A Comparative Analysis of Mercury and Lead in Sindoor from the U.S. and Bangladesh

by
Anna K. Breen

A THESIS

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Abstract approved:____
Molly Kile

Heavy metals have historically been popular for use in cosmetics manufacturing due to the colorful pigmentation of heavy metal compounds. In the case of sindoor, a cosmetic used by married Hindu women for religious purposes, mercury sulfide, lead tetroxide, and lead chromate are compounds suspected to still be involved in its manufacturing due their red and orange coloration. In the United States, federal regulations have established a criteria of allowable lead in cosmetics at 10 parts per million (ppm) and 65 ppm in mercury. In Bangladesh, such regulations have not been implemented. Consequently, it was anticipated that both mercury and lead content would be higher in Bangladesh sourced sindoor than in U.S. sourced sindoor. The metal content in sindoor samples was determined through quantitative analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES). While it was determined that there was no statistical difference in average lead content between U.S. and Bangladesh samples, 35% of Bangladesh samples contained more than 10 ppm of lead, as opposed to 14% of U.S. samples. Because there was no detectable mercury in any of the samples, it is possible that external factors such as cost and accessibility have incentivized use of lead containing compounds in sindoor manufacturing over mercury containing compounds. These findings indicate that sindoor may potentially be a route of lead exposure for Hindu women in Bangladesh, and further investigation into the manufacturing of sindoor and the people it affects is needed.

Key Words: heavy metals, lead, mercury, sindoor, cosmetics, Bangladesh

Corresponding e-mail address: annakathrynbreen@gmail.com

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APPROVED:

____
Molly Kile, Mentor, representing Environmental and Occupational Health

____
William Stubblefield, Committee Member, representing Environmental and Molecular Toxicology

____
Katharine Field, Committee Member, representing BioResource Research

____
Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Anna K. Breen, Author

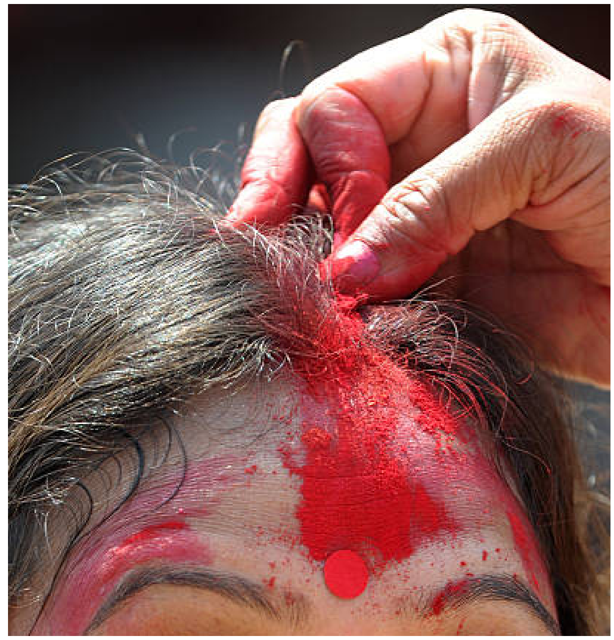
Abstract

Heavy metals have historically been popular for use in cosmetics manufacturing due to the colorful pigmentation of heavy metal compounds. In the case of sindoor, a cosmetic used by married Hindu women for religious purposes, mercury sulfide, lead tetroxide, and lead chromate are compounds suspected to still be involved in its manufacturing due their red and orange coloration. In the United States, federal regulations have established a criteria of allowable lead in cosmetics at 10 parts per million (ppm) and 65 ppm in mercury. In Bangladesh, such regulations have not been implemented. Consequently, it was anticipated that both mercury and lead content would be higher in Bangladesh sourced sindoor than in U.S. sourced sindoor. The metal content in sindoor samples was determined through quantitative analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES). While it was determined that there was no statistical difference in average lead content between U.S. and Bangladesh samples, 35% of Bangladesh samples contained more than 10 ppm of lead, as opposed to 14% of U.S. samples. Because there was no detectable mercury in any of the samples, it is possible that external factors such as cost and accessibility have incentivized use of lead containing compounds in sindoor manufacturing over mercury containing compounds. These findings indicate that sindoor may potentially be a route of lead exposure for Hindu women in Bangladesh, and further investigation into the manufacturing of sindoor and the people it affects is needed.

