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MERCURY LEACHING FROM DENTAL AMALGAM FILLINGS AND ITS ASSOCIATION WITH URINARY ZINC

By

AFAF MOHAMED ZANAGER

Student Number: 3481183

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Medical Biosciences

Faculty of Natural Sciences

University of the Western Cape

Supervisor: **Professor Andre Oelofse**

Co-supervisor: **Professor Yusuf Osman**

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DECLARATION

I, Afaf Mohamed Zanager, declare that the thesis "Mercury leaching from dental amalgam fillings and its association with urinary zinc" for the Masters degree in Medical Bioscience (MSc) at the University of Western Cape is my own work in design and in execution, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references. Also, I have no commercial or associative interest that represents a conflict of interest in connection with the study.

Afaf Mohamed Zanager

Date: 26/03/2019

Signed: 

DEDICATIONS

This thesis is dedicated to my father, Mohamed Zanager, who passed away in 2010. Words cannot describe the pain that is living in my heart. Even though you are by Allah (s), you will always be loved and in my heart. May Allah have mercy on you and rest your soul in eternal peace (Amen). It is also dedicated to my great mother for keeping me in her prayers. Last but not least, to my husband and my children.

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ABSTRACT

Mercury (Hg) is an example of a toxic metal that is not essential for nutrition. It exists in organic and inorganic forms in seafood and vapour from dental amalgam fillings respectively. Elemental mercury (Hg^0) from dental amalgam was the focus of this study. Dental amalgam is one of the most commonly used dental filling materials and has been used for over 150 years. It is composed of Hg^0 (approximately 50%) combined with other metals such as copper and zinc (Zn). These fillings give off Hg^0 vapour throughout their existence, and is further enhanced by activities such as chewing, grinding of teeth and drinking hot liquids. Mercury consumption can lead to Zn loss or deficiency, and is reported to displace Zn and copper. Several European nations have outlawed the use of amalgam as a restorative material due to controversies regarding its safety in children, women of childbearing age and individuals with renal disease. Moreover, various studies have reported correlations between the number of amalgam fillings and Hg concentration in blood plasma, urine, faeces, saliva and different organs. Blood, urine, and hair mercury levels are used to predict possible health effects that may be caused by the different forms of Hg. Urine Hg is used to test exposure to metallic Hg^0 vapour and inorganic Hg forms.

This study aimed to evaluate the effects of Hg^0 from dental amalgam restorations on the status of Zn in the urine. This was done by determining the concentrations of Hg^0 in urine, buccal cells and the oral cavity, and its relationship with urinary Zn concentrations in the same individuals. Samples of urine, buccal tissues, chewing gum and completed questionnaires were collected from the participants (women

and men) at the dental clinics in Tygerberg Hospital (TBH), Cape Town. Samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS).

Findings from this study show that there was a correlation between levels of urinary Hg^0 and urinary Zn ($p=0.02$). However, urinary Hg^0 did not predict the amount of urinary Zn. Also, no relationship was found between levels of Hg^0 in buccal swab or the chew test samples and urinary Zn level. There was a significant difference between females and males in the level of urinary Zn, men had higher levels of Zn excreted in the urine than females ($p=0.05$). However, there was no significant difference in the level of urinary Hg^0 between males and females. The number of fillings (4-7) and age of fillings were significantly associated with urinary Hg^0 level ($p<0.05$), while smoking >15 cigarettes/day increased the level of Hg^0 in buccal swab samples ($p=0.002$). We were not able to demonstrate a causal effect of Hg^0 leaching on urinary zinc levels.

KEYWORDS: Mercury, zinc, dental amalgam, buccal samples, urine, chewing gum test, inductively coupled plasma-mass spectrometry (ICP-MS).

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LIST OF ABBREVIATIONS

Abbreviations	Full Name
AAS	Atomic Absorption Spectrometry
ºC	Degrees Celsius
CAF	Central Analytical Facilities
$CH_3CH_3Hg^+$	Ethyl mercury
Cu^+	Copper
CPOX	Coproporphyrinogen oxidase
EPA	Environmental Protection Agency
EURRECA	European Micronutrient Recommendation Aligned
Fe^{2+}	Iron
FFQs	Food frequency questionnaires
G	Grams
HBM	Human Biological Monitoring
HCL	Hydrochloric acid
Hg	Mercury
Hg^0	Mercury vapor
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
I-Hg, Hg^{2+}	Inorganic mercury
ICP-MS	Inductively coupled plasma mass spectrometer
Kg	Kilograms
MeHg, CH_3Hg^+	Methylmercury

MeSH	Medical Subject Heads
MS	Multiple sclerosis
NAC	N acetyl cysteine
OLL	Oral lichenoid lesion
OLP	Oral lichen planus
PD	Parkinson's disease
RCRA	Resource Conservation and Recovery Act
RDA	Recommended daily allowance
ROS	Reactive oxygen species
SH	Sulfhydryl groups
SNP	Single nucleotide polymorphism
T-Hg	Total mercury
TCLP	Toxicity characteristic leaching procedure
TCV	Thimerosal-containing vaccines
U-Hg	Urinary mercury
WHO	World Health Organization
Zn	Zinc
µg	Micrograms
µg/day	Micrograms per day
µg/L	Micrograms per liter
µg Hg/L	Micrograms Mercury per liter
µg/kg/day	Micrograms per Kilogram per day
>	More than
<	Less than

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CHAPTER ONE

Literature review

1.1 Introduction

Dental amalgam is one of the most commonly used dental filling materials and has been used for over 150 years (Barregard, 2005; Maserejian *et al.,* 2008; Erdal and Orris., 2012) for the restoration of dental caries (Chin *et al.,* 2000; Pizzichini *et al.,* 2003; Derouen *et al.,* 2006;), and it still remains the most widely used dental filling material. It is a compound composed of mercury (Hg) (approximately 50%) combined with other metals including Zn (Maserejian *et al.,* 2008; Erdal and Orris, 2012; Zwicker *et al.,* 2014). These fillings give off Hg^0 vapour, which is toxic (Salonen *et al.,* 1995; Barregard, 2005; Houston, 2007). The amount of Hg^0 vapour depends on the number of fillings, duration of placement and activities such as chewing, grinding of teeth, and drinking hot liquids (Derouen *et al.,* 2006; Svendsen *et al.*, 2010). Mercury has no known physiological role in human metabolism and the human body has no mechanisms to excrete it (WHO, 1990; Houston, 2011). Mercury is stored during life so that the average 70-75 kg person has a total body burden of about 13 mg of Hg (Salonen *et al.,* 1995; Houston, 2011).

Mercury consumption can also lead to Zn loss or deficiency (Dufault *et al*., 2009). Zinc is an essential trace element for all organisms. Additionally, Hg readily binds to metallothioneines, replacing zinc, copper and other trace metals, which serves as the intracellular “sink” for these essential elements (Quig, 1998).

Small amounts of Hg^0 are known to be continuously released from dental amalgam fillings (Risher and DeWoskin, 1999; Baek *et al.,* 2016). However, several studies have reported correlations between the number of amalgam fillings and Hg concentration in blood plasma, saliva, oral air, faeces, urine, pituitary gland, brain, liver and kidneys of patients, and even in mothers of newborn babies. Mercury has been found in fetal and infant brains and livers (Hansen *et al.,* 2004). Saliva has high levels of Hg^0 due to mastication and bruxism associated with the total number of amalgam surfaces (Björnberg *et al.,* 2006). Numerous studies have described the relationship between Hg^0 exposure from dental amalgam fillings and its corresponding excretion in the urine of adults and children (Björnberg *et al.,* 2006). Females who receive amalgam fillings have significantly higher mean urinary Hg (U-Hg) concentrations than males (Berglund 1990). Furthermore, in a randomized clinical trial of amalgam in Portuguese children reported a strong, positive association between urinary U-Hg and both the number of amalgam surfaces and the time of placement of the fillings in children (Maserejian *et al.,* 2008; Woods *et al.,* 2007). More recently, it has been speculated that, relationships between several neurological pathologies (Alzheimer's disease, autism) and exposure of patients to inorganic Hg may exist (Hansen *et al.,* 2004). Several European governments have banned the use of amalgam fillings in children, women of childbearing age, and renal patients (Hansen *et al.,* 2004), due to the uncertainties and controversies that revolve around the possible harm of dental amalgam fillings. For example, countries including France, Denmark and Norway have banned the use of Hg in dental amalgams over worries of health and environmental effects, with Sweden joining this ban on the grounds of both environmental and health concerns (Mackey *et al.,* 2014).

Previous studies have been carried out on Hg^0 from dental amalgams in Europe, Iran, USA, Japan, Portugal, Italy, Germany (Barregard, 2005), Brazil (Obiri *et al.,* 2016) and few studies in Africa (Iwaola *et al.,* 2015). Results showed that dental amalgam was a source of low level exposure to Hg^0, and there was no evidence of adverse health effects at these levels. However, other previous studies have investigated total Hg in urine, blood, hair, saliva and different parts of the human body and even some animals, but no study has looked at or determined Hg levels in buccal cells of humans. In this study, Hg^0 concentrations were determined in urine, buccal cells and Hg^0 release from dental amalgam fillings (using a newly developed, amalgam specific chew test). The aim of this study was to evaluate the effects of metallic mercury (Hg^0) from dental amalgam restorations on the status of Zn to prove whether levels of Zn had been affected by mercury that was absorbed in the body.

Objectives

1- To determine the concentrations of Zn and Hg^0 in urine, and Hg^0 in buccal swabs and chewing gum.

2- To evaluate the relationship between levels of Hg^0 (urine and buccal swabs) with respect to number and age of fillings, size of fillings, habits of hot liquid consumption, chewing gum, smoking, bruxism and brushing teeth and urinary Zn.

3- To determine the relationship between Hg^0 and Zn concentrations in the urine with respect to gender and age of the participants.

4- To determine the relationship between the Hg^0 concentrations in buccal cells or chewing gum and Zn in the urine with respect to gender and age of the participants.

1.2 Dental amalgam fillings and mercury

Dental amalgam is a preferred choice when compared with other restorative materials due to its physical and mechanical properties, ease of use, stability and low-cost, even though it contains mercury (Hg^0) as one of its major components (Chin *et al.,* 2000). There is a particular concern with regard to Hg on human health due to its toxicity and its potentially adverse effects (Lorscheider *et al.,* 1995; Woods *et al.,* 2005; Meyer *et al.,* 2016). Moreover, there are four possible harmful effects of dental amalgam, namely: toxicity, oral galvanism, allergenicity and ecological complaints (Chin *et al.,* 2000).

The typical amalgam is composed of 50% mercury (Derouen *et al.,* 2006; Woods *et al.,* 2007; Fakour *et al.,* 2010; Zwicker *et al.,* 2014), 25% silver, 25% tin, 1-6% copper (Clarkson, 2002; Clarkson *et al.,* 2003; Dye *et al.,* 2005), nickel (Bergdahl *et al.,* 1998; Houston, 2011) and 0-2% zinc (Svendsen *et al.,* 2010; Mackey *et al.,* 2014).

Mercury is ranked third by the US Government Agency for Toxic Substances and Disease Registry as the most toxic element or substance on the planet that continues to be dumped into our waterways and soil, spilled into our atmosphere, and consumed in our food and water (Rice *et al.,* 2014). Mercury poisoning can result in mental retardation, dysarthria, blindness, neurological deficits, loss of hearing, developmental defects, abnormal muscle tone (Rice *et al.,* 2014), kidney failure or damage, nervous-system disorders (Afkhami *et al.,* 2012), intellectual impairment and even death (Martín-Yerga *et al.,* 2013; Houston, 2011).

Hahn *et al* (1990) showed that dental amalgam restorations are associated with risks due to the presence of Hg^0 in various organs and tissues, such as kidneys gastrointestinal tract and jaws. Lorscheider *et al* (1995) and Chin *et al* (2000) confirmed that Hg^0 is released into intra-oral air, and thus Hg^0 can be inhaled and swallowed through the nose and mouth and is distributed throughout the body (Dodes, 2001; Pizzichini *et al.,* 2003; Fakour *et al* 2010; Rice *et al.,* 2014). Research has shown that people with amalgam restorations have higher oral levels of Hg^0 vapour than people who do not have amalgam restorations (Dodes, 2001). In addition, many studies suggest that the levels of exposure to Hg^0 vapor from dental amalgam fillings may be unsafe for certain subpopulations (Geier *et al.,* 2013), particularly for boys with common genetic variants such as coproporphyrinogen oxidase (CPOX4) (Homme *et al.,* 2014). On the other hand, most of the researchers conclude that there is no health risk associated with the exposure to amalgam vapour from fillings for patients (Risher and DeWoskin, 1999; Clarkson, 2002; Derouen *et al.,* 2006; Homme *et al.,* 2014), but from occupational exposures, such as for workers in dental clinics where the inhalation of Hg^0 vapour may cause a variety of adverse effects (Li *et al.,* 2015; Li *et al.,* 2011). Furthermore, in the absence of occupational exposure, Hg^0 release from dental amalgams is a necessary but insufficient condition to obtain a high long term body burden (Hansen *et al.,* 2004).

