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Reconstruction of human exposure to heavy metals using synchrotron radiation microbeams in prehistoric and modern humans

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Key words: enamel, synchrotron radiation microbeams, heavy metals, prehistoric, human

Abstract

Objective Teeth can serve as records of environmental exposure to heavy metals during their formation. We applied a new technology- synchrotron radiation microbeams (SRXRF)- for analysis of heavy metals in human permanent teeth in modern and historical samples.

Methods Each tooth was cut in half. A longitudinal section 200 μm in thickness was subjected to the determination of the heavy metal content by SRXRF or conventional analytical methods (ICP-MS analysis or reduction-aeration atomic absorption spectrometry). The relative concentrations of Pb, Hg, Cu and Zn measured by SRXRF were translated in concentrations (in g of heavy metal/g of enamel) using calibration curves by the two analytical methods.

Results Concentrations in teeth in the modern females (n=5) were 1.2±0.5 μg/g (n=5) for Pb; 1.7±0.2 ng/g for Hg; 0.9±1.1 μg/g for Cu; 150±24.6 μg/g for Zn. The levels of Pb were highest in the teeth samples obtained from the humans of the Edo era (1603-1868 AD) (0.5-4.0 μg/g, n=4). No trend was observed in this study in the Hg content in teeth during 3000 years. The concentrations of Cu were highest in teeth of two medieval craftsmen (57.0 and 220 μg/g). The levels of Zn were higher in modern subjects ($p<0.05$) than those in the Jomon (~1000 BC) to Edo periods [113.2±27.4 (μg/g, n=11)]. Reconstruction of developmental exposure history to lead in a famous court painter of the Edo period (18th century) revealed high levels of Pb (7.1-22.0 μg/g) in his childhood.

Conclusions SRXRF is useful a method for reconstructing human exposures in very long trends.

Introduction

Many toxic heavy metals are found in the environment, and certain levels of exposure are inevitable for the inhabiting human populations. The industrial release of some heavy metals, such as lead and chromium, to the environment is significantly larger than the natural sources of these metals, while the levels of other heavy metals, such as cadmium and mercury, from either natural or industrial sources are the same (1).

Human beings as *Homo sapiens*, have been exposed to various heavy metals from stone age (2). Rapid increase in exposure to levels of the heavy metals in the modern environment, when compared to those in prehistoric periods, may have caused adverse health effects. To investigate such a possibility, reconstruction of the exposure history of humans has recently been explored in a number of studies (3,4,5).

Teeth can serve as records of environmental exposure to heavy metals that are accumulated in the mineral phase of the dental tissues during tooth formation (6,7). In tooth enamel, this mineral phase is not subject to turnover, since it consists of biological mineral hydroxyapatite, where various ions may be substituted into the crystal lattice only during the development. Thus, the enamel encapsulates a permanent record of the trace element environment during the development of a tooth. Migration of ions may occur, but it is confined to the immediate surface exposed to oral environment and burial soils.

In the past, several methodologies have been applied to analysis of heavy metals in the teeth (6,7,8,9,10,11,12,13,14,15). Recently, XRF analysis using synchrotron radiation

(SR) microbeams (SRXRF) has been applied to the analyses of tooth enamel (10,11,12). This method uses microbeams and enables us to provide high spatial resolution with much higher sensitivity (10,11,12).

The aim of the present study is to test applicability of SRXRF to the analysis of heavy metals in human teeth. Specifically, this study has three objectives. First, since the accurate quantification of the amounts of heavy metals is difficult due to the lack of suitable reference materials, we tested whether concentration ratios of various heavy metals are proportional to their absolute concentrations determined by separate analytical methods (11,12). In this way, we aimed to replace a semi-quantitative method typically used, which simply compares ratios of elements in teeth, with a quantitative method. Secondly, we also applied this quantitative method to a series of molar teeth samples from a single individual. We tested whether exposure level can be correlated in several molar teeth with different developmental ages. Finally, we applied this method to the historical reconstruction of exposures to various heavy metals of humans who lived in different times, from the prehistoric era (Jomon era, BC 1000) to present times. In the present study, the targeted heavy metals are lead (Pb), mercury (Hg), copper (Cu) and zinc (Zn). Some of the reasons for selection of these metals are the following: 1) human exposure to lead is reported to be increasingly significant due to recent industrialization in western countries, 2) the major source of mercury in the environment is thought to be from natural release due to geological activities (16) and coal-fired power stations (17), 3) copper is one of the essential metals, which is obtained through diets and is also released by several industrial activities (18), 4) the levels of zinc, an essential metal in the human body, are known to be strongly influenced by nutrition (19). The study of the levels of heavy metals may elucidate the source and effects of long-term environmental exposure to these metals as well as elucidate nutritional conditions of the prehistoric and modern humans.