INTRODUCTION

Heavy metals have been historically used in cosmetics for the purpose of coloration and skin lightening.[1,2] The cosmetics industry continues to utilize heavy metal compounds in their manufacturing processes, and recent studies show there is still a concerning presence of heavy metals in cosmetics globally.[3,4] In the United States, federal regulation of cosmetics has placed acceptable limits of heavy metal content in productions. For mercury content, the allowable amount of mercury in cosmetics used in the eye area or that cannot be processed otherwise without mercury is 65 parts per million (ppm); for lead content, this criteria is 10 ppm in topical cosmetics.[5]

Sindoor is a powder or liquid topical cosmetic of religious significance and use by Hindu women. Specifically, it is used for marriage ceremonies and traditions, and many married women apply sindoor daily. Sindoor is applied to the forehead and the scalp, and though the method of application varies between people, in some cases women will apply sindoor by licking their fingers and repetitively dipping them into sindoor in order to help the powder stick. Hand tools such as brushes or pens are also used in the application of sindoor. Being attached to Hinduism practices, use of sindoor can be most commonly observed in India, Nepal, and Bangladesh.[6(p)] Because sindoor is a vibrant red or orange color, mercury sulfide, lead tetroxide, and lead chromate are popular compounds used in its manufacturing due to their bright red or orange pigments, as well as accessibility.[7] High lead levels have been associated with sindoor in recent studies, indicating the continuation of the use of lead compounds in sindoor.[8,9]



Sindoor being applied to a woman's face and scalp at the Dussehra-Vijaya Dashami Festival in Hyderabad. 2013. Noah Seelman via Getty Images.

Bangladesh is a country involved in many manufacturing industries, cosmetics included. Bangladesh Standards and Testing Institution (BSTI) currently allows small levels of mercury in cosmetic products, with its Environment and Social Development Organization (ESDO) having reported 3,361 to 4,653 ppm of mercury in various cosmetics, including sindoor, in 2012.[10,11] According to the Minamata Convention, which Bangladesh signed in 2013, mercury use in commercial products were to be banned in 2020.[12] This agreement has not been fully phased in by Bangladeshi government.

The form of mercury in the compound suspected to be in sindoor is inorganic. Mercury exposures can lead to damage to the peripheral and central nervous systems, causing neurological effects such as behavioral changes, muscle weakness, spasms, or tremors, and cognitive dysfunction.[13] There can also be damaging effects on the lung and immune systems. Inorganic mercury salts can cause kidney damage and failure, as well as be corrosive to the skin, eyes, and cause damage to the GI system.[13] While organic forms of mercury are bioaccumulative and concentrate in fatty tissues throughout an individual's lifespan, inorganic mercury is water soluble, has a shorter half-life, and does not bioconcentrate as extensively.[13,14]

Similar to mercury, lead exposure is associated with adverse effects in most organ systems; namely, respiratory, digestive, urinary, neurological, and cardiovascular diseases are linked to lead exposure.[15] Lead is a bioaccumulative metal that acts metabolically similarly to calcium ions, allowing it to be stored in the bones. As a consequence, lead can be continuously released back into the blood as the body undergoes normal bone metabolism.[16] As a result, lead bioaccumulation—similar to mercury bioaccumulation—can cause lifelong internal exposure.

Because sindoor is popular in use by Hindu women of reproductive age, and is a cosmetic suspect of heavy metal content, it is of great concern the health effects this route of heavy metal exposure may have within a population. Heavy metal exposure is especially dangerous when an individual is pregnant, as heavy metals are capable of crossing the placental barrier and therefore exposing the fetus.[17] Both mercury and lead are known to cause fetal development issues and gestational complications, making fetal exposure to mercury and lead of concern. Lead exposure in early pregnancy has been linked to premature births and miscarriage,[18] and fetal mercury exposure is associated with neurological and immunological misdevelopment at birth.[19] While heavy metal exposure through sindoor is dangerous for the women exposed, it could also be contributing to generational public health concerns as heavy metal exposure could also be affecting the pregnancies and children of women who use sindoor.