Heavy metals such as Hg create a significant potential threat to human health because they are associated with many adverse effects (Castro-González and Méndez-Armenta, 2008). Mercury damages the nervous system by interfering with energy production which impairs the cellular detoxification processes, thereby causing the cell to either die or live (Lorscheider *et al.,* 1995; Pamphlett *et al.,* 1998; Woods *et al.,* 2007; Fakour *et al.,* 2010;

Rice *et al.,* 2014), but can also affect the immune system (Rice *et al.,* 2014), digestive system, skin and oral tissues (Fakour *et al.,* 2010; Afkhami *et al.,* 2012; Rice *et al.,* 2014) because of their sensitivity to Hg (Insel, 1996). The Hg body burden has been implicated in a number of diseases (Hybenova *et al.,* 2010; Gardner *et al.,* 2010; Rice *et al.,* 2014). Reports indicate that prolonged exposure to Hg causes toxicity to the cardiovascular, renal, immunological, neurological, hematological, pulmonary, endocrine, reproductive and embryonic systems in the human body (Rice *et al.,* 2014, Genchi et al., 2017). Mercury vapour absorbed through the lungs, does not convert to inorganic mercury (I-Hg) and can pass through the blood brain barrier (Rice *et al.,* 2014). Furthermore, once absorbed, Hg^0 is lipid soluble, it can cross the blood brain barrier and placenta and can be oxidised by catalase and hydrogen peroxide into inorganic mercury (Hg^{2+}), which is retained by the brain for years (Clarkson *et al.*, 2003; Rooney, 2007). The half-life of Hg in the brain has been estimated to be as long as 20 years (Rice *et al.,* 2014). Mercury poisoning may also cause chest pain, anemia, including hemolytic anemia and aplastic anemia, leukemia, and Hodgkin's disease (Rice *et al.,* 2014). Mercury vapour has also been well known as a neurotoxin, and thus is a risk to dental personnel due to chronic exposure from the repetition of clinical procedures that are associated with dental amalgam restorations (Langworth *et al.,* 1997). Placement of an amalgam restoration appears to present little risk to the patient. However, polishing and abrading an amalgam restoration should be under high-volume aspiration and moist conditions to avoid a potential risk of neurotoxic damage to both the patient and the operator (Sweeney *et al.,* 2002).

1.3 Mercury forms

The main forms of exposure in the general populations are I-Hg compounds, organic mercury compounds and elemental (or metallic) Hg^0 (Table 1.1) (Dodes, 2001; Houston, 2007; Park and Zheng, 2012; Houston, 2011). Inorganic Hg exists in either the mercuric or mercurous form. It can also purely be absorbed by the gastrointestinal tract, and the half-life of I-Hg is about 40 days (Rice *et al.,* 2014). Inorganic mercury is contained in beauty products and food (McKelvey *et al.,* 2011). Methyl mercury (MeHg) is prevalent in seafood (Choi *et al.,* 2015; Rice *et al.,* 2014) and rice (Li *et al.,* 2015). Metallic Hg is a liquid metal at room temperature that can combine with other metals to form amalgams, due to its low melting point (Martín-Yerga *et al.,* 2013; Zwicker *et al.,* 2014). It is contained in dental amalgam fillings (Björkman *et al.,* 2007; Barregard *et al.,* 2008; Park and Zheng, 2012) and in several other domestic and industrial applications. It is not well absorbed upon ingestion, but is well absorbed upon inhalation (Clarkson *et al.,* 2003; Rooney, 2007). Mercury can be absorbed in tissues of individuals who have been exposed to it, either through consuming seafood such as fish or shellfish (Di Leo *et al.,* 2010; Groth, 2010; Mahaffey *et al.,* 2009; Rice *et al.,* 2014; Li *et al.,* 2015), water (Zhang *et al.,* 2011), leaching from amalgam dental fillings, occupational exposure (Danscher *et al.,* 1994; Li *et al.,* 2015), dissolution in saliva or swallowed as amalgam particles (Dodes, 2001; Fakour *et al.,* 2010). Fish consumption is the main source of human exposure to MeHg (Li *et al.,* 2011; Li *et al.,* 2015), with shark and tuna having the highest concentrations of MeHg (Ouédraogo and Amyot, 2011). The half-life of MeHg is 39 to 70 days depending on the body burden (Dodes, 2001; Rice *et al.,* 2014). Following

ingestion of MeHg, about 85%, 5% and 10% is absorbed in the gastrointestinal tract, blood and brain respectively (Genchi et al., 2017).

Table 1.1: Mercury Types (Houston, 2007 and 2011)

1. Elemental	Mercury vapor (Hg^0), a stable monoatomic gas	Dental amalgams
2. Inorganic	Divalent mercury (Hg^{+2})	Toxic species in human tissue after conversion
3. Organic	Methyl mercury (CH_3Hg^+) Ethyl mercury ($CH_3CH_3Hg^+$)	Fish, sea mammals Thimersol vaccines

During the functional life of an amalgam filling, Hg^0 can be released in the mouth as a vapour or a salt dissolved in saliva. The latter is enhanced by chewing during eating (Derouen *et al.,* 2006; Clarkson, 2002; Mackey *et al.*, 2014), brushing (Dodes, 2001; Mackey *et al.,* 2014), drinking of hot fluids such as tea, low pH of saliva, biological corrosion due to bacteria, electrochemical corrosion (Fakour *et al.,* 2010), nocturnal bruxism, and consumer teeth whitening products (Dye *et al.,* 2005; Mackey *et al.,* 2014). Amalgam corrosion is an oxidation-reduction response in which the metals in the amalgam respond with non-metallic elements to create chemical complexes (Dodes, 2001). Corrosion is a principal factor in determining the amount of Hg^0 that is released into the mouth. It is influenced by factors that disrupt the surface of the fillings such as chewing and brushing teeth, which can cause a rise in Hg^0 release. Moreover, the corrosion is a complex process and decreases the baseline release of Hg^0. The Hg released can be in two forms: mercury vapour or mercuric ions, which can be inhaled or exhaled and passed into the saliva to enter the gastrointestinal tract (Dodes, 2001). The amount released directly depends on the amount of amalgam fillings and their total surface area

(Berglund, 1990; Dodes, 2001; Björnberg *et al.*, 2006). The daily dose of Hg^0 from dental amalgam for the average individual is low, ranging between 1.2 µg (inhaled Hg) and 1.5µg (ingested Hg) (Mackert, 1987), or 1 to 2 µg/day for subjects with more than eight amalgam restorations as determined by other researchers (Dodes, 2001). Nevertheless, low doses can accumulate within the body over time (Houston, 2011), which can result in high metal concentrations and serious health problems (Martín-Yerga *et al.*, 2013). However, the allowable amount or safe intake of Hg from food or non-dental sources has recently been reduced to <0.1 µg/kg/day body weight (Clarkson *et al.*, 2003; Houston, 2011). There are concerns about amalgam fillings, but there is no current scientific evidence that the Hg^0 released from amalgam restorations may cause serious health risks in humans (Järup, 2003; Hansen *et al.*, 2004; Barregard, 2005; Rooney, 2007), except for rare hypersensitivity reactions (Cawson and Odell, 2008). Nonetheless, a general consensus exists about the effect of dental amalgams due to the many factors associated with urinary Hg (Castaño *et al.*, 2012). Additionally, chronic degenerative diseases of the nervous system such as Parkinson's disease and Alzheimer's disease can be caused or exacerbated by Hg^0 released from amalgam fillings (Clarkson, 2002).

Dental amalgams are the most common sources of elemental Hg^0 vapour, which is a stable monatomic gas (Lorscheider *et al.*, 1995; Goyer and Clarkson, 1996; Clarkson, 2002; Houston, 2007; Houston, 2011). Elemental Hg^0 converts to a vapor at room temperature due to its low latent heat of evaporation and its relative absence from ambient air (Rice *et al.*, 2014). It is well absorbed by the lungs and subsequently enters the bloodstream (Clarkson *et al.*, 2003; Rooney, 2007). Approximately 30-50 % of the produced Hg^0 vapour is inhaled, 80% absorbed by the lungs and distributed to the kidneys

and brain, also up to 40% of the absorbed Hg^0 being removed in 30 days after vapour exposure (Dye *et al.,* 2005). Moreover, Hg^0 accumulates in the brain and the kidneys and is excreted in the urine, bile and faeces (Dodes, 2001). I-Hg is a divalent compound, it is a toxic species found in the human body after transformation from the other formulas (Houston, 2007; Houston, 2011). It can be readily transformed into MeHg in aquatic environments (McCall *et al.,* 2000), as certain bacteria in seawater are capable of converting Hg^0 into MeHg (Dodes, 2001). Furthermore, Hg^0 can convert to methyl mercury in the human gastrointestinal tract by certain bacteria (Dodes, 2001).

Mercury is considered the most dangerous of all the heavy metals (Salonen *et al.,* 1995; Woods *et al.,* 2005; Houston, 2011; Afkhami *et al.,* 2012) and its toxicity depends on its chemical forms (Li *et al.,* 2015; Rice *et al.,* 2014). Organic mercury compounds generally being more toxic than inorganic species (Leopold *et al.,* 2010; Li *et al.,* 2011). The Environmental Protection Agency determined that the safe daily intake of Hg to be less than 0.1 μg/kg/day (Houston, 2007). In a previous report, the Environmental Protection Agency (EPA) estimated the total consumption for all forms of Hg to be 5.8 μg/day which is in variance to a report by Clarkson and colleagues estimating it to be 2.3 μg/day (Dodes, 2001). Historically, dental amalgams have been the major source of human exposure (Keating *et al.,* 1997; Guallar *et al.,* 2002; Barregard, 2005; Li *et al.,* 2015) and it is estimated that one dental amalgam filling releases about 3-17 μg Hg/day (Houston, 2011). Moreover, it is estimated that urinary Hg concentrations will increase by 1.8 μg/L for every 10 amalgam filling surfaces (Dye *et al.,* 2005). Nonetheless, concentrations of urinary Hg in individuals with amalgam fillings is about 2 to 4 μg/L which is well below

concentrations found in individuals who are occupationally exposed to Hg (20 to 50 μg/L) (Clarkson *et al.,* 2003).

1.4 Determination of mercury in the human body

Mercury concentrations can be determined in saliva, hair (Fakour *et al.*, 2010), blood samples (Björkman *et al.*, 2007; Park and Zheng, 2012; Martín-Yerga *et al.*, 2013), urine (Černá *et al.,* 2012; Li *et al.,* 2011; Martín-Yerga *et al.,* 2013), breast milk, nail, adipose tissues, and various organs (Xiaojie *et al.,* 2008; Rice *et al.,* 2014). Blood Hg is a useful biomarker after short-term and high-level exposure (Park and Zheng, 2012). Hg can be rapidly removed from the blood, redistributed and sequestered in different tissues (Rice *et al.,* 2014). Nevertheless, urine Hg is the best biomarker for long-term exposure to both elemental and inorganic mercury (Dodes, 2001; Park and Zheng, 2012). Furthermore, Hg analysis in urine is suitable to estimate mercury exposure due to amalgam fillings (Pesch *et al.,* 2002).

Stone *et al.,* (2002) carried out an analysis of the Hg^0 content of different brands of dental amalgam which resulted in the classification of brands into three groups namely: A (Dispersalloy regular set), B (Valliant PhD, Optaloy II, Megalloy and Valliant Snap Set) and C (Tytin regular set double-spill, Tytin FC, Contour, Sybraloy and Tytin regular set single-spill) respectively, in order of decreasing Hg^0 content as shown in Table 1.2. Results indicated that group A, retained the most amount of Hg^0 when compared to groups B and C respectively. Toxicity characteristic leaching procedure (TCLP) analysis of the triturated capsules showed leached Hg^0 at > 0.2 mg/L, which is above the Resource Conservation and Recovery Act (RCRA) limit. This showed that the amount of Hg^0 lost

from the amalgam capsules can be absorbed into the human body through inhaling, swallowing the saliva or through the dental pulp after the dentist places the fillings in the patient's teeth (Fakour *et al.,* 2010). At current RCRA limits, the leaching of Hg^0 from amalgam fillings is not a problem (Stone *et al.,* 2002).

Table 1.2: Groups of mercury capsules (Stone *et al.,* 2002)

Groups	Brands of amalgam capsules	Mercury levels
Group A	Dispersalloy regular set	The most amount of Hg (1.225 mg/capsule)
Group B	Valliant PhD, Optaloy II, Megalloy and Valliant Snap Set	The next highest amount of Hg (0.534-0.770 mg/capsule)
Group C	Tytin regular set double-spill, Tytin FC, Contour, Sybraloy and Tytin regular set single-spill	The least amount of Hg (0.125-0.266 mg/capsule)

1.5 Mercury leaching

Exposure to Hg^0 from amalgam fillings, with potential negative health effects, has commonly been measured to happen either by erosion or evaporation directly from the surface of the filling, followed by ingestion. X-ray fluorescence imaging has been used quantitatively to determine the spatial distribution of Hg^0 and Zn in sections of human teeth that had been filled with amalgam for more than 20 years. The results showed a significant leaching of Hg^0 through the dentine and into the pulp, and migration of Hg^0 through the dentinal tubules of the tooth from where it could enter the blood supply through the pulp, while Zn was evenly distributed (Harris *et al.,* 2008).

1.6 Effect of mercury on human body

Mercury from dental amalgam may be associated with multiple sclerosis (MS). It has been shown that subjects suffering from MS with amalgam fillings have significantly lower levels of red blood cells, hemoglobin and hematocrit compared to MS subjects without amalgam fillings (Siblerud and Kienholz, 1994). In addition, evidence from epidemiological and clinical studies suggest that people with high levels of blood, hair, urine and toenail Hg have an increased risk of cardiovascular diseases (Houston, 2011; Yorifuji *et al.,* 2010).

Guidelines suggesting in several European countries that pregnant women not receive Hg^0-containing dental amalgam fillings, due to a concern raised by many studies that Hg exposure may lead to a decreased birth weight of the newborn infant. A study was carried out in Washington State between 1993 and 2000, to investigate whether placement of Hg^0-containing fillings during pregnancy increased the number of low-birth-weight babies. This study found no evidence that Hg^0-containing dental fillings placed during pregnancy increased the risk of low-birth-weight babies (Hujoel *et al.*, 2005). In another study by Salehi and Esmaili-Sari (2010), 149 pregnant women were invited to participate in a study and provided information about their personal data and the number of dental amalgam fillings was recorded. Samples of hair were taken from these women and treated then analyzed. The results indicated that the geometric mean and range of hair with a total Hg (T-Hg) concentration was 3.52 µg/g (0.44-56 µg/g). About 5.4% of mothers had hair T-Hg levels in excess of 10 µg/g. The maternal hair Hg level was less than the threshold level of WHO (5 µg/g). Women who consumed fish several times per week, had the highest mean hair Hg level of 4.93 µg/g. The results suggested that women needed to

decrease their fish consumption during pregnancy. This finding confirmed that fresh tuna or canned tuna have high concentrations of Hg (Maria *et al.*, 2010).

