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RESEARCH

Biomonitoring of Potentially Toxic Elements in Dyed Hairs and Its Correlation with Variables of Interest

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Abstract

Hair is good bioindicator of exposure, due to its ability to store and retain trace elements for long periods of time. But it can be especially useful when hair dyes are used since they may contain potentially toxic salts in their composition. In this context, analytical methods for the determination of bismuth, cadmium, lead, and silver in scalp human hair by electrothermal atomic absorption spectrometry were successfully validated. A total of 60 samples obtained from women between 18 and 60 years were analyzed: 34 dyed hairs and 26 untreated hairs (control). Average results expressed in dry weight (dyed/control) for each element were 2.34/0.49 μg g^{-1} (silver), 0.142/0.139 μg g^{-1} (bismuth), 0.055/0.054 μg g^{-1} (cadmium), and 2.09/0.99 μg g^{-1} (lead), respectively. These results agreed with those previously reported for non-exposed populations. A statistically significant higher Ag concentration in dyed hairs was observed, suggesting the bioaccumulation of this element. The associations between metal concentration and variables of interest (age, education, smoking habit, dye brand, use of dietary supplements) were investigated. A strong Pearson correlation was found for the pair Ag/Pb ($r = 0.494$, $p < 0.05$). Also, strong associations between lead levels and all the selected variables were observed ($p < 0.05$), while strong associations between silver levels with age and dye brand and association between cadmium levels and smoking habit were found. Furthermore, several commercial hair dye brands were analyzed to verify compliance with cosmetic regulations. This constitutes the first study of such characteristics performed in Uruguay, with worldwide relevance.

Keywords Biomarker · Hair · Hair dyes · Potentially toxic elements · Electrothermal atomic absorption

Introduction

Although hair does not perform a vital function in human beings, it is highly important at the psychological level as it is a fundamental part of the development and identification of body image. Hair color is the most visible human phenotype that plays a significant role in physical appearance and self-perception [1–3]. Since ancient times, products have been developed to change and improve the appearance of hair, including different types of dyes [4]. According to data from the Confederation of Consumers and Users of Europe (CECU), more than 60% of women and between 5 and 10% of men dye their hair, an average of 7 times per year [4, 5]. For several decades, the Cosmetic Ingredient Review (CIR) has evaluated the safety of several chemical compounds used in dyes. However, there is still a lack for a comprehensive review regarding the risk of exposure to these products [6].

Hair is an extension of the scalp, mainly composed of type I and II keratin fibers [7]. These keratins are particularly rich in cysteines, which gives them the ability to form a large number of intramolecular and intermolecular disulfide bridges and to coordinate with exogenously incorporated metals [1, 8, 9]. Hair incorporates endogenous elements from the blood stream during its growth, while it acquires exogenous elements by deposition [10]. Hair follicles act as reservoirs for topically applied substances [11]. This ability to store and retain trace elements for long periods of time makes hair useful as a bioindicator of exposure to contaminants [12].

Hair dyes are classified into temporary, semi-permanent, and permanent, depending on the duration of the coloring on the hair. Permanent dyes have sufficient durability so that the user only requires one application per month. In their formulation, they generally use the

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so-called oxidative hair dyes. These chemicals are often called intermediates, because most of them are colorless and produce colored compounds through a process of oxidative condensation, when mixed with oxidizing products just before its use. In fact, hair color is formed when a dye precursor (called base or primary intermediate) is oxidized by the oxidizing agent (also known as developer) to produce an imine, which quickly reacts with the so-called modifier (also known as coupler) [13]. In addition, dyes are classified into natural, synthetic, or mineral origin. In the latter case, the formulation is based on metallic salts, which cross the hair cuticle and enter the cortex, where they oxidize and bind to keratin to modify hair color. Metallic salts can be accumulated in the hair fiber and even absorbed by the scalp, which can result in harmful health effects when potentially toxic salts are used [11, 14]. These salts determine the type of coloring and the mechanism of action of the preparations, but they do not color the hair by themselves; they require another substance that transforms them into coloring compounds. An activator is used for this task, the most common being pyrogallol (1,2,4-trihydroxybenzene). It is a reducing agent that reacts with the metallic salts and forms the corresponding metal oxides [15]. However, despite the popularity of dyes, its toxicity is not always considered. In fact, the reducers used to break the sulfur bridges of keratin and give rise to the new bond can eventually release the metal ions from the salts, with the risk of being absorbed and producing toxic effects to the user. For this reason, hair cosmetic legislation establishes certain restrictions on commercial products [16].

