# Heavy metals in human teeth dentine: a bio-indicator of metals exposure and environmental pollution

Khandoker Asaduzzaman [a], Mayeen Uddin Khandaker [a, *], Nurul Atiqah Binti Baharudin [a], Yusoff Bin Mohd Amin[a], Mohideen Salihu Farook [b], D.A. Bradley [c, d], Okba Mahmoud[e]

[a] *Department of Physics, Faculty of Science, University of Malaya, Kuala Lumpur, 50603, Malaysia*
[b] *Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, Kuala Lumpur, 50603, Malaysia*
[c] *Department of Physics, University of Surrey, Guildford, Surrey, GU4 8JU, UK*
[d] *Sunway University, Institute for Health Care Development, Jalan Universiti, 46150, PJ, Malaysia*
[e] *College of Dentistry, Ajman University, Ajman, United Arab Emirates*

## ABSTRACT

With rapid urbanization and large-scale industrial activities, modern human populations are being increasingly subjected to chronic environmental heavy metal exposures. Elemental uptake in tooth dentine is a bioindicator, the uptake occurring during the formation and mineralization processes, retained to large extent over periods of many years. The uptake includes essential elements, most typically geogenic dietary sources, as well as non-essential elements arising through environmental insults. In this study, with the help of the Dental Faculty of the University of Malaya, a total of 50 separate human teeth were collected from dental patients of various ethnicity, age, gender, occupation, dietary habit, residency, etc. Analysis was conducted using inductively coupled plasma-mass spectrometry (ICP-MS), most samples indicating the presence of the following trace elements, placed in order of concentration, from least to greatest: As, Mn, Ba, Cu, Cr, Pb, Zn, Hg, Sb, Al, Sr, Sn. The concentrations have been observed to increase with age. Among the ethnic groups, the teeth of the Chinese show marginally greater metal concentrations than those of the Indians and Malays, the teeth dentine of females generally showing greater concentrations than that of males. Greater concentrations of Hg, Cu and Sn were found in molars while Pb, Sr, Sb and Zn were present in greater concentrations in incisors. With the elevated concentration levels of heavy metals in tooth dentine reflecting pollution from industrial emissions and urbanization, it is evident that human tooth dentine can provide chronological information on exposure, representing a reliable bio-indicator of environmental pollution.

**Keywords*:*** Environment, Human teeth, Dentine, Heavy metal, Pollution, ICP-MS

---

## 1. Introduction

Human civilization and the environment (both terrestrial and aquatic) are currently being exposed to the greatest levels of heavy metals within recorded history. Among the sources of exposure are that due to anthropomorphic activities such as the use of coal and oil, oil and gas-exploration and exploitation, disposal of industrial effluents, modern agricultural practices (e.g. use of fertilizers, fungicides, insecticides, herbicides and waste water from use in irrigation), rapid urbanization, atmospheric deposition of dust/aerosols, vehicular emissions, sewage sludge etc (Bhuiyan et al., 2015; Korkmaz Gorur et al., 2012: Barton, 2011).

The discharge of heavy metals into the natural environment, either from geogenic sources or as a result of anthropogenic activities, can be associated with numerous environmental consequences resulting from their non-biodegradability and persistence. Once heavy metals enter into the body, via ingestion, inhalation or dermal contact, they accumulate in various organs including the calcified tissues, bones and teeth (the latter being part of the exo-skeletal system), posing a risk to human health due to their toxicity and long-term persistence (Alina et al., 2012; Millour et al., 2012; Alomary et al., 2006; Amr, 2011; Keshavarzi et al., 2015). As such, it is considered imperative to assess heavy metals in human populations, evaluating the risk, informing the efficacy of systems of control or lack of.

Evaluation of environmental pollution can be performed using physical and chemical methods and through bio-indicators (Kamberi et al., 2012). Bio-monitoring of heavy metal exposure of human beings gives a sign of the current body burden of an individual, which is a function of recent and/or past environmental exposure (Kantamneni, 2010). Thus, the right choice of and development of suitable biomarkers to assess heavy metal exposure is of crucial importance, for primary prevention, health care management and decision-making in public health. Recently, there has been growing interest among researchers in respect of the use of human bodily fluids and organs such as blood, urine, bone, teeth, nails, hair and saliva as bio-indicators, in order to investigate environmental pollution through detection of toxic heavy metals (Kamberi et al., 2012; Kantamneni, 2010; Abdullah et al., 2012; Arruda-Neto et al., 2010).

Each of the detected elements can be associated with particular advantages in respect of physiological function or of risks and limits to normal bodily function. Blood and urine data reflect information on recent exposures, with blood-lead levels being widely used as a marker of lead exposure. Given that the half-life of lead in blood is relatively short (approximately 28–30 days), it does not constitute a reliable indicator of chronic exposure. Conversely, hair and finger-nails are regarded as medium-range bio-monitoring agents, associated with exposure times from a few months to years (Amr, 2011; Kern and Mathiason, 2012). Thus said, they are constantly contaminated by external agents, including from exposure to airborne dust, hair-colouring, shampoo, and nail polish, etc., consequently these samples are often impure and are not ideal as bio-indicators. Calcified tissues such as bone and teeth have a high affinity towards accumulation of heavy metals, particularly when individuals are exposed during early development (Gdula-Argasinska et al., 2004; Zhang et al., 2011).

In contrast to other tissues, bone is typically considered to be suitable as a bio-indicator of long-term exposure, but clearly human bone is usually not readily available for sampling and measurement (Kumagai et al., 2012). On the other hand, dental tissues are also very hard, additionally being similar to the materials making up bone (Arruda-Neto et al., 2010, Webb et al., 2005), they are generally regarded as part of the exo-skeleton, as previously mentioned. Unlike bone, in which the mineral phase is subject to turnover, the dental hard tissues (e.g., dentine and enamel) are not subject to significant turnover and therefore provide a permanent, cumulative and sound record of past and/or recent environmental exposure to heavy metals (Alomary et al., 2006; Appleton et al., 2000; Kolak et al., 2011). These biopsy tissues have recently received considerable attention in support of research into biological modeling, not least because of their easy extraction as a result of medically-indicated needs, and very low rate of pollutant clearance relative to other organs (Kumagai et al., 2012). Consequently, a precise chronological record of exposure to a number of elements is retained in the hard calcified tissues of the teeth (Gdula-Argasinska et al., 2004). Furthermore, teeth of different ages of people can be easily accessed to compare the metal concentrations of multiple generations at one time. Teeth (dentine, enamel or whole-teeth) thus offer particular advantages as suitable bio-indicators of environmental heavy metal exposures (Barton, 2011; Alomary et al., 2006; Kamberi et al., 2012; Kantamneni, 2010; Abdullah et al., 2012; Arruda-Neto et al., 2010; Gdula-Argasinska et al., 2004; Kumagai et al., 2012; Appleton et al., 2000; Kolak et al., 2011).

