BIOMONITORING OF DENTAL STUDENTS EXPOSED TO TOXIC METALS FROM DENTAL MATERIALS WITH SINGLE HAIR STRAND

By

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THESIS ABSTRACT

Dental materials are composed of several metals. During dental procedures, these metals are released as particulate or vapor form in the dental environment. The excess of these metals in the human body may cause chronic or acute adverse effects. New technologies for hair analysis have been used to investigate metal absorption. However, no studies have investigated trace metal elements in the hair of students exposed to the dental environment during dental school programs. First, this thesis evaluated the metal distribution in hair cross-section and developed the Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) technique for single hair analysis. Second, investigated trace metal elements in the hair of dental students during four years of dental school. The final chapter assessed the efficiency of standard barriers against mercury vapor during mixing, condensation and removal procedures with dental amalgam.

Cross-section hair analysis in Back-scattered electron microscopy **(**BSE), Energy dispersive X-ray spectroscopy (EDS), and LA-ICP-MS revealed that most metal elements investigated were more highly concentrated in the cuticle than in the cortex. The results suggest complete ablation of hair strands is an excellent alternative for multiple-segment analysis using LA-ICP-MS. Trace metal elements analysis in the hair of dental students showed an increase of Hg levels during the 2^{nd}, 3^{rd}, and 4^{th} year of dental school compared with Hg levels detected in the 1^{st} year (control). The concentration of gaseous elemental mercury analysis revealed that the mercury vapor released in the mixing procedure of dental amalgam capsules may be reduced by 38 fold if the amalgamator is inside the fume hood turned on. However, an individual near the fume hood is not fully protected against mercury vapor during the mixing procedure of dental amalgam capsules. Finally, the Precision respirator + face shield and Advantage respirator + face shield + level-3 mask were more efficient in blocking the mercury vapor from dental amalgam condensation and

removal than the other PPE set tested.

In summary, work from this thesis contributed to the understanding of the risks of increased metal absorption during dental school. Furthermore, it gives new insights into preventative strategies.

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DEDICATION

To God Almighty who taught me to believe that anything was possible;

To my beloved family for their endless love and support;

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And to my advisor for his guidance, support, and patience throughout this study.

"And the very hairs on your head are all numbered. So don't be afraid; you are more valuable to God than a whole flock of sparrows." (Bible, Luke 12:7)

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LIST OF ABBREVIATIONS

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Solution Nebulization Inductively Coupled Plasma Mass Spectrometry (SN-ICP-MS)

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

Scanning electron microscope (SEM)

Back-scattered electron microscopy (BSE)

Energy dispersive X-ray spectroscopy (EDS)

Gaseous elemental mercury (GEM)

Epoxy resin (ER)

Occupational Safety and Health Administration (OSHA)

National Institute for Occupational Safety and Health (NIOSH)

Association Advancing Occupational and Environmental Health/American Conference of Governmental Industrial Hygienists (ACGIH)

Occupational Health and Safety (AOHS)

Agency for Toxic Substances and Disease Registry (ATSDR)

California Environmental Protection Agency (CA EPA)

Office of Environmental Health Hazard Assessment (OEHHA)

Minimal Risk Levels (MRL)

Time Weighted Average (TWA)

Ceiling limit (C)

The short-term exposure limit (ST)

Reference Concentration (RfC)

Recommended Exposure Limits (REL)

Threshold Limit Value (TLV)

Personal Protective Equipment (PPE)

CHAPTER 1: Introduction

This chapter includes work from the following publication:

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1. Introduction

The use of restorative materials to replace the missing dental structure has been the base of dental care since its beginnings (Sakaguchi, Ferracane, & Powers, 2019). Despite the recent development of new biomaterials such as composites and ceramics, the old metallic restorative materials remain used in current dental practices. One reason is that metallic elements confer excellent mechanical properties and durability for fillings, crowns, bridges, and dental implants (Agnihotri, Gaur, & Albin, 2020; Anusavice, 2013; Sacher & França, 2019). These metallic restorations are presented mainly as alloys containing several metals such as Mercury, Silver, Tin, Copper, Gold, Nickel, Zinc, Aluminum, Chromium, Cobalt, Iron, Manganese, Titanium, Palladium, Platinum, Iridium, Ruthenium, Beryllium, Gallium, Indium, Molybdenum, Beryllium, Lithium, Strontium, Barium, Bismuth, Zirconium, including rare metals Rhodium and Osmium (Camilleri et al., 2020; Givan, 2007; Grech & Antunes, 2019; Nicholson, 2020; Panpisut et al., 2016; Sacher & França, 2019; Zhou, Drummond, & Hanley, 2005), and promising metals as Niobium (Balbinot, Leitune, Ogliari, & Collares, 2020). The examples of metallic components in dental materials are summarized in **Table 1.1**.

With an advance in nanotechnology, the risk of toxicity and metal absorption in the dental environment has increased. Nanometals, ranging from 5 to 260 nm, were incorporated into dental materials to improve physiochemical properties and mechanical purposes. However, the surface area of metal particles was also increased, which contributed to an increase in the reaction rate with the human body when exposed to dental materials containing nanometals. (Agnihotri et al., 2020; Elias, Santos, Garbossa, & Santos, 2017; Paiva et al., 2018). Another technological advance was the introduction of computer-assisted design and computer-assisted manufacturing (CAD/CAM) in dental offices. This technology allows metallic and ceramic restorations to be milled chairside in a same-day visit. Even though there is no available data to prove the toxicity, it is possible to assume that the presence of airborne nano and microparticles originated by the CAD/CAM milling also could aggravate the air quality of the dental environment.

Metal ions from restorations, dental prostheses, implants, and dental appliances may leach out into the patient's saliva due to material degradation in the oral cavity (Bajsman, Vukovic, Zukic, &

Jakupovic, 2010; Berdouses et al., 1995; Mocnik, Kosec, Kovac, & Bizjak, 2017; Nicholson, 2020; Siddharth et al., 2015). However, the exposure to metals is even higher for dentists, dental students, dental technicians, and dental assistants than for patients (Shahi et al., 2019). Dental professionals and students are constantly exposed to aerosols, vapors, and particulates containing metals during dental procedures such as dental materials manipulation, removal, polishing, finishing, trituration, condensation, scaling, and adjustments of dental fillings and cement (Berry, Summitt, Chung, & Osborne, 1998; Camilleri, Kralj, Veber, & Sinagra, 2012; R. Warwick, O'Connor, & Lamey, 2013), prostheses (Keinan, Mass, & Zilberman, 2010) and dental appliances (Gopikrishnan, Melath, Ajith, & Mathews, 2015; Mikulewicz, Chojnacka, Wozniak, & Downarowicz, 2012; Wendl et al., 2017).

The quality of the dentistry environment is affected when invisible metals in nanoscale or metal vapors (e.g., mercury) surround the air and surfaces of dental spaces and laboratories (Liu, Chen, Chuang, Lin, & Wan, 2019; R. Warwick, O'Connor, & Lamey, 2013). Furthermore, the potential for toxicity of metals present in dental materials is unclear, especially for their potentially harmful effects on dental students and professionals in long-term exposure.

Table 1.1 Metallic elements in dental materials.

Element	Atomic Mass	Density (G/Cm^3)	Metal Type	Commonly present in		
				Prostheses	Restorative materials + cement	Implants
Calcium (Ca)	40.08	1.54	Alkaline Earth Metal		X	X
Strontium (Sr)	87.62	2.64	Alkaline Earth Metal		X	
Barium (Ba)	137.33	3.62	Alkaline Earth Metal			X
Magnesium (Mg)	24.30	1.73	Alkaline earth metals			X
Gold (Au)	196.97	19.32	Transition Metal	X		X
Palladium (Pd)	106.42	12.02	Transition Metal	X		
Platinum (Pt)	195.08	21.45	Transition Metal	X		
Iridium (Ir)	192.22	22.65	Transition Metal	X		
Ruthenium (Ru)	101.07	12.48	Transition Metal	X		
Rhodium (Rh)	102.91	12.41	Transition Metal	X		
Copper (Cu)	63.55	8.92	Transition Metal	X	X	
Titanium (Ti)	47.87	4.51	Transition Metal	X		X
Beryllium (Be)	9.01	1.85	Transition Metal	X		X
Chromium (Cr)	51.99	7.15	Transition Metal	X	X	X
Iron (Fe)	55.84	7.87	Transition Metal	X		X
Manganese (Mn)	54.93	7.30	Transition Metal	X		
Molybdenum (Mo)	95.95	10.20	Transition Metal	X		
Nickel (Ni)	58.69	8.91	Transition Metals	X		X
Zinc (Zn)	65.4	7.13	Transition Metal		X	
Mercury (Hg)	200.59	13.53	Transition Metal		X	
Osmium (Os)	190.2	22.57	Transition Metal	X		
Vanadium (V)	50.94	6.0	Transition Metal			X
Zirconium (Zr)	91.22	6.52	Transition Metal	X		X
Niobium (Nb)	92.90	8.57	Transition Metal		X	
Tantalum (Ta)	180.94	16.4	Transition metal			X
Silver (Ag)	107.87	10.49	Post-transition Metal	X	X	X
Aluminum (Al)	26.98	2.70	Post-transition Metal	X	X	X
Gallium (Ga)	69.72	5.91	Post-transition Metal	X		
Indium (In)	114.81	7.31	Post-transition Metal	X		
Bismuth (Bi)	208.98	9.80	Post-transition Metal		X	
Tin (Sn)	118.71	7.28	Post-transition Metal	X	X	
Indium	114.82	7.31	Post-transition Metal	X	X	

1.1 Toxicological potential of metallic elements present in dental materials

1.1.1 Routes for the spread of metals in Dentistry

The ways to spread metallic ions in the dental field can be divided into the following: aerosol, droplets, splatter, and vapor. Dental students and professionals generate them using a slow or high-speed dental drill and ultrasonic scaling dental equipment (Innes et al., 2021). The dental drills are used with diamond or carbide burs and disks for removal, polishing, finishing, and adjustments of restorations, prostheses, dental implants, and orthodontic appliances, while the ultrasonic scaling has a scaling ultrasonic scaler tip coupled. These dental treatment procedures release nanometals and micrometals in a suspension of air-water aerosols containing particles ≤ 5 µm in diameter, droplets in the range of 5–100 µm, and splatter with a size ≥ 100 µm in the dental environment (Han, Li, Walsh, & Ivanovski, 2021; James & Mani, 2016; Leggat & Kedjarune, 2001; Shiu, Leung, & Cowling, 2019; Yan et al., 2018).

Among the metals listed in **Table 1.1**, mercury is a unique liquid metal able to volatilize at room temperature. This characteristic attracts attention to the risk of exposure to materials containing mercury. In dentistry, dental amalgam restoration comprises approximately 50% wt. of elemental mercury (Gelson Luis Adabo, 2018; Estrich, Lipman, & Araujo, 2021). This mercury can be released as liquid metal or mercury vapor during dental amalgam mixing or amalgam filling condensation, placement, polishing, finishing, and removal.

According to Warnick D. et al. 2019 (D. Warwick, Young, Palmer, & Ermel, 2019), the amalgam particulates release significant amounts of mercury vapor after removing the amalgam filling. The literature also shows that mercury vaporization remains in dental instruments, opened capsules, work surfaces and tools, and amalgam residues after procedures with dental amalgam, or spills of bulk mercury may happen with defective amalgamators, mercury dispensers, and leaking from amalgam capsules. (Camilleri et al., 2012; Nagpal, Bettiol, Isham, Hoang, & Crocombe, 2017; R. Warwick, O'Connor, et al., 2013).

1.1.2. Routes of exposure to metals in the dental environment

The three primary routes of occupational and non-occupational exposure to metals in dentistry include 1) inhalation, 2) ingestion, and 3) dermal/ocular exposure. **Figure 1.1** illustrates the possible routes of exposure, pathways to organs, and primary target organs of the human body to metallic elements from the dental environment.

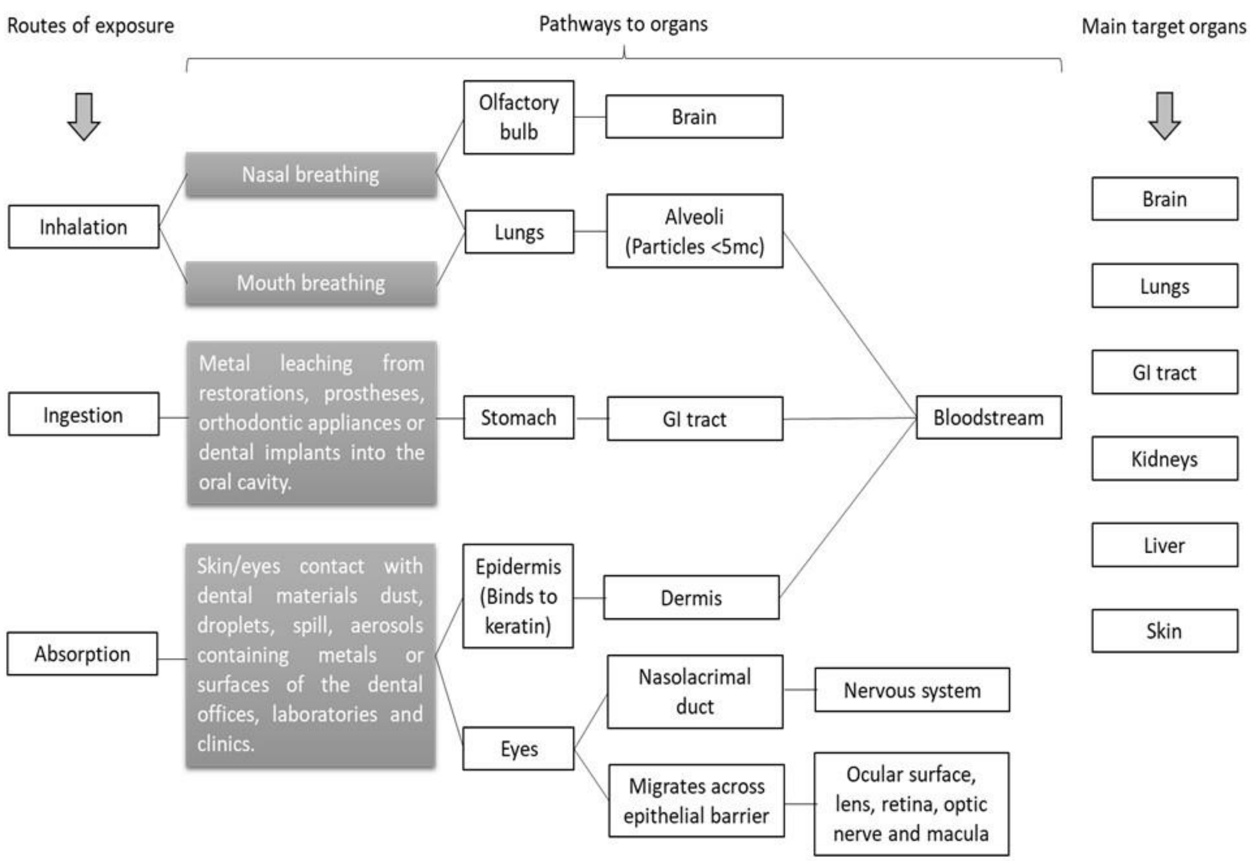


Figure 1.1: Routes of exposure, pathways to organs, and primary target organs of the human body to metallic elements from the dental materials and dental environment.

For dentists, dental students, and dental personnel, inhalation and dermal/ocular absorption are the main routes of exposure to metallic elements from the dental environment. On the other hand, ingestion is a route of exposure to metallic elements that often occur in dental patients. The ingestion of metal ions happens due to the dental materials' degradation in the oral cavity (Bajsman et al., 2010; Berdouses et al., 1995; Mocnik et al., 2017; Nicholson, 2020; Siddharth et al., 2015).

After inhalation and/or dermal/ocular absorption pathway, metal ions reach several organs. Some metal elements (E.g. Cu, Cr, Fe, Mn, Mg, and Zn) are essential for the physiological functions of the human body, while others (E.g. Li, Sr, Ag, Ba, Hg) are non-essential (S. Mitra et al., 2022). It is still controversial in the literature if Chromium is not an essential trace element, as some studies consider Cr to be a vital human activity element (Saikat Mitra et al., 2022; Snitynskyĭ, Solohub, Antoniak, Kopachuk, & Herasymiv, 1999; Vincent, 2015). The metallic elements are non-biodegradable. When the metal ions are in excess, they are widely distributed in different organs, and the human system's bioaccumulation is a concern.

Metals have a long half-life due to the chemical properties of the elements, especially non-essential elements. In general, metal in excess may be trapped in vital organs by covalent chemical bonds with organic groups, which generates intracellular granules in an insoluble form to be excreted in the organism's feces or for long-term storage that may cause toxic effects (Briffa, Sinagra, & Blundell, 2020).

Particularly, mercury toxicity is attributed to its lipophilic and diffusion characteristics to cross the blood-brain barrier, placenta and cellular membranes in addition to the inhibition of enzymatic activity by intracellular thiols reduction, mitochondrial dysfunction, and induction of oxidative stress (Ajsuvakova et al., 2020; Briffa et al., 2020). This heavy metal has a high affinity for sulfhydryl and selenol groups containing cysteine and cystine on metallothionein, erythrocytes, glutathione, or albumin from plasma (Ajsuvakova et al., 2020; Briffa et al., 2020; Song et al., 2021). As elemental mercury (Hg^0), this metal is converted to mercurous (Hg^{1+}) and mercuric ions (Hg^{2+}) in the blood and excreted as mercuric mercury through the urine, faeces, sweat, tears, saliva and breast milk (Briffa et al., 2020). In the dental environment, dental procedures with dental amalgam release mercury vapor (Hg^0), which is commonly inhaled by dental students and professionals. The aggravating risk factor for mercury inhalation in the dental environment is that this heavy metal is odorless and colorless in the vapor form.

1.1.3 Potential Metal Toxicity in the Dental Environment

A dental environment can include a dental clinic, pre-clinical teaching laboratories, dental laboratories, and hospital dental departments. The metal exposure in these places is affected by the type of dental procedure and properties of the environment matrix (air quality and room temperature). However, the properties of the metallic ions (pH, redox potential, and the possible formation of complex metallic compounds) are essential factors in determining the potential toxicity (van Gestel, 2020). In addition, other important factors can affect the exposure-response relationship, such as route and time of exposure, biological factors, skin permeability, and diet of exposed individuals (Hostynek, 2003; L. Li & Li, 2021; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Dentists, dental students, and dental personnel should evaluate the already reported toxic effects of metal exposure in the dental environment (**Table 1.2**) and consider the potential risks of the metallic elements to which they are exposed (**Table 1.3**).

Table 1.2 Adverse health effects on dentists, dental students, and dental personnel associated with metal exposure in the dental environment .

Element	Adverse health effects associated with metal exposure in the dental environment	References
Hg	Risk of neurological and sensory symptoms (memory loss, fatigue, attention deficits, neurobehavioural problems, reduced cognitive flexibility, reduced psychomotor speed and sleep problems)	(Bjørklund, Hilt, Dadar, Lindh, & Aaseth, 2019)

Risk of fertility issues, congenital deficits or abnormalities, and spontaneous abortion	(Bjørklund, Chirumbolo, et al., 2019)
Pathogenetic role in neurological disorders, particularly in pregnant women	(Bjørklund, Bengtsson, Chirumbolo, & Kern, 2017)
Increased risk of tremor	(Anglen et al., 2015)
Risk of reduction in systolic blood pressure	(Goodrich et al., 2013)
Risk of reduction of Th1-type proinflammatory markers in serum	(Björkman, Brokstad, Moen, & Jonsson, 2012)
Risk for miscarriage	(Lindbohm et al., 2007)
Deficit on psychomotor performance (Hand Steadiness)	(Bittner et al., 1998)
The behavioural deficit, tension, fatigue, and confusion	(Echeverria et al., 1995)
Risk of problems in visual memory, verbal memory, visuomotor coordination speed, visuomotor coordination and concentration, optical scanning, and motor speed	(Ngim, Foo, & Boey, 1992)
Risk of reproductive issues, renal function changes, allergies, immunotoxicological effects, and	(Bjørklund, 1991)

	glioblastoma (brain cancer)	
	Risk of headaches, fatigue, malaise, weakness, irritability, depression, loss of memory, a feeling of hopelessness, tremor, decreased reflexes, loss of fine motor control, visual disturbances, lens and retina pigmentation, digestive disturbances, diarrhea, poor appetite, nausea, stomatitis, metallic taste, sore mouth, an increase of nasal secretion and saliva, burning tongue red palms, and Eczema.	(Bauer, 1985)
	Risk of polyneuropathies, mild visuographic dysfunction, and symptom-distress	(Shapiro et al., 1982)
Ni	Allergy	(Warshaw et al., 2022)
	Allergy	(Werfel et al., 2018)
	Contact allergy	(Wrangsjö, Swartling, & Meding, 2001)
	Hand Eczema and allergy	(Wallenhammar et al., 2000)
	Hand Dermatitis	(Hill, Grimwood, Hermesch, & Marks, 1998)
	Allergy, contact dermatitis, and hand eczema	(Rustemeyer & Frosch, 1996)
	Risk of pneumoconiosis	(Okamoto et al., 2017)

Ag	Risk of pneumoconiosis	(Okamoto et al., 2017)
Cu	Contact Dermatitis	(Wrangsjö et al., 2001)
Cr	Toxic to RAW264.7 cells (monocyte/macrophage *cell* line)	(Wang et al., 2020)
	Risk of pneumoconiosis	(Okamoto et al., 2017)
	Risk of lung disorders	(Seldén, Persson, Bornberger-Dankvardt, Winström, & Bodin, 1995)
Co	Allergy	(Wallenhammar et al., 2000)
	Contact allergy	(Wrangsjö et al., 2001)
	Allergy, contact dermatitis, and hand eczema	(Rustemeyer & Frosch, 1996)
	Toxic to RAW264.7 cells (monocyte/macrophage *cell* line)	(Wang et al., 2020)
	Risk of pneumoconiosis	(Okamoto et al., 2017)
	Risk of lung disorders	(Seldén et al., 1995)
Pd	Contact Dermatitis	(Werfel et al., 2018)
	Allergy, contact dermatitis, and hand eczema	(Rustemeyer & Frosch, 1996)
Sn	Contact Dermatitis	(Werfel et al., 2018)

Al	Toxic to RAW264.7 cells (monocyte/macrophage cell line)	(Wang et al., 2020)
	Risk of pneumoconiosis	(Okamoto et al., 2017)
Mo	Toxic to RAW264.7 cells (monocyte/macrophage *cell* line)	(Wang et al., 2020)
	Risk of lung disorders	(Seldén et al., 1995)
In	Risk of pneumoconiosis	(Okamoto et al., 2017)
Ti	Risk of pneumoconiosis	(Okamoto et al., 2017)

Table 1.3 The potentially toxic effects of metallic elements on humans.

Element	Potential toxic effects of metal elements on humans	References
Hg	Risk of lung and eyes irritation, diarrhea, rashes, vomiting Risk of DNA damage Risk of reproductive problems (congenital disabilities, miscarriages, sperm damage in men) Risk of neurological disorders, learning disabilities, speech defects, memory loss, tremors and muscle incoordination, deafness, vision complications, personality changes, Risk of paralysis, insanity, coma, and death	(ATSDR, 1999; Briffa et al., 2020; Haynes, 2011; Kaye & Laby, 1986; R. Li et al., 2017; Martin & Wendy, 2009)

	Risk of congenital disabilities through a toxic effect on an embryo or fetus	
Ni	Allergic reactions Risk of respiratory problems (asthma, lung embolisms, respiratory failure) Risk of heart disorders Ni inhalation may cause: Risk of cancer	(ATSDR, 2017b; Briffa et al., 2020; Denkhaus & Salnikow, 2002; Haynes, 2011; Kaye & Laby, 1986; Sharma, 2013; WHO, 2000)
Ag	Contact with silver liquid may cause: Risk of allergic dermatitis, skin irritation, and argyria Risk of corneal injury Silver inhalation may cause: Risk of dizziness, headaches, drowsiness, confusion, and staggering Risk of respiratory issues Risk of unconsciousness, coma, death Silver ingestion may cause: Risk of nausea and vomiting, diarrhea, stomach discomfort Risk of narcosis, brain damage, and cardiac abnormalities	(ATSDR, 1990; Briffa et al., 2020; Haynes, 2011; Kaye & Laby, 1986; Martin & Wendy, 2009)
Cu	Risk of flu-like symptoms Risk of diarrhea, vomiting, eyes irritation, dizziness, oral	(ATSDR, 2004a; Briffa et al., 2020;

	mucosa irritation Risk of acute gastroenteritis Oral intake will cause hepatic and, Risk of hepatocellular degeneration, kidney disease, insomnia, anxiety, agitation, necrosis Risk of Wilson's disease (symptoms: lack of appetite, fatigue, jaundice, kayser-Fleisher rings, speech impairment, difficulty in swallowing, uncontrolled poisoning, brain damage, demyelination, hepatic cirrhosis Risk of death	Haynes, 2011; Kaye & Laby, 1986; Tsafrir)
Cr	Cr ingestion may cause: Risk of nausea and vomiting, fever, diarrhea, gastrointestinal ulceration, vertigo, toxic nephritis, liver damage, and coma Cr (VI) inhalation or repeated skin contact may cause: Risk of allergic contact dermatitis and eczema, gingivitis, irritation of mucous membranes, bronchitis, liver and kidney disease Risk of respiratory issues (sinusitis, pneumonia, lung cancer) Risk of chrome holes in the forearms, hands, fingers, and nose Risk of cancer	(ATSDR, 2012a; Barnhart, 1997; Briffa et al., 2020; Dayan & Paine, 2001; Martin & Wendy, 2009; WHO, 2000)

	Risk of death	
Co	Risk of skin and respiratory issues allergic dermatitis Inhalation may cause: Risk of congestion, wheezing, asthma, respiratory irritation, lung function reduction, edema, pneumonia, fibrosis, and lung hemorrhage Risk of nausea, vomiting, diarrhea, renal congestion, cardiac and liver disorders	(ATSDR, 2004b; Briffa et al., 2020; Lauwerys & Lison, 1994; Leyssens, Vinck, Straeten, Wuyts, & Maes, 2017; WHO, 2000)
Zn	Risk of nausea and vomiting, fatigue, anemia, neutropenia, stomach cramps, epigastric pain, copper deficiency, decrease in high-density lipoprotein (HDL) cholesterol, pancreatic complications, impaired immune function	(ATSDR, 2005; Briffa et al., 2020; Fosmire, 1990; Haynes, 2011; Kaye & Laby, 1986)
Mn	Risk of weakness, lethargy, decreased blood pressure, dullness, tremors, akathisia, dystonia, anxiety, motor disorders, lack of facial expression Risk of neurological disorders and behavioural changes Risk of manganism Risk of mimicry of Parkinson's' disease Mn inhalation may cause: Risk of reproductive problems (sperm damage, loss of sex drive) and pneumonia	(ATSDR, 2012b; Briffa et al., 2020; Dobson, Erikson, & Aschner, 2004; Haynes, 2011; Iregren, 1990; Kaye & Laby, 1986; Marcus, 2013; Mergler et al., 1994; Roels, Ghyselen, Buchet, Ceulemans, & Lauwerys, 1992; WHO, 2000)

Pd	Risk of allergy or contact dermatitis Risk of eyes and oral mucosa irritation (stomatitis or mucositis) and oral lichen planus	(Kielhorn, Melber, Keller, & Mangelsdorf, 2002; Melber, Keller, Mangelsdorf, & International Programme on Chemical, 2002)
Al	Risk of liver and kidney dysfunction Risk of lung damage and pulmonary fibrosis Risk of leukocytosis, osteomalacia, hypoparathyroidism, colitis Risk of central nervous system damage Risk of Amyotrophic Lateral Sclerosis, Alzheimer's disease, Parkinsonism Dementia Complex (ALS-PDC), and listlessness	(ATSDR, 2008; Briffa et al., 2020; Shaw & Tomljenovic, 2013; WHO, 2000)
Mo	Risk of headache, fatigue, weakness, appetite reduction, hypochromic microcytic anemia, and anorexia Risk of listlessness, chest pain, myalgia, and arthralgia, Risk of testicular atrophy Risk of copper deficiency	(ATSDR, 2017a; Briffa et al., 2020; Haynes, 2011; Kaye & Laby, 1986; Momcilović, 1999)
In	Risk of Interstitial pneumonia, pulmonary and systemic diseases	(Hoet et al., 2012; Nakano et al., 2009)
Ti	Risk of intermittent coughing and respiratory diseases (bronchial asthma, chronic sinusitis, chronic bronchitis, chronic obstructive lung disease, chronic rhinitis, nasal septum deviation, nasal polyposis, recurrent pneumonia,	(Ataya, Kline, Cope, & Alnuaimat, 2015; Decker, Daly, & Scher, 2015; Goutam, Giriyapura, Mishra, & Gupta, 2014; Kim, Eo, Nguyen, &

	recurrent pleural effusion, and acute pulmonary edema) Risk of yellow nail syndrome Risk of inflammation reactions and hypersensitivity Risk of systemic disease, cardiac failure, and death	Kim, 2019; Piraccini, Urciuoli, Starace, Tosti, & Balestri, 2014)
Sn	Risk of interstitial pneumonia	(Nakano et al., 2009)

The potential of metal spreading increases in dental procedures involving high-speed handpiece or ultrasound scaler usage. According to J. Matys et al. 2020 (Matys & Grzech-Lesniak, 2020), these instruments are responsible for intensive dental aerosol generation during dental procedures. In dental clinics and pre-clinical laboratories where a group of dentists or students perform dental practices in the same room, the risk of metal exposure should be evaluated as the metal spread will be more intense in the environment. Thus, a specific room for dental procedures involving dental amalgam is suggested to prevent mercury vapor spread in these cases.

Concerning dental material composition, metallic ion mobility is particularly concerning from a potential toxicological point of view. Unbound nanoparticles in the bulk composition are more reactive and quickly airborne (Elsaesser & Howard, 2012). Furthermore, airborne metal particles are highly insoluble at physiological pH, which can increase the risk of lung toxicity for metal dust from dental materials in dentists, dental students, and dental personnel (Gwinn, 2015).

The experiments of Checchi L et al. 2005 [108] investigated dust with variable dimensions in a range of ~1-300 µm. The results showed that a certified personal respirator could be more effective than high-quality surgical masks in dental settings. However, nanoparticles have been incorporated into dental materials by industry. Therefore, the efficiency of some personal protective equipment (PPE), such as surgical masks, may have been reduced. A study in the U.S.A. that monitored indoor particulates in dental offices in California found that 67% of the particulates had an average particle size of <100 nm in size and there were 33 % ranging from 0.3 µm to 10 µm (Wu, Apte, & Bennett, 2012).

Recently, Liu et al. 2019 (Liu et al., 2019) found that almost all the particles produced by tooth drilling and grinding were ≤1 μm and that the central vacuum systems and protective surgical masks have limited efficacy against these suspended particulate matters. More research about barrier efficiency against ultrafine metal particulates is necessary to protect dentists, dental students, and dental personnel in dental clinics and pre-clinical laboratories.

Thus, dental procedures should not be underestimated in terms of the risk of metal spread in the environment and adverse effects on human health. For better conduct of metal spread, PPE to the operator, patient, and others present in the same room as suggested in **Table 1.4**.

Table 1.4 Guideline of risk of procedure for metal spread.

Low-Risk Procedure	Moderate Risk Procedure	High-Risk Procedure
• Dental procedures without handpiece or ultrasound scaler usage • Dental procedures without dental amalgam • Use the enclosed operatory	• Dental procedures using low-speed handpieces when dental materials containing metal are involved. • Dental procedures non involving dental amalgam • Use an enclosed operatory with air filtration and evacuation unit	• Dental procedures using high-speed handpiece or ultrasound scaler usage • Dental procedures with dental amalgam and cleaning instruments after the clinical session • Use a closed operatory with air filtration and evacuation unit

1.2 General recommendations

Several measures have been proposed to reduce and eliminate occupational and non-occupational hazards from exposure to metallic ions from dental materials in dentists, dental students, and dental personnel ("Canadian Centre for occupational and safety. OSH Answers Fact

Sheets, Mercury," 2023; Colson, 2012; "Occupational Exposure to Elemental Mercury in Dentistry," 2012; D. Warwick et al., 2019; "World Dental Federation. Mercury Hygiene Guidance," 2007). The main ones could be listed as the following:

1.2.1 Air quality precautions

- Use of adequately designed ventilation systems, including local exhaust ventilation.