Exposure to MeHg and Hg^0 was investigated in pregnant women and their newborns in Stockholm and the women were followed for 15 months post-delivery. MeHg, I-Hg, and T-Hg in maternal and cord blood were determined by automated alkaline reduction and cold vapor atomic fluorescence spectrometry. Total Hg in urine was determined by inductively coupled plasma mass spectrometry. Results showed that blood and urine levels of Hg were associated with the number of amalgam fillings (Vahter *et al.*, 2000). Moreover, the levels of Hg and Zn were determined in children's teeth aged 4 to 8 years. Children with autism had significantly higher levels of Hg and similar levels of Zn due to usage of oral antibiotics during their first year of life. They had a higher body burden of Hg during fetal/infant development. Thus, higher use of oral antibiotics may have reduced their ability to excrete Hg, and hence may partially explain the higher levels of Hg found in the baby teeth (Adams *et al.*, 2007).

Urinary biomarkers of kidney integrity were examined by Geier *et al.* in 2013, from a completed clinical trial (parent study), for children of 8 to18 years of age, with and without amalgam fillings. The results suggested that dental amalgam fillings contribute to ongoing kidney damage at the level of the proximal tubules (PTs) in a dose-dependent fashion.

A study was aimed to evaluate a new analytical method for ethyl and MeHg in hair samples of breastfed infants who received the recommended schedule of thimerosal-containing vaccines (TCV). Samples were weighed and leached with an acidic thiourea solution. Leachates were concentrated in a polymeric resin prior to analysis by Hg-thiourea liquid chromatograph/cold vapor atomic fluorescence spectrometry. Results showed a statistically significant difference between hair-Ethyl-Hg concentrations and the time elapsed after the last TCV shot (Dórea *et al.,* 2011).

Fakour *et al.,* in 2010 determined the relationship between Hg concentration in hair and saliva in Iranian women with amalgam fillings and established a relationship with age and the number of amalgam fillings. Furthermore, a significant correlation was also observed between the Hg level in saliva and hair with the number of amalgam fillings. The results showed that amalgam fillings may be an effective source for high Hg concentration in hair and releasing Hg into saliva samples.

Samples of blood were collected, as well as brain, pituitary and thyroid gland, abdominal muscle and toenails during the autopsy of 30 deceased individuals. Mercury in blood and brain cortex was determined by cold vapor atomic fluorescence spectrometry and Hg in the other tissues was determined by sector field ICP-MS. The T-Hg in the thyroid and pituitary glands was associated with the number of dental amalgam fillings present. The I-Hg concentrations in the brain also increased with the number of amalgam fillings surfaces (Björkman *et al.,* 2007).

1.7 Sensitivity to mercury

Several reports have shown the presence of an oral lichenoid lesion (OLL) or a toxic reaction on the buccal mucosa. It was confirmed that the presence of an OLL generally represents a type IV hypersensitivity reaction (Pang and Freeman, 1995; Wong and Freeman 2003; Cawson and Odell, 2008). Amalgam fillings may, in rare instances, cause local side effects or allergic reactions such as OLLs. Mercury salts that accumulate in healthy and damaged oral mucosa will cause this hypersensitivity reaction with resulting reticular white patches, papules and erosions or ulceration similar to that found in oral lichen planus (OLP) – hence the terminology lichenin (Bolewska *et al.,* 1990; Fakour *et al.,* 2010).

The use of buccal swabs has many advantages such as cost effective processing for long-term archiving, postage and easy to obtain ability from widely dispersed participants. Buccal swabs are also comfortable for the patient and tasteless (Wijer *et al.,* 2009). Woods *et al* (2005) collected urine and buccal cell samples (inside cheek scrapes) from dentists and dental assistants during scheduled visits to each participating dental office. On the day of collection, the urine sample was aliquoted and frozen separately to analyze the Hg concentrations. Aliquots for Hg analysis were acidified with 1N HCl by using cold vapor spectrofluorometry. Buccal cells were collected by means of a cotton swab and frozen for subsequent DNA extraction. In addition, personal and health information were collected. Results found that urinary porphyrin concentration exceeded the mean of the value in the overall population by more than 4-fold ($p<0.05$). The findings represented the first report of a polymorphism in a human gene that modifies the effect of Hg on a biological process.

1.8 Path of mercury in the human body

Dental amalgam fillings release elemental Hg^0 vapour in the mouth, resulting in an elevated concentration of Hg^0 in blood plasma, urine (Pizzichini *et al.,* 2003) and brain (Olstad *et al.*, 1987; Björkman *et al.,* 2007). It is also a major source of human exposure to I-Hg. The exposure from dental amalgam occurs mainly by inhalation of Hg^0 evaporation from the fillings (WHO, 1991; Li *et al.,* 2015). The central nervous system and the kidneys are the primary organs affected by Hg poisoning. However, most (about 80%) of Hg^0 enters the bloodstream directly through the lungs, and rapidly moves to the other parts of the body (Risher, 2003; Clarkson and Magos, 2006; Rice *et al.*, 2014; Li *et al.,* 2015). Once Hg^0 enters the body, it can stay for weeks or months and it is easily converted to the inorganic form and it remains trapped in the brain for a long time (40 years) (Risher and DeWoskin, 1999; Rice *et al.*, 2014).

The I-Hg in the U-Hg levels are widely used for screening (Barregard, 1993). Thus, urine is the first indicator to determine whether Hg is absorbed in the human body as a result of dental amalgam or not (Li *et al.*, 2015; Guzzi and Pigatto, 2008). The half-life of Hg in urine is about 2 months (Rice *et al.,* 2014; Li *et al.,* 2015; Martín-Yerga *et al.,* 2013). Most of the Hg^0 absorbed into the body eventually leaves in the urine and feces, while smaller amounts leave the body through exhalation (Risher and DeWoskin, 1999). Woods *et al* (2007) and Dye *et al* (2005) confirmed that dental amalgam is a major determinant for U-Hg. Barregard in 2005 deduced that an increased Hg uptake can easily be shown in urine samples. In addition, urine is one of the most interesting samples that is used to determine Hg concentrations for the body burden analysis (Hansen *et al.,* 2004).

1.9 Hg in urine, hair and saliva

Pesch *et al* (2002) measured Hg levels in urine, hair and saliva of 245 German children (8-10 years old). A basic medical examination was done, as well as a checkup by a dentist to determine the dental status (number of amalgam fillings, condition and age of the amalgam restorations and the number of amalgam filling surfaces). AAS and sodium borohydride were used to determine all forms of the Hg. The results showed that urine is suitable to estimate Hg exposure due to amalgam fillings. Mercury levels increased with the number of amalgam fillings and the number of amalgam filling surfaces but there was no significant difference. There was a weak correlation between the Hg levels in hair and urine. Saliva was not a suitable sample to monitor the Hg burden at low levels of exposure. Results showed that levels of Hg found in urine did not reveal health risks due to exposure to Hg when compared with human biological monitoring (HBM) values.

1.10 Effect of Hg on reactive oxygen species (ROS)

Mercury-induced oxidative damage has been demonstrated observed *in vitro* and *in vivo*. It induced a redox imbalance probably caused by either an increase in the generation of reactive oxygen species (ROS) or by a reduction in the antioxidant defense capacity (Lund *et al.,* 1993), as confirmed in cortex, kidneys and liver of mice exposed to Hg (Goring et al., 2002; Hussain *et al.,* 1999). Moreover, Hg can bind to glutathione (Gatti *et al.,* 2004), which plays a critical role in regenerating vitamins E and C from their oxidized byproducts (Alissa and Ferns, 2011). Furthermore, glutathione-Hg complexes appear to be the main form in which it is transported and removed from the body, thereby reducing cellular defenses against oxidation (Alissa and Ferns, 2011). Naturally, Hg

interacts with most sulfhydryl (SH) groups and produces ROS such as the superoxide anion, hydrogen peroxide and hydroxyl radical, which induce oxidative damage in tissues (Pizzichini *et al.*, 2003).

Metals such as Hg deplete glutathione, and protein bound SH groups, resulting in lesser amount of antioxidants counteracting the activity of ROS (Stohs and Bagchi, 1995). As a result, enhanced lipid peroxidation, DNA damage, altered calcium and SH homeostasis occur. Various studies have suggested that the ability to generate ROS by redox cycling quinones and related compounds may require metal ions. In addition, some mechanisms associated with the toxicities of metal ions increase the production of ROS and oxidative tissue damage (Stohs and Bagchi, 1995). Mercury may bind to a variety of enzyme systems including those of mitochondria and microsomes, producing cell death or nonspecific cell injury (Goyer and Clarkson, 1996).

1.11 Mercury and the essential metals

Mercury is an example of a toxic metal that is not essential in the human body (Park and Zheng, 2012). However, the toxic effect of Hg may be mediated or enhanced by interactions or deficiencies of nutritionally essential metals (Goyer, 1995). Essentially, the interaction of toxic metals with essential metals happens when the chemistry of a toxic metal is similar to that of the essential element (Goyer, 1995). Mercury may interact metabolically with nutritionally essential metals such as selenium. Moreover, nutritionally essential metals modify health risks from exposure to non-essential metals, and its basic role is to provide some component of a vital biochemical or enzymatic

reaction. A number of metabolic interactions between non-essential toxic and nutritionally essential metals reduce the health hazard of the toxic metal (Goyer, 1997).

Dufault *et al* (2009) assert that Hg can disrupt metabolic processes and alter neuronal plasticity. Nutritional deficiencies, including deficiencies in the long chain polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid, the trace minerals such as Zn and the amino acid methionine, have been shown to influence neuronal function and produce defects in neuronal plasticity. Mercury exposure has been shown to alter neuronal function and increase oxidative stress among children with autism (Hansen *et al.,* 2004; Dufault *et al.,* 2009). Its consumption can also lead to Zn loss or deficiency (Quig, 1998), whereas dietary Zn is essential for maintaining the metabolic processes required for Hg elimination (Dufault *et al.*, 2009).

The sulfhydryl-reactive metals (mercury and other toxic metals) are particularly dangerous and can affect a vast array of biochemical and nutritional processes (Quig, 1998; Rice *et al.,* 2014). The metals also inhibit antioxidative enzymes and deplete intracellular glutathione. They also have the ability to interrupt the metabolism and biological activities of several proteins due to their high affinity for free SH groups (Haley, 2007; Agrawal, 2012). Metal exposure affects the essential element status, which can further decrease antioxidation and detoxification processes (Quig, 1998). Furthermore, the SH groups have no metabolic function and their accumulation in the body has serious adverse health effects. On the other hand, enhancement of nutritional status by means of appropriate nutritional support can minimize the daily accumulation and enhance the excretion of toxic metals (Quig, 1998).

Essential element metabolism is also directly affected by the toxic metal burden (Quig, 1998). Mercury is thought to compete with iron for binding to hemoglobin which can result in impaired hemoglobin formation and cause anemia (Pyszel *et al.,* 2005; Rice *et al.*, 2014). Moreover, Hg induces mitochondrial dysfunction and oxidative stress causing displacement of Fe^{2+} and Cu^{+} ions (Houston, 2011). Also, Hg interferes with progesterone metabolism without affecting serum levels. Furthermore, Hg binds to the free SH group on the progesterone receptor and may thereby reduce progesterone binding and cellular response (Quig, 1998).

Mercury has a high affinity for glutathione (GSH), which is the primary intracellular antioxidant and conjugating agent (Kidd *et al.,* 1997), and a single atom of Hg can bind to and cause the irreversible excretion of up to two GSH tripeptides (Zalups and Lash, 1996). Mercury removes GSH from the cell and also inhibits the activities of two key enzymes involved in GSH metabolism (Quig, 1998) namely: GSH synthetase and GSH reductase (Zalups and Lash, 1996). It also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase (Benov *et al.,* 1990), and possibly GSH peroxidase. The inhibition of GSH peroxidase has been attributed to the formation of a Hg-selenide complex (Cuvin-Aralar and Furness, 1991). Furthermore, SH containing molecules such as cysteine, are emerging as necessary factors within the conveyance and spreading of Hg throughout the body owing to the phenomenon of "Molecular Mimicry" and its role within the molecular transport of Hg (Rooney, 2007).

Mercury is the most important metal that has been studied in the primary biochemical processes disrupted by the SH-reactive metals, and much of what has been learned about the toxic effects of Hg holds true for other SH-reactive metals, due to the similarities in

their chemical reactivity (Quig, 1998). However, Hg is much more volatile than other SH-reactive metals and therefore it is highly absorbed in the elemental (Hg^0) form (Lorscheider *et al.*, 1995; Quig, 1998). The primary sources of chronic low-level Hg exposure are dental amalgam fillings and fish, while the two major highly absorbed subspecies of Hg are elemental Hg^0 and MeHg (Barregard, 2005). Figure 1.1 (Quig, 1998) illustrates the processes of assimilation of these two species of Hg. Hg^0 is poorly absorbed if swallowed, but Hg^0 vapor is well absorbed through the lungs and fast passes the blood-brain barrier. Due to its lipophilic nature, Metallic Hg has a high affinity for myelin and lipid membranes. Once inside a cell, Hg^0 is oxidized by catalase to the highly reactive Hg^{2+3}. Once absorbed, mercury has a low excretion rate. A significant proportion of the assimilated Hg is retained and continually accumulates in the kidneys, neurological tissue (including the brain) and the liver. Upon autopsy, high levels of Hg have also been found in cardiac, thyroid and pituitary tissues of dentists (Nylander *et al.*, 1986).