Many studies have been devoted to analyzing metal concentrations in whole-teeth, in order to make correlations between samples and environmental pollution by heavy metals (Barton, 2011; Alomary et al., 2006; Amr, 2011; Arruda-Neto et al., 2010; Zhang et al., 2011; Chew et al., 2000; Kern and Mathiason, 2012). Conversely, data on the heavy metal concentration of tooth dentine is scarce. To increase knowledge of the spatial distribution of elements in each tissue of human teeth, (such as dentine, enamel, pulp and cementum) and their affinity for environmental pollution, it is important to study the elemental concentrations in the dentine and enamel separately.

During the sixth week *in utero*, dental hard tissues, specifically the dentine and enamel compartments, begin to grow and then teeth in each mandible become the deciduous teeth that are later replaced by the permanent teeth (Kohn, Morris, & Olin, 2013; Webb et al., 2005). Dentine which is richer in organic content and biologically more active than enamel, is a typical composite material containing inorganic hydroxyapatite crystals and organic collagen matrix proteins (Arnold & Gaengler, 2007; Webb et al., 2005). Odontoblasts, situated in the pulp adjacent to dentine, continuously produce dentine throughout the whole lifespan of a tooth until shed (Arnold & Gaengler, 2007). Dentine is not affected by the oral environment since it is surrounded by enamel and cementum. (Kumagai et al., 2012). Additionally, heavy metals are easily deposited in tooth dentine during formation and mineralization processes by replacing the mineral tooth compounds throughout the entire human life. There is no active metabolism of elements that occurs after the completion of dental dentine formation (Kumagai et al., 2012). Consequently, the tooth dentine would appear to be an appropriate long-term bio-indicator of exposure to environmental pollution, representing an excellent vehicle for pollution studies (Webb et al., 2005; Zhang et al., 2011).

The aim of present study is to investigate the heavy metal levels in human tooth dentine, further seeking to evaluate and understand correlation with a number of parameters, including the ethnicity of the tooth donor, age, sex, tooth condition and tooth type.

## 2. Materials and Methods

### 2.1. Sample Collection and Preparation

Upon approval by the Ethics Committee of the Dental Faculty, University of Malaya, a total of 50 filling-free permanent teeth (18 molars, 12 premolars, 9 canines and 11 incisors) of various ages and, sex were collected from heavily polluted areas in the Klang valley of Malaysia. The same areas are representative of a multi-ethnic demography.

Immediately after extraction, the teeth were kept in sterile plastic containers with a 30% $H_2O_2$ solution and washed for 2 hours (Arruda-Neto et al., 2010). Soft-tissue, gum-tissue and blood around the teeth were removed by the use of an $H_2O_2$ solution and a brush. Using deionized water, each tooth was rinsed several times and then each tooth crown and root was separated by use of a diamond saw and then, under a magnifying glass and stereoscopic microscope, the enamel was mechanically separated from the crown dentine using a low speed dental hand piece. Finally, the tooth dentine was again rinsed in ultra-pure water (18.2 MΩ-cm), oven dried at 50°C for 1 hour, cooled and placed in a volumetric flask and digested in a mixture of 10 ml of 65% concentrated $HNO_3$ (SpectrosoL grade) and 3 ml of $H_2O_2$ (30%) on a hot plate (about 80°C) for about 1 hour and then left overnight at laboratory room temperature. It was again brought to about 80°C for a further 1 hour, to ensure that the specimens were completely dissolved (Chew et al., 2000). After digestion the sample solution was cooled, filtered with ashless filter paper (Whatman 540). Ultra-pure water (18.2 MΩ-cm) was added to the filtrate in a volumetric flask, for a total volume of 50ml.

Each procedural blank and the standard were prepared using the same volume and acid combinations, following the same procedure used to prepare the tooth samples. Between sample analyses, 2% ultra-pure $HNO_3$ blanks were analyzed, to clean the system and check whether there was any leftover media from the previously analyzed sample.

### 2.2. Sample Analysis

Elemental lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), arsenic (As), copper (Cu), manganese (Mn), barium (Ba), zinc (Zn), strontium (Sr), antimony (Sb), aluminium (Al), and tin (Sn)) analyses were conducted through use of inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7500 Series, USA. Calibration of the ICP-MS was performed using multi-element calibration standard solution 2A (10 mg/l stock solution of each element) (Agilent Technologies, USA, part no. 8500–6940) in 5% pure $HNO_3$ by subsequent dilution within the range 10 ppb to 100 ppb. All of the samples were analyzed in triplicate. In all cases, one blank solution and five standards were run with the same reagents used under the same conditions as a control for possible contamination from the digestion procedures. To validate the ICP-MS results, NIST (National Institute of Standards and Technology) standard reference material SRM 1400 (bone ash) were analyzed in each ICP-MS run. The recovered values for all of the metals ranged from 96% to 104% of the certified value. The limit of detection (LOD) of the ICP-MS was calculated as three times the standard deviation of the uncorrected values for the blanks over the course of the experiment (Jones, 2014). The detection limits (dry weight) for Cr, As, Cu, Mn, Zn, Sr, Sb, Al and Sn was 0.002 µg/g, while for Pb, Hg, Ba and Cd it was 0.001 µg/g.

### *2.2.1. Statistical Analysis*

The experimental data were subjected to statistical analysis employing IBM SPSS version–20 software. Arithmetic mean (AM), Standard error (SE), standard deviation (SD), geometric mean (GM), geometric standard deviation (GSD), median, maximum, minimum, skewness and kurtosis values were estimated by descriptive statistical analysis. The relationships and degree of association that can exist among the measured heavy metal variables were evaluated through Pearson correlation and Dendrogram of hierarchical cluster analysis. Comparison between ethnics group; age; gender, tooth condition and teeth type was done using the ANOVA (One-way analysis of variance) post hoc Tukey test. The level of significance was set at $p<0.05$.

## 3. Results and Discussion

The overall statistics (AM, SD, GM, GSD, medians, maximum and minimum values, skewness and kurtosis) for heavy metal concentrations in the analyzed tooth samples are shown in Table 1. Of the 13 targeted heavy metals, a total of 12 metals were detected in almost all of the tooth dentine samples that were analyzed by ICP–MS (Table 1). In the analyzed samples, no Cd has been detected, while other metals show concentration in tooth dentine in the following order of concentration: As < Mn < Ba < Cu < Cr < Pb < Zn < Hg < Sb < Al < Sr < Sn. The distribution of the concentration of metals against the sample number for each of the metals are presented in Fig. 1. Fig. 1 shows that the concentrations of most of the heavy metals in tooth dentine lie between 0.1 to 10 µg $g^{-1}$.

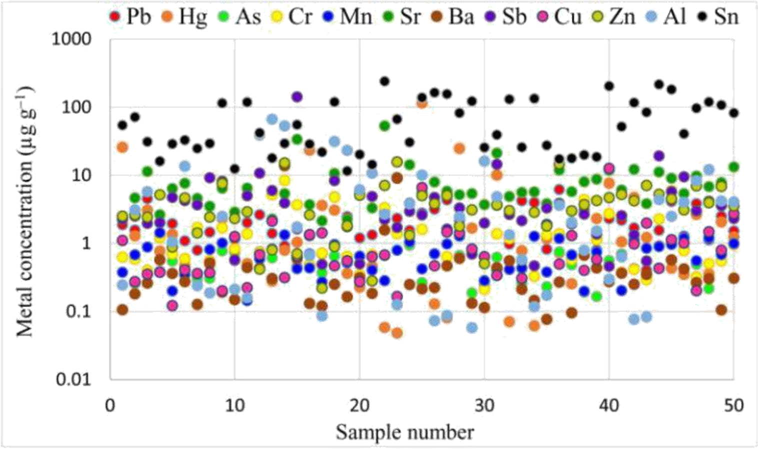

Fig. 1. Distribution of heavy metal concentrations in tooth dentin samples.

