Study of P, Ca, Sr, Ba and Pb Levels in Enamel and Dentine of Human Third Molars for Environmental and Archaeological Research

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Elemental determination of 80 third molars, collected from local dental clinics in Hsinchu City, Taiwan during 2009 to 2010, was conducted using inductively coupled plasma—mass spectrometry (ICP-MS). Results show that the mean concentrations of P, Ca, Sr, Ba and Pb in enamel are respectively 14.63% ± 2.19%, 27.91% ± 4.03%, 108.31 ± 35.71 ppm, 1.96 ± 1.01 ppm, and 0.72 ± 0.49 ppm. The concentrations of P, Ca and Sr are higher in enamel than in dentine, on the other hand, the concentrations of Ba and Pb are higher in dentine than in enamel. In enamel and dentine the concentrations of P, Ca and Ca/P ratio are kept constant. In enamelthe concentrations of Sr and Sr/Ca increase by age statistically but the concentrations of Ba and Ba/Ca are not. Pb concentrations in both enamel and dentine increase by age and also increase with significant differences among each birth era. This may indicate the dates of environmental exposure. The levels of Pb in this study are lower than the previous published findings before 1979. The concentrations and distribution of elements in enamel and dentine of third molars other than deciduous or permanent teeth can provide reliable base references to past and future studies.

Keywords: Elemental Concentration; Third Molars; Enamel; Dentine; ICP-MS

Introduction

The Properties of Teeth

Humans usually have 20 primary (deciduous or "baby") teeth and 32 permanent (adult) teeth. Teeth are classified as incisors, canines, premolars and molars. The development timeline is very different among each type of tooth. Each tooth has its own development stage time line. For example, for third molars, the age of initial calcification, crown completion and root completion is from 7 to 10 years old, 12 to 16 years old and 18 to 25 years old respectively (Ash, 2002). Tooth eruption in humans is a process in tooth development in which teeth enter the mouth and become visible. Each tooth type has its own time of eruption. For example, third molars erupt between 17 to 21 years old (Ash, 2002). Therefore, a third molar's root may be not completed but its crown may have completed development when it erupts in the mouth.

Enamel, dentine, cementum and dental pulp are the four major tissues which make up a tooth. The crown of a tooth is composed of these major tissues and covered in enamel above the neck of the tooth. The crown is visible after eruption. The root of tooth is composed of dentine, cementum and dental pulp, but without enamel. Although both enamel and dentine are essential components of teeth, they are derived from different sources, enamel from ectoderm and dentine from mesectoderm. In addition, the maturation processes of these two components, from initial calcification to completely maturity, occur at different points in time. The enamel and dentine in third molars demonstrate the same phenomenon. Mineralization of enamel in third molars is completed by the age of 12. After the time of mineralization, the enamel remains closed and will no longer perform a significant physiological exchange of elements. However, dentine is vital organ, and the odontoblast in pulp will extend its cell process into dentinal tubular to conduct dentine inside and outside element exchange from blood circulation.

Teeth are a composite of inorganic, organic and water fractions in various amounts. Their inorganic phase consists of the unit cell $(Ca,X)_{10}(P,C)_6(O,OH)_{26}$. The microcations favor coordination with oxygen and do not usually form complex ion species. The common characteristics of these cations are a small ionic radius and high charge/radius ratio. These cations can replace each other. The X in this chemical formula represents a

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variety of possible substitutions for Ca, such as Sr, Ba and Pb (McConnell, 1973). Gruner et al. suggested for enamel and dentine the exact chemical formulas are enamel, $(OH)_2Ca_6[(P_{5.8}C_{0.2})O_{24}](Ca_{3.1}Mg_{0.1}C_{0.5})$, and dentine, $(OH)_2Ca_6[(P_{5.6}C_{0.5})O_{24}](Ca_{2.7}Mg_{0.5}C_{0.6})$. According to these formulas, the Ca/P ratio is about 2.02 (Gruner, 1937).

Teeth Using for Study of Environmental and Archaeological Purposes

In the 1930s, Dreal, Lowater and Murray reported that teeth contain a variety of minor or trace elements (Dreal, 1936; Lowater, 1937). As more sensitive analytical instruments and methods were developed, more information about trace elements and their functions in the different parts of teeth were investigated. Teeth are believed to preserve great information through a life span varying with environmental exposures. Elemental analysis of deciduous and/or permanent teeth has been frequently used for environmental and archaeological purposes (Lowater, 1937; Reitznerová, 2000; Falla-Sotelo, 2005; Malara, 2006; Chao, 2009).

Most of the early dental elements data were obtained by mixing various kinds of teeth or pooling samples. Nowadays we know there are many differences in elemental composition for various tooth types. Brown et al. suggested that in order to use the elemental composition of human primary teeth as environmental indicators, it is necessary to restrict to a single tooth type (Brown, 2002). Thus, third molars are the unique type of teeth selected to be analyzed in this study.

