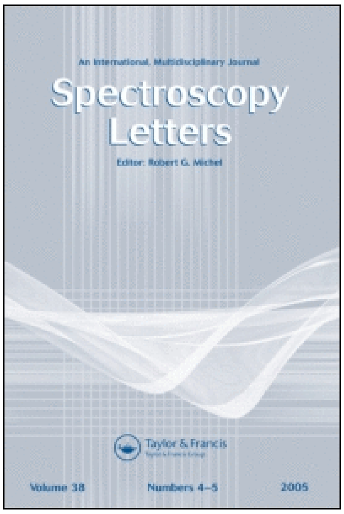

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Evaluation of Laser Ablation Inductively Coupled Plasma Mass Spectrometry for the Quantitative Determination of Lead in Different Parts of Archeological Human Teeth

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Evaluation of Laser Ablation Inductively Coupled Plasma Mass Spectrometry for the Quantitative Determination of Lead in Different Parts of Archeological Human Teeth

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ABSTRACT The lead content of teeth or tooth-parts has been used a biomarker of cumulative lead exposure in clinical, epidemiological, environmental, and archaeological studies. Through the application of laser ablation inductively coupled plasma mass spectrometry, a pilot study of the micrometer-scale distribution and quantification of lead was conducted for two human teeth obtained from an archaeological burial site in Manhattan, New York, USA. Lead was highly localized within each tooth, with accumulation in circumpulpal dentine and cementum. The maximum localized lead content in circumpulpal dentine was remarkably high, almost 2000 $\mu g\,g^{-1}$, compared to the mean enamel and dentine content of about 5 $\mu g\,g^{-1}$. The maximum lead content in cementum was approximately 700 $\mu g\,g^{-1}$. The large quantity of cementum found in the teeth suggested that the subjects had hypercementosis (excess cementum formation) of the root, a condition reported to have been prevalent among African-American slave populations. The distribution of lead in these human teeth was remarkably similar to the distribution that we previously reported in the teeth of present-day lead-dosed goats. The data shown demonstrate the feasibility of using laser ablation inductively coupled plasma mass spectrometry to examine lead exposure in archaeological studies.

KEYWORDS cementum, circumpulpal dentine, hypercementosis, inductively coupled plasma mass spectrometry, laser ablation, lead, tooth

INTRODUCTION

Needleman et al.[1] were the first to demonstrate that the lead content of human teeth could be used to assess lead exposure, in a comparative study of children's deciduous teeth from subjects residing in urban and suburban Philadelphia, Pennsylvania, USA. Lead content ranged from less than 1 $\mu g\,g^{-1}$

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to several hundred $\mu g\,g^{-1}$ and was found to be higher in the urban group. Teeth have subsequently been widely used as indicators of exposure to lead and other toxic metals in clinical, epidemiological, environmental, and archaeological studies.[2–6] Some epidemiological studies have reported statistically significant negative associations between tooth lead and oral health.[7,8] In contrast to lead concentration in blood or urine, which reflects recently ingested or possibly remobilized lead, the lead content of teeth (or bone) is an indicator of long-term exposure. The latter may be a better predictor of adverse neurological effects.[9]

Shapiro et al.[10] recognized that the distribution of lead in teeth was not homogenous by showing that the lead content of circumpulpal dentine was several times higher than that found in the bulk dentine of teeth from children exposed to high concentrations of lead (hundreds of $\mu g\,g^{-1}$ Pb). Lead in circumpulpal dentine resulted from the accumulation of lead from blood in pulp tissue contained within the pulp cavity (root canal) and its incorporation into the hydroxyapatite of proximal circumpulpal dentine. A similar increase in lead content from dentine to circumpulpal dentine was recently shown by Grobler et al.[11] in children with lower tooth lead levels (1 to $10\,\mu g\,g^{-1}$). Enamel lead content in that study was an order of magnitude lower than the lead content of dentine.

