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EVALUATION OF TRACE ELEMENT CONCENTRATIONS IN HUMAN TEETH AS INDICATOR OF ENVIRONMENTAL CONTAMINATION

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ABSTRACT

This study determined the concentrations of trace elements- Pb, Cd, Fe, Cr, Cu, Ni, and Zn in human carious teeth tissues and investigated the effects of sex and age differences on the trace elements as indicators of environmental contamination. Eighty (80) teeth samples were collected from the Dental Clinic of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile Ife, Nigeria. The teeth samples were each divided into three parts: enamel, pulp and dentin by trained dental personnel. These parts were digested in nitric acid and analyzed using Atomic Absorption Spectrophotometric technique. The results of the study showed that the total mean concentrations of trace metals followed the order: Cd < Ni < Cu < Fe < Cr < Pb < Zn. The concentrations of the metals followed the order pulp > enamel > dentin. With respect to sex, the total mean concentrations of the trace metals followed the order Zn > Pb > Fe > Cr > Cu > Ni > Cd for the males and Zn > Pb > Cr >Fe > Cu > Ni > Cd for the females. There was no significant difference in the concentrations of metals with respect to age and sex. The study concluded that the elements were present in the different tissues at varying degrees. The study showed that teeth can be a very useful indicator for heavy metals exposure assessment in biological samples.

Keywords: Trace elements, Carious tooth, Nigerian subjects, Biological samples.

INTRODUCTION

Teeth is the hardest part found in the human body and the only structure that reasonably remains after death. It survives environmental insult, disaster, and plays vital roles in the human body (Brown *et al.,* 2004). In addition to food mastication, teeth are useful in age estimation, particularly in some African locations where birth registration poses a challenge. Presently, teeth are used for forensic study due to the increasing occurrence of mass deaths and disasters globally (Shah *et al.*, 2019). Teeth can also serve as a good indicator in identifying polluted environments concerning heavy metals and nutritional status. (Chew *et al.*, 2000, Brown *et al.,* 2004 and Kaličanin and Nikolić, 2008).

A tooth is made up of four major tissues- the enamel which is the outermost, inert and the hardest calcified layer mainly composed of calcium phosphate. Below the enamel is a sensitive layer called dentin. Dentin is a calcified and strong tissue that consists of microscopic tubes. The pulp is a non-calcified, vital, and highly sensitive tissue located at the center of the tooth. The pulp contains nerves, connective tissues, and blood vessels, while the cementum is a calcified dental tissue that covers the surface of the roots of the teeth.

Trace elements can be defined as mineral elements that are in limited amounts in human organs, irrespective of their richness in the environment. The development of teeth in human body requires the presence of some trace elements. (Shaik *et al.*, 2021). Trace elements in the human teeth are distributed in the dentin and enamel differently. Elements such as Pb, Cu, I, Sr Mn, Co, Al, Se, and Ni are in excess compared to what is obtainable in dentin where Fe and F concentrations are higher than that of the enamel as observed by Lappalaine and Knuuttila (1981). Selenium, copper, cadmium and lead have been considered as caries (a tooth disease) promoter in teeth and the high concentration of copper in drinking water has been linked to the increased occurrence of dental caries (Shaik *et al*., 2021). Also, different layers of enamel have been reported to have different concentrations of trace elements (Reitznerová *et al.,* 2000). Losee *et al.,* (1974) categorized trace metals in dental tissues into elements found in the dental enamel at a median level of more than 10 µg/g dry weight, e.g., Mg, Zn, Sr, and F; elements found in the dental enamel at a median level between 1 µg/g and 10 µg/g dry weight, e.g., Cr, B, Ba and Al and elements found in the enamel at a median level between 0.1 µg/g and 1 µg/g, e.g., Se, Sn, Cu, and Mn.

Dental caries is an oral disease that is globally known to affect individuals of all ages. Many risk factors have been identified in the cause of dental caries such as bad dental hygiene with poor tooth brushing but there are few pieces of evidence linking trace elements with dental caries (Arora *et al.,* 2008a). There is the possibility that trace elements can chelate calcium in teeth thus making them susceptible to dental diseases. For example, an increase in dental caries has been linked to exposure to environmental Cd (Arora *et al.,* 2008a). The knowledge gained from such a study can help identify predisposing and risk factors for oral diseases and help in the prevention of the disease rather than the non-working traditional approach. Furthermore, the level of trace metals in teeth may be a measure of environmental exposure (Arora *et al.,* 2008a). There are disparities in the concentrations of lead, aluminum and strontium in the enamel of ancient Egyptian and contemporary teeth samples as a result of environmental factors and diets (El-Tayeb *et al.,* 2021). Lead concentration in teeth, for example, has been used as a measure of environmental pollution as it has been reported to be integrated and deposited in teeth (Gulson and Wilson, 1994; Martin *et al.,* 2007). Lead toxicity is suggested to occur when the concentration of lead rises above 4 mg/kg in the teeth (Al-Mahroos and Al-Saleh, 1997).

Trace elements can get into the human body via ingestion of contaminated water and food or exposure through the dermal route (Amr and Helal, 2010). Aside from the major routes, other sources of trace elements in tooth enamel include dental prosthesis, dental porcelain, and saliva. The bioaccumulation of toxic metals such as Pb, Ni, and Cd can lead to various toxic effects on different body organs and tissues. These metal toxicities have both acute or chronic effects. Heavy metals disrupt cellular events which include growth, proliferation, differentiation damage repairing, demanding processes, and apoptosis (Balali-Mood *et al.,* 2021).

Considering that tooth trace element levels reflect a measure of environmental pollution, biomonitoring of the elements is a vital tool in the evaluation of environmental hazards posed by these elements. Hence, this study determined the concentrations of some trace elements (Cd, Cu, Cr, Fe, Ni, Pb, and Zn) in carious human teeth obtained from the Dental Clinic Obafemi Awolowo University Teaching Hospitals Complex, (OAUTHC) Ile-Ife, Nigeria. Although some of these metals are essential, but when they occur in samples at above tolerant or permissible levels, they may constitute a health hazard to humans. The study provided useful data on the concentrations of trace metals in human teeth in the study area which are scanty in literature. It also determined the levels of the elements in enamel, dentin, and pulp and with respect to sex and age distributions. This is important as teeth can be a useful indicator of heavy metals assessment in biological samples.