- Use air filtration equipment for dental procedures with dental amalgam and ensure the filters are appropriate for mercury vapors.
- Perform air monitoring periodically to ensure that the Occupational (dental workers) and non-occupational (dental students) exposure limit is not exceeded.

1.2.2 Good hygiene practices

- Clean hands vigorously to create friction in an appropriate sink. Use soap for the hands and a nailbrush to clean the fingernails. After, rinse well to remove all the soap and dry the hands thoroughly using a paper towel.
- Hand hygiene should be performed immediately before starting a clinical session, before placing gloves and following the removal of gloves. Also, perform hand hygiene anytime when the hands are contaminated with visible metal dust or liquid mercury.
- Jewelry is not recommended as it can chemically bond with other metals released during the dental procedure.
- Observe periodically if the working team or dental students follow good hygiene practices for quality control.

1.2.3 Personal Protective Equipment

- Dentists, dental students, dental personnel, and the patient should wear protective clothing (ideally an impermeable full-body covering for procedures with dental amalgam).
- Use a dental dam sealed correctly in the patient's mouth with saliva ejector to remove contaminated saliva or liquid debris from the oral cavity.
- The protective mask or respirator should be selected according to the type of metal involved in the dental material or procedure and the local, national or international regulatory agency of occupational and non-occupational Safety and Health recommendations. Each agency has its guidelines recommending different types of masks or respirators. The barrier choice should be based on the air monitoring results of the dental clinic or laboratory, respecting the Threshold Limit Value (TLV), Permissible Exposure Limit (PEL), Recommended Exposure Limit (REL), Minimal Risk Level (MRL), or a Reference Concentration (RfC) of the agency. The most common agencies used in North America are the National Institute for Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration (OSHA), the American Conference of Governmental Industrial Hygienists (ACGIH), the United States Environmental Protection Agency, California Environmental Protection Agency. The mask or respirator should cover the mouth and nose completely.
- For protective eyewear, prescription safety glasses, safety glasses over corrective lenses, or loupes with side shields should be used during all pre-clinical and clinical procedures.
- Bouffant, impermeable dedicated footwear (covering the entire foot) and a face shield are also recommended.
- Protect the patient's skin and clothing, a whole body, an impermeable barrier and a complete head/face/neck barrier under/around the dam.

- Use high-volume suction and continually add water spray to the site where the amalgam is removed.

1.2.4 Health and educational surveillance

- Perform medical monitoring of levels of metals in the working team or dental students.
- Observe periodically if the working team or dental students follow the local quality control guidelines.
- Review the local guidelines periodically or when updated.

1.2.5 Substitution with less harmful products

- It is reasonable to choose an alternative material when it is applicable. For example, use composite resins instead dental amalgam for dental restorations, use metal-free crowns (porcelain-based ceramic, quartz, glass, or resin, through zirconium and lithium disilicate) instead of metal crowns (made of gold, platinum, copper, or nickel, or chromium) when is indicated, and use cobalt-chromium (Co-Cr) alloy instead Ni-Cr alloy for the fabrication of metallic frameworks of removable partial dentures to reduce allergic reactions. The additional use of allergenic metals should be reduced if possible.

1.2.6 Cleaning dental instruments and surfaces

- For dental procedures involving dental amalgam, use mercury decontaminant to clean instruments, countertops and surfaces after the clinical session.
- For dental procedures involving other metal types, clean instruments with water, soap,

and brush.

- Clean spills of mercury using commercial mercury spill clean-up kits. After, check mercury vapor levels in the dental operatory.
- Clean handpieces according to the manufacturer's instructions. For handpieces contaminated with mercury is recommended to wipe the handpiece with mercury decontaminant before starting the manufacturer's instructions.

The literature shows occupational adverse effects of metal exposure for dental workers, including dentists and dental personnel. However, there is a lack of discussion regarding dental students' exposure to hazardous metals. Even though dental students perform daily dental procedures in pre-clinical laboratories and dental clinics, this population is not considered dental workers. In addition, extensive extra hours of their regular practices are ordinary in pre-clinical laboratories. However, the limits of exposure to metallic elements for this population are not established, as we cannot consider them as dental workers.

CHAPTER 2: Hypothesis and Objectives

2.1 Study Rationale

Most dental procedures produce suspended particulate matter (PMs) and harmful vapor (HV) containing metals from dental materials. High-speed dental equipment usage is responsible for PM and HV spread in the ambient air (Bentley, Burkhart, & Crawford, 1994; Harrel & Molinari, 2004; Liu et al., 2019). These PMs and HV can include hazardous non-essential elements such as Hg, Li, Ag, Sr, and Ba, which have been detected in the air and surfaces of the dental treatment room during dental therapy (R. Warwick, O'Connor, et al., 2013).

Even shortly following dental treatments, the dental treatment area retains a high concentration of Hg vapor and metal dust, which may enter the body through different routes of exposure (inhalation, oral, and dermal), and are stored in different body compartments. Previous studies indicated that PMs and HV exposure was related to the risks of respiratory, liver, renal, and nervous systems dysfunction and other adverse health effects described in **Table 1.2**. Particularly, mercury vapor may cause the formation of free radicals, DNA damage, lipid peroxidation, enzyme inactivation, and oxidative stress (Ajsuvakova et al., 2020; Valko, Morris, & Cronin, 2005; R. Warwick, O'Connor, et al., 2013).

Understanding the dental students exposure to metals from dental materials is crucial to deal with protective measurements for students in the dental environment and during dental procedures. Most studies are related to occupational health effects or isolated analysis of metals absorbed through biomarkers of dental students and professionals. Among biomarkers (e.g. Blood, urine, saliva), the advantages of using single hair samples consist in simple and painless collection, longer sample durability, low cost storage and easy transportation. These are the advantages of using hair as a biomarker in long-term studies, especially when it is necessary to store the sample without risk of decomposition or loss of data. However, biomonitoring of metal absorption through a single hair of dental students and the efficiency assessment of standard barriers against heavy metal during dental procedures have not been investigated thus far.

2.2 Hypothesis

Chapter 4 -The null hypotheses were that (1) there is no significant difference between partial and complete ablation of a single hair strand using the LA-ICP-MS method and (2) there is no significant difference in the metal distribution in the cuticle and cortex of the cross-section hair.

Chapter 5 - The null hypotheses were that (1) there is no significant difference between metal elements concentration in the hair of dental students during 4 years of dental school, (2) there is no significant difference between metal elements concentration in the hair of different dental students from the first- and fourth year of dental school, (3) the class factor would not influence the metal elements concentration in hair of the dental students, and (4) fish consumption would not influence the metal elements concentration in hair of the dental students.

Chapter 6 - The null hypotheses were that (1) there is no significant difference between the PPE sets and the control group during dental amalgam condensation (2) there is no significant difference between PPE sets and the control group during dental amalgam removal (3) Fume hood ON, mercury analyzer location and mixing of dental amalgam capsules would not influence the mercury vapor concentration in the air.

2.3 Objectives

The following objectives were pursued to test the above hypotheses using a combination of approaches, including improving analytical techniques for human biomonitoring using a single strand hair.

2.3.1 Objective 1: To develop Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) techniques for monitoring trace element levels in a single

human hair.

2.3.1.1 Specific objective 1.1: To assess the agreement between complete and partial ablation of a single human hair with bulk hair analyses in Solution Nebulization Inductively Coupled Plasma Mass Spectrometry (SN-ICP-MS).

This objective verified the influence of different ablation parameters in trace element levels for single human hair analysis to reduce analytical errors.

2.3.1.2 Specific objective 1.2: To investigate the internal distributions of trace elements in the cross-section hair.

This objective is to identified the degree and patterns of internal distribution of trace elements in the cortex and cuticle of the human hair.

2.3.2 Objective 2: To investigate the concentration of 13 trace metals in the hair strand of dental students.

2.3.2.1 Specific objective 2.1: To compare the concentration of 13 trace metals in students' hair enrolled in the 1st-year and 4th-year dental school.

This objective investigated the influence of different factors on trace element uptake processes in the hair.

2.3.2.2 Specific objective 2.2: To evaluate the concentration of 13 trace metals in students' hair during four years of dental school.

This objective focused on the levels of 13 trace metals in students' hair during 4 years of dental school and the influence of other factors on trace element uptake processes into hair.

2.3.3 Objective 3: To evaluate mercury vapor exposure in dental amalgam procedures.

2.3.3.1 Specific objective 3.1: To assess the mercury concentration inside and outside the fume hood, when the fume hood is on and off, before and after mixing dental amalgam capsules.

This objective aims to measure mercury concentration in eight conditions involving mixing dental amalgam capsules.

2.3.3.2 Specific objective 3.2: To assess the efficiency of standard barriers against mercury vapor during dental procedures with dental amalgam.

This objective aims to evaluate the efficiency of different surgical masks and respirators during dental amalgam condensation and removal in a simulation of a dental clinical setting. The second focus is to analyze whether the detected mercury levels would exceed the exposure limits to mercury vapor recommended by health, safety, and environmental protection Agencies.

CHAPTER 3: General Materials and Methods

3.1 Materials

3.1.1 Reagents

The following reagents were commonly used in the study: ultra-high purity nitric acid (Sigma Aldrich, Oakville, Ontario, Canada), ultra-high purity hydrogen peroxide (Sigma Aldrich, Oakville, Ontario, Canada), reagent-grade acetone (Sigma Aldrich, Oakville, Ontario, Canada), de-ionized water (18.3 MΩ/cm, Millipore, Bedford, Massachusetts, USA), and alcohol 70%.

3.1.2 Standard, dental amalgam and others

NCS DC73347 human hair standard (Chinese National Analysis Center for Iron & Steel – NACIS (Haidian District, Beijing, China) was purchased for hair analysis.

Two-sided clear 3M tape and epoxy resin (Buehler Ltd., Lake Bluff, IL, USA) were used for mounting hair.

For mercury vapor analysis, Amalgam Permit Regular Set (Southern Dental Industries-SDI, Victoria, Australia), the Vari-mix III™ amalgamator (Caulk Dentsply Co, Toronto, Ontario, Canada), and a fume hood (Purair Advanced Ductless Fume Hood USP 800 Compliant, Air Science, Fort Myers, FL, USA). A head-form model (FloraCraft Corporation, Ludington, Michigan, United States) was used for clinical simulation.

3.1.3 Personal Protective Equipment

Surgical mask levels I, II, III (3M Company, St. Paul, Minnesota, USA), N95 1870 AURA (3M Company, St. Paul, Minnesota, USA), 95PFE – Precision AIR™ (Precision ADM Inc., Calgary, Alberta, Canada), MSA Advantage 200-LS Respirator (MSA - The Safety Company, Cranberry Township, Pennsylvania, USA), and face shield was purchased.

3.1.4 Instruments

Traces of metallic elements were detected in digested hair solution using Element 2 high-resolution ICP-MS (Thermo-Finnigan, Bremen, Germany) and in solid hair was coupled to the ICP-MS a New Wave UP-213 laser ablation system (New Wave Research, Inc., Fremont, CA, USA). A pressed pellet made from human hair standard was made using a pellet maker (SPESS, Metuchen, NJ, USA). FEI Inspect S50 Scanning Electron Microscope with back-scattered electron (BSE) was used for cross-section hair characterization and Energy-dispersive X-ray spectroscopy (EDS) for quantification. Mercury vapors were measured using a Lumex RA-915M mercury analyzer (Lumex Instruments, St. Petersburg, Russia).

3.1.5 Statistical analysis

Statistical analysis was performed using OriginLab data analysis software system version 9.2. (Northampton, Massachusetts, USA). The Shapiro-Wilk test was used for normality. One-way analysis of variance (ANOVA) with Tukey and Kruskal–Wallis was applied. Where applicable, the statistical significance is: ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Pearson correlations coefficient was used to measure the coefficient of the relationship between the amount of dental amalgam removed in each sample and the amount of mercury detected by the mercury analyzer instrument.

Chapter 4: Trace element determination in human hair: comparison between SN-ICP-MS and LA-ICP-MS.

This chapter includes work from the following publication:

Fernandes, Ana Carla B.C.J. ; Yang, Panseok ; Armstrong, Debbie ; França, Rodrigo. "Metal Distributions in Human Hair Strand Cross-Section: Advanced Analysis Using LA-ICP-MS in Dentistry." Talanta (Oxford), vol. 265, 2023, p. 124909–, https://doi.org/10.1016/j.talanta.2023.124909.

4.1 Introduction

Dental practitioners are constantly exposed to noxious metal such as Li, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Ag, Ba, and Hg while preparing dental fillings. Heavy metals such as Cr and Ni are known to have cancerogenic potentials (Hayes, 1997). Elements that can cause allergies and systemic problems, especially in the liver or lungs, include Li, Mn, Fe, Cu, Sr, Ag, and Ba (Syed, Chopra, & Sachdev, 2015). The most hazardous metallic element in dentistry environments is Hg (G. L. Adabo, 2018). Each dental amalgam restoration contains 50% by weight of elemental Hg. In 2015-2016, this kind of restoration was present in more than 50 % of restored teeth in the US population (Estrich et al., 2021). The elemental mercury **(**Hg^0) easily crosses the blood brain barrier in a faster rate compared to the inorganic mercury (Balali-Mood, Naseri, Tahergorabi, Khazdair, & Sadeghi, 2021). Workers exposed to elemental mercury may have defects in sensory and peripheral nerve conduction and the tremor is the early neurological sign of Hg^0 poisoning (Park & Zheng, 2012). High levels of Hg can generate more pronounced symptoms such as tremors, depression, loss of memory, and psychotic reactions (Farina, Avila, da Rocha, & Aschner, 2013). Workers exposed to hazardous environments should be examined periodically (G. L. Adabo, 2018). However, conventional examinations involving body fluids or tissue are time-consuming and expensive. This study uses available human hair as a bio-monitor in dentistry environments because it is easier to sample, store, transport and prepare for various chemical analyses.

Solution nebulization inductively coupled plasma mass spectrometry (SN-ICP-MS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have been utilized in chemical analyses of human hair (Astolfi et al., 2020; Blumenstiel, McDonald, Arriaza, & Amarasiriwardena, 2020; Dressler et al., 2010; Kumtabtim, Matusch, Dani, Siripinyanond, & Becker, 2011; Luo et al., 2017; Sach et al., 2022; Santamaria-Fernandez, Giner Martinez-Sierra, Marchante-Gayon, Garcia-Alonso, & Hearn, 2009; Sela, Karpas, Zoriy, Pickhardt, & Becker, 2007; Stadlbauer, Reiter, Patzak, Stingeder, & Prohaska, 2007). However, SN-ICP-MS is more time-consuming and vulnerable to contamination during sample preparation (Foltz, Andrenyak, & Crouch, 2017). Most importantly, SN-ICP-MS requires more hair samples and cannot reveal chronological information along a single strand. On the contrary, LA-ICP-MS subjects less to contamination because wet chemistry is not involved and can perform micro-local analysis. Single hair capability is essential when large quantities of hair samples are unavailable (Koch & Günther, 2017). Therefore, LA-ICP-MS was employed in this study to monitor trace element uptakes in human hair exposed to dentistry environments.

Human hair has a layered structure named of cuticle, cortex and medulla from rim to core and is composed mainly of protein (65 to 95%, keratin essentially), water (15 to 35%), lipids (1 to 9%) and minerals (<1%)(Harkey, 1993). Keratin is a major structural component of human hair fiber that confers high stability and durability for the hair even after removal from the scalp (Harkey, 1993; Kintz, Villain, & Cirimele, 2006). The scalp hair generally grows 1 cm/month (Astolfi et al., 2020). Therefore, weekly and monthly records of trace elements uptake assimilated can be investigated with the LA-ICP-MS (Sajnog, Hanc, & Baralkiewicz, 2018; Wati M., 2017). Internal structures indicate that trace element distribution may also vary internally. Indeed, hair from individuals intoxicated with Hg and Pt showed elevated levels of the two elements, mostly in the cuticle (Stadlbauer, Prohaska, Reiter, Knaus, & Stingeder, 2005). However, internal distributions of Hg and other toxic metals from healthy individuals exposed to dentistry environments have not been investigated. Internal distributions of trace elements can provide valuable information on trace element uptake processes into hair. Internal distribution of trace elements at the scales of laser beam sizes can also affect accuracy of LA-ICP-MS. The purpose of this study is to develop precise and accurate LA-ICP-MS techniques for determining trace element concentrations in a single hair strand.

4.2 Materials and Methods

4.2.1 Hair sample preparation

According to the protocol approved by the Health Research Ethics Board (HREB HS21107-H2017:299), hair samples were collected from the same individual. The individual was a healthy female exposed to dentistry environment for two years. The hair was not chemically processed in any way (e.g., bleaches, color treatments, dyes, highlights, and ombré hair). The sequences of analyses for each technique used in this study are summarized in **Figure 4.1**.

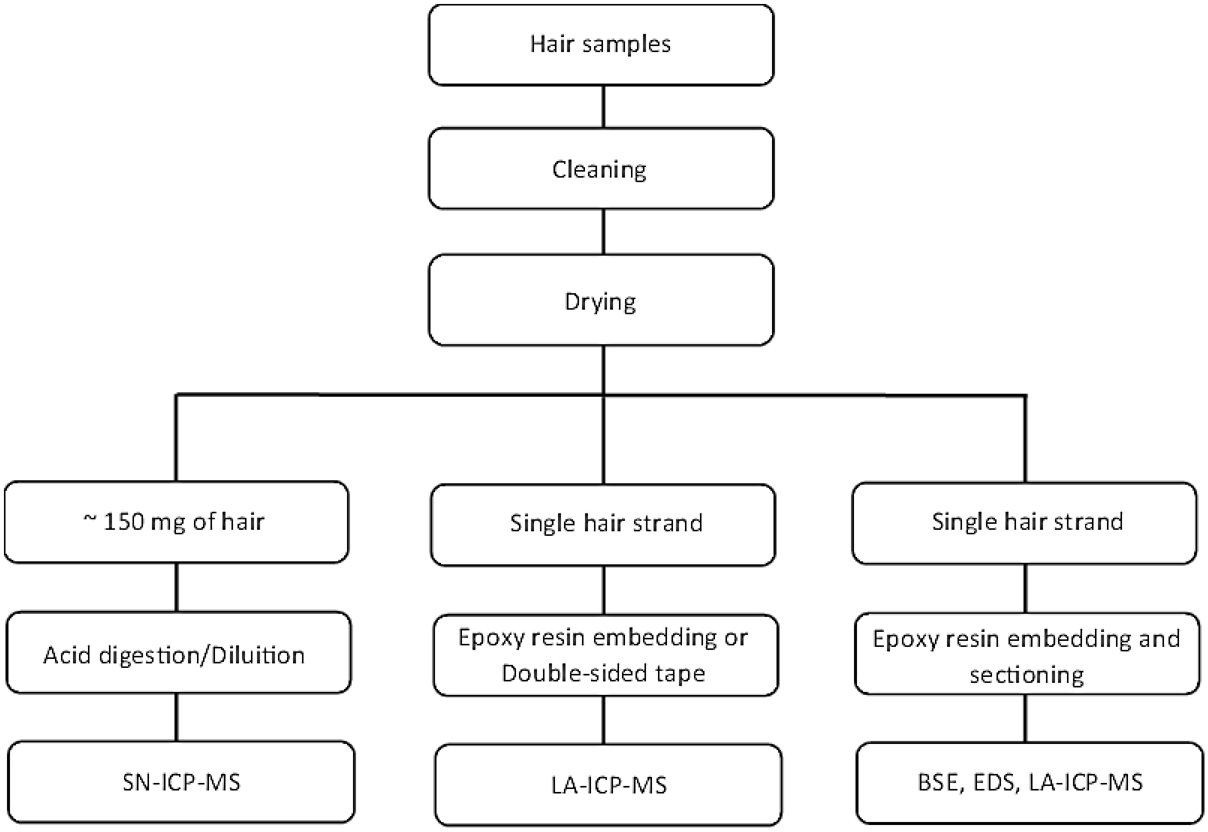


Figure 4.1: Experimental workflow of the hair analysis using SN-ICP-MS, LA-ICP-MS, EDS, and BSE.

4.2.2 Hair Collection

The hair sample was taken from the scalp in the occipital area using a ceramic scissor. All hair strands were cut ~2 cm from the root. The hair samples were labelled, stored in a plastic tube, and kept at room temperature.

4.2.3 Hair cleaning

Following the International Atomic Energy Agency (IAEA) recommendations (Sajnog et al., 2018), the hair was first washed with reagent-grade acetone (Sigma Aldrich, Oakville, Ontario, Canada) for 5 minutes and then rinsed with de-ionized water (18.3 MΩ/cm, Millipore, Bedford, Massachusetts, USA) for 5 minutes to remove surface contaminants. The acetone-water wash was repeated two times and finished with the final water rinse. After cleaning, the hair samples were dried overnight at room temperature under a laminar flow hood to avoid environmental contamination. All laboratory equipment used for the sampling and sample treatment was acid cleaned. Then, ~30 cleaned and dried hair strands were set aside for micro-beam analysis, such as backscattered electron microscopy (BSE), energy dispersive spectrometry (EDS), and LA-ICP-MS. The remaining hair strands were used for SN-ICP-MS.

4.2.4 Acid digestion for SN-ICP-MS

About 150 mg of the cleaned and dried hair strands were acid digested in open vessels containing 3 ml of 70 % (w/w) ultra-high purity nitric acid (Sigma Aldrich, Oakville, Ontario, Canada) and 0.5 ml of 30 % (w/w) ultra-high purity hydrogen peroxide (Sigma Aldrich, Oakville, Ontario, Canada) under a laminar flow hood. The digestion vessels were placed in a hot water bath (~90 °C) for 10 minutes. The resulting solution was colorless, and no residues were found. Afterward, the hair solution was diluted with deionized water until the final volume of 16 ml. The solution was divided into four vials and the multielement standards were added (**Table 4.2)**.

4.2.5 Pressed pellet hair standard for LA-ICP-MS

To use the digested solution as an external calibration standard for LA-ICP-MS, a compressed pellet was made from NCS DC73347 human hair powder (**Figure 4.2**). In the NCS DC73347, Li, Ag, and Mg values are not certified. Approximately 300 mg of the hair powder was pressed for 60 sec at ~10 tons using a pellet maker without adding a binding material. Thin Teflon disks were placed at metal contacts to prevent contamination from the dies.

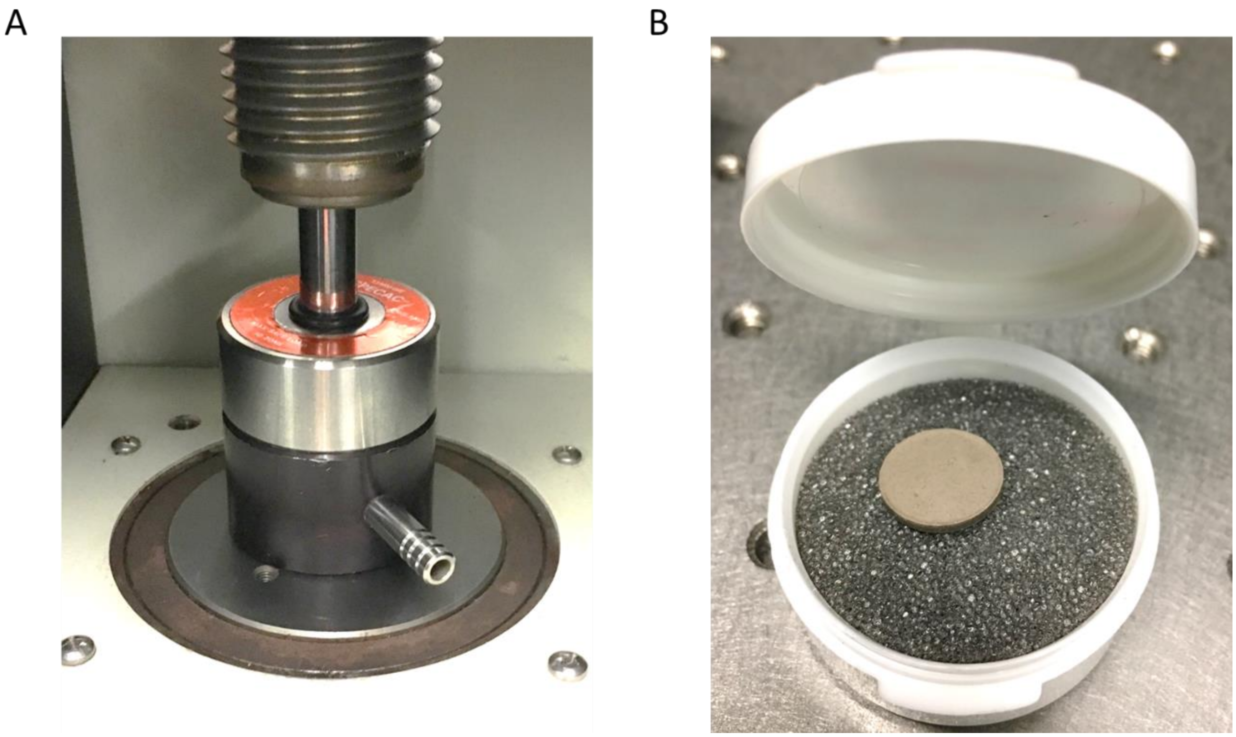


Figure 4.2: Pressed pellet made from NCS DC73347 human hair standard.

A. pellet maker **B.** hair pressed pellet.

4.2.6 Analysis of contaminants in commercial double-sided tapes and epoxy resin slides

In this study, the laboratory protocols to avoid contamination in the hair samples during and after the cleaning was taken. Therefore, it is crucial that the mounting hair option for analysis in LA-ICP-MS be pure to preserve the outcome of data in a laboratory setting. As the double-sided tape and Epoxy Resin (ER) manufacturing environment are unknown, the purity of the double-sided tape and epoxy resin becomes doubtful. To ensure complete laser ablation without contamination from slide glass with double-sided tape or ER slides, two-sided clear 3M tape, and epoxy were submitted to LA-ICP-MS to test the presence of contaminants. The ER (~ 5 mm thick) was prepared in a clean laboratory environment under a laminar flow hood. For all elements investigated in this study, signal intensities from ER were not distinguishable from gas blank except for Fe.

4.2.7 Mounting hair in epoxy resin and double-sided tape

Single hair strands must be fixed onto a substrate to prevent them from being blown away by gas flow inside the sample chamber and shock waves created by the laser ablation. Hair strands have been commonly fixed with double-sided tape or adhesive agents (Cheajesadagul, Wananukul, Siripinyanond, & Shiowatana, 2011; Legrand, Lam, Jensen-Fontaine, Salin, & Man Chan, 2004; Luo et al., 2017; Noel, Christensen, Spence, & Robbins, 2015; Sach et al., 2022; Sajnog et al., 2018; Stadlbauer et al., 2005). Although the mounting techniques are simple, they are prone to contamination, especially for elements common in environments such as Fe and Mn. Detachment of hair strands from the double-sided tape during laser ablation has also been reported (Sajnog et al., 2018). This study also experimented with a clear double-sided tape at the beginning of the study. However, due to impurities and loss of hair strands, especially between two laser ablation scans, we embedded hair strands in epoxy resin (Buehler Ltd., Lake Bluff, IL, USA). The epoxy resin (ER) was poured onto a clean plastic mold to make ~5 mm thick ER slides and cured overnight at room temperature under the laminar flow hood to prevent contamination. A thin layer of ER was added to the surface of ER slides. Then, hair strands hand-picked with a plastic tweezer were placed onto the ER layer and cured under the lamina flow hood. For the cross-section study, ~ 15 hair strands were placed vertically inside a plastic straw and filled with ER. After curing, the straw was cut, mounted in a plastic ring, and polished. Some hair strands were cut obliquely to the hair strands.

4.2.8 Instrumentation

4.2.8.1 EDS analyses and BSE imaging system

A scanning electron microscope (SEM, FEI Inspect S50) housed at the Department of Earth Sciences, University of Manitoba, is equipped with an EDS and a BSE imaging system. The SEM was utilized to investigate compositional variations of major and minor elements in the hair cross-section. An accelerating voltage of 20 kV and beam current of 4.5 nA were employed for the EDS analyses and the BSE imaging.

4.2.8.2 ICP-MS and LA-ICP-MS analyses

Thirteen elements were determined using an Element 2 high-resolution ICP-MS (Thermo-Finnigan, Bremen, Germany) housed at the Department of Earth Sciences, University of Manitoba (**Figure 4.3**), for acid digested hair samples and a New Wave UP-213 laser ablation system (New Wave Research, Inc., Fremont, CA, USA) attached to the ICP-MS for single hair strand analyses. In both SN-ICP-MS and LA-ICP-MS, ^{7}Li, ^{33}S, ^{88}Sr, ^{107}Ag, ^{138}Ba, and ^{202}Hg were measured in low resolution (~300) and ^{24}Mg, ^{33}S, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, and ^{66}Zn were measured in medium resolution (~4000) to avoid spectral interferences. Analytical conditions and data acquisition parameters used in SN-ICP-MS and LA-ICP-MS are summarized in **Table 4.1**.

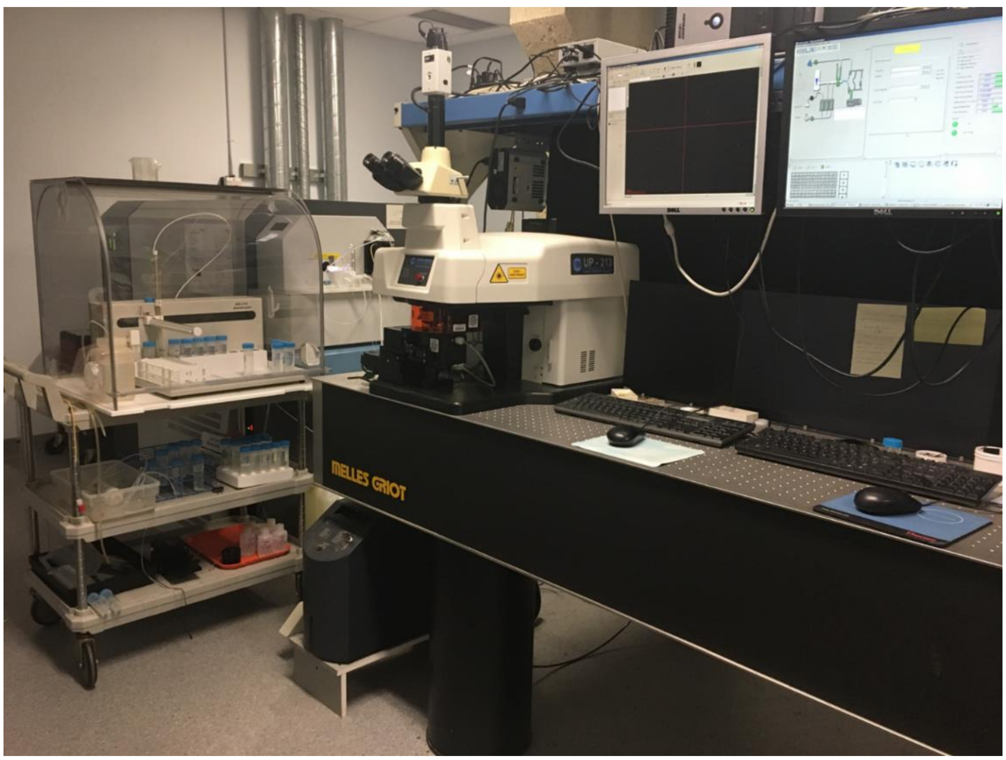


Figure 4.3: Picture of the Element 2 high-resolution ICP-MS and a New Wave UP-213 laser ablation system attached to the ICP-MS for hair strand analyses used in this study.