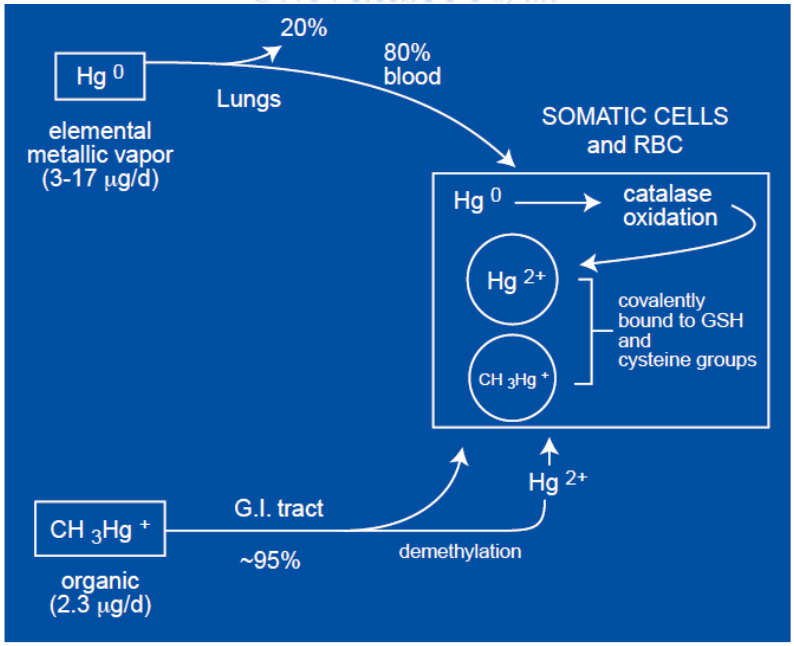

Figure 1.1: Absorption of mercury vapor and organic mercury (Quig, 1998)

Mercury inactivates numerous enzymatic reactions, glutathione, various enzymes, amino acids and sulfur, with following increased oxidative stress and decreased oxidant defense (Salonen *et al.*, 1995; WHO, 1990; Houston, 2011). Mercury's high affinity for -SH, such as glutathione, which comprises much of the antioxidant capacity of plasma, reduces both membrane and plasma antioxidant defense. Mercury also binds to metallothionein and forms a complex in a process that blocks its entry into the mitochondria and triggers the release of Zn. (Rooney, 2007; Quig, 1998). Furthermore, it induces mitochondrial dysfunction resulting in reduction in adenosine triphosphate, depletion of glutathione, and increased lipid peroxidation (Houston, 2011). It has also been shown to induce oxidative stress, which may result in alterations in calcium homeostasis. Additionally, Hg can also increase free radical levels due to its ability to act as a catalyst for Fenton-type reactions (Rice *et al.*, 2014).

1.12 Zinc

Trace elements, such as Zn, are needed in small amounts in the human body (Albergoni and Piccinni, 1983). Zinc has been shown to exert protective effects against mercury toxicity (Rooney, 2007). An adult contains 2–3 g of Zn and about 0.1% or 70 to 130 µg/dL (10.7-19.9 µmol/L) is replenished daily through diet (Maret and Sandstead, 2006) while 60%, 30% and 5% is stored in the skeletal muscle, bone and liver/skin respectively (Kambe *et al.*, 2015). More than 200 metalloenzymes belonging to six major categories including: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases require Zn as a co-factor and a functional component of transcription factor proteins, contributing to gene expression and regulation. Zinc induces the synthesis of metallothionein, which plays a role in regulating the metabolism of Zn including

absorption and storage. It is essential for tissues because it acts as a co-factor for numerous enzymes and proteins (Goyer and Clarkson, 1996), and for the observed biological function of the metalloenzymes (McCall *et al.*, 2000).

Under physiological conditions Zn is not redox active. However, it is a catalytic component of more than 300 enzymes and plays a critical structural role in many proteins, covering all six classes of enzymes (Eide, 1998; McCall *et al.*, 2000). It is also ringed in about 400 enzymes, and over 2000 proteins are believed to contain zinc (Maywald and Rink, 2015). The biological functions of zinc are often associated with proteins. Zinc in proteins can either contribute directly in chemical catalysis or be significant for maintaining protein structure and stability (McCall *et al.*, 2000). It is an essential trace element for all organisms (Maywald and Rink, 2015; McCall *et al.*, 2000), which emphasizes its indispensable role for human health. Furthermore, protein metabolism and optimal nucleic acid, including cell growth, division and function, require sufficient availability of Zn (Vallee and Falchuk, 1993; McCall *et al.*, 2000; Sengupta *et al.*, 2015). Zinc is relatively harmless, but exposure to high doses has toxic effects, making acute Zn intoxication rare (Plum *et al.*, 2010). It is also the second most abundant trace metal with 2-4 grams in organisms and in the human body (Maywald and Rink, 2015); an average 70-kg adult human contains about 2.3 g of Zn, it can also range between 1.5 to 2.5g (McCall *et al.*, 2000; Lee, 2009). In the United States, the recommended daily allowance (RDA) for zinc is 8 mg/day for women and 11 mg/day for men. World Health Organization (WHO) and the European Community recommended values of between 9.4 and 6.5–7.1 mg for women and 10 mg for men are advised. A sufficient daily intake of

Zn is necessary to achieve a steady state for proper immune function, because Zn cannot be stored in the body (Maywald and Rink, 2015).

Zinc also plays a role in DNA synthesis, RNA transcription, cell division and cell activation (John *et al.,* 2010). It is essential in members of all enzyme classes, including over 300 signaling molecules and transcription factors. It is the only metal that is a coenzyme to all enzyme classes. Zinc has also been shown to be important in prokaryotes (Blencowe and Morby, 2003). Furthermore, it also plays an important role in the immune (Maywald and Rink, 2015) and nervous systems, in the optimal metabolism of vitamin A and in the normal calcification of bone (Wu and Chen, 2005). Moreover, Zn deficiency may lead to frequent infections, weight loss, eczema, mental disorders, night blindness, alopecia, hypogonadism, taste perversion, delayed wound healing, and loss of appetite (Ozturk *et al.*, 2014).

A study was done by Roman-Viñas *et al.*, (2010), to review which micronutrients were more accurately assessed with food frequency questionnaire (FFQ) in infants, children and adolescents. An analysis was limited to some minerals, including Zn within the European Micronutrient Recommendation Aligned [EURRECA] Network of Excellence. Three searches were applied for this research, the first one consisted of the identification of articles using Medical Subject Heads (MeSH) terms (nutritional assessment, diet, nutritional status, food intake, validity, validation study, replication study and correlation study). The second stage comprised the evaluation of titles and abstracts by two independent reviewers and applied specific exclusion criteria (studies describing the content of foods in nutrients, additives or contaminants). In stage 3, studies selected from stage 2 that fulfilled the inclusion criteria (i.e. Population group) were analyzed. The

results showed that the articles identified had a calculated quality score ranging from 2.5 to 3.5 for Zn. The mean correlation coefficient weighted by the quality score was 0.56 for Zn and they concluded that the FFQ was a good instrument for estimating intake of minerals in infants, pre-school children and adolescents.

Metals play a key role in the intracellular oxidative balance (Younes-Mhenni *et al.*, 2013). Zinc and some of the other important mineral concentrations in serum of a group of patients were determined. A serum of 3 trace elements, including Zn were investigated in 48 patients with Parkinson's disease (PD a progressive neurodegenerative disease) and matched with 36 controls using plasma Atomic Absorption Spectrometry (AAS). The results showed that the mean Zn in PD patients did not differ significantly from those of controls. Although, Zn participates in the reduction of oxidative stress, but its implication in the onset of PD is not clearly established.

Khalili *et al* (2008) showed in a study of human immunodeficiency virus infected individuals, where the serum level of Zn was measured by graphite furnace atomic absorption, the results showed that the serum level of Zn was significantly lower (p=0. 01).

Whitfield *et al* (2010) estimated effects of personal and socioeconomic characteristics on concentrations of Zn and other minerals in erythrocytes and tested for genetic effects using data from twin pairs in Australia. They used blood samples and determined element concentrations in erythrocytes by ICP-MS. The results of the concentrations of Zn showed substantial correlations with the common genetic effects.

Dickerson *et al* (2011) analyzed the hair of 30 women for Hg and Zn using inductively coupled mass spectrometry. A lock of hair was cut from the nape and blood samples were collected. The hair samples were weighed then washed in acetone to remove external contamination, then left to dry in a vacuum oven at 40ºC overnight. Thereafter, each sample was digested in 0.5ml concentrated nitric acid in a micro-digestion vessel for 24hours, then heated in a microwave. Lastly, it was diluted to a standard volume (5ml) with water incorporating internal standards. A Perkin Elmer Elan ICP-MS was used to analyze the samples of the hair and the blood samples. The results showed that the Zn level in hair correlated positively with oocyte yield after ovarian stimulation ($p < 0.05$), nevertheless, there is no correlation between mercury and Zn in the hair and their corresponding serum levels.

CHAPTER TWO

Materials and methods

2.1 Study Design

This was a quantitative study, it took place at Tygerberg Dental Hospital (Appendix 3). Urinary samples, buccal cells and chewing gum samples were collected. Questionnaires were filled in on the same day of the collection (Appendix 4). Sample collection took three months. Subjects were comprehensively examined by a dentist who counted the number and size of fillings. Questionnaires included questions on the exposure to elemental mercury (how many amalgam fillings were placed or removed), personal mercury exposure from dental amalgam fillings (how many amalgam fillings were present in the mouth), pre-existing diseases, demographics (gender, age, address) and any other factor that may influence their exposure to elemental mercury, such as teeth grinding, gum chewing, hot liquids consumption or smoking. Ethical approval for the study was given by the University of the Western Cape.

2.2 Study Population

Previous studies involved a large number of participants and were carried out over a period of 5 to 10 years. This study included 107 participants. The subjects were 74 females and 33 males, aged between 17 and 60 years. They were recruited from different dental clinics of UWC at Tygerberg Hospital (TBH) in Cape Town. The study included all visitors that have had dental amalgam fillings in their teeth for a period of 1 day to 40 years. Each patient was engaged for over 30 minutes to complete the study. Samples that were collected from the participants included buccal cells, chewing gum and 10 ml urine.

All the tests were carried out at (the Central Analytical Facilities (CAF)), Laboratory of Stellenbosch University, under identical conditions, ensuring comparability of results as shown in appendix 3.

2.3 Inclusion criteria

- The study involved all participants that had at least one or more existing amalgam fillings.
- Participants who had been examined by a dentist.
- Participants who had dental amalgam fillings that ranged from 1day to 40 years in place.

2.4 Exclusion criteria

- Subjects who had serious illnesses or had been ill in the previous month because they might have taken medicines, especially those with kidney diseases as mentioned by Li *et al.,* (2015).
- Participants who were lacking information on their dental restorations or who were edentulous.
- If all surfaces with amalgam fillings were restored with fabricated dental crowns as described by Dye *et al.,* (2005), as over crowned teeth show no measurable Hg release (Hansen *et al.,* 2004).
- Participants who lived in contaminated air near medical incinerators and coal burning power plants as mentioned by Dye *et al.,* (2005).
- Patients that were suffering from mercury poisoning.
- Patients with signs or symptoms of zinc deficiency.

2.5 Study instruments and materials

2.5.1 Trace element analysis procedure

Trace metals were analysed on 7900 ICP-MS from Agilent technologies, using the standard configuration of quartz spray chamber and torch, and Ni-plated sampling and skimmer cones. A 0.4ml/min micromist nebulizer was used to aspirate the sample.

The instrument was optimised for sensitivity and oxide formation before calibration. Instrument parameters were set as follows: (see Table 2.1).

USEPA Methods 6020A and 200.8 guidelines were followed for instrument calibration and data verification protocols. The instrument was calibrated using NIST traceable standards purchased from Inorganic Ventures, and the accuracy of the calibration validated by a separate standard from Merck. A drift monitor standard was analysed after every 12 samples, with internal standard elements added online to correct for drift and matrix differences between samples and standards.

Table 2.1: Instrument: Agilent 7900 ICP-MS

	Value
RF Power (W)	1600
Plasma Mode	HMI
Sample depth (mm)	10
Carrier gas (L/min)	0.68
Dilution gas (L/min)	0.27
Make-up gas (L/min)	0
Robustness (% CeO/Ce)	<1

Cell gas parameters		**Elements**
He flow (ml/min)	4.8	Hg and Zn

Acquisition parameters

Peak mode	1 point
Replicates	3
Integration time (sec)	0.3 - 1

2.5.2 Urine

Determination of urinary Hg concentration was carried out by microwave digestion in a Paar Physica System and by (ICP-MS). During the course of the day a 10ml urine sample was collected from each participant in a 40ml plastic container. Urine samples were immediately put on dry ice and then transported to the main campus (UWC) to be stored at temperatures below −20°C until analysis (Pesch *et al.,* 2002). Samples were 10x diluted in 1% HNO_3 and analysed by ICP-MS under standard conditions. Results were given as µg Hg/L urine.

2.5.3 Amalgam specific chew test (Oral cavity)

Mercury released from dental amalgam fillings was determined using a newly developed amalgam specific chew test. It is generally recommended to quantify oral Hg^0 exposure by measuring Hg^0 concentration in a chewed piece of gum, rather than in the saliva of the patient because of the possibility for selective determination of Hg^0 release from individual fillings. Moreover, it allows direct estimation of the amount of Hg^0 specifically released from amalgam fillings by selective chewing of the gum using a minimum number of teeth. Furthermore, chew tests, no longer exhibit oral Hg^0 exposure after removal of dental amalgam fillings.

All participants were instructed to rinse their mouths with normal water before chewing. One piece of chewing gum (Wrigley's Doublemint chewing gum) was chewed by the patient for 30 minutes, then kept in a 40 ml plastic container and put on dry ice in a cooler box and then transported to the main campus (UWC) to be stored at −20°C until analysis. Each chewing gum sample was weighed before microwave digestion in 6ml HNO_3 + 2ml

H_2O_2. Digestate analysed by ICP-MS and corrected for dilution during digestion. A significant number of tests showed that regular chewing during 30 minutes reduces the mass of the gum from 3.5 g to about 0.9 g; a larger mass is considered a result of insufficient chewing (Hansen *et al.,* 2004). Results were given as micrograms per gram (µg/g).