EXPERIMENTAL METHODS

Sample Collection

Ethical clearance approval was obtained from the Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife with Number IPH/OAU/12/1262. Also, before samples were collected, informed consent and interview forms were administered. The sampling period spanned between April 2019 and December 2019 during which eighty (80) carious teeth samples were collected into a sterilized polyethylene plastic bottle. Carious teeth samples (incisors, canines, premolars, and molars), while taking into consideration patients' age and sex, were collected during teeth extraction with the aid of dental professionals at the Dental Clinic, Obafemi Awolowo University, Teaching Hospitals Complex, Ile-Ife. The teeth samples were then kept in the refrigerator at about 4 °C before digestion.

Sample Preparation

The method of Liu *et al.,* (2013) was employed for the preparation of samples. Teeth samples were put in a clean and sterile sample bottle and taken to the laboratory. Bloodstains were rinsed off with distilled water. The attached soft tissues were removed with the aid of a toothbrush. A diamond-impregnated cutting disc was used to cut the enamel into a horizontal slice of 2 mm thickness from the crown near the incisal and cervical areas of each tooth sample. The enamel, dentin, and pulp were separated with a cutting disc by trained dental personnel at the Faculty of Dentistry, College of Health Sciences, Obafemi Awolowo University Hospitals Complex. Enamel, dentin, and pulp were weighed and put into separate test tubes. After this, 0.5 mL of analytical grade concentrated nitric acid was added to each test tube and soaked for a week for complete dissolution. Distilled water was then added to dilute the sample and made to mark in a 25 mL standard flask. The prepared samples were analyzed using Flame Atomic Absorption Spectrophotometer (FAAS, Varian SpectrAA 55 model, USA) at the Department of Geology, Obafemi Awolowo University, Ile-Ife. The selected wavelengths (nm) and slit width for the metal analyses are presented in Table 1.

Table 1: Selected wavelength and slit width for FAAS determination of the trace elements.

Elements	Wavelength (nm)	Slit Width (nm)	Lamp Current (mA)
Cd	228.8	0.5	4
Cr	357.9	0.2	7
Cu	234.8	0.5	4
Fe	248.3	0.2	4
Ni	232.0	0.2	4
Pb	217.0	1.0	5
Zn	213.9	1.0	10

Quality Control

Recovery of Work

The recovery experiments were carried out by adding 0.5 mL of concentrated nitric acid to 0.3 g Enamel, 0.45 g Dentin, and 0.02 g of Pulp samples in separate Teflon beakers and were allowed to dissolve for a week. The dissolved enamel sample was spiked with 5 mL of 10 mgL^{-1} Pb^{2+}, Cr^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, $Fe^{2+,}$ and Cd^{2+} ions. This step was repeated for the dissolved dentin and pulp samples. The solutions were made up to the mark in a 25 mL volumetric flask with distilled water and stored in a refrigerator before FAAS analysis. Also, unspiked samples were analyzed to calculate the percentage recovery using the formula given in equation 1 (Pamela, 2014).

$$\% \text{ Recovery} = \frac{C_1 - C_2}{C} \times 100 \qquad [1$$

where C_1 = Concentration of metals in the spiked sample,
C_2 = Concentration of metals in the unspiked sample,
C = Concentration of metals in the standard used for spiking

This recovery experiment was necessary to ascertain the efficiency and accuracy of the analytical procedures employed in the study since there were no certified teeth reference samples available to compare our results with when this study was conducted.

Limits of Detection

Limits of detection of the analyzed trace metals (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) were determined from the quantity of the analytes aspirated into the FAAS that gave rise to a reading equal to thrice the standard deviation (SD) of their lowest detectable concentration from the mean of six replicate analyses of the analytes.

Preparation of Standard Solutions

A stock solution of 100 mgL^{-1} of Cu, Pb, Zn, Fe, Ni, Cr, and Cd salts was prepared respectively from which serial dilution was carried out to obtain working concentrations (mgL^{-1}) of 2, 4, 6, 8, and 10 for each metal. The solutions were analyzed on the FAAS and their corresponding absorbance was plotted against concentrations of the metal ions and calibration curves were obtained for each element. This was essential for us to obtain a reliable linear relationship between the absorbance and standard concentrations of the elements (Al-Saleh and Al-Doush, 1996). Linearity was evaluated by calculating the correlation coefficient and relative linearity was obtained from the calibration curve prepared.

Data Analysis

The descriptive statistical analysis was adopted to determine the mean and standard deviation from conventional formulae. This gave rise to the mean values of trace elements in the dentin, enamel, and pulp of the teeth samples. The mean values obtained were used in the general evaluation of data. The inferential statistical analyses (Correlation coefficient, Analysis of Variance (ANOVA), and Principal Component Analysis (PCA) were carried out using SPSS for Windows version 22.0 package.

RESULTS AND DISCUSSION

Validation of Analytical Procedures Used

The results of the quality control study for the teeth samples were obtained as percentage recoveries of Cu, Fe, Ni, Pb, Cr, Cd, and Zn from spiked samples and are presented in Table 2. The recovery work was to verify the reliability of the analytical procedure employed in this study. The percentage recoveries (86.56%, 81.84%, 93.46%, 89.78%, 82.08%, 85.72%, and 90.88%) of the trace elements studied - Cu, Pb, Fe, Cr, Cd, Ni, and Zn respectively were high and validated the procedure used in this study. The percentage recovery values reported in this study agreed with the acceptable 80% obtained by Li *et al.* (1995).

The detection limits for the elements in this study were Cd - 0.02 mg/L, Cr - 0.06 mg/L, Cu - 0.03 mg/L, Fe - 0.06 mg/L, Ni - 0.1 mg/L, Pb - 0.1 mg/L and Zn - 0.01 mg/L (Table 2). There was a good linear relationship between emission intensity and standard concentrations of the calibration curves for the elements which gave correlation coefficients (r^2) of 0.9895 for Cd, 0.9464 for Cr, 1.0 for Cu, 0.9881 for Fe, 1.0 for Ni, Pb and Zn respectively.