Reasons for Selecting Third Molars

The size of a third molar is larger than most of deciduous teeth or permanent teeth. Also, its eruption timing is the latest of all teeth and it is located in the last row of the alveolar ridge. The degree of dental wear and dentine/enamel exposure of a third molar is often less than other teeth in human adults. On the other hand, the amount of material available for study is always a factor affecting the results of trace elements analysis. Third molars are extracted for two general reasons: either the tooth has already become impacted, or the tooth could potentially become problematic if not extracted. Preventive removal of the third molars is a common practice in developed countries and is usually recommended by dentists (Pinkham, 2005). Also, deciduous teeth, specifically third molars, are more available than permanent teeth for research purposes. Availability and quantity are two of the most important reasons for selecting third molars as the material for this study.

Most third molars are removed between the ages of 20 to 30 in recent clinical cases. Once a third molar is studied, it would offer information of a definite time. For example, the trace elements in enamel of a third molar may reflect the subject's diet during the time they were 7 to 16 years old. This implication for third molars is important in the study of environmental changes and human mobility.

The incisal area of human third molars is always kept complete because it is rarely used for grinding foods. Enamel in third molar is rarely exposed to the oral environment. Additionally, some third molars are embedded in bone. The un-erupted third molars have less contamination from soil or water sources. These are specific characteristics of third teeth which are different from other deciduous and permanent teeth for archaeological or paleologic studies.

As described above, third molars are a unique type of tooth suggested as representative material for research. They may provide definite information for supporting evidence for a history of events. Thus this study is devoted to elemental analysis of enamel and dentine of unique third molars. We would like to show the definite characteristics of a single type of tooth using third molars to provide reliable base references for future studies.

Materials and Methods

Sample Collection

Samples of third molars were collected from a local dental clinic in Hsinchu City, Taiwan, from 2009 to 2010. The donors of the samples were 46 males and 34 females, with ages ranging from 17 to 68 years old. The molars are grouped by ages in 10 years increments between 21 years to 40 years. Other groups are included in samples ages 20 years or less and 41 years and older. The molars in the age group of 21 to 30 years are further divided in half to get a more balanced distribution. Distribution of ages in all 5 age groups is shown in **Tables 1** and **2**.

Table 1.

Concentration of elements in enamel of various age groups.

Era of Birth	Before 1969	1970-1979	1980-1984	1985-1989	After 1990
Age	≥41 (n = 11)	31 - 40 (n = 12)	26 - 30 (n = 21)	21 - 25 (n = 21)	≤20 (n = 15)
P (%)	14.70 ± 1.62	15.20 ± 1.85	14.44 ± 2.51	14.21 ± 2.47	15.00 ± 2.03
Ca (%)	28.47 ± 3.69	27.95 ± 3.74	27.81 ± 5.23	27.83 ± 3.70	27.74 ± 3.47
Sr (ppm)	32.22 ± 32.94	131.52 ± 45.44	94.33 ± 24.53	98.85 ± 30.19	105.00 ± 35.25
Ba (ppm)	1.87 ± 0.80	2.33 ± 1.40	1.99 ± 0.87	1.69 ± 0.52	2.07 ± 1.43
Pb (ppm)	1.17 ± 0.62	1.03 ± 0.66	0.77 ± 0.29	0.53 ± 0.31	0.35 ± 0.14
Ca/P	1.94	1.83	1.93	1.96	1.85
Sr/Ca (10^{-5})	46.97	46.97	34.04	35.46	38.0
Ba/Ca (10^{-5})	0.65	0.83	0.72	0.61	0.74
Pb/Ca (10^{-5})	0.42	0.38	0.29	0.20	0.13

Table 2.
Concentration of elements in dentine of various age groups.

Era of Birth	Before 1969	1970-1979	1980-1984	1985-1989	After 1990
Age	≥41 (n = 11)	31 - 40 (n = 2)	26 - 30 (n = 21)	21 - 25 (n = 21)	≤20 (n = 5)
P (%)	11.37 ± 1.32	12.05 ± 2.14	11.00 ± 1.49	11.00 ± 1.82	11.78 ± 2.27
Ca (%)	21.68 ± 2.93	21.48 ± 4.12	21.08 ± 3.79	21.05 ± 4.28	21.64 ± 3.15
Sr (ppm)	104.52 ± 34.75	98.29 ± 28.27	81.18 ± 17.97	82.70 ± 23.67	92.72 ± 31.12
Ba (ppm)	2.24 ± 1.06	3.42 ± 1.87	3.28 ± 1.82	2.28 ± 0.80	2.51 ± 1.17
Pb (ppm)	1.60 ± 0.84	1.25 ± 0.58	1.07 ± 0.46	0.7 ± 0.37	0.49 ± 0.28
Ca/P	1.91	1.78	1.92	1.91	1.84
Sr/Ca (10^{-5})	48.52	48.41	39.24	40.25	43,13
Ba/Ca (10^{-5})	1.05	1.87	1.57	1.11	1.17
Pb/Ca (10^{-5})	0.75	0.62	0.53	0.39	0.23

Sample Preparation

Each third molar was put in a clean tube after extraction and then delivered to the laboratory. The bloodstains were rinsed off with distilled water. The attached soft tissues were eliminated with toothbrushes.