The bulk lead content of teeth or tooth parts has been determined by numerous analytical techniques, including anodic stripping voltammetry (ASV), flame and electrothermal atomic absorption spectrometry (FAAS, ETAAS), X-ray fluorescence (XRF), and inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).[12] K-shell XRF, typically used for noninvasive, *in vivo* measurements of bone lead,[13] was applied to the analysis of shed teeth by Bloch et al.[14] Physical separation of small quantities of different tooth tissues is relatively complex and time consuming, however, and requires specialized procedures to handle and quantify the small sample volume.[15]

There are considerable advantages in using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), which provides *in situ* spatially resolved analysis at micrometer scales.[16–18] Using the LA-ICP-MS methodology, workers have identified substantial lead peaks in enamel at the tooth surface[19] and have examined changes in lead content in pre- and post-natal enamel.[20,21] Kang et al.[22] reported relative elevation of circumpulpal dentine lead by about one order of magnitude over non-adjacent enamel and dentine. Recently, Arora et al.[23] reported quantitative data for lead in pre and post-natal enamel. Bellis et al.[24] recently reported on the linear calibration of LA-ICP-MS for quantitative bone lead measurements based on the use of four candidate-certified reference materials (CRMs) for bone lead; these powder CRMs were pelletized using a binder and pellet press. The approach was validated via analysis of pellets of the reference materials NIST SRM 1486 Bone Meal and NIST SRM 1400 Bone Ash (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The reference value for NIST SRM 1400 Bone Ash was obtained after a correction for the different Ca content or organic (loss on ignition) content that resulted from the dry ashing processes used to create that reference material. In this study, it was noted that NIST SRM 1400 Bone Ash would make a suitable calibration standard for teeth, as the matrices have similar hydoxyapatite content with minimal organic content.

The principal aim of this pilot work was to examine the feasibility of using LA-ICP-MS for quantitative determination of lead in different parts of human teeth recovered from the archaeological remains of adult subjects. The archaeological site is located in Manhattan, New York, USA, and is known to be the burial site of enslaved African/Americans. To our knowledge, this is the first report of quantitative data for lead located in several parts of archeological human teeth obtained by LA-ICP-MS.

MATERIALS AND METHODS

The teeth analyzed in this study were kindly provided by Dr. Alan H. Goodman, Director for Chemical Studies for the New York African Burial Ground (NYABG) Project, a recent study of an 18th-century cemetery in lower Manhattan, New York, USA. The cemetery contained the remains of Africans and American-born Africans, including enslaved and free individuals. In 1991 and 1992, the remains of 419 subjects were excavated and assessed using archaeological methods to provide insight into individual and collective experiences of

Colonial-era Africans in New York.[25] Here, a permanent incisor was selected from Individual ABG101 and a permanent molar of Individual ABG266 for analysis by LA-ICP-MS. Individual ABG101 was a 26- to 35-year-old male and among the best preserved of this skeletal population. Health indicators include enamel hypoplasias, which suggest childhood stress, as well as enlarged and torn muscle attachments and advanced osteoarthritis associated with later work stress. Individual ABG101 had culturally modified teeth (CMT); prior to death, his incisors were intentionally filed or chipped along the distal edges. Individual ABG266 was a 25- to 35-year-old female whose remains were not as well preserved. Advanced dental abscessing was evident in her mandible, however. Individual ABG266 also exhibited CMT, with incisors filed to a (somewhat blunt) point. Bioarchaeologists generally consider CMT to be a marker of early life in Africa, and both patterns observed here were common in western Africa during the period of the trans-Atlantic slave trade.[26–28]

The teeth were fixed in epoxy resin in a cuboid mold and were subsequently sectioned along their longitudinal axis with an Isomet low-speed diamond disc saw (Buehler Ltd., Evanston, Illinois, USA) with a diamond-tipped blade. The blade was cooled and lubricated by Millipore 18M$\Omega \cdot$cm deionized water (Millipore, Billerica, MA). The surface of the analyzed section was etched in 1 M hydrochloric acid for 10 s before quenching with Millipore 18M$\Omega \cdot$cm water. The analytical methodology was developed from the procedure employed previously for the LA-ICP-MS analysis of bone.[26] We used a Model LS $\times$ 100 LA system (CETAC Technologies, Omaha, Nebraska, USA), coupled to a Model ELAN 6000 ICP-MS (Perkin-Elmer, Shelton, Connecticut, USA). LA-ICP-MS analysis was performed with the parameters of a continually firing, 10-Hz, 20-μm diameter laser beam of about 1 mJ energy (energy level setting 13/20). The sample stage of the LA system was moved at a speed of 20 μm s^{-1} along the x-axis to create an ablation track along the horizontal axis of the teeth sections.

The isotopes ^{43}Ca, ^{46}Ca, ^{88}Sr, ^{64}Zn, ^{206}Pb, ^{207}Pb, and ^{208}Pb were monitored by ICP-MS using time-resolved analysis. Each mass was sequentially detected for 100 ms, creating a measurement cycle of 700 ms, corresponding to a distance of 14 μm. The LA was manually initiated after about 30 s of ICP-MS of data acquisition and was automatically ended at least 30 s prior to the end of the data acquisition, depending on the track length.