Table 2: Percentage recovery (%R) and limits of detection of trace metals in teeth samples by AAS.

Sample	Trace Metals	Amount spiked (µg/g)	Amount recovered (µg/g)	% Recovery	Limit of Detection (mgL^{-1})
Teeth	Cu	10	8.88	86.56	0.03
	Pb	10	8.16	81.84	0.1
	Fe	10	9.35	93.46	0.06
	Cr	10	9.21	89.78	0.06
	Cd	10	8.42	82.08	0.02
	Ni	10	8.57	85.72	0.1
	Zn	10	9.09	90.88	0.01

Concentrations ($\mu g.g^{-1}$) of Trace Elements in Human Teeth Samples Analysed

Table 3 presents the total mean concentrations ($\mu g\ g^{-1}$) of the trace elements in human teeth samples analyzed. Cadmium had a mean concentration of 6.35 ± 8.69; Cr: 222.38 ± 174.97; Cu: 69.59 ± 28.03; Fe: 222.01 ± 163.30; Ni: 17.57 ± 24.11; Pb: 412.08 ± 261.87 and Zn: 4706.25 ± 5579.83. The total mean concentration of the trace elements ranged from Cd (6.35 ± 8.69) to Zn (4706 ± 5579.83) and followed the order Cd < Ni < Cu < Fe < Cr < Pb < Zn.

A report by Nowak and Chmielnicka (2000) on the concentrations of metals in human whole teeth of the residents of the polluted region of Beskid and Katowicein, Poland showed Cd; 2.5 ± 1.2 and 3.1 ± 5.8; Cr; 22.9 ± 18.8 and 49.5 ± 22.5, Cu; 5.6 ± 13.7 and 6.2 ± 14.1, Fe; 29.9 ± 16.9 and 40.7 ± 41.6, Ni; 4.9 ± 1.9 and 6.1 ± 3.5, Pb; 36.3 ± 11.5 and 36.5 ± 16.9, Zn; 287 ± 507 and 328 ± 325 $\mu g.g^{-1}$ respectively. Though their research was carried out in a polluted region, the concentrations obtained for each element were lower than what was obtained in this study. The high concentrations in our study suggest that there is a likelihood of trace metal pollution in the environment either through inhalation and or food ingestion apart from historical exposure due to metals deposited into the teeth during the development of the tooth.

Figure 1 shows the comparison of this present study with some other studies. The high levels of Pb (412.08 $\mu g.g^{-1}$), Cr (222.38 $\mu g.g^{-1}$), and Zn (4706 $\mu g.g^{-1}$) reported in this study give cause for concern, particularly because of the toxic effects of Pb and Cr in humans. Lead has been reported to affect the intelligent quotients of children (Onwurah *et al.,* 2020). It has also been reported to have effects on dental caries and also increases the formation of enamel hypoplasia. Lead in saliva has also been reported to have a positive correlation with the development of tooth decay in children (Pathak *et al.,* 2016).

Figure 2 shows the variation in the concentrations of trace metal in enamel, dentin, and pulp. In enamel, mean concentration value ranged from Cd: 0.28 ± 0.47 to Zn: 247.57 ± 333.83. In pulp, the value ranged from Cd: 5.82 ± 7.87 to Zn: 4181.40 ± 4466.15 while in dentine the range was between Cd: 2.5 ± 3.5 and Zn: 1634.47 ± 354.27. The order of the concentration followed dentine<enamel<pulp except for Zn where concentrations in the dentin are greater than enamel. This study, therefore, showed that pulp serves as a repository or sink for most of the trace elements in human teeth. The concentrations of Cd in teeth samples followed the order dentin < enamel < pulp. The mean level of Cd (0.25 ± 0.35) in dentin was higher than (0.005 ± 0.003 $\mu g.g^{-1}$) reported in Spain by Fernandez-Escudero *et al.,* (2000).

The mean concentration of Cd in pulp (5.82 ± 7.8 $\mu g.g^{-1}$) was greater than 1.98 ± 0.27 $\mu g.g^{-1}$ for healthy teeth and lower than 6.70 ± 1.18 $\mu g.g^{-1}$ for carious teeth samples of the inhabitants of El-Kanayat city, Egypt reported by Amr and Helal (2010). Wychowanski and Malkiewicz also reported Cd levels in dentin and enamel for residents of urban central Poland as 0.046 and 0.033 $\mu g.g^{-1}$ respectively (Wychowanski and Malkiewicz, 2017).

Table 3 Total mean concentrations ($\mu g.g^{-1}$) of trace elements in human teeth samples analysed.

Metals	Concentrations
Cd	6.35 ± 8.89
Cr	222.38 ± 174.97
Cu	69.59 ± 28.03
Fe	222.01 ± 163.58
Ni	17.57 ± 27.57
Pb	412.08 ± 261.87
Zn	4706.25 ± 5579.83

