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Analysis of Tooth Enamel Using ICP-MS

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Heavy Metal Exposure in a late 19th and early 20th century San Francisco Bay Population:
Analysis of Tooth Enamel Using ICP-MS

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

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in the

OFFICE OF GRADUATE STUDIES

of the

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DAVIS

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Abstract

Adult tooth enamel is useful for understanding environmental exposure to heavy metal toxicants during childhood tooth development because, unlike bone, enamel does not remodel. Recent construction activity exposed human remains at several historic cemeteries in the cities of San Francisco and San Jose, California. The San Francisco cemeteries were in operation from 1870–1903, and the San Jose cemetery from 1870–1935. Based on historical records, we know that some cemeteries required payment for burial and were associated with higher socioeconomic-status individuals, including Odd Fellows Cemetery (n=8) and Masonic Cemetery (n=2) in San Francisco. By contrast San Francisco’s Golden Gate “City” Cemetery (n=2) and Santa Clara Valley Medical Center in San Jose (n=15) form the lower socioeconomic status population, as these individuals are from free or “pauper” graves. Tooth enamel was analyzed using Inductively Coupled Mass Spectrometry (ICP-MS) to measure the concentrations (ppm) of arsenic, lead, and mercury. Contrary to the study’s hypothesis that lower socioeconomic individuals would have higher concentrations of heavy metals, results of this research show either no statistically significant difference between the populations (for arsenic and mercury), or that the higher socioeconomic group had significantly higher concentrations (for lead).

Table of Contents

Abstract	ii
Table of Contents	iii
List of Tables	iv
List of Figures	v
Acknowledgements	vii
Introduction	1
Background	2
Tooth Formation and Development	2
Heavy Metal Uptake in Teeth	3
Measuring Heavy Metal Concentrations with ICP-MS	4
Research Aim	6
Materials & Methods	6
Tooth Sample Preparation	10
Enamel Digestion	10
ICP-MS Instrumentation	12
Results	13
Discussion	18
Conclusion	26
References	28

List of Tables

Table 1. Historic tooth samples submitted for LA-ICP-MS analysis. Cultural Resource Management Project (CRM) project location in which human tooth samples were recovered, sample catalog number, and tooth type (e.g., MLM3 = upper left third molar; URdc = upper right)..... 8

Table 2. The results of ICP-MS instrumentation, in ppm. The only samples from female adults are ICPMS-6 and ICPMS-11. The deciduous teeth included in the original sample batch, which are also included in figures 1-5, are in bold. These teeth are not included in Table 3, nor Figure 6 onwards. Because of the timing of when deciduous teeth initially form, which is at about five-weeks' gestation, these teeth would reflect a buffering effect from the mother. 13

Table 3. Descriptive statistics and calculated p-values using the Google Sheets calculation command =T.TEST ([range 1], [range 2], 1, 3), for single-tailed data sets, with uneven weight of higher to lower socioeconomic sample sizes as per a Welch's t-test. 18

List of Figures

Figure 1. Arsenic levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.	15
Figure 2. Mercury levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.	15
Figure 3. Lead levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.	16
Figure 4. Comparison of samples from the higher socioeconomic-status individuals across all three heavy metals of interest.	16
Figure 5. Comparison of samples from the lower socioeconomic-status individuals across all three heavy metals of interest.	17
Figure 6. The PCA graph presents the population clusters that help identify our higher socioeconomic group (clustered above 0 value on PC1) and our lower socioeconomic group (clustered below 0 value on PC1).	19
Figure 7. Box-and-whisker plots the median and quartile ranges of arsenic concentrations comparing low- and high-class individuals in our sample. The solid horizontal line presents the mean, error bars present the 25 th and 75 th percentiles, and outliers are shown as data points.....	20
Figure 8. Box-and-whisker plots of the median and quartile ranges of lead and mercury concentrations comparing low- (blue) and high-class (red) individuals in our sample. The solid	

horizontal line presents the mean, error bars present the 25th and 75th percentiles, and outliers are shown as data points. 21

Figure 9. Comparison of arsenic concentrations in M1 and M3 tooth samples from the VMC.. 22

Figure 10. Comparison of mercury concentrations in M1 and M3 tooth samples from the VMC.
..... 23

Figure 11. Comparison of lead concentrations in M1 and M3 tooth samples from the VMC. ... 23

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Introduction

After death, skeletal remains are often all that is left of a person, both in a historical sense and in a forensic one. Bones can carry scars, such as signs of healed injuries or remnants of a death struggle, which can help to piece together the story and identity of the person. For the skeletal structure of a human, only the teeth face daily exposure to one's environment (e.g., calculus or plaque). There are many ways in which teeth help to identify and illuminate a deceased person's life experiences. One method for understanding environmental exposure through the analysis of dental enamel using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to measure traces of heavy metals. Forensic scientists, environmental toxicologists, and archaeologists use tooth enamel to examine an individual's exposure to heavy metals at different points during their lives, especially during childhood.

Enamel in permanent first molars record environmental exposure from birth to about three years of age, as this is the period when the tooth crown is forming; permanent third molar enamel records exposure from about seven to 15 years of age. During tooth formation, trace elements, like lead, can be transported through the bloodstream and deposited in a tooth's hydroxyapatite matrix. For this research, ICP-MS is used to measure heavy-metal concentrations of arsenic, lead, and mercury in tooth enamel samples from late 19th and early 20th century cemetery populations from the San Francisco Bay region. The burial populations come from two different socioeconomic classes: 1) higher status individuals who paid for burial, and 2) lower status individuals interred in free or "pauper" graves. This study adds to a growing dataset on ancient, early industrial, and modern heavy metal levels in human populations (Asaduzzaman et al., 2017; Kamenov et al., 2018; Maoyong et al., 2016; Martinez-Garcias et al., 2005).

Background

Tooth Formation and Development

Human teeth differ from the rest of the skeleton in terms of growth and chemical composition. There are two main parts of teeth: 1) the crown, or the section visible above the gum line, and 2) the roots, or the section set into the bone of the upper and lower jaws (Hillson, 2005: Fig. 2.2, 2.34). Three major dental tissues make up teeth: 1) enamel, 2) dentin, and 3) cementum. Dentin is the inner dental tissue, which is covered by enamel on the crown and by cementum on the root. Overall, each of these tooth tissues is more *inorganic* than any other skeletal tissue in the body (i.e., bone). Enamel, the hardest tissue in the human body, is 97–98% inorganic and is the part of the tooth that engages directly with the environment or oral microbiome. The inorganic component of enamel is made up of a calcium and phosphorus apatite mineral, known as hydroxyapatite (89%), with small amounts of calcium carbonate (4%), calcium fluoride (2%), and magnesium phosphate (1.5%) (Hillson 2005). Dentin comprises the largest portion of a tooth, which is made up of a mix of approximately 70% inorganic hydroxyapatite crystals, about 20% organic collagen matrix proteins, and 10% water by weight (Goldberg et al. 2011). Cementum is about 50% hydroxyapatite and 50% organic materials and water by weight, and thus it is the softest of the three dental tissues (Goldberg et al. 2011; Hillson 2005).

This thesis focuses on the enamel of permanent (adult) teeth, specifically the first and third molars. Enamel formation in first molars starts at around birth and finishes at approximately three years (Hillson 2005: Table 5.1). The second molar starts forming at 3–4 years and ends at 6–7 years (Hillson 2005: Table 5.1). Third molars are more variable, with

enamel formation starting at 7–9 years and finishing at approximately 14–15 years (Jung & Cho 2014).

Because of their specialized anatomy, teeth are more resilient to taphonomic processes or diagenesis, which is the term used to encompass any physical or chemical change that happens to skeletal materials after deposition or burial in sediment (Hillson 2005; Lyman 1994). Multiple studies emphasize that resistance to decomposition and diagenesis is what makes teeth so useful for archaeological and forensic analyses (AlQahtani et al. 2010; Martirosyan et al. 2025). Martirosyan et al. (2025) note that ameloblasts lay down enamel in layers that follow the body's natural circadian rhythm. Because tooth growth follows this natural pattern, it can provide a geochemical profile of a person's diet and environmental exposures during the time of formation. Of importance to this study, unlike bone, there is no turnover or remodeling of enamel, and thus, teeth provide a record of heavy metal exposure during particular windows of time in a person's life.