About 4 g of NIST SRM 1400 Bone Ash were prepared into a pressed pellet or briquette using a manual pellet press without the use of a binder. The pellet was measured in triplicate, using ablation lines and the instrumental parameters described above at the start and end of the analytical run. Lead concentrations in the tooth are quoted as $\mu g\, g^{-1}$ by weight hydroxyapatite. The recorded LA-ICP-MS signal for ^{208}Pb/^{43}Ca in multiple analyses of NIST SRM 1400 was plotted as a linear curve through zero using the certified lead content of NIST SRM 1400 of $9.07 \pm 0.12\, \mu g\, g^{-1}$ dry weight. The concentration was subsequently converted to wt. Hydroxyapatite, assuming a hydroxyapatite content for NIST SRM 1400 Bone Ash of 96% (by weight), calculated from the certified Ca content of NIST SRM 1400 of 38.18 wt% and the general formula for hydroxyapatite of $Ca_5(PO_4)_3OH$. Time-resolved data acquisition in seconds (s) was converted to distance based on the stage translocation speed of $20\, \mu m\, s^{-1}$.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of lead recorded in two human teeth from different individuals obtained from the NYABG in lower Manhattan, New York, USA. Subjects buried at this site had previously been identified as having substantial levels of lead exposure.[25,29] Transects across the sectioned teeth (Fig. 1) showed elevated lead content in circumpulpal dentine surrounding the pulp cavity in both the incisor and molar teeth. The tooth from ABG101 showed more lead, up to $2000\, \mu g\, g^{-1}$. There was relatively little lead in the enamel and primary dentine (about $5\, \mu g\, g^{-1}$). The accumulation of lead in circumpulpal dentine is consistent with its deposition from blood contained within the pulp cavity. Analysis near the root of the tooth from primary dentine into cementum (Fig. 2) showed an accumulation of lead in the cementum to about $700\, \mu g\, g^{-1}$ in both individuals. The large quantity of cementum present in both teeth suggests that the subjects suffered from hypercementosis of the root, a condition of abnormally high level of cementum growth. The accumulation indicates that lead in the blood was incorporated into the growing cementum.

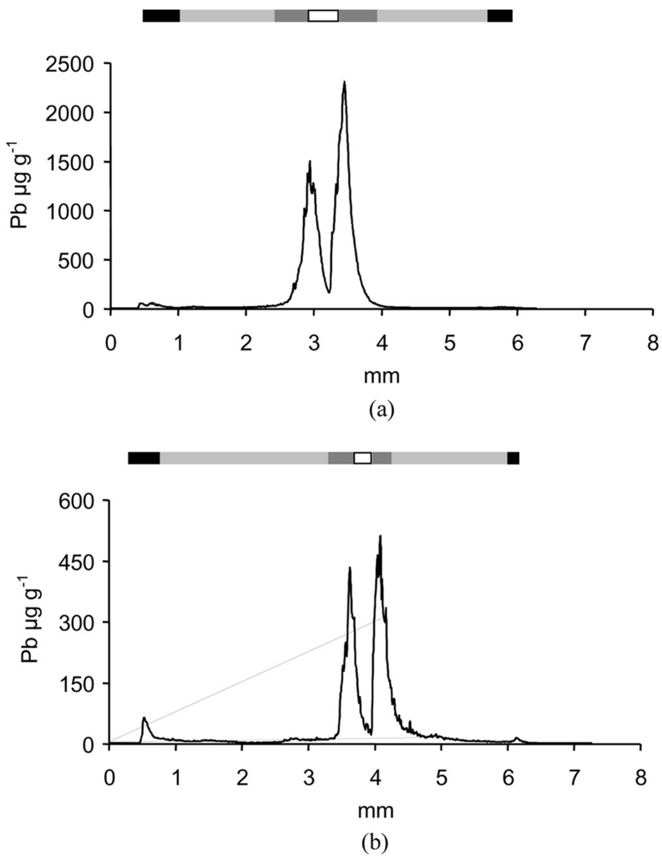

FIGURE 1 **LA-ICP-MS analysis of archeological human teeth: (a) incisor of subject ABG101 and (b) molar of subject ABG266, in cross-section showing lead concentration (µg g^{-1}) in a transect of enamel (black), dentine (light gray), circumpulpal dentine (dark gray), located in either side of the pulp cavity (white) (approximate positions).**