Table 4.1 Operation conditions and data acquisition parameters for SN-ICP-MS and LA-ICP-MS hair analyses.

SN-ICP-MS			
Plasma power (W)	1303		
Cool gas flow rate (L/min)	16		
Auxiliary gas flow rate (L/min)	0.80		
Sample gas flow rate (L/min)	0.89		
LA- ICPMS	*Complete ablation*	*Partial ablation*	*Line profile*
Plasma power (W)	1240	1235	1241
Cool gas flow rate (L/min)	16	16	16
Auxiliary gas flow rate (L/min)	0.81	0.78	0.83
Sample gas flow rate (L/min)	0.62	0.65	0.7
Repetition rate (Hz)	20	10	10
Beam diameter (µm)	100	70	40
Energy density ($J/cm^{2)}$	~6.0	~6.0	~4.5
Scan speed (µm/s)	5	5	1

4.2.8.3 Standard Addition Technique for SN-ICP-MS

To reduce matrix effects, a standard addition technique was employed to analyze hair solution samples. Multi-element standard solution was prepared from an element standard (SCP Sciences, Baie-D'Urfe, Quebec, Canada). The hair sample solution was divided into five vials and then added with four levels of the multi-element standard (**Table 4.2**). Blank solution and the five vials were spiked with In (1 µg/L).

Table 4.2 Multielement standard solutions for hair analyses in SN-ICP-MS. All were spiked with 1 µg/L 115In.

Multielement Standard	Element Concentrations
1	0.2 µg/L of Hg, Li, Cr, Co, Ni, Ag, Zn, Cu, Mn, Fe, Sr, Ba + 2 µg/L of Mg
2	1.5 µg/L of Hg, Li, Cr, Co, Ni, Ag, Zn, Cu, Mn, Fe, Sr, Ba + 8 µg/L of Mg
3	2.8 µg/L of Hg, Li, Cr, Co, Ni, Ag, Zn, Cu, Mn, Fe, Sr, Ba + 14 µg/L of Mg
4	4.0 µg /L of Hg, Li, Cr, Co, Ni, Ag, Zn, Cu, Mn, Fe, Sr, Ba + 20 µg/L of Mg

4.2.8.4 Laser ablation of strand hair

For laser ablation along the hair strand, ten segments (300 µm long) per hair strand were ablated at 5 µm/s; 5 segments in low resolution and another 5 in medium resolution with a ~300 µm gap between the segments. The total length of hair ablated becomes 3 mm spanning ~5.7 mm. This study also compares partial and complete ablation. Complete ablation was performed with a 100 µm beam diameter and a 20 Hz repetition rate. When the hair strand is wholly ablated through, the bottom of the laser pit appears bright under a transmitted light microscope (**Figure 4.4a**). For partial ablation, the beam size and repetition rate were reduced to 70 µm and 10 Hz (**Figure 4.4b**). For laser ablation along the cross-section, the laser beam diameter was further reduced to 40 µm and ablated at the speed of 1 µm/s.

ER adjacent to the hair strands were ablated before and after each hair analysis, and their signal intensities were subtracted from total intensities to correct for background levels and contamination. For all elements investigated in this study, signal intensities from ER were not distinguishable from gas blank except for Fe. The net signal intensities were externally calibrated against the human hair standard and internally standardized against 4.77 wt. % of S (Dressler et al., 2010; Legrand et al., 2004; Pozebon, Scheffler, Dressler, & Nunes, 2014; Rodushkina & Axelsson, 2000; Sela et al., 2007; Stadlbauer et al., 2005; Steely, Amarasiriwardena, Jones, & Yañez, 2007). Data reduction was performed using Iolite 3.7 (Paton, Hellstrom, Paul, Woodhead, & Hergt, 2011). LA-ICP-MS results were verified by analyzing the same hair sample with SN-ICP-MS.

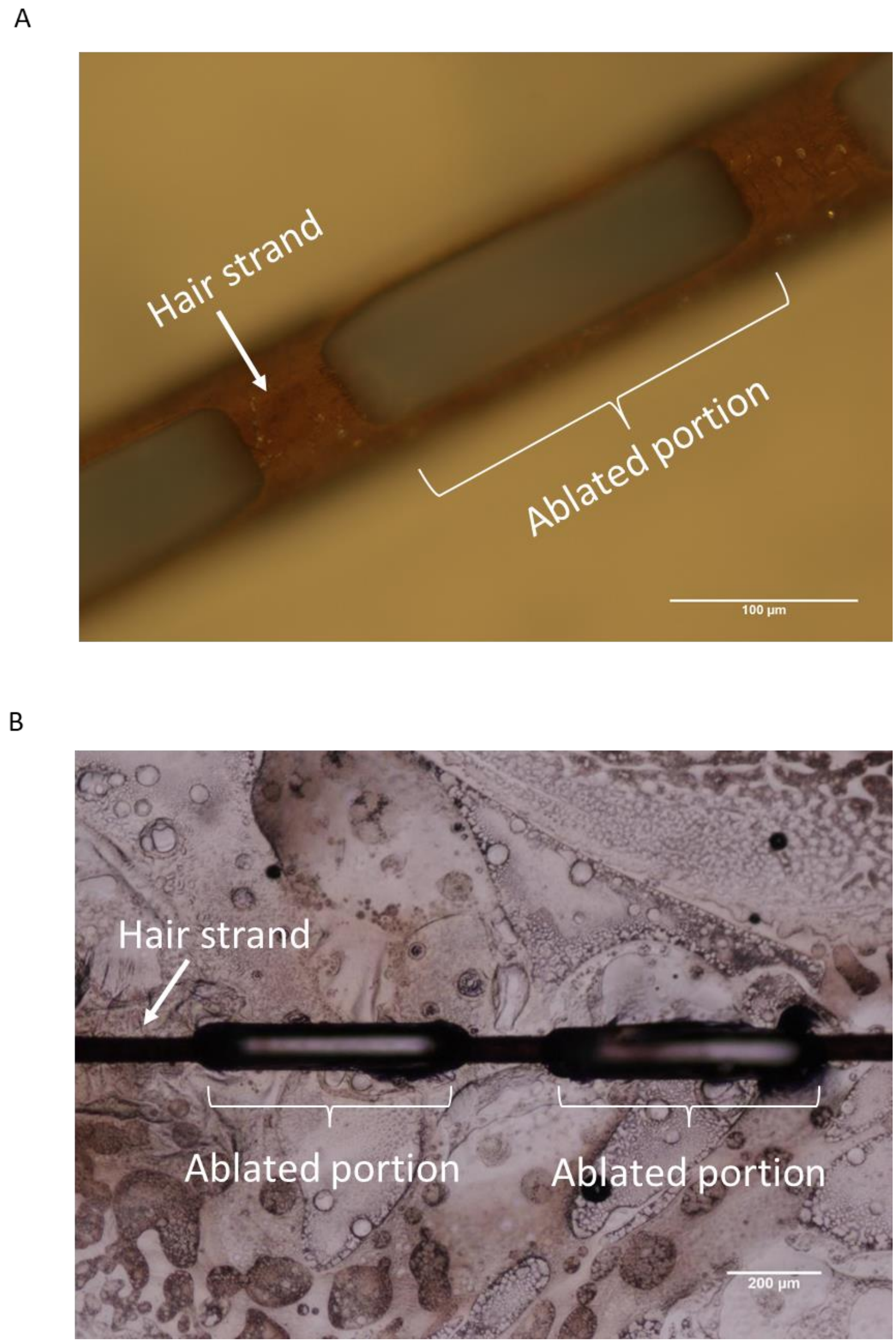


Figure 4.4: Optical microscope images.

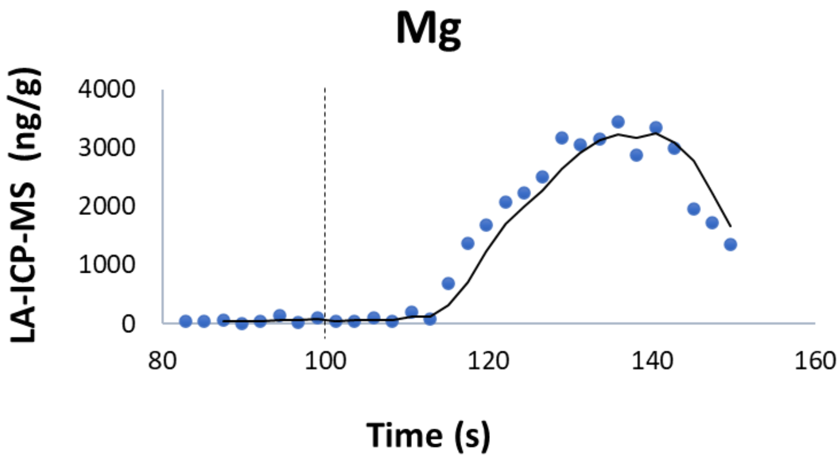
A. partially ablated hair strands **B.** completely ablated hair strands. The bottom of the strand partially ablated is dark, while the bottom completely ablated is bright.

4.3 Results

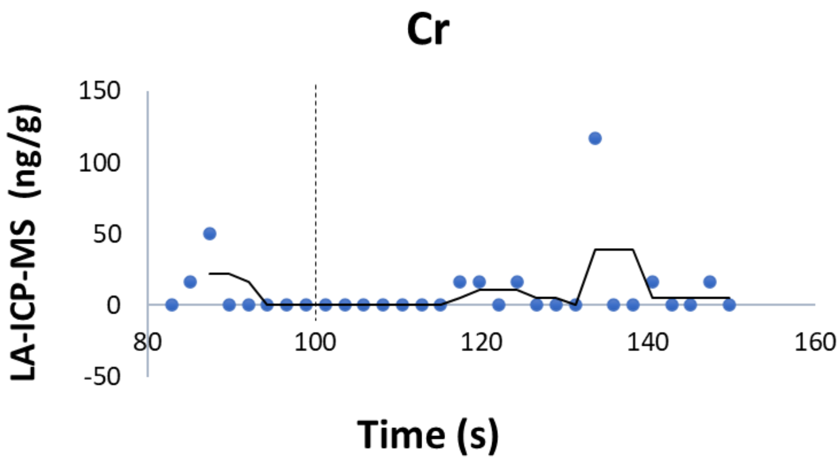
4.3.1 LA-ICP-MS analysis of double-sided tapes and ER

The LA-ICP-MS results show contaminants in the clear tape (**Figure 4.5 A-O**). Epoxy sometime presented a little Fe contamination but no other elements (**Figure 4.6 A-O**). Epoxy signals were subtracted from hair signals to correct for any potential contamination in complete ablation.

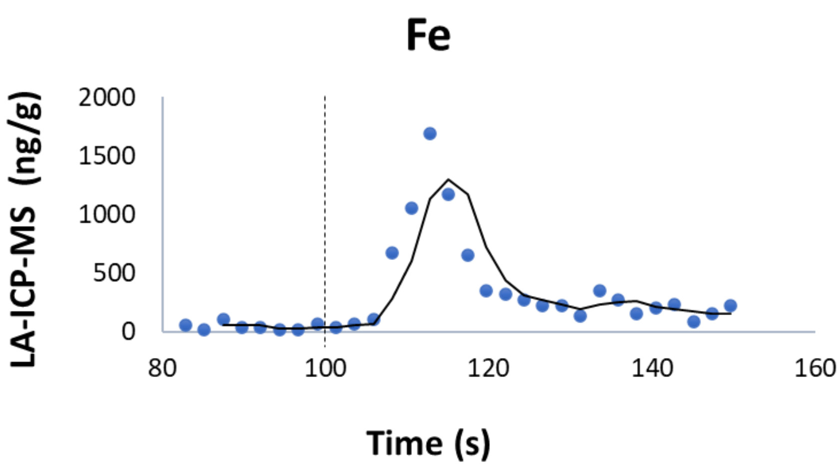
A


Mg
LA-ICP-MS (ng/g)
4000
3000
2000
1000
0
80
100
120
140
160
Time (s)

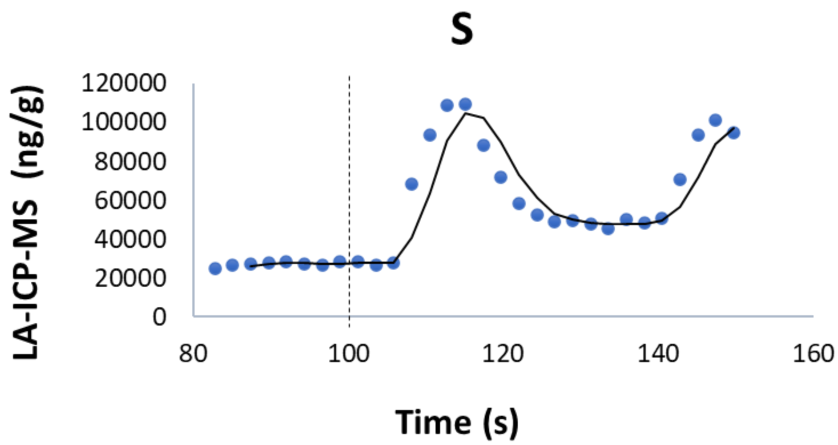
B


Cr
LA-ICP-MS (ng/g)
150
100
50
0
-50
80
100
120
140
160
Time (s)

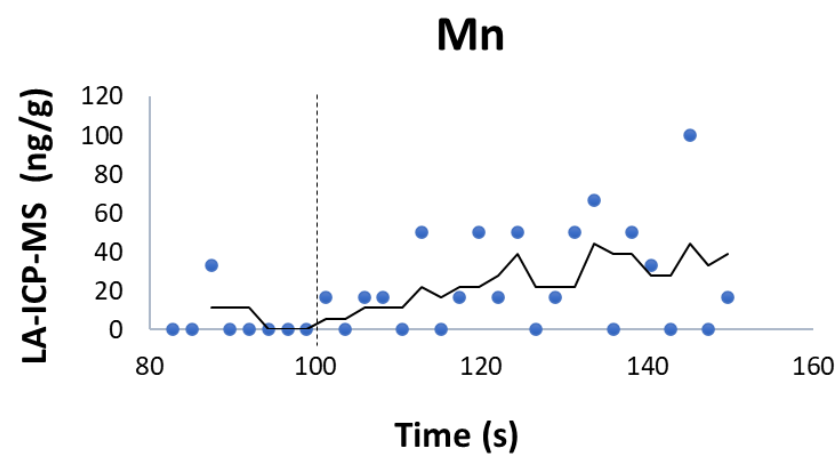
C


Fe
LA-ICP-MS (ng/g)
2000
1500
1000
500
0
80
100
120
140
160
Time (s)

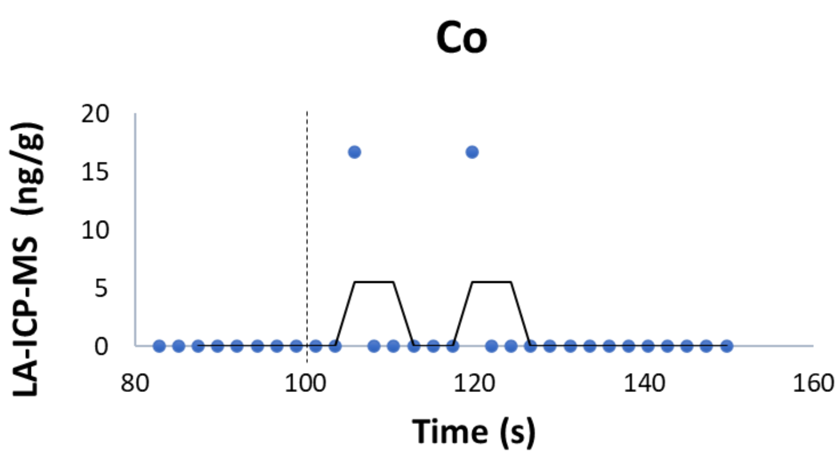
D


S
LA-ICP-MS (ng/g)
120000
100000
80000
60000
40000
20000
0
80
100
120
140
160
Time (s)

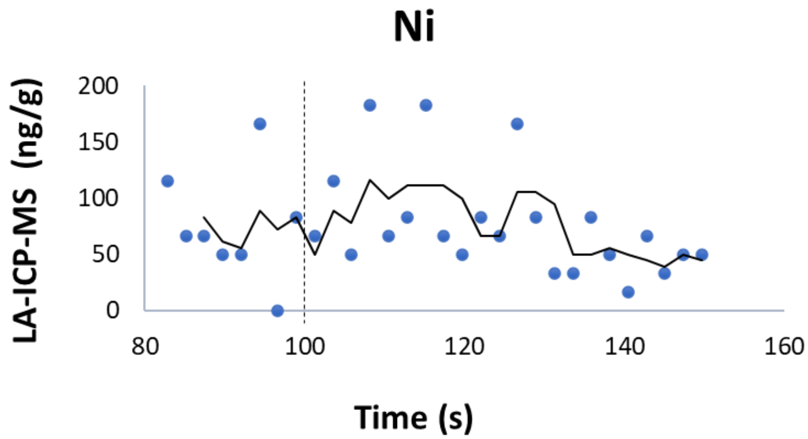
E


Mn
LA-ICP-MS (ng/g)
120
100
80
60
40
20
0
80
100
120
140
160
Time (s)

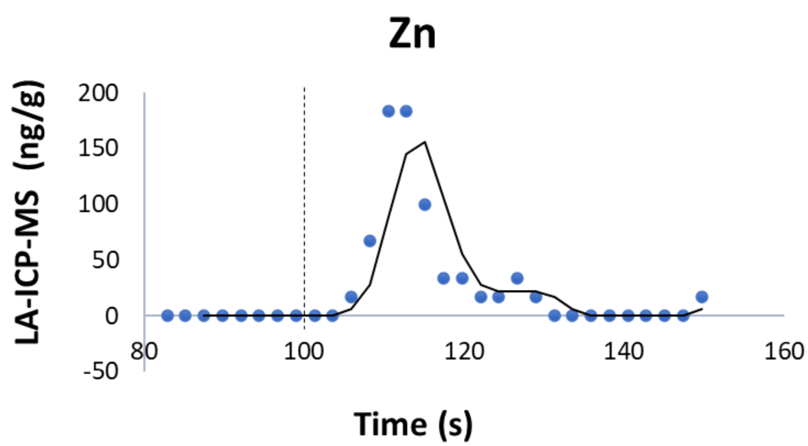
F


Co
LA-ICP-MS (ng/g)
20
15
10
5
0
80
100
120
140
160
Time (s)

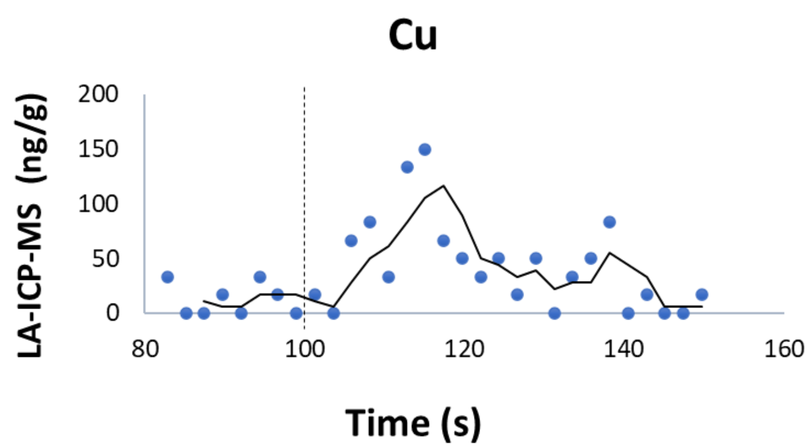
G


Ni
LA-ICP-MS (ng/g)
200
150
100
50
0
80
100
120
140
160
Time (s)

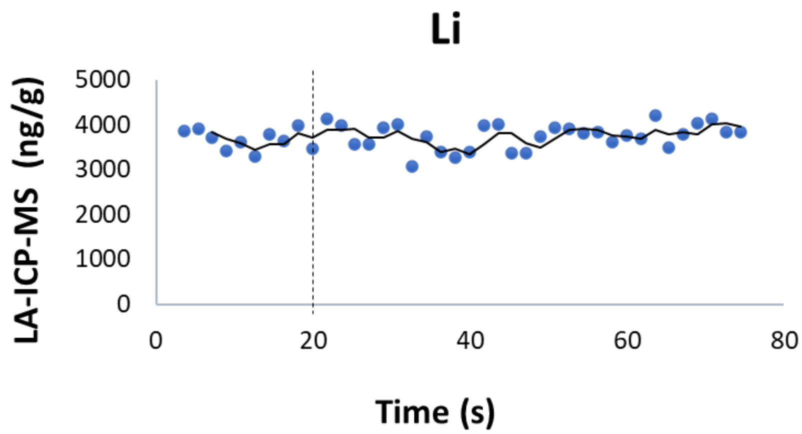
H


Zn
LA-ICP-MS (ng/g)
200
150
100
50
0
-50
80
100
120
140
160
Time (s)

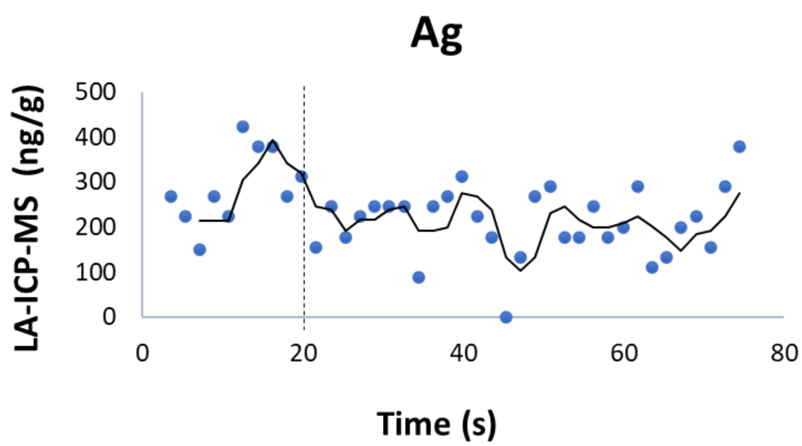
I


Cu
LA-ICP-MS (ng/g)
200
150
100
50
0
80
100
120
140
160
Time (s)

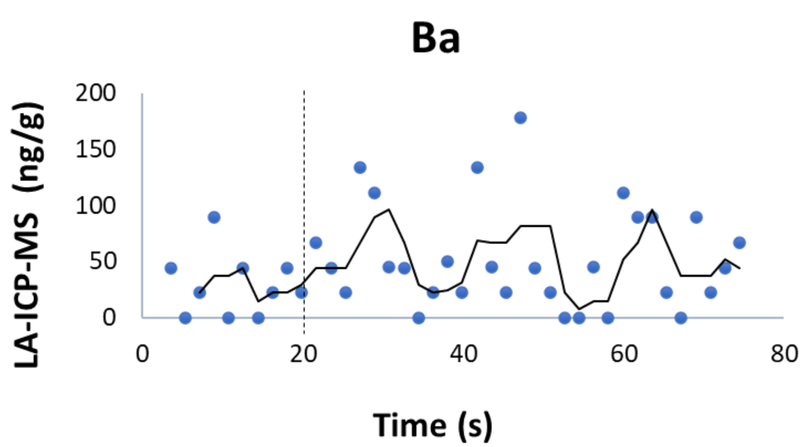
J


Li
5000
4000
3000
2000
1000
0
LA-ICP-MS (ng/g)
0
20
40
60
80
Time (s)

K


Ag
500
400
300
200
100
0
LA-ICP-MS (ng/g)
0
20
40
60
80
Time (s)

L


Ba
200
150
100
50
0
LA-ICP-MS (ng/g)
0
20
40
60
80
Time (s)

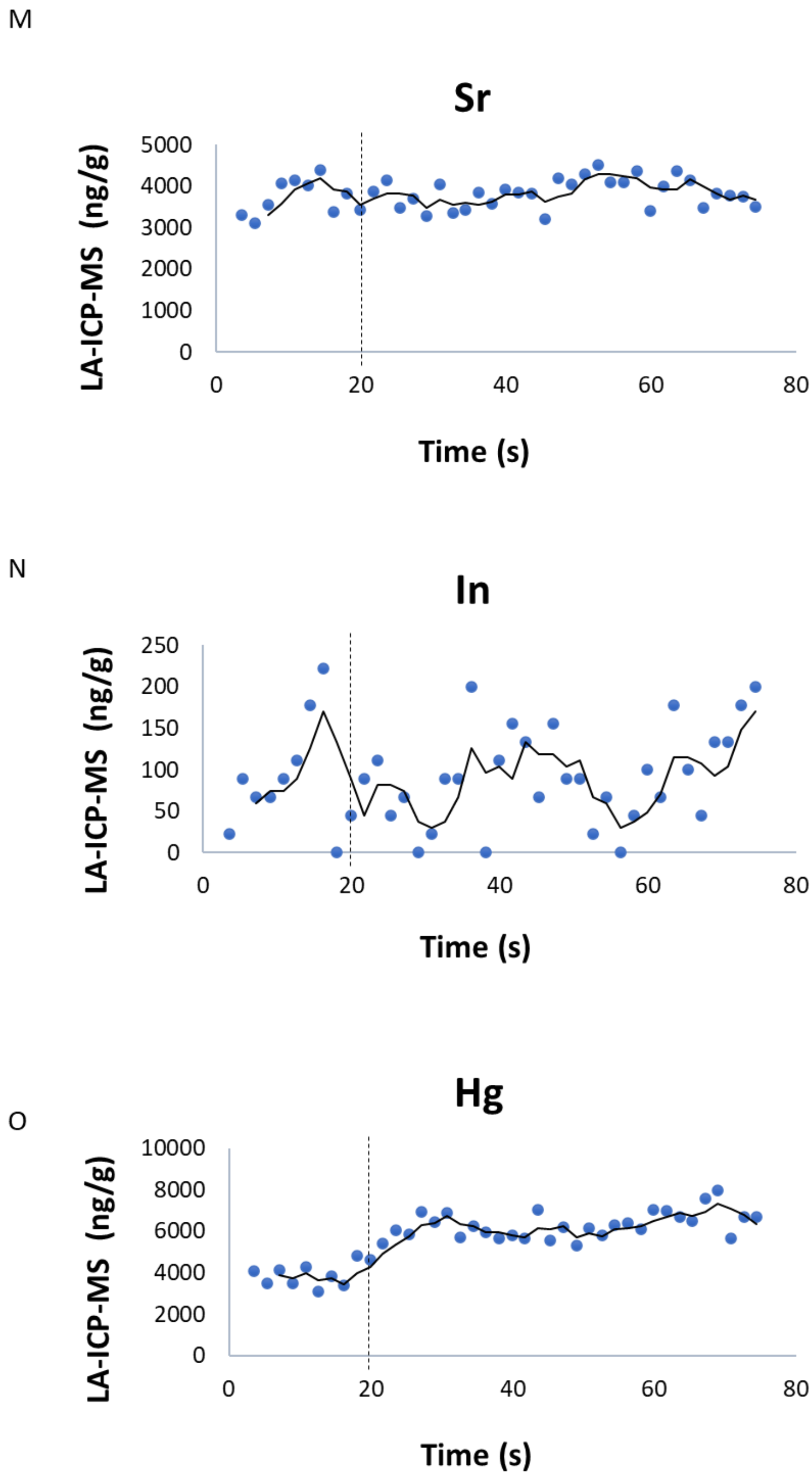
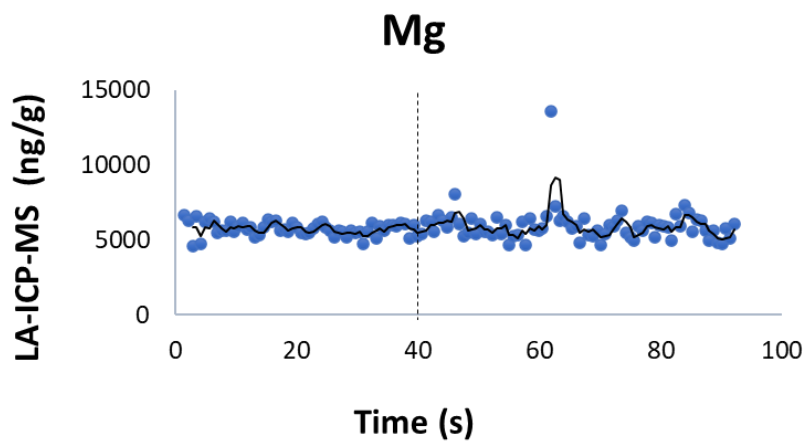
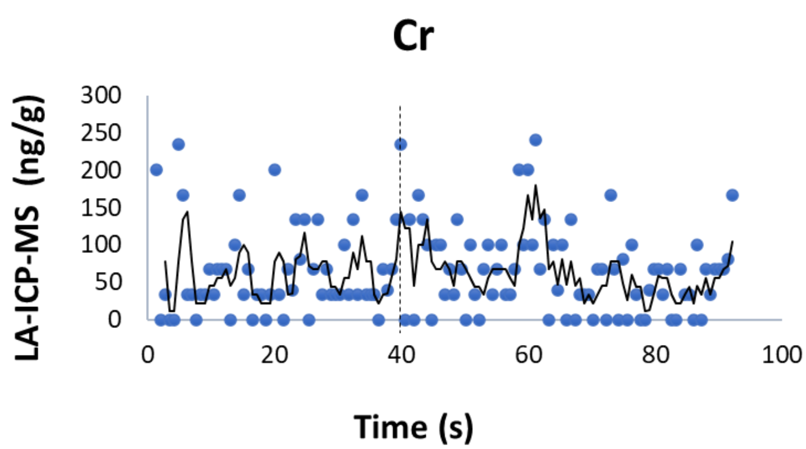


Figure 4.5: LA-ICP-MS linear scan along the clear tape.