Materials and methods

Cases and samples

Permanent teeth samples from modern humans were collected from the donors after we obtained informed consent. The teeth were donated to our study, following an extraction by the dentists. Donors were selected from candidates who had never used dental amalgams. This study protocol was approved by the ethical review board of Kyoto University Graduate School of Medicine.

Archaeological permanent teeth samples were collected during excavations (Table 1). Those teeth were free of caries. The ages of the teeth samples were determined by archaeological criteria except for individual K (1776-1846, AD), who was a court painter. The samples of teeth from subject K were dated based on the documented records from a Buddhist temple in a cemetery where he was buried. Other teeth (E1-E4) were collected from the excavated ruins of a town or a local village of the Edo era (17th C-19th C AD). Subjects E1 and E2 were postulated to be farmers, subjects E3 was postulated to be a merchant's wife and E4 was assumed to be a merchant or a family member of the merchant.

The medieval time of Japan includes the Heian Period (8th C-12th C, AD), the Kamakura Period (12th C-14th C, AD) and the Muromachi Period (14th C-16th C, AD). Teeth samples (C1-C3) were excavated from the cemetery, which was continuously used for burials from 10^{th} C to 16^{th} C, AD. People, who lived in a town across from the cemetery, were buried in this place. No information was available for individuals from the 10^{th} C, AD. However, the 14^{th} C, AD subjects, from whom the teeth samples were collected, are considered to have been craftsmen engaged in casting of a Buddhist statue as ancestral business, as determined from many artifacts (china and white porcelains) buried in the tombs.

The Tumulus period (3^{rd} C - 6^{th} C, AD) is considered to be a period, when the first centralized government was formed. Nomadic people had settled in villages and engaged in agriculture. Human exposure to heavy metals in this period is considered to be mostly attributable to natural sources. Subject (T1) is considered to have been a head of a local clan.

The Jomon period corresponds to the Stone Age and started from BC 16,000 and ended BC 500. Donors of teeth J1 and J2 were buried in a typical Jomon shell mound. The entire skeleton of the donor J3 was found in a ruin in a cave and showed features of a middle aged woman. Exposure to heavy metals during this period is considered to be solely due to ecological sources, since people were primarily engaged in hunting, fishing and gathering of foods.

Sample preparations

Each tooth was cut into half by longitudinal section (Fig. 1). From one piece, a longitudinal section 200 μm in thickness was cut using a diamond saw-cutter. To prevent contamination, diamond wire was immersed in distilled deionized water in a plastic container and the water was replaced after each sample was cut. Fresh water was used for each tooth when grinding and polishing of the samples and all samples were rinsed well with water prior to analysis.

SRXRF Analysis

SRXRF analyses using SR microbeams were performed at the Photon factory, KEK (Tsukuba, Japan) or at SPring-8 (Sayo, Japan) as previously described (Ide-Ektessabi et al., 2004). Briefly, SR from the storage ring (2.5GeV, maximum current 400mA, in the case of KEK) was monochromated using a multilayer film monochromater. The incident X-ray energy was 14.3 keV. Incident X-rays were focused using Kirkpatrick-Baez optics. The incident beam size was about 6 x 5 μm. The incident and transmitted photon flux was monitored with an ion chamber, and the fluorescent X-ray were detected by a solid-state detection (SSD). The measurements were conducted in air. SRXRF imaging was carried out as previously reported (12).