2.5.4 Buccal cells samples

After the chewing process was complete, using the amalgam specific chew test, a special sterile cotton swab was directly rubbed along the inside of the cheeks for 1 minute. There after the cotton swab was placed in a 50 ml tube and then immediately put on dry ice and transported to the main campus (UWC) to be frozen at a temperature below -20°C for three months and then sent to the laboratory for analysis. The swab containers were filled with 1ml 2% HNO_3 + 2% HCl and left to stand for 2 days with the swab tip submerged. Swabs were removed and analysis of the content was done in the swab containers by ICP-MS. The results were taken as the amount of Hg^0 solubilised in 1ml solution, an absolute value cannot be determined. Results were given as micrograms per liter (µg/L).

2.5.5 Zinc concentration in urine

Total concentration of Zn in urine was determined by using inductively coupled plasma mass spectrometer (ICP-MS). Urine samples were stored at -20°C before analysis. Samples were 10x diluted in 1% HNO_3 and analysed by ICP-MS. The reference values of urinary Zn is 75-530 µg/L (Laboratory of Stellenbosch University, CAF). Results were given as micrograms per liter (µg/L).

2.6 Distribution of condition of amalgam fillings and the daily habits groups

Several factors can have a direct or indirect effect on levels of Hg^0 in the samples of urine, chew test, buccal swab and urinary zinc. These include conditions of amalgam fillings (number of fillings, size of fillings and age of fillings) and the daily habits (consumption of hot liquids, smoking, chewing gum, bruxism and brushing teeth). When we applied the multiple regression analysis looking at the relationship between levels of Hg^0 or Zn and condition of fillings or daily habits, we used the smallest group as a reference group (table 2.6.1 and 2.6.2).

Table 2.6.1: Distribution of condition of amalgam fillings groups.

Variables	Groups	Reference group	Number of participants
Number of fillings	1-3 fillings	1-3 fillings	46
	4-7 fillings		51
	8-12 fillings		10
Size of fillings	Small size	Small fillings	5
	Medium size		15
	Large size		14
	Mixed size		73
Age of fillings	≤1 year	≤1 year	3
	>1year to ≤10 years		39
	>10 years		58
	Mixed age		7

Table 2.6.2: Distribution of condition of daily habits groups.

Variables	Groups	Reference group	Number of participants
Consumption of hot liquids	Not much	No hot liquids consumption	32
	A lot		51
	Little		24
Chewing gum	No	No chewing	27
	Daily		10
	Occasionally		70
Smoking	No	No smoking	68
	≤15 cigars/day		30
	>15 cigars/day		9
Bruxism	Yes	No bruxism	34
	No		73
Brushing teeth	Once/day	Once/day	31
	Twice/day		76

2.7 Validity and reliability

The literature has shown that the methods of assessment used by Woods *et al* (2007) and Pingree *et al* (2001) for urine analysis, Hansen *et al* (2004) for specific chew test and urine and Wijer *et al* (2009) and Woods *et al* (2005) for collecting buccal samples are valid and reliable. However, none of the previous studies had analyzed buccal samples. Thus they were analysed using the same procedures that were used for urine and chewing gum analysis. The method of assessment previously conducted by Lowe *et al* (1997) and Lowe *et al* (2004) for Zn is also valid and reliable.

2.8 Ethical issues

- The study protocol was approved by the institute (UWC) with a reference No: SHD EXEC 2014/19.
- Consent Letter from the Dean at Tygerberg Dental Hospital (Appendix 2).
- The participants were informed about the study, explained in full and showed some photos about the amalgam fillings.
- Consent was obtained from prospective participants (Appendix 1).
- Confidentiality was ensured, the participants' names and contact details were not divulged.
- The participants were allowed to opt out of the study at any time if they felt that they could not continue.
- There were no costs related to the study for the participants.

2.9 Statistical analysis

Statistical analyses were done using IBM SPSS for Windows **(**version 24), Armonk, New York, USA. Test for normality was done using the Shapiro-Wilk test, and visual inspection of Q-Q plots and histograms. Non-parametric analysis was performed for abnormally distributed data. The association between variables were analysed using Spearman or Pearson correlation and multiple linear regression. Mann-Whitney U-test and student t-test were used to test the influence of outliers by including and excluding and then compare the results if any significant difference existed between different groups (males and females). Association between categorical variables were performed using Chi-Square test. Box and whisker plots were used to show the distribution of data.

CHAPTER THREE

Results

In this study, urine samples were collected from 107 participants, of which 74 (69%) were females and 33 (31%) males, aged between 17 to 60 years old. Chewing gum and buccal swab samples were collected from 102 participants, of which 70 (69%) were females and 32 (31%) were males. The difference in the number of samples was due to 5 participants who could not provide all the required samples. After normality tests were performed on all data, it was observed that the data (urinary Hg^0, urinary Zn, buccal swabs Hg^0 and chew test Hg^0) was abnormally distributed, thus requiring non-parametric analysis.

3.1 Effect of gender

Low levels of Hg^0 in females and males were observed in all of the samples (see Table 3.1). For instance, the high limit for Hg^0 in urine is 100 µg/L in healthy subject occupationally exposed as proposed by Deutsche Forschungsgemeinschaft in 2001 (Hansen, 2004). In addition, the findings show higher urinary Hg^0 level in males than females. However, the chew test and buccal swab Hg^0 levels were similar in females and males. No significant difference between males and females was observed ($p>0.05$) in urinary, chew test and buccal swab Hg^0 levels.

Additionally, the findings show that urinary Zn levels were significantly higher ($p=0.05$) in males than females (Table 3.1).

Table 3.1: Differences in urinary Zn (µg/L), urinary Hg^0 (µg/L), buccal swab Hg^0 (µg/L) and chew test Hg^0 (µg/g) between males and females.

Variables	Number of samples	Median (Range)		p-value[a]
		Females	Males	
Urinary Hg^0 (µg/L)	107	0.40 (0.19-4.34)	0.60 (0.40-2.27)	0.60
Urinary Zn (µg/L)	107	395.48 (18.93-2829.93)	665.68 (132.95-2337.55)	0.05*
Chew test Hg^0 (µg/g)	102	2.05 (0.05-16.40)	1.99 (0.05-12964)	0.74
Buccal swabs Hg^0 (µg/L)	102	0.10 (0.03-1639.93)	0.09 (0.01-25.43)	0.39

a= p-value calculated from Mann-Whitney U test. * = significant ($p \leq 0.05$).

No significant difference ($p > 0.05$) was observed between males and females in the number of amalgam fillings, size of fillings and age of fillings (see Table 3.2).

Table 3.2: Association between gender and number, size and age of amalgam fillings.

Variables		Gender		Chi-Square value	p-value
		Females	Males		
Number of fillings	1-3 fillings	28	18	2.76	0.25
	4-7 fillings	39	12		
	8-12 fillings	7	3		
Size of fillings	Small fillings	4	1	5.76	0.12
	Medium fillings	8	7		
	Large fillings	7	7		
	Mixed fillings	55	18		
Age of fillings	≤ 1 year	2	1	0.88	0.83
	>1 year to ≤ 10 years	26	13		
	> 10 years	42	16		
	New and old fillings	4	3		

The findings show a significant association ($p<0.05$) between gender and the use of hot liquids, bruxism and brushing teeth. Also, we found that women consumed much more hot liquids compared to men, and had bruxism and brushed teeth more the men. However, no significant association ($p>0.05$) was observed in gender for chewing gum and smoking variables (Table 3.3).

Table 3.3: Association between the daily habits, namely: hot liquids, chewing gum, smoking, bruxism and brushing teeth and gender.

Variables		**Gender**		**Chi-Square value**	**p-value**
		Females	**Males**		
Consumption of Hot liquids	A lot	37	14	6.39	0.04*
	Little	20	4		
	Not much	17	15		
Chewing gum	Occasionally	52	18	3.21	0.20
	Daily	7	3		
	No chewing	15	12		
Smoking	≤ 15 cigarettes/day	22	8	2.89	0.24
	No smoking	48	20		
	> 15 cigarettes/day	4	5		
Bruxism	Yes	28	6	4.07	0.04*
	No	46	27		
Brushing teeth	Once/day	16	15	9.30	0.01*
	Twice/day	58	18		

*= significant ($p\leq0.05$).

3.2 Concentrations of zinc (Zn) and mercury (Hg^0) in urine as well as Hg^0 in buccal swabs and chewing gum

Low levels of Hg^0 were observed in urine, buccal swab and chew test samples when compared to the recommended standard by Deutsche Forschungsgemeinschaft in 2001 (Hansen, 2004). However, high levels of Hg^0 noticed in two chew test samples of participants and one buccal swab sample. Also high levels of urinary Zn were observed when compared to the urinary Zn reference value (75-530 µg/L) (Table 3.4).

Table 3.4: Summary statistics of urinary Hg^0 (µg/L), chew test Hg^0 (µg/g), buccal swab Hg^0 (µg/L) and urinary Zn (µg/L) (genders combined).

Variables	Number of samples	Median (Range)
Urinary Hg^0 (µg/L)	107	0.40 (0.19-4.34)
Chew test Hg^0 (µg/g)	102	2.03 (0.05-12964.00)
Buccal swabs Hg^0 (µg/L)	102	0.10 (0.01-1639.93)
Urinary Zn (µg/L)	107	502.18 (18.93-2829.93)

3.3 The relationship between levels of Hg^0 in urine, buccal swabs and urinary zinc

3.3.1 Spearman correlation

The findings show a significant relationship ($p=0.02$, $r=0.23$) between urinary Hg^0 and urinary Zn levels. However, there was no significant relationship ($p>0.05$) between Urinary Hg^0 and buccal swab Hg^0 as well as between buccal swab Hg^0 and urinary Zn (Table 3.5).

Table 3.5: Correlation between levels of Hg^0 in urine, buccal swabs and zinc (µg/L).

Variables	Number of samples	Spearman's correlation coefficient	p. value
Urinary Hg^0 / Buccal swabs Hg^0 (µg/L)	102	0.13	0.18
Urinary Hg^0 / Urinary Zn (µg/L)	107	0.23	0.02*
Buccal swabs Hg^0 / Urinary Zn (µg/L)	102	- 0.13	0.19

*= significant**.**

3.3.2 Regression analyses

Multiple linear regression analysis showed a significant association (p=0.01, ß=0.42) between the number of amalgam fillings and level of Hg^0 in the urine (r^2=0.19). A significantly higher urinary Hg^0 concentration was observed in the group that had 4-7 amalgam filling when compared to the reference group that had 1-3 fillings (see Figure 3.1). No significant association (p>0.05) was observed in the group that had 8-12 amalgam fillings when compared to the reference group that had 1-3 fillings (Table 3.6). Also, a significant association was observed between the groups that have had fillings for >1 year up to ≤ 10 years (p=0.02, ß=-0.73), > 10 years (p=0.02, ß=-0.75) and mixed age (p=0.04, ß=-0.36) when compared to the reference group that had fillings for ≤1year (Figure 3.2, Table 3.6). However, amalgam filling sizes did not significantly predict urinary Hg^0 levels (p>0.05, see Table 3.6).

Table 3.6: Multiple regression analysis between urinary Hg^0 (µg/L) and conditions of fillings such as number, size and age of amalgam fillings.

Variables		ß	p. value
Number of fillings	4-7 amalgam fillings	0.42	0.01*
	8-12 amalgam fillings	0.21	0.43
Size of fillings	Medium size	0.03	0.94
	Large size	0.17	0.65
	Mixed size	0.08	0.82
Age of fillings	Fillings >1 year to ≤ 10 Years	-1.05	0.02*
	Fillings > 10 years	-1.05	0.02*
	Mixed age of fillings	-1.02	0.04*

● Dependent variable is urinary Hg^0 (µg/L), reference categorical variables (had 1-3 amalgam fillings, small size and ≤ 1 year fillings). *= significant**.**

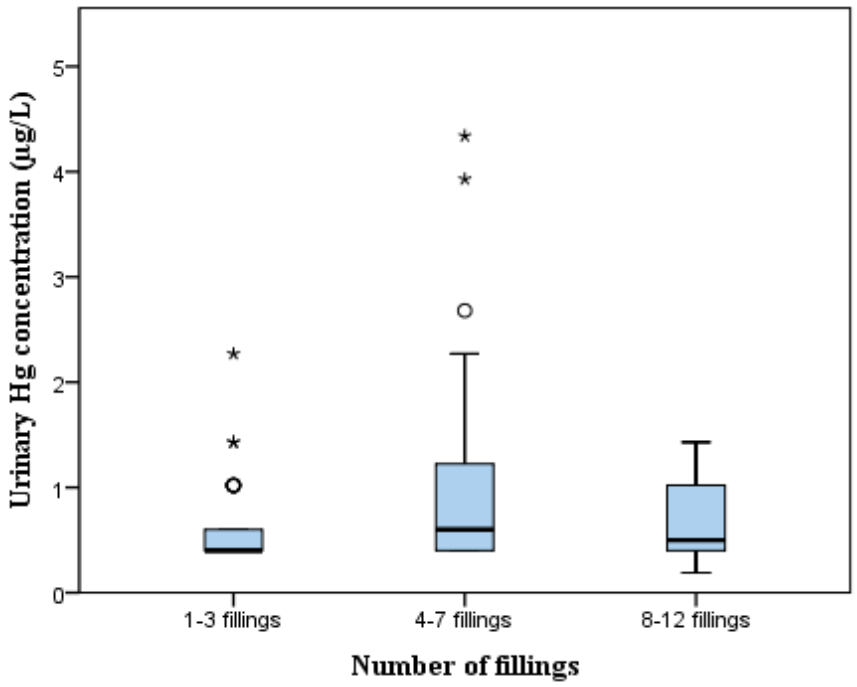

Figure 3.1: Box and whisker plot of urinary Hg^0 (µg/L) in participants who had 1-3, 4-7 and 8-12 dental amalgam fillings. There was a significant association in urinary Hg^0 levels within the groups of number of amalgam fillings, the group with 4-7 amalgam fillings was positively associated with the group that had 1-3 amalgam fillings (p=0.01).