The third molar crown contains two parts: incisal and cervical areas. The incisal area is entirely enamel, and the cervical area contains different quantities of coronal dentine. Using a diamond-impregnated stainless-steel blade the enamel is cut into a horizontal slice of 2-mm thickness from the crown near the incisal and cervical areas of a third molar. If the thin slice contains dentine, it must be fully removed. For example, circum pulpal dentine found in the surroundings of pulp cavity of the tooth must be removed.

Elemental Analyses

The enamel and dentine are separated with diamond blade cuts. After weighing the samples, the enamel and dentine are put into separate 15 ml test tubes (Labcon, green). Then, 5 ml of ultrapure concentrated nitric acid is added to the tubes and settled for a week until complete dissolution. Finally, deionized water is added to dilute the sample to 15 ml.

The ICP mass spectrometer used in the study is an Agilent 7500ce system (Agilent, CA, USA). A Micromist nebulizer (AR35-1-EM04EX, Glass Expansion, Victoria, Australia) is fitted to a Scott-type quartz double-pass spray chamber. Based on the experimental results, the method detection limit obtained for P, Ca, Sr, Ba and Pb are 0.584 ppm, 2.933 ppm, 0.005 ppm, 0.003 ppm and 0.016 ppm, respectively.

Statistical Analysis

The data was analyzed using SPSS for Windows version 13.0. Non-parametric Spearman correlation is used to analyze the correlation of age with the concentrations of P, Ca, Sr, Ba and Pb in enamel and dentine of the third molars. A two-tailed p-value < 0.05 is considered significant.

Results

In **Table 3**, the mean concentrations of P, Ca, Sr, Ba and Pb

respectively in enamel are 14.63% ± 2.19%, 27.91% ± 4.03%, 108.31 ± 35.71 ppm, 1.96 ± 1.01 ppm and 0.72 ± 0.49 ppm, and in dentine are 11.35% ± 1.01%, 21.32% ± 3.68%, 89.52 ± 27.11 ppm, 2.75 ± 1.46 ppm and 0.96 ± 0.61 ppm. The levels of P, Ca and Sr are higher in enamel than in dentine, and Ba and Pb concentrations are higher in dentine than in enamel.

Additionally, correlation coefficients between age and concentration individually in enamel and dentine of third molars are shown in **Table 3**. There are remarked positive correlations between age and dentine Pb, enamel Pb or enamel Sr. There is no correlation between age and concentration in enamel or dentine for P, Ca and Ba. The P and Ca concentrations are kept constant by age.

In this study, Sr, Ba and Pb concentrations in various age groups are shown in **Tables 1** and **2**. Concentrations of Pb present in enamel and dentine increased in all age groups. In regards to Post Hoc tests, there are significant differences for Pb in enamel between the ages of ≤20 and 31 - 40 ($p = 0.002$), ≤20 and ≥41 ($p > 0.001$), 21 - 25 and 31 - 40 ($p = 0.029$), 21 - 25 and ≥41 ($p = 0.003$). And there are significant differences for Pb in dentine between the ages of ≤20 and 26 - 30 ($p = 0.024$), ≤20 and 31 - 40 ($p = 0.006$), ≤20 and ≥41 ($p < 0.001$), 21 - 25 and ≥41 ($p < 0.001$).

Sr concentrations increased from the 26 - 30 age group to the ≥41 age group, but higher concentrations in enamel and dentine were noted in the ≤20 age group. Concentrations of Ba in enamel and dentine increased from the 21 - 25 age group to the 31 - 40 age group. Then the drop of Ba concentration is noted in the ≥41 age group. However, there are no significant differences among them.

There are significant differences for Sr/Ca in enamel between the ages 26 - 30 and 31 - 40 ($p = 0.037$), 26 - 30 and ≥41 ($p = 0.046$).

Discussion

Ca and P Concentrations and Ca/P Ratio in Third Molars

Ca and P are the two main elements in teeth. In this study, the average P and Ca concentration and Ca/P ratio in the dentine and enamel of third molars, shown as **Table 3**, are very closely related to the previous study by Arora et al., which

Table 3.
Mean concentration, correlation coefficients with age, and enamel/dentine ratio of the elements in third molars.