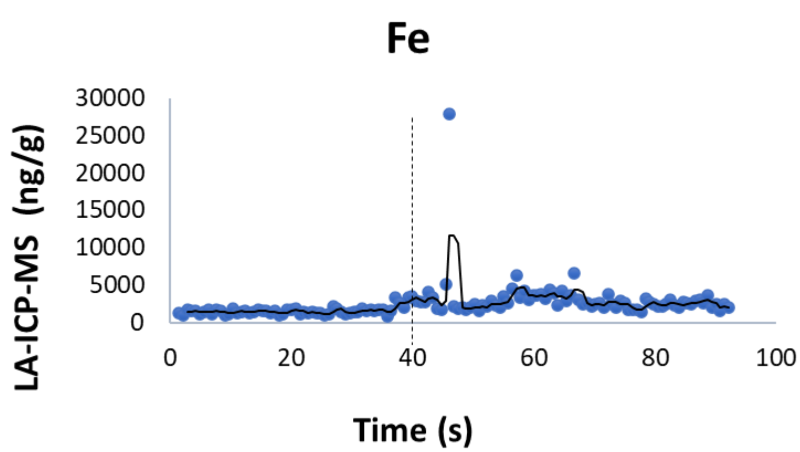
A


Mg
LA-ICP-MS (ng/g)
15000
10000
5000
0
0
20
40
60
80
100
Time (s)

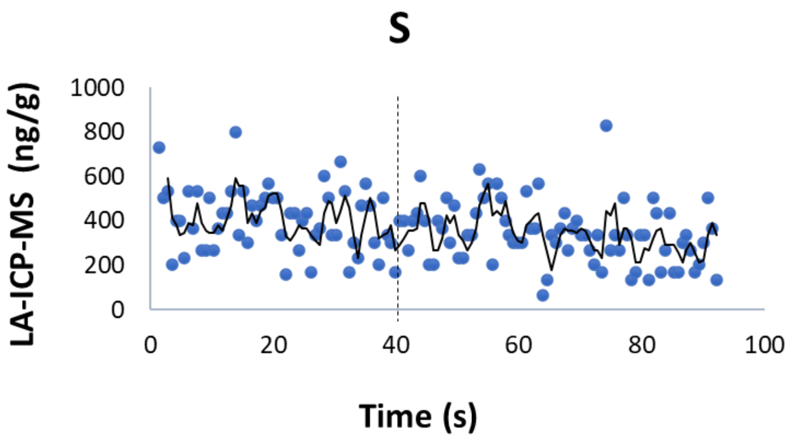
B


Cr
LA-ICP-MS (ng/g)
300
250
200
150
100
50
0
0
20
40
60
80
100
Time (s)

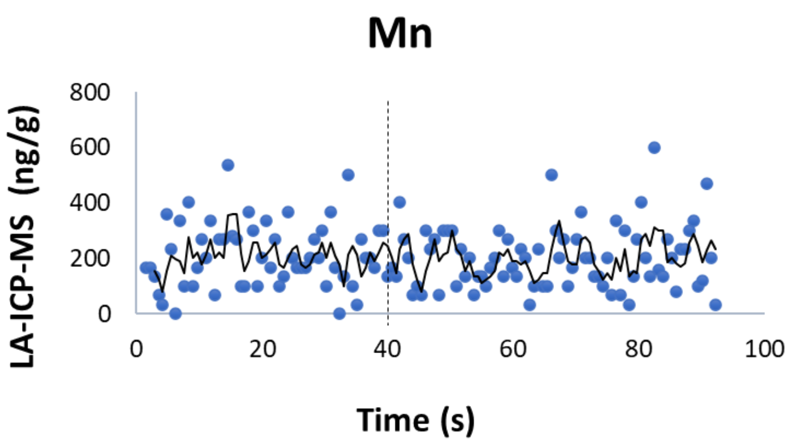
C


Fe
LA-ICP-MS (ng/g)
30000
25000
20000
15000
10000
5000
0
0
20
40
60
80
100
Time (s)

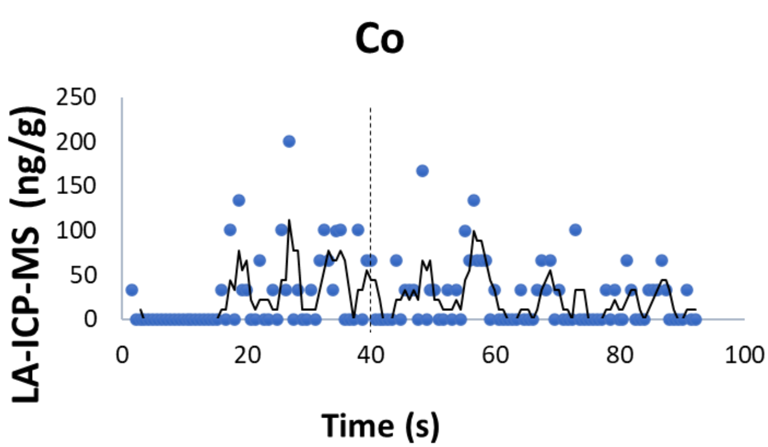
D


S
LA-ICP-MS (ng/g)
1000
800
600
400
200
0
0
20
40
60
80
100
Time (s)

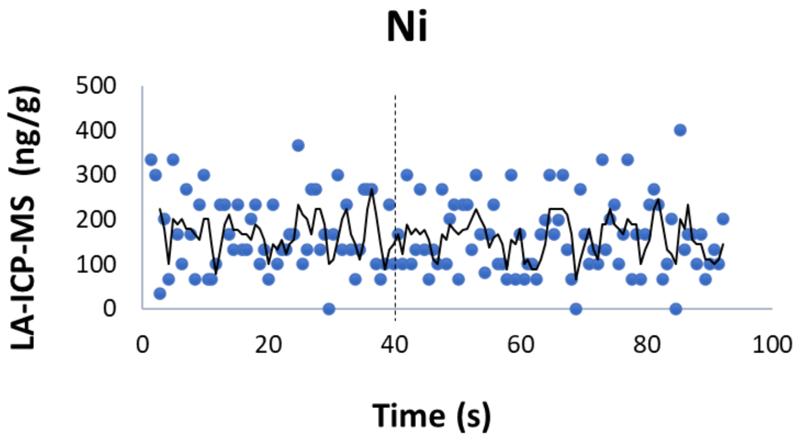
E


Mn
LA-ICP-MS (ng/g)
800
600
400
200
0
0
20
40
60
80
100
Time (s)

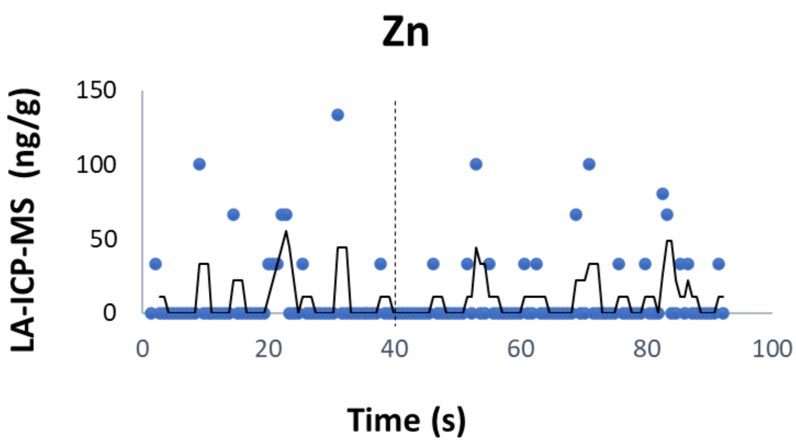
F


Co
LA-ICP-MS (ng/g)
250
200
150
100
50
0
0
20
40
60
80
100
Time (s)

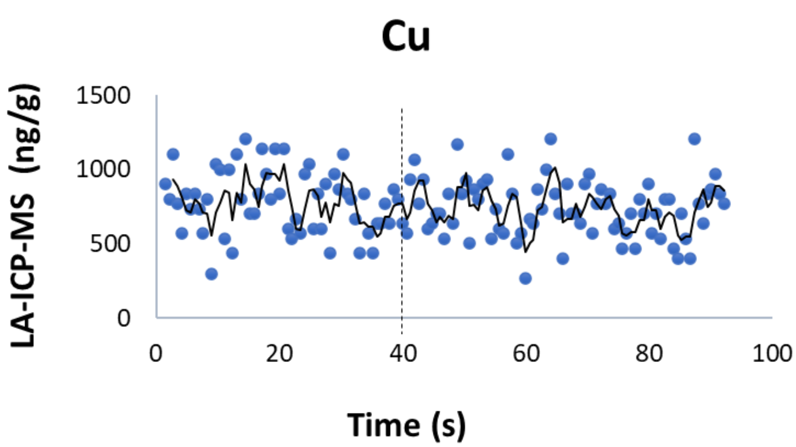
G


Ni
LA-ICP-MS (ng/g)
500
400
300
200
100
0
0
20
40
60
80
100
Time (s)

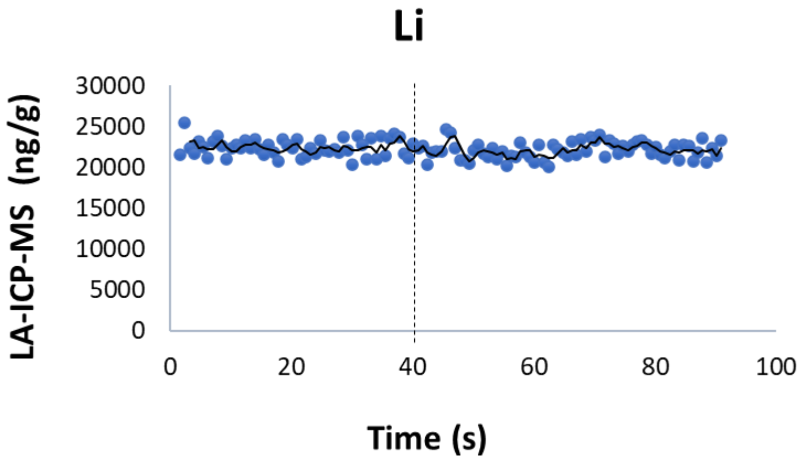
H


Zn
LA-ICP-MS (ng/g)
150
100
50
0
0
20
40
60
80
100
Time (s)

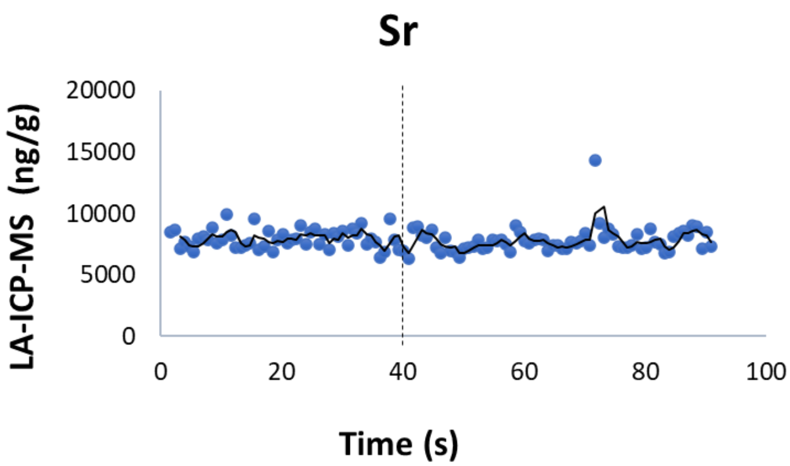
I


Cu
LA-ICP-MS (ng/g)
1500
1000
500
0
0
20
40
60
80
100
Time (s)

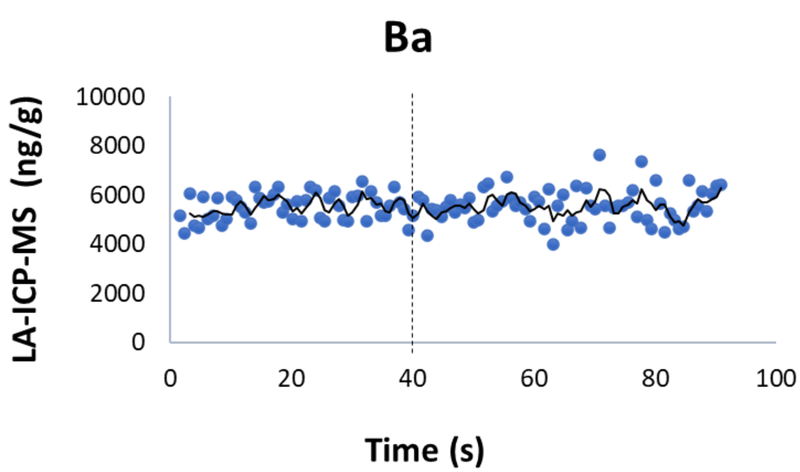
J


Li
30000
25000
20000
15000
10000
5000
0
LA-ICP-MS (ng/g)
0
20
40
60
80
100
Time (s)

K


Sr
20000
15000
10000
5000
0
LA-ICP-MS (ng/g)
0
20
40
60
80
100
Time (s)

L


Ba
10000
8000
6000
4000
2000
0
LA-ICP-MS (ng/g)
0
20
40
60
80
100
Time (s)

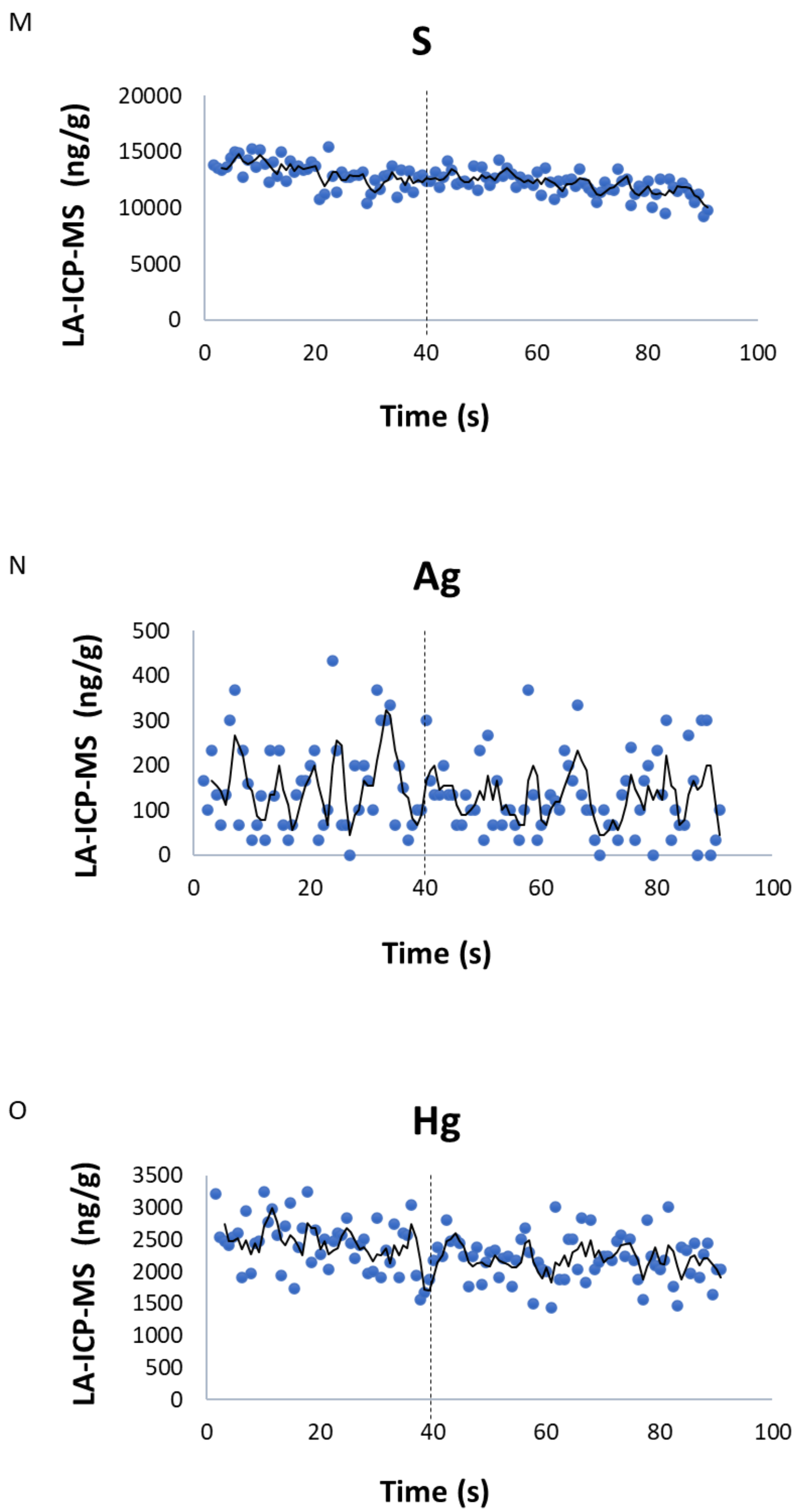


Figure 4.6: LA-ICP-MS linear scan along the ER.

4.3.2 Back-scattered electron image of cross-section hair

A representative Back-scattered electron (BSE) image shows that cross-sections of hair strands are elliptical with ~ 100 μm long semi-major axis and ~50 μm long semi-minor axis (**Figure 4.7**). Cuticle (~5 μm thick) appears brighter than the cortex, and there are several alternating bright and dark layers within the cuticle. The cortex adjacent to the cuticle is brighter than the interior cortex. Because contrast in the BSE image represents mean atomic numbers, bright areas in the image are considered elevated in heavy metals.

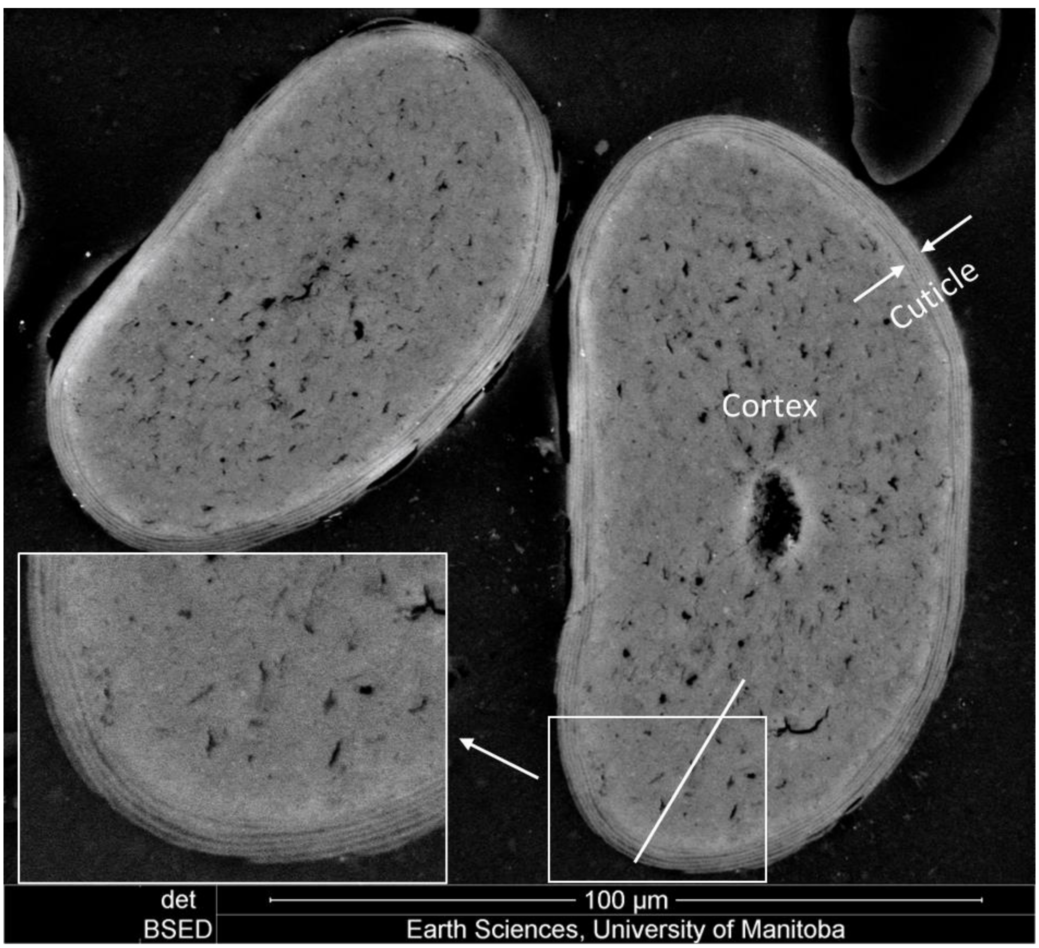


Figure 4.7: A representative BSE image of hair cross-sections. Take note that the cuticle is brighter than the cortex, implying a higher mean atomic number in the cuticle. Inset shows several alternating dark and bright layers within the cuticle.

4.3.3 Analysis of EDS in cross-section hair

In order to identify elements responsible for the contrast in the BSE image, EDS analyses were performed along ~30 μm long line traverse across the cuticle and cortex (**Figure 4.8**).

Major constituents of the hair include C, N, O, and S. However, C is excluded from the discussion because the sample was coated with C. The EDS line profile shows a relatively homogeneous distribution of Sulphur across the cuticle and cortex (**Figure 4.8a**). Background subtracted Calcium is more intense in the cuticle than in the cortex (**Figure 4.8b**). Internal variations of other elements present below the detection limits of the EDS were investigated with LA-ICP-MS.

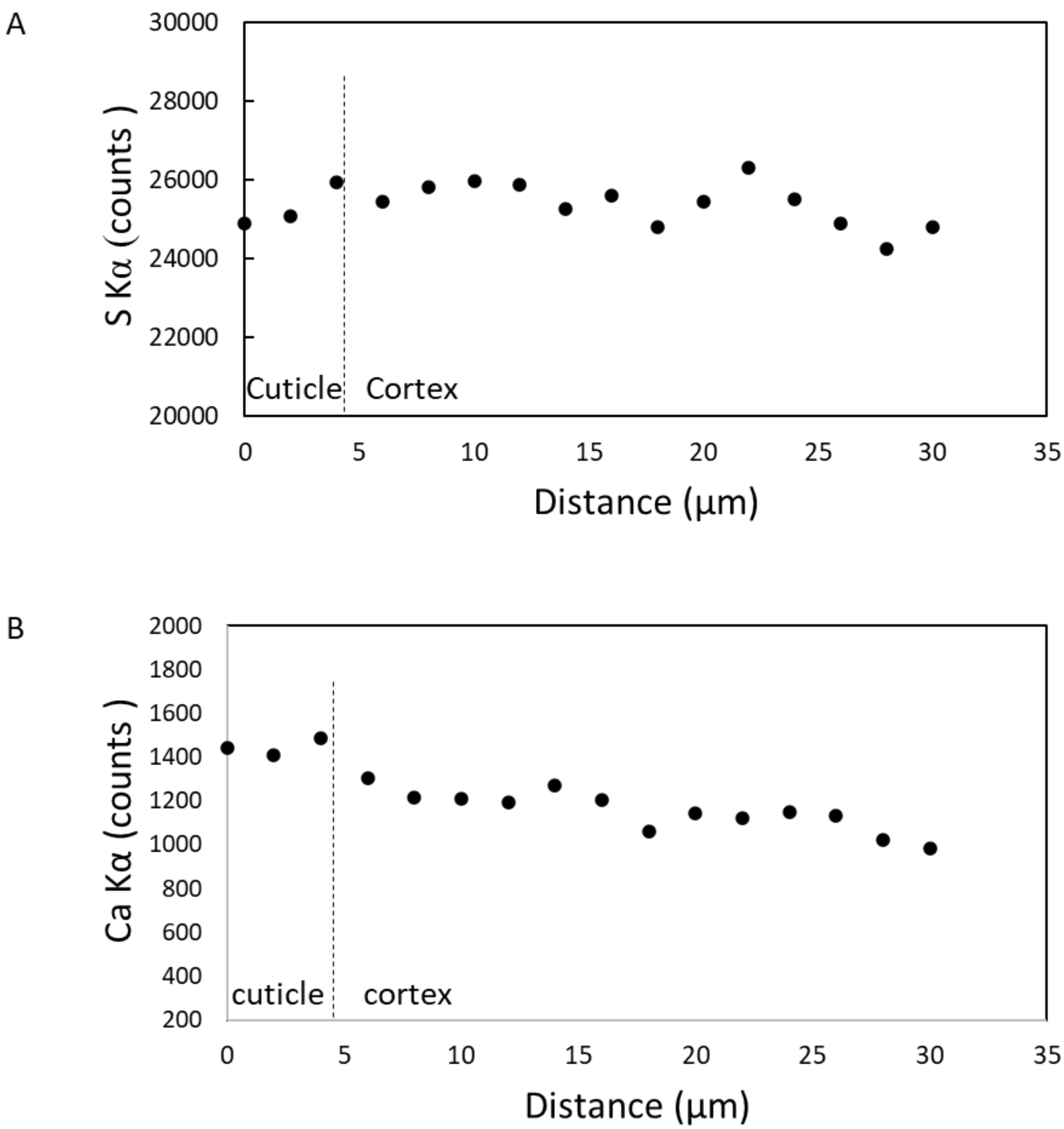


Figure 4.8: EDS line profiles across cuticle and cortex

A. S Kα **B.** Ca Kα. The location of the line profile is indicated in **Figure 4.7**.

4.3.4 ICP-MS and LA-ICP-MS analysis of human hair standard

Figure 4.9 and **Table 4.3** shows a remarkable agreement between most of the certified or reference values and measured values in SN-ICP-MS of the NCS DC73347 human hair standard. Observe that only Li, Ag, and Mg values are reference values (a best estimate of the true value). For this reason, the result of these elements in LA-ICP-MS does not mean a low agreement with the results in SN-ICP-MS. The discrepancies between the certified value of Ni in LA-ICP-MS and SN-ICP-MS are potentially attributed to contamination during open vessel digestion. At the same time, the discrepancy of the Fe and Cr is suggestive of inhomogeneity in the hair standard due to contamination during sample preparation. Ni presented the worst R2 compared to the other elements tested (**Table 4.4**).

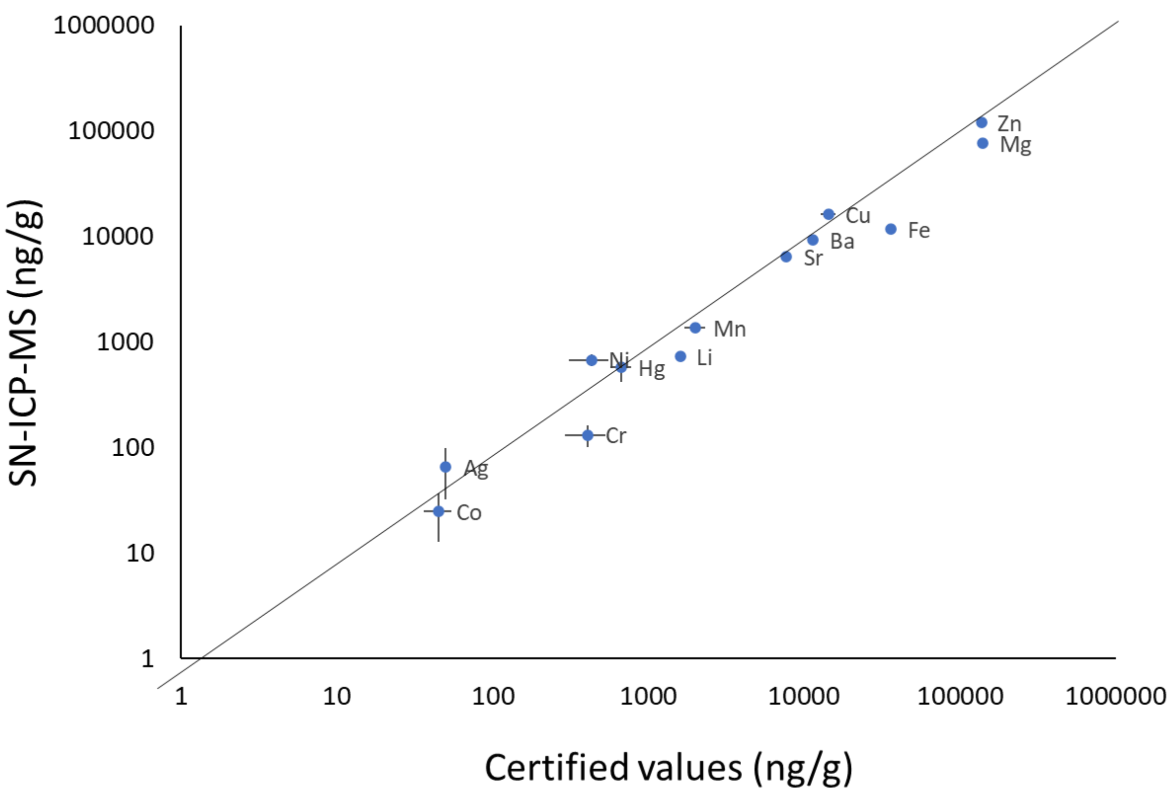


Figure 4.9: Comparison between the certified or reference values and measured values in SN-ICP-MS of the NCS DC73347 human hair standard.

Table 4.3 Compares element concentrations of certified reference values and measured values in SN-ICP-MS of the NCS DC73347 human hair standard.

	SN-ICP-MS				LA-ICP-MS	
	Measured	2SE	DL	R^2	Certified or reference* values	Uncertainty
Li	728	59	0.017	0.9991	1600*	-
Sr	6433	474	0.070	0.9994	7700	400
Ag	65	33	0.048	0.9987	50*	-
Ba	9271	316	0.035	0.9997	11400	600
Hg	579	158	0.320	0.9940	670	100
Mg	76943	2252	1.097	0.9995	140000*	-
Cr	131	30	0.040	0.9998	410	120
Mn	1349	33	0.022	0.9997	2000	300
Fe	11768	356	3.282	0.9997	36000	5000
Co	25	12	0.003	0.9998	45	9
Ni	676	82	0.116	0.9909	430	120
Cu	16198	413	0.038	0.9996	14300	1600
Zn	121109	2198	0.627	0.9996	137000	9000

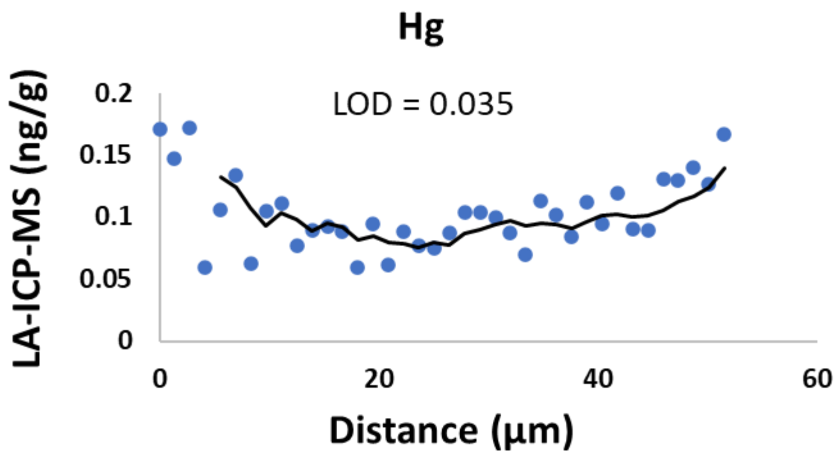
All in ng/g.

4.3.5 Analysis of LA-ICP-MS in cross-section hair

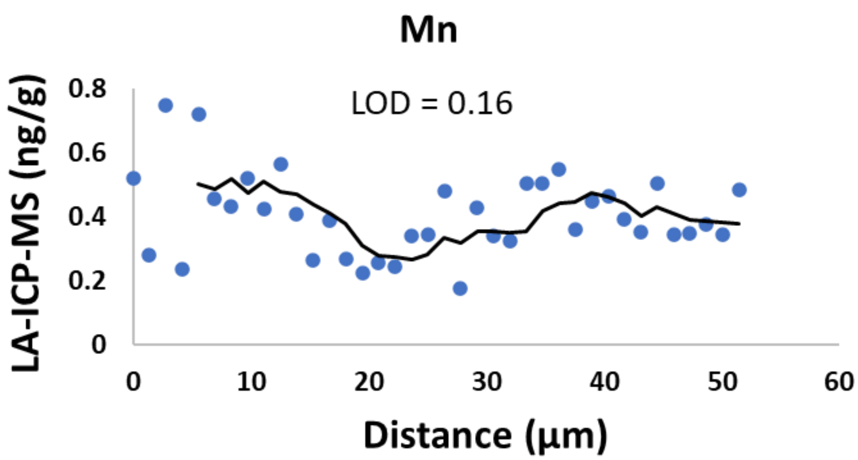
To resolve elemental variations in the cuticle, laser beam size must be at least half of the thickness of the cuticle (~ 5µm). Reducing the beam size to 2 µm, however, is problematic because all trace elements become undetectable. The smallest beam size for the hair cross-section was found to be 40 μm, although Li, Cr, Co, and Ni still fall below the detection limits. Ten cross-sections were ablated from the cuticle to the cortex to the cuticle along the semi-minor axis of the ellipse and a representative cross-section is presented in **Figure 4.7**. Approximately 500 shots of a 40 μm diameter laser beam over a ~50 μm long line inevitably result in substantial signal overlapping. Using this combination, the measured signal intensities are greater, thereby producing a smoother signal.

Despite the smoothing effects, Mn, Cu, Zn, Sr, Ag, and Hg show elevated concentrations in the cuticle compared with the cortex (**Figure 4.10**). In contrast, Mg is more enriched in the cortex than in cuticle. Iron falls mostly below the detection limit. Considering the smoothing effects, trace element enrichment at the cuticle would possibly have been even more dramatic than shown in (**Figure 4.10**). If the majority of heavy metals are concentrated in the cuticle, the analytical results will be heavily influenced by the relative proportions of the cuticle within the ablation volume. To evaluate the possibility, single hair strands were completely and partially ablated, and the results were compared with SN-ICP-MS.

