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ARTICLE

Towards the Development of an Alternative Analysis Method to Determine Lead in Eyeliner Cosmetics

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Previous research has shown that potentially toxic elements may be present in cosmetic products as impurities or for pigmentation and may be linked to adverse health effects. Yet in low-resource countries where cosmetics hold historical and cultural significance, potentially toxic element contamination in cosmetics is often not well studied or regulated. Current 'gold standard' methods for quantifying these contaminates are inductively coupled plasma - optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS). These methods are expensive, time-consuming, and require rigorous sample preparation, making them challenging to perform in low-resource countries. The goal of this research is to develop a field-friendly method of potentially toxic element analysis. Initial studies focused on screening cosmetic samples collected from the low-resource countries; the results showed that lead (Pb) was present in particularly high concentrations. Further analysis showed that 79% of all the collected eyeliner cosmetic samples contained hazardous levels of Pb contamination. Towards the development of a more field-friendly analysis method for Pb contamination, we created and validated a Pb-spiked cosmetic standard that was used to assess different extraction methods. We then optimized an alternative method using citric acid for use with a field-friendly anodic stripping voltammetry analysis method; this method resulted in the detection of 83% of the total Pb present in the fortified standard. However, when this alternative method was used to analyze the collected samples, matrix effects of select cosmetics significantly reduced the Pb detection. Further research will need to be conducted to address the matrix effects of these cosmetics.

Introduction

Exposure to potentially toxic elements, like Pb, As, and Hg, can lead to a cascade of health concerns including, but not limited to cancer, respiratory diseases, kidney disease, and nervous system and skeletal damage.[1, 2] Human exposure to potentially toxic elements can occur from a variety of sources, including water, food, soil, air, and consumer products such as cosmetics.[3-5] The World Health Organization (WHO), Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) set the permitted potentially toxic elements concentrations to 0.5 ppb (μg kg^{-1}) – 50 ppm (mg kg^{-1});[6] however, exposure to these elements from cosmetics has been largely overlooked.

Potentially toxic elements are often present in cosmetic products due to either matrix impurities or color pigmentation ingredients.[7] Cosmetics most often contain Pb, Cd, Fe, Cu, Ni, Ti, Zn, and in rare circumstances, As and Hg.[4] In the United States, the FDA permits a range of concentrations of these elements in cosmetic products, depending on the metal toxicity. For example, the FDA permits Pb concentrations in cosmetics up to 10 ppm (mg kg^{-1}) and As concentrations in cosmetic color additives up to 3 ppm (mg kg^{-1}).[8] Canada and the European Union also set strict limits of potentially toxic elements in cosmetics;[9, 10] however, there is little regulation in most other parts of the world. Due to the lack of regulation, some studies have found potentially toxic element concentrations in cosmetics to be dangerously high in regions of Asia, the Middle East, and Africa.[11-17] As an example, one study showed that Pb and Cd levels in Surma-related cosmetic products ranged between 51.1-4839.5 ppm (mg L^{-1}) and 1-158.6 ppm (mg L^{-1}) respectively, which are substantially higher than FDA permitted levels.[18] Surma eyeliners from Southeast Asia and North Africa are routinely used by women and children as beauty practices and as cultural and medicinal traditions.[19, 20]

Although dermal exposure of potentially toxic elements is generally considered less dangerous than direct ingestion, concentrations exceeding permitted limits can still have a significant impact on human health, as the metals can bioaccumulate over time.[21] The use of contaminated cosmetics can lead to metal accumulation in the skin layers causing dermatitis or enter the blood stream causing a cascade of serious health effects.[22, 23] Thus, the development of a resource- and cost-friendly platform capable of analyzing potentially toxic element concentrations in cosmetics is critical to community health.

Several analytical methods are available for potentially toxic elements quantification in food, water, soil, and cosmetics, including inductively coupled plasma - optical emission

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spectrometry (ICP-OES), atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), and atomic fluorescence spectrometry (AFS).[24, 25] The current gold standard garnered by the EPA and used by the FDA is inductively coupled plasma – mass spectrometry (ICP-MS).[26, 27] Sample preparation for these methods involves traditional extraction methods that use strong acids (or bases) in a controlled, high-temperature environment to destroy the matrix while simultaneously capturing the potentially toxic elements; examples include microwave-assisted or close vessel acid digestions with concentrated nitric acid, hydrochloric acid, perchloric acid, and/or hydrofluoric acid.[27, 28] While all these methods are sensitive, accurate, and suitable for trace metal analysis, they are not field-friendly.

Alternatively, we have developed an electrochemical technique using an alternate sample preparation method towards on-site potentially toxic elements testing. Electrochemical methods, including square wave voltammetry (SWV), anodic stripping voltammetry (ASV), and differential pulse stripping voltammetry (DPSV) have been shown to be suitable for the analysis of potentially toxic elements in cosmetics, with low to sub ppb detection limits.[29-31] Stripping voltammetry techniques are particularly useful in trace metal ion detection for two primary reasons: (1) preconcentration via the accumulation of the metal ions on the working electrode and (2) high sensitivity via the high signal-to-background ratio produced from the voltage pulses during the stripping step.[32] These electrochemical techniques also offer operational simplicity and reduced time to results compared to standard analysis methods.