The most widely used salts are those of bismuth (Bi), lead (Pb), and silver (Ag), with silver nitrate, lead acetate, and bismuth citrate being the most common active principles, which react with the sulfur present in the hair keratin to form insoluble dark-colored sulfides. Sometimes, to vary the tones, salts of other metals are added, such as copper (Cu), iron (Fe), nickel (Ni), or even cadmium (Cd) [15]. These metals can enter the general circulation through the layers of the skin and be transported to various organs within the body [10, 11]. Metals are not biodegradable, having a long half-life which allows them to remain in humans and in the environment for many years [17].

Some potentially toxic elements related to hair dyes are the aforementioned Ag, Bi, Cd, and Pb. Lead bioaccumulates and affects all organs of the human body, being the nervous system the most sensitive target [18]. The main toxic mechanism of Pb is the supplantation of polyvalent cations, especially calcium (Ca) and zinc (Zn), in the molecular machineries of the organism [19]. It binds to sulfhydryl groups and interferes with multiple enzymes. It also binds to mitochondrial membranes, interfering with protein and nucleic acid synthesis [20]. According to the Agency for Toxic Substances and Diseases Registry (ATSDR), Pb occupies the 2nd position of the Substance Priority List [21]. Cadmium poses high bioaccumulative potential as well. One of its main adverse effects is the replacement of Zn in enzymatic active sites [22–24]. While in acute exposures, Cd forms complexes with proteins accumulating in the liver and kidneys, in chronic exposures, it accumulates in bones by displacement and replacement of Ca [22]. The ATSDR places cadmium in the 7th position of the Substance Priority List [21]. Silver, on the other hand, poses relatively low toxicity to humans. It occupies the 229th position of the ATSDR ranking [21]. However, as the intentional use of Ag in pharmaceutical preparations and devices increases, toxic effects may be expected. High doses of Ag can induce neurological and/or psychiatric clinical problems [25]. Silver overexposure can cause low intensity adverse effects on various metabolic parameters by affecting other essential elements, such as selenium (Se) [22]. Although Bi is considered a non-toxic element, its prolonged use could cause side effects and even toxicity to humans. Motor, memory, muscle, encephalopathy, insomnia, and psychiatric issues have been reported [26, 27].

According to the Royal Decree 1599/1997 on cosmetic products, hair dyes must not contain Cd. The Decree also stablishes that hair dyes may contain lead acetate up to 0.6% (expressed in lead) and silver nitrate up to 4%. There are no restrictions regarding Bi compounds [16]. Despite this, after many toxicological studies, lead acetate was prohibited in the European Union (since 2004), in Canada (since 2005), and the USA (since 2018). However, these regulations are not taken into consideration in many developing countries. Since the chronicity of the exposure to Pb and the possible presence of injuries in the scalp increase the toxicological risk, it is important to conduct proper biomonitoring and cosmetic analysis [14]. Therefore, according to the vast amount of information compiled in the scientific literature, concerning the medical afflictions related to the chronic exposure to the aforementioned elements, it is of outmost importance to monitor their levels in presumably exposed populations. In this context, the monitoring of potentially toxic elements in hair can be considered a useful biomarker in health risk assessment screening programs Thus, hair analysis can reconstruct a temporal history of past exposures, being this very convenient for chronic exposure assessment [28]. Besides, it is a non-invasive, easier to preserve, and easier to transport biological sample [29]. In this context, the present study focused on the monitoring of Ag, Bi, Cd, and Pb in hair samples from a group of volunteers with dyed hair, aiming to establish whether there were or were not statistically significant differences when compared to a control group. An

association analysis between metal concentrations in hair and some variables of interest was included. In addition, several hair dye samples obtained from the Uruguayan market were analyzed to verify compliance with international regulations. To the best of our knowledge, this constitutes the first study of such biomonitoring characteristics performed in Uruguay. Although it is a regional study, it acquires importance at a global scale since it allows countries to tailor interventions to their specific contexts and address unique health challenges.