Quantification of the elements

Surface enamel portions (~200 μm) were abraded from the piece of the tooth, from which longitudinal tooth sections were cut for the analysis by SRXRF (12), in order to avoid the potential effects of diagenesis from the enveloping soil that would impact the surface of the tooth (20) (Fig. 2). Dentine was also removed from the tooth fragment to be analyzed. Semi-quantification of the concentration of each element was performed by the integration of the peak areas using software developed by Ide-Ektessabi et al. (20). In this program, the background is estimated from the untreated spectra, and the peak is obtained using Gaussian curve fitting and the least square method. Linear scanning with high resolution was performed from the outside of enamel to pulp to obtain X-ray fluorescence for Ca, Pb Hg, Cu and Zn. The measurements were repeated by a 20-μm interval from the surface of enamel to pulp. The counts at individual points were integrated and standardized by the integrated count of Ca. The linear scanning was repeated for 5 times for different lines per tooth. The mean of relative concentrations by 5-time scanning was taken as a relative concentration for a given heavy metal for a given tooth. The relative concentrations of Pb, Hg, Cu and Zn were standardized by dividing the value of the peak areas of a given element by the peak area of Ca in the sample because its similarity of behavior to that of heavy metals (13). The coefficients of variations were within 20% for this analysis. From the remaining piece of the tooth two enamel blocks were cut (approximately 500 mg each) and washed thoroughly with doubly deionized distilled water. After cleaning, one piece of the enamel section was digested with hydrochloric acid. Digested samples were diluted to appropriate volumes with deionized water. The determinations of the concentrations of Pb, Cu, and Zn were obtained using ICP-MS (Agilent 7500a, Tokyo, Japan) (21). The lowest detection limits were 0.02 mg/L for Zn, 0.01 mg/L for Cu and 0.004 mg/L for Pb, respectively. The second piece of the enamel was subjected to the determination of the mercury concentration by reduction-aeration atomic absorption spectrometry (AAS). The detection limit was 0.002 μg.

Calibrations of SRXRF by ICP-MS and AAS

To determine the concentrations of the heavy metals, we scanned the teeth samples from the oral side to dentin and the pulp through to the enamel (Fig. 1). One dimension and two dimension analyses gave patterns as shown in Fig. 1b and 1c. Concentrations of the elements were means of 20 x 20 pixels. We collected the fragment of the teeth for ICP-MS analyses as shown Fig. 2. From each tooth a sample was collected for the ICP-MS analysis.

Statistics

The collected data was analyzed using the SAS statistical package, version 8.2 (SAS Institute Inc., Cary, North Carolina). *P* values for statistical tests were 2-tailed. *P* value <0.05 was considered to be significant.

Results and discussion

Comparison between SRXRF and ICP-MS.

The comparison between the relative concentrations of the elements obtained by SRXRF and ICP-MS were shown in Fig. 3a-d. The concentrations obtained by these two methods agreed significantly (R^2>0.758, p<0.05). Such significant agreements allowed us to convert the relative concentrations (peak area for a given element divided by the area for Ca) obtained by SRXRF into concentrations (Mass/Mass). In Table 2, the converted values for heavy metal concentrations were presented together with concentrations measured by ICP-MS. The two values agreed well for the concentrations of Pb and Hg. However, for Cu and Zn, the values obtained from the analyses by the two methods were not so in good agreement.

Long-term trend and data interpretation

Limited number of tooth samples made it impossible to draw any definitive conclusions on the long-term trend in the concentration of these four heavy metals in human teeth. However, data presented in Table 2 suggests some very interesting exposure profiles.

Concentrations of Pb are highest in teeth obtained from the skeletons of humans of the Edo era. However, Pb levels are widely scattered among samples, likely reflecting personal life styles or habits. As a matter of fact, it is reported that lead-oxide cosmetic powders were used by females of the Samurai classes or merchants in urban centers in the Edo era (22,23,24,25). Thus, higher exposures to Pb found in teeth samples of two subjects in the merchant class in the Edo era can likely be explained by the use of the lead containing cosmetics by the mother.