It was observed from Table 1 (from its large standard deviation or significant difference between minimum and maximum values) that the heavy metals in permanent teeth dentine samples have a broad range of concentrations. This is mainly due to natural variation in trace element concentration between teeth and can be traced to differences in the soils, food and diet. The differences can be attributed to industrial, urban and agricultural effluents in the environment, and also to the socio-economic condition of individuals, place of residence, profession, age, gender, habits (dietary and smoking), tooth type etc (Barton, 2011; Appleton et al., 2000; Anjos et al., 2004). It has been shown that the presence of heavy metal concentrations in tooth tissues like dental dentine in humans or animals reflect the deposition of pollutant metals in the environment (Appleton et al., 2000).

Table 1. Summary statistics (Sample size 50 teeth in each category) of heavy metal concentrations in human tooth dentine ($\mu g\ g^{-1}$ dry weight).

| Heavy metal | Statistics of elemental concentrations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM | SE | SD | GM | GSD | Median | Min | Max | Skewness | Kurtosis |
| Pb (50) | 2.10 | 0.19 | 1.32 | 1.72 | 1.96 | 1.66 | 0.27 | 6.16 | 1.01 | 0.58 |
| Hg (50) | 5.05 | 2.40 | 16.97 | 0.92 | 5.22 | 0.80 | 0.05 | 115.40 | 5.93 | 38.17 |
| As (33) | 0.50 | 0.04 | 0.20 | 0.45 | 1.67 | 0.48 | 0.17 | 1.10 | 0.52 | -0.45 |
| Cd (0) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cr (49) | 1.51 | 0.24 | 1.68 | 0.99 | 2.38 | 0.78 | 0.21 | 8.38 | 2.36 | 5.85 |
| Mn (49) | 0.69 | 0.05 | 0.35 | 0.59 | 1.80 | 0.67 | 0.14 | 1.44 | 0.36 | -0.97 |
| Sr (50) | 9.01 | 1.34 | 9.48 | 6.73 | 2.02 | 5.97 | 2.14 | 53.0 | 3.23 | 11.4 |
| Ba (50) | 0.77 | 0.32 | 2.26 | 0.31 | 2.60 | 0.27 | 0.08 | 13.8 | 5.10 | 26.4 |
| Sb (50) | 6.61 | 2.78 | 19.8 | 2.88 | 2.87 | 2.67 | 0.46 | 141 | 6.71 | 46.5 |
| Cu (48) | 1.23 | 0.29 | 2.01 | 0.71 | 2.56 | 0.64 | 0.12 | 12.7 | 4.50 | 23.3 |
| Zn (50) | 3.99 | 0.49 | 3.47 | 2.75 | 2.62 | 3.10 | 0.22 | 15.7 | 2.02 | 4.75 |
| Al (50) | 7.34 | 1.92 | 13.6 | 1.62 | 7.30 | 2.48 | 0.06 | 66.7 | 2.98 | 9.34 |
| Sn (50) | 73.1 | 8.55 | 60.5 | 51.2 | 2.40 | 46.8 | 11.6 | 239 | 1.03 | 0.21 |

Values in the parenthesis '()' in the left column indicates that the respective metal was found in '(values)' out of 50 tooth dentine samples. AM: Arithmetic Mean, SE: standard Error of Mean, SD: Standard Deviation, GM: Geometric Mean and GSD: Geometric Standard Deviation.

Pb, the most common heavy metal toxicant, typically associated with environmental pollution, this has relevance in respect of industrial, agricultural and domestic solid and liquid effluents that increase soil and aquatic lead levels. Subsequent uptake by crops and aquatic animals introduces lead into the food chain. Because of its large scale utilisation, human civilization is exposed to lead and lead derivatives, mainly through ingestion of contaminated food, drinking water and also by inhalation of polluted air. Among the donated teeth, the highest concentration of Pb (6.16 μg $g^{-1}$) was obtained in the carious upper left 2nd incisor of a 69 year-old woman, a smoker and factory worker. The lowest concentration (0.27 μg $g^{-1}$) was seen in the healthy lower incisor of a 15-year-old non-smoking male student. Alomary et al. (2006) reported in Jordan that the mean concentration of lead in tooth samples was 28.91 ± 13.70 μg $g^{-1}$ dry weight, with a range of 0.74–69.15 μg $g^{-1}$ dry weight. Previous studies (Chew et al., 2000) showed elevated Pb levels (1.7–40.5 μg $g^{-1}$ with a mean of 4.9 μg $g^{-1}$) in human teeth from Klang valley. In this study we have observed that the concentration of Pb in tooth dentine has experienced a significant decrease in recent years, pointing to the decreased emissions of Pb. The major reasons for this condition have been restrictions against environmental pollution, enforced by Malaysian law, use of new environmental friendly technology in industrial processes, and decrease in the use of leaded gasoline. Kern and Mathiason (2012) reported values of Pb content in teeth (whole teeth) in the range 0.373 ± 0.005–15.78 ± 0.02 μg $g^{-1}$ with a mean of 3.84 μg $g^{-1}$, being slightly more than our values. Mainly accumulated in calcified tissues such as bone, teeth etc. throughout the life span, Pb becomes an endogenous source, released with potential for adverse effects upon the neurologic, hematologic and renal systems of the human body (Florea, & Buesselberg. 2006).

Hg is another highly toxic element among the studied heavy metals. The level of Hg was found to be relatively high in the sampled teeth (Table 1), which may be due to the greater consumption rate of seafood by Malaysians, especially fish (Khandaker et al., 2015). Among the studied teeth, the highest concentration of Hg (115.40 μg $g^{-1}$) was obtained from the carious lower-right 3rd molar of a 59 year-old female smoker (a housewife) while the lowest concentration (0.05 μg $g^{-1}$) was observed in the healthy upper-central right-incisor of a 61 year-old non-smoker (again a housewife). There are numerous anthropogenic sources that release mercury into the environment and contaminate the air, water, food etc. These include coal-based power-plants, crematoria, fossil-fuel burning, gold and silver mining, the health-care sector, various kinds of industrial effluents and also cement production. Considered to be one of the more highly neuro-, nephro- and immuno-toxic elements even at low levels of exposure, such Hg exposure has the potential to cause acute- and chronic-poisoning, with adverse health effects that can manifest at any period during body development (Bose-O'Reilly et al., 2010).