Element/ concentration (n = 80)	Enamel conc. (range) correl. coeff. (p value)	Dentine conc. (range) correl. coeff. (p value)	Enamel/ Dentine ratio
P (%)	14.63 ± 2.19 7.59 - 18.94 r = 0.011 (p = 0.922)	11.35 ± 1.83 6.23 - 16.53 r = 0.015 (p = 0.895)	1.29
Ca (%)	27.91 ± 4.03 13.72 - 36.25 r = 0.067 (p = 0.554)	21.32 ± 3.68 23.06 - 31.88 r = −0.031 (p = 0.785)	1.31
Sr (ppm)	108.30 ± 35.71 51.26 - 199.74 r = 0.301* (p = 0.007)	89.52 ± 27.11 47.73 - 179.0 r = 0.199 (p = 0.077)	1.20
Ba (ppm)	1.96 ± 1.01 0.68 - 6.70 r = 0.116 (p = 0.307)	2.75 ± 1.46 0.33 - 8.12 r = 0.082 (p = 0.471)	0.69
Pb (ppm)	0.72 ± 0.49 0.12 - 2.55 r = 0.719* (p < 0.001)	0.96 ± 0.61 0.1 - 2.77 r = 0.654* (p < 0.001)	0.70
Sr/Ca (10^{-5})	38.87 ± 11.86 r = 0.300* (p = 0.007)	42.88 ± 14.40 r = 0.139 (p < 0.220)	
Ba/Ca (10^{-5})	0.70 ± 0.32 r = 0.135 (p = 0.231)	13.47 ± 9.47 r = 0.082 (p = 0.468)	
Pb/Ca (10^{-5})	0.27 ± 0.19 r = 0.678* (p < 0.001)	0.47 ± 0.31 r = 0.625* (p < 0.001)	

conc.: element concentration. correl. coeff.: Spearman correlation coefficient between element concentration and donator's age. Enamel/dentine ratio: the ratio of element concentration in enamel to that in dentine.

reported Ca and P concentrations in cervical region of 24.35% and 12.41% respectively, with a Ca/P ratio of 1.96 (Arora, 2004).

The enamel itself is an entirely calcified tissue, and is regarded as a non-vital organ. Conversely, dentine is viewed as vital organ because many dentinal tubules exist within it. Ca and P in enamel might participate in body calcium turnover activity for only the first few years of life, and would be isolated from it after tooth eruption. So it is reasonable for constant Ca or P concentrations in enamel of third molars in various age groups (Manea-Krichten, 1991). In **Table 2**, the concentrations of Ca and P in dentine are also constant (with no significant differences) in various age groups. That might mean only minor parts of Ca or P in dentine participating in turnover activity (e.g. dentine eburnation), which does not seem to affect their whole concentration.

Murray reported the Ca/P ratio for human teeth varied from 1.92 (lowest in dentine) to 2.15 (highest in enamel) (Murray, 1936). He suggested the deposition process of calcification is an active and specific cell process, and not merely a precipitation from saturated or supersaturated solutions of a salt of constant composition dependent on the ionic composition of the blood plasma. Hillson discovered biogenic Ca/P ratio ranges

from 1.91 to 2.17 for enamel, and 2.1 to 2.2 for dentine. Different Ca/P ratios mean different nature in enamel and dentine (Hillson, 1996). Arnold and Gaengler reported differences between the Ca/P ratio in predentin and dentin indicating that different calcium phosphate minerals occur during dentinogenesis. They also showed the Ca and P content in enamel of permanent teeth was even higher than in the enamel of developing teeth (Arnold, 2007).

Sr, Ba and Pb Concentrations in Third Molars

Sr

Sr is one of constituent elements of teeth. Steadman et al. (1959) reported Sr concentrations ranging from 80 to 620 ppm in human teeth from different geographic areas (Steadman, 1959). The Sr content of teeth seems to parallel the level of intake by the individual. There are various studies of Sr determination which are summarized in **Tables 4** and **5** (Brown, 2002; Brown, 2004; Soares, 2008). The Sr concentrations determined in this study are close to the Brown's data from dentine of deciduous teeth.

In this study, no significant differences were found in the higher concentration present in the ≤20 age group for enamel and dentine. One possible explanation for this phenomenon is that Sr is one of the component elements of teeth and the Sr^{2+} ion would strongly and rapidly substitute for Ca^{2+} in the ordered apatite lattice during the third molars' growing period.

Ba

Manea-Krichten et al. determined the Ba concentrations in enamel to be 6.4 ppm in dry weight (Manea-Krichten, 1991). Ba may diffuse from a blood-dentine source into enamel, where it replaces Ca and accumulates with age. The distribution does characterize a natural situation for biological occurrences of Ba in humans. There is little research on Ba concentrations in third molars, but more research exists for deciduous and a mixture of permanent teeth. Brown et al. showed Ba concentrations of deciduous and permanent teeth are 6.41 and 4.4 ppm respectively (Brown, 2002; Brown, 2004). Brown's results are higher than this study as shown in **Tables 4** and **5**. In this study, both Ba and Pb are several ppm in concentration, and Ba concentrations are higher than Pb in enamel and dentine. Pb concentration would be much higher than that of Ba if Pb exposure exists (Manea-Krichten, 1991). This indicates the subjects in this study have no exposure to Pb.