High concentrations of Cu in teeth of the people living in the medieval times seem to be associated with their ancestral occupations. Both subjects C1 and C2 were thought to be craftsmen, engaged in the production of the statue of Buddha from copper in cottage industry. In this period, they inherited their occupations from their fathers and conducted their works at home, leading heavy indoor exposure to copper at home. Therefore, subjects C1 and C2 might be exposed to copper through dust or fumes.

The levels of Zn seem to be highest in modern subjects. The levels of Zn in human tissue are known to be associated with nutritional conditions (19). When the results for the Zn content in the teeth were pooled from the Jomon to Edo periods, their mean levels (μg/g, n=11) were 121.6±27.9 as determined by ICP-MS or 113.2±27.4 by SRXRF, significantly lower (p<0.05) (n=5) than those in modern subjects (156.0±27.0 μg/g by ICP-MS or 150.0±24.6 μg/g by SRXRF). Modern increase in the content of Zn in the human teeth probably is associated with increase in the consumption of Zn rich foods such as meats.

A case study for K

K was one of the most famous court painters in Edo era. He was born at the end of the 18th C and died in the middle of the 19th C. He was very active as a leader of the painting school, which was established by his ancestor. The mineralization of his 1st tooth started around year 2 or 3 of his life. The mineralization of his second molar tooth began at the age of 2 and was completed by the age 7. The mineralization of his last molar tooth started at the age of 7 and ended by the age 16. Based on the data collected in this study (Table 2), he was exposed to high levels of lead at the neonatal and early infantile periods and to moderate levels in his childhood period. It should be also pointed out that he was heavily exposed to Cu. On the other hand, his enamel contained only trace amounts of Zn.

In the present study, we have established a method using SRXRF to determine heavy metal concentrations in human tooth enamel collected from humans and human skeletons from 3000 years ago to present times. The values of the concentrations of the analyzed metals (Pb, Hg, Zn and Cu) obtained using SRXRF were compared to the values obtained using ICP-MS or AAS. This process enabled us to translate the relative amounts of heavy metals of interest by dividing the values by the Ca concentrations into absolute concentrations. The calibration method using relative concentrations against Ca has been employed traditionally (9,10). In the present study we also confirmed usefulness of this method.

The dental enamel has been thought to be an ideal material for reconstruction of the exposure histories, because heavy metals incorporated into the enamel are encapsulated as they are chronologically absorbed during the subject's growth (6,7). Therefore, this ability of the enamel can be fully utilized only by *in situ* analysis of the enamel metal content with a high resolution method. For this purpose, a laser abraded method coupled with ICP-MS or SRXRF seems to be promising. SRXRF has some advantages, since this method enables detection of the distribution of heavy metals with high resolution. As an example, in our study this method showed that enamels in K's two teeth, developed in an infantile period, had high levels of Pb presumably due to the levels of Pb contained in the breast milk of his mother, who may have used Pb containing cosmetics.

Preliminary observations in the current studies warrant further studies. Exposures to Pb are highest in the Edo era in Japan as reported by others (22,23,24,25). No trend was observed in this study in the Hg content in teeth during 3000 years in Japan. The copper exposures are considered to be associated with individual's occupation. It is of particular interest that Zn concentrations are highest in modern humans. Since meat and cereal grains are rich in Zn (19), this observed long term trend may result from nutritional improvements in modern humans.

This study lacked solid standard reference materials that are matrix matched for calibration purposes. Alternatively, we calibrated using ICP-MS or AAS, which lost information of special distribution for each heavy metal. This is the major limitation of this study. Thus at present, we cannot fully utilize the advantages of SRXRF. This drawback will be recovered in future.

In conclusion, we have developed a quantitative method using SRXRF with a calibration by ICP-MS and AAS. This method allowed us spatial high sensitivity with high resolution with appropriate external standards. We have applied this method to the reconstruction of the human environmental exposures to the heavy metals as well as determined the nutritional conditions of the humans from the analysis of the heavy metal content of their teeth.