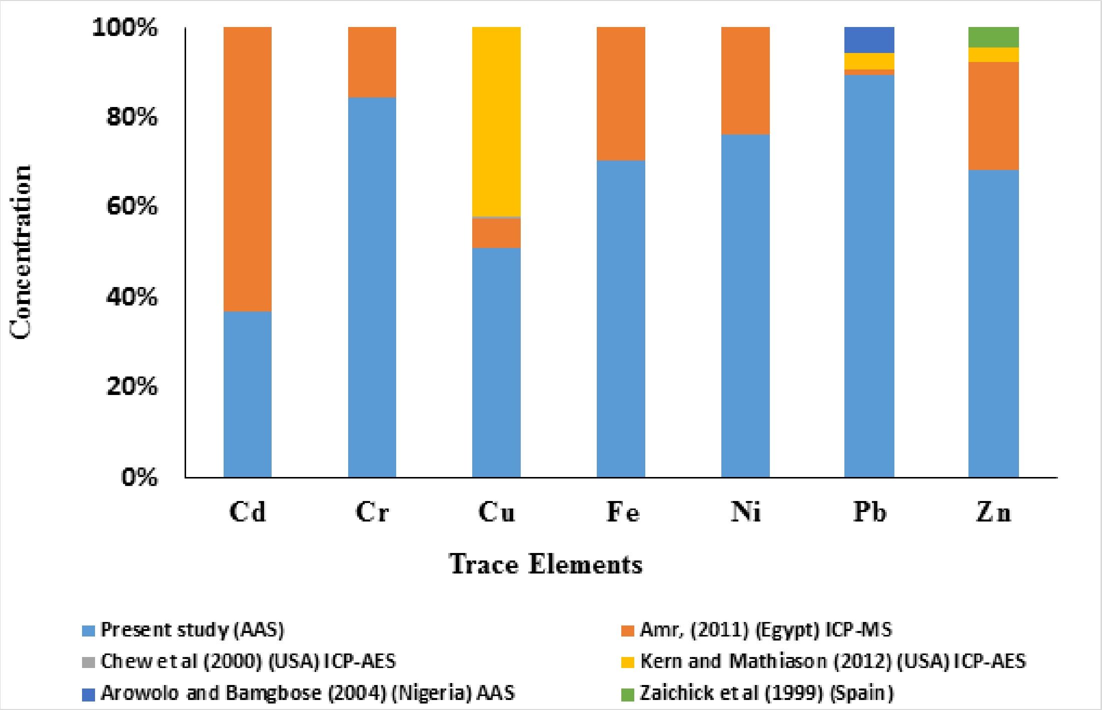

Figure 1: Comparison of the present study with other studies (AAS: Atomic absorption Spectrometer. EDXRF: Energy-Dispersive X-Ray Fluorescent analysis. ICP/AES: Inductively Coupled Plasma Atomic Emission Spectrometer. ICP-MS: Inductively Coupled Plasma Mass Spectrometer).

This study showed higher concentrations for Cd than what was reported by these authors. This might be a result of the trace element pollution the subjects have been exposed to due to improper and non-care of the teeth after food consumption and dental porcelain. Human exposure to Cd can occur through consumption of contaminated food such as some vegetables, potatoes, grains, seeds, and animal parts like liver and kidney; tobacco smoking (active or passive); or through occupational sources (Waalkes *et al.,* 2001; WHO 2001). Once Cd gets into the body system, it accumulates in the liver and bones over some time (Doğan, 2018). Cadmium can also be released from intraoral alloy restoration in dental patients and stored in teeth and mouth tissues which are strictly bound to metallothionein (Guzzi *et al.,* 2009). Cadmium has been implicated in increased tooth decay prevalence (Doğan, 2018). Cadmium enters the food chain when it is taken up by plants from the soil, this may be dangerous as a human is at the top of the food chain. It can also get washed down from the contaminated soil into the water body thereby creating an environmental problem (Doğan, 2018). Many health risks such as kidney failure and cardiovascular disorder are posed in humans as a result of Cd exposure (Arora *et al.*, 2008b). Cadmium has no known health benefits in the human body. The Joint FAO/WHO recommended the Provisional Tolerable Weekly Intake (PTWI) as 0.007 mg/kg BW for Cd (WHO, 2004).

The mean concentrations of Cr followed the order pulp > enamel > dentin. Chromium in human dentin was reported for Spanish to have 0.289 ± 0.196 $\mu g.g^{-1}$ (Fernandez-escudero *et al.,* 2020). This report was far lower than what was obtained from this study. Cr is required in trace quantity in humans. It occurs in the form of Cr (III) which is present in food and active biologically.

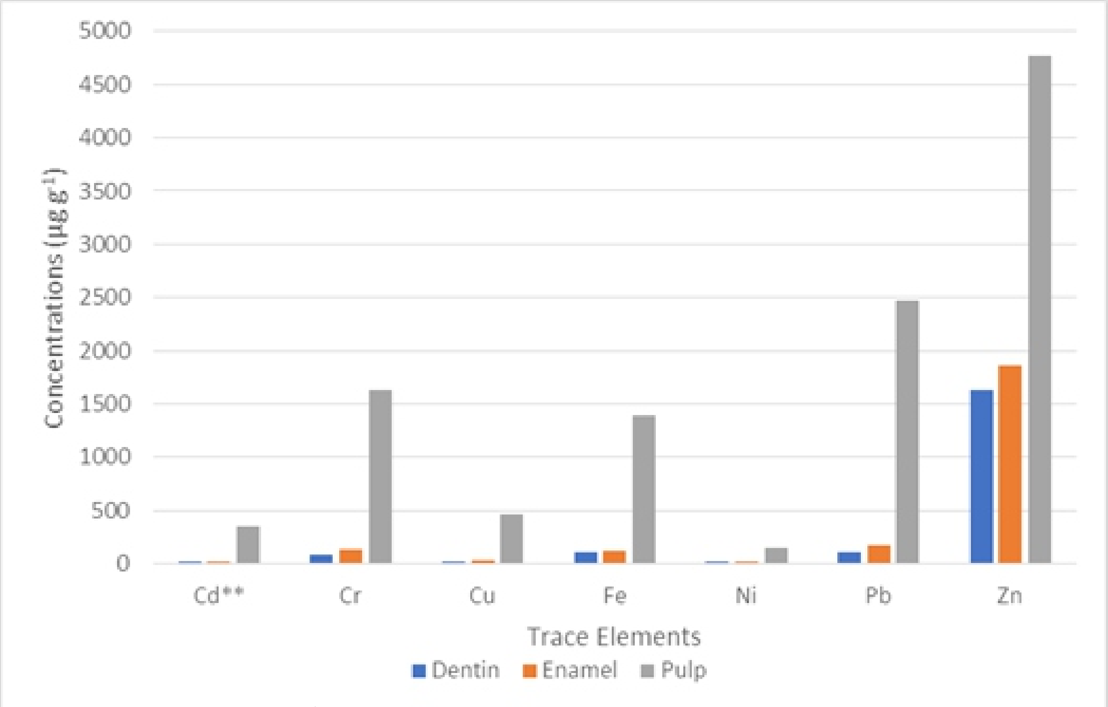

Figure 2: Concentrations (µg.g^{-1}) of trace elements in enamel, dentin, and pulp samples
**The original concentration of Cd was multiplied by a factor of 10 for the clarity of the low level of Cd relative to other elements.