Pb

Pb is a naturally occurring element that is also a ubiquitous environmental pollutant due to its widespread uses. It is well-known that leaded gasoline was a major source of lead exposure in the population. Brudevold and Steadman reported an increased Pb level with age. They found the concentration of Pb in enamel is about 30 ppm in teeth from young adults and about 90 ppm in teeth over 50 years of age (Brudevold, 1956). Derise and Ritchey found the concentration of Pb in enamel and dentine are from 38.9 to 51.5 ppm (Derise, 1974). Thomas et al. showed that reducing lead from gasoline significantly decreased population blood lead from 1978 to 1996 on six continents (Thomas, 1999). Thus, there are no more high levels of Pb in teeth reported in literature within the last ten years (Malara, 2006; Brown, 2002; Brown, 2004; Arora, 2004; Zaichick, 1997). There are few studies about the Pb concentration in third

Table 4.
Concentration of elements in dentine of human teeth reported in previous studies.

Authors	Method	Teeth types	No	P (%)	Ca (%)	Sr (ppm)	Ba (ppm)	Pb (ppm)
this work	ICP-MS	third molar	n = 90	11.37 ± 1.91	21.26 ± 3.74	89.27 ± 28.42	2.78 ± 1.58	1.09 ± 0.91
Soares	INAA	permanent deciuous	10 8		21.40 ± 1.79 25.0 ± 2.2	174.2 ± 118.7 92.1 ± 25.4		
Shi	EMPA	deciduous I	37		34.33 ± 1.33			
Liang	EMPA	deciduous	38	17.42 ± 0.59				
Arnold	EDX	third molar	3	17.2 ± 2.2	37.7 ± 5.9			
Zenóbio	ICP/AES	third molar	30	10.98 ± 0.5	23.10 ± 0.5			
Soremark	INAA			13.5 ± 2.8	28.2 ± 1.2			
Arora	ICP-MS	deciduous molar canine incisor	5 4 7	Cervical 12.41 ± 0.53 12.56 ± 0.33 11.86 ± 0.22	Cervical 24.35 ± 1.29 23.84 ± 1.14 22.56 ± 0.43			Cervical 1.11 ± 0.30 0.87 ± 0.41 1.23 ± 0.39
Arora	ICP-MS	deciduous molar canine Incisor	5 4 7	Apical 11.97 ± 0.54 12.52 ± 0.32 11.28 ± 0.20	Apical 24.21 ± 1.38 24.22 ± 0.68 22.45 ± 0.31			Apical 2.95 ± 2.06 2.17 ± 0.70 3.32 ± 1.56
Malara	AAS	permanent incisor canine pre-molar molar	33 38 43 41		root 55.27 ± 8.52 58.11 ± 10.84 56.90 ± 8.12 57.03 ± 9.73			root 14.08 ± 2.74 9.97 ± 3.57 10.39 ± 2.85 10.11 ± 2.14
Brown	ICP-MS	deciduous whole teeth	UK 27 UG 21		31.4 ± 19.6 31.5 ± 13.7	118 ± 37.8 175 ± 38.0	6.41 ± 2.92 9.87 ± 4.43	1.33 ± 0.89 1.21 ± 0.44

AAS: atomic absorption spectrometer. EDX: energy-dispersive X-ray analysis. EDXRF: energy dispersive X-ray fluorescent analysis. EMPA: electron microprobe X-ray mMicroanalyzer. ICP/AES: ductively coupled plasma spectrometer. ICP-MS: inductively coupled plasma mass spectrometer. INAA: instrument neutron activation analysis.

Table 5.
Concentration of elements in enamel of human teeth from various studies.

Authors	Method	Teeth	No	P (%)	Ca (%)	Sr (ppm)	Ba (ppm)	Pb (ppm)
this work	ICP-mass	third molar	n = 90	14.63 ± 2.21	27.87 ± 4.08	107.57 ± 37.31	1.91 ± 1.01	0.76 ± 0.54
Soares	INAA	permanent deciduous	10 8		31.20 ± 4.76 29.0 ± 5.2	285.8 ± 181.7 84.8 ± 16.7		
Shi	EMPA	deciduous			38.74 ± 1.31			
Liang	EMPA	deciduous	38	18.29 ± 0.44				
Arnold	EDX	third molar	3	19.9 ± 1.8	42.7 ± 5.1			
Zenóbio	ICP-AES	third molar	30	17.5 ± 0.5	36 ± 0.5			
Lakomaa	INAA			18.6 ± 0	34 ± 2.0			
Zaichick	EDXRF	permanent	35		36.3 ± 0.8	240 ± 40		≤3
Brown	ICP-MS	permanent	8			78% ± 5%	4.4% ± 6%	6.0% ± 5%

AAS: atomic absorption spectrometer. EDX: energy-dispersive X-ray analysis. EDXRF: energy dispersive X-ray fluorescent analysis. EMPA: Electron microprobe X-ray mMicroanalyzer. ICP/AES: ductively coupled plasma spectrometer. ICP-MS: inductively coupled plasma mass spectrometer. INAA: instrument neutron activation analysis.

molars. The present results of third molars compare with other studies of permanent teeth are shown in **Tables 4** and **5**.