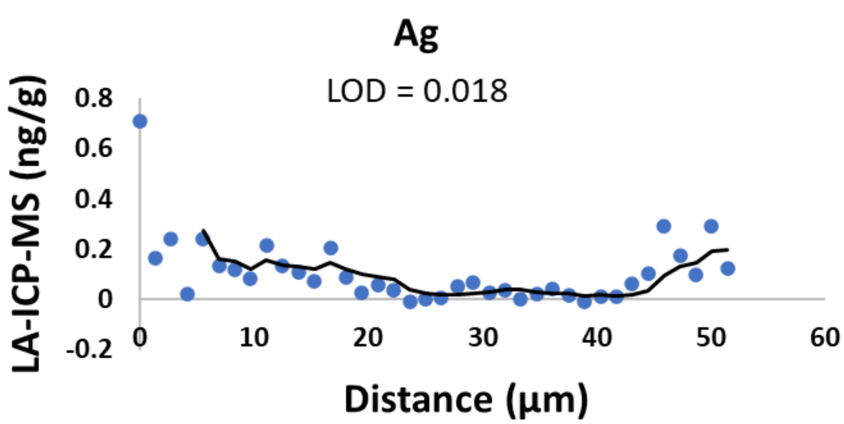
A


Hg
LOD = 0.035
LA-ICP-MS (ng/g)
0.2
0.15
0.1
0.05
0
0
20
40
60
Distance (µm)

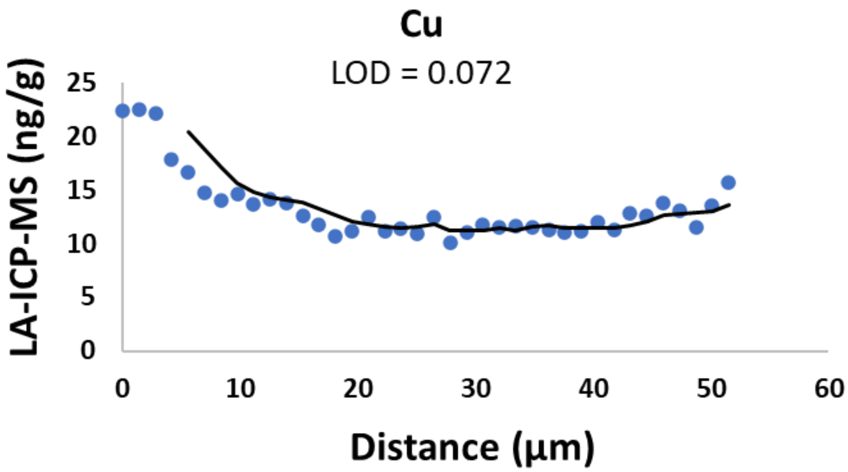
B


Mn
LOD = 0.16
LA-ICP-MS (ng/g)
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Distance (µm)

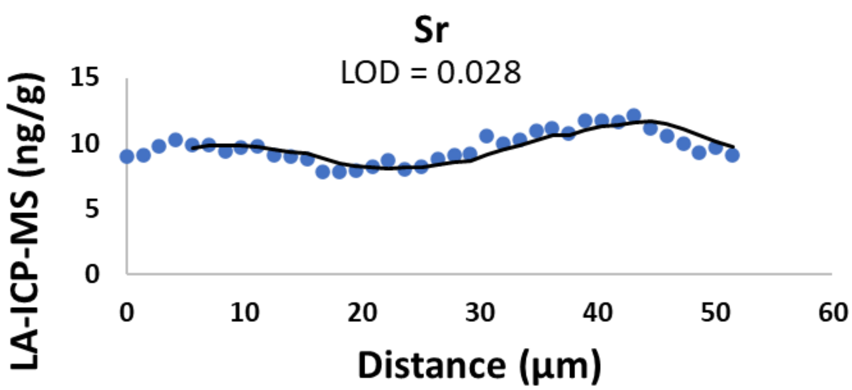
C


Ag
LOD = 0.018
LA-ICP-MS (ng/g)
0.8
0.6
0.4
0.2
0
-0.2
0
10
20
30
40
50
60
Distance (µm)

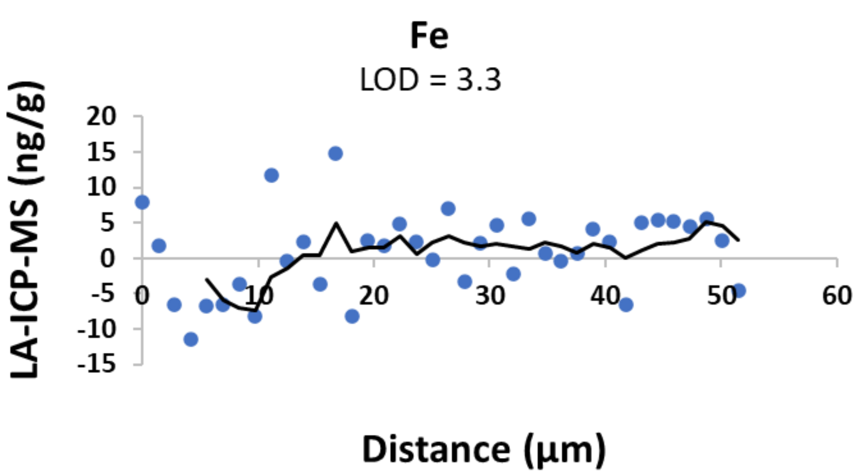
D


Cu
LOD = 0.072
LA-ICP-MS (ng/g)
25
20
15
10
5
0
0 10 20 30 40 50 60
Distance (µm)

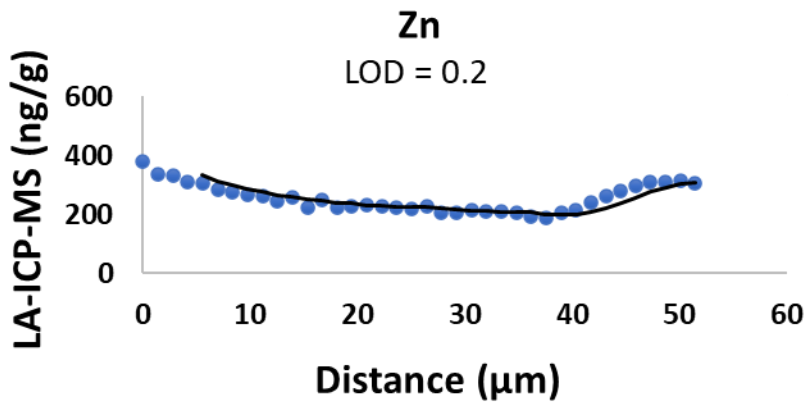
E


Sr
LOD = 0.028
LA-ICP-MS (ng/g)
15
10
5
0
0 10 20 30 40 50 60
Distance (µm)

F


Fe
LOD = 3.3
LA-ICP-MS (ng/g)
20
15
10
5
0
-5
-10
-15
0 10 20 30 40 50 60
Distance (µm)

G



H

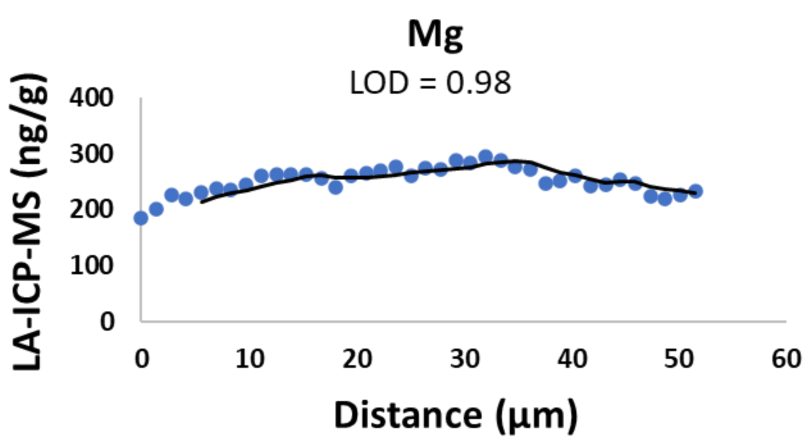


Figure 4.10 LA-ICP-MS linear scan along a cross-section of a hair strand.

4.3.6 Complete and partial ablation of strand hair in LA-ICP-MS compared with SN-ICP-MS

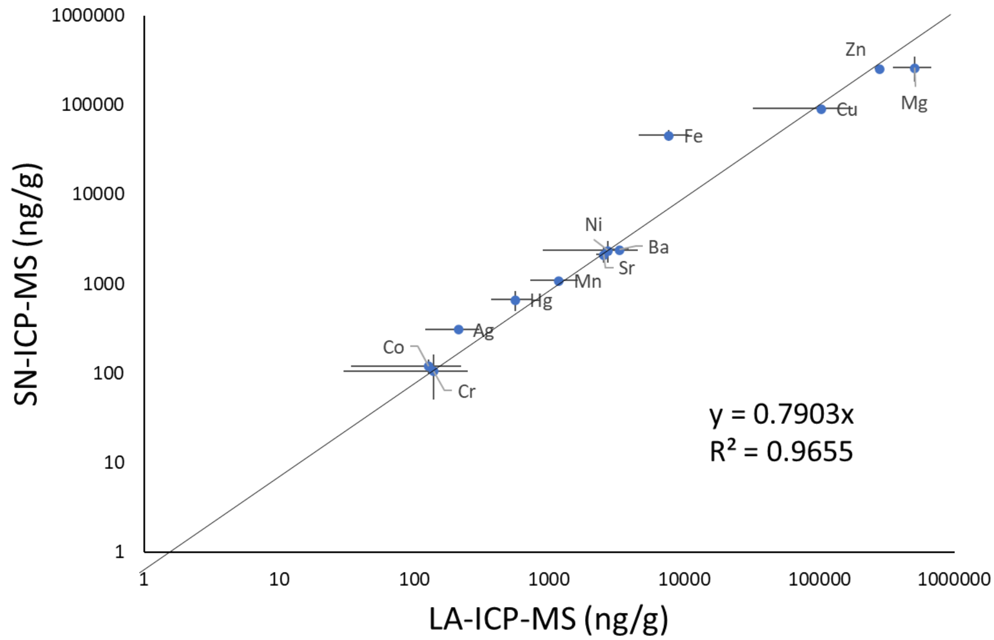
Concentrations of the 13 elements measured by the SN-ICP-MS are given in **Table 4.4**. Detection limits are mostly better than 90 ng/g except for Mg (1.5 µg/g), Fe (3.5 µg/g), and Zn (0.7 µg/g). R^2 values were better than 0.95 except for Mg (0.9322), Fe (0.8934), and Ni (0.6402). Because of the abundance of these three elements in natural environments, the poor R^2 values are attributed to contaminations during sample preparation and ICP-MS analysis.

The analytical results of complete and partial ablation of hair strands from the same individual are also given in **Table 4.4** and graphically compared with SN-ICP-MS in (**Figure 4.11**). Lithium concentrations in both partial and complete analyses are below the detection limits. When the hair strands were ablated completely, the LA-ICP-MS results show a better overall agreement with the SN-ICP-MS (R^2 = 0.9655) than when the hair strands were partially ablated (R^2 = 0.9179) (**Figure 4.11**).

Table 4.4 Comparison between bulk hair (n=3), completely ablated (CA, n=5), and partially ablated hair (PA, n = 5) from the same individual. All in ng/g. 2SE = 2 standard errors.

	SN-ICP-MS				**LA-ICP-MS**			
	Measured	2SE	DL	R^2	CA	2SE	PA	2SE
Li	10	53	0.015	0.9971	<DL	NA	<DL	NA
Sr	2141	436	0.050	0.9980	2596	165	2540	320
Ag	309	22	0.052	0.9979	56	17	212	91
Ba	2424	225	0.027	0.9993	5636	1305	3300	600
Hg	662	164	0.089	0.9748	1694	510	560	190
Mg	263463	83763	1.481	0.9322	368000	110524	510000	160000
Cr	106	55	0.040	0.9968	1664	785	140	110
Mn	1094	84	0.021	0.9938	3574	349	1170	450
Fe	45947	6649	3.462	0.8934	15100	4771	7700	3100
Co	121	22	0.003	0.9970	71.8	32	128	94
Ni	2380	651	0.038	0.6402	2064	379	2700	1800
Cu	90504	6780	0.043	0.9520	51480	1152	104000	72000
Zn	254181	14823	0.665	0.9764	291200	21959	278000	23000

A



B

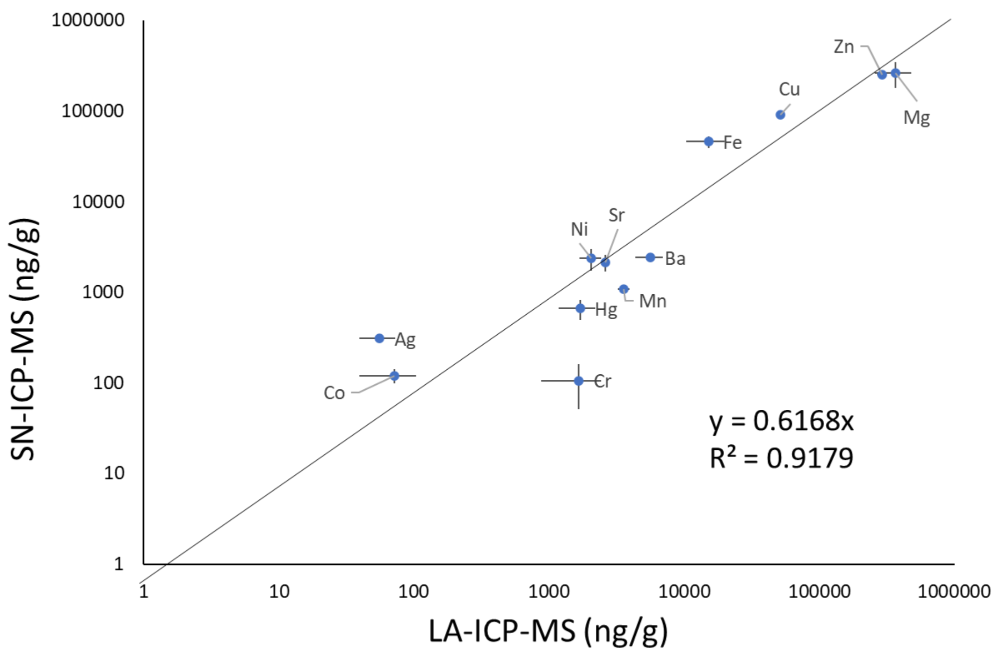


Figure 4.11: Comparison between bulk hair in SN-ICP-MS with complete and partial ablation of a single hair in LA-ICP-MS

A. complete ablation **B.** partial ablation of hair strands from the same individual. The solid lines represent linear regression.

4.4 Discussion

The majority of metal elements investigated in this study was found more concentrated in the cuticle than the cortex. Because of the internal inhomogeneity, complete ablation shows a better agreement with SN-ICP-MS than partial ablation. Furthermore, the results obtained demonstrate that complete ablation of hair strands fixed in ER is an excellent alternative for multiple segment analysis using LA-ICP-MS. Therefore, the first and second null hypotheses were rejected.

In most of the previous LA-ICP-MS work, hair samples have been placed directly on tape or adhesive agents after cleaning steps (Cheajesadagul et al., 2011; Legrand et al., 2004; Luo et al., 2017; Noel et al., 2015; Sach et al., 2022; Sajnog et al., 2018; Stadlbauer et al., 2005). Even though this sample preparation technique is simple, the potential contamination is high, and the detachment of the hair sample from the tape during laser ablation has also been reported (Sajnog et al., 2018). First, to prevent contamination from tape, we had to ensure laser ablation was confined well within the hair samples by partially ablating, leading to loss of sensitivities. Second, the partial ablation of hair can be problematic if the laser penetrates the double-sided tape and ablate slide glass sitting below or if elemental distributions are not internally homogeneous. For example, if an element is preferentially enriched in the cuticle, the results will depend on the sizes of surface areas.

Contrary to the double-sided tape technique (Legrand et al., 2004; Luo et al., 2017; Noel et al., 2015; Sach et al., 2022; Sajnog et al., 2018; Stadlbauer et al., 2005). ER holds the entire hair during ablation and the remaining sample parts for the following segments. Furthermore, the ER-based hair embedding technique allows the complete ablation of hair strands, essential when hair element distribution is inherently non-homogeneous. Indeed, metal distribution in the hair's cortex is known to be heterogeneous (Duncan et al., 2021; Tobin, 2012). Therefore, partial ablation of hair strands can be problematic. In this study, the ER-based hair embedding technique was an excellent alternative to prevent hair sample loss in multiple-segment analysis and prevent analytical errors in complete ablation.

The main limitations of hair analysis in LA-ICP-MS are contamination, memory effects, heterogeneity of standards, and the calibration procedure. (Miliszkiewicz, Walas, & Tobiasz, 2015). In this study, a standard addition technique was employed for the analyses of hair solution samples to reduce matrix effects. Furthermore, the Certified or reference values of the NCS DC73347 human hair standard and element values of complete ablation agree well with digested hair in SN-ICP-MS which suggests a homogeneity of the NCS DC73347 standard, good calibration proceeds with insignificant contamination and memory effects.

To better understand trace element variations along cross-sections of hair strands, we performed a micro-beam analysis along cross-sections of hair strands. The BSE image suggested heterogeneity in the cross-sections of hair strands. The EDS results indicated that the homogeneous distribution of S across the cuticle and cortex (**Figure 4.8a**) agrees with previous studies (Cheajesadagul et al., 2011; Legrand et al., 2004; Noel et al., 2015; Rodushkin & Axelsson, 2003; Sela et al., 2007; Stadlbauer et al., 2005). Due to its homogeneity, S was employed as an internal standard for quantifying LA-ICP-MS results. Because of poor detection limits, however, the only minor element detected with the EDS was Ca. Ca $K\alpha$ signals are more intense in the cuticle than in the cortex (**Figure 4.8b**). The distributions of N and O were also homogeneous (not shown), suggesting similar matrix compositions across the cuticle and cortex. Therefore, the cuticle brighter than the cortex in the BSE image is interpreted to be enriched in heavy metals which also diffuse into the cortex in contact with the cuticle.

Internal variations of other elements present below the detection limits of EDS were investigated with LA-ICP-MS. The LA-ICP-MS analysis in the cross-section hair revealed trace element enrichment at the cuticle, which means the analytical results are heavily influenced by the relative portions of the cuticle ablated. Thus, the bulk hair analyses were compared with complete and partial ablation along the hair strands from the same individual (**Figure 4.11**).

The slopes of liner regression lines are less than the ideal unity in both complete ($y = 0.7903x$) and partial ablation ($y = 0.6168x$), indicating that LA-ICP-MS values are consistently higher than SN-ICP-MS. The systematic discrepancies between the LA-ICP-MS and SN-ICP-MS are attributed to the loss of S which was used as an internal standard in the ablation chamber and during the transportation to the ion source by adsorption to interior walls (Craddock, Rouxel, Ball, & Bach, 2008). In the partial ablation, Hg, Cu, Ba, Mn, Cr, Ag, and Co show greater misfit from the regression line than in complete ablation, suggesting those elements may be more preferentially enriched at the cuticle.

Metals can be incorporated into hair through endogenous and exogenous uptake (Kempson, Skinner, & Kirkbride, 2007; Pozebon, Scheffler, & Dressler, 2017; Sela et al., 2007). In dental environments, nano- and micro-particulates enriched in toxic metals are released into air as vapor. Elemental Hg is particularly an important metal in terms of dental workers' health. This study shows that the majority of metals measured are enriched in the cuticle, consistent with exogeneous origin of the metals. However, the same zoning patterns were found by endogenous absorption of Hg and Pt (Stadlbauer et al., 2005), making the differentiation between the two sources impossible. To distinguish the sources of metals, trace metal concentrations before and after exposure to dental environments should be compared.

In summary, the present study showed that complete laser ablation has a better agreement with the SN-ICP-MS than partial laser ablation, demonstrating the importance of complete ablation in single hair strand analysis by LA-ICP-MS. The developed LA-ICP-MS technique can be applied to monitor the metal levels in the hair of dentists, students, and dental personnel exposed to the dental environment.

Chapter 5 – Biomonitoring of dental students exposed to toxic metals from dental materials.

5.1 Introduction

Particulates matters (PM) and harmful vapors (HV) containing metals are released from dental materials due to high-speed dental equipment usage (Bentley et al., 1994; Han et al., 2021; Krah-Sinan, Adou-Assoumou, Kouakou, Avoaka-Boni, & Kpokpro, 2017; Liu et al., 2019). The issue is that the metals enter the body through different routes of exposure (inhalation, oral, and dermal) and are stored in different body compartments, being a health risk to dental students and workers.

Metal ion (MI) adverse effects in humans have been reported in many parts of the world as a result of the formation of free radicals, DNA damage, lipid peroxidation, and depletion of protein sulfhydryl (e.g., glutathione)(Alissa & Ferns, 2011; Valko et al., 2005). Also, concerns about MI accumulation in the body recently increased because studies have shown that the risk of lung, kidney, brain, and Central Nervous System (CNS) cancers associated with MI among dental workers has amplified (Ahlbom, Norell, Rodvall, & Nylander, 1986; Boffetta, Merler, & Vainio, 1993; Nylander, Friberg, & Lind, 1987; Störtebecker, 1985).

In general, the majority of trace metals present in the human body are investigated using blood and urine analysis (Cheajesadagul et al., 2011; Sela et al., 2007; Stadlbauer et al., 2005). However, hair samples are an excellent biomonitoring substrate for assessing human contaminant exposure. The fact that the scalp has the same blood supply pathway as the brain makes it an even more attractive tool.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is considered a valuable method for hair analysis. This direct solid sampling method provides some advantages when compared to ICP-MS. The hair analysis using LA-ICP-MS allows high sample throughput, does not require previous hair digestion, and the isotope levels can be analyzed along a single hair strand (Cheajesadagul et al., 2011). LA-ICP-MS has already been used to evaluate the long-term variations of trace element levels applied in geological (Chew, Drost, Marsh, & Petrus, 2021), biological (Taylor, Branch, Crews, Halls, & White, 1995), and medical research studies (Sussulini, Becker, & Becker, 2017). However, in dentistry, few studies have used LA-ICP-MS method (Arora et al., 2006; Charadram et al., 2013; Hanć, Olszewska, & Barałkiewicz, 2013; Hare, Austin, Doble, & Arora, 2011).

The health effects of certain PMs and HV from dental materials have been widely reported (Anglen et al., 2015; Bauer, 1985; Bittner et al., 1998; Bjørklund, 1991; Bjørklund et al., 2017; Bjørklund, Chirumbolo, et al., 2019; Bjørklund, Hilt, et al., 2019; Björkman et al., 2012; Echeverria et al., 1995; Goodrich et al., 2013; Hill et al., 1998; Lindbohm et al., 2007; Ngim, Foo, & Boey, 1992; Okamoto et al., 2017; Rustemeyer & Frosch, 1996; Shapiro et al., 1982; Wallenhammar et al., 2000; Wang et al., 2020; Warshaw et al., 2022; Werfel et al., 2018; Wrangsjö et al., 2001) (Seldén et al., 1995; Wrangsjö et al., 2001), however, there is no information regarding the actual contamination of toxic metals in dental students from their exposure to dental materials in the dental school. Also, it is not yet well understood how dietary, lifestyle habits, and the presence of amalgam filling affect the MI accumulation in the body of dental students.

In this study, we used the LA-ICP-MS technique to compare the concentration of 13 MI (Hg, Li, Mg, Cr, Co, Ni, Ag, Zn, Cu, Mn, Fe, Sr, and Ba) in the hair of dental students during four years of dental school. A pilot study was conducted first, a single hair analysis in different populations of dental students from the first- and fourth years of dental school. The null hypotheses were that (1) there is no significant difference between metal elements concentration in the hair of dental students during 4 years of dental school, (2) there is no significant difference between metal elements concentration in the hair of different dental students from the first- and fourth year of dental school, (3) the class factor would not influence the metal elements concentration in hair of the dental students, and (4) fish consumption would not influence the metal elements concentration in hair of the dental students.

5.2 Materials and methods

5.2.1 Ethics statement

The study protocol was approved by the Health Research Ethics Board (HREB HS21107-H2017:299). All Subjects in the present study were adequately instructed and consented to participate by signing the appropriate informed consent paperwork. Also, efforts were made to protect student privacy and anonymity.

5.2.2 Selection of subjects

5.2.2.1 General inclusion criteria

Age between 18 to 40 years old, and good general health.

5.2.2.2 Inclusion criteria for four years of the follow-up study

A student in the first year of Dental School at the University of Manitoba in 2018 and continuing studies in 2019, 2020, and 2021.

5.2.2.3 Inclusion criteria for the pilot study

A dental student in the first or fourth year of Dental School at the University of Manitoba.

5.2.2.4 General exclusion criteria

A student who received metal poisoning treatment, who worked in the metal industry, and who ingested food supplements containing elements analyzed in this study within the last three months.

A previous questionnaire was applied, including questions on their issue (age, sex); lifestyle habits (hair coloring); dietary factors (fish and tinned food consumption); other (amalgam filling).

5.2.2.5 Pilot study population.

A total of 28 volunteer dental students participated in this study, 14 dental students enrolled in the 1st year and 14 in the 4th year of dental school in 2017.

5.2.2.6 Follow-up study population.

A total of 10 volunteer dental students participated in this study, dental students enrolled in the 1st year of 2018 and remained in the dental school until 2022

5.2.3 Sample preparation

The hair sample was taken from the occipital area using a ceramic scissor. All hair strands were cut 2 cm from the root and washed according to the IAEA protocol (46). All samples were stored in separate labeled tube vials and kept at room temperature. In the pilot study, a single hair collection occurred in 2017 (1st YDS) and 2018 (4th YDS). In the follow-up study, the samples were collected in 2018 (1st YDS), 2019 (2nd YDS), 2020 (3rd YDS), and 2021 (4th YDS) from the same individuals.

All hair samples were washed with reagent-grade acetone for 10 min to remove potential contaminants and then washed with de-ionized water (18.3 MΩ/cm, Millipore, Bedford, MA, USA) for 10 min in three consecutive steps followed by a final rinse with acetone. Cleaned hair samples were dried overnight under a laminar flow hood. Dried hair strands were hand-picked and placed on ~ 5 mm thick epoxy resin before curing under the laminar flow hood.

5.2.4 Preparation of pressed pellet made with powder hair standard for LA-ICP-MS

A solid flat pellet was made of a human hair standard in powder (NCS DC73347) from the Chinese National Analysis Center for Iron & Steel – NACIS (Haidian District, Beijing, China) for LA-ICP-MS analysis in this study. Approximately 300 mg of the hair powder was pressed for 60 sec at 10 tons using a pellet maker (SPESS, Metuchen, NJ, USA) without adding a bonding agent. Thin Teflon disks were placed at metal contacts to prevent contamination.

5.2.5 Mounting hair technique in epoxy resin slide (ERS)

The ERS (Buehler Ltda., Lake Bluff, IL, United States) was prepared according to the manufacturer's instruction under a lamina flow hood to prevent contamination and poured onto a mold to make ERS with ~0.5 mm thickness. A thin layer of epoxy resin was added to the ERS surface of the cured ERS. Then, the hair strands were hand-picked with a plastic tweezer and glued with a fresh ERS under the lamina flow hood. ERS adjacent to the hair strands was periodically analyzed with the LA-ICP-MS to obtain blank levels.

5.2.6 Instrumentation

The hair strands were ablated using a New Wave Research UP-213 Nd:YAG laser ablation system (Fremont, CA, USA) attached to a Thermo Finnigan Element 2 ICP-MS (Bremen, Germany). Li, S, Sr, Ag, Ba, and Hg were analyzed in low resolution (~300), while Mg, S, Cr, Mn, Fe, Co, Ni, Cu, and Zn in medium resolution (~4000) to avoid potential spectral interferences. Optimized instrument conditions and data acquisition parameters for LA-ICP-MS are summarized in **Table 5.1**.

Table 5.1 Operation conditions and data acquisition parameters for LA-ICP-MS hair analyses.

SN-ICP-MS			
Plasma power (W)	1303		
Cool gas flow rate (L/min)	16		
Auxiliary gas flow rate (L/min)	0.80		
Sample gas flow rate (L/min)	0.89		
LA- ICPMS	*Complete ablation*	*Partial ablation*	*Line profile*
Plasma power (W)	1240	1235	1241
Cool gas flow rate (L/min)	16	16	16
Auxiliary gas flow rate (L/min)	0.81	0.78	0.83
Sample gas flow rate (L/min)	0.62	0.65	0.7
Repetition rate (Hz)	20	10	10
Beam diameter (μm)	100	70	40
Energy density ($J/cm^{2)}$)	~6.0	~6.0	~4.5
Scan speed (μm/s)	5	5	1

Signal intensities from hair samples were calibrated against the human hair standard and internally normalized to 4.77 wt% of Sulphur (33S isotope) (Dressler et al., 2010; Legrand et al., 2004; Pozebon et al., 2014; Rodushkina & Axelsson, 2000; Sela et al., 2007; Stadlbauer et al., 2005; Steely et al., 2007) in the hair samples. For laser ablation along the hair strands, 10 segments (300 μm long) perhair strand were ablated at the speed of 5 μm/s; 5 segments in low resolution and another 5 in medium resolution with a ~300 μm gap between the segments. The total length of hair ablated, therefore, becomes 3 mm spanning ~5.7 mm. The ER slide near the hair strands was ablated before and after hair analyses, and their signal intensities were subtracted from total intensities to correct for background level. DL for LA-ICP-MS experiments was defined by three standard deviations from the mean (3-sigma) of the background.

Certified Reference Material DC73347 (GBW07601) from the Chinese National Analysis Center was used as an external calibration standard after being prepared as a pressed pellet. Each hair strand mounted in ER slide was ablated entirely, leading to the ablation of a portion of the ER slide. The ablated hair samples were carried by the argon gas to the inductively coupled plasma source. For final results, blank levels from gas and ERS were first subtracted from total counts. Then, the results were internally normalized against ^{33}S and externally standardized against the human hair standard (NCS DC73347). Data reduction was performed using Iolite V. 3.7 (Iolite Softwares Inc., Maryland, United States).

5.2.7 Statistical methods

Data for MI levels were tested for normality and homogeneity. Then, two-way ANOVA with $\alpha=0.05$ was applied to determine possible significant differences between students four years exposed to dental materials exposure vs students non-exposed (first-year dental student). Finally, Tukey's post hoc Test was performed to verify the significance of two factors (Fish consumption and class) upon Hg concentration. Kruskal–Wallis test was applied for the rest of the elements. Differences were considered statistically significant when $p>0.05$. The statistical analysis was performed using OriginLab data analysis software (Northampton, Massachusetts, USA).

5.3 Results

5.3.1 Pilot study

Figure 5.1 shows that normality was rejected for almost all the elements tested except for Hg concentrations (**Figure 5.1**). Furthermore, the results showed that age (**Table 5.2**), sex (**Table 5.3**), hair coloring (**Table 5.4**), tinned food consumption (**Table 5.5**), fish consumption (**Table 5.6**), and presence of amalgam filling (**Table 5.7**) was not a statistically significant factor for increasing Hg concentration ($p<0.05$). On the other hand, the year of study was statistically significant for increasing the Hg concentration ($p<0.05$).

NormalityTest

Shapiro-Wilk

	DF	Statistic	p-value	Decision at level(5%)
Hg	28	0.9559	0.27773	Can't reject normality
Li	28	0.81198	1.74927E-4	Reject normality
Sr	28	0.64938	5.92066E-7	Reject normality
Ag	28	0.33502	3.28592E-10	Reject normality
Ba	28	0.64669	5.46718E-7	Reject normality
Mg	28	0.66025	8.19688E-7	Reject normality
Cr	28	0.70199	3.04009E-6	Reject normality
Mn	28	0.8506	9.61375E-4	Reject normality
Fe	28	0.67949	1.48097E-6	Reject normality
Co	28	0.69872	2.73332E-6	Reject normality
Ni	28	0.899	0.01084	Reject normality
Cu	28	0.67588	1.32331E-6	Reject normality
Zn	28	0.57993	8.45873E-8	Reject normality

Figure 5.1 Image of the normality test result in the Origin software.

Table 5.2 Range and the average age of dental students from the 1st and 4th year of dental school.

YDS	*Range of age*	*Average of Age*
1st	20-27	23
4th	23-35	27

Table 5.3 Sex of the students from the 1st and 4th year of dental school.