Another form is Cr (VI) which is very toxic and occurs as a result of pollution from the industries (Mertz, 1993). Cr is widely found in the food supply such as egg yolk, whole grain products, cereals, coffee, nuts, meat, and green beans (Kozlovsky *et al.,* 1986; Anderson *et al.,* 1992). Long-time exposure to chromium can result in accumulation in the kidney and can result in renal dysfunction (Anderson *et al.,* 1992).

The mean concentration of copper analyzed followed the order dentin < enamel < pulp. A report by Amr (2011) found that the mean concentration of copper in the paramount whole teeth of an Egyptian was 9.2 ± 11.4 mg.kg^{-1}. This result is lower than the findings (69.6 ± 28.03 µg.g^{-1}) for Nigerian subjects recorded in this study. Also, in the study carried out by Chew *et al.*, 2000, the mean concentrations in Malaysian whole teeth were found to be 0.29 ± 0.03 µg.g^{-1} which is lower than the findings in this study. The high concentration of Cu recorded in this study may be linked to ingestion through household emissions and electronic and electrical waste to which the patients might have been exposed.

It should be noted that copper is normally present in a small amount in saliva so continuous pick-up of copper by the enamel is possible. A higher level of copper is found in carious teeth compared to healthy teeth. Higher caries prevalence is found to be related to the presence of Cu in water, food, soil, or vegetables (Pathak *et al.,* 2016).

Over the years, the concentrations of copper in human teeth as determined by scientists have been as high as 320 ± 10 µg.g^{-1} (Kern and Mathiason, 2012). The research carried out by Malara (2016) reported a mean concentration of 17.82 µg.g^{-1} in human teeth. Also, Chew *et al.,* (2000) recorded the mean concentration of copper in human teeth as 6 µg.g^{-1}. The mean values of copper concentrations in people living in Bielsko-Biala and Ruda Slaska in Poland were 8.83 ± 1.30 and 9.13 ± 1.11 µg.g^{-1}, respectively (Malara, 2016). These results were lower than what was obtained in the present study for the concentration of copper in human teeth.

Also, according to the report on the mean concentration of copper by de Cássia *et al.,* (2009), Cu had a very high concentration of 402.47 ± 219.80 ppm in teeth samples. Another result of the mean concentration of Cu in human teeth carried out on the people living in Finland showed the mean concentration to be 10 $\mu g.g^{-1}$. The mean concentration of Cu in human enamel and dentin as reported by Derise *et al.,* (1974) using AAS were found to be 8.1 ± 0.03 $\mu g.g^{-1}$ and 7.3 ± 0.1 $\mu g.g^{-1}$ respectively which are higher than the 2.90 and 4.32 $\mu g.g^{-1}$ reported for dentin and enamel respectively in this study. The concentration of copper in human healthy teeth has been reported to be 0.21 ± 0.10 $\mu g.g^{-1}$ (de Cassia *et al.,* 2009).

The mean concentration of Fe followed the order dentin < enamel < pulp. Iron can be obtained via ingestion of food such as meat, poultry products, fish, green leafy vegetables, nuts, and cereals (Bhattacharya *et al.,* 2016). Iron is an essential element in the human body that ensures that almost all organisms survive. It gets into the body through dietary means. According to Qamar, Fe concentrations are low in enamel compared to dentin and pulp (Qamar *et al.,* 2017). The results of these researchers are at variance with our findings in this study for Fe. The reason for this variation is not clear and is difficult to explain now; further study is required to acquire more data to be able to justify this variation. The concentration of trace elements such as Fe^{2+}/Fe^{3+} is more in the outer layer of dental enamel than in the sub and inner layers and this is related to a report that the concentration of Fe in human enamel is obtained from the external environment and deposited during the process of calcification (Qamar *et al.,* 2017).

The mean concentration of Ni followed the order dentin < enamel < pulp. In a report by Fernández-Escudero *et al.,* (2020), Ni had a concentration of 0.216 $\mu g.g^{-1}$. Ni is a toxic metal. The ionic radius of Ca^{2+} is higher than that of Ni^{2+}. When Ni^{2+} substitutes Ca^{2+}, there will be a decrease in the lattice parameters along the c-axis of the cell in synthetic hydroxyapatite (Qamar *et al.,* 2017). During the substitution process, Ni^{2+} replaces Ca^{2+} and bonds with oxygen and phosphates to form nickel (II) phosphate $Ni_3(PO_4)_2$. Ghadimi *et al*., (2013), also observed a similar inverse association between Ni^{2+} level and the crystal size in tooth enamel.

The use of nickel-chromium (Ni-Cr) based alloy for dental restoration has led to the enhanced excretions of Ni and Cr in patient urine (Chen *et al.,* 2013). Nickel is taken up by the body through contaminated water, air, and food (Kampa and Castanas, 2008). The toxicity of Ni-containing substances such as with other metals is associated with the bioavailability of Ni^{2+} at a systemic or local target site (Buxton *et al.,* 2019). In the case of Pb, the mean concentration followed the order pulp > enamel > dentin. Brudevold and Steadman (1956) reported that young adults have Pb concentration levels in tooth enamel to be 30 ppm and 90 ppm for ages 50 years and above. Also, a report found Pb levels in both enamel and dentin to be from 38.9 to 51.5 ppm (Derise *et al.,* 1974). A review on Pb levels in human teeth showed the concentration of Pb in human surface enamel and secondary dentin of permanent teeth to range from 2 to 2500 $\mu g.g^{-1}$and 18 to 600 $\mu g.g^{-1}$ respectively.