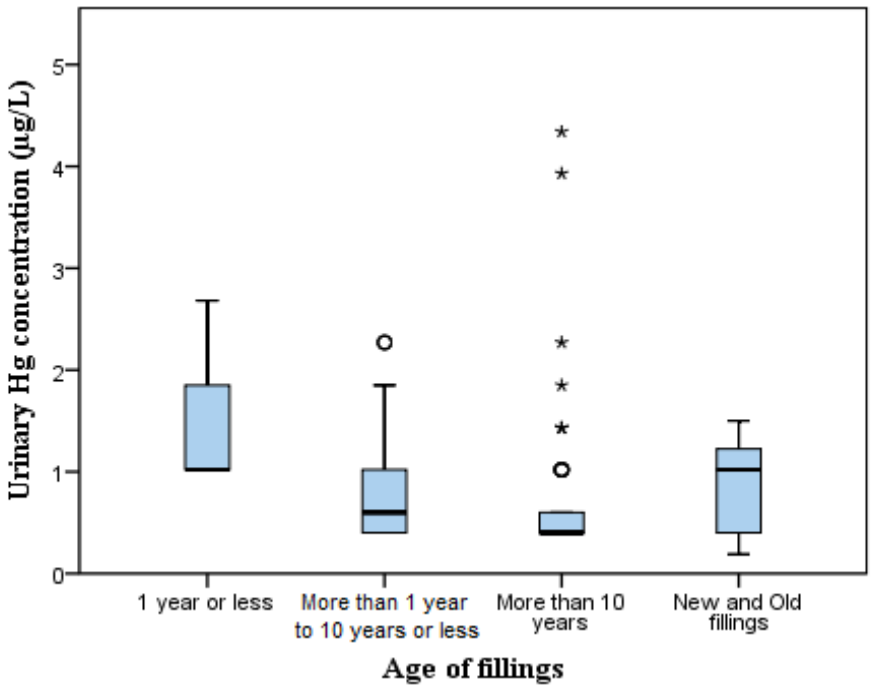

Figure 3.2: Box and whisker plot of urinary Hg^0 (µg/L) in participants who had ≤1 year, >1 year to ≤10 years, >10 years and new and old dental amalgam fillings placed. There was a significant association in urinary Hg^0 levels within the groups of age of amalgam fillings, the group that have had amalgam fillings for >1 year to ≤10 years (p=0.02), >10 years (p=0.02) and new and old fillings (p=0.04) were negatively associated with the group that have had amalgam fillings for ≤1 year.

Multiple regression analysis of daily habits showed that brushing teeth was the only variable that significantly predicted urinary Hg^0 (r^2=0.19). As displayed in Table 3.7, brushing teeth twice a day was negatively associated with brushing teeth once a day (p=0.02, ß=-0.37). The habit of brushing teeth once a day was associated with a higher amount of urinary Hg^0 when compared with brushing teeth twice a day (see Figure 3.3). In addition, further analysis was done between the brushing teeth and number, size and age of fillings, no significant association was found (p>0.05). All other variables (Consumption of hot liquids, chewing gum, smoking and bruxism) did not significantly

predict urinary Hg^0 concentration ($p>0.05$) when compared to the reference variables (did not drink much, did not chew gum, did not smoke and did not have bruxism) (Table 3.7).

Table 3.7: Multiple regression analysis between urinary Hg^0 (µg/L) and the daily habits such as consumption of hot liquids, chewing gum, smoking, bruxism and brushing teeth.

Variables		ß	p. value
Consumption of hot liquids	A lot hot liquids	-0.03	0.89
	Little hot liquids	-0.11	0.63
Chewing gum	Chewed gum daily	-0.03	0.90
	Chewed gum occasionally	0.04	0.83
Smoking	Smoked > 15 cigars/day	-0.28	0.31
	Smoked ≤ 15 cigars/day	-0.16	0.35
Bruxism	Had bruxism	0.02	0.89
Brushing teeth	Brushed twice	-0.37	0.02*

● Dependent variable is urinary Hg^0 (µg/L), reference categorical variables (did not drink much, did not chew gum, did not smoke, did not have bruxism and brushed once a day).

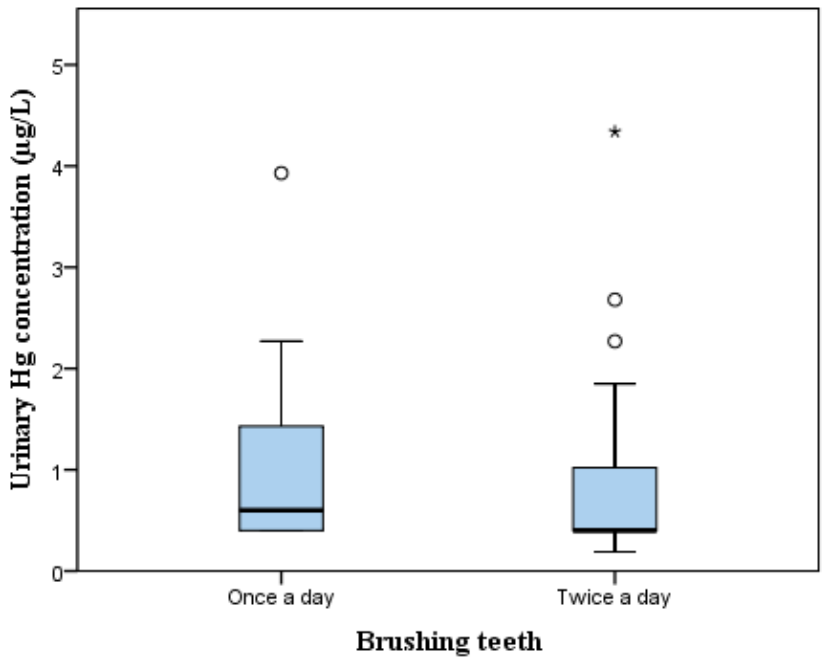

Figure 3.3: Box and whisker plot of urinary Hg^0 in participants who brushed once and twice a day. There was a significant association in urinary Hg^0 levels between the groups of the brushing teeth habit, the group that brushed twice/day was negatively associated with the group that brushed once/day ($p=0.02$).

Additionally, multiple regression analyses showed that number, size and age of fillings did not significantly predict the levels of Hg^0 in buccal swab ($p>0.05$, Table 3.8).

Table 3.8: Multiple regression analysis between buccal swab Hg^0 (µg/L) and conditions of fillings such as number, size and age of amalgam fillings.

Variables		ß	p. value
Number of fillings	4-7 amalgam fillings	33.15	0.40
	8-12 amalgam fillings	-10.60	0.87
Size of fillings	Medium size	-76.132	0.42
	Large size	-5.91	0.95
	Mixed size	-22.19	0.79
Age of fillings	Fillings >1 year to ≤ 10 Years	-38.29	0.72
	Fillings > 10 years	-60.90	0.57
	Mixed age of fillings	-46.44	0.70

● Dependent variable is buccal swab Hg^0 (µg/L), reference categorical variables (had 1-3 amalgam fillings, small size and ≤ 1 year fillings).

Of the daily habits, smoking >15 cigarettes/day was the only variable that significantly predicted Hg^0 in buccal swab after adjusting for hot liquids, chewing gum, bruxism and brushing teeth ($r^2=0.16$). As shown in Table 3.9, the group that smoked >15 cigarettes/day had higher Hg^0 level in the buccal swab samples and significantly predicted level of Hg^0 when compared to the reference group that did not smoke ($p=0.002$, ß=210.47).

Table 3.9: Multiple regression analysis between buccal swab Hg^0 (µg/L) and the daily habits such as hot liquids, chewing gum, smoking, bruxism and brushing teeth.

Variables		ß	p. value
Consumption of hot liquids	A lot hot liquids	48.72	0.32
	Little hot liquids	32.68	0.58
Chewing gum	Chewed gum daily	-50.52	0.47
	Chewed gum occasionally	-3.26	0.94
Smoking	Smoked > 15 cigarettes/day	210.47	0.002*
	Smoked ≤ 15 cigarettes/day	-11.72	0.77
Bruxism	Had bruxism	34.45	0.35
Brushing teeth	Brushed twice	10.62	0.78

● Dependent variable is buccal swab Hg^0 (µg/L), reference categorical variables (did not drink much, did not chew gum, did not smoke, did not have bruxism and brushed once a day).

3.4 The association between urinary Hg^0 and urinary Zn levels

As presented in Table 3.10, the multiple linear regression analyses showed no significant association between urinary Zn and urinary Hg^0, gender, age of participants ($p>0.05$, $r^2=0.04$).

Table 3.10: Multiple regression analysis between levels of Hg^0 (µg/L) and Zn (µg/L) in urine.

Variables	β	p. value
Urinary Hg^0 (µg/L)	73.20	0.35
Females	-133.08	0.26
Age of participants (Years)	-6.43	0.25

● Dependent variable is urinary Zn (µg/L), reference categorical variable (Males).

However, there was a significant association between urinary Zn and the number/size of amalgam fillings (r^2=0.22). The group that had (4-7) amalgam fillings had significantly higher urinary Zn concentration when compared to the reference group that had (1-3) amalgam fillings (p=0.02, ß=297.99, see Figure 3.4 and Table 3.11). When compared to the reference group that had the smallest size of the amalgam fillings, we observed lower urinary Zn levels in the medium size (p=0.01, ß=-737.99) and mixed size (p=0.01, ß=-735.30) of amalgam fillings (see Figure 3.5 and Table 3.11). However, the multiple regression analyses showed no significant association between urinary Zn and age of dental amalgam fillings.

Table 3.11: Multiple regression analysis between urinary Zn and conditions of fillings such as number, size and age of amalgam fillings.

Variables		**ß**	**p. value**
Number of fillings	4-7 amalgam fillings	297.99	0.02*
	8-12 amalgam fillings	145.88	0.49
Size of fillings	Medium size	-737.99	0.01*
	Large size	-329.24	0.26
	Mixed size	-735.30	0.01*
Age of fillings	Fillings >1 year to ≤ 10 years	128.45	0.71
	Fillings > 10 years	-128.02	0.71
	Mixed age of fillings	-221.46	0.57

● Dependent variable is urinary Zn (µg/L), reference categorical variables (had 1-3 amalgam fillings, small size and ≤ 1 year fillings). *= significant.

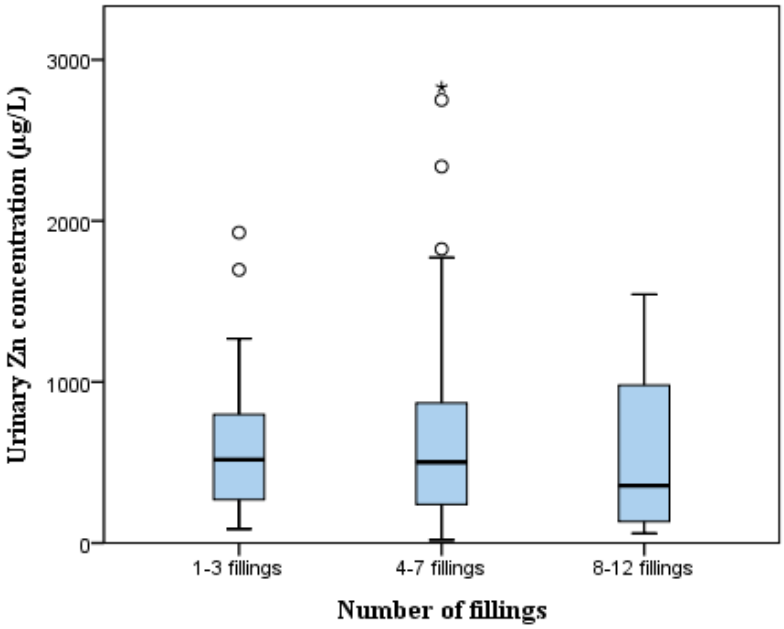

Figure 3.4: Box and whisker plot of urinary Zn (µg/L) in participants who had 1-3, 4-7 and 8-12 dental amalgam fillings. There was a significant association in urinary Zn levels within the groups of number of amalgam fillings, the group with 4-7 amalgam fillings was positively associated with the group that had 1-3 amalgam fillings (p=0.02).

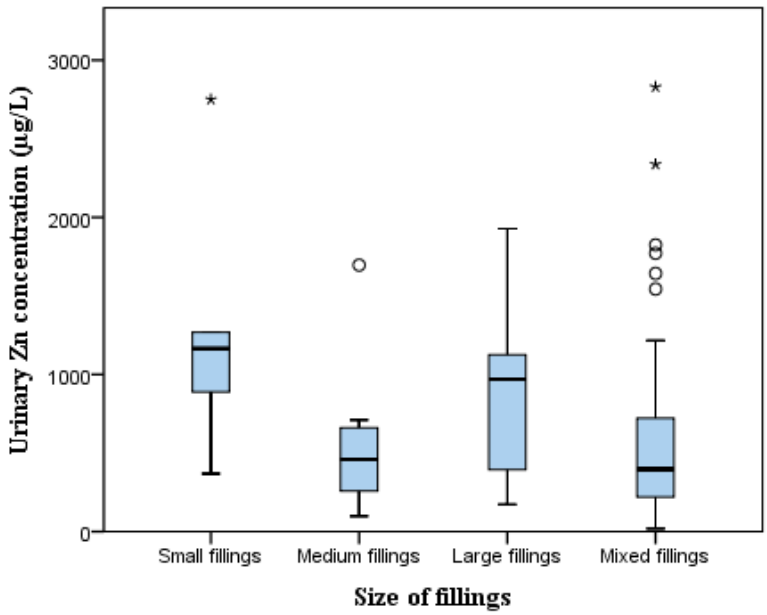

Figure 3.5: Box and whisker plot of urinary Zn (µg/L) in participants who had small, medium, large sizes of dental amalgam fillings. There was a significant association in urinary Zn levels within the groups of amalgam fillings size, the medium and mixed size of amalgam fillings both were negatively associated with the small size of the amalgam fillings (p=0.01).