Arsenic (As), also included among the list of most toxic metals (mainly in its inorganic form), with the greatest concentration (1.10 μg $g^{-1}$) was found in the lower-right 2nd molar of a 57 year-old low-income non-smoking female (but notably living with a chain-smoking husband) while the lowest value of 0.17 μg $g^{-1}$ was observed in the upper-left 3rd molar of a non-smoking female office secretary. Alina et al. (2012) and Khandaker et al. (2015) found the elevated levels of As in the marine fishes in Malaysia. The presence of As in the sampled teeth may be due to the greater consumption rate of seafood by Malaysians, especially marine fishes (Khandaker et al., 2015). Amr (2011) has reported a mean value of As in Egyptian teeth of 0.02 ± 0.007 mg $kg^{-1}$, with a range of 0.014–0.027 mg $kg^{-1}$, which is much lower than our measured values for Malaysian tooth dentine. Jones (2014) from the USA reported a mean concentration for As in human teeth of 0.05 ± 0.0.03 mg $kg^{-1}$ with a range of 0.01–0.16 mg $kg^{-1}$, which is also lower than our results. Anthropogenic sources of arsenic include production of arsenic-containing compounds, advanced agricultural practices (e.g., fungicides, herbicides, pesticides, algaecides, sheep-dips, wood-preservatives, and dyes), production of glass, paper and semiconductors; metal smelting, ore processing, waste disposal and coal-based power plants. Chronic exposure to arsenic may cause various health problems such as cardiovascular and peripheral vascular disease, e.g. blackfoot disease, neurologic and neurobehavioral disorders, damage to the liver, hypertension and carcinoma (Ng et al., 2003; Tchounwou et al., 2012).

Chromium-VI compounds are known carcinogens and therefore there is concern regarding chronic low-level exposure to Cr, both occupationally and environmentally. The highest concentration of Cr (8.38 μg $g^{-1}$) was found in the healthy upper-right 1st premolar of a chain-smoking male truck driver, aged 56. The lowest concentration (0.21 μg $g^{-1}$) was found in the healthy lower-incisor of a 15 year-old non-smoker male school student. By way of comparison, Amr (2011) reported an average concentration of Cr of 0.05 ± 0.03 mg $kg^{-1}$ in Egyptian teeth (whole teeth), with a range of 0.03– to 0.11 mg $kg^{-1}$, lower than our findings in tooth dentine. Jones (2014) from the USA reported a Cr concentration in human teeth enamel of 0.19 ± 0.28 mg $kg^{-1}$ with a range of 0.00– to 1.18 mg $kg^{-1}$, which is within the present range but slightly lower than our results for concentrations of Cr in Malaysian teeth dentine. Cr in the human environment almost entirely originates as a result of anthropogenic activities such as mining, metallurgy, production of chromates and bichromates, stainless-steel, welding, chromium-plating, chrome-pigment, leather tanning, textile production, coal and oil combustion, metal refining, chemical factories and waste incineration, etc. Environmental and occupational exposure to Cr and its compounds is recognized as having multi-organ toxicity, with for instance the potential causation of renal damage, asthma, allergy and lung and respiratory tract cancers, liver and kidney problems (Tchounwou et al., 2012).

Manganese (Mn) is an essential trace element needed for neuronal function, however, have the potential to be toxic at elevated levels, linked to severe movement disorder called 'mangnism' (Dydak et al., 2011). Among the donated teeth, the highest concentration of Mn (1.44 μg $g^{-1}$) was found in the healthy lower-left 3rd molar of a young man aged 19, being an engineering workshop worker and a smoker. The lowest concentration (0.14 μg $g^{-1}$) was seen in the healthy lower-left 3rd molar of a 43 year-old male smoker who works in an office. The concentration of Mn in the studied tooth dentine was found to be somewhat comparable with the reported range values of Amr (2011), of 0.09–1.2 mg kg$^{-1}$ with a mean of 0.27 ± 0.11 mg kg$^{-1}$. Arruda-Neto et al. (2010) reported Mn concentrations of adult (age 18-64) human teeth in São Paulo, in the range 0.3045– to 0.2991 μg g$^{-1}$, being lower but within the range of our findings. The concentrations of Mn in the air are enhanced by human activities such as various industrial activities, steel and alloy production, extraction and processing of ore, chemical synthesis, welding, dry-cell battery fabrication, ceramic production, combustion of fossil-fuels, manganese pesticides etc. Mental disorders, such as loss of memory, apathy and psychosis have been linked to Mn neurotoxicity. Chronic toxicity of Mn may arise from long-term inhalation of dust and fumes. The central nervous system is the main site of damage, which can cause permanent disability (Dydak et al., 2011).

In the studied tooth dentine, Ba existed in practically all samples, with the highest Ba concentration (13.77 μg g$^{-1}$) was obtained from the upper-right 1st premolar of a 56 year-old smoker working as a truck driver. The lowest concentration (0.08 μg g$^{-1}$) was seen in the healthy upper-right 1st premolar of a 45 year-old shopkeeper who is a smoker. The concentration of Ba in our analysed tooth dentine samples was lower than that of Egyptian permanent whole-tooth samples (Amr, 2011), with a mean of 9.5 ± 5.4 mg kg$^{-1}$, obtained from a range 5.11– to 17.97 mg kg$^{--1}$. Ba occurs naturally in most surface waters and also is released in the terrestrial and aquatic environments by industry. Due to its large-scale application in industry, anthropogenic sources add significantly to the release of Ba in the environment and pollute the soil, air and water bodies. Ba has a minimal toxicity for the general public, but accidental exposure to a high level of Ba may cause acute Ba intoxication. Chronic exposure to Ba oxide through inhalation may cause bronchitis, along with coughs, phlegm and shortness of breath (CICAD, 2001).

Quite measurable levels of Sr was found in all dentine samples in which, the highest Sr concentration (52.97 μg g$^{--1}$) was seen in the healthy upper-left 3rd molar of a 44 year-old woman who was a hotel worker and non-smoker. The lowest concentration (2.14 μg g$^{-1}$) was found in the carious lower-left 1st molar of a 36 year-old student who was also a non-smoker. By way of comparison, Amr (2011) reported levels in Egyptian teeth in the range 70.2– to 130 mg kg$^{-1}$ with a mean of 101.2 ± 24.3 mg kg$^{-1}$, while Brown et al. (2004) reported Sr concentration in teeth sampled in the UK within the range 52– to 262 mg kg$^{-1}$ and 97– to 244 mg kg$^{-1}$ in Ugandan primary teeth, both values much greater than those in our present study of tooth dentine samples in Malaysia. The disposal of electronic/electrical devices containing a variety of materials, including Sr (e-waste), in landfill possibly contaminates the ecosystem and represents a potential threat to human health. The levels of stable Sr in air due to burning of coal and oil and the concentration of Sr in soil may increase as a result of the generation of coal-ash, incinerator-ash and industrial waste. Sr can accumulate in the tissues, more so in the bones, and chronic or excess exposure may cause metabolic dysfunction and other harmful effects on human health; one example concerns osseous mineralization issues.

Excessive ingestion of Sr can also diminish the content of Ca in bone and lead to hypocalcemia (Chen et al., 2015).