Heavy Metal Uptake in Teeth

Ingestion of heavy metals in drinking water or food can result in subsequent deposition in teeth. Archaeologists, environmental toxicologists, and forensic scientists can use tooth enamel to examine an individual's exposure at different points during their childhood. During matrix production and maturation by ameloblasts, cationic and anionic centers form between hydroxyapatite layers of enamel (Kang et al. 2004). These centers are normally fitted between the regular incremental growth layers and usually consist of calcium (Ca^{2+}) and phosphate (OH^- , PO_4^{3-}), but they can be substituted with various other strong cationic and anionic elements (Kang

et al. 2004). Because enamel does not turn over like other organic tissues in the body, these growth layers and trace elements remain in readable lines of chronology.

The body absorbs trace elements (e.g., lead, arsenic, mercury) through ingestion, inhalation, or epidermal contact. As with bone, trace elements like Pb^{2+} from the bloodstream can substitute for Ca^{2+} in the hydroxyapatite matrix. Thus, teeth can provide a durable record of heavy metal exposure (e.g., Asaduzzaman et al., 2017; Malara et al., 2016). Further, chemical exchange with the environment may affect a small layer – 30 μm – of enamel, which can lead to enhanced Pb concentrations (Budd et al. 1998). It should be noted as well, the differences in heavy metal storage of the three trace elements of focus here; while lead is typically stored in the bones and teeth, mercury is more often stored in proteins like musculature, and arsenic is quickly broken down in the body, leading to it having a short half-life of ~24 hours. These differences are reflected in the overall higher levels of lead in teeth and bones than mercury and arsenic.

Measuring Heavy Metal Concentrations with ICP-MS

ICP-MS measures analyte concentrations in a matrix by detecting ions' mass-to-charge ratios. The front end of the instrument breaks larger molecules into smaller, charged fragments via pressurized argon plasma, which the MS detector then reads to determine the concentration of elements within the sample. ICP-MS instruments have low detection limits, making them a common choice for the study of trace elements in tooth enamel.

In archeological tooth studies, ICP-MS has been used to identify differences in environmental lead contamination of modern versus ancient populations. Webb et al. (2005), compare modern dental samples from Mexico and Egypt to those from Bronze Age (ca. 2200 BC) deposits at Tell Abraha on the Persian Gulf. In their study, ICP-MS analysis in conjunction

with atomic emission spectroscopy (ICP-AES) confirmed that modern populations have significantly higher lead concentrations than humans from the Bronze Age.

Asaduzzaman et al. (2017) studied 50 permanent teeth from Malaysia using ICP-MS to understand levels of environmental pollutants and to examine heavy metal concentrations across multiple parameters, including sex, age, ethnicity, tooth condition, and tooth type. They found that metal concentrations increased with age, but also that females had generally higher concentrations in tooth dentin than males. By comparison, Budd et al. (1998) evaluated teeth from six archeological sites around the UK from different historical periods and compared their lead concentrations to samples of modern, shed, deciduous teeth and one unerupted permanent tooth spanning from the 1960s to late 1990s. Using Laser Ablation ICP-MS (LA-ICP-MS), they scanned individual enamel and dentin layers to create a chronological timeline of lead exposure. They found that lead concentrations within the surface layer of enamel was more likely from chemical exchanges *in vivo*; in other words, historical surface layer concentrations were more likely from similar environmental exposures, rather than post-mortem diagenesis from the burial environment. This shows that teeth retain information on environmental exposure during the time those teeth formed.

This research into the structure of human teeth allows us to glean a better understanding of both past and contemporary environments and behaviors. Because we know how teeth are developed within the body (both *in vivo*, and *in vitro*), we can use ICP-MS to investigate environmental toxicant exposures in different populations over time.

Research Aim

This research aims to use ICP-MS to measure lead (Pb), mercury (Hg), and arsenic (As) levels in tooth enamel, and to compare the relative concentrations of these three heavy metals between two different socioeconomic populations from the late 19th and early 20th century in the San Francisco Bay area. One population comes from a cemetery where people paid to be buried and, thus, are presumed to be higher in socioeconomic status. The other population comes from “pauper” cemeteries, where people are presumed to be from a lower socioeconomic status. These samples provide an environmental marker for metal exposure by different socioeconomic populations residing in the San Francisco Bay region 100 to 150 years ago.

Hypothesis: During the late 19th and early 20th century in the greater San Francisco Bay area, individuals of lower socioeconomic status will have greater heavy-metal concentrations of arsenic, lead, and mercury in their tooth enamel than individuals of higher socioeconomic status. We predict this difference will be because they are more likely to have been living and/or working in more heavy-metal contaminated environments than higher socioeconomic status individuals.

Materials & Methods

Human tooth samples for this thesis project come from archaeological mitigation efforts during cultural resource management projects (King 2020) undertaken in advance of construction work in the cities of San Francisco and San Jose, California. These dental samples are part of a larger dissertation research project by UC Davis Anthropology PhD candidate Diana

Malarchik on human diet and health of historic populations living in the San Francisco Bay area. During modern construction monitoring, archaeologists exposed human remains from late 19th and early 20th century cemeteries. Based on historical records, we know that some cemeteries required payment for burial and were associated with higher socioeconomic status individuals. One of these higher status cemeteries was located at Arguello and Anza Streets in San Francisco—under what is now San Francisco Parks and Recreation’s Rossi Pool (**Table 1**). This cemetery was formerly part of the Odd Fellows Cemetery, which was in operation between ~1870-1900. Here we have tooth samples from eight adult individuals (incisor, n=1; first molar, n=2; second molar, n=1; third molar, n=4). Tooth samples from an additional four individuals were recovered from under the University of San Francisco’s War Memorial Gymnasium (deciduous molar, n=1; deciduous canine, n=1; incisor, n=1; premolar, n=1). Historically, this cemetery was known as the Masonic Cemetery and like the Odd Fellows Cemetery it was also in operation between ~1870–1900. Together there are 10 adults and two juveniles from higher socioeconomic status cemeteries. Although the focus of analysis is on adults across higher and lower socioeconomic classes, we sampled the two juveniles to see if we could discern any differences between those who died young versus those who survived to adulthood within the higher socioeconomic class.

In addition to the 10 higher socioeconomic status adults described above, the study also includes 17 tooth samples from lower socioeconomic status cemetery populations (**Table 1**). Under Baker Beach in what was San Francisco’s Golden Gate “City” Cemetery (1871–1901), archaeologists recovered two adult individuals during construction by the San Francisco Public Utilities Commission (third molar, n=2). The majority of the lower socioeconomic status samples are from a cemetery associated with the Santa Clara Valley Medical Center (SCVMC)

in San Jose. The SCVMC cemetery was created in the early 1870s after a local cholera outbreak, and the medical center and cemetery remained in use until 1935. Despite approximately 60 years of use, few records remain, and the gravesite itself was forgotten after it stopped appearing on city maps and was paved over to become a parking lot in the 1940s. During construction efforts in 2012, the gravesite was rediscovered, and during excavation, remains of over 1000 individuals were unearthed. Most individuals were likely in “pauper graves,” some even buried with surgical equipment remaining inside. We include tooth samples from 15 adults from the SCVMC (paired first and third molars). Here we can test the hypothesis that individuals of lower socioeconomic status would have been exposed to higher concentrations of lead, mercury, and arsenic by virtue of their working and/or living conditions.

Table 1. Historic tooth samples submitted for LA-ICP-MS analysis. Cultural Resource Management Project (CRM) project location in which human tooth samples were recovered, sample catalog number, and tooth type (e.g., MLM3 = upper left third molar; URdc = upper right).