Lead is a toxic metal and harmful to the human body. At low concentrations, Pb^{2+} replaces Ca^{2+} in the hydroxyapatite (Ghadimi *et al.,* 2013). Lead is transferred to teeth through the environment or diet. Lead has been determined to have a positive effect on dental caries and increase the formation of enamel hypoplasia (Dogan, 2018). Also, in children, a positive correlation exists between the level of Pb in saliva and the development of tooth decay. It also plays important role in the development of caries lesions (Dogan, 2018). Lead in the body is rapidly transported through the blood to bones and teeth where it is stored (Arowolo and Bamgbose, 2004). The presence of lead in drinking water may be as a result of leaching of the elements through the distribution system or the plumbing. A permissible limit of concentration 5 $\mu g.L^{-1}$ is proposed by Federal-Provincial-Territorial Committee on Drinking Water, Canada for total lead in drinking water (Sahirou *et al.,* 2022). The WHO proposes a guide value of 0.010 $mg.L^{-1}$ (Sahirou *et al.,* 2022). Water is a route by which lead gets into human body. Lead bioaccumulation occurs when animals feed on plants from a lead-contaminated environment and human beings eat such animals and vegetables from such a contaminated environment. Drinking lead-contaminated water is another source of lead in the human body. Lead in the human body gets stored in the teeth and bones and gets accumulated over time (WHO, 2019). About 90%

of Pb contamination in the human body is stored in the bone, blood, and teeth. Lead in the bone can be lost due to mobility. Pb in the teeth is considered a better assessment as an index of environmental pollution and an indicator of the burden of lead (Arowolo and Bamgbose, 2004).

In a study carried out on teeth of Nigerian subjects in three cities of Lagos, Abeokuta, and Ibadan, the levels of lead in carious teeth of residents across the three cities respectively reported a mean concentration of 1.91 to 26.8 $\mu g.g^{-1}$ for Lagos, 2.05 to 21.6 $\mu g.g^{-1}$ for Ibadan and 1.95 to 18.08 $\mu g.g^{-1}$ for Abeokuta (Arowolo and Bamgbose, 2004). Arowolo and Bamgbose, (2004) only studied and reported concentrations of lead in teeth samples. Another report on lead concentrations in teeth from people living in Kosovo and Austria showed that Mitrovica residents had 23.3 mg/kg, Klina residents had 3.2 mg/kg and Graz residents had 1.7 mg/kg Kamberi *et al.* (2011). These figures are lower than what is reported in this study.

The mean concentration of Zn followed the order enamel < dentin < pulp. Zinc concentrations in human teeth have been reported to range from 44 ± 20 to 227.23 ± 0.02 $\mu g.g^{-1}$ (Kern and Mathiason, 2012) while 320 ± 10 $\mu g.g^{-1}$ was reported by Zaichick *et al.,* (1999). In the present study, Zn had the highest concentration of metals probably due to its important role in the human body. Researchers have reported that Zn can be easily incorporated as a replacement for Ca^{2+} ions. Many publications have described the important role Zn plays in dental plaque and oral tissues in reducing the ability of bacteria to cause tooth decay, most especially anaerobic bacteria (Sejdini *et al.,* 2018).

3.3 Concentrations of Trace Elements ($\mu g.g^{-1}$) in Male and Female

Table 4 presents the concentrations ($\mu g.g^{-1}$) of trace elements in enamel, dentin, and pulp based on sex. The total mean concentrations of ($\mu g.g^{-1}$) in teeth samples for male were: Cd, 4.97 ± 8.73; Cr, 178.95 ± 187.55; Cu, 65.7 ± 22.42; Fe, 232.79 ± 199.52; Ni, 13.95 ± 25.11; Pb, 482.01 ± 1130.78 and Zn, 1249.16 ± 1615.79, while in female, the total concentrations were: Cd, 7.28 ± 8.63, Cr, 241.8 ± 157.98; Cu, 68.15 ± 27.8; Fe, 214.14 ± 133.47; Ni, 20.09 ± 28.23; Pb, 363.53 ± 269.82 and Zn, 1205.99 ± 1672.94.

Table 4: Concentrations ($\mu g.g^{-1}$) of trace elements in male and female teeth samples.

Trace Metals	**Males (38)**				**Females (42)**			
	Parts of Tooth				Parts of Tooth			
	Dentin	**Enamel**	**Pulp**	**Total**	**Dentin**	**Enamel**	**Pulp**	**Total**
Cd	0.20 ± 0.33	0.37 ± 0.45	4.40 ± 7.95	**4.97 ± 8.73**	0.25 ± 0.36	0.24 ± 0.48	6.79 ± 7.79	**7.28 ± 8.63**
Cr	8.20 ± 7.24	13.24 ± 10.08	157.51 ±170.23	**178.95 ± 187.55**	11.74 ± 6.43	16.96 ± 9.35	213.10 ± 142.20	**241.8 ± 157.98**
Cu	2.73 ± 1.50	3.85 ± 2.03	59.12 ± 18.89	**65.7 ± 22.42**	2.84 ± 1.39	4.22 ± 3.58	61.09 ± 22.83	**68.15 ± 27.8**
Fe	13.15 ± 7.05	16.18 ± 8.85	203.46 ± 183.62	**232.79 ± 199.52**	12.22 ± 14.51	13.38 ± 8.62	188.54 ± 110.34	**214.14 ± 133.47**
Ni	0.87 ± 1.34	0.86 ± 1.37	12.22 ± 22.40	**13.95 ± 25.11**	0.82 ± 1.30	1.07 ± 2.65	18.20 ± 25.28	**20.09 ± 29.23**
Pb	15.86 ± 12.25	22.22 ± 17.25	443.93 ± 207.30	**482.01 ± 1130.78**	12.66 ± 10.67	19.56 ± 16.72	331.31 ± 242.43	**363.53 ± 269.82**
Zn	259.81 ± 497.26	351.05 ± 481.72	648.30 ± 636.81	**1259.16 ± 1615.79**	263.14 ± 958.55	169.96 ± 99.45	772.89 ± 614.94	**1205.99 ± 1672.94**
Mean ± Sd.	**42.97 ± 75.28**	**58.25 ± 74.54**	**218.42 ± 178.17**	**319.53 ± 455.70**	**43.38 ± 141.89**	**32.20 ± 20.12**	**227.40 ± 166.54**	**302.99 ± 328.55**