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Figure legend

Fig. 1. Anatomy of a tooth and two dimensional distributions of Ca and Zn by SRXRF

A. Anatomy of a tooth by horizontal section

B. Distribution of Ca by SRXRF

C. Distribution of Zn by SRXRF

Relative signal intensities were expressed in gradient colors.

Fig. 2. Surface ablation and removal of dentin

The figure shows a half tooth after longitudinal section.

Fig. 3. Correlations between the concentrations obtained using SRXRF(x10^{-6}) (X) and ICP-MS or AAS (Y)

The X axis represents relative concentrations of metals by SRXRF (x10^{-6}), while Y axis represents actual concentrations per gram of tooth enamel:

a: Y axis is μg of Pb/g

b: Y axis is ng of Hg/g

c: Y axis is μg of Cu/g

d: Y axis is μg of Zn/g

Table1

Summary of permanent teeth samples

Period	ID	Tooth type	Gender	Personal Information	Residential area
Modern	M1	Rt Mandibular 3rd molar	Female	Born in 1983	Kyoto
	M2	Rt Maxillary 3rd molar	Female	Born in 1975	Kyoto
	M3	Rt Mandibular 3rd molar	Female	Born in 1969	Kyoto
	M4	Rt Mandibular 3rd molar	Female	Born in 1958	Kyoto
	M5	Rt Mandibular 3rd molar	Female	Born in 1934	Kyoto
Edo Era					
18C	K	Rt Maxillary 1st Molar - 3rd Molar	Male	Court Painter	Tokyo
17C	E1	Lt Maxillary Canine	Unknown	Farmer	Ibaragi
17C	E2	Lt Maxillary1st Molar	Unknown	Farmer	Ibaragi
17C	E3	Rt Maxillary Cutting	Female	Merchant wife	Tochigi
17C	E4	Lt Maxillary 1st Molar	Unknown	Merchant associated	Tochigi
Medieval					
14C	C1	Maxillary Front	Male	Craftsman	Kyoto

14C	C2	Lt Maxillary 1st Molar	Male	Craftsman	Kyoto
10C	C3	Rt Mandibular Molar	Unknown	Town people	Kyoto
Tumulus					
6C	T1	Maxillary Front	Unknown	Local head of a clan	Nagano
Jomon					
-5C	J1	Mandibular 1st Molar	Unknown	Unknown	Chiba
-5C	J2	Rt Maxillary 3rd Molar	Unknown	Unknown	Chiba
-10C	J3	Lt Maxillary 1st Molar	Female	Middle aged	Miyagi

Rt: Right

Lt: Light

Table 2

Estimated concentrations of elements in enamels

		Pb (μg/g)		Hg (ng/g)		Cu (μg/g)		Zn (μg/g)		
Time	ID	ICP	SRXRF	AAS	SRXRF	ICP	SRXRF	ICP	SRXRF	Tooth Age at AD 2000
Modem	M1	1.0	0.7	1.8	1.7	0.4	0.1	170.0	130.4	17
	M2	0.3	1.0	1.5	1.5	0.6	2.7	190.0	173.0	25
	M3	0.4	1.3	2.0	1.9	0.7	0.0	140.0	171.5	31
	M4	1.2	1.2	1.8	1.7	0.3	0.8	120.0	118.5	42
	M5	3.7	2.0	1.7	1.6	0.1	1.0	160.0	156.6	66
	Mean	1.3	1.2	1.8	1.7	0.4	0.9	156.0	150.0	
	SD	1.4	0.5	0.2	0.2	0.2	1.1	27.0	24.6	
Edo	E1	0.1	0.5	1.5	1.4	0.5	3.0	170.0	128.0	300
	E2	0.1	0.5	1.2	1.5	0.4	0.4	130.0	98.5	300
	E3	7.3	4.0	1.2	1.3	0.5	0.0	110.0	72.8	300
	E4	3.9	2.8	1.0	1.4	0.3	1.4	130.0	104.3	300