Materials and Methods

Reagents

Triton X-100 (Sigma-Aldrich, St. Louis, MI, EUA) and reagent grade acetone ($CH_3(CO)CH_3$) (Dorwil, Buenos Aires, Argentina) were used for sample cleaning. A 3.5 mol L^{-1} nitric acid (HNO_3) solution prepared from concentrated HNO_3 (67 % w/v, Merck, Darmstadt, Germany) was used for sample preparation. Calibration solutions were prepared from commercial 1000 mg L^{-1} stock solutions of each analyte (Merck, Darmstadt, Germany). Ultrapure water of 18.2 MΩcm resistivity (ASTM type I) was obtained from a Millipore™ Direct Q3 UV water purification system (Millipore, Bedford, MA, USA). Monobasic ammonium phosphate ($NH_4H_2PO_4$) (Carlo Erba, Milan, Italy) was used as a matrix modifier for Pb determinations. A palladium nitrate ($Pd(NO_3)_2$) 10 g L^{-1} commercial solution (Merck, Darmstadt, Alemania) was used as a matrix modifier for Cd determinations. Nickel nitrate hexahydrate ($Ni(NO_3)_2 \bullet 6H_20$) (Sigma-Aldrich, St. Louis, MI, EUA) was used as matrix modifier for Bi determinations. A certified reference material (CRM) of human hair (NIES No. 13, Tsukuba, Japan) was used for accuracy and precision evaluation of the analytical methods.

Studied Population

The studied population was composed of adult women, between 18 and 60 years of age, from Montevideo, Uruguay. An informed consent was obtained from all the participants involved. The volunteers also completed a questionnaire which included age, education level achieved, use of dietary supplements, smoking habits, and hair dye brand, in order to establish potential correlations between the obtained metal concentrations in hair and these variables. This study was approved before its start by the Human Research Ethics Committee of Faculty of Chemistry, Universidad de la República, File Number 101900-001374-18, in accordance with the Declaration of Helsinki and the National Decree No. 158/019 [30].

Sample Collection and Cleaning

A total of 60 samples were collected, of which 34 corresponded to hair treated with permanent dyes and 26 to untreated hair (control group), randomly selected. Age distribution was similar in both groups, both involving healthy Caucasian women with an active lifestyle. Also, smoking habit and education level were similarly distributed in both groups. Hair samples were taken according to Menezes-Filho et al. [31]. A tuft of 0.5 cm diameter and 7-cm-long hair was cut near the scalp from the occipital lobe region, using clean stainless-steel scissors, and stored in individual polypropylene bags with zips and properly labeled. Hair samples were previously cleaned to remove exogenous contamination, without affecting the dye layer. First, samples were washed with acetone to remove the primary oiliness. Then, they were then rinsed with ultrapure water. Afterwards, each sample was individually placed in a clean beaker with 1% m/v Triton X-100 and sonicated for 10 min in an ultrasonic bath (Cole Parmer, Vernon Hills, IL, USA) at 47 kHz, in order to remove the more attached oiliness. Then, samples were rinsed again with ultrapure water to remove the foam, placed on a clean watch glass, and dried in an electric oven (Daihan Scientific, Seoul, South Korea) at 85 °C for 4 h [32, 33]. Finally, samples were transferred to a silica gel desiccator and allowed to cool for 30 min, and subsequently stored in properly labeled polypropylene tubes. Samples were handled with special care to avoid external contamination.