YDS	*Male*	*Female*
1st	50 %	50%
4th	36%	64%
Total of participants	43%	57%

Table 5.4 Percentage of hair coloring in students from the 1st and 4th year of dental school.

YDS	*Yes*	*No*
1st	18%	32%
4th	18%	32%

Table 5.5 Tinned food consumption by students from the 1st and 4th year of dental school.

YDS	*Yes*	*No*
1st	46%	4%
4th	39%	11%

Table 5.6 Percentage of fish consumption (g/month) by students from the 1st and 4th year of dental school.

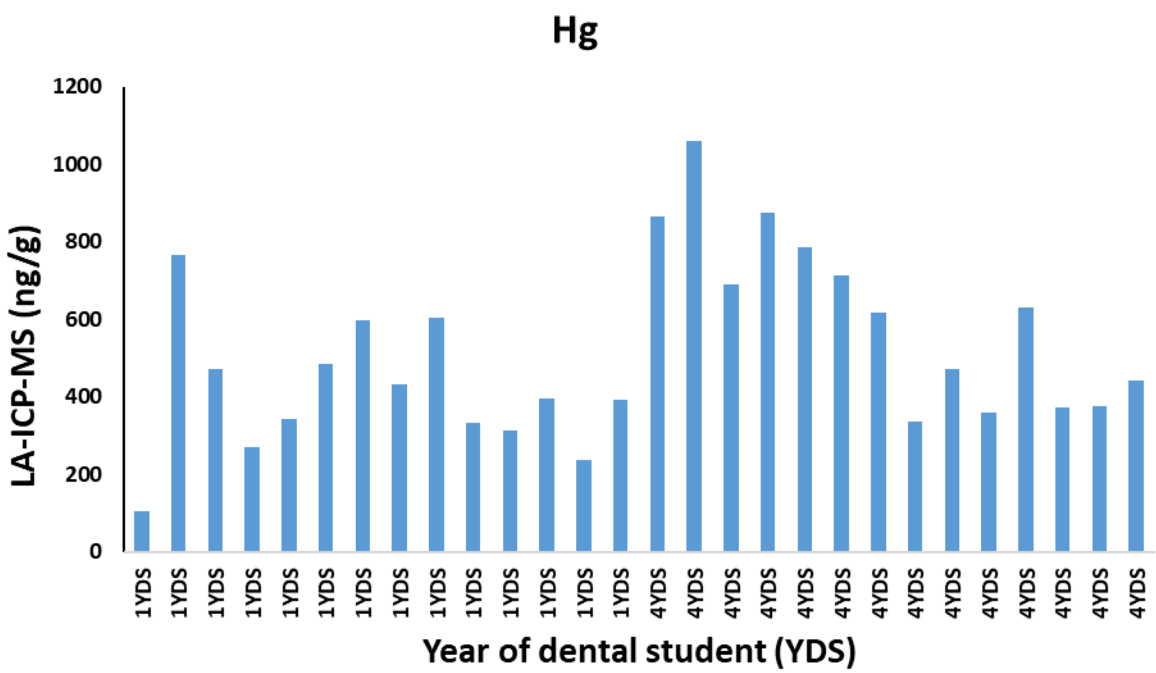
Fish consumption (g/month)	*YDS*	*Percentages of fish consumption*
0g	*1st*	12%
	4th	16%
500g	*1st*	20%
	4th	8%
1kg	*1st*	20%
	4th	16%
1.5kg	*1st*	4%
	4th	4%

Table 5.7 Percentage of amalgam fillings in the mouth of students from the 1st and 4th year of dental school.

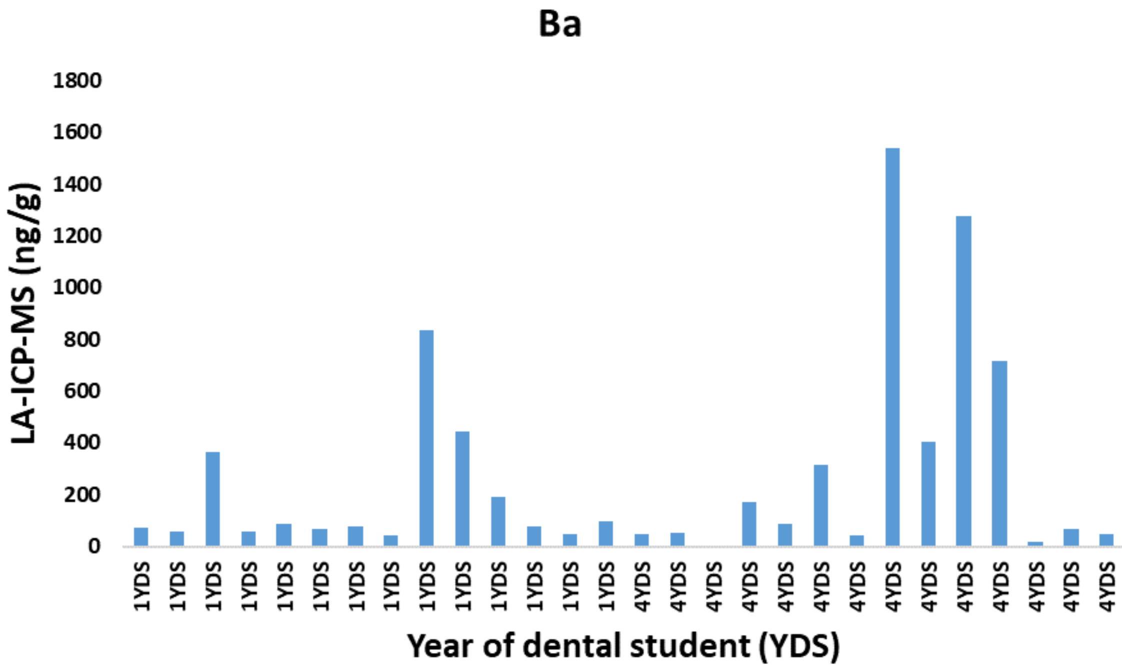
YDS	*Presence of amalgam filling*	
	yes	**No**
1st	14%	36%
4th	7%	43%

Among 13 metal ions (Li, S, Sr, Ag, Ba, Hg, Mg, S, Cr, Mn, Fe, Co, Ni, Cu, Zn) tested in this study, Hg showed a higher concentration in the hair of 4YDS compared with the 1YDS ($p<0.05$). The results of the Hg concentration (ppb) from first-year dental students (1YDS) and fourth-year dental students (4YDS) are presented in **Figure 5.2, Figure 5.3,** and **Table 5.8**.

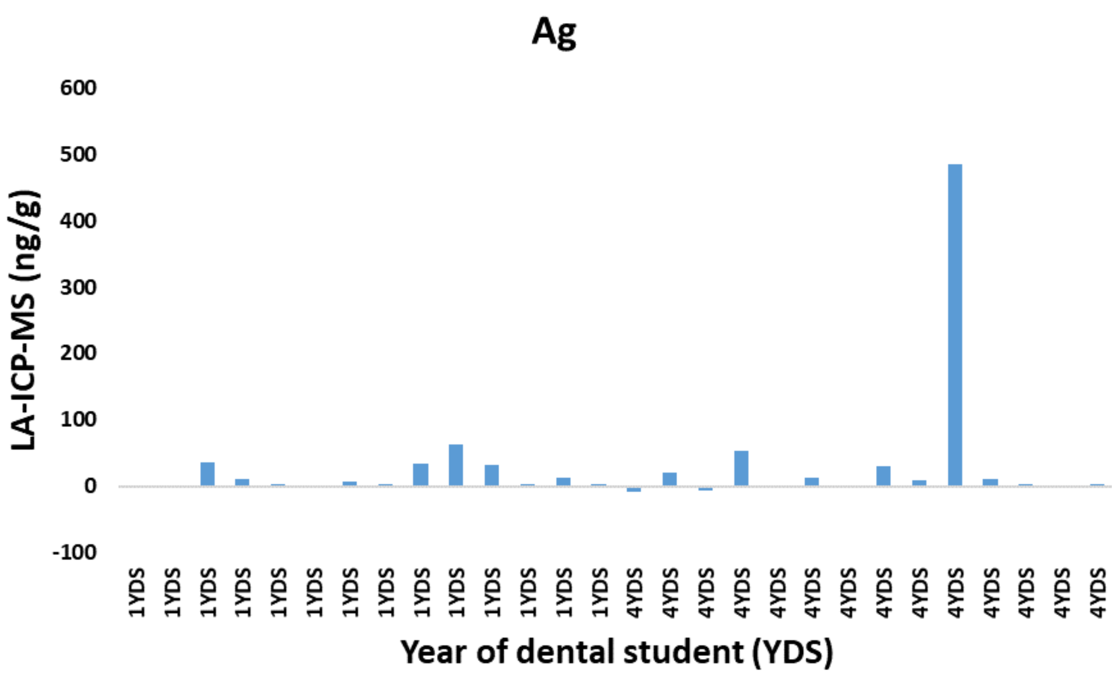
A



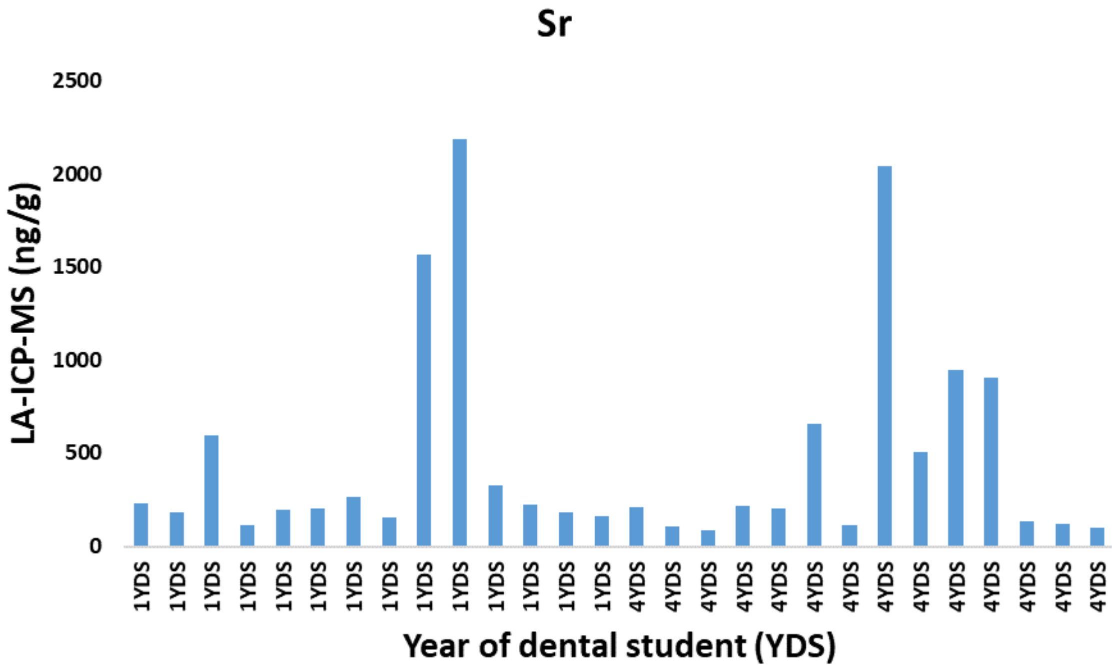
B


Ba
LA-ICP-MS (ng/g)
1800
1600
1400
1200
1000
800
600
400
200
0
1YDS
4YDS
Year of dental student (YDS)

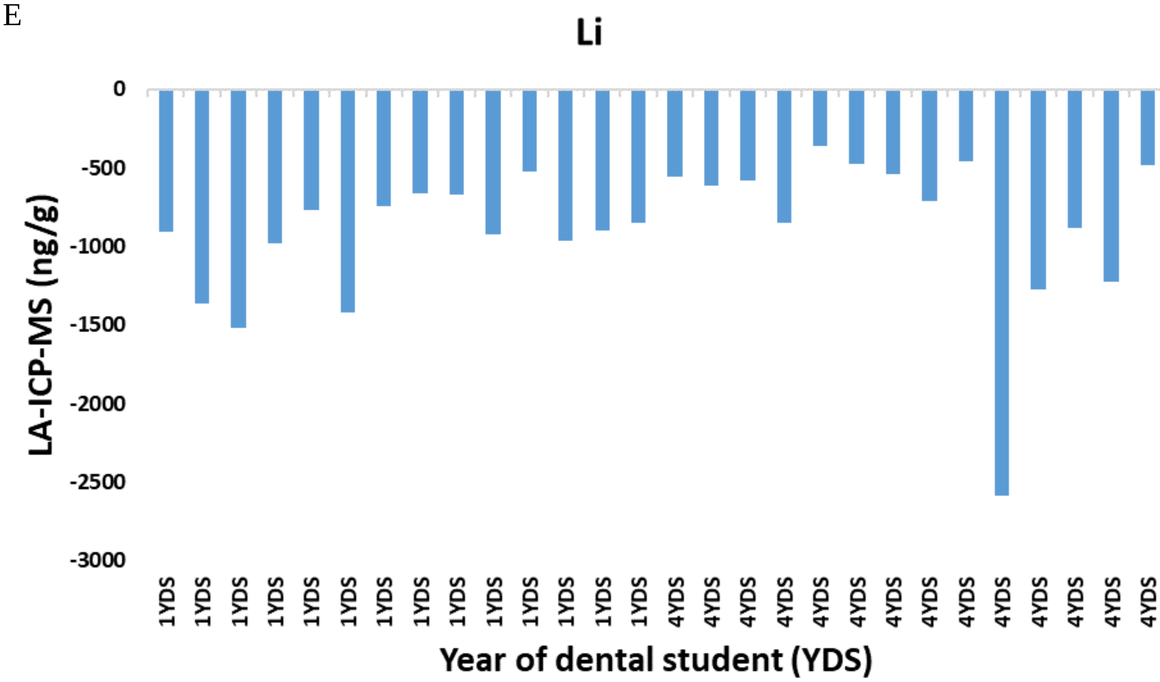
C


Ag
LA-ICP-MS (ng/g)
600
500
400
300
200
100
0
-100
1YDS
4YDS
Year of dental student (YDS)

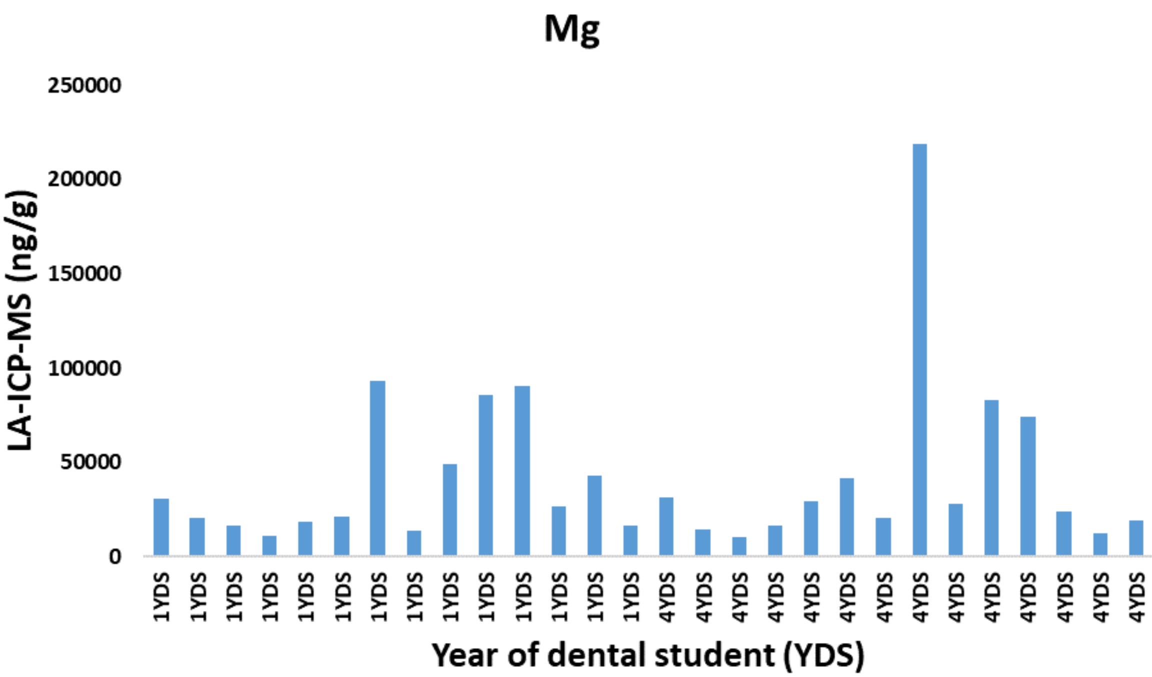
D


Sr
2500
2000
1500
1000
500
0
LA-ICP-MS (ng/g)
1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS
Year of dental student (YDS)

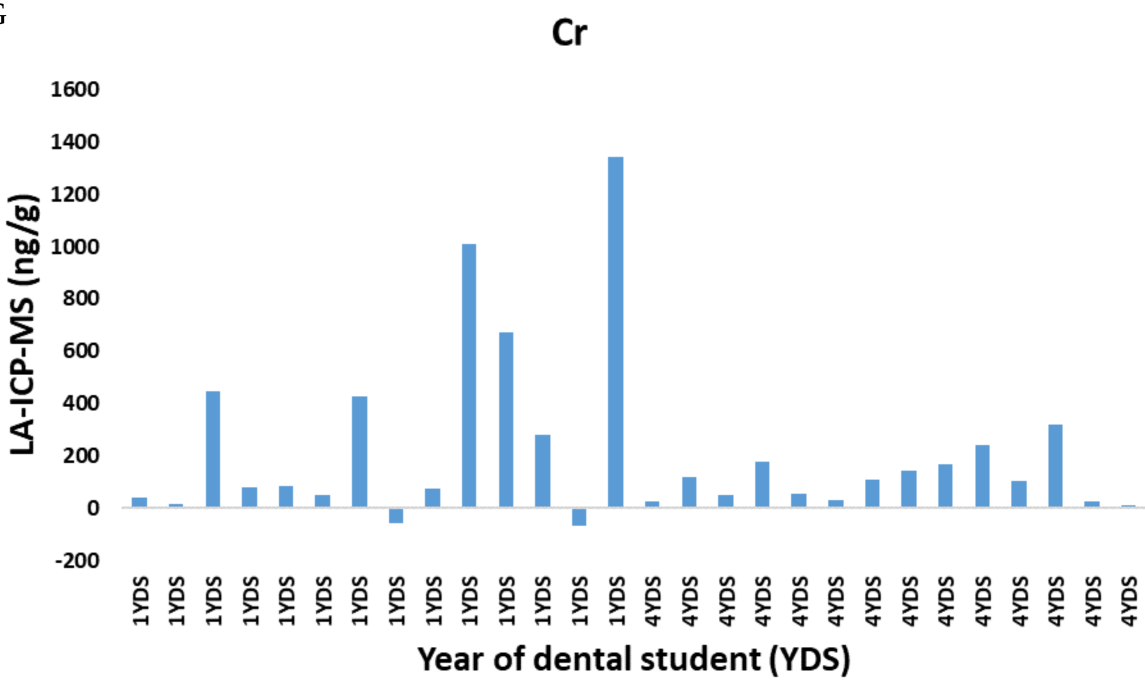
E


Li
0
-500
-1000
-1500
-2000
-2500
-3000
LA-ICP-MS (ng/g)
1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 1YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS 4YDS
Year of dental student (YDS)

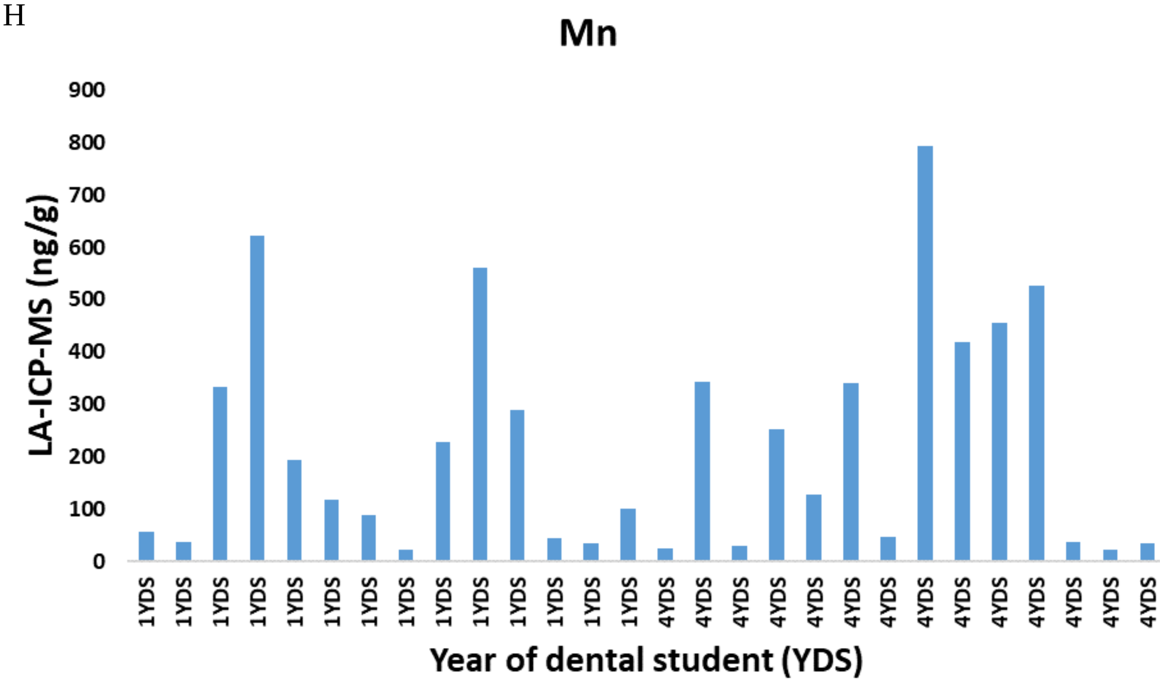
F


Mg
250000
200000
150000
100000
50000
0
LA-ICP-MS (ng/g)
1YDS
4YDS
Year of dental student (YDS)

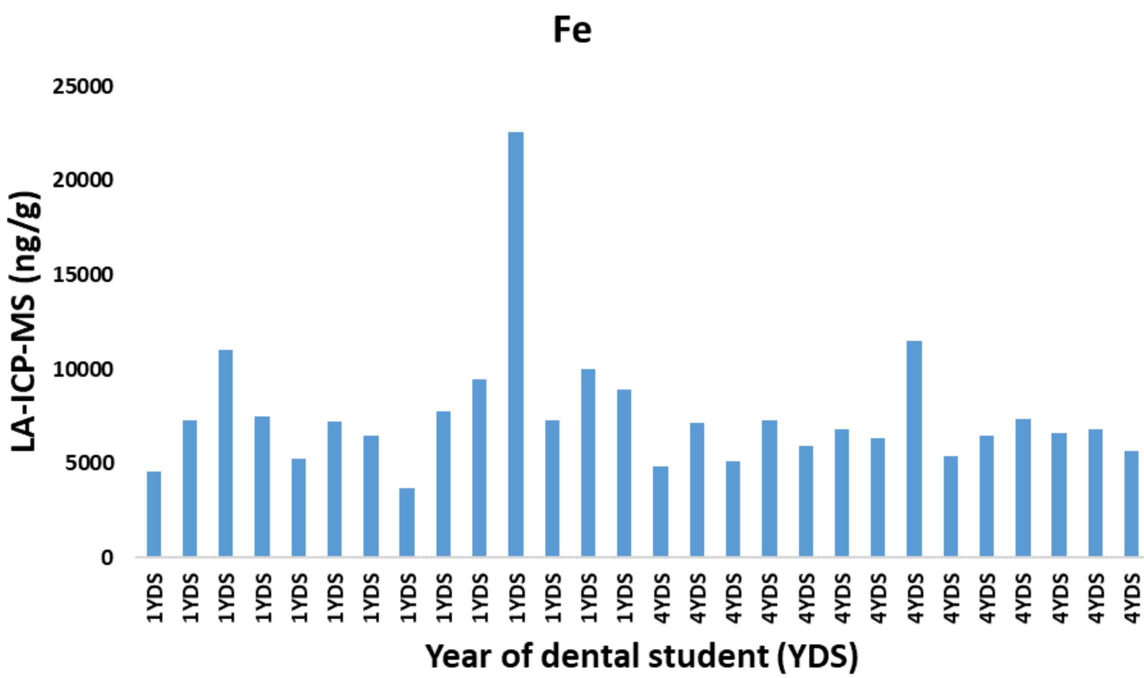
G


Cr
1600
1400
1200
1000
800
600
400
200
0
-200
LA-ICP-MS (ng/g)
1YDS
4YDS
Year of dental student (YDS)

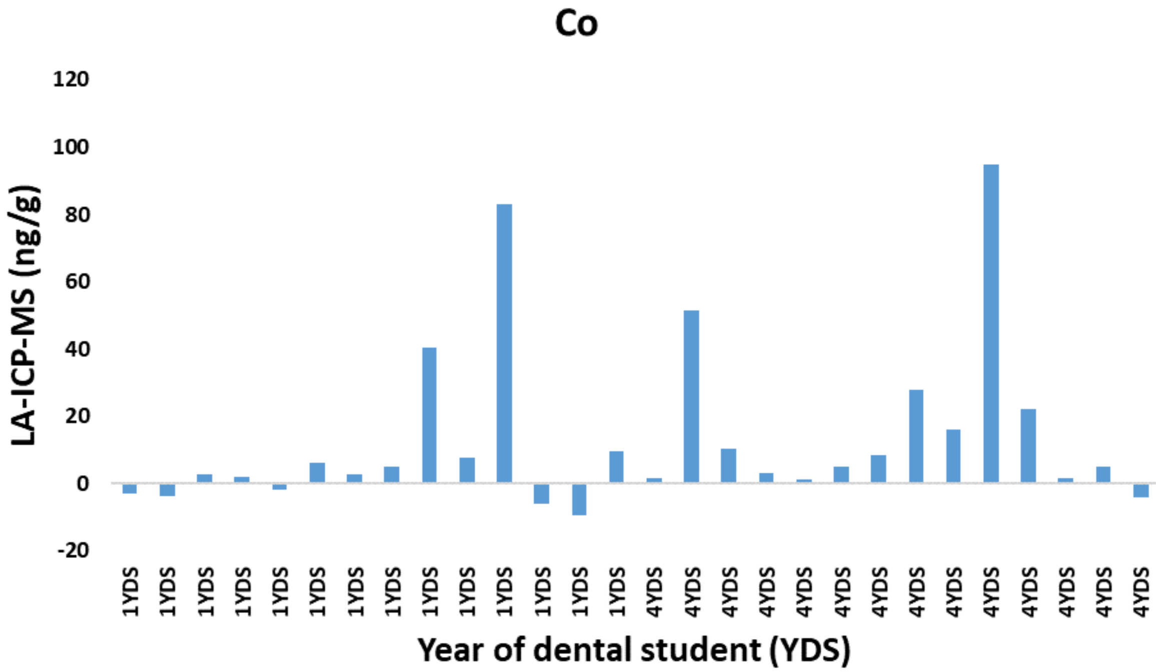
H


Mn
LA-ICP-MS (ng/g)
900
800
700
600
500
400
300
200
100
0
1YDS
4YDS
Year of dental student (YDS)

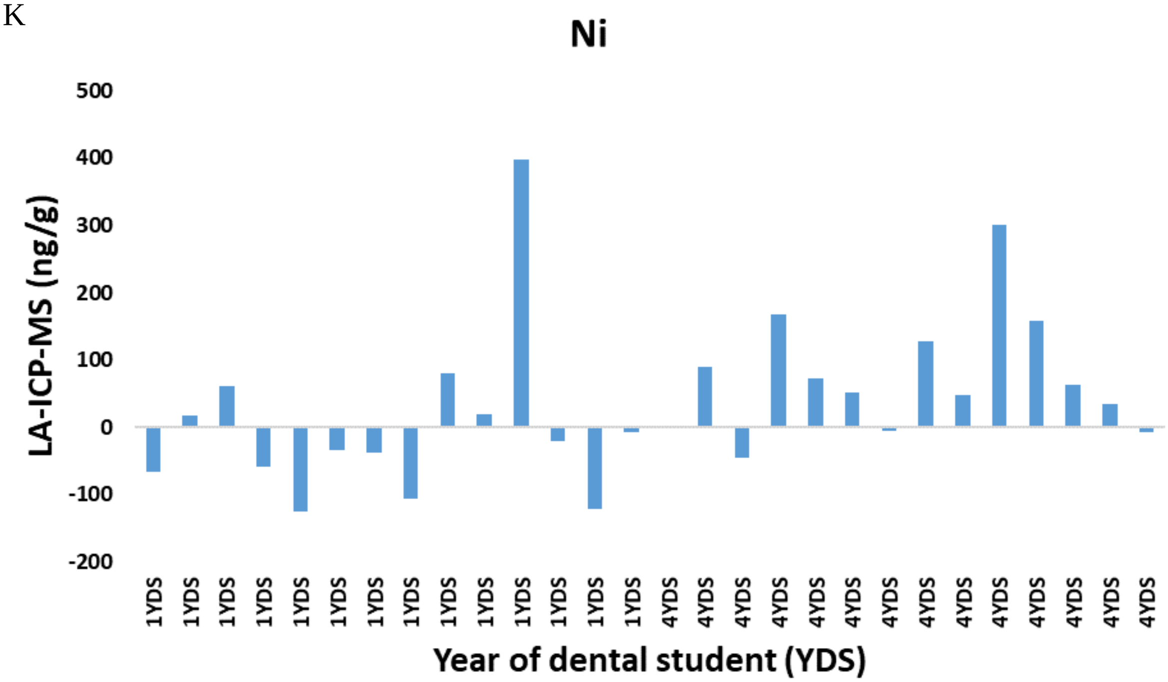
I


Fe
LA-ICP-MS (ng/g)
25000
20000
15000
10000
5000
0
1YDS
4YDS
Year of dental student (YDS)

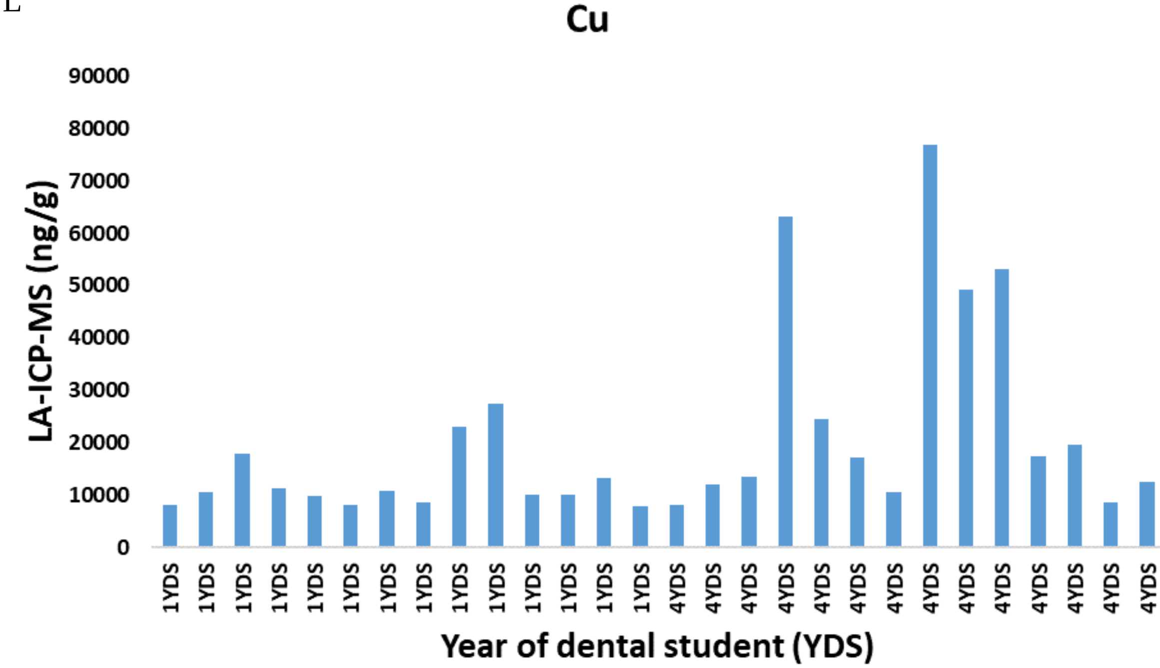
J


Co
LA-ICP-MS (ng/g)
120
100
80
60
40
20
0
-20
1YDS
4YDS
Year of dental student (YDS)

K


Ni
LA-ICP-MS (ng/g)
500
400
300
200
100
0
-100
-200
1YDS
4YDS
Year of dental student (YDS)

L



M

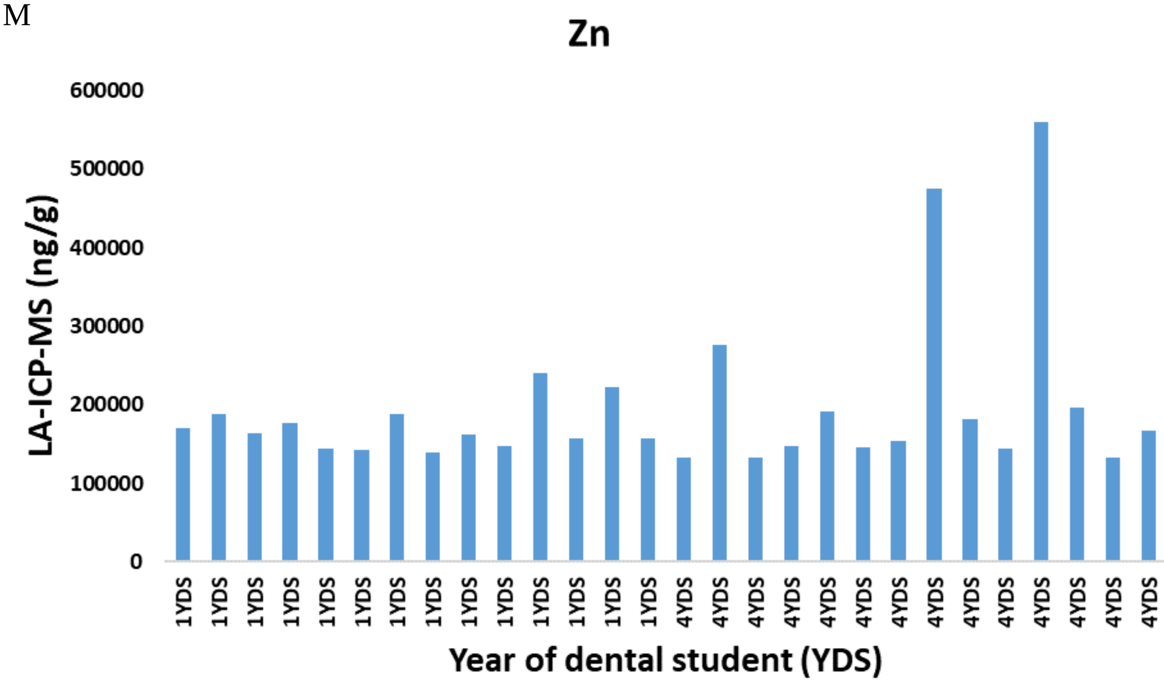


Figure 5.2 LA-ICP-MS results (ng/g) from 13 metal elements in hair of dental students in the 1st year and others in the 4th year of dental school.