Given the differences in federal regulation and enforcement of heavy metal use in cosmetics, it is anticipated that sindoor sourced from Bangladesh will have higher mercury and lead content than sindoor sourced from the U.S. Additionally, it is suspected that Bangladesh sourced sindoor will likely have mercury and lead content greater than that of the FDA criterias.

METHODS

Sample Collection

For sindoor sourced in the U.S., sampling was conducted based on most popular purchases on Amazon. Samples imported from India were excluded, leaving seven U.S. sourced samples for study. Seventeen samples were sourced from a popular central market in Bangladesh.

Acid Digestion Procedure

Samples were transferred from their original containers into acid clean ultraWAVE vials using disposable polypropylene spatulas and 5 mL pipette tips acting as funnels, using new spatulas, pipette tips, and gloves between samples in order to prevent cross-contamination. Prior to sample transfer, vials were placed on the calibrated microbalance and tared. Approximately 100 mg of sample was transferred into each vial and mass was measured with accuracy up to the fourth decimal place. Two vials each containing approximately 200 mg of hair standard of known mercury and lead concentration were included in each digestion batch, following the same procedures.

Following sample transfer, 3 mL water and 2 mL nitric acid were added to each vial, in addition to three blank vials containing only water and nitric acid. Vials were capped and placed in the Milestone UltraWAVE microwave digestion system. The standard operating procedure (SOP) for Milestone "UltraWAVE" Microwave Digester Operation was conducted using the parameters described in **Figure 1**. The first batch of samples had visible particulate remaining after digestion, and so the second batch of samples' digestion temperature was increased by 30℃. Following this change, the second batch contained less particulate than the first.

Figure 1. Acid UltraWAVE Digestion Method Parameters.

Load Pressure	40 bar
Vessel Cooling Beyond	Activated 40℃
Release Rate	5 bar/min
Temperature Below	50℃
Run Temperature (batch 1)	220℃
Run Temperature (batch 2)	250℃
Run Pressure	120 bar

After digestion, samples were quantitatively transferred to acid clean Falcon tubes. Prior to transfer, the mass of the empty Falcon tubes was recorded. Digest contents were poured into falcon tubes. Water was added to vials which were inverted several times in order to rinse the vial, poured into the falcon tubes. This process was repeated at least two times for a minimum of three rinses, ending with 25 mL of transferred digest at a nitric acid conentration of 6%. After quantitative transfer, the mass of falcon tubes with the digest were recorded to obtain the mass of the digested sample.

OES Analysis

Figure 2. ICP-OES instrument parameters for determination of mercury and lead in sindoor.

Parameters	Assigned value
Power	1200V
Sample uptake rate	3.1 mL/min
Coolant flow	13.00 L/min
Nebulizer flow	0.80 L/min
Auxiliary flow	1.00 L/min
Rinse Time	45 sec

The linearity of the method was evaluated by analysis of eight elemental mercury standard solutions with concentrations of 0, 2.6, 7.79, 15.58, 36.35, 77.9, 129.83, and 207.73 parts per billion (ppb) and eight elemental lead standard solutions with concentrations of 0, 2.5, 7.5, 15, 35, 75, 125, and 200 ppb. To create these standard solutions, the stock standard of inorganic mercury (1000000 µg/L) was diluted to 1039 µg/l by 6% nitric acid. Then, a second dilution was conducted of the first dilution and the stock standard of lead (1000 µg/L) using 6% nitric acid to create a mercury and lead working stock solution with concentrations of 208 µg/L for mercury and 200 µg/L for lead. This working stock solution was diluted with 6% nitric acid in order to reach the final eight working standard solutions.