Sample Treatment

Samples were previously cut in approximately 1-cm-long pieces. Sample treatment consisted in the digestion of 0.25 g of clean and dried hair with 10.0 mL of 3.5 mol L^{-1} HNO_3 in a CEM Mars 6 microwave digestor (Matthews, NC, USA), provided with 12 Easy Prep Plus® vessels. The program consisted of a 15-min ramp time until 180 °C, holding for 15 min, and then cooling to room temperature. Power varied between 400 and 1800 W, with a maximum pressure of 3.45 MPa [32]. After mineralization, samples were quantitatively transferred into a volumetric flask and filled up to 10.0 mL with ultrapure water. The obtained solutions were used for analytical determinations without further dilution. Samples and reagent blanks were run in triplicate. The human hair CRM was treated in the same way, starting from 0.25 g of dried material. In addition, cream hair dyes were also treated using the same digestion procedure, starting from 0.25 g of fresh material.

Analytical Determinations

Samples were analyzed by electrothermal atomic absorption spectrometry (ETAAS) using a Thermo

iCE 3500 (Thermo Scientific, Waltham, MA, USA) spectrometer with Zeeman background correction. Analytical lines were 328.1 nm (Ag), 223.1 nm (Bi), 228.8 nm (Cd), and 283.3 nm (Pb), respectively. A 10 g L^{-1} $Ni(NO_3)_2$ solution was used as a matrix modifier for Bi, a 500 mg L^{-1} $Pd(NO_3)$ solution was used for Cd, while a 5 g L^{-1} $NH_4H_2PO_4$ solution was used for Pb. No matrix modifier was used for Ag determinations. Optimized pyrolysis/atomization temperatures were 600/1900 °C (Ag), 1000/1600 °C (Bi), 800/1800 °C (Cd), and 800/2200 (Pb), respectively. The signal used for quantification was integrated absorbance (peak area) [34]. Pyrolytically coated graphite tubes (Thermo Scientific, Cambridge, UK) were used. Argon 99.998% of purity (Linde, Montevideo, Uruguay) was used as purge and protective gas. Sample injections were of 20 µL.

Statistical Analysis

Data analysis was carried out using IBM SPSS Statistics 28.0.0.1 and JMP 17.0 software. For Pearson correlation analysis, differences at a 5% significance level ($p < 0.05$) were considered as statistically significant. Kolmogorov–Smirnov test was used for testing the normality of distributions. The differences between the analyte concentrations in the samples were tested by a one-way analysis of variance (ANOVA) followed by *t*-test to evaluate the relationship between them. Differences among mean concentrations at a 5% significance level ($p < 0.05$) were considered statistically significant [35].

Results and Discussion

Analytical Method Validation

Analytical methods were validated following the recommendations of the Eurachem Guide [36]. The figures of merit evaluated were linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. Linearity was verified in a suitable range, using the lack-of-fit test [35]. For all calibration functions, the determination coefficient values (R^2) were greater than 0.999. The LOD and LOQ were evaluated using the 3*s* and 10*s* criteria, where *s* was the standard deviation (SD) of 10 independent reagent blanks [35]. A Student *t*-test was performed for accuracy evaluation, to prove if there were statistical differences between the experimental and the certified or informed values of the CRM, which showed no differences at the 95% confidence level, being the experimental *t*-values below the theoretical $t\ (0.05, 5) = 2.57$ [35]. Recoveries were in the range 97.8–102.3%. Precision (repeatability) expressed as relative standard deviation (RSD (%)) after the analysis of the CRM was less than 5%. The validated methods proved to be reliable tools for the biomonitoring of the aforementioned elements, which employ an easily accessible analytical technique. Obtained figures of merit are summarized in Table 1.

Analysis of Hair Samples

The concentrations obtained for Ag, Bi, Cd, and Pb in hair samples are summarized in Table 2, for both dyed and control groups. As can be observed, the concentrations followed

Table 1 Figures of merit obtained after the validation of the employed analytical methods

Parameter	Silver (Ag)	Bismuth (Bi)	Cadmium (Cd)	Lead (Pb)
Linearity (µg L^{-1})	0.8–400.0	4.6–60.0	0.3–4.0	0.2–40.0
LOD (µg g^{-1}) (dry weight)	0.0096	0.055	0.0034	0.0024
LOQ (µg g^{-1}) (dry weight)	0.0336	0.184	0.0112	0.0080
Accuracy* (% recovery, $n = 10$)	99.7	97.8	98.5	102.3
Precision* (% RSD, $n = 10$)	3.9	4.3	1.2	2.5

LOD limit of detection, *LOQ* limit of quantification, *RSD* relative standard deviation.