Recent work has shown that portable and non-toxic stencil-printed carbon electrodes (SPCEs) for ASV can be successfully used for trace metal ion measurements;[33-38] these electrodes are fabricated using glassy carbon particles (a form of graphitic carbon) and conductive ink, and when used in combination with a Bi film, generate well-resolved voltammograms for the trace metal ions.[33, 34] Bi readily forms a stable alloy with trace metal ions, demonstrating similar electrochemical performance to traditional Hg-film electrodes, but without any added toxicity.[34, 39] Additionally, SPCEs are low cost, disposable, and have high stability, making field deployment of these electrochemical methods feasible when paired with portable potentiostats. While traditional potentiostats are bulky and expensive, numerous small and inexpensive alternatives, which can be controlled using Wi-Fi, Bluetooth, or near-field communication, have been developed.[40-42]

To analyze the cosmetic samples in low-resource settings, an alternative sample preparation protocol that avoids using any hazardous reagents is necessary. An additional challenge lies in the need to extract labile potentially toxic elements ions from the complex cosmetic matrix that generally consists of lipids, organic absorbers, and other organic additives without coextracting other matrix components. Several soil studies have demonstrated successful implementation of less hazardous extraction protocols for analysis of potentially toxic elements, including the use of extraction solutions containing weak or diluted acid/bases, chelating agents, and redox manipulators.[43] These soil studies served as a starting point in our development of the safer extraction method of potentially toxic elements from cosmetic samples.

Our research focused on answering the following research questions: (Q1) Do cosmetic samples from select low-resource countries contain toxic levels of potentially toxic element contamination? (Q2) If so, then which metal presents the greatest risk to human health? (Q3) Can we develop a field-friendly, reliable analytical platform to analyze the contamination? The work described herein details the varying levels of potentially toxic element contamination in eyeliner samples collected from low resource countries in Southeast Asia and Africa using a traditional extraction method, highlighting the need for routine analysis of these cosmetics. A safer extraction method coupled with ASV analysis is then described and verified using a Pb-spiked cosmetic standard to mimic a contaminated cosmetic sample. This alternative method was then applied to many of the collected eyeliner cosmetics, highlighting the need for further improvements to the proposed method to increase extraction efficiency and reduce matrix effects.

Experimental

Cosmetic Sample Collection

Cosmetic samples were collected in Nepal in June of 2019, in Ghana in December of 2019, and Uganda in January of 2020 (see Figure S1 and Table S1). A variety of sample types were chosen, including those with different matrices (e.g., waxy paste, solid/powder, and liquid). Commercial samples of particular interest included any product labeled with “surma” or “kohl”, as those ingredients are banned in the US and have been shown to contain high levels of Pb, among other toxic metals. Culturally significant (natural) samples were collected as well. Altogether, 35 samples were collected and stored at ambient temperatures prior to analysis.

X-Ray Fluorescence (XRF) Spectrometry

An Orbis Micro-XRF Analyzer was used with a 30 mm^2 Silicon Drift Detector, rhodium x-ray source, and 30 µm PolyCap sensor. Samples did not undergo any preparation steps other than creating uniform physical surface for detection of the stage platform. All XRF analyses were done under ambient conditions.

ICP-MS and ICP-OES

Dr. Loretta Corcoran used a Nu Instruments AttoM High Resolution (HR) inductively coupled plasma mass spectrometer (ICP-MS) to collect preliminary data of potentially toxic elements present in six eyeliner cosmetic samples. Traditional extraction procedures were followed using strong acids; ICP-MS instrument details can be seen in the Supplementary Information.

ICP-OES Sample Preparation: Approximately 50 mg of sample was weighed and placed into a CEM MARS6 Microwave Digester tube. 5 mL of trace-metal grade concentrated (16 N) HNO_3 (J.T.

Baker) was then added and placed into the CEM carousel. The pre-programmed CEM "USP RM Organic" digestion method (1030-1800 Watt power, 20-25 min ramp time, 15 min hold time at 210°C) was used to microwave digest the samples. Following digestion, the samples were cooled. The tubes were carefully opened, and the contents were transferred to a Falcon tube and diluted with 20-25mL of deionized (18 MΩ cm^{-2}) water.

ICP-OES Analysis: Pb^{2+} concentrations of all solution aliquots were collected on the Perkin Elmer Optima 8000 ICP-OES with Prep3 instrument. Plasma conditions: Argon gas flow of 8 L min^{-1} for the plasma, 0.2 L min^{-1} for auxiliary, and 0.7 L min^{-1} for nebulizer; Rf of 1400 Watts and pump speed of 0.44 mL min^{-1}. At the start of each analytical session, the instrument optics were optimized (axial view mode) using a Mn standard and the instrument was calibrated using standard Pb^{2+} (Atomic Absorption Lead Standard, Sigma-Aldrich) solutions, 0-10 mg L^{-1}. Yt (wavelength of 371.029 nm) was used as an internal standard to monitor and correct for instrument drift and matrix effects. Lead was detected at a wavelength of 220.353 nm, and lead concentrations were calculated based on an external calibration technique ($R^2 > 0.999$). Each sample (n) was analyzed in triplicate. The results reported in mg L^{-1} were converted to mg kg^{-1} to account for mass of the cosmetic; mass and volume of the sample were considered when calculating concentrations. All results are reported in ppm for consistency.