Pb Concentrations in Various Age Groups Indicate Eras of Exposure

There are significant positive correlations between age and Pb in enamel and dentine. Pb is believed to have a cumulative effect. This study shows the Pb concentration in teeth increases from younger groups to older groups. This means that Pb is deposited in teeth year by year.

The various age groups may be looked upon as eras of birth groups. Eras of birth indicate the eras of environmental exposure. Initial calcification of human third molars occurs between

the ages of 7 to 10. That means if more Pb exists in one's third molars, he may have been exposed to Pb before 7 to 10 years ago. The eras are defined by birth year of the donor as follows: ≤20 age group is 1990 and later, 21 - 25 age group is 1985-1989, 26 - 30 age group is 1980-1984, 31 - 40 age group is 1970-1979, and ≥41 age group is before 1969.

Figure 1 shows Sr, Ba and Pb concentrations in various eras of birth. Pb concentrations are seen to increase by age group. More importantly, this shows Pb concentration increases by eras of birth. In Taiwan, unleaded gasoline was supplied starting in 1986, which decreased the extent of cord blood lead level decline in the Taipei area from 1985 to 2002 (Hwang, 2004). It is reasonable that donors born before 1979 will have higher concentrations of Pb in their teeth.

Sr/Ca and Ba/Ca

The ratio of Sr to Ca concentration is an indicator for bio-puri-fication of calcium. The Sr/Ca ratio in teeth is an interesting issue (Chao, 2009; Arora, 2004; Webb, 2005). Data for Sr/Ca and Ba/Ca in teeth enamel may offer much information for researchers in many fields of study, such as environmental sci-ence, ecology, biology, paleontology and archaeology (Wang, 2010).

In this study, the Sr/Ca ratio is 3.86×10^{-6} and 4.20×10^{-6} in enamel and dentine of third molars, respectively. In **Tables 1** and **2**, Sr/Ca ratios increase with age except in the under the
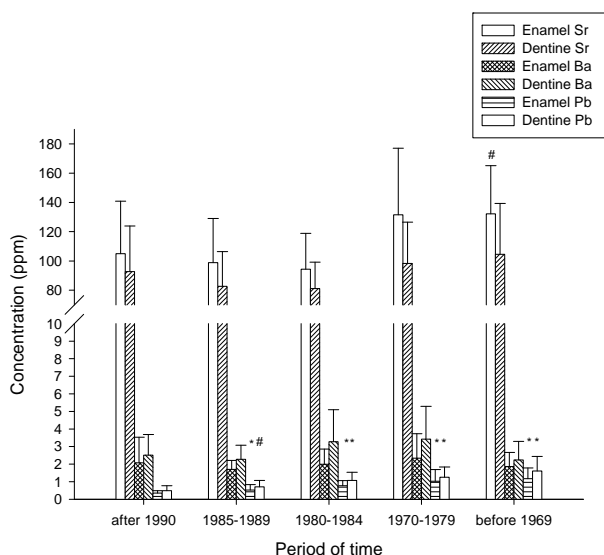
Figure 1.
Sr, Ba and Pb concentration in in enamel or dentine of third molars at various eras of birth year. [#]: *p* value, $0.05 < p < 0.1$. [*]: *p* value, $p < 0.05$. Sr concentrations are higher in enamel than that in dentine. Ba and Pb concentrations are higher in dentine than that in enamel. Sr concentrations in enamel or dentine are higher in after 1990, and increased gradually from 1985-1989 to before 1969. However there are no significant differences among each group. Pb concentrations increased gradually by age in both enamel and dentine. For enamel there are significant differences between after 1990 and 1970-1979 ($p = 0.002$), after 1990 and before 1969 ($p < 0.001$), 1985-1989 and 1970-1979 ($p = 0.029$), 1985-1989 and before 1969 ($p = 0.003$). For dentine there are significant differences between after 1990 and 1980-1984 ($p = 0.024$), after 1990 and 1970-1979 ($p = 0.006$), after 1990and before 1969 ($p < 0.001$), 1985-1989 and before 1969 ($p < 0.001$). Ba concentrations decay in older age groups in both enamel and dentine. There are no significant differences among each group.

20-year-old age group. Chao et al. also indicated that Sr/Ca ratios in teeth are dependent on age (Chao, 2009). The information for third molars in this study provides a reference for the diet of modern adults.

Element Concentration Ratio of Enamel to Dentine

In **Table 3**, elements with enamel/dentine ratio >1 means that the element concentration in enamel is higher than that in dentine. Enamel is the hardest tissue in the human body. P and Ca are major component elements in teeth. Enamel is closed construction where no more elemental exchange since P and Ca are deposited at about the age of 16. It is reasonable that their concentrations are higher in enamel than those in dentine. In this study, both of P and Ca concentrations are always higher in enamel than those in dentine (**Table 3**). These results of third molars are compared with other studies of different teeth types in spite of fewer references in the past (**Tables 4** and **5**).