CRM Project Location	Historic Cemetery	Sample Number	Sex	Tooth	Lab Number	Socioeconomic Status
Rossi Pool, San Francisco	Odd Fellows	Feature 2, Sample 2	M	MLM3	ICPMS-1	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 8.1b, Sample 1	M	MLI1	ICPMS-2	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 8.4b	M	LRM3	ICPMS-3	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 9.52	M	LRM2	ICPMS-4	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 10, Sample 1	M	LRM3	ICPMS-5	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 11, Sample 1	M	LRM1	ICPMS-7	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 15, Sample 1	M	LRM3	ICPMS-8	Higher
Rossi Pool, San Francisco	Odd Fellows	Feature 17, Sample 2	F	LRM1	ICPMS-6	Higher

University of San Francisco War Memorial Gym	Masonic	SP265	M	MLdm1	ICPMS-9	Higher
University of San Francisco War Memorial Gym	Masonic	Burial 1, Cat. 2	M	LRPX	ICPMS-10	Higher
University of San Francisco War Memorial Gym	Masonic	Burial 3, Cat. 74	F	MLI1	ICPMS-11	Higher
University of San Francisco War Memorial Gym	Masonic	SP254	M	URdc	ICPMS-12	Higher
San Francisco Public Utilities Commission, Baker Beach	City Cemetery	Burial 4, Ind. 1	M	MLM3	ICPMS-13	Lower
San Francisco Public Utilities Commission, Baker Beach	City Cemetery	Burial 7, Ind. 1	M	LCM3	ICPMS-14	Lower
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-116	M	URM3	ICPMS-17	Lower
				URM1	ICPMS-18	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-175	M	LLM3	ICPMS-19	Lower
				LLM3	ICPMS-20	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-274	M	LRM1	ICPMS-43	Lower
				LRM3	ICPMS-44	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-415	M	LRM1	ICPMS-37	Lower
				LRM3	ICPMS-38	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-426	M	LLM3	ICPMS-23	Lower
				LLM1	ICPMS-24	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-434	M	MLM1	ICPMS-25	Lower
				MLM3	ICPMS-26	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-437	M	URM3	ICPMS-27	Lower
				URM1	ICPMS-28	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-440	M	MLM1	ICPMS-29	Lower
				MLM3	ICPMS-30	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-314	M?	LLM3	ICPMS-21	Lower
				LLM1	ICPMS-22	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-494	M	MLM3	ICPMS-33	Lower
				MLM1	ICPMS-34	
		VMC-576	M	URM1	ICPMS-41	Lower

Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery			URM3	ICPMS-42	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-611	M?	URM3	ICPMS-35	Lower
				URM1	ICPMS-36	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-613	M	MLM1	ICPMS-31	Lower
				MLM3	ICPMS-32	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-685	M	URM3	ICPMS-15	Lower
				URM1	ICPMS-16	
Santa Clara Valley Medical Center, San Jose	SCVMC Cemetery	VMC-718	M	LLM1	ICPMS-39	Lower
				LLM3	ICPMS-40	

Tooth Sample Preparation

In the Archaeometry Lab at UC Davis, each tooth sample (**Table 1**) had been prepared by sectioning them into vertical halves using a low-speed Isomet saw, thereby creating a complete crown to root segment. Powdered enamel was removed from one-halved section of each tooth using a Foredom drill with a stainless-steel bit.

Enamel Digestion

Enamel digestion started by labeling a series of 2mL microcentrifuge tubes and recording the empty container weights. Using a precision balance scale with 0.001mg (1 μ g) precision, we weighed approximately 30mg of each powdered enamel sample into a microcentrifuge tube. Using the UC Davis ICPMS lab protocols for enamel digestion, the suitable mass range for digestion was 27–33mg. We also prepared a similar mass of NIST1486 bone meal, which is a certified reference material to ensure element recovery. We prepared one Method Blank (MB1) with 30 μ L MilliQ Water (MQW), and two control standards: a first Laboratory Control Standard (LCS1) with 300 μ L NIST1643F Trace Elements in Water, and a second Laboratory Control

Standard (LCS2) with 30 μ L 10ppm ICV custom mix and 30 μ L 1ppm custom mix of single element standards of gallium (Ga), rubidium (Rb), cesium (Cs), and mercury (Hg).

To all samples, standards, and blanks in the batch, we added 500 μ L 50% HNO₃, which was a 1:1 ratio of concentrated trace metals in HNO₃ to MilliQ water. Next, we added 167 μ L of concentrated trace metals in HCl, as well as 125 μ L 100ppm gold (Au). These two additions were needed for the Hg measurements. After adding these to each microcentrifuge tube, we left the batch uncapped in the fume hood for 30 minutes, to allow for release of gasses. After this first half hour, we checked the samples to make sure no solids had been pushed up the tube by digestion bubbles. As there was no remaining solid material, we placed the uncapped microcentrifuge tubes into a hot-block water bath, and heated them for 15 minutes at 95° C. We allowed the samples to cool before moving on to the next additions.

The next addition was 50 μ L 30% H₂O₂, which was added gently to not cause sample spill-over or spitting. After we added the first 50 μ L, we returned each tube to the hot-block bath for five minutes, then added another 100 μ L of the same 30% H₂O₂. We repeated this addition of 100 μ L and waited five minutes two more times, and then after the fourth, five-minute period, added 150 μ L of H₂O₂. This gave us a total addition of 500 μ L of 30% H₂O₂ over the course of 25 minutes. Once all the H₂O₂ had been added to the batch, we left the tubes in the hot block to digest at 95° C for another 30 minutes. We then checked the samples for active bubbling, and clarity of the liquid to indicate whether the samples had finished digesting.

Once the batch showed adequate signs of having finished digestion, we removed the samples from the heat, let them cool, and centrifuged as needed to collect all droplets off the sides of the tube and concentrated fluid into the bottom of the tube. At this point, the samples were left alone overnight. The next day, we added MQW to each of the microcentrifuge tubes to

bring the volumes to just below the 1mL mark on the tubes. We then transferred each sample to an appropriately labeled 15mL Falcon centrifuge tube, making sure to measure the sample volume for each using a pipette. We used digital micropipettes to calculate and add an appropriate volume of MQW for the final volume in the Falcon tube to be 3.35mL.

This digestion process resulted in a 3.35mL final volume of digested sample with a 7.5% concentration HNO₃, 5% concentration HCl, and 3.7ppm Au matrix.

ICP-MS Instrumentation

Digested samples were analyzed with the ICP-MS instrument at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry. Within the instrument, the dissolved samples were aerosolized and heated to produce an ionized gas that could be characterized for elemental concentrations by the mass spectrometer. Due to the detection limits of the instrument, each sample needed to be diluted to mitigate possibilities of over-saturation of an element, or under-saturation to the point of unreadability. Because of this, we ran both 5x and 25x dilutions of the samples. The ICP-MS analysis provided concentrations for 34 different elements. However, for this project we focus on the results of arsenic, lead, and mercury.

Results

Results are reported in ppm by multiplying the instrument measurements by the dilution factor (25x) and the digestion factor, the latter of which differs by sample (**Table 2**).

Table 2. The results of ICP-MS instrumentation, in ppm. The only samples from female adults are ICPMS-6 and ICPMS-11. The deciduous teeth included in the original sample batch, which are also included in figures 1-5, are in bold. These teeth are not included in Table 3, nor Figure 6 onwards. Because of the timing of when deciduous teeth initially form, which is at about five-weeks' gestation, these teeth would reflect a buffering effect from the mother.

Archaeological Sample Number	ICPMS Sample Number	Arsenic (ppm)	Mercury (ppm)	Lead (ppm)
RPF2 UCM3	1	0.598	1.851	42.476
RPF8.16 SI MLI1	2	0.413	0.348	61.212
RPF8.46 LRM3	3	268.300	3.470	131.849
RPF9.52 LRM2	4	0.423	0.692	104.991
RPF10 LRM3	5	71.160	0.393	180.855
RPF17 LRM1	6	0.179	1.203	16.247
RPF11 LRM1	7	0.520	0.581	33.021
RPF15 LRM3	8	0.238	0.178	55.307
USFW SP265 MLdm1	9	6.339	0.064	239.739
USFW B1 C2 LRPX	10	0.311	33.013	100.898
USFW B3 C74 MLI1	11	45.753	3.116	44.650
USFW SP254 URdc	12	19.785	0.102	877.607
SFPUC B4.1 MLM3	13	0.512	0.088	5.543
SFPUC B7.1 LCM3	14	0.077	0.545	48.066
VMC 685 M3	15	0.067	2.214	17.944
VMC 685 M1	16	0.046	1.336	20.971
VMC 116 M3	17	0.084	0.403	21.000
VMC 116 M1	18	0.208	0.318	79.606
VMC 175 M3	19	0.229	0.546	13.315
VMC 175 M1	20	0.122	0.322	16.241
VMC 314 M3	21	0.059	1.248	12.107
VMC 314 M1	22	0.088	1.125	24.302
VMC 426 M3	23	0.114	0.120	31.269
VMC 426 M1	24	0.132	0.236	24.039
VMC 434 M1	25	0.203	0.099	10.355
VMC 434 M3	26	0.077	0.119	17.274
VMC 437 M3	27	0.095	0.462	10.392