Pb-Spiked Cosmetic Standard

A Pb-spiked cosmetic standard was not commercially available, so one was made using the cosmetic Coty Airspun Loose Face powder. 2.0 g of the Airspun cosmetic was mixed with 5 mL of 50 mg L^{-1} Pb^{2+} standard (from 1000 ppm Atomic Absorption Lead Standard, Sigma-Aldrich) for 48 hrs while rotating at ~40 rpm. An aqueous solution of Pb^{2+} was chosen to ensure the Pb^{2+} was fully solubilized, increasing the likelihood of it chelating with the ingredients in the powder; rotation was used to evenly mix the powder with the Pb^{2+} solution. The samples were gravity filtered with W40 filter paper; both the filtrate and filtride conserved. The filtride, or lead-spiked cosmetic standard, was allowed to dry completely (~12 hrs).

Safer Citric Acid Extraction Method

An extraction solution was adapted from previous literature with modifications.[43] Briefly, 250 mL of 0.5 mol L^{-1} citric acid, pH 2.0 was prepared by adding solid citric acid (Sigma-Aldrich) to deionized water. The solution was pH adjusted using either HCl or NaOH until desired pH was reached, and solution was brought to volume. 0.2 g of cosmetic sample was added to 5 mL of 0.5 mol L^{-1} citric acid, pH 2.0, and incubated for 2 hrs at ~40 rpm. Samples were gravity filtered with W40 filter paper; both the filtrate and filtride conserved. The filtride was allowed to dry completely (~12 hrs).

Alternate Electrochemical Method

Stencil Printed Carbon Electrode (SPCE) Fabrication: SPCEs were fabricated according to previous literature with no modifications.[33] Briefly, 2 g of glassy carbon (Sigma-Aldrich) was combined with 1.8 g of commercial carbon ink (Ercon). Using a precut transparency stencil cut with a CO_2 Epilog laser, the electrodes were printed onto a separate transparency sheet and dried at 65°C for 30 min. Ag/AgCl ink (Sigma Aldrich) was painted onto the right electrode to serve as the reference electrode and dried at 65 °C for 30 min.

Electrochemical Measurements: ASV parameters for the metal deposition step, including in situ plating of the Bi-film, and the subsequent stripping step were adapted from previous work.[33] All buffer, pH and Bi concentrations were replicated with minor modifications to the electrochemical parameters. 0.1 mol L^{-1}, pH 4.0 acetate buffer was made using sodium acetate (Sigma Aldrich) and trace-metal grade acetic acid (Fischer Scientific). Atomic Absorption Lead and Bismuth Standards (1000 mg mL^{-1}, Sigma-Aldrich) were used to create various concentrations for the calibration curves. Before deposition, electrodes were cleaned using chronoamperometry at 0.4 V for 120 s, using 50 µL of 0.1 mol L^{-1} acetate buffer, pH 4.0. 50 µL of Pb^{2+} standards and cosmetic samples were used for all measurements.

Standard Addition Curve: A 10 ppm Pb^{2+} standard, made from an Atomic Absorption Lead Standard (1000 ppm, Sigma-Aldrich) were added to the unknown sample, in 10 µL or 20 µL aliquots up to a total of 40 µL. All generated curves were fit to a linear regression model, and the lines of best fit were used to calculate the x-intercept. The unknown concentration was calculated using Equation 1, where V_s is the x-intercept, C_s is the concentration of the standard, and V_x is the volume of the standard.

$$C_x = -\frac{(-(V_S)_0)(C_s)}{V_x} \qquad \textbf{Equation 1}$$

Results and Discussion

Preliminary eyeliner cosmetic analysis for potentially toxic element contamination via XRF and ICP-MS

To answer our first two research questions, Q1 and Q2, we collected 35 eyeliner cosmetic samples from various regions of Nepal, Ghana, and Uganda and conducted a preliminary analysis of these samples for potentially toxic element contamination.

We analyzed all the solid/powder eyeliner cosmetic samples for potentially toxic element contamination via XRF (Table S1); liquid and waxy paste samples were not analyzed due to the presence of large organic background noise. Preliminary analysis was done using XRF because it is a non-destructive, qualitative method that is often used to understand the composition of trace metals in environmental samples.[44, 45] As seen in Figure 1, these representative cosmetic samples contain a variety of potentially toxic elements and other inorganic