*CRM: NIES No. 13, human hair

Table 2 Mean concentrations obtained in analyzed dyed and control hair samples (dry weight)

	Silver (Ag)		Bismuth (Bi)		Cadmium (Cd)		Lead (Pb)	
	Dyed	Control	Dyed	Control	Dyed	Control	Dyed	Control
Mean (µg g^{-1})	2.34	0.49	0.142	0.139	0.055	0.054	2.09	0.99
SD (µg g^{-1})	0.68	0.19	0.025	0.022	0.014	0.015	0.52	0.21
RSD (%)	29.1	38.6	17.6	15.8	25.4	27.8	24.9	21.2

SD standard deviation, *RSD* relative standard deviation

the order Cd < Bi < Pb < Ag. Cadmium and Pb results in control samples agreed with those previously reported by Rodushkin and Axelsson in Sweden [37] and Liang et al. in China [38] for non-exposed populations, as shown in Table 3. Furthermore, Ag, Bi, Cd, and Pb levels in control samples agreed with those reported by Chojnacka et al. in Poland [40]. A Student *t*-test was performed to evaluate if there were statistically significant differences between the means obtained for the dyed and control groups. Although the means corresponding to dyed hairs were higher than those of control hairs, results showed statistically significant differences at the 95% confidence level only for Ag, being the experimental *t*-value above the theoretical $t\,(0.05, 58) = 1.67$ [35]. Therefore, Ag concentrations in dyed hairs turned out to be statistically higher than in control hairs. The SD values reported in Table 2 show the variability obtained for each element in each studied group. As it can be observed, the highest RSD values were obtained for Ag determinations, which can be attributed to different grades of exposure to this element. It is important to emphasize that the use of hair dyes is not the only source of Ag exposure. The wide use of Ag allows exposure through various mechanisms. It is worth mentioning that Ag compounds have been used in the medical field to treat burns and a variety of infections. Elevated Ag concentrations in hair could be due to chronic exposure to this element at low concentrations, owing to its vast use as an antimicrobial agent in recent years. Inhalation of dust or fumes containing Ag may also occur at working places. Silver can also access the body through the use of acupuncture needles, catheters, dental amalgams, jewelry, and the consumption of seafood [40]. Therefore, since Ag presence in hair can pose many origins, some correlations between metal concentrations and certain variables of interest were incorporated in the next section, aiming to enrich the discussion.

Although there are no universally accepted reference values in hair samples for the elements studied here in this work, obtained concentrations turned out to be comparable with those previously reported by other authors in non-exposed populations from other countries as shown in Table 3. Especially, in the case of Pb, the content in hair above 10.0 μg g^{-1} may indicate significant exposure [43]. In this regard, all samples presented Pb levels below the aforementioned limit, which is an important piece of information, since Pb occupies the 2nd position of the ATSDR ranking due to its high toxicity. Regarding Ag and Bi determinations, scarce information is available on the literature, so the results obtained in this work constitute novel information at the national and international level. The biggest difference between means was observed for Bi, when compared to Sweden, being the Swedish mean more than 7 times lower than the Uruguayan, as shown in Table 3, which is probably related to the occurrence of this element in the environment. This element is naturally present in the Earth's crust, but not as widely distributed or as toxic as the other heavy metals. Besides, anthropic activities can release it into the environment as well. Furthermore, it is also used in pharmaceuticals [27].

The lack of universal reference values in hair is mainly due to variabilities in populations. Different populations and individuals may have varying baseline levels of heavy metals in their hair due to differences in genetics, dietary habits, environmental exposures, and many other factors. Thus, creating a single reference range that applies to all populations can be challenging.