VMC 437 M1	28	0.059	0.318	4.692
VMC 440 M1	29	0.086	0.188	11.660
VMC 440 M3	30	0.156	0.036	2.819
VMC 613 M1	31	0.081	0.274	15.878
VMC 613 M3	32	0.044	0.217	9.922
VMC 494 M3	33	1.124	1.302	44.622
VMC 494 M1	34	1.348	0.709	24.986
VMC 611 M3	35	0.117	1.624	5.167
VMC 611 M1	36	0.041	4.382	5.676
VMC 415 M1	37	0.071	0.413	39.334
VMC 415 M3	38	0.062	0.332	37.825
VMC 718 M1	39	0.053	0.081	3.921
VMC 718 M3	40	0.051	0.074	3.171
VMC 576 M1	41	0.065	0.147	83.510
VMC 576 M3	42	0.040	0.348	194.938
VMC 274 M1	43	0.055	0.385	19.946
VMC 274 M3	44	0.093	0.983	36.362

Figures 1-3 show the metal concentrations from all individuals for each of the three HM elements of interest and use a log scale on the vertical axis to enhance readability. Figures 4 and 5 compare all three sets of HM data across the two socioeconomic groups: higher and lower.

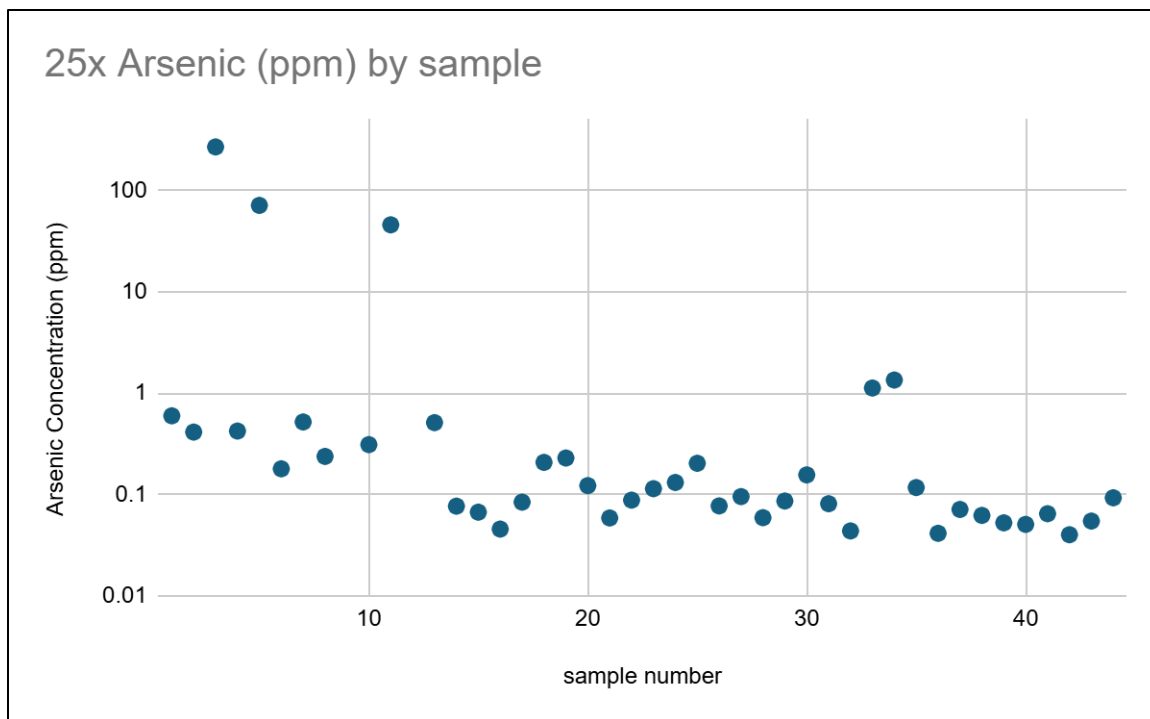


Figure 1. Arsenic levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.

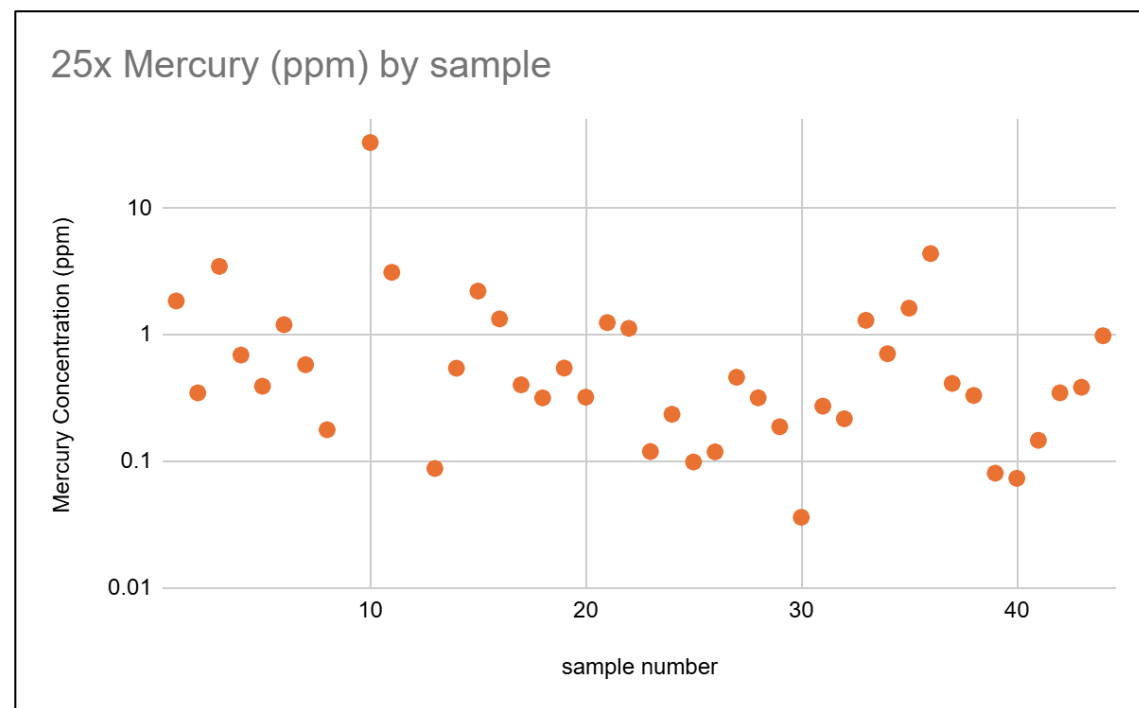


Figure 2. Mercury levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.