The maximum localized lead concentrations recorded here are at least an order of magnitude higher than the lead content reported in whole teeth or in physically separated tooth parts in other studies.[1–15] However, it does not necessarily indicate a higher level of lead exposure for these individuals compared to those reported in other studies. The high levels of lead occurred in localized regions of the teeth. Whole tooth analysis or physical separation of tooth parts lack the spatial resolution of LA-ICP-MS and would thus lead to lower lead concentrations as the areas enriched in lead are, in effect, diluted. Neither tooth was available for destructive analysis, preventing measurement of the whole tooth lead concentration. It is possible that subject ABG101 had higher lead exposure than ABG266, as ABG101 had considerably higher circumpulpal lead, but this speculation is difficuly to verify.

The distribution of lead is remarkably similar to that previously observed in the teeth of lead-dosed goats,[30] which showed elevated levels in circumpulpal dentine and cementum formed during hypercementosis. Our findings are in contrast to those of Budd et al.[19] who, using LA-ICP-MS, recorded Pb/Ca intensity ratios that were higher in the enamel than in the dentine in prehistoric and Romano-British teeth from the UK. Accumulation of lead in circumpulpal dentine or cementum would indicate internal lead exposure (ingestion) during adulthood when the circumpulpal dentine was being re-worked and the (hyper) cementum was forming. Accumulation of lead in enamel could indicate either internal lead exposure during the period of enamel formation during childhood, or external lead contamination of the tooth surface.

The sources of lead found in human remains excavated from the NYABG are not well established, but in the Americas, these likely included lead-contaminated rum and drinking water, food stored

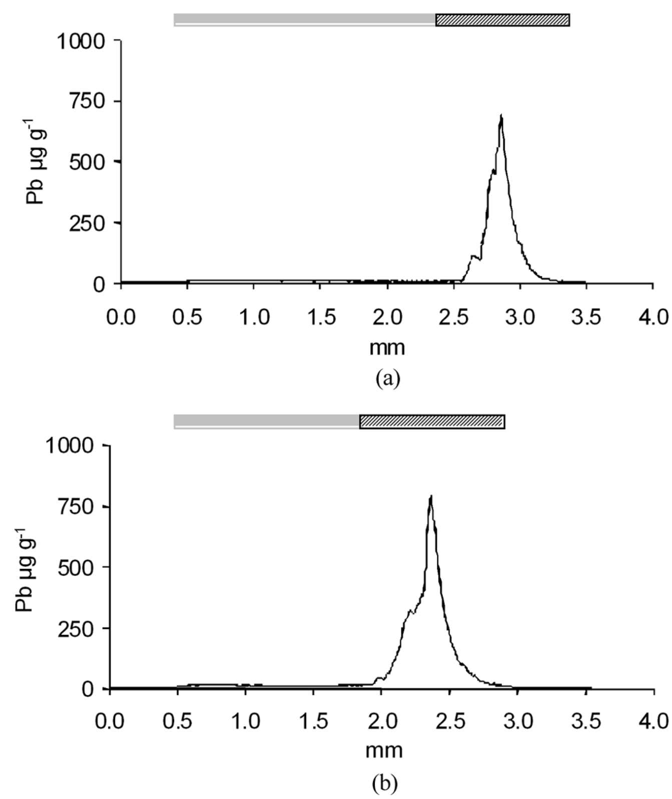

FIGURE 2 **LA-ICP-MS analysis of archeological human teeth: (a) incisor of subject ABG101 and (b) molar of subject ABG266, in cross-section showing lead concentration (µg g^{-1}) in a transect through dentine (light gray) and cementum (hatched) in the root (approximate positions).**

in pewter (traditionally a tin/lead alloy) containers, and labor involving the melting and casting of lead (e.g., fishing and printing).[31] Corrucini et al.[32] and Rathbun[33] reported high skeletal lead and high rates of hypercementosis for enslaved individuals in Barbados and South Carolina, respectively. Corrucini et al.[33] further suggested that poor nutrition and periodontal disease influenced the development of hypercementosis within this population, although the precise etiology remains uncertain and may be linked to other health conditions, e.g., Paget's disease.[34]

Lead may be associated with direct impact on dental health, being associated with dental caries.[35] The pilot data in this study show a considerable accumulation of lead in the circumpulpal dentine and cementum of two archaeological teeth. The relationship between lead and hypercementosis, in particular, would appear to warrant further study.