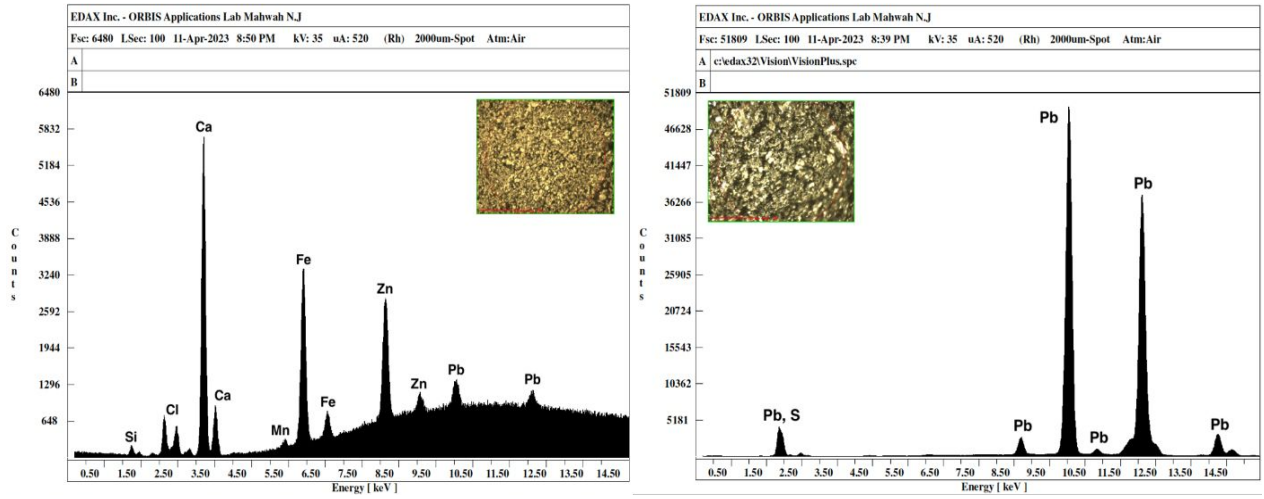

Figure 1. Representative XRF spectra of eyeliner cosmetics from Nepal (left, commercial sample) and Ghana (right, natural rock sample). Spectra represents relative abundances of inorganic species at the location of the X-ray source.

matter, including toxic metals of Pb and As. Micronutrients such as Fe, Ca, Zn, and Cu were detected in high abundances, which is not unexpected as they are often used as additives. The XRF analysis of the natural rock obtained in Ghana (note, rock is used as a natural cosmetic because it can be crushed and mixed with water for application as an eyeliner cosmetic) showed high abundances of Pb and S, consistent with galena rock (PbS). Drawing from these results, six black eyeliner samples of various matrixes (waxy paste, solid/powder, and liquid) were selected for ICP-MS analysis. Black eyeliners were chosen since darkly pigmented cosmetics have a higher probability of containing Pb contamination.[46]

As seen in Table 1, Pb, Cr, and As were found in the samples. In two samples, the Pb^{2+} concentrations were 503 ppm and 1543 ppm, respectively; this is 50x and 150x the amount of Pb permitted in cosmetic products by US regulations.[18] Since the Cr and As concentrations were below the permitted levels for all samples and Pb concentrations were well above the 10 ppm levels for some samples, further analysis of the commercial cosmetic samples was focused solely on Pb contamination via ICP-OES analysis.

Table 1. Results of ICP-MS analysis, in ppm, of six eyeliner samples collected from Nepal. Samples were only run n=1, due to limited sample inventory.

	Cr, ppm	As, ppm	Pb^{2+}, ppm
Sample 1	1.44	0.75	1543
Sample 2	1.07	1.09	9.20
Sample 3	0.58	0.04	27.5
Sample 4	0.60	0.04	3.36
Sample 5	0.01	-	1.08
Sample 6	1.30	0.34	503

ICP-OES analysis for Pb contamination of all collected eyeliner cosmetic samples

24 eyeliner cosmetic samples from various regions of Nepal, Ghana, and Uganda were analyzed for Pb^{2+} contamination via ICP-OES (Table S1) (Note, only one of four natural rocks collected in Ghana was analyzed via ICP-OES). 67% of the samples collected in Nepal, 85% of the samples collected in Ghana, and both samples collected in Uganda were above permitted levels of Pb (> 10 ppm). In total, 79% of the eye-liner cosmetic samples showed high levels of Pb^{2+} contamination, clearly demonstrating a community health concern.

Development of a Pb-spiked cosmetic standard for potentially toxic element analysis

To develop a method that can analyze cosmetics in a field setting, a cosmetic standard containing Pb first needed to be developed, as there are none commercially available. To avoid any initial Pb contamination that could be present in darkly pigmented cosmetics,[46] a light cosmetic powder, Airspun, was chosen as the standard matrix. ICP-OES analysis of the Airspun cosmetic did not detect Pb above the instrument noise (0 ± 1 ppm, n=4), indicating Pb concentrations well below the FDA level of 10 ppm. The spike efficiency was determined by digesting the cosmetic standard and measuring the total Pb^{2+} content using ICP-OES. Both the filtrate and filtride of the cosmetic standard were analyzed, so that the percent loss of Pb^{2+} could be determined (Table 2). The majority of the Pb^{2+} should remain in the filtride if the spiking protocol was successful.

On average, a ~22% loss of the total Pb^{2+} spiked into the cosmetic standard was observed; this equates to the cosmetic standard having a concentration of ~40 ppm Pb^{2+}. The loss is likely a result of the manual filtration process. This spiking method proved reproducible within a batch of spiked cosmetic (n=5) and thus was used to create cosmetic standards for all future experiments (Note, there was batch-to-batch variability

observed when quantifying the [Pb^{2+}]. To account for this, the standard was routinely quantified before use).