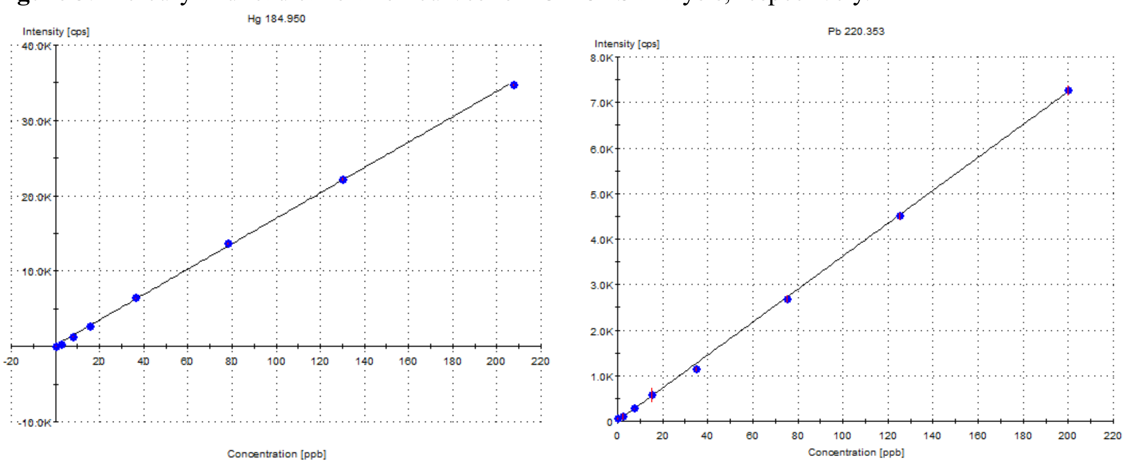
The limits of quantification (LOQ) were determined by the second lowest standard concentrations and the highest standard concentrations, with the lower LOQ defined as 2 ppb and the upper LOQ as 200 ppb. The parameters by which the samples were analyzed on the ICP-OES can be viewed in **Figure 2**.

A NIST certified water standard of known elemental lead concentration was included in the ICP-OES analysis. The NIST standard was diluted by half in order to reduce the concentration to that within the LOQ using 6% nitric acid and analyzed in triplicates at the end of the run, after all samples—including duplicates—had been analyzed.

RESULTS

Quality Control Analysis

Figure 3. Mercury and lead calibration curves for ICP-OES analysis, respectively.



Prior to sample analysis by ICP-OES, a calibration curve was established using eight certified standards at concentrations of 0, 2.6, 7.79, 15.58, 36.35, 77.9, 129.83, and 207.73 parts per billion (ppb) of mercury. The resulting curve had a correlation coefficient (R^2) of 0.9998. Similarly, a calibration curve for lead analysis was established using eight certified standards at concentrations of 0, 2.5, 7.5, 15, 35, 75, 125, and 200 ppb. The resulting curve had a R^2 value of 0.9998. The linear nature of the resulting calibration curves, as expressed by R^2 values of 0.9998, indicates precision in the sample preparation methods and reliability in the sample concentrations derived from the ICP-OES analysis. The linearity of these curves can be examined in **Figure 3**.

Figure 4. Quality control results.

Percent Recovery of NIST Water Standard				
Sample ID	**Hg (ppb)**	**Pb (ppb)**	**% Recovery Hg**	**% Recovery Pb**
NIST 2x Dilution	N/A	30.30	N/A	164%
NIST 2x Dilution	N/A	20.52	N/A	111%
NIST 2x Dilution	N/A	22.94	N/A	124%
Duplicate Recovery				
Sample ID	**Hg (ppb)**	**Pb (ppb)**	**% Recovery Hg**	**% Recovery Pb**
B1.V4	<LOQ	8.91	N/A	111%
B1.V4 Duplicate	<LOQ	9.89	N/A	
B1.V8	<LOQ	5.02	N/A	165%
B1.V8 Duplicate	<LOQ	8.27	N/A	
B1.V11	<LOQ	37.21	N/A	108%
B1.V11 Duplicate	<LOQ	40.09	N/A	
B2.V3	<LOQ	65.04	N/A	101%
B2.V3 Duplicate	<LOQ	65.39	N/A	
B2.V7	<LOQ	3855400	N/A	100%
B2.V7 Duplicate	<LOQ	3856250	N/A	
B2.V10	<LOQ	9.93	N/A	391%
B2.V10 Duplicate	<LOQ	38.81	N/A	
Certified Hair Standard Recovery				
Sample ID	**Hg % Recovery**		**Pb % Recovery**	
B1.V13	102%		223%	
B1.V14	93%		226%	
B2.V13	100%		312%	
B2.V14	94%		291%	

The NIST water standard was included in analysis in order to determine the precision of sample handling when preparing for ICP-OES analysis, as well as to identify any sample contamination that may have occurred in the spectroscopy laboratory. The percent recoveries of some of the water standard samples were acceptable, being within 20-25% of total recovery, though one sample recovered high, and overall the samples favored a higher percent recovery, indicating there may have been some lead contamination during ICP-OES sample preparation and analysis.