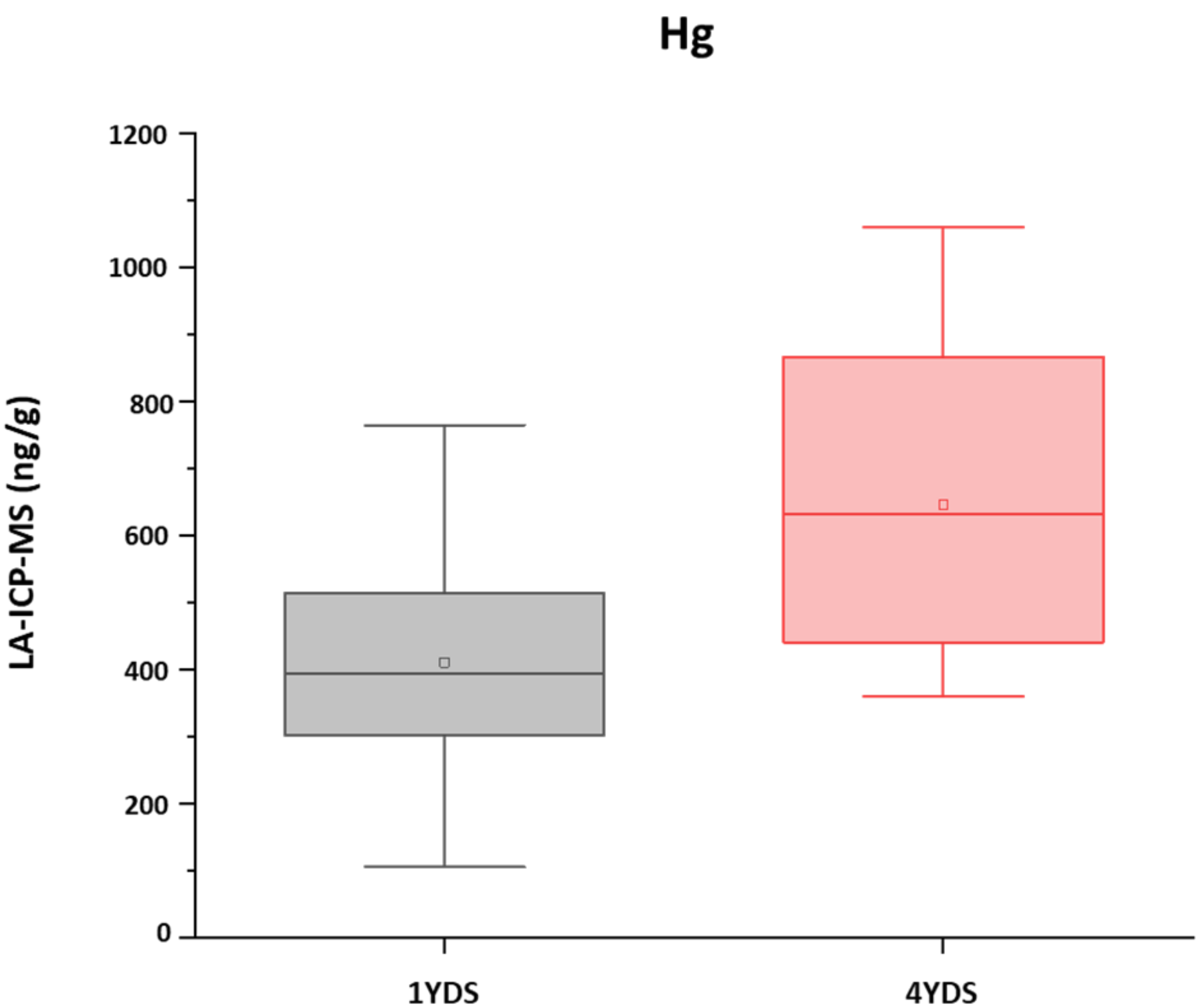


Figure 5.3 Hg concentration (ng/g) ± standard deviation in hair of dental students in the 1st year (1YDS) and others in the 4th year (4YDS) of dental school.

Table 5.8 Mean ± standard deviation (SD) of mercury concentration (ng/g) in hair of dental students in the 1st year and others in the 4th year of dental school

YDS	*Mean*	*SD*
1st year	410.31	169.05
4th year	645.71	225.06

5.3.2 Follow-up of 4 years study

The questionnaire reveled the participants average age (**Table 5.9**), percentage of sex (**Table 5.10**), hair coloring (**Table 5.11**), tinned food consumption (**Table 5.12**), fish consumption (**Table 5.13**), and presence of amalgam filling (**Table 5.14**).

The results in **Figure 5.4** and **Table 5.15** show that mercury concentration in the hair of dental students in the 2nd, 3rd, and 4th years was significantly higher than in the dental students in the 1st year. There is a peak of mercury concentration in the hair of dental students in the 2nd year of dental school. Furthermore, statistically, there is no difference between the mercury concentrations found in dental students in the 3rd and 2nd years, and in the 4th and 3rd years. However, mercury concentrations in the hair of dental students in the 4th year were significantly lower than in the dental students from the 2nd year.

Table 5.9 Range and average age of dental students.

	Range of age	***Average of Age***
Participants	20-23	22

Table 5.10 Sex of the dental students.

	Male	***Female***
Participants	70%	30%

Table 5.11 Percentage of hair coloring in dental students.

	Yes	***No***
Participants	30%	70%

Table 5.12 Tined food consumption by dental students.

	Yes	*No*
Participants	90%	10%

Table 5.13 Percentage of fish consumption (g/month) by dental students

Fish consumption (g/month)	*Percentages of fish consumption*
0g	40%
500g	50%
1kg	0
1.5kg	10%

Table 5.14 Percentage of amalgam fillings in the mouth of dental students.

	Presence of amalgam filling	
	yes	**No**
Participants	20%	80%

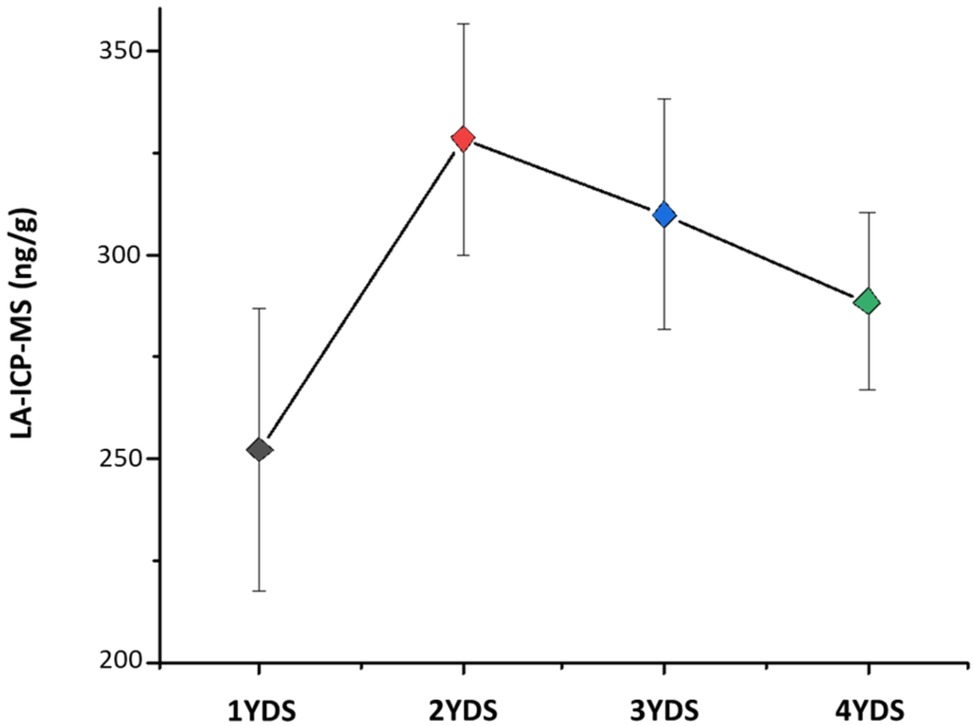


Figure 5.4 Hg concentration (ng/g) ± standard error (SE) in the hair of the same dental students by year of dental school

Table 5.15 Mean ± standard error (SE) of mercury concentration (ng/g) in hair of the same student during 1st, 2nd, 3rd, and 4th year of dental school.

YDS	*Mean*	*SE*
1st	252.19	34.34
2nd	321.12	25.59
3rd	304.59	28.61
4th	289.61	22.60

5.4 Discussion

In this study, we considered the exposure to metal elements relevant for students during dental school programs. Dental students have non-occupational exposure to several metal elements from dental materials, mainly the toxic mercury vapor from dental amalgams, which consist of approximately 50% Hg (Gelson Luis Adabo, 2018; Estrich et al., 2021). Furthermore, dental students are also exposed to other sources of metals through diet (methylmercury via fish consumption), lifestyle habits (hair coloring) and others (elemental Hg via dental amalgam fillings in the mouths). The pilot and the four years of follow-up study demonstrate that exposure to dental materials during dental school affects only Hg concentration in students´ hair. The first and second null hypotheses were rejected, while the third null hypothesis was accepted.

Despite studies pointing to a positive correlation between Hg concentrations in hair and fish consumption (Hui-Wen Hsiao et al., 2011, Santos et al., 2002; Bjornberg et al., 2003; IP et al., 2004; Legrand et al., 2005; Budtz-Jorgensen et al., 2007)., the results from pilot study showed that fish consumption was not a significant factor to increase Hg in hair of dental students, as well as tinned food consumption, hair coloring, and presence of amalgam filling. These finds suggest a high exposure of dental students to elemental mercury vapor during dental procedures, mainly mixing, condensation, and removal. This concerns the dental community due to the possible health effects of chronic exposure to elemental mercury vapor (Park & Zheng, 2012).

In the follow-up study, the 2nd, 3rd and 4th-year dental students exhibited higher hair Hg levels (**Figure 5.4** and **Table 5.15**) compared to the 1st year when they were not exposed to dental materials. This finding emphasizes the importance of using personal protective equipment and safety protocols to reduce Hg vapor in the dental environment. Interestingly, a decrease in the Hg levels in the 4th year can be observed, likely because of a decrease in laboratory and clinical practices. Furthermore, these results may also reflect improvements in protective measures after the Covid-19 Pandemic situation occurred in the dental school where the present study was performed.

A peak of Hg levels was observed in the 2nd YDS. Interestingly, the pre-clinical classes occur in the 2nd Year of dental school. Therefore, the high amount of dental fillings in artificial teeth performed in the pre-clinical classes and the practicing during the extra class hours is a possible explanation for this result.

Overall, the hair mercury level is usually <1 μg/g in individuals who do not eat fish frequently (Nuttall, 2006). Even the results found in this study showed an increase of mercury in the hair of students during dental school, the mercury levels detected did not exceed the common average or above the U.S. EPA Reference Concentration for human hair of 1 µg/g and World Health Organization (WHO) reference level of 2.0 μg/g for mercury in human hair (Reuben et al., 2020). However, WHO has also stated: “Recent studies suggest that mercury may have no threshold below which some adverse effects do not occur” (WHO, 2005). Furthermore, Studies point to susceptible subsets of the population may be more affected by chronic mercury exposure due to genetic components and polymorphisms (Echeverria et al., 2006; Woods et al., 2014; Kall et al., 2016; Warwick, 2019).

Changes in the time and routine of dental practices and the improvement of personal protective equipment for dental students may have affected considerably the results in the follow-up study. Furthermore, the lower number of participants in the follow-up study compared with the pilot study possibly influenced the mean of Hg concentration in the hair of dental students. Even so, in the follow-up study, the level of mercury in the dental students' hair was higher in all years compared to the control group. A similar result was found in a previous study with 180 dentists in the West of Scotland (Burke et al., 2004). Therefore, the results of this present study are relevant from a public health perspective.

It is noteworthy that the pilot study was primarily concerned with discerning disparities in metal concentration within the hair of students in their 1st and 4th academic years. Given the heterogeneous nature of the cohort in the initial study, it would have been exceedingly intricate to compare outcomes from both the pilot and subsequent studies, taking into account multifarious factors such as age, gender, hair pigmentation, consumption of canned food and fish, and the existence of dental amalgam fillings. Consequently, this investigation juxtaposed outcomes from the same subjects at different time points. Notwithstanding, the study findings are available to enrich comprehension of the extent to which dental trainees are exposed to metals from dental materials and to stimulate further research and discourse.

Evaluating the health risks related to mercury exposure in the dental environment becomes difficult when lower exposure levels are involved, and symptoms shift to more chronic patterns. Therefore, this study highlights the importance of long-term biomonitoring of mercury in dental students and professionals and the single hair analysis as an alternative to whole blood and urine analysis.

The high exposure to elemental mercury during dental school is a concern because elemental mercury vapor has a direct and indirect pathway to the CNS. The classic symptoms associated with chronic exposure to elemental mercury vapor (Hg °) involve the central nervous system (CNS) (Aschner & Aschner, 1990). The common symptoms are tremors, psychological disturbances or erethism, gingivitis, stomatitis, and excessive salivation (Langworth et al., 1997; Ngim, Foo, Boey, & Jeyaratnam, 1992; Park & Zheng, 2012). To prevent possible health effects associated with mercury exposure, the dental community should encourage using alternative dental restoration materials in dental clinics and pre-clinical training of students.

Hence the findings from this study point to a possible increase in mercury levels in students during dental school. This interesting finding highlights the importance of preventing and controlling mercury vapor in the dental environment.

Chapter 6 – Efficiency of standard barriers against mercury vapor from dental procedures.

6.1 Introduction

Mercury (Hg) is confirmed to be a hazardous element to human health (United Nations Environment Programme. Global Mercury Assessment. Geneva, Switzerland. 2002. Retrieved from https://wedocs.unep.org/handle/20.500.11822/12297Programme, 2002; World Health Organization.March 31, 2017.Mercury and health. Retrieved from https://www.who.int/news-room/fact-sheets/detail/mercury-and-health.WHO, 2017, March 31). Research has proven that inhaled gaseous Hg penetrates and accumulates in human tissues and cells, causing several pathophysiological impacts (Andreoli & Sprovieri, 2017; Bernhoft, 2012). Mercury is the most hazardous element for occupational health and environmental exposure to dental students and personnel. Even alternative materials (e.g., composite resin, glass ionomer, porcelain, and gold) dental amalgam remains the preferred restorative material in many countries, especially low- and middle-income nations (Nagpal et al., 2017). The market for Hg-containing dental amalgam increases approximately 5% during 2017-2023 with expectations to reach USD 500 million in 2023 (Bjorklund, Hilt, Dadar, Lindh, & Aaseth, 2019; Medgadget, 2018). Thus, the mercury exposure in the dental environment should increase following the market estimations. For this reason, understanding the mercury levels released during dental procedures with dental amalgam is essential for the prevention must take place.

During dental practices with amalgam, the aerosol containing Hg vapor and particulates are released into ambient air (Han et al., 2021; Krah-Sinan et al., 2017; D. Warwick et al., 2019). High-speed and low-speed handpieces are responsible for generating a large volume of aerosol and increase the risk of mercury spread. Furthermore, a study of dental student training revealed that, without the use of suction or water spray, mercury vapor levels released in the air with highspeed handpieces exceeded the absolute ceiling level for occupational exposure for an 8-hour time-weighted period (Warwick et al., 2013). This proves that the production of mercury vapor found when performing amalgam removal makes the air and surfaces of the dental treatment room during dental therapy a hazardous environment.

In addition to amalgam removal, other dental procedures or accidents may release mercury vapor into the air, such as during dental amalgam condensation, trituration, mercury spills and from mercury particles (Harrel & Molinari, 2004; Nagpal et al., 2017; D. Warwick et al., 2019; R.

Warwick, O'Connor, et al., 2013). For this reason, Dental students must be trained in mercury hygiene by using personal protective equipment to reduce their risk of mercury exposure through inhalation or skin exposure (R. Warwick, O'Connor, et al., 2013). However, Liu et al., 2019 (Liu et al., 2019) evaluated the efficiency of surgical masks and NIOSH-approved N95 respirators during drilling and grinding teeth restored with dental amalgam using an artificial head and an artificial breathing system. However, no reduction of mercury particles was observed.

Since there is limited evidence of the effectiveness of specific personal protective equipment to prevent inhalation of mercury vapor during dental procedures, more research is indicated to advise dental personnel to prevent health issues.

In this study, we quantify mercury vapor produced in a preclinical setting before and during amalgam removal and condensation to verify the effectiveness of varied specific personal protective equipment for operator protection from mercury exposure. The personal protective equipment studied included ASTM level 1, ASTM level 2 + face shield and ASTM level 3 + face shield, NIOSH-approved disposable 3M Aura 1870+ N95 respirator + face shield, a NIOSH-approved Precision Air reusable N95 respirator + face shield, a NIOSH-approved reusable Advantage 200 LS half mask N95 respirator + ASTM level 3 + face shield. Furthermore, the mercury vapor concentration was quantified inside and outside the fume hood, when it was on and off, before and after mixing the dental amalgam capsule.

6.2 Materials and methods

6.2.1 Materials

Amalgam Permit Regular Set (Southern Dental Industries- SDI, Victoria, Australia) was mixed in the Vari-mix III™ amalgamator (Caulk Dentsply Co, Toronto, Ontario, Canada) for mixing and condensation procedures, as well as filling the pre-modelled cavities for amalgam removal procedures. A head-form model (FloraCraft Corporation, Ludington, Michigan, United States) was coupled to the mercury analyzer during dental amalgam condensation and removal experiments to simulate dental personnel wearing a protective mask (**Figure 6.1**). The mercury analyzer detector tube was inserted into the head model oral cavity through the neck. The opening mouth of the head model was customized following the average dimension of 52mm, according to Xiao-Yan Li, 2017 (X. Li, Jia, & Zhang, 2017). During dental amalgam condensation and removal experiments, the head model was donned with different PPE sets according for each group.

The head model was donned with different PPE set for dental amalgam condensation and removal according to each group. The groups are described in **Table 6.1**. The PPE used in this study was ASTM Level 1 Mask (PRIMED Medical Products Inc., Edmonton, Alberta, Canada), ASTM Level 2 Mask (PRIMED Medical Products Inc., Edmonton, Alberta, Canada), ASTM Level 3 Masks (Ronco, Concord, Ontario, Canada), N95 1870 AURA (3M Company, St. Paul, Minnesota, USA), 95PFE – Precision AIR™ (Precision ADM Inc., Calgary, Alberta, Canada), and MSA Advantage 200-LS Respirator (MSA - The Safety Company, Cranberry Township, Pennsylvania, USA) and face shield. Only 95PFE – Precision AIR™ and 95PFE – Precision AIR™, and MSA Advantage 200-LS are reusable respirators, the first with disposable filters. All the surgical masks and respirators tested are approved by NIOSH and CAN/CSA3 standards.

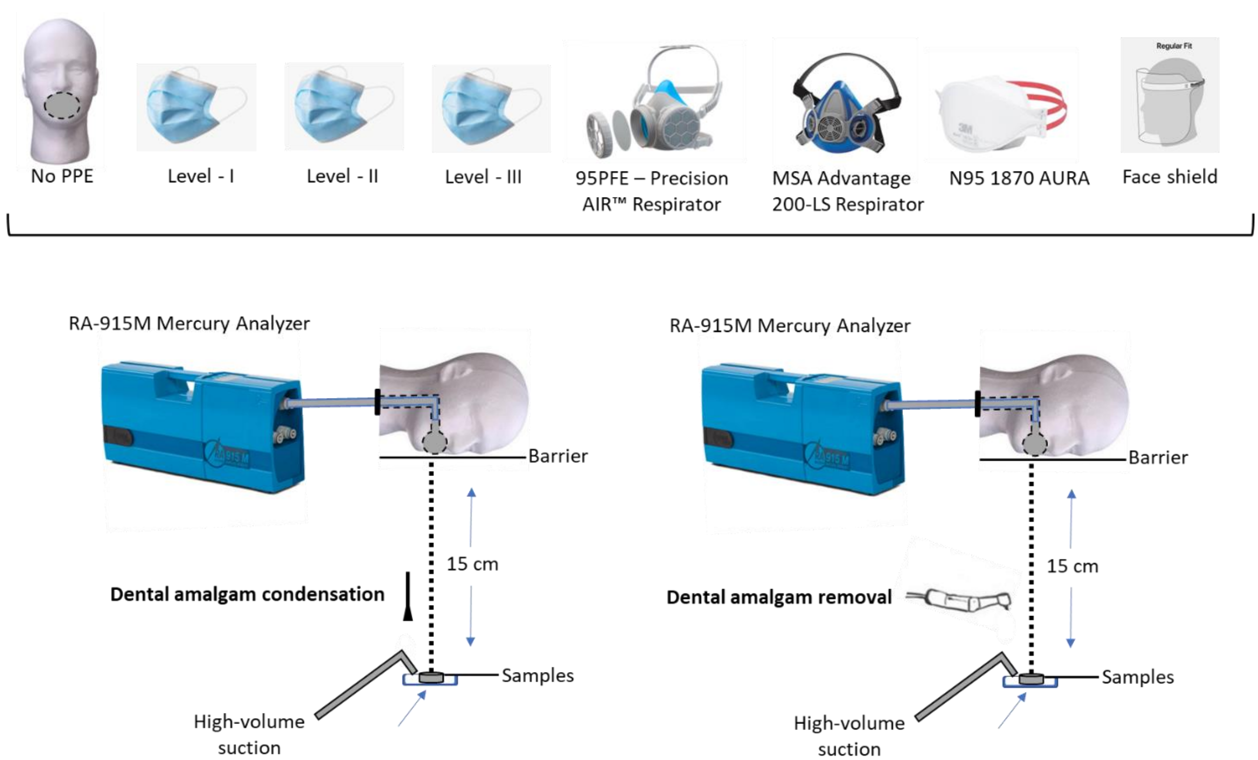


Figure 6.1 Illustration of dental amalgam condensation and removal and PPE type used.

Table 6.1 Description of PPE used in each group.

Groups	PPE
1	Without PPE (control)
2	Level-1 mask
3	Level-2 mask + faceshield
4	Level-3 mask + faceshield
5	Precision respirator + faceshield
6	Advantage respirator + level-3 mask + faceshield
7	N95 1870 AURA mask + faceshield

6.2.2 Sample preparation

Seventy dental amalgam samples were prepared in heavy silicon molds with dimensions of 2mm (Height) x 2mm (width) x 4mm (length) as a standard of 1-surface molar restoration involving the occlusal surface. After 24 hours, the samples were weighted and fixed in gypsum to facilitate the dental amalgam removal procedure in seven experimental conditions (n=10). Seventy molds with empty cavities were designed for dental amalgam condensation in seven experimental conditions (n=10).

The removals were performed using a standard high-speed handpiece equipped with a #556 bur, and the dental amalgam condensation was performed with a dental amalgam condenser. The dental amalgam fillings were removed using water spray from the high-speed handpiece and a high-volume suction beside the sample. For the dental amalgam condensation procedure, only the high volume suction beside the sample was used (**Figure 6.1**).

6.2.3 Elemental mercury analysis

6.2.3.1 Mercury analyzer

The concentration of gaseous elemental mercury (GEM) in this study was monitored by the Lumex RA-915M mercury analyzer (Lumex Instruments, St. Petersburg, Russia). Lumex is a portable atomic absorption spectrometer that gives real-time GEM readings. The mercury analyzer detector was coupled and sealed in the oral cavity through the neck of the head model, as illustrated in **Figure 6.1**. The Lumex pumped the air through the instrument's multi-path detection cell at 10 L min–1 flow rate, and GEM concentration data was acquired every 5 s. An automatic zero correction (60 s duration) was initiated every 30 min to correct for baseline drift during sampling. Instrumental precision was measured before and after sampling using the instrument's self-test function; standard deviation (D) values were within ± 5% of the check value for all self-tests. The instrument's detection limit with accuracy is 50 μg/m^3.

6.2.3.2 Dental amalgam mixing procedure

GEM was measured under eight experimental conditions detailed in **Table 6.2**. In each experiment, data were acquired every 5 s for 1.8 minutes (n=22). The mercury analyzer detector was positioned inside the fume hood (15 cm far from amalgamator) or near the fume hood (15cm far from fume hood access, 1.5 m height, and 30 cm far from the amalgamator) as illustrated in **Figure 6.2** and **6.3**. The mixing time was 8s according to the dental amalgam capsule manufacture´s recommendations on the amalgamator type, age, and line voltage.

Table 6.2 Description of experimental conditions for each group.

Groups	Experimental conditions		
	Fume hood	**Lumex Distance**	**Mixing (Timer)**
1	On	Inside	0s
2	On	Inside	8s
3	On	Near	0s
4	On	Near	8s
5	Off	Near	0s
6	Off	Near	8s
7	Off	Inside	0s
8	Off	Inside	8s

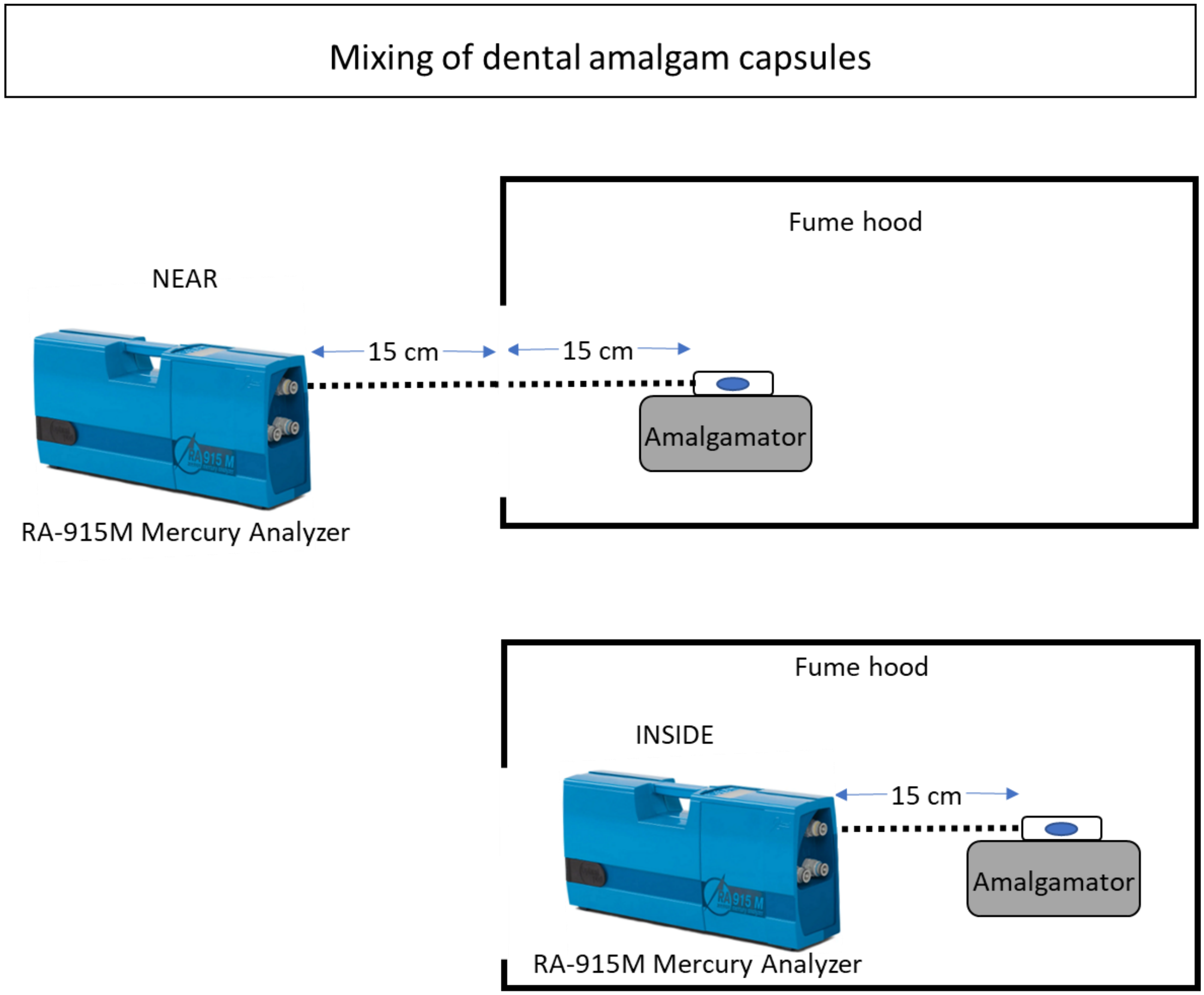


Figure 6.2 Illustration of mercury analyzer position related to the fume hood and amalgamator.