Table 2. Results of the ICP-OES analysis of a representative cosmetic standard. The filtride was digested for analysis of total [Pb^{2+}] conjugated to the Airspun matrix; the filtrate determined the total [Pb^{2+}] not conjugated to the sample; the difference determined the percent loss.

	Cosmetic Standard
Theoretical [Pb^{2+}], ppm	1249 ± 1
Measured [Pb^{2+}] in Filtride, ppm	970 ± 40
Measured [Pb^{2+}] in Filtrate, ppm	0.60 ± 0.01
[Pb^{2+}] Loss, ppm	278
Percent Loss, %	22

Development of a safer extraction solution using the Pb-spiked cosmetic standard

To answer the final research question, Q3, and develop a field-friendly sample extraction solution, it is necessary to balance the ability of the potentially toxic elements (Pb^{2+} for this study) to both associate with the ligand and consequently dissociate for electrochemical analysis. Citric acid (CA), a biodegradable metal chelator and naturally available organic acid, was chosen as the extraction solution. CA has three carboxyl groups, which all act as potential coordination centers for potentially toxic elements and can form stable metal complexes with Pb^{2+} ions.[47] Additionally, CA is capable of releasing the Pb^{2+} ions back into the solution by adjusting solution conditions such as pH.

A 0.5 mol L^{-1} CA solution at pH 2.0 was chosen for study. The 0.5 mol L^{-1} concentration was used because it is concentrated enough to release metal ions into solution without being too hazardous to bring into the field, and pH 2.0 was chosen to resemble that of traditional extraction solutions. The cosmetic standard was extracted using the solution and analyzed on ICP-OES, to understand the total Pb^{2+} concentration that could be extracted, regardless of lability. Both the filtrates and filtrides were analyzed to assess the efficiency of the extraction method (Table 3). The majority of the Pb^{2+} should be released from the cosmetic matrix and into the filtrate if the extraction solution was successful.

Seen in Table 3, the total amount of Pb^{2+} that was extracted on average was 914 ppm, with 47 ppm left in the cosmetic. Considering the mass of the extracted sample and the volume of the extraction, this yields an extraction efficiency of 94%. This value is comparable to other CA techniques applied to lead remediation in soil and other environmental sources, while also taking less time and fewer resources.[48, 49] However, this result does not account for the lability of Pb. Small coordination complexes could be extracted using the 0.5 mol L^{-1} CA, pH 2.0 solution, decreasing lability but having no effect on the total Pb^{2+} concentration. Since ICP-OES reduces most sample matrix effects, we consider this extraction efficiency to be maximized; other techniques that rely on metal lability could result in smaller extraction efficiencies.

Table 3. ICP-OES analysis of a representative CA-extracted cosmetic standard (n=5). The filtride was digested for analysis of total [Pb^{2+}] remaining in the sample after extraction; the filtrate determined the total [Pb^{2+}] extracted from the sample. Extraction yield was calculated using the difference between theoretical and measured concentrations.

	Cosmetic Standard
Theoretical [Pb^{2+}], ppm	970 ± 40
Measured [Pb^{2+}] in Filtride, ppm	47 ± 2
Measured [Pb^{2+}] in Filtrate, ppm	914 ± 15
[Pb^{2+}] Loss, ppm	57
CA Extraction Yield (%)	94

Development of an alternate electrochemical method

Electrochemical techniques for metal analysis have been widely seen in the literature,[50-52] in particular, square wave ASV.[53-55] The SPCE fabrication, solutions, and ASV conditions were adapted from previously published work.[33] The adopted protocol was designed for sub-ppb analysis, and adjustments were made to produce a wider working linear range, making it more suitable for cosmetic, rather than water, analysis. Kava et al demonstrated that a pH of 3.6 and a frequency of 14 Hz was optimal for Pb^{2+} detection, due to the uniformity and thickness of the Bi film that is formed on the surface in an acidic environment. Bi is critical to the Pb deposition on carbon electrodes, as it forms an amalgam with the Pb during deposition, increasing stability and deposition efficiency.[56] Despite this, when using higher concentrations of Pb^{2+}, pH 4.0 showed the best result (Figure 2A). It is hypothesized that at pH 4.0, the Bi film is thinner. When using higher concentrations of Pb^{2+}, there was more Pb^{2+} present undergoing diffusion and a thicker Bi layer could hinder the electron transfer of the Pb^{2+} during the stripping step.[36]

It was also determined that by lowering the frequency to 10, the linear correlation was improved at higher Pb^{2+} concentrations (Figure 2B). Frequency can impact peak sharpness and background characteristics.[57] As frequency increases, the oxidative peak current is increased, but the peaks suffer from widening, making them less reproducible. By lowering the frequency, we improved the peak definition, allowing the higher concentration peak currents to be captured more accurately.

Using the optimized conditions described above, a calibration curve was constructed in 0.1 mol L^{-1} acetate buffer pH 4.0 and was used to quantify the labile Pb^{2+} ions extracted from the cosmetic samples throughout the remainder of the study (Figure 3). By fitting the peak currents of the extracted samples to the calibration curve (represented in red on Figure 3) and accounting for dilution factors, the labile Pb^{2+} concentration, in ppm, can be determined.