Tin has no known biochemical function in the human body. The values (Table 1) of Sn are markedly greater than any of the other studied metals. Malaysia was once the third largest producer of Sn in the world. (achieving 40% of world production). One possible reason for the high concentration of Sn in human teeth may be the contamination of soil and vegetation via tin mining activities. Among the tooth donors, the maximum concentration of Sn (239.05 µg $g^{-1}$) was found in the healthy upper-left 3rd molar of a 44 year-old woman who was a non-smoker and hotel worker. The lowest concentration (11.57 µg $g^{-1}$) was found in the healthy upper-left 1st premolar of a 47 year-old woman who was a non-smoker and housewife. Jones (2014) reported that the concentration of Sn in Medellin, Colombia was in the range of 0.00 – 230.81 mg $kg^{-1}$ with a mean of 4.51 ± 29.26 mg $kg^{-1}$, which is lower than our findings. Sn may be discharged into the ecosystem from different anthropogenic sources of human utilization. As an example, tinplate is used extensively for food and beverage packaging, possibly entering into the dietary intake, especially when plain uncoated internal surfaces are used for cans. The consumption of excessive canned food can cause digestive disturbances. Tin poisoning also alters the activities of some enzymes, affecting the metabolism of Zn, Cu, Fe and Ca and modifying the concentration of several other elements in the tissue of organs (Blunden & Wallace, 2003).

Antimony is regarded as a non-essential element. Among the investigated teeth the greatest Sb concentration (141.32 µg $g^{-1}$), was found in the carious upper-left 1st incisor of a 71 year-old woman who was a non-smoker as well as being a jewelry worker. The lowest concentration (0.46 µg $g^{-1}$) was seen in the carious lower-right 1st molar of a 51 years man who was a non-smoker and office worker. In comparison, Jones (2014) reported that the concentration of Sb in tooth enamel in Medellin, Colombia was in the range 0.00–1.01 mg $kg^{-1}$, with a mean of 0.05 ± 0.15 mg $kg^{-1}$, which is very much lower than our findings. In the environment, antimony occurs naturally, but it also contaminates the environment through various human activities: waste incineration, mine sites, metal processing (smelters), refining, coal burning or fly ash when ores containing Sb are smelted. Chronic ingestion of antimony (oral) may cause nausea, vomiting, liver damage and cardiotoxic effects, and also increased occurrence of lung, liver and bile cancers (SEPA, assessed at 2015).

In this study, notwithstanding the important homeostatic co-enzymatic roles that it plays, Cu has been because of the possibility that in overload Cu may cause threats to life, one possibility being through a potential link to Wilson's disease (Chew et al., 2000). The greatest concentration of Cu (12.65 µg $g^{-1}$) was found in the carious lower-right 1st molar of a 51 year-old male office worker non-smoker. The lowest concentration (0.12 µg $g^{-1}$) was seen in the carious lower-right 2nd molar of a 62 year-old business-man who is a smoker. Amr (2011) reported that the concentration of Cu in Egyptian permanent whole-teeth was in the range of 1.4–26.1 mg $kg^{-1}$, with a mean of 9.2 ± 11.4 mg $kg^{-1}$, being greater than our findings. Chew et al. (2000) reported Cu concentrations in Malaysian teeth (whole-teeth) in the range 0.1 – 6.0 µg $g^{-1}$ with a mean of 0.29 ± 0.03 µg $g^{-1}$, slightly lower than our results. Airborne Cu is mostly from the combustion of fossil fuels, household emissions, mining, metallurgy, electroplating etc. Cu, albeit an essential structural constituent of many metalloenzymes, its overdose may result in anemia, allergies, hair loss, arthritis, autism, acne, adrenal hyperactivity and insufficiency, cancer, depression, diabetes, dyslexia, fatigue, bone fracture, heart attacks, headaches, anxiety, hypertension, infections, kidney and liver dysfunction, strokes, tooth decay, vitamin C and other vitamin deficiencies (Lokeshappa et al., 2012).

Among the donated teeth the highest concentration of Zn (15.66 µg $g^{-1}$) was seen in the healthy upper right 1st premolar of a 56 year-old man who is a smoker and truck driver. The lowest concentration (0.22 µg $g^{-1}$) was found in the carious lower-left 1st molar of a 36 year-old non-smoker male student. Amr (2011) reported that the concentration of Zn in Egyptian permanent whole-teeth was in the range 124.6 – 235.7 mg $kg^{-1}$ with a mean of 178 ± 44.6 mg $kg^{-1}$, very much greater than our findings. Chew et al. (2000) reported that the concentration of Zn in Malaysian teeth (whole-teeth) was in the range 93.4 – 182.5 µg $g^{-1}$ with a median value of 123 µg $g^{-1}$, being very much greater than values found herein for Malaysian tooth dentine. In comparison to Kern and Mathiason (2012) (with a reported concentration range of 44 ± 2 to 227.23 ± 0.02 µg $g^{-1}$ and a mean value of 100.49 µg $g^{-1}$), our Zn concentration values are very much lower. Zn can enter the environment by way of natural processes, as well as by its utilization in anthropogenic activities. Zn is released into the atmosphere from industrial processes such as galvanization, smelting or welding. Exposure to high doses of Zn causes acute intoxication, prolonged high doses of Zn inhibiting the uptake of Cu and Fe, also interfering with δ-aminolevulinase more greatly than Pb. The brain is the main organ in which Zn is highly involved in cell death (Plum et al., 2010).

Among the studied metals Al, observed in all analyzed tooth dentine samples, typically in moderate quantities. The greatest Al concentration (66.70 µg $g^{-1}$) was found in the healthy lower-left 2nd premolar of a 30 year-old male mechanic and smoker. The lowest concentration (0.06 µg $g^{-1}$) was found in the healthy lower-left 2nd premolar of a 15 year-old non-smoking male student. Amr (2011) reported Al concentrations in Egyptian whole-teeth in the range 27.5 – 84 mg $kg^{-1}$ with a mean of 51.4 mg $kg^{-1}$, greater than in our present findings. Al enters the environment due to acidic precipitation and direct anthropogenic discharges of it and compounds of it that are related to industrial processes. It is found in air, water and foodstuffs. Due to its large-scale utilization (from home to office to industries), it can contaminate environmental media such as soil, plants, foodstuffs, drinking-water, aquatic organisms etc. Those exposed to higher amounts of Al may develop Alzheimer's disease while exposure to lower levels of this metal are known to contribute to Shaver's disease (Krewski et al., 2007).

### *3.1. Correlation Matrix*

The degree of association and linear relationship that may exist among the heavy metal parameters for the studied tooth samples were evaluated by means of Pearson's correlation analysis and presented in Table 2. Some metal shows (bold in Table 2) positive correlation between them but none of these correlations were observed to be particularly strong, thus said the correlations are suggestive of similar anthropogenic sources of activity of these metals. Whereas, a very weak negative correlation (Table 2) indicating that the anthropogenic source of origin of these metals are dissimilar in the environmental media. The negative correlation of some metals can be attributed to the antagonism of the heavy metals during their absorption in human teeth.