Figure 6.3 Picture of the fume hood, amalgamator, and the mercury analyzer near to the fume hood access.

6.2.3.3 Amalgam removal and condensation

GEM was measured in each group (**Table 6.1**) before (background checking) and during dental amalgam condensation and removal. In each experiment, data was acquired every 5 s for 30 s. For each group, ten repetitions of 30 seconds were performed (n=10). After the removal procedure, the remaining dental amalgam of the sample was weighed.

Each PPE set was adjusted to the head model face. Only 95PFE – Precision AIR™ and MSA Advantage 200-LS Respirator were sealed with impermeable tape. Packages were only opened immediately prior to mercury analysis and were handled carefully. The PPE sets were handled using nitrile gloves, touching only the straps of the surgical masks, respirators, and face shield to reduce the potential for further contamination. The face shield was washed and cleaned with a 70% Isopropyl Alcohol pad (Alliance Alcohol Prep Pads, Medical Mart Supplies Ltd, Mississauga, Ontario, Canada) after each analysis.

The amalgam removal occurred with the simultaneous use of water spray and suction, during the condensation was with the use of suction only.

6.2.3.4 Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for the results of the mixing procedure, Shapiro Wilkes with Kruskal-Wallis ANOVA for results of the condensation procedure, and Shapiro Wilkes with one-way analysis of variance (ANOVA). Pearson correlations coefficient was used to measure the coefficient of the relationship between the amount of dental amalgam removed in each sample and the amount of mercury detected by the mercury analyzer instrument. The background was measured before each analysis and subtracted from the result. Differences were considered statistically significant when $p<0.05$. All statistical analyses were carried out using the OriginLab software system version 9.2.

6.3 Results

6.3.1 Mixing

The results of GEM concentrations for the seven experimental conditions are described in **Figure 6.4** and **Table 6.3**. The highest mean of Hg vapor concentrations (µg/m3) was recorded in group 8 (57.8 ± 9.9). This result was statistically different from other groups. The mean of group 7 (9.1 ± 1.8) was higher than the other groups, except to the group 8 (57.8 ± 9.9). The mean of group 7 was 6 times lower than the group 8. The group 7 means are significantly different from all the other groups. The mean of group 1, 2, 3, 4, 5, and 6 are not statistically different.

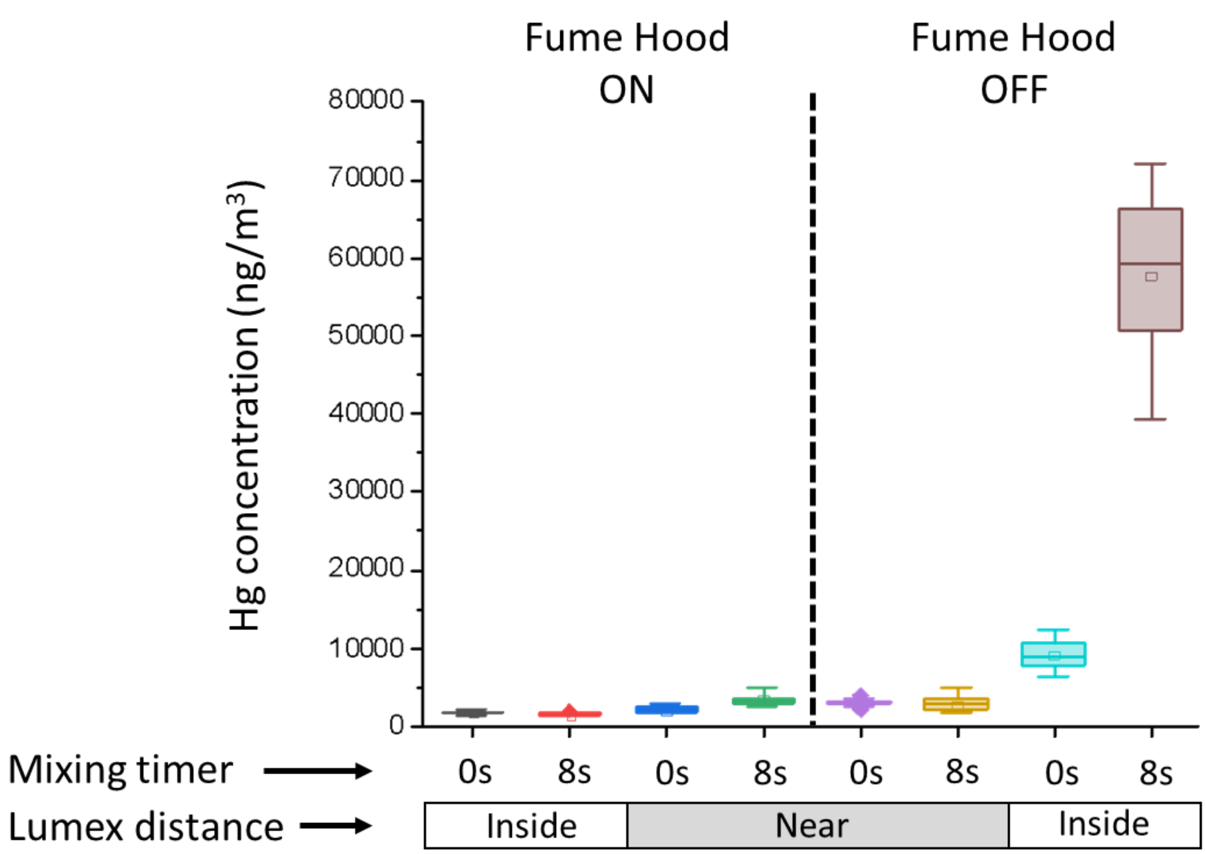


Figure 6.4. Graph of Hg vapor concentrations (ng/m^3) under eight experimental conditions.

Table 6.3 Measured Hg vapor concentrations (μg/m^3) under eight experimental conditions. Different letters show that, at the 0.05 level, the population means are significantly different.

Group	*Experimental conditions*			*Mean of Hg concentration (μg/m3)*	*Standard Deviation (μg/m3)*
	Fume hood	Lumex Distance	Mixing (Timer)		
1	On	Inside	0s	1.7[a]	± 0.2
2	On	Inside	8s	1.5[a]	± 0.2
3	On	Near	0s	2.1[a]	± 0.3
4	On	Near	8s	3.3[a]	± 0.6
5	Off	Near	0s	3.1[a]	± 0.4
6	Off	Near	8s	2.9[a]	± 0.9
7	Off	Inside	0s	9.1[b]	± 1.8
8	Off	Inside	8s	57.8[c]	± 9.9

6.3.2 Condensation

The results of GEM concentrations during the dental amalgam condensation procedure under seven experimental conditions are described in **Figure 6.5** and **Table 6.4**. The lowest median and max of Hg vapor concentrations (µg/m^3) were recorded in group 8 (0.1 and 0.1, respectively). For, the PPE set from group 7 showed the lowest median (0.1 µg/m^3), but statistically no differences were found in the efficiency of the PPE set of groups 7 (0.1 µg/m^3) and 6 (0.6 µg/m^3). Even though the highest median was found in the control group, statistically, no differences were found in group 2 (1.0 µg/m^3), 4 (1.2 µg/m^3), and 5 (0.8 µg/m^3). Also, no statistical differences between group 3 (0.5 µg/m^3), 5 (0.8 µg/m^3), and 6 (0.6 µg/m^3) were observed.

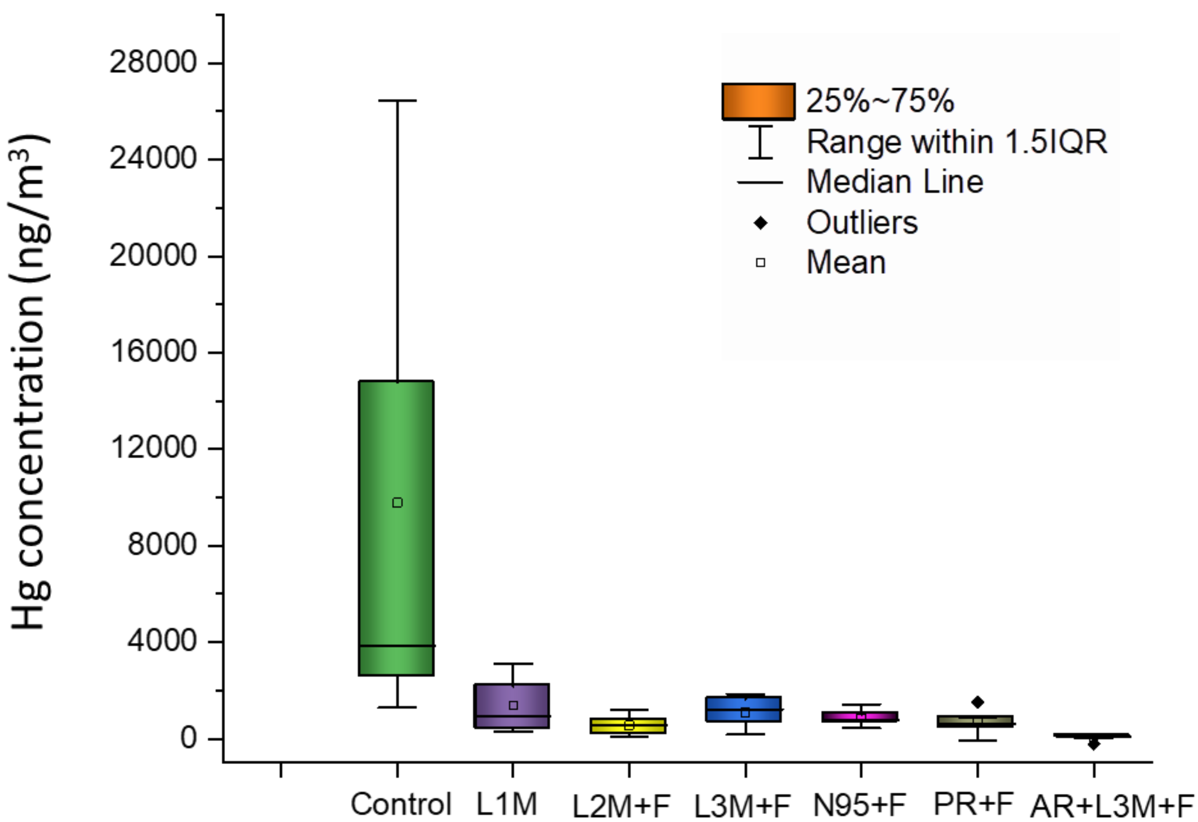


Figure 6.5: Graph of Hg vapor concentrations (ng/m^3) during dental amalgam condensation under seven experimental conditions.

Table 6.4 Measured Hg vapor concentrations (μg/m^3) during dental amalgam condensation under seven experimental conditions. Different letters show that, at the 0.05 level, the population medians are significantly different.

Group	*PPE*	*Median (μg/m3)*	*Max of Hg concentration (μg/m3)*
1	*Without PPE (control)*	3.9^{a}	26.5
2	*Level-1 mask (L1M)*	1.0^{ab}	3.1
3	*Level-2 mask + faceshield (L2M+F)*	0.5^{b}	1.2
4	*Level-3 mask + faceshield (L3M+F)*	1.2^{b}	1.9
5	*N95 1870 AURA mask + faceshield (N95+F)*	0.8^{abc}	0.1
6	*Precision respirator + faceshield (PR+F)*	0.6^{bcd}	1.4
7	*Advantage respirator + level-3 mask + faceshield (AR+L3M+F)*	0.1^{d}	1.5

6.3.3 Amalgam removal

The results of GEM concentrations during dental amalgam removal for the seven experimental conditions are described in **Figure 6.6** and **Table 6.5**. The greatest mean and maximum of GEM concentration were recorded in group 1 (20.4 ± 13.3 μg/m^3 and >50 μg/m^3, respectively. The control group's mean was statistically different from the other groups. The PPE set of groups 5 (1.7 μg/m^3) and 6 (2.4 μg/m^3) presented the lower mean among the groups. Groups 5 and 6 are not statistically different, likewise groups 2 (4.2 μg/m^3), 3 (2.6 μg/m^3), 4 (2.8 μg/m^3), and 7 (3.4 μg/m^3).

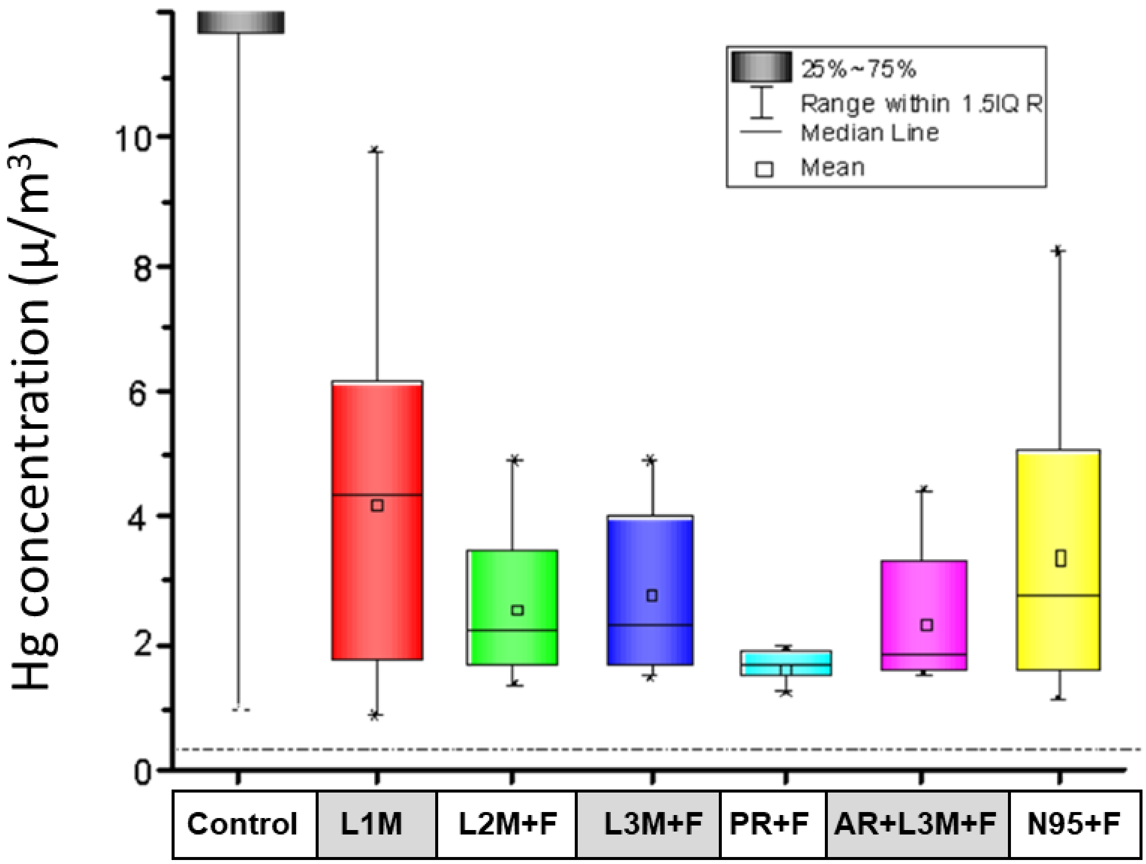


Figure 6.6: Graph of Hg vapor concentrations (μg/m3) during dental amalgam removal under seven experimental conditions.

Table 6.5 Measured Hg vapor concentrations (μg/m^3) during dental amalgam removal under seven experimental conditions. Different letters show that, at the 0.05 level, the population means are significantly different.

Group	*PPE*	*Mean of Hg concentration*	*Standard Deviation*	*Max of Hg concentration*	*Median*
1	*Without PPE (control)*	20.4[a]	± 13.3	>50	17.0
2	*Level-1 mask (L1M)*	4.2[b]	± 2.9	24.8	4.3
3	*Level-2 mask + faceshield (L2M+F)*	2.6[b]	± 1.2	8.8	2.2
4	*Level-3 mask + faceshield (L3M+F)*	2.8[b]	± 1.3	14.6	2.3
5	*N95 1870 AURA mask + faceshield (N95+F)*	1.7[c]	± 0.2	3.9	1.7
6	*Precision respirator + faceshield (PRF+F)*	2.4[c]	± 1.0	8.4	1.8
7	*Advantage respirator + level-3 mask + faceshield (AR+L3M+F)*	3.4[b]	± 2.4	20.0	2.8

6.3.4 Threshold levels

Figure 6.7 shows that the mean values of mercury vapor concentration found in the dental amalgam condensation, removal procedures, and median values of the mixing of dental amalgam capsules inside the fume hood turned off exceed the occupational threshold limit of the ACGIH (8hour TWA, Skin), AOTLV (8hour TWA, Skin), and the non-occupational chronic inhalation threshold limit of the ATSDR –MRL (0.3 μg/m^3), US EPA – RfC (0.3 μg/m^3), CA EPA – REL (0.03 μg/m^3), CA EPA – REL Intermittent 8 hrs-inhalation (0.06 μg/m^3), CA EPA – REL Intermittent 1 hr-inhalation (0.06 μg/m^3). Only the median values of the mixing procedure of dental amalgam capsules inside of the fume hood turned off exceed the NIOSH (8-hour TWA, Skin).

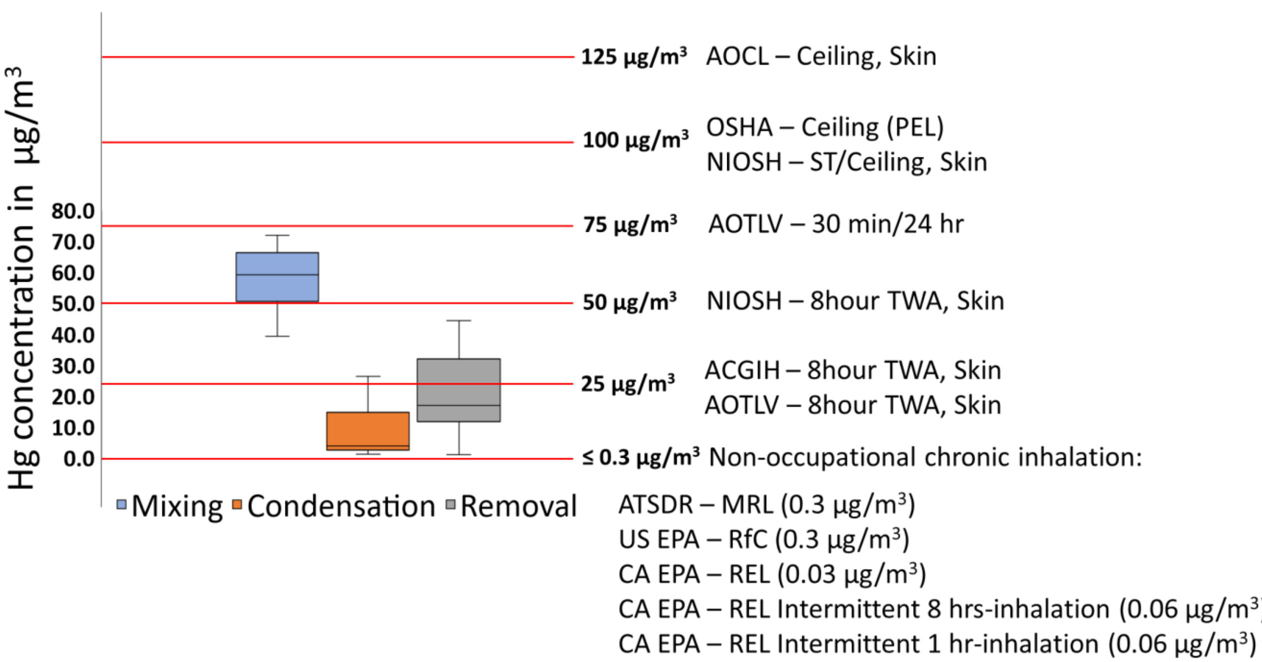


Figure 6.7 Mercury vapor concentrations from the mixing procedure of dental amalgam capsules inside the fume hood turned off, the condensation and removal procedure without PPE relative to occupational (NIOSH, ACGIH, OSHA, AOHS) and non-occupational (ATSDR, US EPA, CA EPA) threshold levels. Legend: Occupational Safety and Health Administration (OSHA, U.S.); National Institute for Occupational Safety and Health (NIOSH, U.S.); Association Advancing Occupational and Environmental Health/American Conference of

Governmental Industrial Hygienists (ACGIH, U.S.); Occupational Health and Safety (AOHS, Alberta, Canada); Agency for Toxic Substances and Disease Registry (ATSDR); California Environmental Protection Agency Office of Environmental Health Hazard Assessment (CA EPA - OEHHA); Minimal Risk Levels – MRL (ATSDR a chronic-duration inhalation MRL for elemental mercury was derived based on neurological effects observed in epidemiological studies); Time Weighted Average (TWA) is the average limit of the substance concentration that shall not exceed in usually 8 hours in the workday; Ceiling limit (C) is the exposure level that shall at no time be exceeded; The short-term exposure limit (ST) is a 15- 8 minute TWA exposure that should not be exceeded at any time during a workday; Reference Concentration (RfC) estimate of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime; Recommended Exposure Limits – REL (NIOSH REL is the NIOSH-recommended time-weighted average exposure concentration for up to a 10-hour workday during a 40-hour workweek that should not be exceeded; CA EPA REL is the airborne concentration of a chemical that is not anticipated to result in adverse noncancer health effects for specified exposure durations in the general population, including sensitive subpopulations; CA EPA Chronic REL is designed to address continuous exposures for up to a lifetime: the exposure metric used is the annual average exposure); Threshold Limit Value – TLV (ACGIH TLV is the recommended time-weighted average concentration to which most workers can be exposed over a lifetime without adverse effects); Permissible Exposure Limits – PEL (OSHA PEL is the time-weighted average concentration that must not be exceeded during any 8-hour work shift of a 40-hour workweek).

6.3.5 Samples weight

This study showed a 95% correlation between the amount of dental amalgam removed in each sample and the amount of mercury detected by the mercury analyzer instrument **(Figure 6.8)**.

Pearson Correlations

		A	B
A	Pearson Corr.	1	0.95482
	p-value	--	0.01145
B	Pearson Corr.	0.95482	1
	p-value	0.01145	--
2-tailed test of significance is used			

Figure 6.8. Image from Pearson correlations test result in the Origin software showing 95% of correlation (p < 0.01) between the amount of dental amalgam removed in each sample and the amount of mercury detected by the mercury analyzer instrument.**

A. the amount of dental amalgam removed in each sample **B.** the amount of mercury detected.

6.4 Discussion

The dental amalgam is still an option of restorative material in many countries. Even banned or under restrictions in some Nordic countries in Northern Europe, such as Norway, Sweden, Scandinavia, and Denmark, the dental amalgam restoration removal procedure is still a reality in dental clinics (Kopperud, Staxrud, Espelid, & Tveit, 2016). Thus, the protective measurements for dental students, dentists and dental personnel against mercury vapor released from mixing, condensation and removal procedures with dental amalgams should not be underestimated. In this present study, the results obtained showed that the mercury vapor released in mixing procedure of dental amalgam capsules may be reduced by 38 fold if the amalgamator is inside of fume hood turned on. However, an individual near the fume hood is not fully protected against mercury vapor during this dental procedure. In addition, the Precision respirator + face shield and Advantage respirator + face shield + level-3 mask was more efficient in blocking the mercury vapor from dental amalgam condensation and removal than the other PPE set tested. Therefore, the first and second and third null hypotheses were rejected.

The mixing procedure of dental amalgams can be done with or without fume hood equipment. When the amalgamator is inside of this equipment, the fume hood is able to draw air containing mercury vapors emitted from the mixing process of dental amalgam. Then, the aspirated air is filtered and released into the external environment. The results showed that the mercury concentration inside of the fume hood reduced from 57.8 $\mu g/m^3$ (fume hood off) to 1.5 $\mu g/m^3$ (fume hood on) with mixing procedure and from 9,1 $\mu g/m^3$ (fume hood off) to 1.7 $\mu g/m^3$ (fume hood on) without mixing procedure of dental amalgam capsules. Even the fume hood is on or off and with or without mixing procedure, the range of mercury concentration was between 1.5 $\pm$ 2 $\mu g/m^3$ and 3.3 $\pm$ 0.6 $\mu g/m^3$. However, statistical variations in mercury concentration were not observed near the fume hood, where the operator (dental student, dentist, professor, researcher, and dental personnel) is commonly positioned. These results reveal to the dental community the necessity of the dental student, dentist, professor, researcher, and dental personnel to don appropriated PPE for mercury exposure when mixing dental amalgam capsules even near a fume hood.

A high mean of mercury vapor was found inside of the fume hood even before the mixing procedure of dental amalgam capsules. This result suggests internal contamination of the amalgamator. The findings are in alignment with a previous study (Roberts, Leonard, & Osborne, 2001), in which different amalgamator units presented mercury contamination below the capsule mixing area, on the electric drive motor casing, and within the electrical motor wiring. In addition, some units had mercury vapor levels that exceeded the OSHA permissible exposure limit (0.1 mg/m^3). The amalgamator contamination is attributed to mercury leakage from amalgam capsules inside the amalgamator (Anderson, Kuhl, & Schloyer, 1988; Roberts et al., 2001). Therefore, the ideal was to use a new amalgamator for all the analysis in the present study. However, the available amalgamator for this study was a unit that has been used by dental students in dental clinics and pre-clinical teaching laboratories, which is more representative of possible of the clinical setting.

Interestingly, all the medians of mercury concentration in the condition near the fume hood with and without mixing procedure was similar or lower than the median found in the control group (without PPE) of condensation. These results indicate that the same PPE set can be used for mixing near to the fume hood and condensation procedures. Therefore, possibly the Precision respirator + face shield and Advantage respirator + face shield + level-3 mask is also more efficient in blocking the mercury vapor from mixing of dental amalgam capsules.

It is known that for procedures with dental amalgam, a critical component for mercury vapor spreading is the usage of the high-speed handpiece (Matys & Grzech-Lesniak, 2020). This agrees with the results of the present study that the dental amalgam removal procedure released approximately five times more mercury vapor than the mixing procedure near to fume hood and the condensation procedure. In addition, the efficiency of all the PPE set tested is reduced for the removal procedure when compared with the condensation procedure. However, the mixing procedure inside the fume hood turned off release approximately twice more mercury vapors than dental amalgam removal. Considering that the mercury exposure from the mixing procedure inside of the fume hood turned off corresponds to the same mercury exposure from a mixing procedure without the fume hood, these results reveal the following sequence for risk of procedure for mercury vapor exposure: mixing procedure without fume hood > removal procedure > condensation procedure. If the individual is near the fume hood turned on, the sequence for risk of mercury vapor exposure will change for removal procedure > condensation procedure = mixing procedure without fume hood.

Based on the findings, the mercury vapor released in dental procedures with amalgams such as mixing, condensation, and removal may exceed some occupational and non-occupational safety levels from different human health and safety protection agencies followed by jurisdictions of states or provinces in the U.S.A. and Canada (**Figure 6.7**). Furthermore, the risk of mercury vapor may be increased in cases where multiple dental procedures are performed in the same room simultaneously or in sequence. Some clear examples are the pre-clinical teaching laboratories, where many dental students practice amalgam restorations or dental clinics, where several appointments occur in the same room simultaneously. In these cases, the mercury vapor released may accumulate in the same ambient air and easily reach safety limits for human health.

Therefore, before dental procedures with dental amalgam it is important follow general recommendation for mercury exposure in dental environment, such as provide a guideline of risk for each specific procedure with dental amalgam, use properly designed room with ventilation systems and air filtration equipment with a particular filter for mercury, perform air monitoring and clean internally the amalgamator periodically, use appropriated PPE for mercury levels compatible with the type and volume of dental procedure to be performed with the dental amalgam, choose an alternative material to dental amalgam when it is applicable, use non-latex dental dam with saliva suction on the patient, use entire body barrier for patient, use air supply to patient, clean dental instruments and surfaces with precautions including wipe the handpiece with mercury decontaminant before starting the manufacturer's instructions ("Canadian Centre for occupational and safety. OSH Answers Fact Sheets, Mercury," 2023; Colson, 2012; "Occupational Exposure to Elemental Mercury in Dentistry," 2012; D. Warwick et al., 2019; "World Dental Federation. Mercury Hygiene Guidance," 2007).

Chapter 7 – Conclusion and future directions

7.1 Conclusion

The dental environment is contaminated with metals released from dental materials in dental procedures. The intensity of this contamination is increased with aerosol generation by high-speed handpiece usage. This aerosol may contain invisible particles and odorless vapors that may be inhaled or absorbed through the skin. In general, this is highly relevant for the safety and health protection of the dental community because studies have shown adverse health effects related to metal exposure in Dentistry.

The first study evaluated internal distributions of trace elements in the cross-section hair. The BSE images clearly revealed inhomogeneous distribution of trace elements across the cuticle and cortex. LA-ICP-MS line transactions across hair cross-section confirm Mn, Cu, Zn, Sr, Ag, and Hg are more elevated in the cuticle. Because of the inhomogeneity, complete laser ablation shows a better agreement with the SN-ICP-MS than partial laser ablation, demonstrating the importance of complete ablation in single hair strand analysis by LA-ICP-MS. The developed LA-ICP-MS technique will be applied to monitor the health of students exposed to dental environments.

In the second study, the concentration of 13 trace metals in the hair strand of dental students was investigated. The results showed that the exposure to dental materials during dental school significantly affected Hg concentration in hair of dental students who participated in the pilot and those in the 4-year follow-up study. In addition, fish consumption, tinned food consumption, hair coloring, and the presence of amalgam filling in the mouth were not a significant factor in increasing Hg concentration in the pilot study. These finds suggest a high exposure of dental students to elemental mercury vapor during dental procedures, mainly mixing, condensation, and removal.

In the third study, we evaluated mercury vapor exposure in dental amalgam procedures. The results showed that the mercury vapor released in the mixing procedure of dental amalgam capsules may be reduced by 38 fold when the amalgamator is inside of the fume hood turned on. However, an individual near to the fume hood is not fully protected against mercury vapor during this dental procedure. For mixing, condensation, and removal, the Precision respirator + face shield and Advantage respirator + face shield + level-3 mask was more efficient in blocking the mercury vapor among all PPE sets tested. Considering that the mercury exposure from mixing procedure inside of the fume hood turned off corresponds to the same mercury exposure from a mixing procedure without fume hood, these results reveal the following sequence for risk of procedure for mercury vapor exposure: mixing procedure without fume hood > removal procedure > condensation procedure. The results also revealed that the mercury vapor released in dental procedures with amalgams such as mixing, condensation, and removal exceed some occupational and non-occupational safety levels from different human health and safety protection agencies followed by jurisdictions of different states or provinces in the U.S.A. and Canada.

This was the first study of biomonitoring trace element levels in strand hair of dental students using a state-of-the-art technique such as the LA-ICP-MS. Findings from this study corroborate with the hypothesis that trace metal elements concentration increases in the students' hair during the years of exposure to dental materials in dental school. In addition, these results will guide agencies, dental associations, and dental schools to improve the occupational and non-occupational guidelines for the health and safety protection of dental students, dentists, and dental personnel to elemental mercury vapor related to dental procedures.

7.2 Future directions

Knowing several adverse effects may be attributed to short- or long-term exposure to elemental mercury depending on a load of exposure, the interest in understanding the risk of mercury exposure for the dental community is a public health issue.

The results suggest complete ablation of hair strands in LA-ICP-MS is an excellent tool for this purpose. However, it would be essential to investigate the association between mercury concentration through the biomonitoring of biomarkers and health effects on dental students, dentists and dental personnel in the short and long term. For example, whether mercury vapor absorbed from dental procedures can influence cognitive performance (memory, integrative function) and subjective, physiological symptoms (mood swings, irritability, nervousness, timidity, loss of confidence). Animal studies will also be useful to investigate the renal and reproductive effects of the mercury levels released from dental procedures in the short and long-term of exposure.

Findings from this thesis showed that none of the masks and respirators tested blocked 100% of the mercury vapor released during the dental amalgam removal procedure. Therefore, future studies testing the efficiency of other PPE’s may help find the more appropriate PPE set to be donned during dental amalgam removal.

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