	Mean	2.6	1.7	1.0	1.2	0.4	1.2	113.4	85.6	
	SD	3.1	1.7	0.5	0.5	0.1	1.2	53.0	39.4	
Medieval	C1	1.1	1.3	1.5	1.4	57.0	56.2	110.0	119.7	600
	C2	0.7	1.4	1.3	1.2	220.0	215.6	120.0	170.7	600
	C3	0.2	0.5	2.0	1.5	0.4	2.2	82.0	72.1	1000
	Mean	1.5	1.3	1.3	1.2	55.6	55.3	95.7	97.5	
	SD	1.2	0.5	0.6	0.4	95.1	92.7	27.9	50.0	
Tumulus	T1	0.1	0.4	1.2	1.6	0.2	0.8	170.0	130.3	1400
Jomon	J1	0.4	0.4	1.5	1.4	0.7	2.7	96.0	117.9	2500
	J2	0.2	0.4	1.2	1.3	0.2	1.0	120.0	114.3	2500
	J3	0.4	0.9	2.5	2.2	0.5	0.0	100.0	117.1	3000
	Mean	0.3	0.6	1.7	1.7	0.5	1.2	105.3	116.4	
	SD	0.1	0.3	0.7	0.5	0.3	1.4	12.9	1.9	

Edo	K 1st	26.0	25.8	0.6	0.4	15.0	11.8	0.5	19.3	204
	K 2nd	24.0	22.0	1.9	1.8	20.0	19.2	2.5	24.9	204
	K 3rd	7.1	7.1	0.7	0.6	16.0	11.3	0.9	18.9	204

ICP: Values measured by ICP-MS

AAS: reduction-aeration atomic absorption spectrometry

SRXRF: Relative concentrations were converted to concentrations using correlation equations

Figure 1. Anatomy of a tooth and two dimensional concentrations of Ca and Zn by SRXRF

