	0.856	0.233
Cd	0.039	0.112	0.067	0.016
Cu	-0.038	0.664	0.076	0.112
Pb	0.157	0.853	0.512	0.171

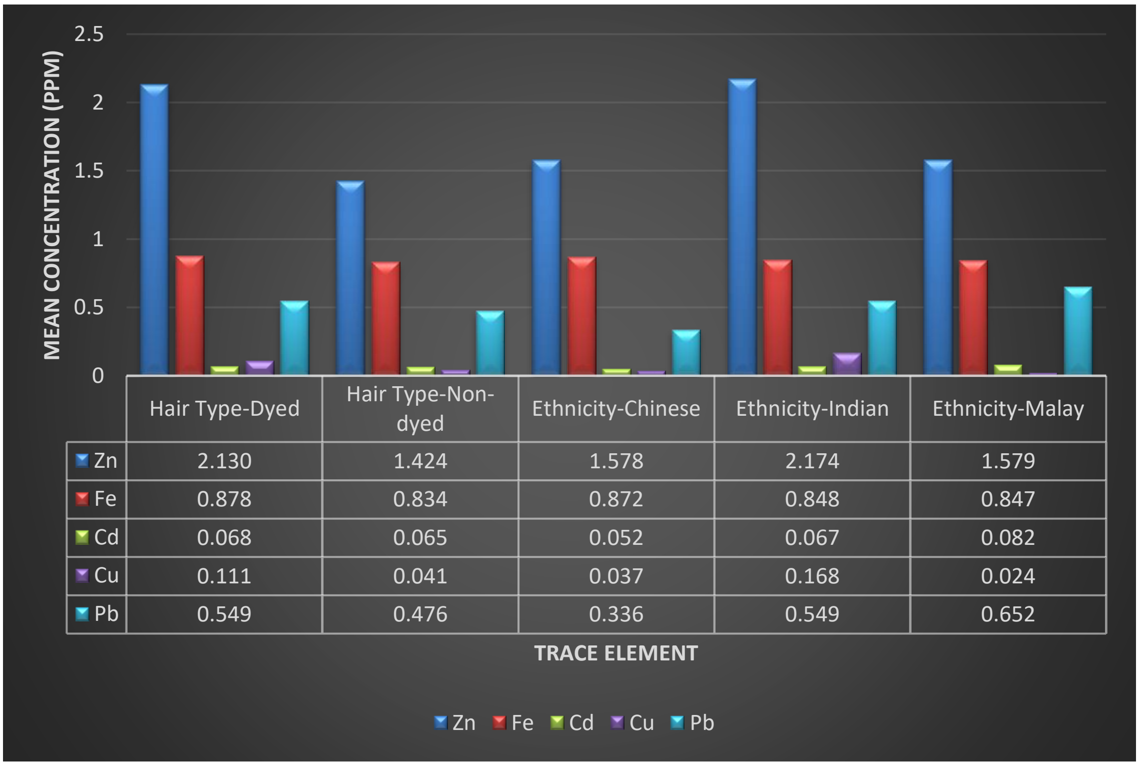

Figure 2: Dual presentation highlights both the effect of hair dyeing on heavy metal content and potential ethnic variations in heavy metal levels. (1) Comparison of heavy metal concentrations between dyed and non-dyed

hair, combining all ethnic groups to emphasize the effect of hair dyeing. (2) Combined heavy metal levels (dyed and non-dyed) within each ethnic group, demonstrating potential ethnic variations in heavy metal accumulation.

The first 2 columns in Figure 2 compares the concentrations of the heavy metals between dyed and non-dyed hair, combining all ethnic groups to show overall differences related to hair dyeing. The last 3 columns in Figure 2 represents the combined heavy metal levels (including both dyed and non-dyed samples) within each ethnic group, allowing for a comparison of metal accumulation patterns across ethnicities. The mean concentration of zinc was elevated in dyed hair samples across all ethnic groups. Indian females had the highest mean level (2.174 ppm), while Malay females and Chinese females have a very small difference (0.001 ppm), revealing that zinc accumulation in hair is nearly identical between Malay and Chinese female participants. This also suggests the individual differences in zinc accumulation in hair possibly due to personal use of hair dyes, dietary intake and environmental exposure. While for iron (Fe), the mean concentrations disclosed a modest increase in dyed hair samples (mean = 0.878 ppm) compared to non-dyed hair samples (mean = 0.834 ppm) which hinted that dyeing may not significantly affect the amount of Fe metal in hair. When study by ethnicity, Chinese females showcase the slightly higher mean concentration (0.872 ppm), while Malay (0.847 ppm) and Indian females (0.848 ppm) had nearly identical levels.