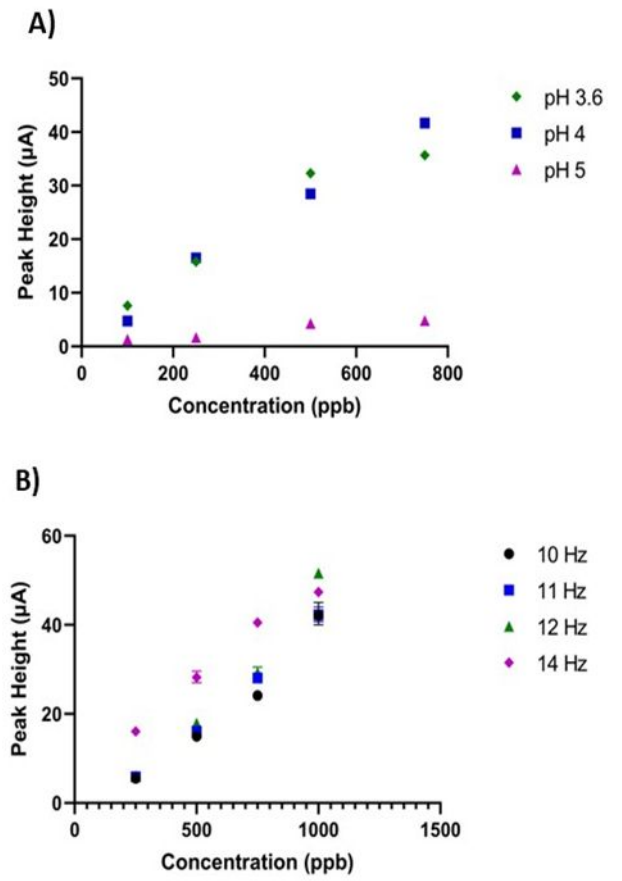

Figure 2. ASV response curves of Pb^{2+} to assess the optimal working linear range for A) pH and B) frequency. Optimal pH was determined using Pb^{2+} concentrations from 100-750 ppb and frequency was determined using Pb^{2+} concentrations from 250-1000 ppb.

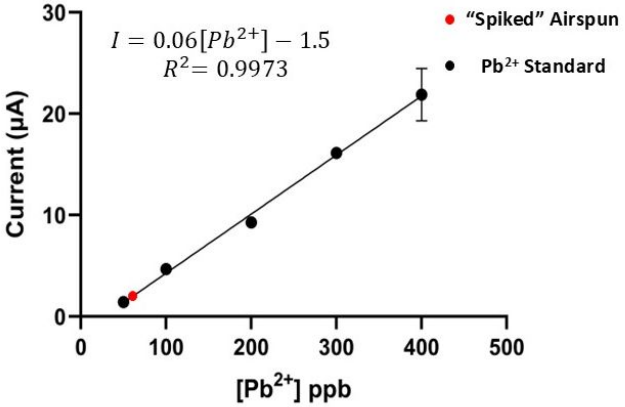

Figure 3. ASV calibration curve of the peak heights of standard solutions, normalized to the background buffer solution, versus Pb^{2+} concentrations in the range of 50-400 ppb, giving a limit of detection (LOD) of 27 ppb and limit of quantification (LOQ) of 50 ppb. All standard solutions contained 2 ppm Bi. The red dot represents the average peak height of the extracted cosmetic standard and an extracted Pb^{2+} concentration of 21 ± 2 ppm was calculated using the line of best fit. All dilutions factors were considered.

As the ASV method can only measure labile Pb^{2+}, rather than total Pb^{2+}, it is likely that the Airspun matrix contains organic ingredients that act as chelators that are not affected by the CA and are permeable through the filtration step. It is also possible that a pH adjustment from 2.0 to 4.0 (dilution of the standard into the acetate buffer) is not enough to fully release the metal ions from the CA coordination centers. Despite this extraction efficiency being half of what is observed with ICP-OES, the data are reproducible with small error margins (21 ± 2 ppm). Most importantly, the ASV method was able to quantify Pb^{2+} concentrations above what is permitted by the FDA, and thus, the alternative CA extraction method coupled with ASV has demonstrated the capability of analyzing Pb content in a Pb^{2+}-spiked cosmetic standard.

To further optimize the electrochemical method in hopes of maximizing the amount of labile Pb^{2+} extracted from the cosmetic standard, an acid study and a pH study were conducted. For the acid study, cosmetic standards were extracted using four different acids, nitric acid (HNO_3), hydrochloric acid (HCl), acetic acid (AA), and citric acid (CA), and then run on the optimized ASV system (Figure 4A). Of the strong acids, HCl extracted the most labile Pb^{2+}, which is expected due to its ability to break any covalent interactions. Of the two organic acids, CA outperforms AA, providing further evidence that CA is the best organic acid for the extraction of Pb^{2+} from cosmetic samples.