The certified reference hair standard was included in analysis in order to determine the precision of sample handling during the sample transfer, acid digestion, and quantitative transfer procedures. Additionally, the hair standard served to identify any contamination during these processes.

Sample Analysis

Analysis determined that none of the samples contained detectable mercury, and 35% (n=17) of Bangladesh samples contained lead content higher than the FDA criteria of 10 ppm. 14% (n=7) of the U.S. sourced samples contained more than 10 ppm of lead. Two samples were below the ICP-OES LOQ of 2 ppb in lead content, and were excluded from analysis. Two samples were above the LOQ of 200 ppb in lead content, but were included in the analysis with outliers included. Outliers were defined as samples with values greater than three times the standard deviation, and were excluded from one set of analysis in order to decrease the variability in the dataset and allow for analysis that may be more representative of the population. Statistical analysis of mercury content in samples could not be conducted because all samples were below the LOQ. Descriptive statistics of the lead content in the samples, with and without outliers included in analysis, can be found in **Figure 5**.

Figure 5. Descriptive statistics including mean and standard deviations of lead concentrations in sindoor samples purchased in the United States and Bangladesh.

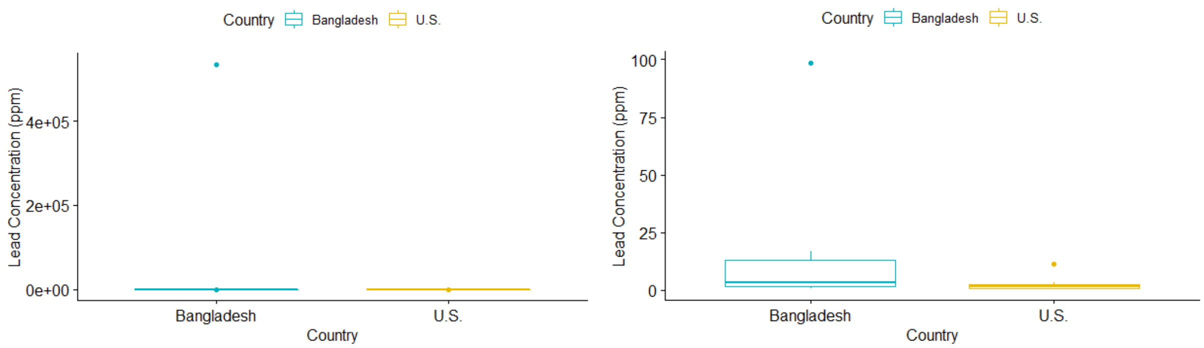
Data Analysis Without Outliers Included		
Descriptive Statistic	U.S. Sindoor (n=7)	Bangladesh Sindoor (n=13)
Minimum lead detected (ppm)	0.4679	0.8020
Mean lead levels (SD) (ppm)	2.9220 (3.8879)	5.9776 (5.7651)
Median lead levels (ppm)	1.5409	3.2239
25%-75% IQR	2.7266	10.6145
95th percentile of lead content	2.9220 ± 2.88	5.9776 ± 3.13
Maximum lead detected (ppm)	11.4373	16.9191
p-value	0.2274	

Data Analysis With Outliers Included		
Descriptive Statistic	U.S. Sindoor (n=7)	Bangladesh Sindoor (n=15)
Minimum lead detected (ppm)	0.4679	0.8020
Mean lead levels (SD) (ppm)	2.9220 (3.8879)	35618 (137899.293)
Median lead levels (ppm)	1.5409	3.4676
25%-75% IQR	2.7266	12.6942
95% Confidence Interval	2.9220 ± 2.88	35618 ± 69800
Maximum lead detected (ppm)	11.4373	534,094
p-value	0.5078	

Statistical analysis using an unpaired two-tailed t-test showed there was no statistical significance in lead content between Bangladesh and U.S. samples, regardless of inclusion of outliers in analysis. When comparing the interquartile range (IQR) between samples without outliers included in analysis, there are notable differences in the third and fourth quartiles as can be observed in **Figure 6**.