Table 2. Correlation matrix for human teeth samples giving values of Pearson's correlation coefficients for pairs of heavy metals (the bold values represent significantly correlated parameter).

| | Pb | Hg | As | Cr | Mn | Sr | Ba | Sb | Cu | Zn | Al | Sn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pb | 1 | | | | | | | | | | | |
| Hg | .057 | 1 | | | | | | | | | | |
| As | **.338**$^{*}$ | -.048 | 1 | | | | | | | | | |
| Cr | .136 | -.026 | .195 | 1 | | | | | | | | |
| Mn | .164 | -.114 | .110 | .155 | 1 | | | | | | | |
| Sr | **.244**$^{*}$ | -.084 | **.229**$^{*}$ | .188 | -.122 | 1 | | | | | | |
| Ba | -.079 | -.063 | .144 | **.532**$^{**}$ | **.246**$^{*}$ | .025 | 1 | | | | | |
| Sb | -.026 | -.045 | .012 | .222 | -.084 | **.410**$^{**}$ | -.017 | 1 | | | | |
| Cu | -.043 | **.417**$^{**}$ | .030 | .078 | -.105 | -.005 | -.109 | -.081 | 1 | | | |
| Zn | .221 | -.005 | .147 | **.265**$^{*}$ | **.341**$^{*}$ | .050 | **.674**$^{**}$ | -.144 | -.045 | 1 | | |
| Al | -.033 | -.022 | .119 | **.534**$^{**}$ | .219 | .054 | **.367**$^{**}$ | -.018 | -.045 | .042 | 1 | |
| Sn | **.269**$^{*}$ | .135 | .022 | .189 | .085 | **.317**$^{*}$ | -.057 | -.012 | **.333**$^{*}$ | .169 | -.211 | 1 |

$^{**}$Correlation is significant at the 0.01 level (2-tailed).
$^{*}$Correlation is significant at the 0.05 level (2-tailed).

In addition to Pearson's correlation analysis, Hierarchical cluster analysis was performed to confirm the association between the metal parameters. Cluster analysis categorize the objects of the system into clusters or groups according to their similarities to find a best grouping for which the objects or variables within each cluster are identical. The dendrogram of hierarchical cluster analysis shows the order in which parameters combine to form clusters with identical properties. An interesting link among the metal variables can be judged from the dendrogram. It can be seen from the dendrogram (Fig. 2) that all heavy metal parameters are grouped into two statistically significant clusters, which are formed based on the existing similarities of the parameters.

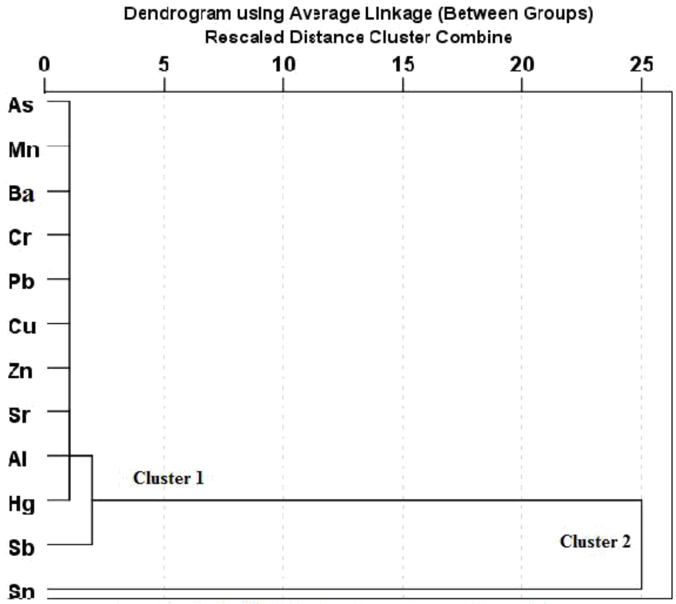

Fig.2 Dendrogram of hierarchical cluster analysis

In Fig.2, cluster 1 comprises of Pb, As, Cr, Mn, Sr, Ba, Cu, Zn, Al and Hg with similarity and closely correlated with each other, which suggests that these metals come from similar anthropogenic or natural source of origins. While, only Sn is grouped into cluster 2 and has less similarity with other metals, indicating that different mechanism control the source of Sn in the environment.

Several articles have reported that a number of factors influence the heavy metal concentration in human teeth, including socioeconomic status, age, gender, tooth type and condition etc. (Alomary et al., 2006; Arruda-Neto et al., 2010; Kumagai et al., 2012). Since sample concentrations of each element were diverse, the average concentration for each factor was calculated. In regard to the three main ethnic groups living in Malaysia (Malay, Chinese and Indian), no significant distinct pattern of As and Sn concentration was observed among the three ethnic groups, whereas for the remainder of the metals (Pb, Hg, Cr, Mn, Sr, Ba, Sb, Cu, Zn and Al), some distinct patterns of concentration were observed (Fig. 3a). Greater levels of Pb, Hg, Cr, Sr and Sb were found in Chinese teeth, while, Mn, Cu and Al were noticed to be a little greater than in Indian teeth. Conversely, Ba concentrations were greater in Malay teeth, whereas similar concentrations were observed in the rest of the metals. This pattern of metal concentrations among the ethnic groups may be related to their socioeconomic status, dietary habits, profession etc (Alomary et al., 2006; Kumagai et al., 2012). The food habits and occupations also differ somewhat among the three races. Indians are also mostly vegetarians, the Malays are chicken and fish lovers and the Chinese tend to favour the consumption of pork and alcoholic beverages. Thus said, statistical analysis (ANOVA–one way analysis of variance) showed that with the exception of Pb, there are no other significant differences ($p > 0.05$) in metal concentration among the ethnic groups. Only Pb is found to be significantly greater ($p < 0.05$) in Chinese teeth than in Malay teeth.