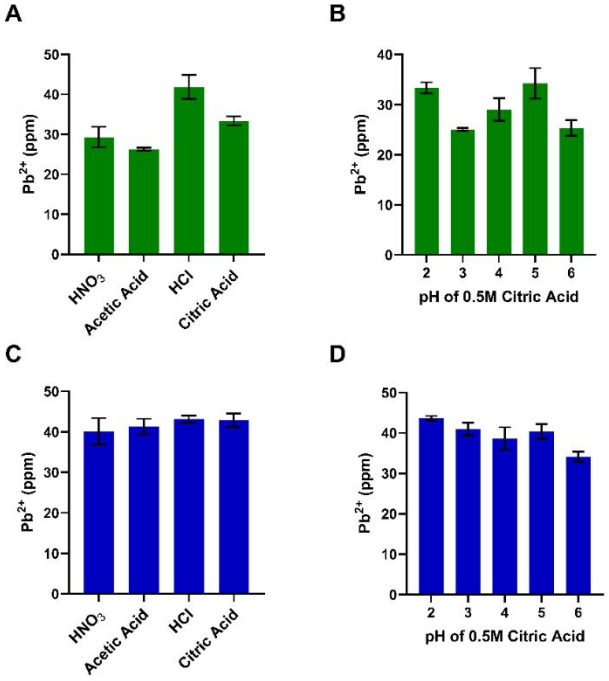

Figure 4. Final Pb^{2+} concentrations of the cosmetic standards after extractions using different acids and different pHs. Labile $[Pb^{2+}]$ measured from cosmetic standards using ASV after extraction from A) 0.5 mol L^{-1} acids and B) 0.5 mol L^{-1} citric acid, pH 2.0-6.0. Total $[Pb^{2+}]$ measured using ICP-OES for C) 0.5 mol L^{-1} acids and D) 0.5 mol L^{-1} citric acid, pH 2.0-6.0.

Since relevant literature showed that pH can impact Pb^{2+} extraction efficiency,[49, 58-60] a pH study was conducted whereby cosmetic standards were extracted using 0.5 mol L^{-1} CA solutions, pH range of 2.0-6.0 (Figure 4B). pH 2.0 showed the best extraction efficiency with 83% labile Pb^{2+} extracted, whereas pH 6.0 showed the worst with an extraction efficiency of 62.5%. Recall the average concentration of the cosmetic standard is 40 ppm, but due to batch-to-batch variability, higher or lower [Pb^{2+}] were used throughout the study. These results were validated using ICP-OES (Figure 4C and Figure 4D) and follow the same trends as shown with ASV; thus, the 0.5 mol L^{-1} CA, pH 2.0, solution was the most optimal for use with the alternative ASV analysis method.

Assessment of the safer CA extraction method of collected eyeliner cosmetic samples

Of the 24 samples, seven commercial and natural eyeliner cosmetics contained exceptionally high concentrations of Pb^{2+} and were not tested further via the alternate CA extraction method. The ICP-OES results of the remaining 17 commercial samples, digested and extracted with 0.5 mol L^{-1} CA at pH 2.0, are shown in Figure 5. As seen in Figure 5, there is a large range of extracted Pb^{2+} concentrations, and the agreement between the extracted versus digested Pb^{2+} is sample dependent. For example, samples 2 and 9 have good agreement between extracted versus digested concentrations whereas samples 3 and 15 have a large discrepancy. This can be attributed to differences in the cosmetic matrix itself.

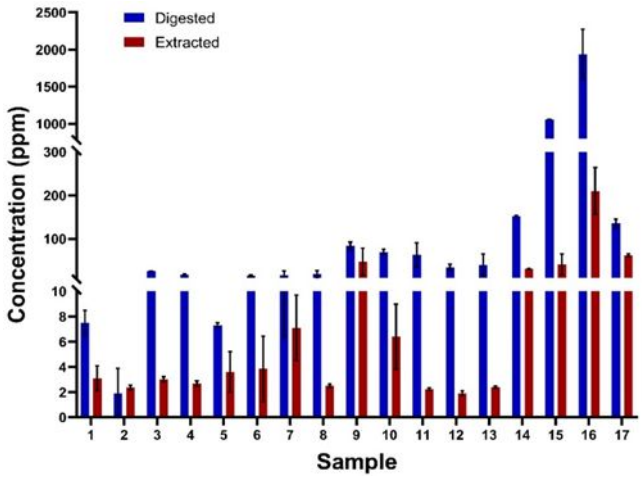

Figure 5. ICP-OES results of 17 real eyeliner cosmetic samples collected in Nepal, Ghana, and Uganda showing the comparison of the measured Pb^{2+} concentrations of the digested (blue) versus the extracted (red) samples.

As discussed previously, the CA extraction method had a 94% extraction efficiency for the Pb-spiked cosmetic powder. However, cosmetic matrices can vary drastically in their composition, with common ingredients including various chelators, preservatives, antioxidants, and pH adjusters. Additionally, liquid or emulsified cosmetics will likely contain more oils, humectants, and thickeners than powdered cosmetics, which could hinder Pb extraction.[61] As CA is a weak acid, it is likely unable to break several of the metal-ligand interactions, reducing the overall amount of labile Pb^{2+} extracted post sample treatment. To address this problem, a mixture of chelators could be tested to improve extraction independent of matrix differences for various cosmetics. Additionally, additives such as $CaCl_2$ or $FeCl_3$ could be added to the CA to enhance metal-exchange with the matrix via substitution reactions, where the Pb^{2+} ions could then be released back into solution for electrochemical analysis with a pH adjustment step. The addition of low concentrations of hydrogen peroxide during extraction could also be examined to increase the oxidizing capacity of the acid.[62]