Analysis of Cd levels exhibited a slightly increased mean concentration in dyed hair samples (0.068 ppm) compared to non-dyed samples (0.065 ppm), point to that the effect of dyeing on Cd accumulation is insufficient. When comparing across ethnic groups, Malay females showed the highest Cd levels with a mean of 0.082 ppm, followed by Indian females (0.067 ppm) and Chinese females (0.052 ppm). The mean concentration of Cu was excessive in dyed hair samples (0.111 ppm) compared to non-dyed samples (0.041 ppm), indicating that hair dyeing may contribute to increased Cu accumulation in hair. Ethnic group comparisons revealed the lowest Cu concentration in Malay females (0.024 ppm), followed by Chinese females (0.037 ppm), with Indian females exhibiting the highest levels (0.168 ppm). Figure 2 also revealed that dyed hair samples exhibited a higher mean Pb concentration (0.549 ppm) compared to non-dyed hair samples (0.476 ppm), suggesting that hair dyeing may contribute to increased lead accumulation. Among the ethnic groups, Malay females exhibited the highest Pb levels (0.652 ppm), followed by Indian females (0.549 ppm) and Chinese females (0.336 ppm).

A notable difference was identified especially with Pb, Cu and Cd in the levels of heavy metals in the hair samples from different ethnic groups possibly influenced by environmental exposure, lifestyle, dietary, hair product composition or hair care factors may lead to exposure to detrimental trace metals. Conversely, hair samples that have not been dyed generally exhibit lower concentrations of these metals, with zinc and iron levels showing relative similarity between the two ethnic groups.

Statistical analysis

A multivariate analysis of variance (MANOVA) was conducted to examine the effects of hair type (dyed vs non-dyed) and ethnicity (Malay, Chinese, Indian) on the levels of Zn, Fe, Cd, Cu, and Pb in female hair samples. Statistical analysis revealed that there is a statistically significant multivariate effect of hair dyeing on the levels of heavy metals. Hair dyeing significantly alters the concentrations of Zn, Fe, Cd, Cu, and Pb in hair. The concentration of heavy metals in hair vary significantly by ethnicity. Different ethnic groups show different metal profiles. The results revealed a significant effect of hair type (Wilks' $\Lambda = 0.758$, $F(5, 82) = 5.233$, $p = 0.000$) and ethnicity (Wilks' $\Lambda = 0.134$, $F(10, 164) = 28.347$, $p < 0.0001$) as shown in Table 3. This research indicates that both hair dyeing and ethnic background have significant effects on the accumulation of heavy metals in hair.

Table 3: A two-way MANOVA analysis

Source	Wilks' Lambda	F-Observed	DF1	DF2	F-Critical	p-value
Hair Type	0.758	5.233	5	82	2.326	0.000
Ethnicity	0.134	28.347	10	164	1.889	<0.0001

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Mean concentration of heavy metals in dyed and non-dyed hair samples in comparison with Regulatory Standards

Essential elements like Zn, Fe, and Cu are vital for normal biological functions, but it can become harmful when present at levels exceeding the body's requirements [15]. In contrast, non-essential elements such as Cd and Pb, which have no known beneficial role in living organisms, are dangerous even at very low concentrations [16] and can ultimately pose significant risks to both human health and the environment [17]. Hair analysis is recognized as an important tool for monitoring heavy metals [18;19] and is regarded by the United States Environmental Protection Agency (USEPA) as one of the key biomarkers [20]. Hair dye is a widely used cosmetic product applied directly to the scalp, exposing users to potentially harmful elements in the dye, primarily through absorption via the skin pores to other body organs [21]. Women tend to dye their hair more often and more frequent than men, leading to greater lifetime exposure to toxic metals [22].

Previous studies revealed that in comparison to non-dyed hair, dyed hair consistently exhibits elevated levels of trace elements, suggesting that the dyeing procedure induce the buildup of the heavy metals [23] and the hair care product and hair dyeing can caused changes in hair construction and composition [24;25]. It has been disclosed that the oxygen content in melanin granules was increased by bleach treatment [26] and perms can reduce the levels of certain metals in hair [27]. It is proven that dyeing human hair can introduce heavy metals into hair, but the levels are different for different element and depends on the duration of the exposure [28]. In this current study, Table 4 shows that dyed hair samples from all the female ethnic groups revealed higher mean concentrations almost for all the heavy metals studied in this research except for Zn and Pb in Malay ethnic group and Cd in Chinese ethnic groups which show elevated mean concentration in non-dyed hair samples. In comparison to the acceptable ranges defined by the United States Environmental Protection Agency (USEPA) [29], World Health Organization (WHO) [30] and Singh et al. [31], it is observed that in majority, all five heavy metals exhibited concentrations exceeding the permissible limits. However, the indicated permissible limit values were established based on environmental matrices such as soil, rather than on biological matrices like blood or urine because hair is recognised as one of the most important biomarkers for assessing environmental contamination [20].