We observed no significant association between urinary Zn and daily habits such as hot liquids, chewing gum, smoking, bruxism and brushing teeth ($p>0.05$, see Table 3.12).

Table 3.12: Multiple regression analysis between urinary Zn (µg/L) and the daily habits such as hot liquids, chewing gum, smoking, bruxism and brushing teeth.

Variables		ß	p. value
Consumption of hot liquids	A lot hot liquids	25.86	0.87
	Little hot liquids	155.01	0.40
Chewing gum	Chewed gum daily	-50.52	0.47
	Chewed gum occasionally	-3.26	0.94
Smoking	Smoked > 15 cigars/day	191.73	0.38
	Smoked ≤ 15 cigars/day	-62.22	0.63
Bruxism	Had bruxism	-88.23	0.46
Brushing teeth	Brushed twice	-76.05	0.53

● Dependent variable is urinary Zn (µg/L), reference categorical variables (did not drink much, did not chew gum, did not smoke, did not have bruxism and brushed once a day).

3.5 The association between the Hg^0 concentrations in buccal swab or chewing gum and zinc in the urine

Three outliers in the levels of Hg^0 (two values in chew test and one value in buccal swab sample) were taken of the regression analysis because when included, it showed a significant association between urinary Zn and buccal swab Hg^0. In addition, multiple linear regression analyses showed no significant association between levels of Hg^0 in chew test/buccal swab and urinary Zn ($p>0.05$, $r^2 = 0.06$). Furthermore, gender and age of participants showed no significant association with urinary Zn ($p>0.05$, Table 3.13).

Table 3.13: Multiple regression analysis between levels of Hg^0 in the chew test or buccal swab and urinary Zn.

Variables	B	p. value
Chew test Hg^0 (µg/g)	0.08	0.58
Buccal swab Hg^0 (µg/L)	-50.70	0.45
Females	-107.55	0.37
Age of participants (Years)	-9.84	0.09

● Dependent variable is urinary Zn, reference categorical variable (Males).

CHAPTER FOUR

Discussion and conclusion

4.1 Introduction

To our knowledge, this is the first study that evaluates the effect of Hg^0 from dental amalgam fillings on the status of urinary Zn to prove whether Zn had been affected by Hg^0 that was absorbed in the body. The level of Hg^0 in urine, buccal swabs and chew test was also investigated while conditions of the filling and daily habits which may affect Hg^0 leaching were studied.

Elemental Hg^0 is the main type of metal in dental amalgam fillings and represents approximately 50% of the total number of metals found in these fillings (Derouen *et al.*, 2006). It can be combined with other metals such as silver, copper and zinc (Maserejian *et al.*, 2008; Zwicker *et al.*, 2014). It does not have any beneficial function in the human body, and any amount of this toxic element could be harmful (Fakour *et al.*, 2010). The amalgam fillings give off Hg^0 as vapour throughout their existence in the mouth, which can be released by some activities such as chewing, grinding of teeth, and drinking hot liquids (Svendsen *et al.*, 2010). There are anecdotal reports confirmed that Hg at very low concentrations can produce multiple adverse health effects because of its ability to accumulate in the human body (Obiri *et al.*, 2016). Furthermore, several studies have reported that there is a correlation between the number of amalgam fillings present in the teeth and mercury concentration in saliva, blood plasma, urine, brain, liver and kidneys of patients (Hansen *et al.*, 2004).

Mercury is reported to compete and replace Zn and other trace metals such as copper (Quig, 1998). In addition, consumption of Hg can lead to Zn loss or deficiency, whereas dietary Zn is essential for maintaining the metabolic processes required for Hg elimination and all organisms (Dufault *et al*., 2009).

4.2 Concentrations of mercury (Hg^0) in urine, Hg^0 in buccal swabs and chewing gum as well as zinc (Zn) in urine

To estimate the influence of Hg^0 released from dental amalgam fillings, we can compare the data obtained from the urine, the chew test and buccal swabs with other non-occupational sources of Hg exposure. The average Hg concentration in drinking water and ambient air is believed to be 0.5 µg Hg/L, and 10 ng Hg/m^3 respectively, leading to a daily intake of 1 and 0.2 µg Hg, respectively (WHO, 1996). Food is believed to be the most important environmental source of Hg with an extra intake of 2-20 µg Hg/day (WHO, 1996). The provisioned tolerable weekly intake (PTWI) of Hg, established by the WHO in 1972, and confirmed in 1980 and 1988, is 5 µg Hg/kg of body weight (WHO, 1996), which corresponds to 350 µg Hg/week for a subject of 70 kg (Hansen *et al.*, 2004). The high limit of weekly Hg exposure from air, food and drinking water is 148.4 µg Hg/week using the WHO data calculation. Thus, the weekly Hg intake of most patients should be well below the PTWI, unless extremely Hg rich food is consumed on a regular basis (Hansen *et al.*, 2004). For instance, if one individual performs about 30 minutes of chewing/day, a total weekly intake of 358 µg Hg is calculated for a subject with a chew test value of 2.03 µg Hg/g gum (that is, the median value or the 50^{th} centile value). However, few participants had high values of Hg^0 in the chew test, which exceeded the PTWI. Therefore, the results showed that, in most cases, the amount of Hg^0 released from

amalgam fillings determined whether Hg^0 intake is above or below the PTWI. Similarly, the median levels of Hg^0 levels in urine and buccal swabs were below the PTWI. However, Hg^0 levels in buccal swabs and the chew test for most of the samples were significantly below toxic levels except for two participants that had high levels of mercury in the chew test and one participant who had a high level of mercury in buccal swab samples. We believe that might be due to amalgam fillings which were placed in or removed from the teeth in the same day of taking the samples or a day before.

Furthermore, in this study and in line with other findings, maximum urinary Hg^0 concentration was significantly below toxic levels (Hansen *et al.,* 2004). According to the Deutsche Forschungsgemeinschaft in 2001, the maximum limit for healthy subjects occupationally exposed, for Hg^0 in urine is 100 µg/L (that is, about 100 µg/g) (Hansen *et al.,* 2004). The urinary Hg^0 concentration up to the 99th centile lie below 4 µg Hg/g creatinine (4µg/L), which has been defined as the lower limit of environmental exposure (Schiwara *et al.,* 1999) while the exposure limit was considered to be 5 µg Hg/g creatinine (5 µg/L). All of the participants included in this study did not exceed these values (Gerhard et al., 1992; HBM Commission, 1999; Risher, 2003). Therefore, no patients suffering from mercury poisoning were included in this study. This study is in agreement with a previous study that showed no significant association between urinary Hg/24 hours and age or gender of participants (Akerstrom *et al.,* 2017).

Zinc level in a standard human body is 2-3 g, and about 700 to 1300 µg/L is replenished daily through food (Maret and Sandstead, 2006). In this study, the median level was high, but showed the amount of Zn excreted in the urine in line with the reference values of urinary Zn is 75-530 µg/L (the Laboratory of Stellenbosch University, CAF).

Surprisingly, that level of urinary Zn excreted was higher in males than females, which brings to mind that most of the men might have had chronic diseases as most of the males were older than female, however, the difference was not significant. For instance, urinary zinc is positively associated with the risk of developing diabetes and hyperglycemia (Liu *et al.,* 2016). Zn is lost through sweat, hair and nail growth, and skin shedding however, in certain disease states, only 2% of zinc is lost in the urine (Lee, 2009). Trace elements such as zinc, accumulate in hair at concentrations that are generally at least ten times higher than that present in blood, serum and urine. Often the trace element content of the hair reflects the total body trace element status (Ozturk *et al.,* 2014). Additionally, excessive zinc may be lost in the urine, in cases of alcoholism, B-thalassemia, diabetes mellitus, diuretic therapy, nephrotic syndrome, sickle cell anemia, and treatment with parenteral nutrition. Furthermore, severe prolonged diarrhea may lead to significant zinc loss in the stool (Lee, 2009). The present study did not include participants who were ill or had serious illnesses, however, a few individuals reported to be diabetic.

The present study is in agreement with a study by Pfrimer and colleagues demonstrating that urinary Zn concentrations of elderly subjects were not significantly different from that of younger adults (Pfrimer *et al.,* 2014). Besides, an ideal biomarker for zinc status has not yet been determined (Bogale *et al.,* 2015), which makes it difficult to find a relationship between males and females with regards to levels of urinary Zn as demonstrated in our study.

4.3 The relationship between levels of Hg0 in urine, buccal swabs and urinary Zn

Hg^0 can be absorbed in tissues of individuals who have been exposed to it through leaching from amalgam dental fillings (Li *et al.*, 2015), dissolution in saliva or swallowed as amalgam particles (Fakour *et al.,* 2010). However, the ingested Hg^0 from amalgam fillings is insignificant and therefore contributes very little to the total amount of Hg^0 in the human body (Dodes, 2001). In this study, a correlation was found between the levels of urinary Hg^0 and urinary Zn. In other words, the incremental increase of Hg^0 in the urine was associated with an incremental increase in urinary Zn. However, no correlation was found between urinary Hg^0 and the buccal swab Hg^0 as well as between buccal swab Hg^0 and urinary Zn for these participants. Hence, the high concentrations in the buccal swab samples of some subjects in this group might partially be as a result of recent amalgam fillings removal or placement. However, there might be a direct correlation between urinary Hg^0 and urinary Zn and the other tissues in the body but not buccal cells samples, as up to 80% of Hg^0 can be absorbed through the lungs rapidly to the blood and the body organs such as the brain, the kidneys and the liver (Dye *et al.*, 2005). In addition, 7-15% of Hg^0 can be absorbed from the gastrointestinal tract after the oral cavity, and is excreted in the urine, saliva, breast milk, sweat and exhalation (Abass *et al.*, 2018).

This study was in agreement with Hansen *et al* (2004), as we observed no strong correlation between number of fillings and urinary Hg^0 levels. However, in many other studies the number of amalgam fillings correlates with Hg^0 levels in saliva (Krauss *et al.*, 1997), and that the amount of mercury in organs and body liquids is a function of the number and quality of amalgam fillings (Schiwara *et al.*, 1992 and Kleber *et al.*, 1995). However, the level of urinary Hg^0 in our study in the group that had 4-7 fillings showed

a positive association with the reference group that had 1-3 fillings. Additionally, no association was found between the sizes of fillings and urinary Hg^0, although the amount of mercury released from the dental amalgam fillings is dependent on the surface area of each filling (Björnberg *et al.,* 2006). It has been shown that the amount of mercury contained on the surface decreases due to chemical changes (George *et al.,* 2009). However, we found an association between urinary Hg^0 and the time of amalgam fillings placement, which is in line with a study by Woods *et al* (2007). In other words, the urinary Hg^0 level was influenced by the age of fillings (new or old fillings), but not by the number and the size of the fillings. The youngest fillings aged ≤1year had higher levels of Hg^0 than the older fillings and the group with mixed age of fillings had higher Hg^0 than the two groups with fillings that aged >1 year to ≤10 years and >10 years, due to the effect of the new fillings.

People who chewed gum often showed an increased Hg^0 in their urine samples (Barregard, 2005). In addition, Hg^0 can be released in the mouth as a vapour or a salt dissolved in saliva. The latter is enhanced by chewing during eating (Derouen *et al.,* 2006), brushing (Mackey *et al.,* 2014), drinking of hot fluids, electrochemical corrosion (Fakour *et al.,* 2010), nocturnal bruxism, and consumer teeth whitening products (Dye *et al.,* 2005). The results of this study showed no relationship between daily habits (hot liquid consumption, smoking, chewing gum and bruxism) and levels of urinary Hg^0. It is possible that the participants consumed liquids that were warm rather than very hot, thus, there was inadequate heat to release Hg^0 from the surfaces of the fillings. Furthermore, no significant association was observed in the level of urinary Hg^0. This could be attributed to participants chewed the gum softly and for a short period of time, also

possibly due to lack of patience by the participants, as this was noticed during the chew test procedure. Also, it is possible that there was no direct contact point between the amalgam filling surface and the opposite teeth, and the fillings position might be on the mesial or distal side rather than the occlusal surface. Therefore, no friction between the surfaces of the teeth, and this may explain the lack of significant association for the habit of chewing and the bruxism influence. However, there was a significantly negative association in urinary Hg^0 levels between individuals who brushed once and twice a day. Possibly because frequent brushing of filled teeth is linked to higher amounts of Hg^0 which can be released in the mouth and absorbed into the different parts in the body (Dodes, 2001). In this study, the group that brushed teeth once had higher urinary Hg^0, which shows that there was probably another reason affecting the level of Hg^0 in the urine rather than the habit of brushing teeth. Furthermore, the number, size and age of the amalgam fillings were included in our analyses when the effect of brushing teeth was analysed. However we observed no relationship between the fillings conditions and the brushing teeth habit. Individuals who brushed twice a day might have taken care of their teeth and as a result they had less number of amalgam fillings or had older amalgam fillings. Also, the participants may not have reported their brushing habit correctly, thus leading to the under estimation of the actual habit.

Surprisingly, there was no relationship between levels of Hg^0 in the buccal swab samples and condition of amalgam fillings, even though there is a direct contact between the fillings and cells of the cheek in the mouth. The reason could be due to various factors such as food or liquids affecting the level of Hg^0 in the mouth. For instance, Hg^0 is converted to a vapour at room temperature due to its low latent heat of evaporation (Rice

et al., 2014), is inhaled and absorbed by the lungs (Rooney, 2007) and is then distributed to the kidneys and other parts of the body (Dye *et al.,* 2005). From this point, we can conclude that the life of Hg^0 vapour in the mouth is short and can be affected by different factors, thus explaining our finding of no relationship between level of Hg^0 in buccal cells and condition of the amalgam fillings.