In both males and females, Zn had the highest concentration which was found in the pulp to be 648.30 ± 636.81 in males and 772.89 ± 614.94 in females. The least concentrated metal was Cd which was found in dentin; 0.20 ± 0.33 for males and in enamel 0.25 ± 0.36 for females. The concentrations of metals in male followed the order: Zn > Pb > Fe > Cr > Cu > Ni > Cd while the concentrations in female are in the order: Zn > Pb > Cr > Fe > Cu > Ni > Cd. Although Pb was not observed to have the highest concentration of the trace elements obtained, the concentrations in this study in both males (482.01 ± 1130.78) and females (363.53 ± 269.82) tend to serve as a source of health concern to the patients since Pb is toxic to the human body at low concentrations and can lead to various health challenges.

3.4 Concentrations (μg.g^{-1}) of Trace Elements in Human Teeth with respect to Age distribution

Figure 3 shows the mean concentrations (μg.g^{-1}) of trace elements in human teeth and with age distribution. The concentrations of trace metals in teeth samples in the age group 10–19 category ranged from 8.52 for Cd to 1196.99 for Zn with a mean concentration of 285.1 ± 183.91. For age brackets 20 to 29, the metal concentrations ranged from 6.84 for Cd to 1568.19 for Zn, and the mean value of 367.3 ± 105.74. Age brackets 30–39 have concentrations ranging from 8.76 for Cd to 837.61 for Zn with a mean value of 256 ± 182.62. For ages 40–49, we have the range of metal concentration from 2.16 to 1064.40 with the mean value of 333 ± 221.21. Also, for ages 50 - 59, the mean concentration of 315.4 ± 255.02 was obtained. The concentrations recorded for the age range 60 to 69 were 6.35 Cd to 958.43 Zn with an average value of 263.5 ± 229.00. For ages 70 to 79, the concentrations ranged from 0.48 Cd to 1181.88 Zn, and the mean value of 300.5 ± 19.31. The concentrations for the age range 80 to 89 were from 0.86 Cd to 169.71 Pb with a mean value of 53.78 ± 0.01. Cd had the lowest concentration (0.48) in the age bracket of 70–79 while Zn had the highest concentration (1568.19) within the age brackets of 20–29 years. From the results obtained from this present study, total metal concentration in human teeth did not follow any particular pattern with respect to age. It can be deduced that individual exposure to trace metals may be responsible for the levels of metals found in the teeth samples irrespective of the age group. Generally, the results of this study were relatively higher than those reported by other researchers except for Cd (234 ± 59) and Cu (10.9 ± 2.56) (Amr, 2011) which were higher than the concentrations of the same trace elements Cd (6.85 ± 8.89) and Cu (69.5 ± 28.03) reported in this study.

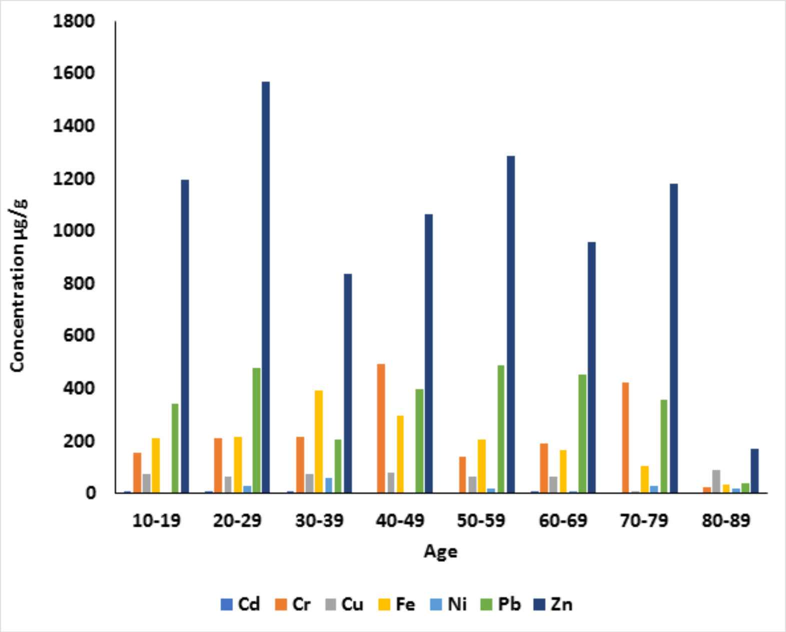

Figure 3: Chart representation of concentrations (μg.g^{-1}) of trace elements in human teeth with respect to age

3.5 Analysis of Variance

3.5.1 Analysis of Variance of Trace Elements in Male and Female Teeth Samples

One-way parametric analysis of variance (ANOVA) was used to compare the concentrations of trace metals present in teeth samples. The result is presented in Table 5 at $p \leq 0.05$ significant level, $f_{critical}(4.747) > f_{calculated}(0.005182)$. This implies that there was no significant difference in the concentrations of (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in the teeth samples collected.

3.5.2 Analysis of Variance of Trace Elements with Respect to Age of Subjects

One-way parametric analysis of variance (ANOVA) was also used to compare the concentrations of trace elements present in teeth samples with respect to age. The result is presented in Table 6 at $p \leq 0.05$ significant level, $f_{critical}$ (2.2074) > $f_{calculated}$ (0.04179). This also implies that there was no significant difference in the concentrations of (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) with respect to age in the teeth samples collected.