The results highlight some substantial distinctions and potential health hazards associated with trace element exposure, particularly in both dyed and non-dyed hair samples from all the female ethnic groups considerably exceeding the acceptable range for Zn, which is 0.5 ppm and Pb with permissible limit of 0.1 ppm. Exposure to high levels of Pb can lead to many health problems such as high blood pressure, kidney and brain damage, cognitive decline, cardiovascular diseases, irritability, headaches, and hallucination, which can later progress into convulsions, infertility in males, damage to the nervous system, miscarriage in pregnant women, paralysis, and even death [32]. Individuals can be susceptible to zinc toxicity due to excessive zinc supplementation [33]. Symptoms of zinc toxicity include headache, nausea, vomiting, diarrhea, abdominal discomfort and, in rare cases, can also cause metabolic imbalances and severe neurological symptoms [34]. Cd mean concentration in both dyed and non-dyed hair samples of Malay and Indian females exceeds the acceptable limit of 0.06 ppm. In contrast, for Chinese females, the mean concentration of Cd in both dyed and non-dyed hair samples remains below the permissible limit. Cd is a very toxic metal to humans and could lead to several diseases such as cardiovascular disease, lung damage, kidney disease, fragile bones and arthritis [35].

Mean concentration of Fe in dyed hair slightly exceed the acceptable range of 0.8 ppm, while those in non-dyed hair remain marginally below the threshold for Malay and Indian female ethnic groups. While for the Chinese female group the mean concentration for Fe in both dyed and non-dyed hair samples are slightly above the permissible limit. Ingestion of less than 20 mg/kg of elemental iron is non-toxic, 20 mg/kg to 60 mg/kg results in moderate symptoms and more than 60 mg/kg can result in severe toxicity and lead to severe morbidity and mortality [36]. Ingested iron can cause direct caustic injury to the gastrointestinal mucosa, resulting in nausea, vomiting, abdominal pain, and diarrhea [37]. Cu levels in both dyed and non-dyed hair are generally below the permissible limit of 0.1 ppm across all ethnic groups, expect for dyed hair of Indian females showed elevated Cu concentrations. Copper toxicity often results from intentional self-poisoning and can be life-threatening because of damage to red blood cells, the liver, and the kidneys [38;39]. Stomach and intestinal irritation, anemia, adrenal hyperactivity and insufficiency, allergies, hair loss, hypertension, and strokes are associated with exposure to high concentration of Cu. Long term exposure to Cu may lead a way to kidney and liver damage (35).

Table 4: Trace elements and their acceptable ranges.

Element	Acceptable Range (ppm)	Mean Concentration (ppm) for Malay females		Mean Concentration (ppm) for Chinese females		Mean Concentration (ppm) for Indian females	
		Dyed Hair	Non-dyed Hair	Dyed Hair	Non-dyed Hair	Dyed Hair	Non-dyed Hair
***Zn	0.5	0.8990 ± 0.0108	**2.2593 ± 0.0288**	1.7539 ± 0.9578	1.4016 ± 0.6718	2.3755 ± 1.3844	1.9716 ± 1.1449
*Fe	0.8	0.9320 ± 0.0057	0.7619 ± 0.0045	0.9253 ± 0.1966	0.8192 ± 0.3072	0.9470 ± 0.2301	0.7499 ± 0.1134
**Cd	0.06	0.0820 ± 0.0477	0.0810 ± 0.002	0.0516 ± 0.0060	**0.0529 ± 0.0151**	0.0728 ± 0.0058	0.0613 ± 0.0079
**Cu	0.1	0.0340 ± 0.0011	0.0130 ± 0.0014	0.0520 ± 0.0482	0.0223 ± 0.0445	0.2683 ± 0.1486	0.0682 ± 0.0437
**Pb	0.1	0.6480 ± 0.0131	**0.6560 ± 0.0081**	0.3972 ± 0.0783	0.2739 ± 0.0555	0.5929 ± 0.1565	0.5053 ± 0.0844

*Source from (*30, **31 & ***29)

The health concerns are raised by the fact that almost all the heavy metals in both types of hair groups exceeded the acceptable range. The necessity for additional research into the potential health implications and trace element accumulation in human hair is emphasised by this data. Rapid industrialization and urbanization have caused heavy metals to start increasing in soil, water and air pollution through improper dumping of industrial wastes on land, air and into water (29). These heavy metals in the environment can cause many environmental problems and be absorbed by crops, affecting food security and potential health risks [40].

CONCLUSION

The present study provides the concentrations of selected heavy metals in dyed and non-dyed hair samples collected from 90 females from different ethnic groups in Malaysia. The results indicate that the elevated levels of heavy metals in dyed hair may be attributed to hair dye use while high levels in non-dyed hair highlight the environmental exposure, largely caused by industrialization and urbanization. There is no globally standardised "normal" range for heavy metals in hair due to large variability influenced by geography, age, sex, ethnicity, hair treatments and analytical methods. Therefore, comparisons should ideally be made with matched population data from similar geographical location, ethnicity, gender, lifestyle and working environment as recommended in the study conducted in Penang state of Malaysia [41]. Since heavy metals occur naturally in the environment, complete avoidance is impossible. Therefore, additional monitoring and stricter regulation of environmental heavy metals levels are necessary to safeguard public health.

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