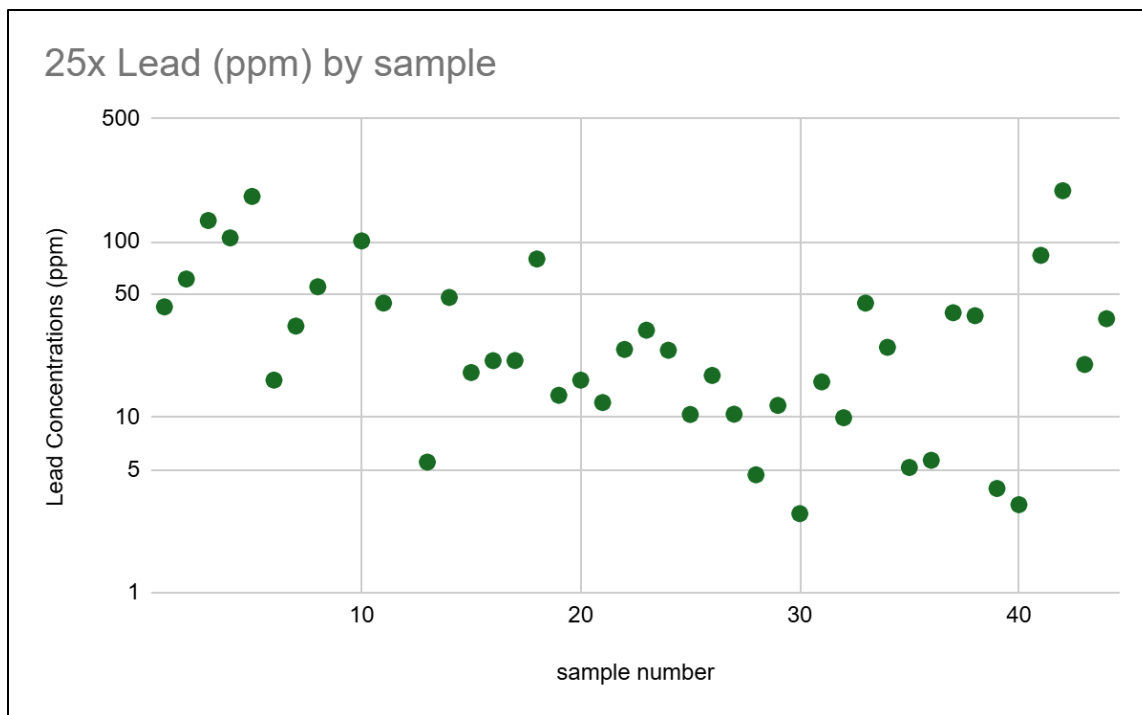


Figure 3. Lead levels across all sampled individuals in ppm, with a log10 scale on the vertical axis.

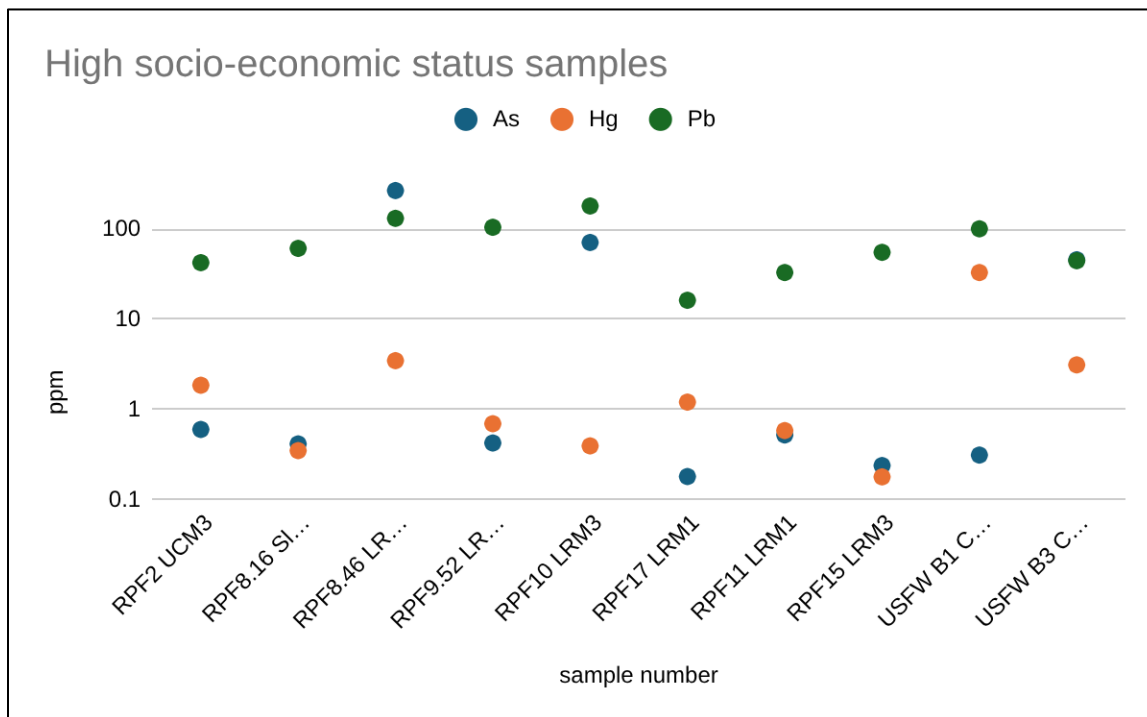


Figure 4. Comparison of samples from the higher socioeconomic-status individuals across all three heavy metals of interest.

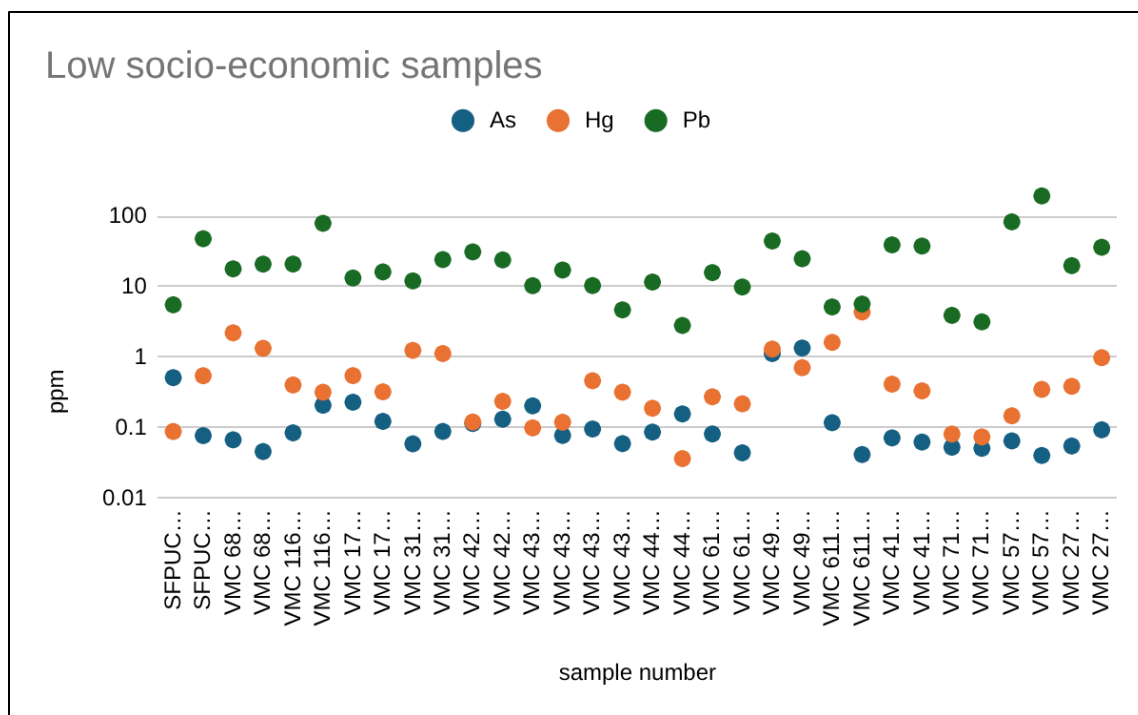


Figure 5. Comparison of samples from the lower socioeconomic-status individuals across all three heavy metals of interest.

Based on descriptive statistics (**Table 3**), the kurtosis value for lead is closer to a normal distribution (Kurtosis of 4.67) than for either arsenic or mercury. Table 3 also presents calculated p-values from a Welch’s t-test of the two data sets (higher and lower socioeconomic status individuals). The two deciduous teeth highlighted in Table 2 were removed from the higher socioeconomic sample because of issues related to fetal development and maternal buffering effects. In this study, a “significant” result represents a p-value less than 0.05, which means that there is less than a 5% chance the difference is due to random effects (given observed variation within the data). A p-value of 0.0368 for lead indicates a significant difference in lead exposure between higher and lower socioeconomic status. These results do not support the original hypothesis that adults from lower socioeconomic backgrounds should have higher lead values. Rather the opposite appears to be the case, with those of higher socioeconomic status having

significantly higher levels of lead exposure. By contrast, arsenic values nearly meet the 0.05 level of significance, and mercury shows the least difference between socioeconomic classes ($p=0.137$).

Table 3. Descriptive statistics and calculated p -values using the Google Sheets calculation command =T.TEST ([range 1], [range 2], 1, 3), for single-tailed data sets, with uneven weight of higher to lower socioeconomic sample sizes as per a Welch's t -test.