CONCLUSION

LA-ICP-MS is an effective tool for the quantitative determination of the micrometer-scale distribution lead in archaeological human teeth. It was demonstrated that lead was accumulated in the circumpulpal dentine and cementum of two teeth obtained from the NYABG.

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REFERENCES

1. Needleman, H. L.; Tuncay, O. C.; Shapiro, I. M. Lead levels in deciduous teeth of urban and suburban American children. *Nature* **1972**, *235*, 111–112.
2. Needleman, H. L.; Gunnoe, C.; Leviton, A.; Reed, R.; Peresie, H.; Maher, C.; Barrett, P. Deficits in psychologic and classroom performance with elevated dentine lead levels. *N. Engl. J. Med.* **1979**, *300*, 689–695.
3. Grobler, S. R.; Rossouw, R. J.; Kotze, D. Lead levels in circumpulpal dentine of children from different geographical areas. *Arch. Oral. Biol.* **1985**, *30*, 819–820.
4. Outridge, P. M.; Evans, R. D.; Wagemann, R.; Stewart, R. E. A. Historical trends in heavy metals and stable lead isotopes in beluga (*Delphina leucas*) and walrus (*Odobenus rosmarus rosmarus*) in the Canadian Arctic. *Sci. Total Environ.* **1997**, *203*, 209–219.
5. Budd, P.; Millard, A.; Chenery, C.; Lucy, S.; Roberts, C. Investigating population movement by stable isotope analysis: a report from Britain. *Antiquity* **2004**, *78*, 127–141.
6. Gulson, B. L. Tooth analysis of sources and intensity of lead exposure in children. *Environ. Health Perspect.* **1996**, *104*, 306–312.
7. Davies, B. E.; Anderson, R. J. The epidemiology of dental caries in relation to environmental trace elements. *Experientia* **1987**, *43*, 87–92.
8. Bowen, W. H. Exposure to metal ions and susceptibility to dental caries. *J. Dent. Educ.* **2001**, *65*, 1046–1053.
9. Graziano, J. H. Validity of lead exposure markers in diagnosis and surveillance. *Clin. Chem.* **1994**, *40*, 1387–1390.
10. Shapiro, I. M.; Dobkin, B.; Tuncay, O. C.; Needleman, H. L. Lead levels in dentine and circumpulpal dentine of deciduous teeth of normal and lead poisoned children. *Clin. Chim. Ac.* **1973**, *46*, 119–123.
11. Grobler, S. R.; Theunissen, F. S.; Kotze, T. J. V. W. The relation between lead concentrations in human dental tissues an in blood. *Arch. Oral. Biol.* **2000**, *45*, 607–609.
12. Outridge, P. M.; Hughes, R. J.; Evans, R. D. Determination of trace metals in teeth and bones by solution nebulization ICP-MS. *Atom. Spectros.* **1996**, *17*, 1–8.
13. Todd, A. C.; Chettle, D. R. *In vivo* X-ray fluorescence of lead in bone: Review and current issues. *Environ. Health Perspect.* **1994**, *102*, 172–177.
14. Bloch, P.; Shapiro, I. M.; Soule, L.; Close, L.; Revic, B. Assessment of lead exposure of children from K-XRF measurements of shed teeth. *Appl. Radiat. Isotop.* **1998**, *49*, 703–705.
15. Grünke, K.; Stärk, H.-J.; Wennrich, R.; Franck, U. Determination of traces of heavy metals (Mn, Cu, Zn, Cd, and Pb) in microsamples of teeth material by ETV-ICP-MS. *Fresenius J. Anal. Chem.* **1996**, *354*, 633–635.
16. Evans, R. D.; Richner, P.; Outridge, P. M. Micro-spatial variations of heavy-metals in the teeth of walrus as determined by laser-ablation ICP-MS. The potential for reconstructing a history of metal exposure. *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 55–60.
17. Cox, A.; Keenan, F.; Cooke, M.; Appleton, J. Trace element profiling of dental tissues using laser ablation-inductively coupled plasma mass spectrometry. *Fresenius J. Anal. Chem.* **1996**, *354*, 254–258.
18. Uryu, T.; Yoshinaga, J.; Yanagisawa, Y.; Endo, M.; Takahashi, J. Analysis of lead in human tooth enamel by laser ablation-inductively coupled plasma mass spectrometry. *Anal. Sci.* **2003**, *19*, 1413–1416.
19. Budd, P.; Montgomery, J.; Cox, A.; Krause, P.; Barreiro, B.; Thomas, R. G. The distribution of lead within ancient and modern human teeth: Implications for long-term and historical exposure monitoring. *Sci. Total Environ.* **1998**, *220*, 121–136.
20. Lee, K. M.; Appleton, J.; Cooke, M.; Keenan, F.; Sawicka-Kapusta, K. Use of laser ablation inductively coupled plasma mass spectrometry to provide element versus time profiles in teeth. *Anal. Chim. Ac.* **1999**, *395*, 179–185.
21. Lochner, F.; Appleton, J.; Keenan, F.; Cooke, M. Multi-element profiling of human deciduous teeth by laser ablation-inductively coupled plasma-mass spectrometry. *Anal. Chim. Ac.* **1999**, *401*, 299–306.
22. Kang, D.; Amarasiriwardena, D.; Goodman, A. H. Application of laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) to investigate trace metals spatial distributions in human tooth enamel, dentine growth layers and pulp. *Anal. Bioanal. Chem.* **2004**, *378*, 1608–1615.