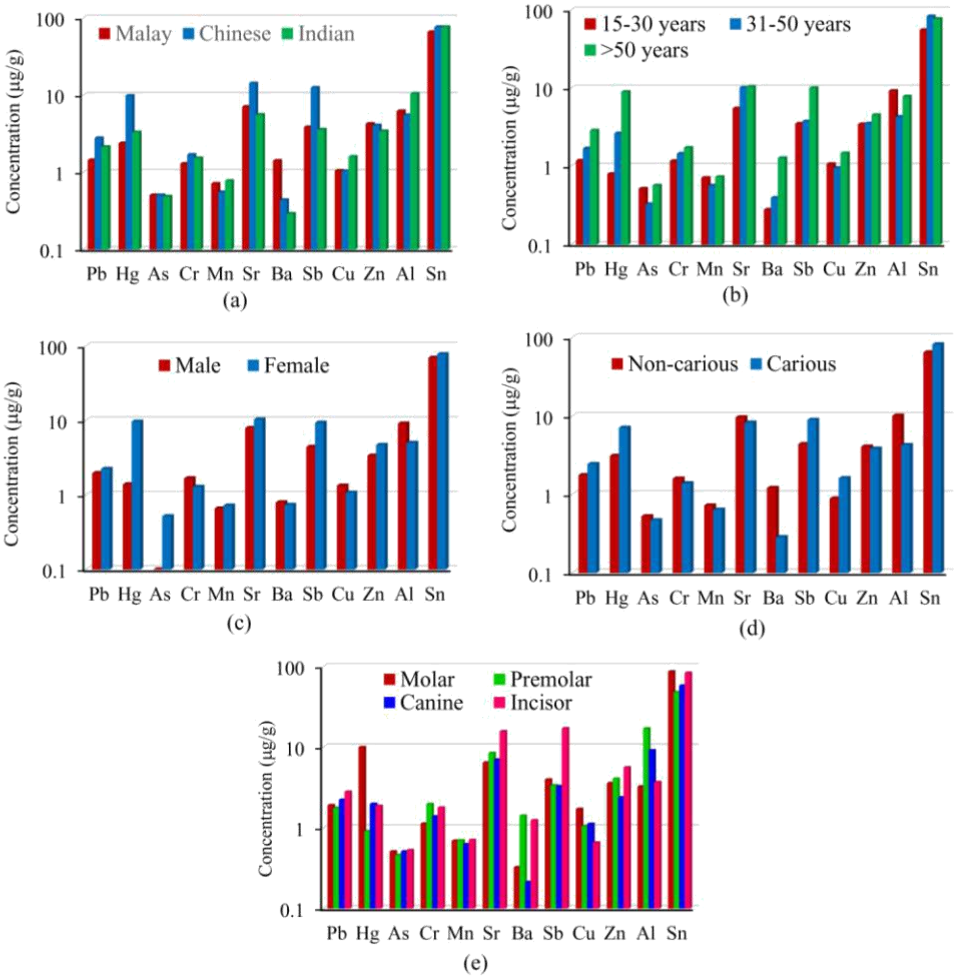

Fig.3 Distribution of heavy metal concentrations in teeth dentine in relation to (a) ethnicity, (b) age, (c) sex, (d) teeth condition and (e) teeth types.

With older age, the concentrations of most of the heavy metals increase (Fig. 3b). Pb, Hg, As, Cr, Mn, Sr, Ba, Sb, Cu, and Zn were observed to be higher for ages > 50 years. Conversely, Sn was noticed to be slightly elevated in the age group 31-50 years, wherea, only Al was found to be slightly elevated in the age group 15-30 years. The results of age-dependency of metal accumulation in human teeth agrees with some similar studies (Alomary et al., 2006; Kumagai et al., 2012; Kern, and Mathiason, 2012). While the implication is of accumulation of previous exposures to metals in calcified tissues, statistical analysis (ANOVA) showed that only the concentration of Pb gives rise to a significant difference ($p < 0.05$) among the age groups (50 years > 31-50 years > 15-39 years), while for the other heavy metals the age-related concentration difference is not statistically significant ($p > 0.05$).

Figure 3c showed the accumulation of Pb, Hg, As, Mn, Sr, Sb, Zn and Sn to be greater in female teeth i.e. gender dependent, while Cr, Cu and Al were found to be greater in male teeth. Similar concentrations were observed in the case of Ba. In regard to the health status of teeth, Pb, Hg, Sb, Cu and Sn concentrations in carious teeth were comparatively higher than those of non-carious (healthy) teeth, as shown in Fig. 3d. As, Cr, Mn, Sr, Ba and Al were found to be slightly elevated in non-carious teeth. An almost similar pattern of concentration was observed in the case of Zn in both types of teeth.

Figure 3e showed that the accumulation of metals in different types of teeth varied. We found differences in metal concentrations between the tooth groups. Relatively higher concentrations of Hg, Cu and Sn were found in molar groups and Pb, Sr, Sb and Zn in incisors. The differences in relative contents of heavy metals result from complex processes based on structural and developmental differences between the teeth types. Statistically (by ANOVA), the difference of metal concentration among the tooth type (position) was not found to be significant ($p > 0.05$).

## 4. Conclusions

Accurate determination of heavy metal levels in human teeth is important due to their pontential for toxic and sub–toxic effects in the human body. This study identified a total of 12 heavy metals in almost all tooth dentine samples. No Cd was detected in any of the analyzed samples. Among the analyzed samples and metals, As < Mn < Ba < Cu < Cr (i.e. in descending order of concentration) had the lower concentrations in tooth dentine, moderate levels of the heavy metals were found in Pb < Zn < Hg < Sb < Al < Sr (again in descending order of concentration), while Sn recorded the greatest levels. Pearson's correlation and the dendrogram of hierarchical cluster analysis showed Pb to be correlated with As, Cr, Mn, Sr, Ba, Cu, Zn, Sb and Al, which suggests that exposure to these metals arise as a result of similar anthropogenic origin. Among the ethnic groups, higher levels of Pb, Hg, Cr, Sr and Sb were found in Chinese teeth, while, Mn, Cu and Al were noticed to be slightly greater in Indian teeth. Overall, Chinese teeth were found to have slightly greater concentration of heavy metal compared to the Indian and Malay teeth that were sampled. The concentration of most of the heavy metals (Pb, Hg, As, Cr, Mn, Sr, Ba, Sb, Cu, and Zn) has been observed to increase among the more advanced age group (> 50 years), concentrations of Sn were slightly elevated in the age group 31-50 years, while Al was found to be slightly greater in the age group 15-30 years. In most of the cases, female tooth dentine showed higher metal concentrations than that of male tooth dentine. In regard to tooth groups, relatively greater concentrations of Hg, Cu and Sn were found in molars and Pb, Sr, Sb and Zn in incisors. Some of the elevated levels of heavy metals in the tooth dentine are reflective of pollution from industrial emissions and urbanization. The results of present study suggest that human tooth dentine can be used to obtain chronological information of heavy metal exposure and is a stable bio-indicator of environmental pollution by heavy metals.

## Acknowledgment

This research has been carried out under the following University of Malaya Research Grants: PG020-2013A, RP006D-13AFR.

## References

Abdullah, M.M., Ly, A.R., Goldberg, W.A., Clarke-Stewart, K.A., Dudgeon, J.V., Mull, C.G., et al. 2012. Heavy metal in children's tooth enamel: related to autism and disruptive behaviors? J. Autism Dev. Disord. 42, 929-936.

Alina, M., Azrina, A., Mohd Yunus, A.S., Mohd Zakiuddin, S., Mohd Izuan Effendi, H., Muhammad Rizal, R. 2012. Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. Int. Food Res. J. 19, 135-140.

Alomary, A., Al-Momani, I.F., Massadeh, A.M., 2006. Lead and cadmium in human teeth from Jordan by atomic absorption spectrometry: Some factors influencing their concentrations. Sci. Total Environ. 369, 69-75.

Amr, M.A., 2011. Trace elements in Egyptian teeth. Int. J. Phys. Sci. 6, 6241-6245.

Anjos, M.J., Barroso, R.C., Pérez, C.A., Braz, D., Moreira, S., Dias, K.R.H.C., et al., 2004. Elemental mapping of teeth using μSRXRF. Nucl. Inst. and Meth. in Phys. Res. B 213, 569-573.

Appleton, J., Lee, K.M., Sawicka-Kapusta, K., Damk, M., Cooke, M., 2000. The heavy metal content of the teeth of the bank vole (Clethrionomys glareolus) as an exposure marker of environmental pollution in Poland. Environ. Pollut. 110, 441-449.

Arnold, W. H., Gaengler, P. 2007. Quantitative analysis of the calcium and phosphorus content of developing and permanent human teeth. Ann. Anat. 189(2), 183-190.