Table 5: Analysis of Variance (ANOVA) with respect to Male and Female

Sources of Variation	Sum of Squares	df	Mean Square	$F_{calculated}$	P(Same)	$F_{critical}$
Between Groups	970.279	1	970.279	0.005182	0.9438	4.747
Within Groups	2.24669E-07	12	187224			
Total	2.24766E-06	13				

Table 6: Analysis of Variance (ANOVA) with respect to Age

Sources of Variation	Sum of Squares	df	Mean Square	$F_{calculated}$	P(Same)	$F_{critical}$
Between Groups	445295	7	63613.5			
Within Groups	7.31	48	152232	0.4179	0.8864	2.2074
Total	7.75	55				

3.6 Correlation of Trace Elements in Teeth Samples with Respect to Age

Table 7 presents the Pearson correlation matrix among different age groups. It was observed from the table that there are strong correlations among all the age groups with respect to the concentration of the trace elements at $p \geq 0.087$. There was no antagonism in the correlations reported in respect of the age groups studied. Strong correlations of between 0.994 and 0.901 were recorded for the 10–19 to 70–79 age distributions whereas the age range of 80–89 gave correlations of between 0865 and 0.755 compared to all other age distributions studied.

3.7 Principal Component Analysis (PCA) of Trace Elements in Teeth Samples

Figure 4 shows the projection of all the age groups in the factor space and the two-dimensional map showing trends between the concentrations of trace elements in teeth samples along with age distributions. It was observed that there were strong associations among the age groups just as observed in the correlation matrix (Table 7). Age groups (10–19, 20–29, 50–59, 60 –69, and 70 –79) had distributions along the same axis while age groups (30–39, 40–49, and 80–89) had distributions along the same axis which indicated a strong association with one another. Figure 4 also shows that there is a relationship and hence the association between Cd and Ni; Cu, Cr, and Fe that are aligned within the same axis respectively, and also Pb and Zn are associated as they showed a relationship along the same axis in terms of their distributions

Table 7: Correlation of trace elements in teeth samples with respect to age.

Variables/Age	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89
10-19	**1***							
20-29	0.998*	**1***						
30-39	0.947*	0.933*	**1***					
40-49	0.939*	0.939*	0.932*	**1***				
50-59	0.994*	0.996*	0.922*	0.928*	**1***			
60-69	0.980*	0.985*	0.904*	0.945*	0.993*	**1***		
70-79	0.959*	0.966*	0.901*	0.979*	0.951*	0.959*	**1***	
80-89	0.865*	0.852*	0.794*	0.757*	0.842*	0.813*	0.780*	**1***

n = 209, r ≥ 0.087 at 95% confidence interval

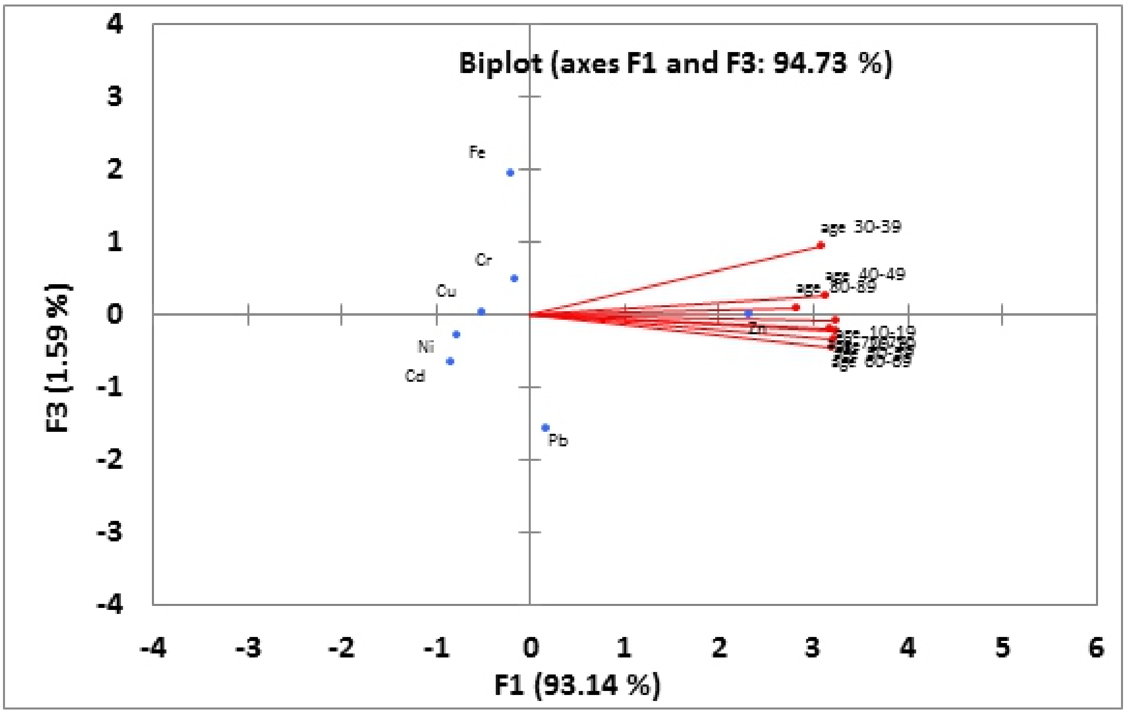

Figure 4: Principal Component Analysis (PCA) of trace elements in teeth samples with respect to age

CONCLUSION

This study reported varied concentrations of trace elements Cd, Cr, Cu, Fe, Ni, Pb, and Zn in carious human teeth samples after acid digestion using Atomic Absorption Spectrometer (AAS) with respect to age distribution and sex. The high percentage recoveries of the trace metals in this study showed that the sample preparation methods and the analytical procedures employed were satisfactory. The total mean concentrations ($\mu g.g^{-1}$) of the trace elements in the samples followed the order: Cd < Ni < Cu < Fe < Cr < Pb < Zn. The mean concentrations ($\mu g.g^{-1}$) of elements in dentin, enamel, and pulp samples showed that pulp had the highest concentrations and served as a repository or sink for most of the trace elements in human teeth studied. The total mean concentrations of the elements in human teeth samples followed the same decreasing order with respect to male and female subjects and across the age groups studied. Zinc had the highest concentration while Cd was the lowest. The study has demonstrated that teeth can be very useful in the environmental exposure assessment of trace elements.

Declaration of Conflict of Interest:

On behalf of the authors, the corresponding author states that there is no conflict of interest or competing financial interest.

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