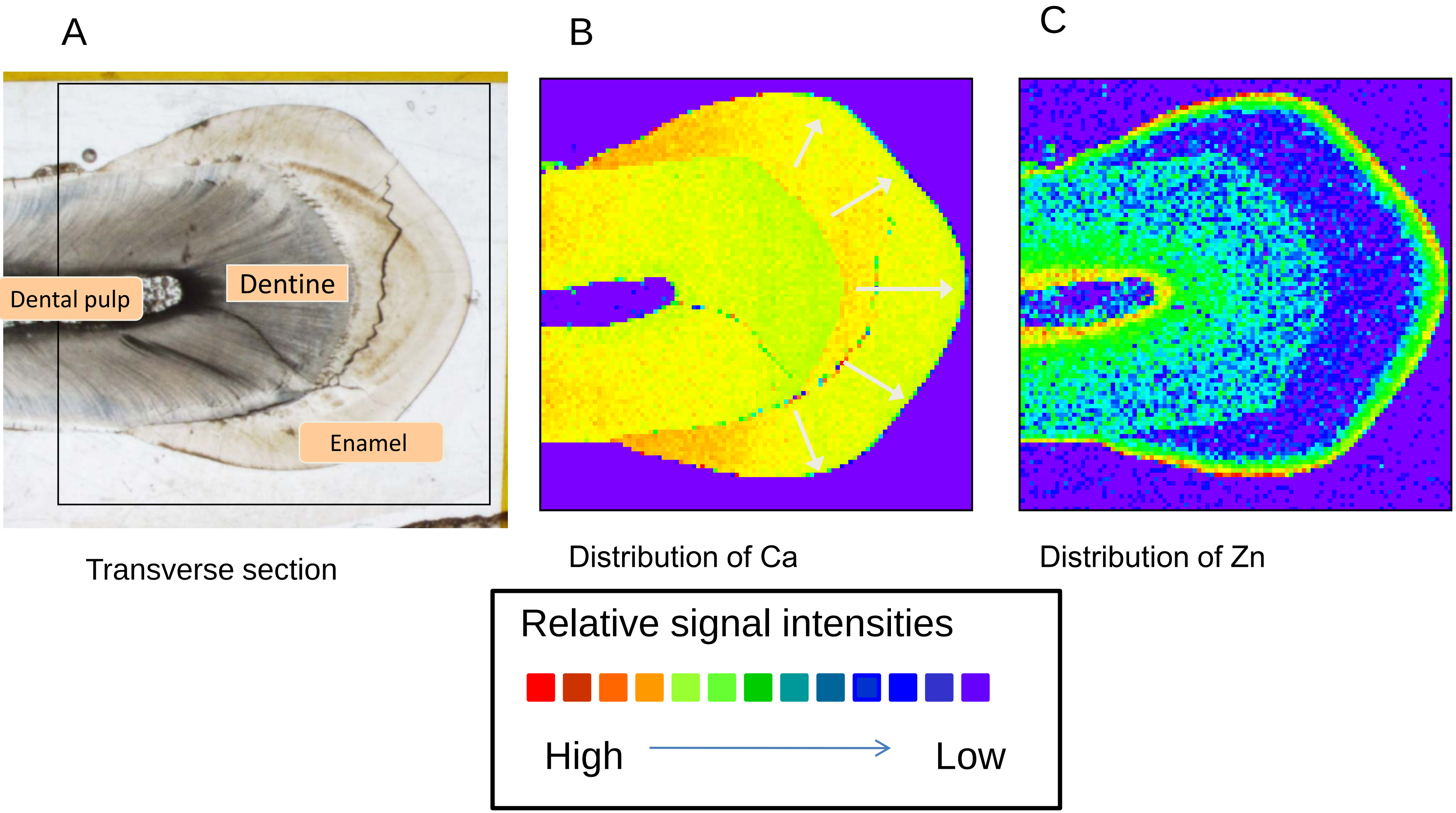
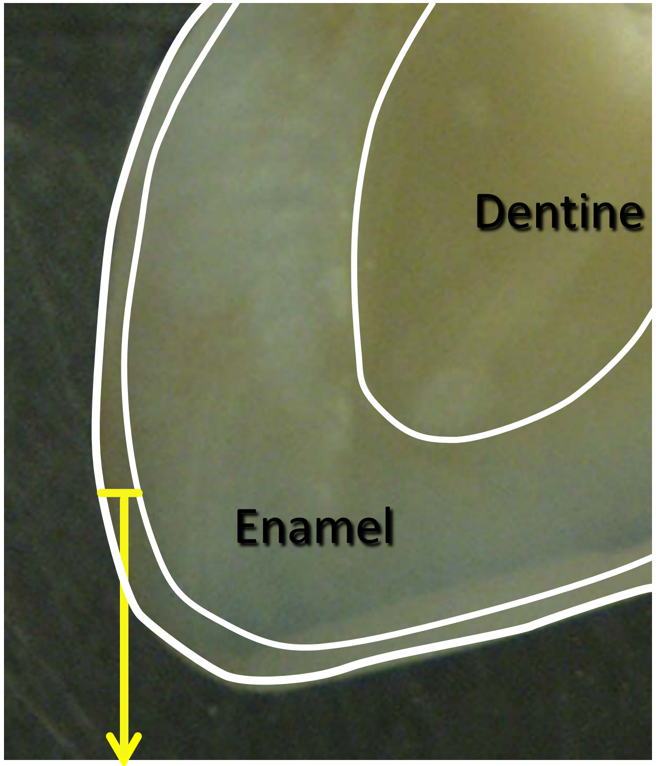
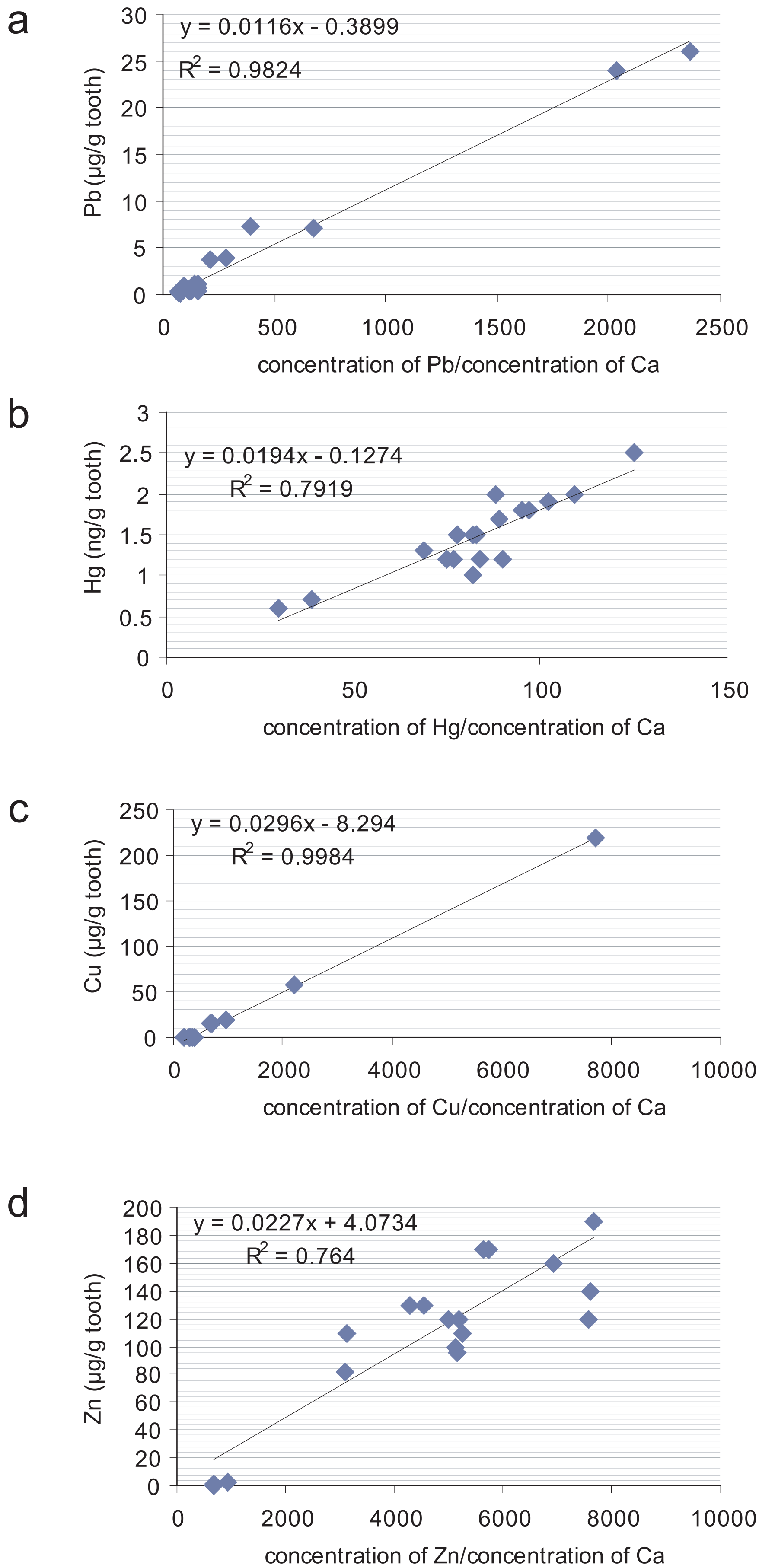


Figure 2.



200µm from the surface was abraded.

Fig. 3



a
y = 0.0116x - 0.3899
R2 = 0.9824
Pb (µg/g tooth)
30
25
20
15
10
5
0
0
500
1000
1500
2000
2500
concentration of Pb/concentration of Ca
b
y = 0.0194x - 0.1274
R2 = 0.7919
Hg (ng/g tooth)
3
2.5
2
1.5
1
0.5
0
0
50
100
150
concentration of Hg/concentration of Ca
c
y = 0.0296x - 8.294
R2 = 0.9984
Cu (µg/g tooth)
250
200
150
100
50
0
0
2000
4000
6000
8000
10000
concentration of Cu/concentration of Ca
d
y = 0.0227x + 4.0734
R2 = 0.764
Zn (µg/g tooth)
200
180
160
140
120
100
80
60
40
20
0
0
2000
4000
6000
8000
10000
concentration of Zn/concentration of Ca