Correlation Analyses

Pearson correlation analyzes were performed in order to study the correlation and interaction between the four studied elements in hair. A statistically significant positive correlation was obtained for the pair Ag/Pb ($r = 0.494$, $p < 0.05$) as shown in Table 4. This fact could reflect a similar chemical interaction with hair keratin for these two elements. In this regard, some authors have suggested that certain metal ions could suppress or promote the binding of a particular element to the human hair fiber and that they behave differently in the presence of other ions that affect their reactivity [9].

In addition, a statistically significant positive association ($p < 0.05$) between Pb concentrations and the smoking habit was obtained. All dyed hair samples containing Pb levels above 2.0 μg g^{-1} corresponded to smokers, as shown in Fig. 1. A statistically significant higher concentration was also observed for Cd in the smoker group. Both Cd and Pb positive associations between its concentration in hair and

Table 3 Comparison between mean silver (Ag), bismuth (Bi), cadmium (Cd), and lead (Pb) levels in hair from Uruguayan population and those previously reported in other countries for non-exposed populations

Country	Silver (Ag)	Bismuth (Bi)	Cadmium (Cd)	Lead (Pb)	Reference
China	-	-	0.071 ± 0.032	1.56 ± 0.78	[38]
France	0.67	0.072	0.087	2.35	[39]
Poland	0.65 ± 1.45	0.190 ± 0.421	0.079 ± 0.042	2.55 ± 1.54	[40]
Russia	-	-	0.030	1.05	[41]
Spain	0.61 ± 0.39	-	0.022 ± 0.054	1.46 ± 0.21	[42]
Sweden	0.231 ± 0.298	0.019 ± 0.025	0.058 ± 0.056	0.96 ± 0.85	[37]
Uruguay	0.49 ± 0.19	0.139 ± 0.022	0.054 ± 0.015	0.99 ± 0.21	This work

Table 4 Pearson correlation coefficient matrix of element-to-element interactions in the analyzed dyed hair samples ($n = 34$)

	Silver (Ag)	Bismuth (Bi)	Cadmium (Cd)	Lead (Pb)
Silver (Ag)	1.000	0.1659	− 0.1518	**0.4941***
Bismuth (Bi)		1.0000	− 0.0849	0.3517
Cadmium (Cd)			1.0000	0.0677
Lead (Pb)				1.0000

*Value in bold corresponds to statistically significant correlation ($p < 0.05$)

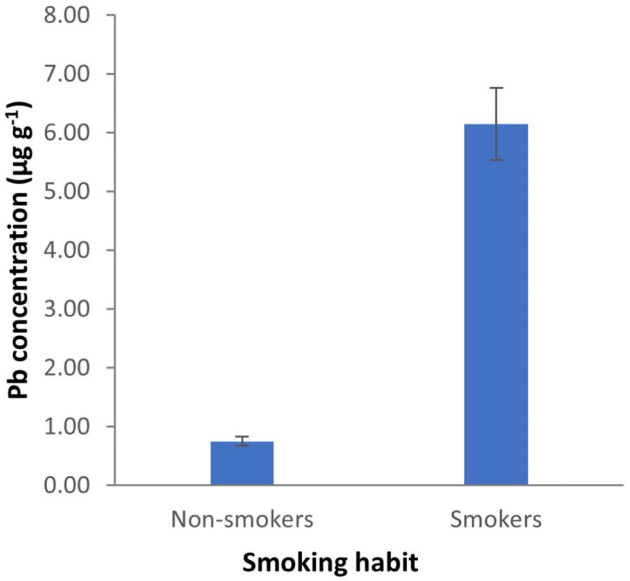

Fig. 1 Lead (Pb) concentration distribution in dyed hairs between non-smokers and smokers

the smoking habit were previously described in the literature [44]. This is directly related to the content of these elements in cigarettes since the tobacco plant is well-known to accumulate heavy metals that can be brought to the cigarette during processing. Heavy metals in tobacco enter the human body through the smoke in the form of aerosol or metal oxides and may accumulate in the hair [45]. No statistically significant associations were observed between Ag and Bi levels with smoking habit.