Element of Interest	Mean	Median	Skewness	Kurtosis	Welch's t -test p -value
As	9.371	0.105	5.704	34.257	0.077
Hg	1.567	0.398	6.142	38.909	0.137
Pb	29.053	22.519	2.159	4.670	0.037

Discussion

To further evaluate the differences between these two datasets, a Principal Component Analysis (PCA) was performed (**Figure 6**). This PCA takes the principal components of the data set and correlates them across new axes to maximize the spread of the data, thus creating clusters of similar data points that correlate with the two populations (higher socioeconomic status and lower socioeconomic status). By considering the patterns of multiple element concentrations, we observed the higher socioeconomic status group clusters *above* the 0-value of PC1 (32.7% variance), while the lower socioeconomic status group clusters below the 0-value of PC1.

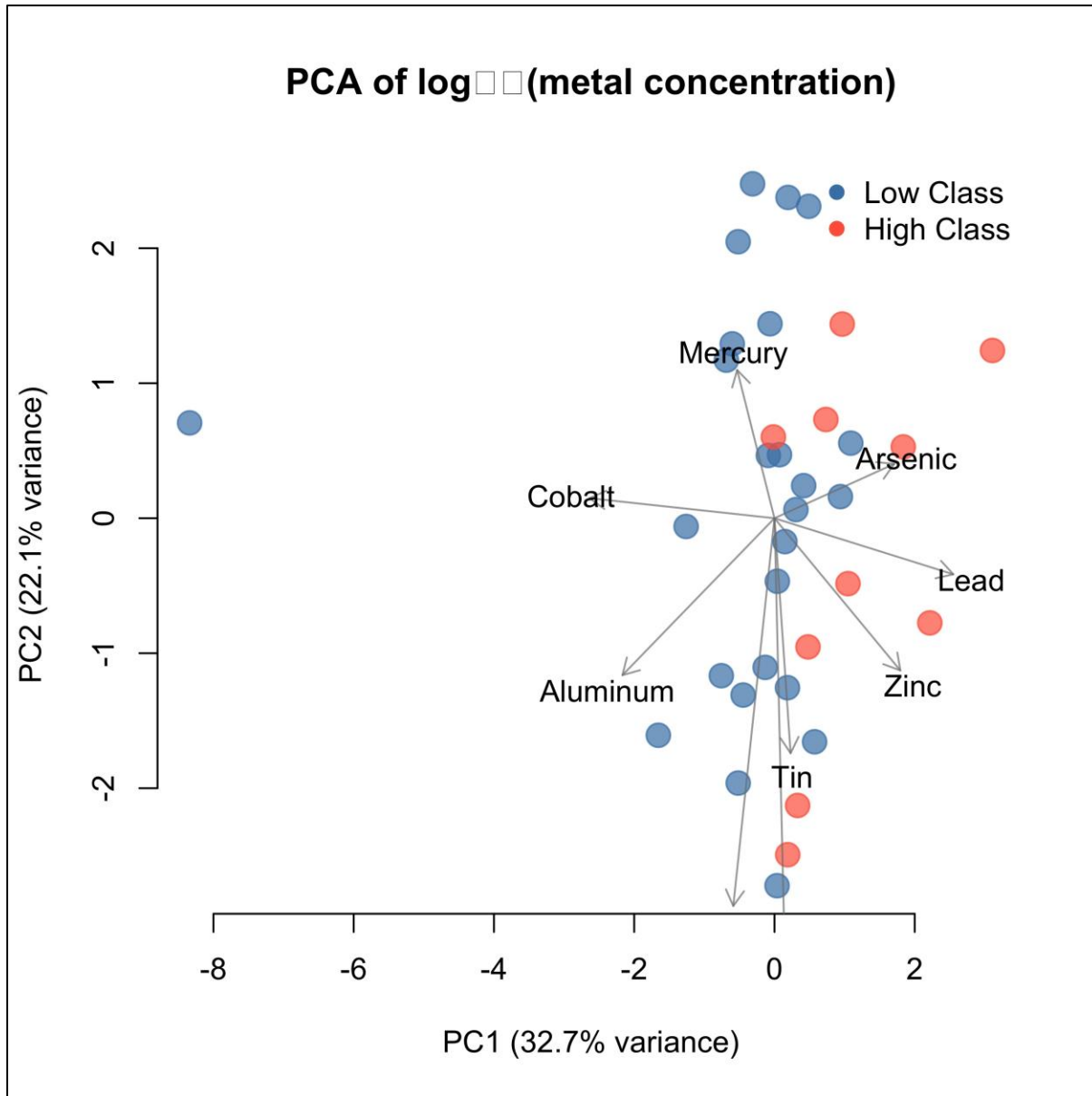


Figure 6. The PCA graph presents the population clusters that help identify our higher socioeconomic group (clustered above 0 value on PC1) and our lower socioeconomic group (clustered below 0 value on PC1).

To better understand the differences in socioeconomic status, **Figure 7** and **Figure 8** present box-and-whisker plots prepared using R, comparing the low-status data to the high-status data. All graphs have a log scale on the vertical axis for readability.

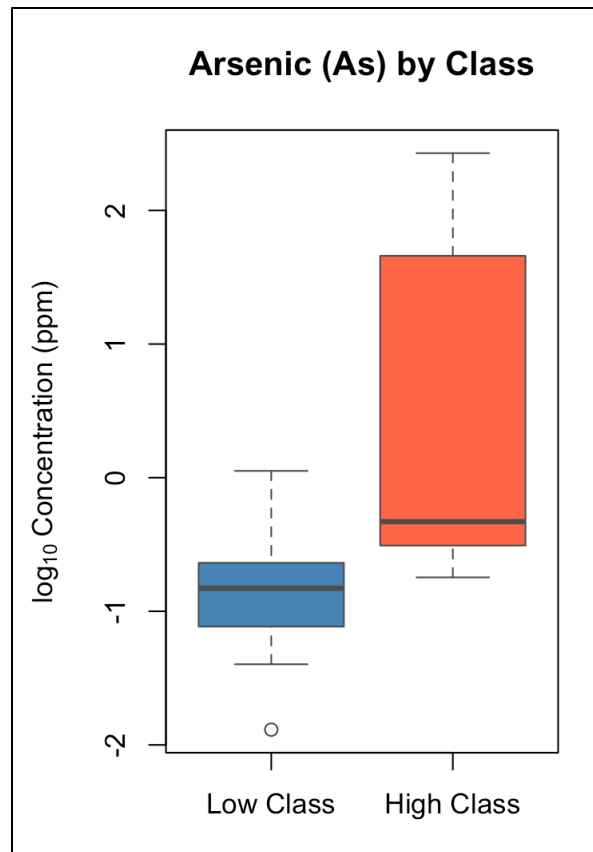


Figure 7. Box-and-whisker plots the median and quartile ranges of arsenic concentrations comparing low- and high-class individuals in our sample. The solid horizontal line presents the mean, error bars present the 25th and 75th percentiles, and outliers are shown as data points.