23. Arora, M.; Chan, S. W. Y.; Kennedy, B. J.; Sharma, A.; Crisante, D.; Walker, D. M. Spatial distribution of lead in roots of primary teeth. *J. Trace Elements Med. Biol.* **2004**, *18*, 135–139.
24. Bellis, D. J.; Hetter, K. M.; Jones, J.; Amarasiriwardena, D.; Parsons, P. J. Calibration of laser ablation inductively coupled plasma mass spectrometry for quantitative measurements of lead in bone. *J. Anal. At. Spectrom.* **2006**, *21*, 948–964.
25. Goodman, A. H.; Jones, J. L.; Reid, J.; Mack, M.; Blakey, M. L.; Amarasiriwardena, D.; Burton, P.; Coleman, D. Isotopic and elemental chemistry of teeth: Implications for places of birth, forced migration patterns, nutritional status, and pollution. In *New York African Burial Ground Skeletal Biology Final Report, Volume 1: A Report for the United States General Services Administration, Northeast and Caribbean Region*; Blakey, M. L.; Rankin-Hill, L., Eds.; United States Government Printing Office: Washington, DC, 2004; 217–265.
26. Handler, J. S. Determining African birth from skeletal remains: A note on tooth mutilation. *Hist. Archaeol.* **1994**, *28*, 113–119.
27. Handler, J. S.; Aufderheide, A. C.; Corruccini, R. S.; Brandon, E. M.; Wittmers, Jr., L. E. Lead contact and poisoning in Barbados slaves: Historical, chemical, and bioanthropological evidence. *Soc. Sci. Hist.* **1986**, *10*, 399–425.
28. Gould, A. R.; Farman, A. G.; Corbitt, D. Mutilations of the dentition in Africa: A review with personal observations. *Quint. Int.* **1984**, *15*, 89–94.
29. Webb, E.; Amarasiriwardena, D.; Tauch, S.; Green, E. F.; Jones, J.; Goodman, A. H. Inductively coupled plasma-mass (ICP-MS) and atomic emission spectrometry (ICP-AES): Versatile analytical techniques to identify the archived elemental information in human teeth. *Microchem. J.* **2005**, *81*, 201–208.
30. Bellis, D. J.; Hetter, K. M.; Jones, J.; Amarasiriwardena, D.; Parsons, P. J. Lead in teeth from lead-dosed goats: microdistribution and relationship to the cumulative lead dose. *Environ. Res.* **2008**, *1*, 34–41.
31. McCord, C. P. *Lead and Lead Poisoning in Early America*. Industrial Medicine Publishing Company: Ann Arbor, 1953; 1954.
32. Corrucini, R. S.; Jacobi, K. P.; Handler, J. S.; Aufderheide, A. C. Implications of tooth root hypercementosis in a Barbados slave skeletal collection. *Am. J. Phys. Anthropol.* **1987**, *74*, 179–184.
33. Rathbun, T. A. Health and disease at a South Carolina plantation: 1840–1870. *Am. J. Phys. Anthropol.* **1987**, *74*, 239–253.
34. Hillson, S. *Dental Anthropology*. Cambridge University Press: Cambridge, 1996.
35. Gil, F.; Facio, A.; Villanueva, E.; Perez, M. L.; Tojo, R.; Gil, A. The association of tooth lead content with dental health factors. *Sci. Total Environ.* **1996**, *192*, 183–191.