Figure 6a-b. Lead distribution by median with versus without outliers included in analysis.

a. Lead distribution with outliers **b.** Lead distribution without outliers



DISCUSSION

Given the linearity of the calibration curves established for this analysis, as well as the percent recovery data, we can assume that the values derived from spectroscopy analysis are overall reliable. There was an incident of bleedover in the case of one of the outlier samples, in which the concentration of lead in that sample was great enough that lead carried over in the spectrometer and contaminated neighboring samples. This was determined by the presence of lead in blanks positioned next to the high lead concentrate sample, and likely caused lead to measure higher for several samples than was accurate. One of the duplicate samples likely underwent this process based on its placement in the ICP-OES, and so the percent recovery for that duplicate is high as a result. The high lead percent recoveries—at one or two times greater than the ideal percent recovery of 100%—observed in the hair standard indicates there may have been lead contamination in the sample transfer and digestion process, or during quantitative transfer. We can assume that our quantification of mercury is accurate, given the quality control data showing hair standard percent recoveries within ten percent of 100% recovery.

In future sample analysis, it would be beneficial to manually run samples on the ICP-OES to allow for additional rinses in order to prevent situations such as bleedover. Additionally, samples below the LOQ could be concentrated and ran again in order to obtain a quantitative value for metal content. This would be particularly valuable for mercury content analysis, given that mercury content was so low comparisons between sample groups could not be conducted. Samples above the LOQ could similarly be diluted and ran again in order to obtain a more accurate concentration, as well as prevent contamination of other samples through bleedover.

There was not a significant difference in lead content between sindoor samples sourced from the U.S. and those sourced from Bangladesh, as the p-values for both methods of comparison were greater than 0.05. However, given that a considerable percentage of Bangladesh samples had lead content greater than 10 ppm, it cannot be fully ruled out that lead content in sindoor in Bangladesh is not a concern. Additionally, this percentage was much greater in Bangladesh than in U.S. samples, indicating there may be higher lead content in sindoor from Bangladesh as was originally suspected. It is also important to note that about half of Bangladesh samples contained lead at concentrations greater than that of U.S. samples, as we can observe in **Figure 4**; again indicating a difference in lead content between sample groups. A small sample size of less than 30 samples in each group likely contributed to the insignificant result, and the results may have been significant had there been a greater number of samples included in analysis.

Similarly, our hypothesis cannot be confirmed that there is a significant difference between sample groups in mercury content, because all sample concentrations were below the LOQ in the ICP-OES analysis. However, we can confirm that all samples are acceptable by FDA criteria for mercury content in cosmetics, given that the LOQ is much lower than the FDA criteria.

Given this difference in lead and mercury content in samples, it is possible that lead-containing compounds are more favorable in the manufacturing process currently. Where mercury sulfide may have previously been used in achieving red pigmentation in sindoor, lead tetroxide, also a red pigmented compound used in cosmetics manufacturing, may be preferred. While there are several factors that likely contribute to this preference, cost likely plays a large role given the price differences between mercury sulfide at $1.85 USD per gram and lead tetroxide at $0.34 USD per gram.[20,21]

Based on these data, we can assume that sindoor is unlikely to be contributing to mercury poisoning in women in Bangladesh. However, it is possible that sindoor may be contributing to lead exposure in women in Bangladesh, especially those who use it daily or are orally exposed. While some of these products are safe for use, over a third of the Bangladesh samples are not due to their lead content being greater than the FDA standard. Given these findings, future research of the manufacturing and distribution of sindoor in Bangladesh would be beneficial in understanding how it is contributing to lead exposure in women in Bangladesh. Additionally, tracing the consumers that are using sindoors brands known to have unsafe lead levels and educating the public about heavy metals in cosmetics might prove effective in protecting those in Bangladesh currently being exposed to toxic heavy metals.

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