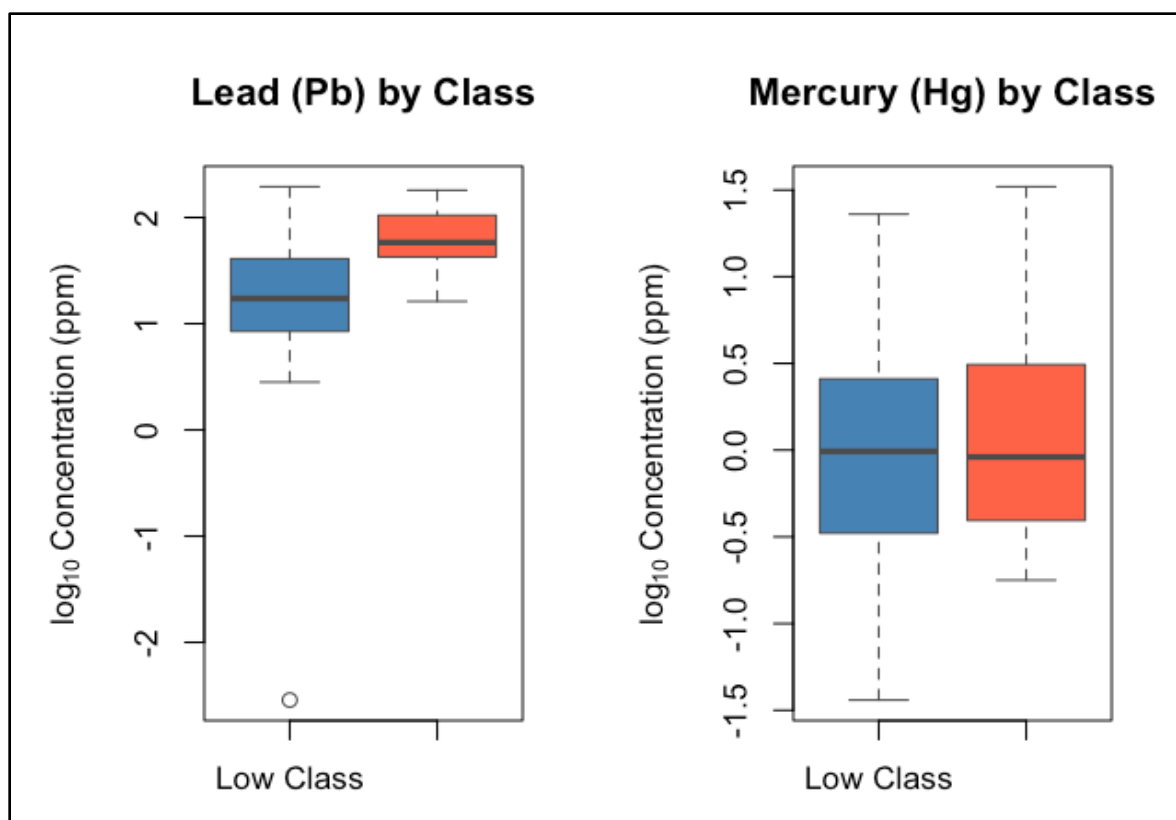


Figure 8. Box-and-whisker plots of the median and quartile ranges of lead and mercury concentrations comparing low- (blue) and high-class (red) individuals in our sample. The solid horizontal line presents the mean, error bars present the 25th and 75th percentiles, and outliers are shown as data points.

As seen in figures 7 and 8, and in Table 3, we do not support the hypothesis that lower socioeconomic class individuals have significantly higher concentrations of heavy metals. Either there is no statistically significant difference in the case of arsenic or mercury, or in the case of lead, the *higher* socioeconomic individuals have greater concentrations, on average. The two deciduous teeth in our sample were excluded from statistical analysis because, as stated in the background section, deciduous teeth form during pregnancy, and so reflect the mother's intake of heavy metals, rather than the sampled individual. These levels would also be affected by the buffering effect of the mother's body on the element concentrations found in the sampled tooth.

For the lower socioeconomic status individuals recovered from excavations at the VMC in San Jose, we had both M1 and M3 data points. Thus, for this sample we had the opportunity to further examine differences in heavy metal exposure over these individual's childhood years versus early adolescence years, when these teeth formed (**Figure 9, 10, and 11**).

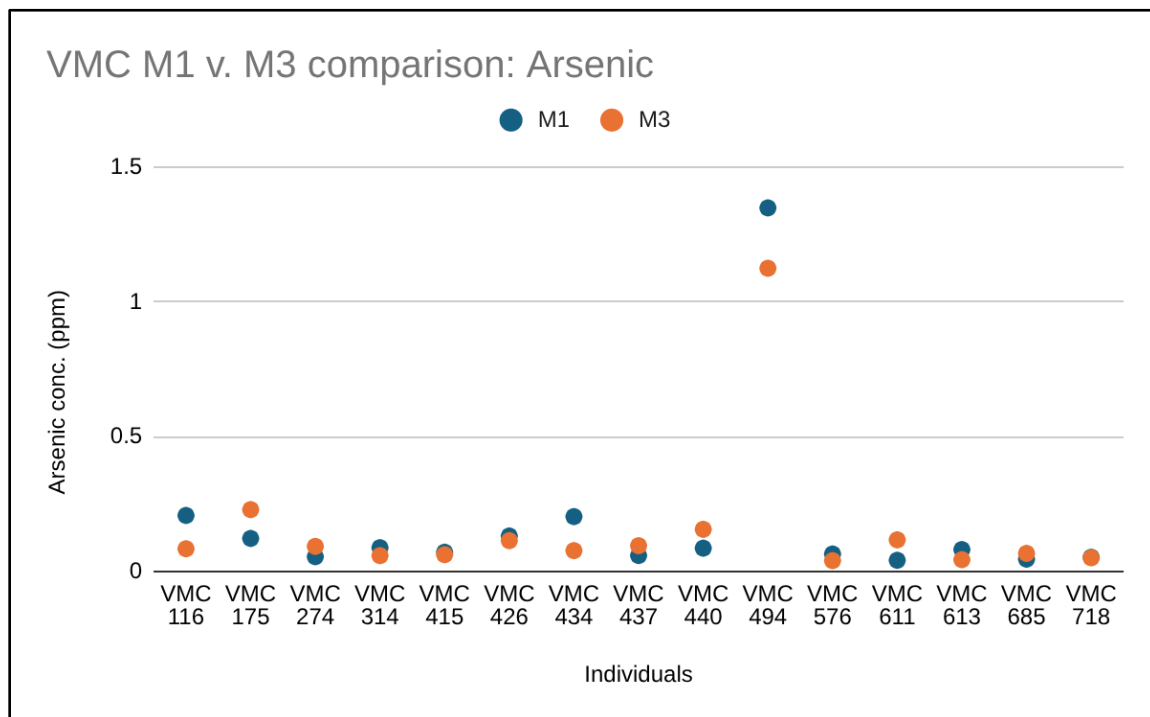


Figure 9. Comparison of arsenic concentrations in M1 and M3 tooth samples from the VMC.

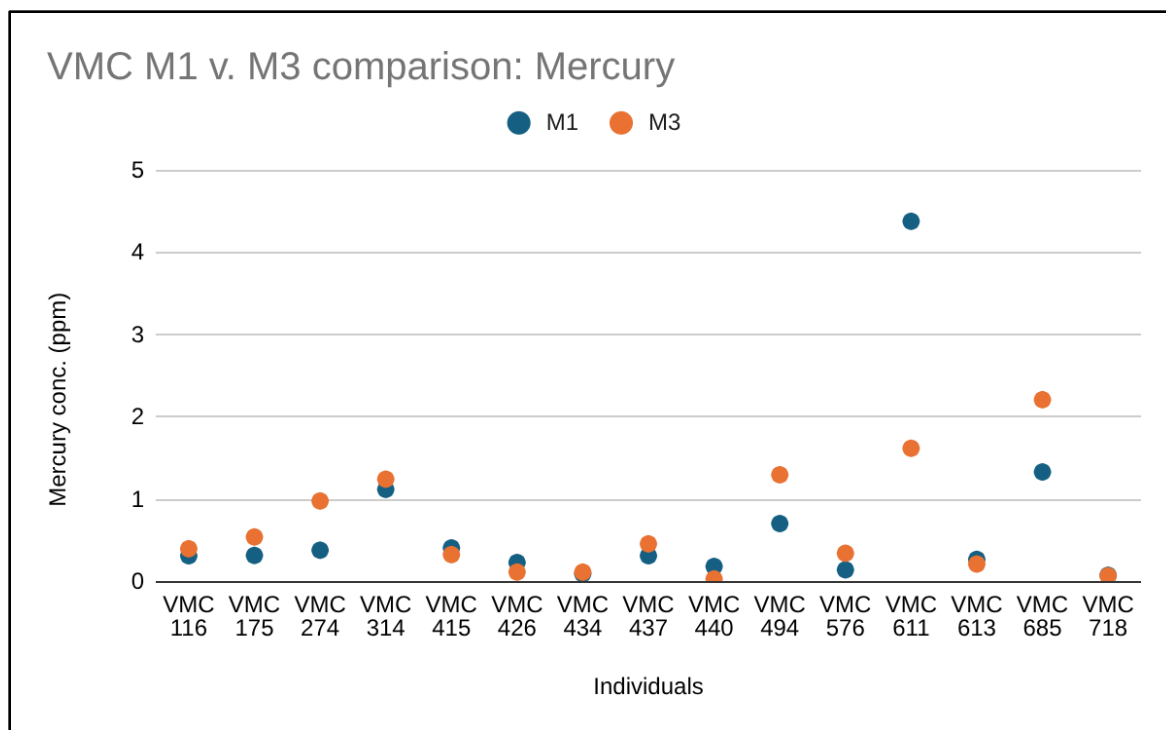


Figure 10. Comparison of mercury concentrations in M1 and M3 tooth samples from the VMC.