Alternate electrochemical method of analysis of collected eyeliner cosmetic samples

To determine the efficacy of the alternate electrochemical method on "real" samples, four powder eyeliner cosmetic samples were chosen, as their matrix most closely resembles that of the Pb-spiked cosmetic standard. For all four samples, Pb^{2+} was measured and quantified (Table 4), but the matrix had a large impact on the lability of the metal ion in the sample. Preliminary testing (not shown) of the sample using ASV showed that the labile Pb^{2+} concentration was below what was quantifiable using the original calibration curve (Figure 3). To combat this, a 10 ppm Pb^{2+} standard was added to the unknown sample in increasing volume to generate a 3- or 4-point standard addition calibration curve for each sample. No more than 40 µL of standard was added, to preserve the integrity of the calibration curve and not mask the matrix effect. The standard addition plots for the four samples tested can be seen in Figure S3.

The calculated Pb^{2+} concentrations from the standard addition curves were 8.6 ppm, 330 ppb, 1.32 ppm, 240 ppb for Samples 1-4, respectively. The extracted samples were also measured with ICP-OES, to determine the total Pb^{2+} concentration extracted using the solution. Comparing the measured Pb^{2+} concentrations between ICP-OES and ASV, Samples 2 and 4 had the most matrix interference with 0.5% of extracted Pb^{2+} being labile, whereas Sample 1 had the least amount of matrix interference with 20% of the extracted Pb^{2+} being labile. Collectively, all ASV measurements were at least an order of magnitude below what was determined with the ICP-OES. Based on the low concentrations quantified by both the ASV and ICP method using the CA extraction method, the complexity of the cosmetic matrixes has a great impact on the utility of the approach. There are likely several potential interferents that have strong binding constants with Pb^{2+}, decreasing the lability. Therefore, CA alone is not enough to extract Pb from cosmetics, and the extraction solution requires further optimization. However, all samples tested were able to be quantified to some degree using both methods.

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Table 4. ICP-OES and ASV results of Pb analysis of four cosmetic samples. All samples were extracted with the CA extraction solution. Extraction efficiency was calculated by comparing the total Pb^{2+} concentration to the Pb^{2+} concentration measured after extraction. Concentrations were normalized to the mass of the sample and volume of extraction solution. Sample numbers do not correspond to Table 1.

		ICP-OES Measurement		ASV Measurement	
	Total [Pb^{2+}], ppm	**[Pb^{2+}], ppm**	**Extraction Efficiency (%)**	**[Pb^{2+}], ppm**	**Extraction Efficiency (%)**
Sample 1	1058	41.3	3.9	8.60	0.81
Sample 2	135.6	62.3	46.9	0.33	0.24
Sample 3	1938.6	210.2	10.8	1.32	0.07
Sample 4	152.6	30.9	20.2	0.24	0.16

Conclusions

The work detailed here verifies potentially toxic element contamination in cosmetics, particularly Pb in eyeliner cosmetics, in low-resource countries. ICP-OES analysis using a traditional acid extraction of 24 eyeliner cosmetic samples showed that 79% of the natural and human-made eyeliner cosmetic samples contained hazardous levels of Pb contamination, concentrations well above the FDA-regulated levels of 10 ppm, which may yield serious community health effects. Preliminary development of a safer extraction method and alternative electrochemical analysis method demonstrates its potential utility in cosmetic analysis; however, further optimization is needed before either the CA extraction solution or the ASV method can be successfully implemented in field environments. Despite this, the CA solution is suitable for simple matrices, such as the Airspun cosmetic standard. Potential alternations to make the solution more robust include pH adjustment and the addition of $CaCl_2$ or $FeCl_3$ to the solution. An increase in the pH could promote more ion-exchange between the matrix and the CA by reducing the available OH groups, thus opening more binding sites for the Pb^{2+} ions and promoting stronger chelation. The addition of additives, such as $CaCl_2$ or $FeCl_3$, could enhance metal-exchange reactions through substitution reactions, increasing the extraction of Pb from the matrix. To re-release the Pb^{2+} ions into solution for the electrochemical analysis, a pretreatment step could be incorporated to increase the lability. Since chelation is strongly dependent on pH, an adjustment in either direction could promote dissociation. Broadly, overarching efforts in cosmetic analysis need to continue to develop alternative methods that steer away from using hazardous reagents and expensive instrumentation. Lastly, although this work focuses on the presence of Pb^{2+} in eyeliner cosmetics, several other potentially toxic elements, such as Cr, As, Cd, Hg, could be of concern in eyeliner or other cosmetics.

Author contributions

Catherine J. McMahon: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft, Writing – Review and Editing, Visualization. **Toni L. O. Barstis:** Conceptualization, Methodology, Investigation, Formal Analysis, Resources, Validation, Writing - Review and Editing. **Rae A. Bellows:** Investigation, Writing - Review and Editing. **Charles S. Henry:** Resources, Supervision, Project Administration.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data available on request from the authors.

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Data available on request from the authors.