Cate also showed that the composition of enamel is 96 wt% inorganic material and 4 wt% organic material and water, while 70% of dentin consists of the mineral hydroxylapatite, 20% is organic material and 10% is water (Cate, 1998). Therefore, P and Ca elements are more concentrated in enamel than in dentine.

However, for trace elements in teeth the concentrations of Pb and Ba are higher in dentine than those in enamel as shown in **Table 3**. The reasonable explanation is that dentine is a vital organ where trace elements, like Ba and Pb, could enter dentine from blood circulation, resulting in an increasing in concentration year after year.

In this study, the level of Sr is higher in enamel than in dentine. This may be due to the fact that Sr, as well as Ca and P, are essential elements for tooth structure, which deposits during the enamel formation process. These observations in **Tables 4** and **5** are consistent with the results reported by Soares et al. (Soares, 2008).

Conclusions

Enamel and dentine of 80 third molars were analyzed by ICP-MS in this study. The concentrations of P, Ca and Sr are higher in enamel than in dentine of third molars, and the concentrations of Ba and Pb are higher in dentine than in enamel. In enamel and dentine, the concentrations of P, Ca and Ca/P ratio are stable and persist. In enamel the concentrations of Sr and Sr/Ca increase by age statistically but the concentrations of Ba and Ba/Ca do not. In spite of increasing with age, the concentration of Sr is higher in the under 20-year-old age group. Pb concentrations in enamel and dentine are increased by birth eras, which may indicate the years of environmental exposure. The levels of Pb in this study are lower than the previous published findings before 1979.

Teeth are believed to be a particularly rich source of information on life history and environmental exposures. A single type of teeth such, as third molars, may be suitable for demonstrating the definite characteristics of teeth. These results show some basic data that may offer information for supplemental evidence to the history of events.

REFERENCES

Arnold, W. H., & Gaengler, P. (2007). Quantitative analysis of the

calcium and phosphorus content of developing and permanent human teeth. *Annals of Anatomy, 189,* 183-190. doi:10.1016/j.aanat.2006.09.008

Arora, M., Chan, S. W. Y., Kennedy, B. J., Sharma, A., Crisante, D., & Walker, D. M. (2004). Spatial distribution of lead in the roots of human primary teeth. *Journal of Trace Elements in Medicine and Biology, 18,* 135-139. doi:10.1016/j.jtemb.2004.07.001

Ash, M. M., & Nelson, S. J. (2002). *Wheeler's dental anatomy, physiology and occlusion* (pp. 32,45,53). Philadelphia, PA: W.B. Saunders.

Brown, C. J., Chenery, S. R. N., Smith, B., Tomkins, A., Roberts, G. J., Sserunjogi, L., & Thompson, M. (2002). A sampling and analytical methodology for dental trace element analysis. *Analyst, 127,* 319-323. doi:10.1039/b109066f

Brown, C. J., Chenery, S. R. N., Smith, B., Mason, C., Tomkins, A., Roberts, G. J., Sserunjogi, L., & Tiberindwa, J. V. (2004). Environmental influences on the trace element content of teeth—Implications for disease and nutritional status. *Archives of Oral Biology, 49,* 705-717. doi:10.1016/j.archoralbio.2004.04.008

Brudevold F., & Steadman, L. T. (1956). The distribution of lead in human enamel. *Journal of Dental Research, 35,* 430-437. doi:10.1177/00220345560350031401

Cate, A. R. (1998). *Oral histology: Development, structure, and function* (5th ed., pp. 1,150). Maryland Heights, MO: Mosby Publisher.

Chao, J. H., Liu, M. T., Yeh, S. A., Huang, S. S., Wu, J. M., Chang, Y. L., Hsu, F. Y., Chuang, C. Y., Liu, H. Y., & Sun, Y. C. (2009). Using medical accelerators and photon activation to determine Sr/Ca concentration ratios in teeth. *Applied Radiation and Isotopes, 67,* 1121-1126. doi:10.1016/j.apradiso.2009.02.089

Derise, N. L., & Ritchey, S. J. (1974). Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: II. Microminerals. *Journal of Dental Research, 53,* 853-858. doi:10.1177/00220345740530041601

Dreal, W. F. (1936). Spectrum analysis of dental tissues for trace elements. *Journal of Dental Research, 15,* 403-406. doi:10.1177/00220345350150060401

Falla-Sotelo, F. O., Rizzutto, M. A., Tabacniks, M. H., Added, N., & Barbosa, M. D. L. (2005). Analysis and discussion of trace elements in teeth of different animal species. *Brazilian Journal of Physics, 35,* 761-762. doi:10.1590/S0103-97332005000500010

Gruner, J.W., McConnell, D., & Armstrong, W.D. (1937). The relationship between crystal structure and chemical composition of enamel and dentin. *The Journal of Biological Chemistry, 121,* 771-781.