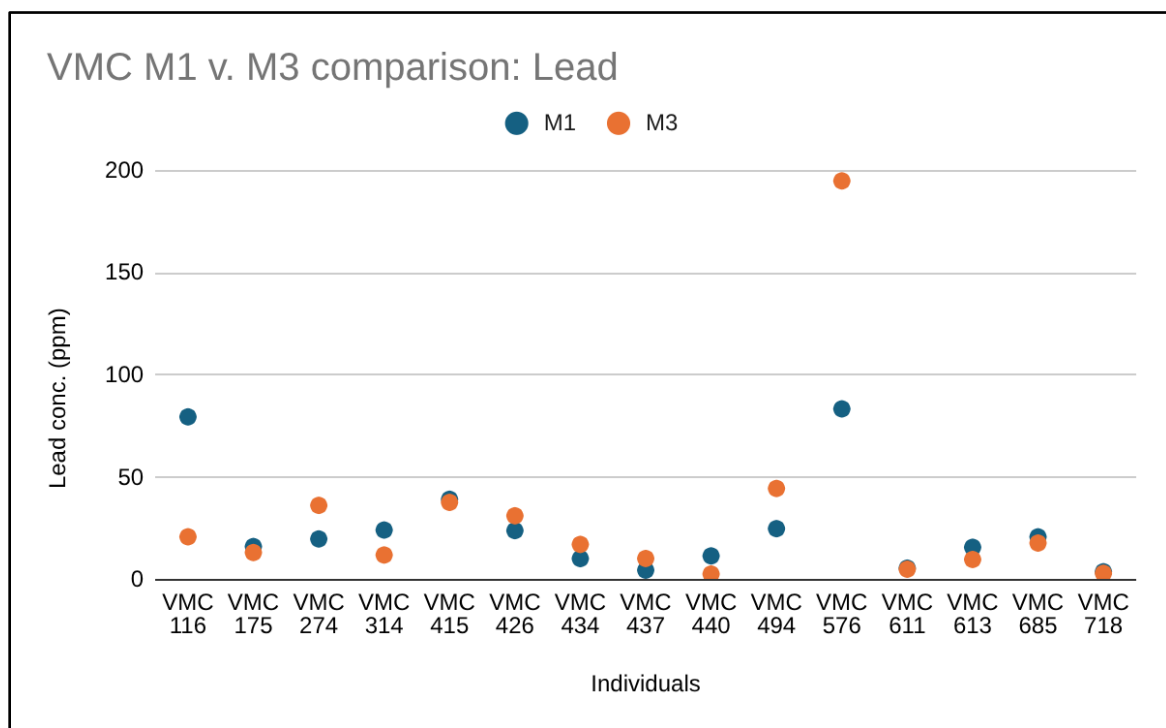


Figure 11. Comparison of lead concentrations in M1 and M3 tooth samples from the VMC.

As shown in figures 9–11, there is not a particular pattern of either increasing or decreasing heavy metal intake over any individual's childhood to early adolescence. In arsenic, eight of the 14 individuals had some degree higher concentrations in their M1 teeth; in mercury the count is only five out of 14; and in lead the count is nine out of 14. Of these individuals, even fewer have consistently higher M1 concentrations across the three heavy metals (n=2) and the same number (n=2) had lower M1 concentrations compared to their M3 concentrations. Across the individuals sampled there is no pattern of increasing concentrations in later adolescence, decreasing concentrations from early childhood, or noticeable repeated ratios of growth/decay in concentration across these age periods.

In a study comparing tooth enamel samples from the Cartagena region of Spain across the Neolithic, Roman, Byzantine, Islamic, and 18th century (Martinez-Garcias et al. 2005), researchers noted the lowest levels of lead in the Neolithic (6.9 ppm) period prior to metallurgical production, followed by the Roman (101.4 ppm), Byzantine (295.9 ppm), Islamic (108.1 ppm), and 18th century (23.6 ppm). Although it shows an increase in lead exposure since the Neolithic, there is considerable variation and higher averages for the Roman, Byzantine, and Islamic individuals. Across all historic San Francisco Bay samples, the average lead concentration is 29.1 ppm, which is comparable to the 18th century samples from Spain.

Average lead concentrations in tooth enamel from the historic San Francisco population is much lower than those of Hui (129.3 ppm) and Han (144.6 ppm) ethnic groups in contemporary China (Maoyong et al. 2016). However, Asaduzzaman et al. (2017), found much lower lead levels in tooth enamel of only 2.1 ppm on average with a maximum of only 6.2 across various ethnic groups in contemporary Malaysia. Thus, our historical population had an average almost 14x higher than the latter two contemporary populations, but just a quarter of

contemporary Chinese. In general, our average lead values are higher than both historical or archaeological tooth enamel data reported by Kamenov et al. (2018) for Florida, Peru, and the Philippines (0.63 +/- 1.14 ppm), and from modern populations in Europe, Africa and the Americas (6.55 +/- 8.6 ppm). The San Francisco Bay lead concentrations are, on average, 46x higher than the archaeological samples, and 4x higher than the modern samples in this dataset. These various comparisons show that lead levels are highly variable over time and across space, with some of the highest levels recorded for the Byzantine period in Spain and for ethnic Han peoples in modern China. Thus, environmental, historical, and cultural contexts are important to understanding the significance of these lead concentrations.

There are fewer studies with which to compare the mercury and arsenic concentration averages for tooth enamel. For the San Francisco Bay individuals in this study, the averages were 1.57 ppm for Hg, and 9.37 ppm for As. In modern Malaysian tooth enamel across various ethnic groups, Asaduzzaman et al. (2017) report averages for both heavy metals: Hg is 5.05 ppm, and As is 0.05 ppm. The 19th century San Francisco populations had an average mercury concentration of about 3x less than the modern Malaysian samples, while the average arsenic concentration is 18x greater. There are three arsenic concentration outliers in the higher socioeconomic status sample that inflate the average, notably the three samples which measured 45.8 ppm, 71.1 ppm, and 268.3 ppm, whereas all other samples were under 1.3 ppm. Clearly, these high-status individuals experienced excessive arsenic exposure during tooth development.

Conclusion

This research examines historical concentrations of arsenic, lead, and mercury in dental enamel to understand the relative heavy metal exposure in humans during the late 19th and early 20th century in the San Francisco Bay area. Comparing the relative elemental levels in lower socioeconomic and higher socioeconomic individuals resulted in data contradictory to the hypothesis that those of lower socioeconomic classes were exposed to higher levels of environmental pollutants. Rather, it appears that higher socioeconomic classes had more access and exposure to lead during their childhoods. The late 19th century coincided with the California Gold Rush and rapid colonization and industrialization. This period also overlapped with Victorian-era use of mainly lead-based commodities, such as in plumbing, paint, and manufacturing (Lewis 1985; Reh et al. 2021). Greater access to such lead-laden commodities may explain the higher lead concentrations among individuals buried in higher status cemeteries. By comparison to lead, mercury use decreased after the California Gold Rush (Curry 2015). However, mercury was still being used in the medical field for dental fillings or as cures for syphilis, and it was used in products such as mirrors, for gold plating, and in dyes (Curry 2015). Similarly, arsenic was common in household products such as wallpapers, cosmetics, and fabric dyes—specifically a very vibrant green (Hughes et al., 2011).

The research presented here adds to a body of archaeological, environmental toxicology, and forensic literature on the uptake of heavy metals during tooth development or odontogenesis (Rojas-Torres et al. 2024), and illustrates how teeth can act as chronological, environmental, and socioeconomic markers for metal exposure. This study also reminds us that assumptions about socioeconomic status and exposure to toxicants should always be tested rather than simply assumed, given that wealthier individuals appear to have had greater exposure to heavy metals

during tooth development than poorer individuals in the late 19th and early 20th century of the greater San Francisco Bay area.

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