Arruda-Neto, J.D., Geraldo, L.P., Prado, G.R., Garcia, F., Bittencourt-Oliveira, M.C., Sarkis, J.E., et al., 2010. Study of metals transfer from environment using teeth as biomonitor. Envirn. Int. 36(3), 243-246.

Barton, H.J., 2011. Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland). Biol. Trace. Elem. Res. 43, 637-658.

Bhuiyan, M.A.H., Dampare, S.B., Islam, M.A., Suzuki, S., 2015. Source apportionment and pollution evaluation of heavy metals in water and sediments of Buriganga River, Bangladesh, using multivariate analysis and pollution evaluation indices. Environ. Monit. Assess. 187, 4075.

Blunden, S., Wallace, T., 2003. Tin in canned food: a review and understanding of occurrence and effect. Food Chem. Toxicol. 41, 1651-62.

Bose-O’Reilly, S., M. McCarty, K., Steckling, N., Lettmeier, B., 2010. Mercury Exposure and Children’s Health. Curr. Probl. Pediatr. Adolesc. Health Care. 40, 186-215.

Brown, C.J., Chenery, S.R., Smith, B., Mason, C., Tomkins, A., Roberts, G.J., et al., 2004. Environmental influences on the trace element content of teeth--implications for disease and nutritional status. Arch. Oral Biol. 49, 705-717.

Chen, J.Y., Luong, H.V.T., Liu, J.C., 2015. Fractionation and release behaviors of metals (In, Mo, Sr) from industrial sludge. Water Res. 82, 86-93.

Chew, L.T., Bradley, D.A., Amin, Y.M., Jamil, M.M., 2000. Zinc, lead and copper in human teeth measured by induced coupled argon plasma atomic emission spectroscopy (ICP-AES). Appl. Radiat. Isot. 53, 633-638.

CICAD, 2001. Barium and barium compounds. Concise International Chemical Assessment Document 33 (CICAD). World Health Organization, Geneva. Available at: <http://www.who.int/ipcs/publications/cicad/en/cicad33.pdf>.

Florea, A.M., Buesselberg, D., 2006. Occurrence, use and potential toxic effects of metals and metal compounds. BioMetals 19, 419-27.

Gdula-Argasinska, J., Appleton, J., Sawicka-Kapusta, K., Spence, B., 2004. Further investigation of the heavy metal content of the teeth of the bank vole as an exposure indicator of environmental pollution in Poland. Environ. Pollut. 131, 71-79.

Jones, M.E., 2014. Trace element analysis of human tooth enamel by laser ablation-inductively coupled plasma-mass spectrometry for estimation of region of origin. M.Sc thesis, School of Medicine, Boston University, USA.

Kamberi, B., Kocani, F., Dragusha, E., 2012. Teeth as Indicators of Environmental Pollution with Lead. J. Environ. Anal. Toxicol. 02, 1-5.

Kantamneni, P.P., 2010. Lead levels in exfoliated teeth of children from the twin cities of Hyderabad and Secunderabad, India. Master of Public Health Thesis, Faculty of San Diego State University, USA.

Kern, J., Mathiason, L., 2012. The determination of copper, zinc, and lead in human teeth using inductively coupled plasma atomic emission spectrometry (ICP-AES). Concordia College Journal of Analytical Chemistry 3, 33-39.

Keshavarzi, B., Moore, F., Ansari, M., Rastegari Mehr, M., Kaabi, H., Kermani, M., 2015. Macronutrients and trace metals in soil and food crops of Isfahan Province, Iran. Environ. Monit. Assess. 187, 4113.

Khandaker, M.U., Asaduzzaman, Kh., Nawi, S.M., Usman, A.R., Amin, Y.M., Daar, E., Bradley, D. A., Ahmed, H., Okhunov, A.A., 2015 Assessment of Radiation and Heavy Metals Risk due to the Dietary Intake of Marine Fishes (Rastrelliger kanagurta) from the Straits of Malacca. PLoS ONE 10 (6) 1-16.

Khandaker, M.U., Olatunji, M.A., Shuib, K.S.K., Hakimi, N.A., Nasir, N.L.M., Asaduzzaman, Kh., Amin, Y.M., Kassim, H.A., 2015. Natural radioactivityand effective dose due to the bottom sea and estuaries marine animals in the coastal waters around Peninsular Malaysia. Radiat. Prot. Dosim. 167, 196–200.

Kohn, M. J., Morris, J., Olin, P. 2013. Trace element concentrations in teeth – a modern Idaho baseline with implications for archeometry, forensics, and palaeontology. J. Arch. Sci. 40(4), 1689-1699.

Kolak, V., Melih, I., Pesic, D., Mileusnic, I., Ristic, T., Pejanovic, D., et al., 2011. Hard dental tissue lesions in inhabitants of an industrial zone. Sci. Res. Essays 6, 5315-4323.

Korkmaz Gorur, F., Keser, R., Akcay, N., Dizman, S., 2012. Radioactivity and heavy metal concentrations of some commercial fish species consumed in the Black Sea Region of Turkey. Chemosphere 87, 356-361.

Krewski, D., Yokel, R.A., Nieboer, E., Borchelt, D., Cohen, J., Harry, J. et al., 2007 Human Health Risk Assessment for Aluminium, Aluminium Oxide, and Aluminium Hydroxide. J. Toxicol. Environ. Health, Part B. 10, 1-269.

Kumagai, A., Fujita, Y., Endo, S., Itai, K., 2012. Concentrations of trace element in human dentin by sex and age. Forensic Sci. Int. 219, 29-32.

Lokeshappa, B., Shivpuri, K., Tripathi, V., Dikshit, A.K., 2012. Assessment of Toxic Metals in Agricultural Produce. Food Pub. Health. 2, 24-29.

Millour, S., Noël, L., Chekri, R., Vastel, C., Kadar, A., Sirot, V., et al. 2012. Strontium, silver, tin, iron, tellurium, gallium, germanium, barium and vanadium levels in foodstuffs from the Second French Total Diet Study. J. Food Comp. Anal. 25, 108-129.

Ng, J.C., Wang, J., Shraim, A., 2003. A global health problem caused by arsenic from natural sources. Chemosphere. 52, 1353-1359.

Plum, L.M., Rink, L., Haase, H., 2010. The Essential Toxin: Impact of Zinc on Human Health. Int. J. Environ. Res. Public Health. 7, 1342-65.

SEPA (Scottish environment protection agency), 2015. Antimony. Scottish pollutant release inventory. Available at: <http://apps.sepa.org.uk/spripa/Pages/SubstanceInformation.aspx?pid=98>. Assessed on October, 2015.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy Metals Toxicity and the Environment. NIH Public Access Author Manuscript. 101, 133-164.

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. Microchem. J. 81, 201-208

Zhang, C., Wang, Y., Cheng, X., Xia, H., Liang, P., 2011. Determination of Cadmium and Lead in Human Teeth Samples Using Dispersive Liquid-liquid Microextraction and Graphite Furnace Atomic Absorption Spectrometry. J. Chin. Chem. Soc. 58, 919-924.