On the other hand, individuals that consumed dietary supplements presented the lowest Pb levels in hair. Statistically significant differences were found at the 5% significant level. It is known that minerals such as Ca, Mg, and Zn are associated with a lower accumulation of Pb in the human body. In addition, genetic factors could be the cause of a lower accumulation of Pb in hair, such as a different protein composition of the hair fiber [1]. No statistically significant associations were observed between Ag, Bi, and Cd levels and the use of dietary supplements was observed.

Also, a statistically significant negative association ($p < 0.05$) was found between the concentration of Pb in hair and the education level achieved. The highest Pb levels were observed for those participants with only middle school completed, with mean concentrations exceeding 6.0 µg g^{-1} as shown in Fig. 2. On the other hand, the lowest Pb levels were observed in participants with postgraduate degrees, with mean concentrations below 0.5 µg g^{-1}. The association between higher Pb levels in hair and lower education levels is a complex issue influenced by various socioeconomic variables. It is important to note that correlation does not imply causation, and multiple factors could contribute to this observed pattern. One potential explanation for this finding is that people with lower education levels may be more likely to work in environments with higher Pb exposure, such as certain industries or occupations where Pb is used. Also, they may be more likely to live in areas with higher environmental pollution. Furthermore, people with lower education levels may be less informed about potential sources of Pb exposure and may not take adequate precautions to avoid them. No statistically significant associations were observed between Ag, Bi, and Cd levels with education level achieved. The lack of association between Cd concentrations in hair and education was also reported by Kim and Kim [44].

As previously mentioned, one of the questions included in the volunteers' questionnaire was the hair dye brand used. All participants used one of the two most popular commercial brands in Uruguay, namely, brand number 1 and brand number 2. Hair samples corresponding to participants who applied brand number 2 presented statistically higher Ag levels ($p < 0.05$), being mean concentrations 7.6 µg g^{-1} (brand 2) and 2.5 µg g^{-1} (brand 1), respectively. Likewise, statistically higher Pb levels ($p < 0.05$) were observed in hair samples corresponding to participants using brand

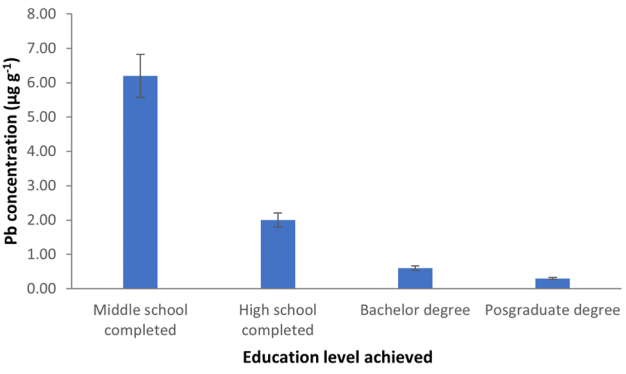

Fig. 2 Association between lead (Pb) concentration in hair and education level achieved

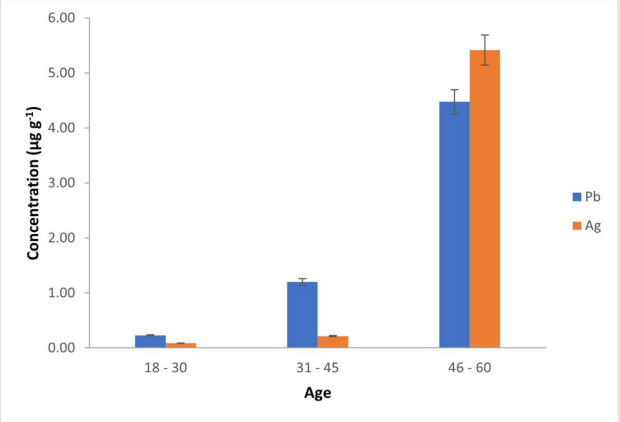

Fig. 3 Association between silver (Ag) and lead (Pb) concentration in hair and age

number 2, being mean concentrations 5.4 µg g^{-1} (brand 2) and 1.4 µg g^{-1} (brand 1), respectively. These findings may suggest that brand number 2 is actually adding statistically significant amounts of Ag and Pb, respectively. No statistically significant differences ($p < 0.05$) were observed for Bi and Cd levels between the groups treated with different hair dye brands.