Hillson, S. (1996). *Dental anthropology* (pp. 217-225). Cambridge, UK: Combridge University Press. doi:10.1017/CBO9781139170697.010

Hwang, Y. H., Ko, Y., Chiang, C. D., Hsu, S. P., Lee, Y. H., Yu, C. H., Chiou, C. H., Wang, J. D., & Chuang, H. Y. (2004). Transition of cord blood lead level, 1985-2002, in the Taipei area and its determinants after the cease of leaded gasoline use. *Environmental Research, 96,* 274-282. doi:10.1016/j.envres.2004.02.002

Lakomaa, E. L., & Rytomaa, I. (1977). Mineral composition of enamel and dentine of primary and permanent in Finland. *Scandinavian Journal of Dental Research, 85,* 89-95.

Liang, Q., Shi, S. Z., & Liu, Y. (2005). Microanalysis of phosphorus in enamel and dentin of deciduous teeth. *Journal of PractStomatol, 21,* 455-459.

Lowater, F., & Murray, M. M. (1937). Chemical composition of teeth. V. Spectrographic analysis. *Biochemical Journal, 31,* 837-841.

Malara, P., Kwapulinski, J., & Malara, B. (2006). Do the levels of selected metals differ significantly between the roots of carious and non-carious teeth? *Science of the Total Environment, 369,* 59-68. doi:10.1016/j.scitotenv.2006.04.016

Manea-Krichten, M., Patterson, C., Miller, G., Settle, D., & Erel, Y. (1991). Comparative increases of lead and barium with age in human tooth enamel, rib and ulna. *Science of the Total Environment, 107,* 179-203. doi:10.1016/0048-9697(91)90259-H

McConnell, D. (1973). *Apatite, its crystal chemistry, mineralogy, utilization and geologic and biologic occurrences, applied mineralogy.* Wien/New York: Springer-Verlag.

Murray, M. M. (1936). *The Chemical composition of teeth. IV. The calcium, magnesium and phosphorus contents of the teeth of different animals. A brief consideration of the mechanism of calcification* (pp.1567-1571). London, UK: University of London.

Pinkham, J. R. (2005). *Pediatric dentistry: Infancy through adolescence* (4th ed.). Alexandria, VA: Mosby Publisher.

Reitznerová, E., Amarasiriwardena, D., Kopčáková, M., & Barnes, R. M. (2000). Determination of some trace elements in human tooth enamel. *Fresenius' Journal of Analytical Chemistry, 367,* 748-754. doi:10.1007/s002160000461

Shi, S. Z, Liang, Q., & Lai, H. (2005). Study on the calcium content of enamel and dentin in deciduous teeth. *Journal of Oral Science, 21,* 226-229.

Soares, M. A. B., Adachi, E. M., & Saiki, M. (2008). INAA of enamel and dentine samples of a group of children and adults: A comparative study. *Journal of Radio Analytical and Nuclear Chemistry, 276,* 49-52. doi:10.1007/s10967-007-0408-6

Soremark, R., & Samsahl, K. (1962). Gammaray spectrometric analysis of elements in normal human dentin. *Journal Dental Research, 41,* 603-606. doi:10.1177/00220345620410031201

Steadman, L. T., Brudevold, F., Smith, F. A., Gardner, D. E., & Little, M. F. (1959). Trace elements in ancient indian teeth. *Journal of Dental Research, 38,* 285-292. doi:10.1177/00220345590380021001

Thomas, V. N., Socolow, R. H., Fanelli, J. J., & Spiro, T. G. (1999). Effects of reducing lead in gasoline: an analysis of the international experience. *Environmental Science & Technology, 33,* 3942-3948. doi:10.1021/es990231+

Wang, C. H., Hsu, C. C., Chang, C. W., You, C. F., & Tzeng, W. N. (2010). The migratory environmental history of freshwater resident flathead. Mullet mugilcephalus L. in the Tanshui river, Northern Taiwan. *Zoological Studies, 49,* 504-514.

Webb, E., Amarasiriwardena, D., Tauch, S., Green, E. F., Jones, J., & Goodman, A. H. (2005). Inductively coupled plasma-mass (ICP-MS) and atomic emission spectrometry (ICP-AES): Versatile analytical techniques to identify the archived elemental information in human teeth. *Microchemical Journal, 81,* 201-208. doi:10.1016/j.microc.2005.04.002

Zaichick, V., Ovchjarenko, N., & Zaichick, S. (1997). In vivo energy dispersive X-ray fluorescence for measuring the content of essential and toxic trace elements in teeth. *Applied Raidiation and Isotopes, 50,* 283-293. doi:10.1016/S0969-8043(97)10150-6

Zenobio, M. A. F., Nogueira, M. S., & Zenóbio, E. G. (2011). Chemical composition of human enamel and dentin.Preliminary results to determination of the effective atomic number. http://biblioteca.cdtn.br/cdtn/arpel/adobe/Art-01_Madelon_AFZenobio.pdf