Similarly, for the level of Hg^0 in buccal samples and the daily habits, no relationship was observed. Once again, we assume that the participants did not consume very hot fluids and that the action of other habits was moderate and for a short time. The smoking habit showed a positive association with the level of Hg^0 in buccal swab samples, which confirmed that the high heat with excessive smoking led to Hg^0 release. The amount released can be absorbed into the tissues of the mouth, nose and into the body through the food and drinks (Fakour *et al,* 2010; Rooney, 2007).

4.4 The association between Hg^0 and zinc levels in the urine

When data is abnormally distributed (non-parametric), the analysis becomes weak and can result in a difference between correlation and multiple regression analysis, thus only median and range can be discussed for this kind of data. In the present study we had a linear relationship between the amount of urinary Hg^0 and urinary zinc. However, the multiple regression analysis showed no relationship between the levels of the two elements in the urine. The Hg^0 in the urine did not determine or affect the amount of Zn in the urine indicating that urinary Hg^0 is not a good predictor of Zn in the urine. In addition, trace elements such as zinc, accumulate in hair at concentrations that are generally at least ten times higher than those present in blood, serum and urine. It is often believed that the trace element content of the hair reflects the total body trace element

status (Ozturk *et al.*, 2014), thus, the amount of zinc found in the urine samples could be due to the indirect effects of Hg^0 (Quig, 1998) or chronic diseases (Lee, 2009). For instance, Hg is one of the sulfhydryl-reactive metals that can disrupt the structure and function of several essential proteins via direct binding to free sulfhydryl groups (Quig, 1998). Previously research has demonstrated that the interaction between Hg and sulfhydryl groups is destructive (Falconer *et al.*, 1994; Pendergrass *et al.*, 1997). Also, it is believed that the metabolism of zinc is directly affected by toxic metal burden since Hg readily displaces zinc metallothionein, which serves as the intracellular "sink" for this essential element (Quig, 1998).

The level of urinary Zn was in agreement with the level of urinary Hg^0 with respect to the condition of amalgam fillings. The group that had 4-7 fillings predicted and had higher levels of Zn in the urine, which led to a positive association between fillings and levels of urinary Zn in this group. Furthermore, the medium size and mixed size of amalgam fillings negatively predicted level of Zn in the urine and had lower amount of urinary Zn compared to the small size. However, there were fewer large fillings which might explain the lack of the association with regards to urinary Zn level.

4.5 The association between the Hg^0 concentrations in buccal swab or chewing test and urinary Zn

Although Hg^0 vapor is poorly absorbed if swallowed (Quig, 1998), it is well absorbed through the nose mucosa and lungs and rapidly enters the bloodstream and subsequently passes to the other tissues of the body (Li *et al.*, 2015; Rice *et al.*, 2014). It accumulates in the brain and the kidneys and is excreted in the urine, bile and faeces (Dodes, 2001). It

can be oxidised by catalase and hydrogen peroxide into inorganic mercury (Hg^{2+}), which is retained by the brain for years (Clarkson *et al.*, 2003). About 80% of the inhaled Hg^0 is absorbed by the lungs and distributed to the kidneys and brain, and up to 40% of the absorbed Hg^0 can be removed in 30 days after vapour exposure (Dye *et al.*, 2005). This may explain not finding relationships between levels of Hg^0 in buccal swab samples/chew test and urinary Zn level in this study. Hg^0 in these samples is not a good predictor of the Zn amount in the urine, as no correlation was found between the three samples. Thus, it seemed as if urinary Zn level was not affected by the amount of Hg^0 absorbed in the body. Additionally, multiple regression analysis showed no relationship between the chew test and buccal swab Hg^0 and urinary Zn and gender or age of participants.

4.6 Limitations of the study

The limitations of this study is that the participants did not have time to stay for the study after a long visit with their dentist. In addition, some of the participants did not perceive the importance of the study hence did not always want to complete all the tests. There were many difficulties in identifying patients with amalgam fillings, which made the data collection of the study unnecessarily lengthy. Collecting blood samples might have added value to the study. However, blood samples could not be taken due to ethical and financial constraints. Some of the participants could not remember the details of their amalgam fillings accurately. There is very little information on the effect of Hg on zinc as the study is novel in many ways. Moreover, there was only one body fluids was assessed for Hg^0 and Zn. It is suggested that in a future study one should include more body fluid matrixes.

4.7 Conclusion

This study found that there was a correlation between levels of urinary Hg^0 and urinary Zn ($p=0.02$). However, urinary Hg^0 did not predict the amount of urinary Zn. Our findings showed no correlation between levels of Hg^0 in buccal swab samples or chew test and urinary Zn level ($p>0.05$). In addition, there was a significant difference between females and males in the level of urinary zinc. Men had higher level of urinary Zn than females ($p=0.05$). However, there was no significant difference in the level of urinary Hg^0 between males and females. The number of fillings (4-7) and age of fillings were significantly associated with urinary Hg^0 levels ($p<0.05$). In addition, smoking >15 cigarettes a day increased the level of Hg^0 in buccal swab samples ($p=0.002$). We were not able to demonstrate a causal effect of Hg^0 leaching on urinary zinc levels.

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APPENDICES

APPENDIX 1

Information and consent of participants

I, (Name…….) have been informed about the study entitled mercury leaching from dental amalgam fillings and its association with urinary zinc, by Afaf Zanager.

I understand the purpose and procedures of the study.

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at cell phone number (0744065099) or via e-mail 3481183@myuwc.ac.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact: The Dean of Dentistry.

Francie van Zyl Drive

Private Bag Xl

Tygerberg 7505

Cape Town, SOUTH AFRICA

_____ _____

Signature of Participant Date References:

Bylae 1.1

Ingeligte Toestemming

Hiermee bevestig ek, (Naam…….), dat ek ten volle ingelig was oor die navorsingstudie, genaamd: *Kwik uitloging van tandheelkundige amalgaamvulsels en die assosiasie met urinêre sink",* deur Afaf Zanager

Ek verstaan die doel en prosedures van bogenoemde studie.

Ek is 'n geleentheid gegee om vrae oor die studie te vra en is tevrede met die antwoorde.

Ek verklaar dat my deelname aan hierdie studie heeltemal vrywillig is en dat ek te eniger tyd kan onttrek sonder dat enige behandeling of sorg, wat ek gewoonlik geregtig op sou wees, beinvloed sou wees.

Ek verstaan dat ek die navorser enige tyd kan kontak as ek enige verdere vrae / knelpunte of navrae het, wat verband hou met die studie. Selfoonnommer: **074 4065 099** of per e-pos: **3481183@myuwc.ac.za**

As ek enige vrae of kommentaar oor my regte as 'n studie deelnemer het, of as ek bekommerd is oor 'n aspek van die studie of die navorsers kan ek kontak:

Dekaan: Fakulteit Tandheelkunde
Tygerbergkampus
Francie van Zyl Rylaan
Privaatsak X1
Tygerberg 7505
Kaapstad, Suid-Afrika

_____ _____

Vrywillige deelnemer **Datum**

APPENDIX 2

Information and consent letter from the dean at Tygerberg dental Hospital

The Dean of dentistry department in

Tygerberg Hospital in Cape Town

Dear Sir

Re: Request for permission to conduct research at Tygerberg hospital TH

I am a Full-time MSc student in the Department of Medical Biosciences at the University of Western Cape. To fulfill the requirements of the degree, I am undertaking research into mercury leaching from dental amalgam fillings and its association with urinary zinc. This research study will take place over the period of 1 month. Therefore, I believe that your hospital would be a suitable place to conduct my study.

Data for this one-month quantitative action research project will be collected through post-samples results, examination, and interviews. Research participants will be asked for their permission for the data collection.

I undertake that my study will cause no harm to the hospital or any of the staff or patients. None of the participants will be asked to pay any money. I also ask you to allow me to use the instruments that TH using by the dentists help.

I would be very grateful if you could allow me to conduct this research at your hospital.

If you require any information about this study or any other questions regarding your rights as the place where the study will be conducted, please do not hesitate to ask.

Yours sincerely

Afaf Zanager

0744065099

3481183@myuwc.ac.za

I have read the above information regarding this research study on effect of mercury from dental amalgam on the metabolic function of zinc, and allow Afaf Zanager to conduct her study at TH and to use the instruments that TH is using by the dentists help.

_____ (Name)

_____ (Signature)

_____ (Date)

APPENDIX 3

Structural work to collect samples at Dentistry of Tygerberg Hospital (TBH)

03/05/2016

Time

Work time was from 6 am to 6 pm and 9 am to 2 pm every day

1- Study explanation and dental examination were done for every person according to the study criteria as following:

a- How many amalgam fillings and surfaces.
b- How old are the amalgam fillings.
c- How old is the patient.
d- Does the patient have a chronic disease or taking any medicine or not.

2- After the examination, patient were informed about the study then sent to the next clinic to sign the consent letter, fill in the questionnaire and to do the interview to collect more information about the participants.

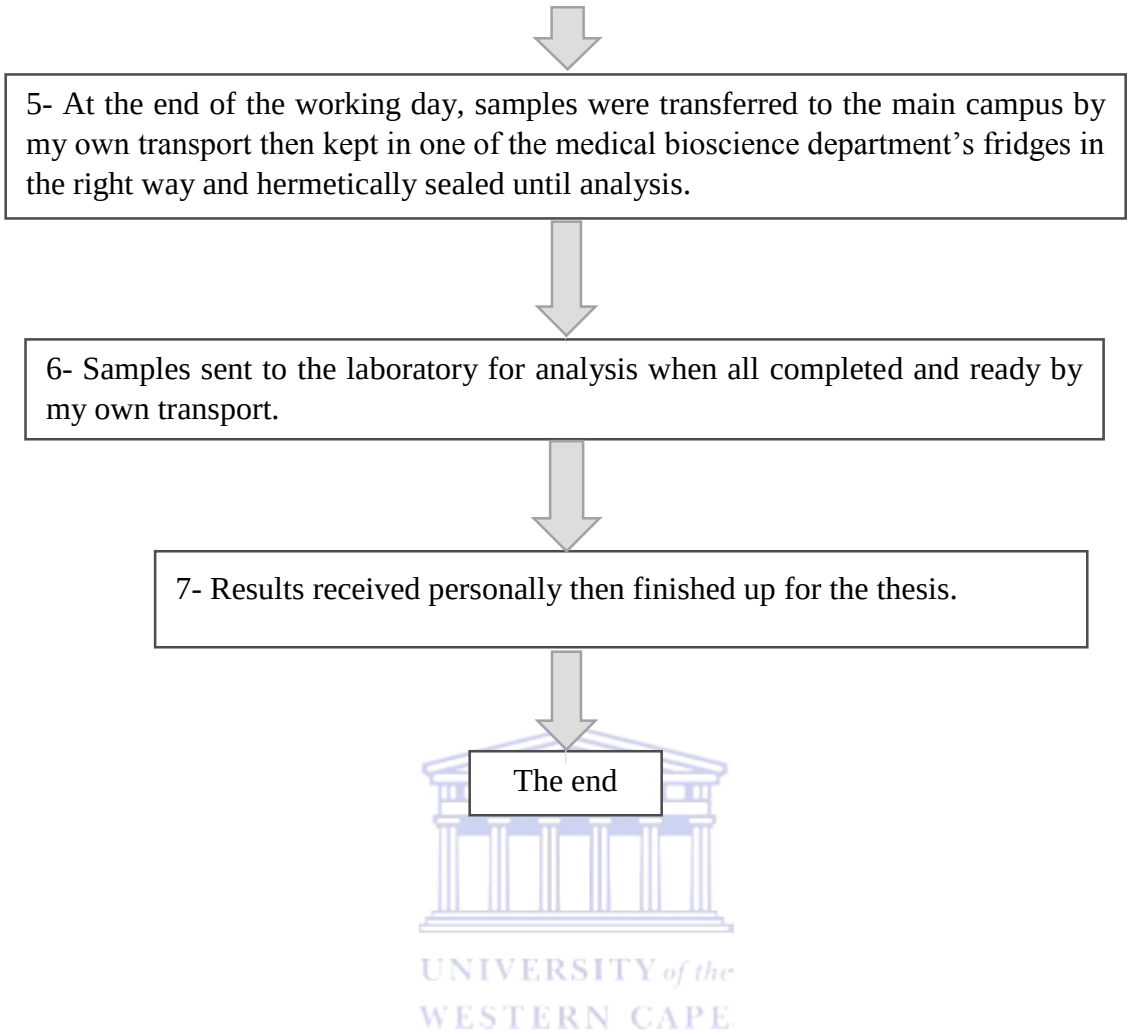
3- Start taking samples

a- A piece of chewing gum was given to each person to chew for 30 minutes.

b- Participants sent out to get urine samples in 50 ml plastic containers.

c- After finishing chew the gum, buccal samples were taken from each parson by special sterile cotton swabs.

4- All samples were kept in an Ice container after the collecting directly.



APPENDIX 4

Questionnaire on the participants and their amalgam fillings

Patient Name: _____ Cell phone: _____

Age: **Sex:**

Date of visit: / / **Date of sample collection: / /**

Address: _____

1- Is the patient with a serious illness or taking any medication?

□ Yes □ No

2- How many amalgam fillings does the patient have?

□ 2 □ 3 □ 4 □ more

3- For how long does the patient have these fillings?

□ Days □ Months □ Years

4- What is the size of the fillings?

□ Small □ Medium □ Large

5- How much does the patient consume of hot beverages?

□ Little □ Not much □ A lot □ Not

6- How much does the patient consume the chewing gum?

□ Daily □ Occasionally □ Not at all

7- How much does the patient smoke?

□ No □ >15 cigarettes/day □ <15 cigarettes/day

8- Does the patient have teeth grinding while is sleeping (bruxism habit)?

□ Yes □ No

9- How many times does the patient brush teeth?

□ 1/day □ ≥2/day □ <1/day □ Never

10- Does the patient live near to medical incinerators and coal burning power plants?

□ Yes □ No

CONSENT OF VISITOR TO THE QUESTIONS SUPPLIED

I hereby declare that I have agreed to participate in this study and all the information supplied is true and correct.

Signature (participant) _____