Finally, statistically significant associations ($p < 0.005$) between metal concentration in dyed hairs and age were observed for Ag and Pb. Fig. 3 shows how Ag and Pb levels increase with age. The Pb increase with time was previously reported [45]. Metal ions present in hair dyes can permeate into the hair during the dyeing process and react with the sulfur of the cysteine residues from hair proteins. Therefore, heavy metal levels in hair tend to increase and accumulate with successive dyeing. Furthermore, hair gradually turns white with age because of the gradual death of the melanin cell, which usually ends in women dyeing their hair more often. Thus, there is a direct correlation between age and dyeing times that may lead to bioaccumulation of certain elements. The fact that some authors have found a decrease in Pb concentration with increasing age for non-dyer populations [46] could indicate a certain degree of Pb bioaccumulation for hair dyes users, especially in the case of older women that have been dyeing their hair for almost 30 years, considering that cosmetic regulations were not very strict in the past due to the lack of toxicological evidence.

Analysis of Commercial Hair Dyes

Ten highly consumed commercial hair dye samples from the Uruguayan market were analyzed using the previously validated analytical methods. Recovery studies (spiking experiments) were performed to evaluate the accuracy. The relative spike recovery (R (%)) was calculated by comparing the difference between the mean spiked value and the mean value of the sample, with the added concentration. The method proved to be reliable for this application, being R (%) between 98.9 and 100.6% for the studied elements. Results showed non-detectable levels for Bi (LOD = 0.055 µg g^{-1}), Cd (LOD = 0.0034 µg g^{-1}), and Pb (LOD = 0.0024 µg g^{-1}). However, quantifiable limits for Ag were obtained in the range 0.54–0.92 µg g^{-1}. This correspond to 0.00009–0.00014% $AgNO_3$, which is far below the maximum limit of 4% $AgNO_3$ established in the Royal Decree [16]. This information shows how the analyzed hair dyes comply with international regulations. On the other hand, the fact that Ag was the only analyte that could be quantified can shed some light to this element being the only one statistically higher than the control group. Although Pb levels were non-detectable in the samples, still very low amounts might be present that may bioaccumulate over time.

Conclusions

The validated analytical methods proved to be efficient and accurate strategies for the determination of Ag, Bi, Cd, and Pb in scalp human hair. Analyte concentration in hair samples followed the order Cd < Bi < Pb < Ag. Obtained values agreed with those previously reported in the international literature for non-exposed populations. Statistically significant differences in Ag concentrations between dyed and control hairs were observed, suggesting that hair dye consumers may suffer from Ag accumulation in hair, although this metal can be uptaken by other sources as well. High Pb concentrations were linked to age, dye brand, smoking habit, education level achieved, and dietary supplement use. Also, strong associations between Ag levels with age and dye brand and associations between Cd levels and smoking habits were found. Human hair can be considered an alternative and complementary biological sample that reflects a prolonged unidirectional deposition of elements, unlike classical matrixes such as blood, which reflects a short equilibration period between different biological tissues and compartments. Biomonitoring provides unequivocal analytical evidence of exposure and can generate quantitative information. However, metal sources may have several origins. Thus, it is important to continue and deepen this research line. It is important to understand the risks associated with the exposure to potential contaminants that can be found in hair dye formulations. The toxicity associated with chronic exposure to certain substances, even at low concentrations, should be evaluated to provide evidence and assure consumer safety.

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Author Contribution A.G.: data curation, formal analysis, and writing original draft; L.F.: data curation and writing original draft. F.I.: supervision, methodology, and writing original draft; I.M.: supervision, methodology, writing original draft, review, editing, validation, and project administration.

Declarations

Competing Interests The authors declare that there is no conflict of interest regarding the publication of this article.

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